The effects of drought, heat and elevated atmospheric CO₂
on physiology and growth of *Eucalyptus* –
does climate-of-origin matter?

Anita Wesolowski

BSc, MSc

A thesis submitted in fulfilment of the requirements for the degree of

*Doctor of Philosophy*

August 2017
Acknowledgements

I am grateful to my principal supervisor David Tissue for his great guidance and support. He has given me fantastic advice due to his large repertoire of scientific knowledge and experience. He has consistently made time to read and quickly give back feedback on drafts and at the same time found kind and positive words for me, making sure I am on the right track.

I owe a great debt of gratitude to Sebastian Pfautsch who has allowed me to stalk him for more than seven years now and without whom I might have never started my PhD program. He has always given me a boost of confidence that I can finish this degree and inspired me to become a better version of myself by knowing how passionate he is about his work and how much he cares for his students, always going the extra mile to see them succeed. Thanks for being my friend.

Special thanks to Mark Tjoelker, Víctor Resco de Dios and Chris Blackman for reading my thesis and providing helpful comments over the last months.

I am thankful to John Drake and Mike Aspinwall for statistical advice. I was fortunate enough to work with and getting amazing technical support from Renee Smith, Carrie Drake, Burhan Amiji, Craig Barton and Gavin McKenzie who have a great pool of expertise, making this institute so successful. My thanks particularly go to Renee for running the glasshouse so perfectly and always having an answer to my many questions with a confident smile, it would have been impossible without her. Special thanks to Wen Shi and Danielle Creek for sharing, developing and working on some of the projects with me.

I am grateful to have had so many additional helping hands: Joey Kerr, Giles Ross, Will Balmont, Mati Simoes, Scott Bevins, Chelsea Maier, Norbert Klause and Morgan Ryan. They have always tried to achieve the best possible result which made my research so much easier and very enjoyable.

I am thankful for my wonderful friends that I met in Australia and who have so many diverse qualities making this experience even more amazing and
unforgettable. Thanks to Peter Kern and Kirk Barnett for reading my thesis and spending so many hours to this day with me discussing life and its ups and downs. I am grateful for Kirk’s enthusiasm for R and his patience when teaching others how to use it. Thanks to my dear friend Chelsea Maier who has always made me feel loved and has been through so many crazy adventures with me. Thanks to my office mates Juan Piñeiro and Lisa Bromfield for not making me feel alone in these challenging times and thanks to Juan for enjoying discussions about statistics and R. I am thankful for the time I spent with Robert Müller, Agnieszka and Norbert Klause over the last few months, playing board games and cooling off in the pool during these epic heat waves, it was key to keeping my sanity. The list of friends I am grateful to goes on, but I am sure they know who they are, it would have never been so much fun without them. I am so grateful that they have been around and hope we will always be in each other’s lives. I am also thankful to my friend Luise Schmidt for calling me over all these years I have spent in Australia on a regular basis, listening to me and giving me all the news from home.

Last but not least, I am grateful to my parents for always supporting and taking care of me no matter how far I am away, for skyping with me uncountable times and sending me care packages even after the third time of me moving to Australia.

Thanks to the Science and Industry Endowment Fund (SIEF) and Higher Degree Research Scholarship from the Hawkesbury Institute for the Environment for funding this project.
Statement of Authenticity

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.
# Table of Contents

List of Figures ......................................................................................................................... i

List of Tables ............................................................................................................................. iii

List of Abbreviations ................................................................................................................ iv

Abstract ................................................................................................................................. vi

Chapter One: General Introduction ......................................................................................... 1
   1.1 Elevated [CO$_2$] and tree growth ................................................................................. 3
   1.2 Drought stress and tree growth ............................................................................... 6
   1.3 Heat stress and tree growth ..................................................................................... 9
   1.4 Combined effects of eCO$_2$, drought and heat stress on tree growth .............. 11
   1.5 Phenotypic plasticity ............................................................................................. 14
   1.6 Harnessing phenotypic plasticity for future tree growth .................................. 17
   1.7 Thesis objectives ................................................................................................... 20
   1.8 Thesis outline ........................................................................................................ 21

Chapter Two: The impact of climate of origin – responses of E. camaldulensis
provenances grown at elevated [CO$_2$] during and after heat and water stress .......... 25
   2.1 Introduction ............................................................................................................ 25
   2.2 Material and Methods ............................................................................................ 32
   2.3 Results .................................................................................................................... 40
   2.4 Discussion .............................................................................................................. 58
   2.5 Conclusion .............................................................................................................. 67

Chapter Three: Intra-specific variation of physiological response to elevated CO$_2$
and water limitation in Eucalyptus grandis ............................................................................ 69
   3.1 Introduction ............................................................................................................. 69
   3.2 Material and Methods ............................................................................................... 75
   3.3 Results ....................................................................................................................... 83
   3.4 Discussion ............................................................................................................... 99
   3.5 Conclusion .............................................................................................................. 107
Chapter Four: Drought-hardiness of *Eucalyptus melliodora* and *E. coolabah* is linked to leaf area and carbohydrate reserves

4.1 Introduction ................................................................. 109
4.2 Material and Methods ..................................................... 114
4.3 Results ........................................................................... 122
4.4 Discussion ...................................................................... 139
4.5 Conclusion ...................................................................... 147

Chapter Five: Synthesis and Conclusion ........................................ 149

5.1 Thesis aim ....................................................................... 149
5.2 Intra-specific variation of traits in *E. camaldulensis* and *E. grandis* grown in aCO₂, ambient temperature and non-limiting water and nutrient conditions ........................................ 150
5.3 Intra-specific variation of traits in *E. camaldulensis* and *E. grandis* grown in eCO₂ ......................................................................... 151
5.4 Intra-specific differences of *Eucalyptus* to heat and drought .......... 152
5.5 Intra-specific differences of *Eucalyptus* to a combination of environmental factors ............................................................................. 155
5.6 Conclusion ........................................................................ 156

Bibliography ........................................................................ 158
Appendix ............................................................................. 190
List of Figures

Figure 1.1: Effects of water limitation on carbon assimilation, storage and use...............8
Figure 1.2: Generalised relationship of tree morphological and physiological responses to eCO₂, water and heat..................................................14
Figure 1.3: Plant genotype (G) by environment (E) interaction under current and future climate conditions.............................................16
Figure 2.1: Sum of heat wave days in Australia defined by the Excess Heat Factor......26
Figure 2.2: Typical 24-hour progression of glasshouse environmental parameters during the heat wave and drought experiment..................................................41
Figure 2.3: Mean volumetric soil water content (VWC) during the experiment.........42
Figure 2.4: Physical appearance of *E. camaldulensis* saplings during the heat wave and drought experiment...............................................................43
Figure 2.5: Days until leaf abscission across *E. camaldulensis* provenances and [CO₂] treatment averaged for heat and water stressed trees..............................................43
Figure 2.6: Midday leaf water potentials (Ψₘd) over different experimental phases for the six *E. camaldulensis* provenances.........................................................45
Figure 2.7: Leaf gas exchange of *E. camaldulensis* during different experimental phases.................................................................................................46
Figure 2.8: Rate of photosynthesis at saturating light (Aₛₘₙ) at the final stage of the heat wave and water limitation treatment...............................................................47
Figure 2.9: Pools of non-structural carbohydrates (NSC) of *E. camaldulensis* after heat and water stress across [CO₂] treatments.................................50
Figure 2.10: Non-structural carbohydrate (NSC) concentrations of *E. camaldulensis* after heat and water stress related to new foliage (LAjuveniles) after recovery........51
Figure 2.11: Midday leaf water potential (Ψₘd) related to leaf water content (MCleaf, A-B); and leaf osmotic potential (Ψₙ) related to leaf water content (C-D)........52
Figure 2.12: Leaf water content (MCleaf; A) and osmotic potential (Ψₙ; B) before (26°C, WW), during (42°C, WW/WL) and after heat/water treatment (26°C, WW)........53
Figure 2.13: Stem dry weight (DWstem) of *E. camaldulensis* during recovery related to mean annual temperatures (MAT) of seed origin.................................57
Figure 3.1: Climate parameters and location for the four selected *E. grandis* sites......77
Figure 3.2: Time-course of volumetric water content (VWC) of soil before and during water limitation in *E. grandis*.  

Figure 3.3: Stomatal conductance ($g_s$), light-saturated photosynthesis ($A_{sat}$) and carbon isotope discrimination ($\Delta^{13}C$) of four *E. grandis* provenances.  

Figure 3.4: Relative decrease of $A_{sat}$ in water-limited (WL) compared to well-watered (WW) tress on day 25, related to mean annual temperature (MAT).  

Figure 3.5: Stomatal conductance ($g_s$; panel A-B) and light-saturated photosynthesis ($A_{sat}$; panel C-D) in relation to midday leaf water potentials ($\Psi_{md}$).  

Figure 3.6: Root-mass-fraction at harvest of *E. grandis* for well-watered (WW) and water-limited (WL) trees.  

Figure 3.7: Relative growth rate (log-transformed) of stem volume per day.  

Figure 3.8: Percentage change of non-structural carbohydrates (NSC) in three tissues of water-limited relative to well-watered *E. grandis*.  

Figure 4.1: Schematic overview of the experimental design in the poly tunnel.  

Figure 4.2: Pot arrangement on pallets inside the poly tunnel.  

Figure 4.3: Volumetric water content (VWC) of the growing medium.  

Figure 4.4: Physiological traits of *E. coolabah* and *E. melliodora* during 2016.  

Figure 4.5: Relative changes in leaf physiological traits of water-limited *E. coolabah* and *E. melliodora* compared to well-watered controls at the end of drought 1.  

Figure 4.6: Predawn leaf water potential ($\Psi_{pd}$) related to leaf physiological traits of *E. coolabah* and *E. melliodora* at the end of drought 1.  

Figure 4.7: Predawn leaf water potential ($\Psi_{pd}$) related to leaf physiological traits of *E. coolabah* and *E. melliodora* at the end of the recovery phase.  

Figure 4.8: Predawn leaf water potential ($\Psi_{pd}$) related to leaf physiological traits of *E. coolabah* and *E. melliodora* at the end of drought 2.  

Figure 4.9: Relative change of leaf area (LA) in water-limited compared to well-watered trees of *E. coolabah* and *E. melliodora* during drought 1 and 2.  

Figure 4.10: Non-structural carbohydrate (NSC) concentrations of branch and root tissue at the end of drought 1 (left panels) and drought 2 (right panels).  

Figure 4.11: Photosynthesis ($A$) related to concentrations of soluble sugars (Ss, left panel) and starch (St, right panel) in branches and roots.
List of Tables

Table 1.1: Definition of terminology used ............................................................................. 15
Table 2.1: Spatial and climate information for provenances of *E. camaldulensis* ........ 33
Table 2.2: Temperature settings for the glasshouse rooms in ambient (*T*_{amb}) and heat wave (*T*_{heat}) conditions ......................................................................................................................... 35
Table 2.3: Average stem volume means (± one standard error) for each *E. camaldulensis* provenance (prov.) and [CO\(_2\)] level .............................................................................................................. 54
Table 2.4: Total plant dry weight and leaf area of *E. camaldulensis* before heat and water limitation (pre-treat.) and after recovery .............................................................................................................................. 55
Table 2.5: Relative ranking of selected plant traits across *E. camaldulensis* provenances ......................................................................................................................................................... 59
Table 3.1: Location and climate parameters characterising the origin of *E. grandis* seed provenances .................................................................................................................................................................. 76
Table 3.2: Mean predawn and midday leaf water potentials of *E. grandis* before and 25 days after water limitation commenced .................................................................................................................. 85
Table 3.3: Dry mass (g) of *E. grandis* at the end of experiment ........................................ 92
Table 3.4: Leaf characteristics of *E. grandis* seedlings grown in ambient (aCO\(_2\)) and elevated [CO\(_2\)] (eCO\(_2\)) ................................................................................................................................................................. 95
Table 4.1: Location and environmental characteristics of the selected tree populations of *E. coolabah* and *E. melliodora* ........................................................................................................................................ 115
Table 4.2: Leaf water potential of *E. coolabah* and *E. melliodora* during the last days of drought 1 .......................................................................................................................................................... 125
Table 4.3: Days until complete leaf mortality was reached during drought 2 .............. 133
Table 4.4: Biomass and leaf area (LA) of *E. coolabah* and *E. melliodora* trees at the end of drought 2 ......................................................................................................................................................... 136
List of Abbreviations

[CO₂] CO₂ concentration of the atmosphere
A photosynthesis
aCO₂ ambient atmospheric carbon dioxide
Aₘₐₓ light- and CO₂-saturated photosynthesis
Aₙₛₜ light-saturated photosynthesis
CHO osmotically active carbon compounds
cᵢ CO₂ concentration of the intercellular leaf space
d stem diameter at base
DW dry weight
E environment
eCO₂ elevated atmospheric carbon dioxide
ET evapotranspiration
G genotype
gₛ stomatal conductance
gₛₘₐₓ stomatal conductance at saturating CO₂ concentration
h main stem height
iWUE intrinsic water use efficiency estimated from A and gₛ
LA leaf area
MAP mean annual precipitation
MAT mean annual temperature
MCₙₑᵃᶠ leaf moisture content
NSC non-structural carbohydrates
NSW New South Wales
Pearson’s r Pearson’s r correlation coefficient
PPFD photosynthetic photon flux density
QLD Queensland
r stem radius
Rₗₐ₅ dark leaf dark respiration
RGR relative growth rate
RH relative humidity
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMF</td>
<td>root-mass-fraction</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SLA</td>
<td>specific leaf area</td>
</tr>
<tr>
<td>Ss</td>
<td>soluble sugars</td>
</tr>
<tr>
<td>St</td>
<td>starch</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>TDR</td>
<td>Time Domain Reflectometry</td>
</tr>
<tr>
<td>VIC</td>
<td>Victoria</td>
</tr>
<tr>
<td>VPD</td>
<td>vapour pressure deficit of the atmosphere</td>
</tr>
<tr>
<td>$\nu_{stem}$</td>
<td>stem volume</td>
</tr>
<tr>
<td>VWC</td>
<td>soil volumetric water content</td>
</tr>
<tr>
<td>WL</td>
<td>water-limited</td>
</tr>
<tr>
<td>WUE</td>
<td>water use efficiency</td>
</tr>
<tr>
<td>WW</td>
<td>well-watered</td>
</tr>
<tr>
<td>$\delta^{13}C$</td>
<td>ratio of $^{13}C$ to $^{12}C$ stable carbon isotopes</td>
</tr>
<tr>
<td>$\Delta^{13}C$</td>
<td>carbon isotope discrimination</td>
</tr>
<tr>
<td>$\Delta\Psi_l$</td>
<td>difference between $\Psi_{pd}$ and $\Psi_{md}$</td>
</tr>
<tr>
<td>$\Delta\Psi_{md}$</td>
<td>difference between $\Psi_{md}$ of WL and $\Psi_{md}$ of WW trees</td>
</tr>
<tr>
<td>$\Psi_l$</td>
<td>leaf water potential</td>
</tr>
<tr>
<td>$\Psi_{md}$</td>
<td>midday leaf water potential</td>
</tr>
<tr>
<td>$\Psi_{pd}$</td>
<td>predawn leaf water potential</td>
</tr>
<tr>
<td>$\Psi_r$</td>
<td>osmotic potential</td>
</tr>
</tbody>
</table>
Abstract

This thesis assessed the effects of future climate factors (i.e. [CO₂], heat waves and soil water availability) on growth and physiology of Eucalyptus species originating from different climates-of-origin. The main aim was to test intra-specific variation of plant traits to climate change. Four tree species native to Australia were selected due to their national ecological and international economic importance: Eucalyptus camaldulensis, E. grandis, E. melliodora and E. coolabah. I tested the response of E. camaldulensis to elevated atmospheric [CO₂] (eCO₂), heat and drought stress; E. grandis to eCO₂ and drought stress; and the acclimation response of E. melliodora and E. coolabah to wetting and drying cycles with final drought to mortality.

Phenotypic plasticity in leaf gas exchange, growth and non-structural carbohydrate (NSC) reserves was significantly different in E. camaldulensis and E. grandis when subjected to heat and/or water stress. In E. grandis, the tallest trees from cool temperatures had the greatest growth reductions during stress. In E. camaldulensis, trees originating from semi-arid climates initiated leaf abscission early and conserved NSC, which led to faster stem and leaf area recovery than trees from more mesic climates. Moreover, eCO₂ ameliorated stress responses related to photosynthesis when trees were either heat stressed or water-limited; time-to-leaf-death was extended in one provenance of E. camaldulensis in eCO₂. There was no acclimation of leaf gas exchange to variable water availability during the series of droughts in E. melliodora and E. coolabah. Yet, species had contrasting water use strategies linked to their distributional range across Australia. E. coolabah originating from semi-arid climate reduced its leaf area to prevent hydraulic failure, while E. melliodora originating from mesic climate utilized NSC reserves to tolerate water limitation. These results highlight the importance of soil water availability for physiological functioning and growth, but also show that intra-specific differences exist in response to heat and drought. In conclusion, my PhD research extends information on inter- and intra-specific differences in phenotypic plasticity of trees to the main and interactive effects of climate factors, which can be used to identify plantation trees for future climate regimes.
Chapter One: General Introduction

In the last 400,000 years before the industrial age, carbon dioxide concentrations ([CO$_2$]) in the atmosphere ranged between 200 to 280 µmol mol$^{-1}$ (Global Climate Change NASA 2016), but with industrialisation and land use change, it increased to 407 µmol mol$^{-1}$ in 2017 (NOAA ESRL 2017). By the end of this century, [CO$_2$] is conservatively predicted to increase to ca. 600 µmol mol$^{-1}$ and global mean surface temperatures are predicted to increase by at least 1.5°C (IPCC 2013). Rising [CO$_2$] and air temperature are predicted to generate changes in rainfall patterns and heat wave events in some terrestrial ecosystems (Fischer & Knutti 2015; Trenberth et al. 2014). Australian ecosystems are not exempt from these changes and south eastern Australia is predicted to experience lower precipitation as well as more frequent and longer heat waves (Dowdy et al. 2015; Hope et al. 2015; IPCC 2013).

Water shortage and extremely hot temperatures are two of the most challenging factors affecting the survival of trees (McDowell et al. 2008), including plantations with young forests (Battaglia et al. 2004). Despite the predicted beneficial impacts of elevated [CO$_2$] (hereafter eCO$_2$) on carbon assimilation and water use efficiency (Sherwin et al. 2013), heat and drought can potentially offset positive effects on growth (Lewis et al. 2013; Saxe et al. 1998). Primary productivity declines of up to ~30% have been estimated for European forests after a severe heat wave combined with water limitation in 2003 (Ciais et al. 2005) and carry-over effects on primary productivity were still observed one year later (Granier et al. 2007). Extreme climatic events have been associated with forest and woodland mortality in the past (e.g. Allen et al. 2010; Breshears et al. 2005, 2009; Matusick et al. 2013; Williams et al. 2013) and it is anticipated that further die-back will occur in the near future (Anderegg et al. 2013, 2015; McDowell et al. 2008).

The main effects of high temperature, altered precipitation or eCO$_2$ on growth and physiology are variable and sometimes antagonistic. In nature, trees will experience
the combined effects of drought, heat stress and eCO₂. How trees will respond to these interactive environmental factors is not well understood (e.g. Battaglia et al. 2009). It is thus critical to study how trees cope and possibly adapt to these interactive factors.

The genus *Eucalyptus* consists of ca. 700 different species (Brooker 2000), nearly all of them native to Australia where they are key species in managed and native ecosystems across all climate zones; worldwide, eucalypts are the most important hardwood plantation species. Currently, 26% of the global plantation area consists of *Eucalyptus* with the majority found in Europe, Asia and South America (FSC 2012). Only nine species qualify as high-yielding plantation trees, including their hybrids (Harwood 2011): *Eucalyptus camaldulensis*, *Eucalyptus dunnii*, *Eucalyptus globulus*, *Eucalyptus grandis*, *Eucalyptus nitens*, *Eucalyptus pellita*, *Eucalyptus saligna*, *Eucalyptus tereticornis* and *Eucalyptus urophylla*.

Biomass modelling of eucalypt plantations in Brazil showed that more frequent and intense droughts could reduce yield by one-third (Almeida et al. 2010). The creation of hybrid species and clonal forestry have improved drought tolerance of plantation eucalypts (Eldridge et al. 1993; Harwood 2011), in response to predicted future growing conditions. To ensure future biomass yield of plantation trees, efforts are now undertaken to identify highly productive genotypes in native populations. These targeted genotypes are used for intensive tree breeding programs (Booth 2013; Wang et al. 2010). Therefore, the present work investigates the combined effects of heat, drought and eCO₂ on selected eucalypt species. Experiments that simulate stressful and re-occurring events of abiotic factors will be used to assess physiology, metabolism and growth. I will investigate intra-specific differences across trees from various climates-of-origin (i.e. provenances) to identify trees that have superior growth and physiological functioning in future climate. The tree species used in this study grow naturally in temperate, sub-tropical and semi-arid Australia, where they are exposed to a wide range of environmental conditions. Here, I present novel information that may be used for tree selection in economically viable tree plantations adapted to future climate conditions.
1.1 Elevated [CO₂] and tree growth

Tree growth is derived from net carbon uptake where the process of assimilation of atmospheric CO₂ via the biochemical reactions associated with photosynthesis (A) exceeds the demand for carbon use by metabolic processes, including respiration (R; Lambers et al. 2008). To understand the effect of eCO₂ on tree growth, a brief review of the processes involved in assimilation, transport and catabolism, including storage and growth, is necessary.

Carbon economy of plants

Stomata facilitate CO₂ diffusion from the atmosphere into the intercellular space of the leaf, where the carboxylating enzyme Rubisco binds with CO₂ to form sugars which are the first products of photosynthesis. Sugars are metabolically active, but can be converted to starch, which may be stored as starch granules in chloroplasts (Chapin et al. 1990). Starch reserves mostly function as storage substance, but can be hydrolysed into sugars during periods of lower photosynthetic activity (e.g. low light conditions) or to meet higher energy demands (e.g. following stress events or seed production; Chapin et al. 1990; Kozlowski 1992; Lambers et al. 2008). Starch can also be transformed into soluble sugars for growth, respiration, metabolites for other biochemical pathways, osmolytes or transformed into sucrose for phloem transport (Martínez-Vilalta et al. 2016). Excess sucrose is loaded into the phloem of leaves from where it is transported to sites that require energy (e.g. growing cells, fruits, seeds, etc.), which is known as source-to-sink transport (Dietze et al. 2014; Savage et al. 2016; Thalmann & Santelia 2017). Phloem transport is assumed to be facilitated via a concentration gradient (i.e. osmotic gradient) between the source and the sink.

In eucalypts, carbon reserves are crucial to support plant function, in particular when photosynthesis is limited by drought or canopy scorch by bush fire (O’Brien et al. 2014). Carbon allocation to sinks may be actively regulated and favoured over stem growth after drought stress (Galiano Pérez et al. 2017). During abiotic stress, including water limitation or high temperatures, starch can be mobilised and
transformed into compatible solutes for protein or cell membrane protection and osmotic adjustment, though the magnitude of this response may be species and stress dependant (Thalmann & Santelia 2017). Furthermore, carbohydrate reserves can be utilised during recovery after stress; hence, access to a large carbohydrate pool after a stress event may increase recovery and survival (Bucci et al. 2003; Dietze et al. 2014; Sala et al. 2012). Plants most likely require a minimum carbohydrate pool size to survive the stress event, but pool sizes vary from plant to plant, among species, and as a function of environmental conditions. To date, minimum carbohydrate pool sizes required for plant survival have not been determined (Martínez-Vilalta et al. 2016).

eCO₂ effects on trees

Elevated atmospheric [CO₂] can impact many tree traits related to carbon assimilation and utilisation of water, subsequently affecting tree growth. The most commonly observed response is that eCO₂ stimulates photosynthesis (Curtis 1996; Curtis & Wang 1998; Ghannoum et al. 2010; Will & Ceuleman 1997). Resco de Dios et al. (2016) found a 58% enhancement of photosynthesis in eCO₂ in a meta-analysis of tree species grown in different facilities, including greenhouses, open top chambers and free-air carbon dioxide enrichment experiments (FACE). The basis for this ‘fertilising effect’ is that the enzyme Rubsico is less substrate-limited under eCO₂, which leads to an increase in carboxylase activity, resulting in greater assimilation of CO₂. Moreover, eCO₂ alters the ratio of CO₂ to O₂ inside the chloroplast, thereby competitively inhibiting the oxygenase reaction of Rubisco, which reduces photorespiration and increases A (Ceulemans & Mousseau 1994; von Caemmerer 2000). eCO₂ (up to 640 µmol mol⁻¹) can also have either no effect (Crous et al. 2012; Gauthier et al. 2014; Will & Ceulemans 1997) or a positive effect (Crous et al. 2011) on leaf respiration.

Increased carbon assimilation results in growth enhancement of above-ground biomass and thus has a beneficial effect on plantation trees. Mean stem biomass of eight tree species including Eucalyptus of different ages was found to increase by 36% from aCO₂ to eCO₂ (Resco de Dios et al. 2016). Eucalyptus dry mass has been
found to increase by 14–74% (Duan et al. 2014; Ghannoum et al. 2010), highlighting the influence of species and growth conditions on eCO$_2$ (640 and 650 μmol mol$^{-1}$) effects. Photosynthesis rates on a leaf area basis are often subject to greater enhancement than above-ground growth, suggesting that carbon is allocated to other sinks (Resco de Dios et al. 2016). Carbon in the form of simple carbohydrates (e.g. sucrose, fructose) may be directed to root growth for further nutrient uptake, cell respiration, defence mechanisms e.g. osmotic potential or cell wall strengthening, or directed to soil organisms. Hence, biomass yield in eCO$_2$ not only varies across species, but is also influenced by limiting growth conditions including soil nutrients. Accordingly, when comparing biomass production of natural ecosystems in long-term experiments, growth enhancement between aCO$_2$ and eCO$_2$ (550 and 560 μmol mol$^{-1}$) declined over time, arguably as consequence of nitrogen or phosphorus deficiency in the soil (Ellsworth et al. 2017; Luo et al. 2006; Norby et al. 2010; Reich et al. 2006). Nevertheless, when woody tissue mass increases, leaf area is also positively affected by eCO$_2$ due to either increasing leaf size or greater leaf number. For example, in *Eucalyptus* grown in greenhouses under eCO$_2$ (up to 650 μmol mol$^{-1}$), leaf area increased by up to 80% (Ghannoum et al. 2010; Phillips et al. 2011; Sherwin et al. 2013).

Carbon storage, in form of non-structural carbohydrates (NSC), particularly in starch granules, may be increased in eCO$_2$ with greater carbon assimilation. Since NSC are utilised during times of low carbon assimilation, eCO$_2$ may promote greater tolerance to abiotic stress (Hartmann & Trumbore 2016). Several studies show that NSC reserves in tree species were greater in eCO$_2$ (Kitao et al. 2007; Körner 2003 and references within; Rey & Jarvis 1998; Will & Ceulemans 1997). Absolute concentrations of NSC are usually highest in leaves which also show the greatest and most immediate response to eCO$_2$, yet on a mass basis more NSC are stored in woody tissue (Wong et al. 1992). Leaf starch in *Eucalyptus* is often increased under eCO$_2$ (640 and 650 μmol mol$^{-1}$), while leaf soluble sugars may or may not show the same response (Ayub et al. 2011; Gauthier et al. 2014; Smith et al. 2012). Thus, on a whole-plant basis, total NSC (NSC$_{total}$) reserves may be similar for plants grown in aCO$_2$ and eCO$_2$ when starch is transformed into sugars (Duan et al. 2013a, 2014).
In addition to stimulation of the carbon economy of trees, their water use can also benefit from eCO$_2$, which may be crucial during intervals of low soil water availability. For example, eCO$_2$ has been shown to decrease stomatal conductance ($g_s$); in a meta-analysis of FACE experiments, $g_s$ declined by ca. -20% due to eCO$_2$ (550 and 567 µmol mol$^{-1}$) in various tree species (Ainsworth & Rogers 2007; Leakey et al. 2012). Despite small reductions of $g_s$, ameliorating effects of eCO$_2$ on carboxylation rates and photorespiration still resulted in enhanced $A$. Guard cells of stomata sense [CO$_2$] and with rising intercellular concentrations, stomata may partially close (Mott 1988; Rico et al. 2013). Reductions in $g_s$ often lead to reductions in transpiration in eCO$_2$. Moreover, intrinsic water use efficiency (iWUE, the ratio of carbon fixed via $A$ to water lost through stomata) in different tree species was also found to increase with eCO$_2$ by over 70% in forest FACE experiments (Battaglia et al. 2013). This increase in iWUE was either the result of lower $g_s$ or higher $A$ (Albert et al. 2011; Curtis & Wang 1998; Ellsworth et al. 2017; Faralli et al. 2016, Leakey et al. 2009; Wullschleger et al. 1997) and may be beneficial during water limitation. Overall, additional CO$_2$ available for trees will generally increase carbon assimilation and storage, and reduce water loss through stomata; however, these responses may only occur during favourable growing conditions and may be substantially different when eCO$_2$ is combined with abiotic stressors that are expected to commonly occur in future climate conditions.

1.2 Drought stress and tree growth

Water is one of the most limiting factors for plant growth (Correia et al. 2014; Costa e Silva et al. 2004; Li et al. 2000; Maseda et al. 2016). Photosynthesis requires an influx of atmospheric CO$_2$ through the stomata, which simultaneously results in water lost through transpiration, thereby creating a water potential gradient (i.e. tension) in the xylem from the canopy to the roots, where water is taken up from the surrounding soil (McElrone et al. 2013). During periods of soil water deficit, xylem tension can become severe, leading to rupture of water miniscii inside the xylem. The subsequent formation of air bubbles can lead to xylem cavitation and in severe cases to a collapse of water transport (McElrone et al. 2013). However,
across species and populations, trees have evolved numerous mechanisms to tolerate different regimes of water availability and resist cavitation commonly encountered in their environment; hence, selecting different seed material adapted to these environments may increase tree performance in future forest plantations (Ashraf et al. 2011; Blackman et al. 2017; Niinemets & Valladores 2006; Viger et al. 2016). This can be achieved by testing plant traits like leaf gas exchange, carbon storage or allocation to roots or osmotic adjustment during soil water deficit.

Drought stress leads to a broad variety of plant responses from molecular to physiological scales and these responses depend on the intensity and duration of the stress. Maintenance of cell turgor during more negative water potential (Ψ) is essential to support metabolic processes. Hence, osmotic potential (Ψ_π) can be actively regulated by accumulating osmotically active solutes (i.e. osmolytes) such as inorganic ions, sugars or amino acids in cell cytoplasm to create a force triggering water influx (Lambers et al. 2008; Sanders & Arndt 2012; Shvaleva et al. 2006; White et al. 1996). Furthermore, stomata will close to reduce transpiration rates and hence also limit CO₂ influx, photosynthesis and growth (Fig. 1.1; Correia et al. 2014; Costa e Silva et al. 2004, Flexas & Medrano 2002).

Under these circumstances concentration of NSC in leaves is likely to increase, at least over short time periods, since the decline in growth is often greater than the decline in rates of photosynthesis and rates of photosynthesis decline faster than those of respiration (Fig. 1.1; Farooq et al. 2009; McDowell 2011). Yet, with continuing drought stress where carbon assimilation may have ceased, these NSC reserves will predominately be used for maintenance respiration and osmotic adjustment (Dietze et al. 2014). Martínez-Vilalta et al. (2016) found that NSC declined only by around −40% of maximum seasonal concentrations in field studies, showing that NSC reserves are unlikely to be depleted entirely, even during extreme stress. However, the same group of researchers showed that on a global scale, starch was more likely to be reduced than soluble sugars, in agreement with earlier studies (Arndt et al. 2008; Duan et al. 2013a, 2014; Mitchell et al. 2014; Quick et al. 1992), highlighting that starch is predominately a storage compound while soluble sugars are used immediately during water stress responses. However,
utilization of NSC and osmotic adjustment are only partly determining drought tolerance of trees. Stomatal sensitivity to water deficit and xylem cavitation resistance are also key traits for survival. All traits and their responses can vary depending on species and/or genotype (Blum 2017; Rodríguez-Calcerrada et al. 2017; Sevanto et al. 2014; Sparks & Black 1999; Wikberg & Ögren 2004).

Figure 1.1: Effects of water limitation on carbon assimilation, storage and use. Shown are rates of photosynthesis (A) and respiration (R), concentration of non-structural carbohydrates (NSC), osmotically active carbon compounds (CHO) and resulting tree growth. Arrow 1: Given that growth generally slows down before A and R, the NSC pool increases with carbon assimilation in the first days of water deficit before A starts to decrease. Arrow 2: Growth R is negligible and NSC are only utilised for maintenance R, however stress might increase R due to defence mechanism and thus deplete NSC pools faster. Arrow 3: Concentrations of CHO to maintain cell turgor and phloem pressure gradient increase with duration of water limitation. Taken from McDowell (2011).

_Eucalyptus_ species have the potential for high photosynthesis and growth rates, yet are often restricted in their natural habitat by low availability of nutrients and/or water (Whitehead & Beadle 2004). Many species are also well adapted to the dry climate of Australia by having thick leaves that will be shed when drought becomes too severe and root systems that can reach deep soil moisture stores when necessary (Bucci et al. 2012; Pita et al. 2005; Whitehead & Beadle 2004). Lowering osmotic potentials during drought is also a common response with variable
adjustment capacities across *Eucalyptus* species (Callister et al. 2008; Merchant et al. 2007). Negative effects of severe water limitation (for up to eight weeks) on leaf gas exchange, NSC or above-ground growth have been reported (e.g. Ayub et al. 2011; Correia et al. 2014; Gauthier et al. 2014; Warren et al. 2011a), yet some species survive in arid locations and can experience very negative leaf water potentials due to high cavitation resistance (Duan et al. 2014; Whitehead & Beadle 2004). Stomatal sensitivity to declining water potentials is one of the key limitations for growth and can vary considerably across species (Whitehead & Beadle 2004). Moreover, eucalypts have been shown to vary in their capacity to tolerate drought. Species from drier climates often have smaller canopies, higher capacities for osmoregulation and greater water use efficiency (WUE) and consequently are able to maintain greater \( A \) during more negative \( \Psi \) (Cano et al. 2014; Searson et al. 2004). Accordingly, *Eucalyptus* species have developed biochemical, physiological and morphological strategies to survive water limitation, making them attractive candidates for hardwood plantations. Less clear, however, is how variation in biomass, especially on an intra-specific level, can be harnessed for improving productivity under mild and severe water shortages. Therefore, I will investigate relationships between \( A \), leaf dark respiration \( (R_{\text{dark}}) \), NSC and osmotic potential in trees originating from contrasting climates-of-origin and assess if mechanisms exist that increase tolerance, but also maintain high relative rates of growth during periods of drought.

### 1.3 Heat stress and tree growth

Heat waves can have many different definitions which vary in intensity and duration (see Perkins & Alexander 2013). However, the World Meteorological Organisation defines heat waves as periods of daily maximum temperatures exceeding the 90\(^{\text{th}}\) percentile average by 5°C for more than five days. Heat waves in Australia are defined as three or more days of high temperatures abnormal for a specific location (Bureau of Meteorology). Heat stress in plants may involve a temperature increase of 10–15°C above expected ambient air temperatures (Wahid et al. 2007). Such extreme temperatures can severely damage plant cells, as well as
slow down growth, depending on the severity and duration of heat stress (Wahid et al. 2007). Heat stress will increase transpiration of trees if sufficient soil water is available. High air temperatures usually lead to high vapor pressure deficit of the atmosphere (VPD), which is the driver for the rate of phase transition from liquid to gaseous water inside the leaf and the subsequent rate of loss of water vapor out of the leaf into the atmosphere. Besides accelerating transpiration, heat stress can affect many different functions of a tree ranging from leaf development, carbon allocation, growth, photosynthesis, respiration, secondary biochemical pathways and cell membrane and enzyme stability (Teskey et al. 2015; Wahid et al. 2007).

The photosynthetic apparatus is affected by heat in several different ways. For C₃ plants, the temperature optimum for photosynthesis is usually below 30°C since the activation of Rubisco, electron transport along the thylakoid membrane and sucrose synthesis are temperature sensitive (Ameye et al. 2012; Berry & Bjorkman 1980; Craft-Brandner & Salvucci 2000; Rennenberg et al. 2006; Schrader et al. 2004; Wise et al. 2004). As a consequence, rates of photosynthesis decline when temperatures increase. Also, the carboxylase activity/affinity of CO₂ to Rubisco and solubility of CO₂ relative to O₂ decreases with greater temperature, leading to relative greater oxygenase activity, higher photorespiration and lower A (Brooks & Farquhar 1985; von Caemmerer & Quick 2000). Protein denaturation and aggregation is a common consequence of heat stress, hence leading to dysfunction in chloroplast and mitochondria (Wahid et al. 2007). Phospholipids of plasma membranes show increased fluidity which can cause instability and alteration of permeability for ions like Ca²⁺ and larger molecules (Sung et al. 2003).

Plants may experience oxidative stress due to excess light energy at high temperatures (Foyer et al. 1994). Reactive oxygen species (ROS) can form during periods of excess heat and soil water deficit and may damage DNA, proteins, membrane lipids and photosynthetic pathways (Lawlor & Tezara 2009). Heat (up to 46°C) was also shown to deplete existing carbohydrate pools potentially due to increased utilisation for ROS scavenging and osmotic adjustment (Crous et al. 2012; Hüve et al. 2012).
Field studies on tree response to heat waves are rare (Niu et al. 2014; Pfautsch & Adams 2013). To better understand the effect of heat waves on trees, it is necessary to design and implement new experiments that simulate very hot conditions and assess how these conditions impact tree physiological traits. Here, I will study the effect of extreme heat (42°C) on the re-sprouting potential of different provenances of *E. camaldulensis* as a function of post-stress NSC pool dynamics. This eucalypt species represents one of the most economically valuable hardwood plantation species.

**1.4 Combined effects of eCO$_2$, drought and heat stress on tree growth**

In nature, changes in environmental conditions rarely involve only one factor, but rather occur in combination with others (Niinemets 2010). For example, heat waves often occur during periods of low precipitation and associated soil water limitation, which in turn increases the chance for tree mortality (De Boeck et al. 2010, 2011; O’Grady et al. 2013; Teskey et al. 2015). Studying the combined effects of eCO$_2$, heat and drought stress is required to more accurately predict physiological functioning and biomass yield in future climates. For instance, growth models for *Eucalyptus globulus* plantations in Australia predict that eCO$_2$ will increase growth in wetter regions, whereas drier areas will suffer from increasing tree mortality (Pinkard et al. 2014). When subjecting *Quercus rubra* and *Pinus taeda* to a combination of heat (+12°C above ambient temperature) and water stress (~20% of soil field capacity) as well as eCO$_2$ (700 µmol mol$^{-1}$), biomass reduction due to heat was less severe in oak in eCO$_2$ compared to aCO$_2$ whereas this effect was absent in pines (Bauweraerts et al. 2014a). In a FACE experiment using two deciduous tree species, *Populus tremuloides* and *Betula papyrifera*, eCO$_2$ (1000 and 1200 µmol mol$^{-1}$) counteracted negative effects of summer drought and heat (up to 39°C) on photosynthesis by enhancing Rubisco carboxylation activity, electron transport capacity and triose phosphate use (Darbah et al. 2010). The authors also found leaf temperatures to be cooler in trees grown in eCO$_2$ and proposed that this was likely the result of reduced soil water use before the onset of heat stress and thus greater remaining soil water availability that could be utilised for evaporative cooling during
hot temperatures until soil water stores were exhausted. Additionally, eCO₂ might improve tolerance to heat waves or water limitation of trees by increasing A while decreasing or maintaining transpiration rates and thus improving WUE (Faria et al. 1996; Liang & Maruyama 1995).

Evidence shows that eCO₂ increases the temperature optimum of photosynthesis in a variety of C₃ plants. It enables these plants to efficiently operate photosystem II in higher temperatures and maintain greater net photosynthesis rates than plants grown in aCO₂ (Ameye et al. 2012; Faria et al. 1996; Hamilton et al. 2008; Long 1991; Taub et al. 2000; Wang et al. 2008). In a meta-analysis of effects of eCO₂ and heat stress on plant traits, Wang et al. (2012) found that A in C₃ plants increased by 29% and above- and below-ground biomass increased by around 20%. When well-watered Quercus rubra seedlings were subjected to a heat wave (+12°C above ambient temperature), A decreased by −41% in eCO₂ (700 µmol mol⁻¹) compared to −61% in aCO₂ suggesting greater acclimation capacity through regulation of Rubisco activity, heat shock proteins or ROS scavengers (Bauweraerts et al. 2014b). These effects demonstrate that different plants can respond differently to a combination of eCO₂ and heat stress.

Overall, eCO₂ has been shown to have opposing impacts on physiology and growth of water-limited or heat stressed trees since coping mechanisms of species, stress intensity or experimental conditions can vary widely. Studies involving eCO₂ and water limitation are plentiful. Positive effects of eCO₂ during water stress have been observed for growth (Atwell et al. 2007), photosynthesis and hence WUE (Anderson & Tomlinson 1998; Wertin et al. 2010), while other studies found negative effects (Bobich et al. 2010; Warren et al. 2011a) or no difference (Gauthier et al. 2014). Moreover, root mass generally increases with above-ground mass in eCO₂. A root system that can reach deeper water sources or a denser fine root system that can extract more water may be beneficial during periods of water shortage (Crookshanks et al. 1998; Wullschleger et al. 2002). However, greater leaf area in eCO₂ can lead to increased transpiration on a whole-tree level, which is a critical trait in dry climates (Warren et al. 2011b). In addition, reduced transpiration during water limitation can further increase leaf temperatures and accumulation of ROS.
Chapter One

General Introduction


However, greater carbon assimilation can also lead to greater pools of osmolytes, which can be used for osmotic adjustment and ROS scavenging during water deficits (Hüve et al. 2006; O’Brien et al. 2014; Tognetti et al. 1998).

Depending on drought severity, tree growth might still be enhanced under eCO₂ and mild water limitation, as a result of higher A and lower gs. For example, eCO₂ (640 µmol mol⁻¹) was shown to increase biomass, A and NSC (due to sink limitation) in Eucalyptus globulus seedlings during mild drought stress; however, negative effects of severe drought could not be overcome (Duan et al. 2013a). Similarly, in studies on other Eucalyptus species grown under eCO₂ and severe water limitation, positive effects of eCO₂ on drought response were minor (Duan et al. 2014; Kelly et al. 2016; Lewis et al. 2013). Furthermore, eCO₂ (640 µmol mol⁻¹) did not affect A or leaf R in long-term water-limited Eucalyptus saligna compared to aCO₂, but leaf starch was increased by 20% as carbon was allocated to storage rather than biomass production (Ayub et al. 2011; Crous et al. 2012). These results highlight the importance of drought severity and duration on the impact of eCO₂ on leaf gas exchange and suggest that water regulates the effects of [CO₂] (Tschaplinski et al. 1995). In support of this argument, responses of three tree species (Platanus occidentalis, Liquidambar styraciflua and Acer saccharum) grown under aCO₂ (340 µmol mol⁻¹) and eCO₂ (650 µmol mol⁻¹) in well-watered and water-limited conditions, showed unanimously that water reduction triggered stronger negative effects in all three species compared to smaller positive effects of eCO₂ (Tschaplinski et al. 1995).

Information contained in the cited literature will guide my experiments to test if trees from different climates-of-origin have variable plastic responses in terms of water status, leas gas exchange, carbon storage, osmotic adjustment and biomass allocation, in response to eCO₂ and abiotic stress. A generalised overview of tree morphological and physiological traits potentially impacting growth is shown in Fig. 1.2 and will be discussed in detail in the following sections.
Figure 1.2: Generalised relationship of tree morphological and physiological responses to eCO$_2$, water and heat. Green arrows indicate a positive relationship (greater value for trait X correlates with greater value for Y or lower X correlates with lower Y), red arrows represent a negative relationship (greater value for trait X correlates with lower value for trait Y or lower X correlates with greater Y). In general, water limitation and heat stress have a negative effect on tree growth, while elevated CO$_2$ has a positive effect on growth. Thick arrows show the strongest trait correlations with tree growth. Grey text identifies traits that are important in whole-plant responses to eCO$_2$, water and heat but could not be measured in the current project.

1.5 Phenotypic plasticity

As established earlier, temperature, water and [CO$_2$] are important factors influencing tree growth and physiological functioning (Aspinwall et al. 2015). Tree responses to changing environmental conditions can be long-term adaptation or short-term acclimation processes of physiological traits (Anderson et al. 2012; Aspinwall et al. 2017; Bussotti et al. 2015). While adaptation relies on natural selection within tree populations, acclimation is reversible after a stress event has ended. Provenances of species growing in distinct environmental conditions can display local adaptations, often related to environmental gradients (Kawecki & Ebert 2004). The degree of variation in intra-specific responses to environmental stress is indicative of genetic diversity within a given population, commonly termed phenotypic plasticity (see Table 1.1; Aspinwall et al. 2015; Baquedano et al. 2008; Drake et al. 2017; Moran et al. 2016).
Depending on genotype, phenotypes can have altered traits in any direction or degree to the environmental factor, called ‘genotype by environment interaction’ (Des Marais et al. 2013). For example, mean annual temperature of seed origin across the western United States was the main driver for phenotypic variation in *Pinus contorta* (Wang et al. 2010). Phenotypic plasticity can be very effective and quick in response to changing environmental conditions (Nicotra et al. 2010; Valladares et al. 2007). Greater plasticity of physiological or morphological traits enables a tree to survive in a wide range of biotic and abiotic conditions and can play a key role in improving plantation productivity in future climates (Booth et al. 2013; Grime & Mackey 2002; Nicotra et al. 2010; Valladares et al. 2007). The capacity to respond to environmental change is genetically regulated and can be passed from one generation to the next (Bradshaw 2006). Epigenetic alteration of genes is a key mechanism for phenotypic plasticity (Nicotra et al. 2010). The degree of plasticity is species and population dependant, but also varies across plant traits (Duan et al. 2013b).

### Table 1.1: Definition of terminology used.

<table>
<thead>
<tr>
<th>terminology</th>
<th>definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>species</td>
<td>group of individuals that potentially interbreed and exchange genes</td>
</tr>
<tr>
<td>population</td>
<td>group of individuals of the same species that live in a certain area</td>
</tr>
<tr>
<td>provenance</td>
<td>group of trees with the same origin of seeds</td>
</tr>
<tr>
<td>genotype</td>
<td>set of genes inherited by an organism</td>
</tr>
<tr>
<td>phenotype</td>
<td>expression of plant traits of a genotype influenced by its environment</td>
</tr>
</tbody>
</table>

Tree responses to environmental stresses are dependent on genotype (*G*), environmental conditions (*E*) and genotypic responses to the environment (*G* × *E*) (see Aspinwall et al. 2015). A *G*-response refers to genotypes that have the same phenotype despite varying environmental conditions (Fig 1.3A), while a *G + E* - response indicates that genotypes will have phenotypes that respond to the environment, but in similar ways (Fig. 1.3B). In the case of *G × E*, genotypes display plasticity in their response to environmental conditions. While some genotypes may improve their trait response, others are non-responsive to new environmental conditions (Fig 1.3C). Lastly, some genotypes may display an improved trait...
response, while others showing a declining trait response to the shared future environmental conditions (Fig. 1.3D).

Figure 1.3: Plant genotype ($G$) by environment ($E$) interaction under current and future climate conditions. A: No genotypic variation to environmental change. B: Different genotypes respond in similar patterns to environmental change. C: Some genotypes improve their trait response in new environment. D: Some genotypes profit from new environment, others do not. Modified after Grishkevich & Yanai (2013).

The degree of phenotypic plasticity to environmental factors (i.e. $G \times E$) has been identified as a critical characteristic for future tree survival (Anderson et al. 2012; Nicotra et al. 2010). For example, some genotypes could respond more strongly to eCO$_2$ producing greater biomass while also being more drought tolerant relative to other genotypes of the same species. Identifying the degree of phenotypic plasticity when selecting trees for future forest plantations is crucial when aiming to maintain or even improve biomass yield in future climates (Booth et al. 2015). In general, tree populations adapted to drier and hotter environments tend to cope better with water and heat stress compared to populations adapted to wetter and cooler environments (e.g. Nguyen-Queyrens & Bouchet-Lannat 2003; Richards et al. 2014). However, it has been suggested that populations from colder climates will cope better with climate warming than populations from warmer areas, because populations from warmer environments may be closer to their upper temperature
tolerance (Drake et al. 2015; Rehfeldt et al. 2002). Accordingly, populations of Corymbia calophylla from cool and wet climates exhibited significant phenotypic plasticity and high rates of A due to acclimation when grown at warmer temperatures compared to plants from provenances originating from warm climates (Aspinwall et al. 2017; Blackman et al. 2017). However, the temperature responses of A, R and growth in different populations of Eucalyptus tereticornis representing different climates-of-origin did not differ, suggesting no local adaptation in this species (Drake et al. 2017). It becomes clear that many unknowns remain when selecting candidate tree species based on their phenotypic plasticity to different environmental variables. Hence, my research focused on ecological and economically important Eucalyptus species and assessed their phenotypic plasticity when grown under the same environmental conditions. This will expand our knowledge on the potential use of intra-specific differences to improve forest plantations of tomorrow.

1.6 Harnessing phenotypic plasticity for future tree growth

Elevated CO₂ and phenotypic plasticity

The magnitude of biomass increase in eCO₂ can vary substantially between provenances (e.g. Cantin et al. 1997; Ceulemans et al. 1996; Dickson et al. 1998; Isebrands et al. 2001; Mohan et al. 2004). Wong et al. (1992) documented varying plasticity in four eucalypt species to [CO₂]. In their study, fast-growing species exhibited higher rates of A in eCO₂ compared to slow-growing species. The authors suggested that the highly plastic response to substrate supply is favourable on fertile soils where fast-growing species may grow naturally. In contrast, the poor soils where the slow-growing species naturally occur may have limited the capacity to increase tree performance. This demonstrates how natural selection may not necessarily always support high A or growth. In support of this interpretation, greater A in Eucalyptus camaldulensis that originated from cool relative to those that originated from warm climates were apparent in aCO₂. However, when grown under eCO₂ this advantage of cool-climate genotypes disappeared (Loik et al. 2017).
Variation in growth stimulation among genotypes can also be related to different strategies for carbon allocation to plant organs (Ceulemans et al. 1996; Dickson et al. 1998; Isebrand et al. 2001; Mohan et al. 2004). Despite evidence for intra-specific differences of growth under eCO$_2$, analyses of genotypes from several tree species showed that intra-specific ranking of growth responsiveness to ambient or elevated CO$_2$ treatments is likely to remain unchanged (Resco de Dios et al. 2016; Temme et al. 2015), indicating that tree genotypes growing well under current [CO$_2$] may also be the candidates that grow well under eCO$_2$.

**Water limitation, heat waves, eCO$_2$ and phenotypic plasticity**

Few studies have investigated the plasticity of intra-specific responses to combined impacts of environmental parameters (i.e. eCO$_2$, water limitation and heat) in regards to climate-of-origin. Specifically, the degree of intra-specific variation of physiological traits like $A$, $g$, and $R$ in response to eCO$_2$, in combination with water and heat stress, has rarely been tested. Since such studies are scarce, I will summarise findings on crops before mainly focusing on intra-specific responses to either water limitation or heat stress in trees in combination with eCO$_2$.

Interactions of $G \times E$ have been shown for two genotypes of *Triticum aestivum* when grown under eCO$_2$ (700 µmol mol$^{-1}$), increased temperatures of +6°C and severe water limitation (Dias de Oliveira et al. 2013). Biomass reductions under heat stress could not be overcome by eCO$_2$, yet biomass reductions due to drought initiated a decline of $A$ that was dampened under eCO$_2$. Importantly, this response to eCO$_2$ was more pronounced in the genotype that had lower above-ground biomass under ambient conditions, but greater above-ground biomass under water limitation and eCO$_2$. Contrastingly, *Prosopis glandulosa* genotypes from xeric and mesic climates responded similarly when grown under severe water limitation and eCO$_2$ (700 µmol mol$^{-1}$; Polley et al. 1999), showing increased growth and leaf area and decreased transpiration rates in eCO$_2$. These studies highlight that there is potential for ameliorated responses during abiotic stress when [CO$_2$] is increased. However, not all species may display $G \times E$ interactions.
Plastic responses have also been reported for *Eucalyptus* when grown with limited access to water. Widely distributed species like *E. camaldulensis* have been shown to vary in their response to severe water limitation depending on seed origin (Gibson *et al.* 1995). Some trees increased their biomass allocation to roots, some shed their leaves and others only reduced $A$ and $g_s$. Two different strategies to cope with severe water stress were observed in *E. globulus* clones (Granda *et al.* 2014). One group conserved water during stress by closing stomata and reducing leaf growth, thereby maintaining leaf and stem water content to continue cell function. The other group reduced their leaf water content, and hence lowered $\Psi_w$, while also maintaining $A$, resulting in greater growth rates than the water-saving group. Other studies on this species found additional intra-specific differences to water deficit, where one genotype had greater hydraulic conductance, greater root and leaf growth, as well as greater $g_s$ compared to a different genotype (Costa e Silva *et al.* 2004). Furthermore, with varying accumulation of ROS scavenger compounds and osmotic adjustment, *Eucalyptus* genotypes were shown to respond differently to mild water limitation (Bedon *et al.* 2012), indicating a high variability in responses of some *Eucalyptus* species and genotypes to environmental stresses.

This variability seemed absent in other species of the same genus or for other plant traits. For example, no interactions for high temperature (up to 40°C) sensitivity of $R_{dark}$ were found for provenances of *E. grandis* when grown in eCO$_2$ (640 µmol mol$^{-1}$; Aspinwall *et al.* 2017). Similarly, no interactions for $A$ or $g_s$ were found when *E. camaldulensis* genotypes were subjected to eCO$_2$ (640 µmol mol$^{-1}$) and a spring heat wave (35°C; Loik *et al.* 2017). Other studies testing only one environmental factor have found that responses of *E. microtheca* provenances to severe water deficit were similar for biomass and specific leaf area (SLA; Li *et al.* 2000; Li & Wang 2003). Also, no interactions of iWUE, $R_{dark}$ or growth rates to a heat wave were found for two provenances of Douglas-fir from drier and wetter climates (Jansen *et al.* 2014). These diverse findings underscore the great variability in acclimation mechanisms to environmental stress depending on species and traits tested.

Studies on intra-specific responses to combined effects of eCO$_2$, water and high temperatures are lacking, especially for woody species. Thus, my research will test
the effects of combinations of these factors on physiological and morphological traits.

1.7 Thesis objectives

Productivity of *Eucalyptus* plantations of the southern hemisphere and Mediterranean countries will be affected by eCO$_2$, more frequent heat waves and altered precipitation regimes. To remain economically viable, it is imperative to identify eucalypt species that can remain productive across a wide range of environmental conditions. Knowledge gaps remain concerning phenotypic plasticity of fast-growing *Eucalyptus* species and their responses to climate change. The following research projects are designed to test whether physiological and morphological traits of eucalypt species and their provenances reflect adaptation to the climate-of-origin, and if so, how they might explain intra-specific variation of tree productivity. Most published studies have assessed tree responses following a single stress event, whereas trees in their natural habitat are exposed to repeated stress events. Little is known how re-occurring stress events may impact tree functioning or whether the degree of plasticity of traits results in modification of responses to re-occurring stress.

The objective of my PhD thesis was to evaluate the effect of environmental parameters – eCO$_2$, high temperatures and variable soil water availability – on plant physiology, morphology and biomass yield of four different *Eucalyptus* species native to Australia. Water limitation for this study will be defined as mild and severe. Although there are many different definitions, mild water limitation describes a reduction of $A$, $g_s$ and $\Psi_l$ with trees having functional leaves attached, whereas severe water limitation will result in $g_s$ of $< 0.1 \text{ mol m}^{-2} \text{ s}^{-1}$ and leaf wilting. Populations from contrasting climates were selected from each species and intra-specific differences to a combination of environmental factors were tested. A large number of traits ($A$, $g_s$, $R$, WUE, NSC, $\Psi_l$, $\Psi_n$, LA, root-mass-fraction, biomass and growth) were used to characterise these responses.
This research provides new insight as to how tree populations may cope under future climatic conditions. This knowledge is critical to forest industries, particularly plantation management, as current species and provenance selection has a major impact on revenue returns in the future. Thus, my research was designed to address the following questions:

1. Does intra-specific variation in phenotypic plasticity to environmental parameters (i.e. $G \times E$) exist within selected trees species?

2. If phenotypic plasticity is present, can responses be related to climate-of-origin to identify superior trees for future climates?

3. Do responses to repeated events change via acclimation, or are physiological and morphological traits genetically fixed and hence are predictable for long-lived organisms like trees?

1.8 Thesis outline

Chapter 1 presents a general introduction to my field of research.

Chapter 2 assesses the phenotypic plasticity of six provenances of *E. camaldulensis* originating from a climate gradient (warm/dry to cold/wet) to eCO$_2$, heat and drought stress as single factor or in combination.

- Trees were grown in a glasshouse under aCO$_2$ (400 µmol mol$^{-1}$) and eCO$_2$ (640 µmol mol$^{-1}$). Once established, tree saplings were exposed to a combination of very high daytime temperatures (42°C) and subjected to severe water limitation.

- Under non-stressful conditions, eCO$_2$ positively affected plant water status ($\Psi_l$), leaf physiological traits (i.e. leaf gas exchange) and biomass (i.e. 35% increase in total plant mass). No $G \times E$ interaction for biomass yield was detected under these conditions (i.e provenances with high yield in aCO$_2$ had high yield in eCO$_2$).
• Trees originating from cold/wet climates displayed a greater capacity than provenances from warm/dry climates to cope with heat and water stress, regardless of [CO₂] treatment. However, this superior resistance resulted in greater depletion of NSC, and hence slower recovery from the stress event, resulting in faster re-sprouting capacity and hence greater stem and leaf biomass in trees from warm/dry climates.

• General trends in growth did not change from aCO₂ to eCO₂, but water-limited trees from warmer/drier climates displayed a different plasticity in A and growth.

This chapter highlights the dominant role of water availability compared to [CO₂] on plant physiological function. Results of this work suggests that mechanisms leading to faster recovery, rather than stronger resistance to the stress event, may be more successful for biomass production during long-term water deficit.

Chapter 3 investigates phenotypic plasticity of four provenances of E. grandis from distinctly different climates-of-origin to eCO₂ and mild water limitation.

• Trees were grown under aCO₂ (400 µmol mol⁻¹) and eCO₂ (640 µmol mol⁻¹) and were subjected to a prolonged, 50% reduction of soil water content.

• Despite interactions between [CO₂] and water treatments in leaf gas exchange traits, trees from cooler climates generally exhibited higher rates of A and gₛ compared to trees from warmer climates, resulting in greater final biomass and leaf area.

• Some minor G × water interactions were detected leading to the largest trees under well-watered conditions having greater growth reductions under water limitation. Hence, the second largest trees in well-watered conditions had the greatest stem biomass at the end of the treatment.

• Trees originating from warmer climates displayed a conservative water use strategy that resulted in lower leaf area and rates of leaf gas exchange as well as greater RMF and low growth rates.

These results suggest a trade-off between growth rates and drought tolerance and highlight the importance of water availability on plant response to eCO₂.
Chapter 4 tests the capacity for physiological acclimation of *E. coolabah* originating from a semi-arid climate and of *E. melliodora* originating from a temperate climate, when subjected to re-occurring drought events of different length and severity.

- The first drought was slowly implemented over three months and maintained for additional six weeks after dividing trees into three treatment groups with either sufficient irrigation, mild or severe water limitation. After this period, all trees were re-watered and allowed to recover for three weeks before the second severe and rapid drought was introduced by taking trees off irrigation until leaf death.
- Different physiological acclimation processes in the two species were observed during recovery from the first, prolonged drought; unexpectedly, *E. coolabah* displayed upregulation of \( g_s \) during recovery.
- During the second and terminal drought, neither species showed any signs of acclimation to water stress originating from the previous long interval of water limitation; leaf mortality was reached at a similar time interval in both species and independent from having a history of water limitation or not.
- The species displayed different survival strategies during water limitations that involved clear morphological changes; *E. coolabah* reduced its leaf area by up to \(-60\%\) depending on drought severity to conserve water and avoid xylem cavitation, while *E. melliodora* utilised stored NSC for osmotic adjustment.

This research provides evidence for the relatively low phenotypic plasticity of two eucalypt species in response to short-term changes in environmental conditions and suggests fixed long-term adaptation, highlighting the vulnerability of some eucalypt species to rapid climate change.

Chapter 5 synthesises the main findings of my PhD research and provides a range of conclusions.

- Overall, eCO\(_2\) had positive effects on \( A \) and growth in well-watered as well as water or heat stressed trees. Yet, some trees were more responsive than others confirming \( G \times E \) interactions.
• Significant $G \times$ water interactions were found for biomass yield of
  *E. grandis*, whereas *E. camaldulensis* had further interactions when adding
  $[\text{CO}_2]$ resulting in small changes. *E. grandis* trees from cooler climates and
  *E. camaldulensis* trees from semi-arid climates were the largest. Thus, $G \times E$
  interactions for $[\text{CO}_2]$ and abiotic stress are confirmed and suggest that
  responses are related to climate-of-origin, answering my first and second
  research question.

• A key driver for variation in physiology and biomass was water availability.
  Mechanisms to cope with water deficit were related to long-term
  adaptations of *Eucalyptus* species to their climate-of-origin with only minor
  phenotypic plasticity when stress was repeated. Thus, my third research
  question can be addressed with these findings suggesting that there is no
  acclimation capacity for leaf gas exchange traits, yet some adjustments
  were found for morphological traits i.e. leaf area in *E. coolabah*.

My PhD research provides evidence for $G \times [\text{CO}_2] \times$ abiotic stress interactions in
eucalypt species, with soil water availability having the biggest effect on these
responses. Mechanisms to cope with water deficit were related to long-term
adaptation of *Eucalyptus* species to their climate regime at seed origin. The limited
physiological plasticity of trees to respond/cope with water limitation indicated that
rapid climate change with associated shifts in water availability will have negative
effects on trees, potentially increasing tree mortality in the future. Yet, some
provenances have better adaptations to future environmental conditions due to
natural selection and hence can be used to improve biomass yield of future
plantations.
Chapter Two: The impact of climate of origin – responses of *Eucalyptus camaldulensis* provenances grown at elevated [CO$_2$] during and after heat and water stress

2.1 Introduction

Increasing mean surface temperatures in Australia result in reduced rainfall in the South East, leading to more frequent and possibly longer droughts, as well as heat waves that are becoming hotter, longer and more frequent (Fig. 2.1 B–C). This trend is expected to continue throughout the coming decades (IPCC 2014; Perkins *et al.* 2012; Perkins & Alexander 2013). There is no unified definition of heat waves. However, the World Meteorological Organisation defines heat waves as periods of daily maximum temperatures exceeding the 90$^{th}$ percentile average by 5°C for more than five days. Heat waves in Australia are defined as three or more days of high temperatures abnormal for a specific location (Bureau of Meteorology). They vary in time of year, frequency, intensity and duration. Currently, Australia experiences on average nine heat wave days every year; however, values can be highly variable, depending on location, with most of the western, central and eastern Australian regions experiencing at least eleven days of abnormally high temperatures per year (Fig. 2.1A).
Chapter Two Intra-specific differences during eCO$_2$, heat and water stress

Figure 2.1: Sum of heat wave days in Australia defined by the Excess Heat Factor (Nairn et al. 2009). Average sum of heat wave days per year from 1951–2008 are shown in panel A and colour coded from 0 to 14 days. Trends for the years 1951–2008 (panel B) as well as 1971–2008 (panel C) are shown as change in number of heat wave days per decade ranging from 5 days below to 5 days above average. Hatched area represents statistical significance of temporal trends. Figures by Perkins & Alexander (2013).

In Australia, tree species of the genus *Eucalyptus* are widespread and often dominant. They are adapted to a wide range of climates and occupy xeric, mesic, wet, lowland and sub-alpine habitats (Austin et al. 1990; Brooker et al. 2006). Climate change will likely impact most ecosystems, as plants are affected by their environmental conditions. Furthermore, heat waves are usually accompanied by more sunshine, less rainfall and hence greater vapour pressure deficit (VPD; De Boeck et al. 2010), which can increase tree water stress and have a negative impact on tree growth and survival (Allen et al. 2010; Klos et al. 2009). Moreover, elevated [CO$_2$] (eCO$_2$) affects stem growth and tree productivity (Bazzaz et al. 1990; DeLucia et al. 1999; Saxe et al. 1998), but these effects also depend on temperature, water availability, soil type and species (Körner et al. 2005; Phillips et al. 2011; Wang et al. 2012).

Trees often have some capacity to adjust to new abiotic conditions by changing physiological traits, enabling the tree to survive in a range of biotic and abiotic conditions (Bradshaw 2006). This acclimation process, referred to as phenotypic plasticity, can result in different phenotypes of the same genotype depending on environmental factors (Aspinwall et al. 2015). In periods of rapid climate change, the fastest way for populations of long-lived organisms such as trees to adapt to new environmental conditions is through intra-specific phenotypic plasticity (Nicotra et al. 2010). Hence, intra-specific variation of naturally occurring tree
populations may prove important for maintaining productivity of trees in future climate conditions. However, our knowledge about phenotypic plasticity regarding climate change, especially in species that are valuable for the plantation industry, such as selected species of the genus *Eucalyptus*, is very limited (Aspinwall *et al.* 2015).

*Heat and water stress in plants*

Under natural conditions, heat stress is usually coupled with water stress, so it is difficult to identify the independent impact of these two climate variables. However, plant responses to these stress factors are often very similar (Wahid *et al.* 2007) and can vary with tree age, developmental stage and size (Niinemets 2010). In addition, responses may also vary if the tree is affected by a single stressor (e.g. drought) or simultaneously by multiple stressors (e.g. drought combined with a heat wave). The responses of trees to heat waves coupled with water limitation are rarely tested (De Boeck 2010), yet heat can aggravate the effects of water limitation by increasing water loss through the leaf cuticle (Riederer & Schreiber 2001). With sufficient water, effects of heat waves alone can be small due to leaf transpirational cooling (De Boeck *et al.* 2011). Yet, when transpirational cooling is suppressed, high temperatures can generate toxic reactive oxygen species (ROS). Stem growth, leaf area and stomatal conductance ($g_s$) has been shown to be more negatively affected by heat and drought combined than the sum of response to these factors tested separately (De Boeck *et al.* 2011; Ruehr *et al.* 2015), highlighting that testing interactive effects is crucial to understand tree responses.

Genotypes may respond differently to heat and water stress due to different adaptations of morphological or physiological traits i.e. displaying genotype by environment interactions ($G \times E$; Aspinwall *et al.* 2015). Some genotypes may have more sensitive stomatal responses, and thus control transpirational loss better, while reducing transpirational cooling at the same time. Other genotypes may have a more efficient or sensitive system to scavenge harmful substances (e.g. ROS) or produce heat shock proteins, and thus reduce the damage to photosynthesis components and cell membranes (Iba 2002). Ideally, breeders are looking for
genotypes with excellent biomass yield during favourable environmental conditions, whilst maintaining some productivity and high survival rate during disturbances, including heat waves and droughts (Aspinwall et al. 2015).

Recovery

Recovery is possible, if the stress event was not too severe or long enough to kill the tree. Defoliation is a common response of trees after a major disturbance like severe and/or long drought, fire or pest infestation. In particular, starch reserves in trees are used to maintain plant metabolism and supply cell respiration with fuel in form of sugars as energy source during times of low carbon fixation. Furthermore, starch reserves are utilised during recovery and regrowth (Bamber & Humphreys 1965; Mitchell et al. 2013; Myers & Kitajima 2007; Nzunda et al. 2008). Leaf area recovery has been shown to be positively correlated with carbohydrate reserves before defoliation (Myers & Kitajima 2007). Thus, non-structural carbohydrate (NSC) pools are especially important in the genus *Eucalyptus* which has a high portion of re-sprouting species (Zeppel et al. 2015). If starch reserves become depleted due to intense or prolonged abiotic or biotic stress, tree mortality increases (Bamber & Humphreys 1965; Mitchell et al. 2013). Reserves can recover as seen within twelve months in *Angophora costata* after defoliation by fire, yet if recovery time is not long enough to refill NSC pools prior to the next stress event, survival of trees can be severely impeded (Bamber & Humphreys 1965; Palacio et al. 2012).

Plant stress and elevated CO₂

Examples for the positive effect of eCO₂ on plant growth are plentiful (see Chapter 1). In theory, eCO₂ could increase plant tolerance to heat and drought by increasing A and hence NSC available for osmotic adjustment, resulting in more negative osmotic potential to maintain cell turgor (Wullschleger et al. 2002). In an experiment on two well-watered *Eucalyptus* species grown under eCO₂ (640 µmol mol⁻¹), plant dry mass was shown to increase by up to 74% due to leaf area increasing by up to 50% and enhanced A by up to 40% (Ghannoum et al. 2010). Yet,
responses of trees to \( \text{eCO}_2 \) during stress can vary depending on species or severity and responses to \( \text{eCO}_2 \) in combination with multiple abiotic stressors (e.g. heat or drought) may not be the same if stress factors were tested separately (Shaw et al. 2002). Transpiration per unit leaf area may be reduced in \( \text{eCO}_2 \) due to decreased \( g_s \) and slightly delay the onset of severe plant stress, yet increased canopy size due to greater growth rates in \( \text{eCO}_2 \) can still increase whole canopy transpiration (Ainsworth & Rogers 2007; Long et al. 2004; Warren et al. 2011a). At the same time, reduced transpiration during extreme heat can intensify stress by reducing passive leaf cooling (Urban 2003). In many instances, \( \text{eCO}_2 \) does not alleviate reductions in photosynthesis and growth induced by severe water deficit (Duan et al. 2013a; Lewis et al. 2013).

Regardless of \([\text{CO}_2]\) treatment, severe water limitation increased leaf \( R \) during heat stress (up to 65°C) in \textit{Eucalyptus globulus} (Gauthier et al. 2014). Moreover, neither leaf area nor \( g_s \) or whole tree water use was different across \([\text{CO}_2]\) treatments in severely water-stressed \textit{E. radiata} (Duan et al. 2014). Similarly, \( \text{eCO}_2 \) (640 \( \mu \text{mol mol}^{-1} \)) did not affect \( A \) or leaf respiration in long-term water-limited \textit{E. saligna} compared to a\( \text{CO}_2 \), but leaf starch was increased by 20% as carbon was allocated to storage rather than production of biomass (Ayub et al. 2011; Crous et al. 2012). Overall, even though leaf traits in \textit{Eucalyptus camaldulensis} were found to vary across eleven genotypes from six provenances grown together in a glasshouse, \( \text{eCO}_2 \) (640 \( \mu \text{mol mol}^{-1} \)) did not alter relationships between these traits in a PCA relative to observations in a\( \text{CO}_2 \) (Blackman et al. 2016). Hence, the interactive effects of \( \text{eCO}_2 \) with other environmental stressors are still unclear (Becklin et al. 2017) and more multifactorial studies are needed to address this issue.

\textit{Intra-specific variation in stress tolerance}

Studies on the combined effects of heat, drought and \( \text{eCO}_2 \) in different genotypes of the same species are very limited and sometimes contradicting. It has been shown for \textit{Eucalyptus camaldulensis} that temperatures at which heat damage occurs vary across provenances (Karschon & Pinchas 1971). A commonly observed trend is that trees originating from more xeric environments display a greater
capacity to cope with drought or heat stress compared to trees of the same species originating from mesic environments (Anderson et al. 1996; Aspinwall et al. 2015; Choat et al. 2007; Gibson et al. 1995; Tuomela 1997). As proof-of-concept, a drought-stress experiment used four different *Betula pendula* genotypes (planted in pots outside) that originated from a pronounced rainfall gradient (Aspelmeier & Leuschner 2004). The authors were able to show that genotypes from drier climates had greater osmotic adjustment and capacity to regulate $g_s$ than genotypes from wetter climates when exposed to severe water limitation. Several other studies have shown that water-limited trees from drier climates had less negative leaf water potentials ($\Psi$), lower $g_s$, greater water use efficiency (WUE) and greater root-to-shoot ratio, but also lower biomass yield due to drought adaptation than trees from wetter climates, which displayed greater rates of productivity when grown at the same location (Li 1998; Li & Wang 2003; Tuomela 1997). In contrast, leaf dark respiration ($R_{\text{dark}}$) appears to remain stable across provenances or [CO$_2$] treatments (Aspinwall et al. 2017).

Costa e Silva et al. (2006) found significant $G \times E$ interactions for stem diameter growth of 600 *Eucalyptus globulus* genotypes planted at 15 Australian field sites. These interactions were likely related to soil water availability at seed origin. For the same species, trees which were planted in common gardens had less leaf damage during drought when originating from areas with greater temperature, but less rainfall and radiation seasonality (Dutkowski & Potts 2012). Regarding genotypic differences to heat stress, fast-growing *Picea glauca* exposed to short-term heat of 42–50°C had greater needle damage than slower growing individuals of the same species, confirming the trade-off between fast growth and drought resistance (Bigras 2000). However, *Eucalyptus tereticornis* trees grown in open top chambers in western Sydney, but originating from warmer climates in Queensland, sustained greater biomass with and without summer heat wave (up to 47°C) relative to trees from three other provenances. This suggests that there was no $G \times E$ interaction triggered by higher temperatures (Hancock & Hughes 2014). Furthermore, when *E. camaldulensis* provenances were subjected to a spring heat wave of 35°C and eCO$_2$ (640 µmol mol$^{-1}$), $g_s$ and $A$ were similar in both [CO$_2$]
treatments and provenances. However, electron transport in photosystem II was increased by 17% during heat stress from aCO₂ to eCO₂ (Loik et al. 2017). Thus, whether or not G × E interactions exist may depend on species, provenance as well as the environmental factor that is tested.

Research objectives

This study assessed G × E effects related to eCO₂ and the capacity of different E. camaldulensis provenances to cope with heat stress and water limitation. Trees were grown in aCO₂ and eCO₂ and selected physiological (e.g. A, gₛ, Ψᵋ, biomass) and biochemical traits (i.e. NSC and Ψₚ) were monitored to assess physiological functioning. Importantly, this project assessed the capacity of the selected provenances to recover from severe stress. It further examined whether increased carbon supply due to eCO₂ improves or exacerbates stress tolerance. The main questions addressed by this study were:

1) Do trees from provenances of E. camaldulensis originating from different climates-of-origin vary in their growth, gas exchange or osmotic adjustment? More specifically, do trees from cool and wet climates have higher rates of growth and photosynthesis than trees from warm and dry climates, when well-watered and grown under aCO₂ (G response)?

2) Do provenances differ in their growth response to eCO₂ ([CO₂] response or G × [CO₂] interaction)?

3) Are trees of provenances originating from drier and warmer climates more tolerant to heat and water stress, and if so, which are key traits regulating tolerance (G × heat & drought interaction)?

4) Does eCO₂ ameliorate heat and water stress and/or shorten recovery times due to greater carbon supply and lower plant water loss (G × heat & drought interaction)?
2.2 Material and Methods

2.2.1 Species provenance

*Eucalyptus camaldulensis* (River Red Gum) is one of the most widespread eucalypts in Australia. Several subspecies, including *E. camaldulensis* Dehnh. var. *camaldulensis*, are distributed along the East coast of Australia from South East Australia to South Queensland. This woodland tree grows up to 50 m tall along the Murray-Darling river system in moist clay soil. It has a pronounced capacity to cope with waterlogging, salinity, heat and drought (Brooker *et al.* 2006; Lemcoff *et al.* 1994; McMahon *et al.* 2010b), although due to its wide distribution, this capacity may vary among provenances (McMahon *et al.* 2010b). *E camaldulensis* can also re-sprout from basal and epicormic buds after disturbance. Due to its fast growth, the species is very popular as plantation tree in countries with mild winters. It is planted in southeast Asia, most African countries and Central and South America (CABI 2016).

Six provenances of *Eucalyptus camaldulensis* var. *camaldulensis* Dehnh. were selected along a gradient of temperature and rainfall (Table 2.1). This gradient spanned from cool, humid south western Victoria to warm, arid central New South Wales. Note that mean annual precipitation (MAP) is inversely related to mean annual temperature (MAT). Climate data were obtained from eMAST (www.emast.org.au) which is part of the Terrestrial Ecosystem Research Network (TERN). Long-term climate parameters were calculated using daily observations from 1970 to 2012 (Table 2.1).
Table 2.1: Spatial and climate information for provenances of *E. camaldulensis*. Annual information for mean maximum as well as mean air temperatures (\(T_{\text{max}}\), MAT, °C), mean precipitation (MAP, mm) and ratio of evapotranspiration/precipitation (ET/MAP) are shown. Abbreviations (abb.) for provenances are shown and will be used throughout the text.

<table>
<thead>
<tr>
<th>site name</th>
<th>abbrev.</th>
<th>coordinates</th>
<th>(T_{\text{max}})</th>
<th>MAT</th>
<th>MAP</th>
<th>ET/MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yass River</td>
<td>YR</td>
<td>34.53° S 149.02° E</td>
<td>20.5</td>
<td>13.9</td>
<td>669</td>
<td>2.07</td>
</tr>
<tr>
<td>Coonawarra</td>
<td>CW</td>
<td>37.20° S 140.42° E</td>
<td>20.4</td>
<td>14.4</td>
<td>631</td>
<td>2.18</td>
</tr>
<tr>
<td>Avon River</td>
<td>AN</td>
<td>36.52° S 143.11° E</td>
<td>21.6</td>
<td>15.1</td>
<td>441</td>
<td>3.59</td>
</tr>
<tr>
<td>Narrandera</td>
<td>ND</td>
<td>34.45° S 146.33° E</td>
<td>23.6</td>
<td>16.7</td>
<td>431</td>
<td>4.06</td>
</tr>
<tr>
<td>Lake Cargelligo</td>
<td>LC</td>
<td>33.12° S 146.21° E</td>
<td>24.7</td>
<td>18.0</td>
<td>404</td>
<td>4.59</td>
</tr>
<tr>
<td>Wilcannia</td>
<td>WC</td>
<td>31.28° S 143.39° E</td>
<td>27.1</td>
<td>19.9</td>
<td>275</td>
<td>8.93</td>
</tr>
</tbody>
</table>

### 2.2.2 Plant material

Plants were grown from cuttings which were established by germinating seeds from different open-pollinated mother trees i.e. half-sib families of each provenance. Details about germination and propagation can be found in Blackman *et al.* (2016). Multiple cuttings (replicates) were taken from several trees of each provenance and each replicate (ramet) was grown separately in pots from January 2014.

### 2.2.3 Growing conditions

Seedlings were grown in pots constructed from PVC pipes (15 cm in diameter, 40 cm long) filled with approximately 7.5 kg soil. The soil (Australian Native Landscapes Pty Ltd, NSW, Australia) had a coarse sandy texture with a pH below 6.5.

**Environmental conditions**

Seedlings were grown in two glasshouse rooms located at Western Sydney University in Richmond, NSW at 33.61° S 150.75° E under natural sunlight and additional artificial light (LumiGrow Pro 325 and Pro 650, LumiGrow, CA, USA). Each room was fully temperature controlled. Temperature and relative humidity of the rooms were continuously monitored and logged every 15 minutes (HMP60, Vaisala Oyj, Finland fitted with radiation shields). Vapour pressure deficit (VPD) was
calculated using temperature (T, °C) and relative humidity data (RH, %) following Snyder and Shaw (1984):

\[
\text{VPD} = \left( 0.6108 \exp \left( \frac{17.27 T}{T + 237.3} \right) \right) \left( 1 - \frac{\text{RH}}{100} \right) \quad \text{Equation 1}
\]

\(\text{CO}_2\) treatment

Atmospheric \(\text{CO}_2\) concentration in each room was controlled automatically (Lambda T, ADC BioScientific Ltd., Herts, UK). An average [\(\text{CO}_2\)] of 400 µmol mol\(^{-1}\) (a\(\text{CO}_2\)) and 640 µmol mol\(^{-1}\) (e\(\text{CO}_2\)) was maintained in the two glasshouse rooms for each [\(\text{CO}_2\)] treatment. Each of the six provenances was represented by eight replicate seedlings per [\(\text{CO}_2\)] treatment (total of 96 plants), which were randomly assigned to rooms; trees were rotated within rooms weekly and between rooms on a monthly basis.

Watering regime

For the first 19 weeks of the experiment, pots were watered every morning until soil was saturated. Approximately 90 mg of liquid fertiliser (500 mL Aquasol, at 1.6 g l\(^{-1}\); 23% N, 4% P, 18% K, 0.05% Zn, 0.06% Cu, 0.013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B; Yates Australia, NSW, Australia) was added to each pot every fortnight. The water limitation treatment started on 30 May 2014 and ended with the last tree to be rewatered on 15 June 2014. Two trees per provenance and [\(\text{CO}_2\)] treatment remained well watered (WW) throughout the entire experiment; the other six trees received progressively less water to impose water limitation (WL). Provision of water was slowly reduced every day by 100 mL (starting with 600 mL) until no water was supplied. This state of water stress was maintained until seedlings exhibited a minimum of 80% reduction of leaf area. The exact time when this level of leaf abscission was reached was noted for each tree individually, using total initial leaf number as an indicator. Immediately following 80% leaf loss, trees were re-watered to facilitate tree recovery and new leaf production. Soil volumetric water content (VWC) of two water-limited and one well-watered tree (per [\(\text{CO}_2\)])
treatment) was continuously monitored using Time Domain Reflectometry sensors (TDR; CS616, Campbell Scientific Australia, QLD, Australia).

**Heat wave**

Seedlings were grown under ‘ambient’ temperature with day- and night-time oscillation (Table 2.2) that approximated a 30-year summer-time temperature average from November to May of the local area. On 26 May 2014, before water limitation treatment commenced, midday maximum temperatures were increased in 4°C increments over four days to reach a maximum of 42°C. Table 2.2 displays the temperature regime for the diel cycle of the ambient and heat wave phase of the experiment. Water limitation was initiated on day 5 of the heat wave and continued in combination with high temperatures until 80% leaf abscission was reached. Note that both water-limited and well-watered trees were exposed to the heat wave.

**Table 2.2: Temperature settings for the glasshouse rooms in ambient (T<sub>amb</sub>) and heat wave (T<sub>heat</sub>) conditions. Temperatures were slowly increased in 4°C increments over four days to attain T<sub>heat</sub>.**

<table>
<thead>
<tr>
<th>time of day</th>
<th>T&lt;sub&gt;amb&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;heat&lt;/sub&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>06:00–10:00</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>10:00–16:00</td>
<td>26</td>
<td>42</td>
</tr>
<tr>
<td>16:00–20:00</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>20:00–06:00</td>
<td>15</td>
<td>31</td>
</tr>
</tbody>
</table>

Once 80% leaf abscission was reached, trees within each [CO₂] treatment were immediately returned to ambient temperature conditions and allowed to recover. Since no leaf abscission was expected for heat-exposed, well-watered trees (based on previous experience), one individual well-watered tree was moved out of heat wave conditions at the same time when moving one water-limited tree of the same provenance.
2.2.4 Leaf water potential

Midday leaf water potential ($\Psi_{md}$) was measured four times: (1) before commencing heat and water treatments (21 May 2014); (2) at 42°C when plants were well-watered (29 May 2014); (3) at 42°C when plants received a reduced amount of water (2 June 2014); and (4) at 42°C when plants had received no water for several days but had not started to shed leaves (5 June 2014). One leaf per tree was collected during midday, placed into moisturised plastic bags and kept cool inside an ice box before taking it to the lab. To slightly increase humidity and limit further leaf transpiration, air was exhaled into the plastic bag before sealing. In the lab, $\Psi_{md}$ was measured using a pressure chamber (1505D, PMS Instrument Company, OR, USA).

2.2.5 Leaf gas exchange

Leaf gas exchange was determined on the same days as $\Psi_{md}$. Measurements were taken between 9:30–15:30 hours on a minimum of three trees per treatment combination. Leaf light-saturated photosynthetic rates of CO$_2$ assimilation ($A_{sat}$, μmol m$^{-2}$ s$^{-1}$) and stomatal conductance of water vapour ($g_{s}$, mol m$^{-2}$ s$^{-1}$) of one newly developed, fully expanded leaf were recorded with an infrared gas analyser (LI-6400XT, Li-Cor Inc., NE, USA) using the 2 × 3 cm chamber. Measuring conditions inside the chamber cuvette were set to saturating light (1600 μmol m$^{-2}$ s$^{-1}$) generated by a red/blue light-emitting diode light source. Block temperature was set to midday room temperatures (i.e. 26°C or 42°C) and mean ambient room [CO$_2$] (400 or 640 μmol mol$^{-1}$) for $A_{sat}$ and saturating [CO$_2$] (1000 μmol mol$^{-1}$) for $A_{max}$. Vapour pressure deficit (VPD) inside the cuvette was regulated to be 1.2–1.6 kPa via adjustment of RH. Leaves were allowed to equilibrate in the cuvette for a minimum of five minutes before recording data. Gas analysers were matched every time prior to logging data. A minimum of five data points per plant were logged at 5 second intervals and later averaged. Intrinsic water use efficiency (iWUE, μmol CO$_2$ mol H$_2$O$^{-1}$) was calculated by dividing $A_{sat}$ by $g_s$. 

36
2.2.6 Non-structural carbohydrates

Plant materials (leaves, stem, and roots) were sampled during morning hours immediately after the heat and drought treatment to assess the concentration of non-structural carbohydrates (NSC). Two replicates of water-limited trees per [CO₂] and provenance were harvested following the heat wave/water treatment. A detailed protocol for NSC extraction can be found in Tissue & Wright (1995). In short, subsamples of plant material were dried and ground to a fine powder before extracting NSC. Stem sections (5 cm long) including bark were cut at 10-15 cm above the base and the entire root material was carefully washed free from soil. After drying to constant weight at 70°C, all plant materials, including the entire root bulb, were coarsely ground, homogenized and a representative subsample was ground to fine powder. Starch (St) was separated from soluble sugars (Ss), lipids and amino acids using a methanol, chloroform and water solution. Water soluble sugars were partitioned from remaining cell components by phase separation. Furthermore, St was digested into sugars with perchloric acid. Sugar content of both fractions was quantified colorimetrically in a spectrophotometer (DU800, Beckman Coulter Australia Pty Ltd, NSW, Australia) via the phenol-sulfuric acid reaction. A 3-point calibration standard made from glucose was analysed with every batch of samples. Extractions were repeated when absorptions of the standard solutions were not as anticipated or the samples exceeded the glucose standard range.

2.2.7 Water content and osmotic potential of leaves

Leaf samples were collected before commencing the treatment, at 42°C and water limitation, but before leaf abscission as well as during recovery. One leaf per tree was removed, kept on ice and microwaved for ten seconds to stop enzyme activity before storing samples at −80°C. Prior to leaf sap extraction, a small piece (approximately 10 × 5 mm) was cut out and weighed (fresh weight, FW) on a three-point balance before drying for three days at 40°C to determine dry weight (DW). Leaf water content on a dry weight basis (MC, %) was calculated as:
A mixture of apoplastic and protoplastic sap was extracted from the remaining thawed leaf tissue using the metal chamber of a leaf sap press (NTS High-Pressure Sap Extractor, Nutri-Tech Solutions Pty Ltd., QLD, Australia). Aluminium foil was used to fill empty space inside the cylinder. Up to 5 tons of pressure was applied with a hydraulic press (Atlas Manual Hydraulic Press, SPECAC Inc., NJ, USA). Excess leaf sap was collected in a 1.5 mL PCR tube and frozen at −80°C until further analysis. Osmotic potential of sap was measured using a thermocouple psychrometer (PSY-1 Stem Psychrometer, ICT International, NSW, Australia) by soaking a filter paper disc (Whatman filter paper Grade 1, GE Healthcare Australia Pty. Ltd., NSW, Australia). Three readings per disc were taken and averaged for the sample.

2.2.8 Tree biomass and leaf area

Tree height ($h$, cm), diameter ($d$, mm) and leaf number were recorded before and after the heat and water treatment, as well as during the recovery phase, to assess differences in growth rates. Main stem volume ($v_{stem}$, cm$^3$) was calculated from $h$ and $d$ (in cm) of each tree by assuming the shape of a cone:

$$v_{stem} = \frac{1}{3} \pi \left(\frac{d}{2}\right)^2 h$$  \hspace{1cm} \text{Equation 3}

Trees were harvested at the end of the experiment when trees had produced new foliage at the end of July/early August 2014. To account for individual tree differences in the duration of recovery after leaf abscission, trees were divided into two groups (early and late leaf abscission). The two groups were harvested one week apart from each other. Within groups, the sequence of harvested trees depended on the number of days each tree had been exposed to the heat and drought treatments.

At the final harvest, leaf area (LA) of the whole canopy was measured by placing all leaves into a leaf area area meter (LI-3100C, Li-Cor Inc., NE, USA). Stem material was cut into smaller pieces, roots washed, and all tissues dried separately at 70°C.
for three days before recording dry weight. Stem and plant dry weight (\(DW_{\text{stem}}\) and \(DW_{\text{plant}}\)) for missing data was estimated from \(v_{\text{stem}}\) using a linear correlation between \(DW_{\text{stem}}\) or \(DW_{\text{plant}}\) to \(v_{\text{stem}}\). In order to compare variation before and after treatments across provenances and [\(CO_2\)], data for biomass and LA were extracted from a separate experiment on the same species and provenance groups and conducted at the same location a few weeks before this study (Blackman et al., in preparation). Root-mass-fraction (RMF) was calculated by dividing \(DW_{\text{root}}\) by \(DW_{\text{plant}}\).

### 2.2.9 Statistical analyses

Data were analysed with R-3.3.3 software (R Core Team 2017) using analysis of variance (ANOVA) for plant traits including leaf gas exchange, leaf osmotic potential, carbohydrates and water content as well as plant biomass, leaf area and days until leaf death (Supplementary Table S2.1). Multivariate analysis consisted of a combination of the three fixed factors: \(CO_2\) concentration, water treatment and provenance with all interactions. Data taken at one time point were tested for meeting statistical assumptions of an ANOVA by graphically checking for normal distribution using the ‘hist’ and ‘plot’ function. Equal variance across test groups was checked by conducting Levene’s test using the ‘leveneTest’ function. Where data were not normally distributed, they were log-transformed. The ‘Anova’ function of the ‘car’ package (Fox & Weisberg 2011) was used to reduce Type III errors. Effects of \([CO_2]\) and water treatment on soil water content over time was analysed with the lm() function of the nlme package (Pinheiro et al. 2017).

The ‘lm’ function for linear models was used to establish the strength of the relationship between days until leaf death and plant dry weight (\(DW_{\text{plant}}\)). A two-sample t-test was used to test if osmotic potential of leaf sap varied significantly during recovery from those apparent during pre-water and pre-heat treatments. The same test was used to compare means of \(A_{\text{sat}}, g_s\) and iWUE of well-watered trees before the stress event with means at the end of the heat wave, separated for \([CO_2]\) treatments.
Graphs and regression fits with 95% confidence intervals were generated using OriginPro 2017 (OriginLab, MA, USA). Values for coefficient of determination ($R^2$), as well as testing for significance of regression fits using ANOVA, was determined using this software. Mean values are presented with one standard error (SE). Correlations between $DW_{\text{plant}}$, $DW_{\text{stem}}$ and carbohydrates to climate parameters as well as carbohydrate concentrations to new leaf production, were analysed with Pearson’s correlation coefficient.

2.3 Results

2.3.1 Growing conditions

*Environmental conditions*

Climatic conditions inside the glasshouse rooms were similar across [CO$_2$] treatments. Before commencement of the heat treatment, daytime (10:00–16:00 hours) temperatures averaged 28.0 ± 0.01°C and vapour pressure deficit (VPD) was 1.4 ± 0.02 kPa. During the heat wave, average daytime temperature in the aCO$_2$ room was 41.5 ± 0.1°C and in the eCO$_2$ room was 41.3 ± 0.1°C (Fig. 2.2A). With rising room temperatures, VPD increased to 4.9 ± 0.03 kPa in aCO$_2$ and 4.8 ± 0.03 kPa in eCO$_2$ (Fig. 2.2B), with maximum VPD peaking at 7.1 kPa in aCO$_2$ and 6.7 kPa in eCO$_2$. Average daily CO$_2$ concentrations for aCO$_2$ reached a mean of 437 ± 1 µmol mol$^{-1}$ and 632 ± 2 µmol mol$^{-1}$ in eCO$_2$ (Fig. 2.2C). During the heat wave and recovery phase, photosynthetic photon flux density (PPFD) at midday (1200–1300 hours) at a nearby weather station averaged 918 ± 9 µmol m$^{-2}$ s$^{-1}$. Trees inside the glasshouse experienced 10–15% less light due to absorption by the glass.
Figure 2.2: Typical 24-hour progression of glasshouse environmental parameters during the heat wave and drought experiment. A: temperature (T); B: vapour pressure deficit (VPD); C: CO$_2$ concentration in air ([CO$_2$]). The morning peak in aCO$_2$ was the result of someone entering the room to water the trees. Grey lines represent conditions in 400 µmol mol$^{-1}$ atmospheric CO$_2$, black lines in 640 µmol mol$^{-1}$.

Soil volumetric water content fluctuated throughout the experiment (Fig. 2.3). Soil of well-watered pots averaged 33.5 ± 0.002% in aCO$_2$ and 30.6 ± 0.002% in eCO$_2$ conditions. Soil in water-limited pots reached a mean minimum VWC of 12.3% before plants were taken out of the heat wave to recover and values were not significantly different between [CO$_2$] treatments ($p = 0.71$; Fig. 2.3B).
Chapter Two  Intra-specific differences during eCO$_2$, heat and water stress

Figure 2.3: Mean volumetric soil water content (VWC) during the experiment. Panel A shows VWC of well-watered control trees, panel B shows VWC of water-limited trees. Grey lines show mean of all pots in ambient [CO$_2$], black lines in elevated [CO$_2$]. Water limitation commenced on the 30 May. Moisture probes were removed once trees started wilting so that pots could be transported into a different glasshouse bay with ambient temperature conditions; hence, VWC for this period was not monitored. Pots were fitted with moisture probes for plant recovery on the 16 June.

**Treatment effects**

Heat and water limitation resulted in leaf wilting and eventually in leaf abscission. The interval from the beginning of the stress treatment to the point of complete leaf loss varied among trees from 4–18 days. Once sufficient stress levels were reached, individual trees lost all leaves within one day, without any obvious relationship between leaf age and time of abscission (Fig. 2.4B). In contrast to water-stressed trees, well-watered trees retained their full canopy during the stress event (Fig. 2.4A). Heat- and water-stressed trees which lost their leaves re-sprouted and formed a new canopy during recovery (Fig. 2.4C).
Chapter Two

Intra-specific differences during eCO$_2$, heat and water stress

Figure 2.4: Physical appearance of *E. camaldulensis* saplings during the heat wave and drought experiment. Panel A shows trees before heat and drought stress. Panel B shows one individual tree after leaf senescence with dry leaves. Panel C shows these trees re-sprouting and forming juvenile leaves.

Neither [CO$_2$] nor seed origin had a sufficiently strong impact on the time interval required for 80% of the leaves to be dead ($p = 0.09$ for [CO$_2$] and $p = 0.14$ for provenance; Fig. 2.5). The exception for this general observation is provenance WC, where leaves grown under eCO$_2$ coped with heat and drought stress several days longer, compared to the same leaves grown under aCO$_2$. Across all provenances, leaves lasted $11 \pm 0.4$ days until death. Notably, in aCO$_2$ trees from WC reached leaf death four days earlier than trees from YR.

Figure 2.5: Days until leaf abscission across *E. camaldulensis* provenances and [CO$_2$] treatment averaged for heat and water stressed trees. Solid columns show trees in atmospheric [CO$_2$] (400 µmol mol$^{-1}$), while hatched columns represent trees grown in elevated [CO$_2$] (640 µmol mol$^{-1}$). Provenances are abbreviated as per Table 2.1. Error bars represent one standard error of means ($n = 6$). Different lower case letters indicate significant differences across provenances pooled across [CO$_2$] treatments, with $p < 0.05$. 

43
Days until leaf abscission was influenced by plant size, namely plant dry weight at harvest ($p < 0.001$), with smaller plants lasting longer in heat and drought. However, the fitted linear regression was weak ($R^2 = 0.15$).

### 2.3.2 Leaf water potential

Leaf water potentials were strongly impacted by water treatment, while [CO₂] did not have a strong effect. Leaf water potentials varied most strongly among the six provenances during severe heat and water stress. In well-watered, ambient temperature conditions, $\Psi_{md}$ did not differ across provenances. However, with a marginal $p = 0.05$, $\Psi_{md}$ was more negative in aCO₂ (mean: $-1.3 \pm 0.1$ MPa) compared to eCO₂ (mean: $-1.2 \pm 0.1$ MPa) at this point in time.

At the end of the heat wave/water limitation treatment, $\Psi_{md}$ in stressed trees decreased significantly compared to well-watered trees ($p < 0.001$). $\Psi_{md}$ averaged $-2.5 \pm 0.1$ MPa in aCO₂ and $-2.2 \pm 0.1$ MPa in eCO₂. Average $\Psi_{md}$ in well-watered trees was $-1.3 \pm 0.1$ MPa in aCO₂ and $-1.4 \pm 0.1$ MPa in eCO₂. Further, $\Psi_{md}$ became significantly more negative in trees coming from warmer temperatures during severe water limitation ($p = 0.04$). The provenance originating from the coldest and wettest climate (YR) had a mean $\Psi_{md}$ of $-2.0 \pm 0.3$ MPa, whereas the provenance from the warmest and driest climate (WC) averaged $-2.8 \pm 0.2$ MPa (Fig. 2.6).
Chapter Two  
Intra-specific differences during eCO$_2$, heat and water stress

Figure 2.6: Midday leaf water potentials ($\Psi_{md}$) over different experimental phases for the six *E. camaldulensis* provenances ($n = 2$–6 per provenance). Trees were first grown at 26°C daytime maximum temperature and were well-watered (WW). In the next experimental phase, trees were exposed to 42°C daytime maximum temperature and separated into groups of well-watered and water-limited (WL) trees. Data are shown for aCO$_2$ in panel A and for eCO$_2$ in panel B with intervals between phases not related to actual time between measurements. Dashed lines show means across provenances of well-watered trees (closed symbols), while dotted lines represent means across provenances of water-limited trees (open symbols). Provenances are represented as different symbols and colours: YR: ◇, CW: ●, AN: ▼, ND: □, LC: ▲, WC: ◆. Provenances are abbreviated as per Table 2.1. Error bars represent one standard error of means pooled across provenances.

2.3.3 Leaf gas exchange

Generally, eCO$_2$ had a positive effect on $A_{sat}$ across provenances at all stages of the experiment (Fig. 2.7A–B), yet $A_{sat}$ did not vary consistently among provenances ($p > 0.29$). However, before water or heat stress was initiated, mean $A_{sat}$ of trees grown in aCO$_2$ was 23.2 ± 0.7 µmol m$^{-2}$ s$^{-1}$, while trees in eCO$_2$ reached 27.0 ± 1.1 µmol m$^{-2}$ s$^{-1}$, which was a 16% increase ($p < 0.006$). At severe water limitation and heat, trees maintained a greater rate of $A_{sat}$ in eCO$_2$ with 6.0 ± 1.2 µmol m$^{-2}$ s$^{-1}$ compared to 3.4 ± 0.9 µmol m$^{-2}$ s$^{-1}$ in aCO$_2$. Regardless, the slight positive effect of eCO$_2$ was negligible when severe water limitation reduced $A_{sat}$ by 75% ($p < 0.001$; Fig. 2.7 A–B). Interestingly, at the end of the heat wave, $A_{sat}$ of well-watered trees grown in aCO$_2$ was significantly lower than before the stress (10.4 ± 1.3 µmol m$^{-2}$ s$^{-1}$; $p < 0.001$; Fig. 2.7), whereas $A_{sat}$ of well-watered trees grown at eCO$_2$ did not change (27.2 ± 1.3 µmol m$^{-2}$ s$^{-1}$).
Figure 2.7: Leaf gas exchange of *E. camaldulensis* during different experimental phases. Photosynthesis rate at saturating light ($A_{\text{sat}}$), stomatal conductance ($g_s$) and intrinsic water use efficiency (iWUE) were averaged before and during the heat and drought treatment ($n = 2–6$ per provenance). Trees were first grown at 26°C daytime maximum temperature and were well-watered (WW). In the next experimental phase, trees were exposed to 42°C daytime maximum temperature and separated into groups of well-watered and water-limited (WL) trees. Data are shown for aCO$_2$ in left-hand panels and for eCO$_2$ in right hand panels. Dashed lines show means across provenances of well-watered trees (closed symbols), while dotted lines represent means across provenances of water-limited trees (open symbols). Provenances are represented as different symbols and colours: YR: ▲, CW: ○, AN: ▼, ND: □, LC: △, WC: ◆. Provenances are abbreviated as per Table 2.1. Error bars represent one standard error of means pooled across provenances.

At the end of the heat and drought treatments, $A_{\text{sat}}$ across [CO$_2$] treatments was significantly different between stressed trees from the warmest climate, and trees from the coldest climates ($p = 0.02$; Fig. 2.8). Here, WC had the lowest $A_{\text{sat}}$ with
–0.6 ± 1.1 µmol m⁻² s⁻¹ and YR and CW from the coolest climate averaged ca.
6 µmol m⁻² s⁻¹ in aCO₂. The highest photosynthetic rates in a water-limited
provenance in eCO₂ was measured in provenance CW and averaged 11.1 ± 4.4 µmol
m⁻² s⁻¹. In contrast, in eCO₂ trees from AN and WC had mean A_sat of 2.4 ± 1.1 and
3.0 ± 0.7 µmol m⁻² s⁻¹, respectively, a difference of at least 73%.

Figure 2.8: Rate of photosynthesis at saturating light (A_sat) at the final stage of the heat wave
and water limitation treatment. *E. camaldulensis* was grown in ambient [CO₂] (aCO₂, panel
A) and elevated [CO₂] (eCO₂, panel B). Closed columns represent means of well-watered trees
(WW) and open columns represent means of water-limited trees (WL). Provenance names
are abbreviated (see Table 2.1). Error bars represent one standard error. For reasons of
display, the minimum y-axis value was set at -1.

[CO₂] had a major impact on gₛ before and during stress conditions. During ambient
temperatures and well-watered conditions, gₛ significantly decreased from 1.05 ±
0.11 mol m⁻² s⁻¹ in aCO₂ to 0.64 ± 0.08 mol m⁻² s⁻¹ in eCO₂ (p = 0.007; Fig. 2.7 C–D).
This effect was reversed in well-watered trees towards the end of the heat wave
and drought. As with A_sat in aCO₂, well-watered trees showed a reduction of gₛ
during the heat wave, averaging 0.38 ± 0.07 mol m⁻² s⁻¹ (p < 0.001), while well-
watered trees in eCO₂ had a mean of 0.80 ± 0.07 mol m⁻² s⁻¹. These rates were
similar to the pre-treatment conditions (p = 0.14). Water limitation during high
temperatures eliminated the positive effect of eCO₂. Water-limited trees reduced gₛ
close to zero, regardless of [CO₂] levels or provenance. Values for gₛ did not differ
across provenances (p > 0.20), except for well-watered trees under heat stress (p =
0.04). Here, trees from AN had the largest means, whereas trees from YR had the
Intra-specific differences during eCO$_2$, heat and water stress

smallest means of $g_s$. Nevertheless, these differences could not be linked to climate of seed origin ($p > 0.45$).

Similarly, iWUE across provenances did not differ at any point in time ($p > 0.33$). Throughout the experiment, trees grown in eCO$_2$ exhibited higher iWUE than trees in aCO$_2$ ($p < 0.001$; Fig. 2.7 E–F). Water limitation and heat increased iWUE ($p < 0.001$) when compared to well-watered trees. However, this was only true for trees in eCO$_2$ ($p = 0.004$) with a mean of 100.5 ± 7.4 µmol mol$^{-1}$, which represented a 174% increase relative to well-watered trees. In aCO$_2$, water-limited trees were not significantly different compared to well-watered trees at the end of treatment (Fig. 2.7E–F).

Maximum carbon assimilation under saturating CO$_2$ concentration ($A_{max}$) was not different across provenances nor affected by growing [CO$_2$] at any time during the experiment (data not shown). With progressive water limitation and heat, $A_{max}$ declined in water-limited trees (9.6 ± 1.1 µmol m$^{-2}$ s$^{-1}$) compared to well-watered trees (31.2 ± 1.5 µmol m$^{-2}$ s$^{-1}$), which was a −69% decrease ($p < 0.001$). Stomatal conductance at saturating [CO$_2$] ($g_{smax}$) exhibited down-regulation from 1.10 ± 0.16 mol m$^{-2}$ s$^{-1}$ in aCO$_2$ to 0.61 ± 0.08 mol m$^{-2}$ s$^{-1}$ in eCO$_2$ before water limitation and heat treatment ($p = 0.009$). With water availability decreasing during the heat wave, positive effects of eCO$_2$ on $g_{smax}$ vanished and declined to 0.11 ± 0.02 mol m$^{-2}$ s$^{-1}$ ($p < 0.001$).

2.3.4 Non-structural carbohydrates

Concentrations of non-structural carbohydrates (NSC) in stem and roots did not change as result of eCO$_2$. However, NSC varied significantly among provenances (Fig. 2.9). At the end of the heat wave and water limitation treatment and before recovery, trees from different provenances varied in their root ($p = 0.04$) and marginally ($p = 0.08$) in their concentrations of soluble sugars (Ss) in stems.

Moreover, Ss concentrations had an overall tendency to be higher in trees from populations with higher mean annual temperatures at seed origin ($Pearson’s r = 0.67, p < 0.001$ for Ss in stem and $Pearson’s r = 0.70, p < 0.001$ for Ss in roots; Fig. 2.9).
2.9 A–B). Concentration of Ss in roots of provenance WC, originating from the warmest climate (7 ± 0.4%) was twice that contained in roots of provenance YR from the coolest climate (4 ± 0.7%). The same trend was found for Ss in stem tissue where trees from WC were found to have twice as much Ss (4 ± 0.4%) as trees from YR. Although St concentrations among provenances were significantly different in stems ($p < 0.001$), but not in roots, there was no correlation of St concentrations and climate-of-origin. Trees from CW, ND and YR had similar St concentrations in stems, ranging from 3–4%, whereas concentration of St in trees from LC was 6%.

The relationship between NSC concentration and total NSC per dry mass (NSC$_{\text{total}}$) of roots and stems was assessed. However, regressions for all combinations of Ss and St in stem and roots pooled for [CO$_2$] treatments indicated that NSC$_{\text{total}}$ were not correlated with concentrations of NSC. Accordingly, despite significant differences among provenances for NSC$_{\text{total}}$ in roots ($p < 0.05$; Fig. 2.9 C–D), correlations with climate parameters were weak and mostly driven by provenance WC (detailed statistical results can be found in Supplementary Table S2.2).
Figure 2.9: Pools of non-structural carbohydrates (NSC) of *E. camaldulensis* after heat and water stress across [CO$_2$] treatments (n = 2). Panel A and B show mass of NSC per mass of tissue, C and D the estimate of total stored NSC (NSC$_{total}$) for stem or root tissue. Left-hand panels show NSCs in stem, right hand panels in root tissue. NSCs were separated into soluble sugar (Ss) and starch concentrations (St). Error bars represent one standard error of the means. Lower case letters above columns indicate significant differences among provenances for St, upper case letters for Ss for tree stems and roots separately. Provenances are abbreviated as per Table 2.1.

Assessing NSC remaining in stems and roots after the stress event relative to the trees ability to form new leaves during recovery from stress showed that trees with greater concentrations of Ss produced more LA$_{juveniles}$ (Fig. 2.10). This trend was significant for NSC in stems ($p = 0.005$) and roots ($p = 0.03$). Trees from warmer climates (WC and LC) tended to have greater concentrations of NSC available and generated greater LA$_{juveniles}$ than trees from colder climates (YR or CW). This climate-related trend was stronger for stem NSC compared to root NSC ($R^2 = 0.56$ and 40). [CO$_2$] treatments did not alter the observed patterns and regression fits shown in Fig. 2.10 were pooled for aCO$_2$ and eCO$_2$. 

---

Chapter Two  
Intra-specific differences during eCO$_2$, heat and water stress
Chapter Two

Intra-specific differences during eCO$_2$, heat and water stress

2.3.5 Leaf water content and osmotic potential

Osmotic potential of leaf sap was influenced by soil water availability. Water content in leaves (MC$_\text{leaf}$) was related to midday leaf water potentials (Fig. 2.11) in aCO$_2$ ($p < 0.001$) and eCO$_2$ ($p < 0.001$). As trees became more water-limited, MC$_\text{leaf}$ decreased with more negative $\Psi_{\text{md}}$. Consequently osmotic potential of leaf sap ($\Psi_r$) declined. Neither MC$_\text{leaf}$ nor $\Psi_r$ were affected by [CO$_2$] treatments during the experiment, nor were differences across provenances detected. Yet, MC$_\text{leaf}$ and $\Psi_r$ declined with water limitation and heat ($p < 0.001$). Thus, water-limited trees had a $-9\%$ decrease in MC$_\text{leaf}$ averaging $68.4 \pm 0.8\%$ compared to $75.2 \pm 0.5\%$ in well-
watered trees and a −53% decline in $\Psi_\pi$ averaging −2.07 ± 0.09 MPa compared to −1.35 ± 0.03 MPa across [CO$_2$] treatment and provenances.

Figure 2.11: Midday leaf water potential ($\Psi_{md}$) related to leaf water content (MC$_{leaf}$, A–B) and leaf osmotic potential ($\Psi_\pi$) related to leaf water content (C–D). All data of the four selected provenances of E. camaldulensis are shown for well-watered conditions (closed symbols) plus water-limited trees (open symbols) only during water reduction phases. Lines represent regression fits across all provenances with 95% confidence intervals as grey areas. Provenances are shown as different symbols and colours: YR: ▲, CW: ●, ND: □, WC: ◈. Provenances are abbreviated as per Table 2.1.

Previous trends in MC$_{leaf}$ and $\Psi_\pi$ reversed during the recovery phase (Fig. 2.12). Water-limited trees had with 72.8 ± 0.6% greater MC$_{leaf}$ in newly developed leaves compared to leaves from trees that were continuously well-watered (68.6 ± 0.8%; $p < 0.001$). Osmotic potential in previously water-limited trees was also less negative ($−1.58 ± 0.02$ MPa) compared to well-watered trees ($−1.71 ± 0.04$ MPa; $p = 0.001$). $\Psi_\pi$ was less negative in water-limited trees during recovery compared to trees.
before water and heat treatment commenced \((p = 0.03)\). Finally, \(\Psi_n\) remained unchanged in well-watered trees throughout the different experimental phases.

![Figure 2.12: Leaf water content (MC\(_{\text{leaf}}\); A) and osmotic potential (\(\Psi_n\); B) before (26°C, WW), during (42°C, WW/WL) and after heat/water treatment (26°C, WW). Well-watered control trees (WW) are shown as black, water-limited trees (WL) as white columns. Data were pooled for [CO\(_2\)] treatments \((n = 23–67\text{ per treatment})\). Error bars represent one standard error of means.](image)

### 2.3.6 Biomass and leaf area

Stem volume \((v_{\text{stem}})\) did not differ among provenances \((p > 0.15; \text{Table 2.3})\). eCO\(_2\) significantly increased \(v_{\text{stem}}\) before the heat treatment commenced \((p = 0.04)\) and was 33.6 ± 1.8 cm\(^3\) in eCO\(_2\) compared to 28.2 ± 1.4 cm\(^3\) in aCO\(_2\). However, this growth enhancement disappeared during recovery \((p = 0.76)\). Water limitation and heat substantially resulted in a dramatic reduction of \(v_{\text{stem}}\) by almost 50%. At the end of the experiment, \(v_{\text{stem}}\) of well-watered trees was with 108.2 ± 6.7 cm\(^3\) significantly greater \((p < 0.001)\) compared to \(v_{\text{stem}}\) of water-limited trees with 52.5 ± 3.3 cm\(^3\).
Table 2.3: Average stem volume means (± one standard error) for each *E. camaldulensis* provenance (prov.) and [CO₂] level. Data are presented for before commencing of water treatment (pre-treat.) as well as separated for water-limited (WL) and well-watered (WW) trees after heat and water treatment during recovery.

<table>
<thead>
<tr>
<th>prov.</th>
<th>water treat.</th>
<th>pre-treat.</th>
<th>recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCO₂</td>
<td>eCO₂</td>
<td>aCO₂</td>
</tr>
<tr>
<td>YR</td>
<td>WW</td>
<td>27.5 ± 4.3</td>
<td>31.1 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>49.2 ± 14.6</td>
<td>46.1 ± 17.3</td>
</tr>
<tr>
<td>CW</td>
<td>WW</td>
<td>27.8 ± 3.3</td>
<td>30.3 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>34.6 ± 7.8</td>
<td>21.2 ± 3.7</td>
</tr>
<tr>
<td>AN</td>
<td>WW</td>
<td>27.5 ± 3.6</td>
<td>38.1 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>55.5 ± 8.1</td>
<td>62.7 ± 8.4</td>
</tr>
<tr>
<td>ND</td>
<td>WW</td>
<td>28.1 ± 3.1</td>
<td>34.7 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>54.0 ± 10.0</td>
<td>47.5 ± 23.3</td>
</tr>
<tr>
<td>LC</td>
<td>WW</td>
<td>28.6 ± 3.1</td>
<td>35.1 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>59.7 ± 10.9</td>
<td>62.6 ± 5.8</td>
</tr>
<tr>
<td>WC</td>
<td>WW</td>
<td>29.3 ± 4.1</td>
<td>32.6 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>69.4 ± 14.4</td>
<td>55.7 ± 9.8</td>
</tr>
</tbody>
</table>

Additionally, DW<sub>plant</sub> of trees harvested in the precursory experiment before heat and water stress commenced was significantly different across provenances (*p* = 0.03); trees from provenance WC were the largest and trees from CW and LC were the smallest trees (Table 2.4). These differences were due to significant differences in DW<sub>root</sub> across provenances (*p* = 0.003), but not DW<sub>stem</sub> or DW<sub>leaf</sub>. Moreover, DW<sub>plant</sub> at the end of experiment ranged from 19–304 g (separate means for leaves, stem and roots can be found in the Supplementary Table S2.3). At the end of the experiment, trees from colder climates were generally smaller than trees from warmer climates (Fig. 2.13A–B). Water-limited trees from YR (cooler climate) had a mean DW<sub>plant</sub> of 64 ± 22 g in aCO₂, whereas trees from WC (warmer climate) weighed on average 100 ± 22 g in aCO₂ (Table 2.4).
Table 2.4: Total plant dry weight and leaf area of *E. camaldulensis* before heat and water limitation (pre-treat.) and after recovery. Means are aggregated weights of leaves, stem and roots and given with ± one standard error in brackets for each provenance (prov.) and [CO\(_2\)] level as well as for water-limited (WL) and well-watered (WW) trees (*n* = 2 or 4 per provenance). Provenances are abbreviated as per Table 2.1.

<table>
<thead>
<tr>
<th>prov.</th>
<th>water treat.</th>
<th>pre-treat. DW(_{plant})</th>
<th>recovery</th>
<th>pre-treat. leaf area</th>
<th>recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>aCO(_2)</td>
<td>eCO(_2)</td>
<td>aCO(_2)</td>
<td>eCO(_2)</td>
</tr>
<tr>
<td>YR</td>
<td>WW</td>
<td>17.3 (1.9)</td>
<td>25.3 (2.5)</td>
<td>167.1 (71.9)</td>
<td>216.1 (46.2)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>63.6 (21.5)</td>
<td>68.0 (25.2)</td>
<td>1245 (492)</td>
<td>1249 (243)</td>
</tr>
<tr>
<td>CW</td>
<td>WW</td>
<td>15.5 (1.7)</td>
<td>23.0 (2.2)</td>
<td>136.6 (56.0)</td>
<td>267.7 (25.4)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>49.9 (12.4)</td>
<td>36.9 (5.1)</td>
<td>1322 (445)</td>
<td>625 (459)</td>
</tr>
<tr>
<td>AN</td>
<td>WW</td>
<td>21.9 (2.8)</td>
<td>25.1 (3.1)</td>
<td>136.1 (5.4)</td>
<td>1429 (144)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>60.9 (7.9)</td>
<td>92.1 (25.6)</td>
<td>1442 (231)</td>
<td>1823 (764)</td>
</tr>
<tr>
<td>ND</td>
<td>WW</td>
<td>16.9 (2.0)</td>
<td>24.8 (2.4)</td>
<td>194.6 (1.0)</td>
<td>208.6 (1.5)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>68.7 (16.8)</td>
<td>66.4 (40.9)</td>
<td>1603 (402)</td>
<td>1336 (922)</td>
</tr>
<tr>
<td>LC</td>
<td>WW</td>
<td>15.7 (2.1)</td>
<td>22.0 (3.6)</td>
<td>182.2 (39.4)</td>
<td>203.9 (100.2)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>74.9 (21.1)</td>
<td>119.8 (29.1)</td>
<td>1665 (150)</td>
<td>3079 (1016)</td>
</tr>
<tr>
<td>WC</td>
<td>WW</td>
<td>23.6 (3.1)</td>
<td>29.6 (2.2)</td>
<td>147.0 (36.3)</td>
<td>143.6³</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>99.6 (21.7)</td>
<td>88.1 (14.4)</td>
<td>2173 (223)</td>
<td>1829 (432)</td>
</tr>
</tbody>
</table>

³Data of only one tree.
Similar to stem volume, eCO$_2$ caused DW$_{\text{plant}}$ to increase by 35% before heat and drought stress ($p < 0.004$), but had no significant effect on DW$_{\text{plant}}$ after recovery. Yet, at the end of the recovery period, well-watered trees had a total mass of 160.6 ± 14.7 g in aCO$_2$ and 210.7 ± 18.8 g in eCO$_2$, representing a 31% increase. Water-limited trees in eCO$_2$ were slightly bigger than water-limited trees in aCO$_2$, but still 60% smaller than their well-watered counterparts (Fig. 2.13). Correlation analysis for DW$_{\text{stem}}$ of water-limited trees revealed positive relationships to mean annual temperature at seed origin (Pearson’s $r = 0.40$, $p = 0.01$). When comparing the magnitude of decrease in DW$_{\text{stem}}$ of water-limited to well-watered trees, water-limited trees from colder climates had a greater decrease in DW$_{\text{stem}}$ compared to trees from warmer climates. This trend was more obvious in aCO$_2$ than eCO$_2$ (Fig. 2.13C–D). Root-mass-fraction (RMF) was only significantly different among provenances before the onset of stress ($p = 0.03$), likely due to variation in DW$_{\text{root}}$ as mentioned earlier with trees from YR, AN and WC having the greatest ratios among the six tested provenances. Yet, with the greatest means found for trees from the coolest and warmest climate, RMF was not related to climate-of-origin. During recovery, no such differences could be confirmed, but trees in eCO$_2$ had a greater RMF of 0.35 ± 0.02 compared to trees in aCO$_2$ with 0.29 ± 0.02 ($p = 0.05$). Water treatment had no effect on this plant trait.
Leaf area (LA) was also increased by 31% in eCO$_2$ before stress treatment commenced ($p = 0.04$) averaging $1584 \pm 73$ cm$^2$ compared to $1209 \pm 58$ cm$^2$ in aCO$_2$, this effect was lost during recovery. During recovery, water-limited trees had lower LA ($1672 \pm 171$ cm$^2$) compared to well-watered trees ($4082 \pm 285$ cm$^2$; $p < 0.001$). By the time of final harvest, water-limited trees had recovered about 40% LA of the well-watered trees (Table 2.4). Leaf area varied across trees from different origins during pre-treatment conditions ($p = 0.04$), but no trends related to climate-of-origin were detected.
However, trees originating from higher mean annual temperatures produced more LA since leaf death than trees from cooler climates \((\text{Pearson’s } r = 0.34, p = 0.03)\). Thus, water-limited trees from CW and YR had the smallest canopy, and trees from WC and LC had the largest canopy. Leaf area ratio per unit plant dry weight was non-significant for \([\text{CO}_2]\), provenance or water treatment effects before and after the heat wave.

### 2.4 Discussion

This study was designed to test \textit{genotype by environment interactions} of \textit{Eucalyptus camaldulensis} provenances grown in elevated \([\text{CO}_2]\) and exposed to future extreme climate events (i.e. heat wave and water limitation). Water limitation triggered the strongest negative responses in trees, while eCO\(_2\) had a minor positive effect on growth and physiology that mostly disappeared with water stress. During optimal environmental conditions, neither physiological traits like \(A\) nor biomass were correlated to climate of seed origin (Table 2.5). However, differences in physiology and growth related to mean annual temperature became apparent when trees were exposed to heat and water stress. In contrast to expectations based on previous studies, trees from more mesic climates were generally less affected by heat and water stress than trees from semi-arid climates. This effect was evidenced by higher \(A\) and longer time-to-leaf-death during stress.

A commonly observed trend is that trees originating from warmer and drier environments display greater tolerance to drought or heat stress relative to trees originating from cooler and wetter environments (Aspinwall \textit{et al.} 2015; Choat \textit{et al.} 2007; Gibson \textit{et al.} 1995; Tuomela 1997). Yet, previous work has shown that the genetic structure of \textit{E. camaldulensis} was relatively uniform across provenances that originated from locations with contrasting history of drought severity (Dillon \textit{et al.} 2015). In the present study, however, trees from semi-arid climates contained greater quantities of stored carbohydrates after heat and water stress that possibly enabled them to develop a new canopy and regain stem growth more rapidly compared to trees from mesic climates.
Table 2.5: Relative ranking of selected plant traits across *E. camaldulensis* provenances. Means were ranked for measurements before (pre-treatment), during (42°C, no added water) and after heat and water stress (recovery). Ranking was separated for ambient (400 µmol mol⁻¹) and elevated (640 µmol mol⁻¹) [CO₂] conditions. Provenances ranking higher had greater mean values for photosynthesis rates at saturating light (\(A_{sat}\)), leaf area (LA), stem dry weight (DWstem), number of days after onset of water limitation until leaf death (days leaf death), midday leaf water potential (\(\Psi_{md}\)) and concentrations of non-structural carbohydrates in roots (NSCroots). Provenances are abbreviated and highlighted in colours according to their climate-of-origin (red: warm and dry; blue: cold and wet; green: intermediate). Provenances are abbreviated as per Table 2.1.

<table>
<thead>
<tr>
<th></th>
<th>(A_{sat})</th>
<th>(LA)</th>
<th>DWstem</th>
<th>days leaf death</th>
<th>(\Psi_{md})</th>
<th>(A_{sat})</th>
<th>NSCroots</th>
<th>LA</th>
<th>DWstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>high, (less negative)</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
</tr>
<tr>
<td>WC</td>
<td>CW</td>
<td>AN</td>
<td>WC</td>
<td>WC</td>
<td>YR</td>
<td>YR</td>
<td>CW</td>
<td>WC</td>
<td>WC</td>
</tr>
<tr>
<td>CW</td>
<td>LC</td>
<td>WC</td>
<td>AN</td>
<td>AN</td>
<td>YR</td>
<td>ND</td>
<td>NC</td>
<td>CW</td>
<td>ND</td>
</tr>
<tr>
<td>YR</td>
<td>AN</td>
<td>YR</td>
<td>ND</td>
<td>YR</td>
<td>ND</td>
<td>ND</td>
<td>YR</td>
<td>CW</td>
<td>LC</td>
</tr>
<tr>
<td>AN</td>
<td>ND</td>
<td>ND</td>
<td>CW</td>
<td>AN</td>
<td>LC</td>
<td>AN</td>
<td>LC</td>
<td>AN</td>
<td>YR</td>
</tr>
<tr>
<td>ND</td>
<td>YR</td>
<td>YR</td>
<td>CW</td>
<td>YR</td>
<td>LC</td>
<td>AN</td>
<td>LC</td>
<td>YR</td>
<td>AN</td>
</tr>
<tr>
<td>low, (more negative)</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
<td>400 640</td>
</tr>
<tr>
<td>LC</td>
<td>WC</td>
<td>LC</td>
<td>CW</td>
<td>LC</td>
<td>WC</td>
<td>LC</td>
<td>AN</td>
<td>YR</td>
<td>CW</td>
</tr>
<tr>
<td>YR</td>
<td>CW</td>
<td>CW</td>
<td>AN</td>
<td>CW</td>
<td>AN</td>
<td>CW</td>
<td>AN</td>
<td>YR</td>
<td>CW</td>
</tr>
<tr>
<td>AN</td>
<td>ND</td>
<td>ND</td>
<td>AN</td>
<td>YR</td>
<td>AN</td>
<td>AN</td>
<td>YR</td>
<td>AN</td>
<td>AN</td>
</tr>
<tr>
<td>CW</td>
<td>YR</td>
<td>YR</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Intra-specific variation

Tree species with a wide distributional range like *Eucalyptus camaldulensis* usually show phenotypic variation in different plant traits related to their climate-of-origin in order to maximise carbon gain and minimise water loss under locally predominant environmental conditions. However, in *E. camaldulensis* seedlings investigated here, leaf physiological traits were not generally related to climate-of-origin when grown in favourable conditions (ambient temperature and sufficient water supply). Leaf gas exchange, as well as leaf and osmotic potentials in these provenances were relatively similar.

Similarly, when several provenances of *Eucalyptus tricarpa* were grown in a common garden, naturally occurring differences in water use efficiency vanished (McLean *et al.* 2014). However, differences in leaf morphological and anatomical traits were still detectable and trees from drier climates exhibited greater plasticity in leaf thickness than trees from wetter climates. Additional intra-specific differences were found in other studies using *Eucalyptus* spp. These included growth rates, traits related to leaf gas exchange and associated water use efficiency (Johansson & Tuomela 1996; Li *et al.* 2000). The observed differences may be the result of a less conservative water use strategy in climates where soil water is abundant and can be utilised to maximise growth. In contrast, trees growing naturally in drier climates must adapt their physiological functioning to remain functional during periods of minimal water availability and as such ensuring survival (Mäkelä *et al.* 1996; O’Brien *et al.* 2007).

Intra-specific variation under eCO$_2$

Elevated [CO$_2$] had a positive effect on plant water status, leaf physiology traits and biomass under optimal growing conditions before heat and water treatments commenced. The magnitude of increases in $A$ (+16%) and total plant mass (+35%) of *E. camaldulensis* as result of eCO$_2$ (over 560 µmol mol$^{-1}$) was similar to that estimated for a broad range of tree species (Wang *et al.* 2012) and matched the results of a similar study on the same species (Loik *et al.* 2017). However,
provenances of *E. camaldulensis* that displayed high growth rates and A in aCO$_2$ also displayed higher responses in eCO$_2$, indicating that there was no $G \times [\text{CO}_2]$ interaction. This result was further supported by an analysis on a broad variety of trees species, including *E. camaldulensis* seedlings, that found relative rankings of growth traits in eCO$_2$ across genotypes and species were similar to those observed under aCO$_2$ (Resco de Dios *et al.* 2016; Temme *et al.* 2015). Contrary to growth traits, ranking of photosynthesis rates across provenances was not consistent between aCO$_2$ and eCO$_2$ in my study which was also found by Resco de Dios *et al.* (2016). However, photosynthesis per leaf area was not linked to biomass outcome and on a whole plant basis, may generate different rankings.

Additional carbon generated by increased photosynthesis in eCO$_2$ may be used for other purposes than above-ground growth, including synthesis of osmolytes (Morse *et al.* 1993; Pérez-López *et al.* 2010; Tognetti *et al.* 2000). With root-mass-fraction increasing by 21% in *E. camaldulensis* when grown in eCO$_2$, this is indicative of shifts in carbon allocation to the roots in order to increase water and nutrient uptake since carbon fixation is already increased (Franklin *et al.* 2012). In this study, eCO$_2$ did not have an impact on osmotic potential of *E. camaldulensis* suggesting that newly-fixed carbon was not utilised to optimise osmotic adjustment. Accordingly, Wullschleger *et al.* (2002) concluded that eCO$_2$ would have a relatively minor effect on osmotic potential in leaves since carbon is likely used to enhance growth performance, especially in young fast-growing trees, rather than generate osmotically active solutes.

*Intra-specific variations to heat and water stress*

In the present study, heat and water stress triggered a substantial and negative impact on tree functions. Both heat and water stress resulted in marked $G \times E$ interactions related to growth, A and NSC concentrations. This indicates that trees from the selected provenances operated similarly during optimal growing conditions, but responded differently to heat and water stress depending on climate-of-origin. Biomass yield after heat and drought stress differed significantly among provenances of *E. camaldulensis*, leaving trees from semi-arid climate to
have the greatest stem dry weight. This result suggests that differences in the allocation of carbon to growth, carbohydrate storage, cell respiration or defence mechanisms exist – similar to other studies on plasticity of tree proveances (e.g. Körner 2003; Resco de Dios et al. 2016; Sala et al. 2012). It follows that phenotypic differences in selected plant traits do exist and utilisation of these traits in breeding programs can be harnessed (see Booth 2013).

Midday leaf water potentials during heat and drought were more negative in trees from semi-arid climate (WC), resulting in lower A and a shorter time to reach 80% leaf mortality. These intra-specific differences were likely a result of variation in canopy size. In particular, trees from provenance WC had a larger canopy area (+22%) when compared to the other five provenances. This larger canopy is likely to have depleted available soil water more rapidly compared to trees with smaller canopies. Similar to the present study, Gibson et al. (1991) showed that within a different subspecies of E. camaldulensis, proveances had similar rates of photosynthesis when well-watered; however, smaller trees maintained relatively high rates of photosynthesis even when water was severely limited, whereas rates of photosynthesis declined up to −30% in larger trees.

Despite being more affected by heat and drought, trees from the semi-arid climate (WC and LC) had a greater capacity to recover more quickly from these stress events due to greater carbohydrate reserves, which supported relatively faster production of new foliage. This faster recovery of photosynthetically active tissue also led to the overall smallest growth reduction after re-watering had commenced, compared to continued growth of well-watered control plants (Table 2.5). Since embolism recovery requires extra energy resources (Brodersen & McElrone 2013; Zwieniecki et al. 2009), it is reasonable to speculate that greater carbohydrate reserves in these proveances supported a fast recovery of hydraulic functions and subsequently allows for a faster development of a new canopy (O’Brien et al. 2014).

E. camaldulensis, like many other eucalypts, re-sprouts from epicormic buds after disturbances like fire or drought (Zeppel et al. 2015). Its capacity to re-sprout is mainly dependent on the form and degree of damage and the ability to mobilise carbon resources (Knox & Clarke 2005). Not surprisingly, species with high re-
sprouting capacity have high root and stem NSC concentrations (Myers & Kitajima 2007; Nzunda et al. 2008). Given that trees originating from WC and LC live in semi-arid conditions in Australia’s outback and regularly experience heat waves and water shortages, it is very likely that these provenances had adapted to sustain such conditions by dedicating a large proportion of assimilates to carbohydrate storage. Thus, *E. camaldulensis* trees originating from semi-arid climate with long drought periods may have adapted to these conditions by initiating leaf abscission earlier to prevent xylem cavitation and rather utilise more energy when conditions improve.

Arguably, trees originating from more xeric climates are often found to be smaller than trees from more mesic climates due to adaptations to their limitations from heat and water stress as well as low nutrient availability (e.g. Li et al. 2000; Li & Wang 2003; Viger et al. 2016). My findings that provenance WC from semi-arid climate was relatively larger when grown inside the glasshouse, may have been the result of greater plastic responses when conditions were ideal. Similar results were obtained for *Eucalyptus globulus* genotypes grown at numerous sites across Australia (Costa e Silva et al. 2006). Here, significant *G × E* interactions for stem growth between field sites have been reported, resulting in greater relative growth enhancement of smaller trees at dry sites with increasing water availability at other growing location.

Despite intra-specific differences in carbon related traits, stomatal conductance was regulated in similar ways across provenances during water limitation. White et al. (2000) showed that *E. camaldulensis* has control over transpirational water loss via regulation of stomatal opening, which explains the uniform decline across all provenances observed here during water limitation. The study further reported great cell wall elasticity for this species. With more flexible cell walls, turgor pressure can further decrease, which would theoretically result in more negative water potentials that in turn would allow an increase of water uptake from drying soil. Since osmotic potential was correlated with leaf water status, the more negative osmotic potential during heat and water stress was likely a product of decreasing volume of water rather than an active accumulation of osmotic solutes.
even though these two mechanisms don’t have to be exclusive (Sanders & Arndt 2012; Blum 2017). However, osmotic potential was not different across provenances, which was in agreement with a previous study on this species (Lemcoff et al. 2002), while other studies on different eucalypts found evidence for provenance differences (Guarnaschelli et al. 2003; Li 1998; Merchant et al. 2007; Ngugi et al. 2003). Clearly, future research is required to uncover if species-specific differences in osmotic adjustment are related to climate-of-origin for a given species or provenance.

Response to heat and water stress and eCO$_2$

Water stress was the main environmental driver of plant responses in the current study. eCO$_2$ may ameliorate some genotypic responses, yet such responses were insufficient to fully compensate for the overall effect of drought on time-to-leaf-death, leaf gas exchange or most of the growth reduction. This observation matches well with other studies (Duan et al. 2014; Gunderson et al. 2002). Despite the near complete lack of significant $G \times E$ (heat, water and [CO$_2$]) interactions, eCO$_2$ had the most noticeable positive influence on leaf gas exchange during heat stress. At the end of the heat and water treatment, eCO$_2$ ameliorated rates of photosynthesis and hence iWUE in water-limited $E$. camaldulensis. Moreover, $E$. camaldulensis trees in eCO$_2$ had less negative midday water potentials at the end of the stress event and similar results were found for other eucalypts grown in eCO$_2$ (700 µmol mol$^{-1}$; Atwell et al. 2007; Roden & Ball 1996). This indicates that increased water use efficiency in trees grown in eCO$_2$ ameliorated responses to heat and drought. Elevated CO$_2$ also helped maintain photosynthesis and stomatal conductance in seedlings that were heat stressed while well-watered, whereas the same treatment in aCO$_2$ showed a decline in these parameters. It is likely that the positive effect of eCO$_2$ during heat stress has its origin in a greater suppression of carbon losses due to photorespiration (Loik et al. 2017). These observations match findings from a similar experiment that used Quercus rubra (Bauweraets et al. 2013) and suggest that, while the impact of eCO$_2$ during water limitation may be small, heat tolerance can be greatly improved in eCO$_2$. 
One recorded plant trait involving time-to-leaf-death was found to have $G \times \text{heat, drought and eCO}_2$ interaction. Overall, five of the six provenances were not affected by eCO$_2$ regarding time-to-leaf-death during heat and water stress due to reduced stomatal conductance and transpirational water loss. With $g_s$ decreasing close to zero, effects of eCO$_2$ are negligible and similar results have been found in other studies on *Eucalyptus* (Duan *et al.* 2014). However, trees originating from the warmest and driest climate (WC) did delay leaf death by 4 days in eCO$_2$ – a clear $G \times E$ interaction. In another study, eCO$_2$ (650 µmol mol$^{-1}$) delayed the onset of wilting as a response to severe water limitation in *Eucalyptus saligna*, but not in *E. sideroxylon*, confirming a complex response to eCO$_2$ (Lewis *et al.* 2013). Different genotypes of *Pinus silvestris* also showed extended time to mortality when A and NSC in woody tissue were greater before the onset of severe drought suggesting that drought tolerance is related to carbon metabolism (Garcia-Forner *et al.* 2016).

In conclusion, among the six provenances, trees originating from semi-arid Australia were the ‘winners’ in recovering from heat and water stress. Trees growing naturally near Wilcannia developed the greatest biomass in aCO$_2$, whereas those from Lake Cargelligo were the largest trees in eCO$_2$. Hence, as a plantation tree, these populations may generate the highest volumes of biomass when environmental conditions are benign, but also cope well when recovering from abiotic stress events that involve a combination of drought and very high temperatures.

*Limitations and suggestions for future research*

Due to a limited number of available trees, replication was very low and individuals might not fully represent the true population mean. A sample number $> 5$ is advised for future studies that assess effects of eCO$_2$ and abiotic stresses on tree provenances.

Furthermore, it has been suggested that C$_3$ species originating from cooler climates would be more heat tolerant when grown at low temperatures rather than warmer temperatures before the stress event (Wang *et al.* 2008). Thus, growing all
provenances under the same temperature regime of 26°C midday temperatures with relatively mild night time temperatures might have skewed results in support of provenances originating from warmer climates in the present study.

Another limitation of this study is tree age since responses may vary with developmental stage as a result of morphological and physiological alterations with age and size (Saxe et al. 1998). Despite ameliorating effects of eCO₂ on growth seen in young trees, there is evidence that this effect diminishes in mature trees (e.g. Hättenschwiler et al. 1997, Tognetti et al. 2000). Furthermore, *E. camaldulensis* in natural field conditions was shown to avoid drought stress by increasing its root system into deeper soil layers, which is not possible in pot studies (White et al. 2000). Nevertheless, this is only the case for mature trees, whereas smaller saplings in pots as used in this study cannot reach deeper soil layers. Moreover, duration of the drought treatment for a maximum of 16 days was short and severe, which may not mimic natural conditions of slowly evaporating soil moisture. This may have affected concentration changes of NSC, osmotic and cell wall elasticity adjustment. Future studies should be designed to slowly reduce soil water content and multiple heat waves of short duration.

Low irradiation during the drought implementation in winter may be another limitation for this study. Excess light energy in summer commonly causes increased concentrations of ROS, which is unlikely to happen in winter. However, since soil moisture reduction of potted plants is often faster than in natural conditions due to the small volume of soil, low irradiation and associated slow rates of transpiration may have been beneficial, as these conditions would have resulted in a slowed release of soil water.

Due to limited replication within provenances, NSC concentrations were not measured before the heat and drought treatment. Hence, data do not allow conclusions about existing NSC storage before the treatment and the extent of utilisation of this storage during high temperatures and low water availability. In theory, trees may have had similar amounts of NSC available before the treatment, but utilised variable proportions during stress; alternatively some trees may have
had more NSC available than others before the treatment, but all trees utilised similar quantities of NSC.

In addition, eucalypt plantations have a rotation frequency of six to ten years (Booth 2013). In their lifetime, plantation trees may also encounter multiple stress events before their final harvest, which underlines the importance to study and understand carry-over effects and acclimation capacity of preconditioned trees, which might alter the response to re-occurring stresses. Furthermore, it needs to be pointed out that results from this study originated from controlled environments as well as young potted trees. Natural conditions and responses of mature trees may vary from reported results, making it essential for future studies to focus on older trees in outdoor experiments like common gardens.

2.5 Conclusion

A major focus in forestry is the identification of tree species and their provenances that display high growth rates and high stress resilience in plantations in future climate regimes. The six provenances of *E. camaldulensis* in this study displayed a considerable degree of plasticity to increased [CO₂], heat stress and water limitation. Physiological responses to stress were related to mean annual temperature and precipitation at climate-of-origin of tree seeds. Populations originating from cooler and wetter climates were less affected by heat and water stress for longer periods of time, regardless of [CO₂]. However, this resistance comes at the expense of depleting their stored carbon resources and hence recovering slower after the stress event. On the other hand, provenances from warmer and drier climates tolerated stress for a shorter period, but had greater carbohydrate resources remaining after the stress event, which they could use to generate more leaf area and regain stem growth faster. Thus, two different survival strategies appear to have been developed for adaptation to environmental conditions at the local seed locations (Mitchell *et al.* 2013). Trees growing in semi-arid Australia experience long and severe heat and drought events throughout their life cycle. Hence, shorter periods of resistance may minimise the risk of severe or lethal water stress and conserve carbohydrates, which maximises recovery rates.
once conditions are more favourable. In contrast to this strategy, trees originating from environments with higher rainfall and fewer heat waves may gain advantage by resisting this short-term stress and maintaining productivity, at the cost of slower recovery. In conclusion, selecting provenances within plantation species on the basis of their response to multiple stresses represents an innovative strategy to establish high yielding plantations in the novel climates of the future.
Chapter Three: Intra-specific variation of physiological response to elevated CO$_2$ and water limitation in *Eucalyptus grandis*

3.1 Introduction

In Australia, species of the genus *Eucalyptus* are widespread and adapted to a wide range of climates (Austin *et al.* 1990; Brooker *et al.* 2006). Yet, with changing patterns of precipitation, particularly with longer and more frequent water scarcity, growth rates are likely to decline (Whitehead & Beadle 2004). However, trees have a capacity to adjust to new abiotic conditions by changing anatomical, morphological or physiological traits. Intra-specific variation exists either due to heritability of specific trait characteristics or due to phenotypic plasticity (Moran *et al.* 2016). Greater plasticity of traits can have a positive effect on tree performance during abiotic stress (Bradshaw 2006). Furthermore, eCO$_2$ affects tree productivity and stem growth (Bazzaz *et al.* 1990; DeLucia *et al.* 1999; Saxe *et al.* 1998), but these effects are modulated by environmental variables such as temperature, water availability and many others (e.g. Körner *et al.* 2005; Wang *et al.* 2012). Moreover, CO$_2$ effects are species-specific and differences may also exist within genotypes of the same species, in particular when combined with environmental stresses, such as water limitation (Aspinwall *et al.* 2015). In present times of rapid changes in climate, the survival, and consequently fitness, of long-lived organisms such as trees may depend on the capacity to adjust to the new environment through phenotypic plasticity (Nicotra *et al.* 2010). Hence, understanding and harnessing intra-specific variation among naturally occurring tree populations may prove important for maintaining productivity of trees in the future. However, our knowledge about phenotypic plasticity to water limitation and elevated [CO$_2$], especially in tree
species that are valuable for the plantation industry, is very limited (Aspinwall et al. 2015).

**Intra-specific variation**

Species can have high genetic variation across and within populations, thus enabling intra-specific differences in their phenotypic response to biotic and abiotic stress (Bradshaw 1965; Jump & Peñuelas 2005; Moran et al. 2016). Genotypes dominating a population may be successful due to their adaptation to local environmental conditions (Booth et al. 2015; Kawecki & Ebert 2004; Mitton & Duran 2004; Stutz & Mitton 1988). In a changing climate, it is important to understand if trees have plastic responses to improve drought tolerance in order to select plantation trees with the highest possible growth and survival rate at the same time (‘winners’) and also identify and avoid trees that are more drought susceptible (‘losers’).

Selected *Eucalyptus* species from Australia were found to have great intra-specific differences in growth performance when grown in a common environment in China and Japan (Liang et al. 1994: Wang et al. 1994; Wenlong & Kunnan 1992; Wu et al. 1994). However, *E. nitens* did not show a distinct relationship between climate-of-origin and growth, but provenances from warmer New South Wales grew faster than provenances from cooler Victoria (Wang et al. 1994). Therefore, assessments of growth rates for commercially relevant tree species may benefit from examining intra-specific variation in different physiological and morphological traits.

**Physiological response to elevated [CO$_2$]**

Elevated [CO$_2$] can have major effects on physiological traits of trees, including increased biomass, leaf area (LA), photosynthesis (A) and water use efficiency (WUE). However, the magnitude of this response is substantially dependent on tree type (angiosperm or gymnosperm), species, nutrient and water availability, temperature, as well as tree size and age (Ainsworth & Long 2005; Ellsworth et al. 2017; Nowak et al. 2004; Pritchard et al. 1999; Reich et al. 2006; Resco de Dios et
Therefore, Free-Air CO₂ Enrichment (FACE) experiments can be useful to understand tree responses to eCO₂. FACE experiments operated in the northern hemisphere showed that \( A \) of tree species increased by 47% and \( g_s \) decreased by \(-20\%\), resulting in 28% greater above-ground dry mass when grown in eCO₂ (480–600 \( \mu\text{mol mol}^{-1} \); Ainsworth & Long 2005). Declining stomatal conductance \((g_s)\) has also been reported in many studies (Ainsworth & Long 2005; Gunderson et al. 2002; Medlyn et al. 2001; Wang et al. 2012; Will & Ceulemans 1997), however this response is not universal and dependant on the species and environmental conditions (Ainsworth & Rogers 2007; Curtis & Wang 1998; Norby & Zak 2011; Saxe et al. 1998). Moreover, plants can alter carbon allocation to plant organs, for example more carbon can be allocated to root growth in a nutrient limited soil or to canopy growth to fix more carbon when sufficient nutrients are available (Pritchard et al. 1999).

When grown in eCO₂, greater biomass (including greater stem diameter and height) and greater LA is observed, originating from greater stimulation of cell division and expansion in the apex and vascular cambium (Ainsworth & Long 2005; Curtis & Wang 1998; Pritchard et al. 1999; Resco de Dios et al. 2016). Growth increase in eCO₂ is not necessarily tightly correlated with \( A \), as shown in a meta-analysis on young Populus trees. This finding suggests that extra fixed carbon is allocated to other sinks potentially below-ground, respiration or stress tolerance (Resco de Dios et al. 2016). Importantly, growth enhancement in eCO₂ can only be continued if soil nutrients like nitrogen or phosphorus are not limiting (Ellsworth et al. 2017; Norby et al. 2010). Greater root-to-shoot ratios in trees grown in eCO₂ have been observed, which may be the result of nutrient deficiency in glasshouse studies (Luo et al. 2006; Norby 1994). In different FACE experiments, however, evidence was found that rooting depth and mass of trees increases in eCO₂ (see review of Norby & Zak 2011). Regardless of eCO₂ effects on tree physiology, greater rooting depth is often related to tree size, improving excess to deeper soil water and maintaining water status during drought (Duursma et al. 2011).
Intra-specific differences during eCO$_2$ and mild water limitation

Physiological response to water limitation

In addition to increasing concentrations of [CO$_2$], longer and more severe droughts are predicted to occur in the future. Increasing soil water deficit leads to declining plant water potentials and thus an increased risk of hydraulic failure due to xylem embolism (McDowell et al. 2008). Therefore, plants will have various physiological responses to limit their water consumption. Growth is sensitive to water limitation and is often one of the first plant traits that declines due to reduction in cell turgor and thus reduced cell division (Hsiao et al. 1976). Photosynthesis is also reduced since stomatal conductance declines during water stress to limit transpirational loss.

Furthermore, declining carbon assimilation but continuous carbon catabolism can alter NSC concentrations in a tree experiencing water limitation. As a first response, NSC concentrations, especially St, will increase due to a relatively greater reduction in growth than in A. However, as drought continues, NSC concentrations eventually decline due to the relatively larger decline in A, relative to that of respiration and osmotic adjustment (Ayub et al. 2011; Körner 2003; McDowell 2011). A common finding during long and severe water limitation is a decrease of starch concentrations due to transformation into soluble sugars (Ss; Thalmann & Santelia 2017). Duan et al. (2013a) found an increase in Ss and decrease in St concentrations in long-term water-limited Eucalyptus globulus seedlings compared to controls suggesting that with a decline in leaf respiration available carbon was used for osmotic adjustment or maintenance of hydraulic conductance.

Genotypic variation to water limitation

At the intra-specific level, when grown together in common gardens, provenances of the Eucalyptus, Populus and Quercus genus originating from drier climates are often more drought-tolerant than those from wetter climates. These trees had higher WUE and thicker leaves (Gratani et al. 2003; Li et al. 2000; McLean et al. 2014; Viger et al. 2016), lower mortality rates (Thiel et al. 2014), higher osmotic adjustment (Nguyen-Queyrens & Bouchet-Lannat 2003) and may also maintain A at
more negative water potentials (Abrams 1994). At the same time, they showed lower growth rates compared to provenances from wetter climates. *Eucalyptus globulus* exhibited significant genotype by environmental (*G* × *E*) interactions when grown in mild drought conditions (Osório & Pereira 1994). Total biomass of faster-growing genotypes was reduced by 37%, whereas the slower-growing genotypes exhibited only a reduction of 18%. Similarly, WUE increased by 19% in the slower-growing genotypes and 31% in the faster-growing genotypes during drought. When three provenances of *E. microtheca* seedlings were exposed to mild and severe water limitation, trees originating from dry environments were smaller, but had enhanced water use strategies with greater root-to-shoot ratios, greater WUE and tolerated more negative water potentials than trees from wetter areas (Li & Wang 2003). This indicates that a conservative water use strategy inevitably leads to slow rate of growth and is in agreement with the suggested conservative water use strategies of trees growing in drought-prone areas.

*Combining effects of eCO₂ and water limitation on genotypic response*

While some studies have investigated phenotypic responses of trees to eCO₂ or water limitation separately, few studies have assessed their interactive effects. However, separately observed tree responses to these two factors may not simply be additive when tested together and hence results are highly variable in the literature. The stimulating effect of eCO₂ on *A*, *gₐ*, NSC or osmotic adjustment thought to be beneficial during drought might be overridden due to declines of photosynthetic capacity (Gunderson *et al.* 2002). Depending on drought severity, tree growth might still be enhanced in eCO₂ and mild water limitation due to improved *A*, *gₐ* and/or water use which then can become problematic during severe water stress when canopy size increases whole plant transpiration (Atwell *et al.* 2007; Ayub *et al.* 2011; Poorter & Pérez-Soba 2001; Warren *et al.* 2011a; Wertin *et al.* 2010; Xu *et al.* 2013; Zeppel *et al.* 2012).

As an example, eCO₂ was shown to increase biomass, *A* and increased NSC concentrations due to sink limitation in *Eucalyptus globulus* seedlings during mild drought stress, however negative effects of severe drought could not be overcome.
Intra-specific differences during eCO$_2$ and mild water limitation

(Duan et al. 2013a). Similarly, in studies on other tree species grown under eCO$_2$ (640–750 μmol mol$^{-1}$) and severe water limitation, effects of eCO$_2$ on drought response were minor and hence there was no detectable interaction between these two factors (Duan et al. 2014; Kelly et al. 2016; Lewis et al. 2013; Perry et al. 2013; Wertin et al. 2012). Moreover, drought tolerance was rather related to tree growth and higher water loss in trees with greater leaf area (Lewis et al. 2013). In five-months old *Eucalyptus tereticornis* seedlings grown in the glasshouse under aCO$_2$ and eCO$_2$ (700 μmol mol$^{-1}$) and subjected to drought stress, growth responses varied depending on developmental stage and water treatment (Atwell et al. 2007). At the end of that experiment, the largest trees were those grown under eCO$_2$ and ample water. Yet, the relative effect of eCO$_2$ on growth was larger in water-limited compared to well-watered trees due to ameliorated water savings.

In addition to these generalised responses, different species and genotypes may respond at different magnitudes to eCO$_2$ and water limitation. In *Platanus occidentalis*, *Liquidambar styraciflua* and *Acer saccharum*, grown in open-top chambers under aCO$_2$ and eCO$_2$ (650 μmol mol$^{-1}$) as well as well-watered and water-limited conditions, species displayed different magnitudes of responsiveness (Tschaplinski et al. 1995). Here, eCO$_2$ increased the relative growth rate of well-watered trees by up to four-fold. During water limitation, this enhancement was reduced (but not eliminated) in sugar maple, while the other two (presumably faster-growing) species did not respond to eCO$_2$. Moreover, LA was reduced during water limitation in all three species, but to a lesser extent in eCO$_2$ than aCO$_2$. Overall, water reduction triggered stronger, more negative effects in all three species, compared to the smaller, positive effects of eCO$_2$.

*Research objectives*

To this day, only little research has been done on the response of trees representing different provenances to combined factor of eCO$_2$ and drought. The main research objectives of this study were to identify provenances of *Eucalyptus grandis*, a relatively drought sensitive plantation species (Fan et al. 1994), that show increased growth in eCO$_2$ while maintaining relatively fast growth during periods of
water limitation. While increased growth in eCO$_2$ (660 µmol mol$^{-1}$) has been shown for *E. grandis* in the past (Conroy *et al.* 1992), the interactive effect of eCO$_2$ and water limitation has not been assessed for different provenances. Evaluating how eCO$_2$ and drought impacts physiological and growth responses of trees from different climates-of-origin may provide a tool to determine ‘winners’ and ‘losers’ in a drier and eCO$_2$-world, thereby improving tree selection for plantations over the next decades. This study addresses the following questions:

1) Are there differences between provenances of *E. grandis* in growth, photosynthesis, stomatal conductance, water use efficiency, and carbohydrate accumulation when grown in aCO$_2$ under well-watered conditions (*G* response)?

2) If the provenances express intra-specific differences under well-watered conditions in aCO$_2$, do these differences persist in eCO$_2$ (*E* response)?

3) Once soil water becomes limiting, do intra-specific differences among provenances grown in eCO$_2$ remain similar to those observed under aCO$_2$ (*G* × *E* interaction)?

### 3.2 Material and Methods

#### 3.2.1 Climate of seed locations

*Eucalyptus grandis* Hill ex Maiden (common name: Flooded Gum) is a fast-growing, tall hardwood species widely used in Australia and the southern hemisphere as a plantation tree for production of timber, fuel and fibre. Moreover, *E. grandis* naturally inhabits the coastal regions of eastern Australia. The natural distribution of this species occurs from North of Newcastle (New South Wales) northwards to Gympie in Queensland with some isolated populations in Northern Queensland from Townsville to the Daintree (Brooker *et al.* 2006). It primarily grows in wet climates with fertile soil. It does not tolerate severe water limitation (McMahon *et al.* 2010a).
Genotypes from four provenances of *E. grandis*, representing different mean annual precipitation (MAP) and/or temperature (MAT) conditions, were selected for this study. Two provenances (Kilcoy Creek and Coffs Harbour) originated from similar MAT, but different long-term water availability, while two sets of provenances originated from similar MAP (Woondum and Kilcoy Creek; and Coffs Harbour and Mount Lindsay), but different temperature regimes (Table 3.1, Fig. 3.1). Climate data was obtained from eMAST (www.emast.org.au) which is part of the Terrestrial Ecosystem Research Network (TERN). Monthly mean temperature, MAP and evapotranspiration (ET) were averaged for the periods of 1971–2010. Altitude was taken from data shown on Google Earth (Google Earth 7.1. 2016) at site coordinates.

Table 3.1: Location and climate parameters characterising the origin of *E. grandis* seed provenance. Mean annual air temperatures (MAT, °C), altitude (ALT, m), mean annual precipitation (MAP, mm) and the ratio of evapotranspiration/MAP (ET/MAP) are shown. Note that higher ET/MAP represents greater water stress.

<table>
<thead>
<tr>
<th>site name</th>
<th>site coordinates</th>
<th>MAT</th>
<th>MAP</th>
<th>ALT</th>
<th>ET/MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt Lindsay (L)</td>
<td>28.21° S 152.45° E</td>
<td>13.6</td>
<td>1314</td>
<td>1022</td>
<td>0.76</td>
</tr>
<tr>
<td>Kilcoy Creek (K)</td>
<td>26.45° S 152.35° E</td>
<td>17.3</td>
<td>974</td>
<td>603</td>
<td>1.22</td>
</tr>
<tr>
<td>Coffs Harbour (C)</td>
<td>30.10° S 153.07° E</td>
<td>17.5</td>
<td>1549</td>
<td>345</td>
<td>0.75</td>
</tr>
<tr>
<td>Woondum (W)</td>
<td>26.18° S 152.49° E</td>
<td>20.2</td>
<td>1021</td>
<td>216</td>
<td>1.34</td>
</tr>
</tbody>
</table>
3.2.3 Growing conditions

Seeds were obtained from five mother trees (family) within each provenance, germinated and individuals grown to enable collection of vegetative cuttings. After development of sufficiently strong roots, cuttings were transplanted into pots made of PVC pipe (15 cm in diameter, 40 cm long) with holes in the bottom and filled with a customised medium starting on 12 March 2015. The medium consisted of 90% coarse washed river sand, 10% diatomaceous earth (intermediate grade: 0.9–2 mm from Mt Sylvia, QLD) and mineral soil (Zeogreen, premium turf grade 0.5–1.6 mm from ZeoGreen, VIC, Australia). Pots were watered daily to saturation. Liquid fertiliser (500 mL Aquasol, at 1.6 g l⁻¹; 23% N, 4% P, 18% K, 0.05% Zn, 0.06% Cu, 0.013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B; Yates Australia, NSW, Australia) was added to the pots on a fortnightly basis.

Trees were grown in four temperature and [CO₂] controlled glasshouse rooms located on the campus of Western Sydney University in Richmond, NSW at 33.61° S 150.75° E. Two glasshouse rooms per [CO₂] were used. Trees were grown under
natural light and constant ambient [CO$_2$] (aCO$_2$; 400 µmol mol$^{-1}$) and eCO$_2$ (640 µmol mol$^{-1}$) for 14 weeks. Twenty pots per provenance were randomly assigned to glasshouse bays and rotated monthly between bays. Temperature, relative humidity, light conditions and [CO$_2$] inside the glasshouse bays were continuously monitored with the same instruments as in Chapter 2. Trees inside the glasshouse experienced 10–15% less light due to absorption by the glass. Diurnal temperature set points were based on average climate conditions at the glasshouse location: 22°C from 6:00–10:00 hours, 26°C from 10:00–16:00 hours, 24°C from 16:00–20:00 hours and 15°C from 20:00–06:00 hours.

Ten seedlings (half-sibs of five mothers) representing each of the four provenances and two [CO$_2$] treatments (total of 80 plants) were initially grown in the glasshouse under aCO$_2$ and eCO$_2$ in well-watered conditions; subsequently, half of the plants were grown in limited water conditions 8 weeks after planting. All five mothers per provenance were represented within the well-watered and water-limited group except for trees from provenance Woondum which had two families which were only represented in either the well-watered or water-limited treatment, respectively.

Over a period of three weeks (starting 18 May 2015), trees received 50% of their initial daily maximal water use which was recorded one week before water limitation started and assessed for each seedling using a gravimetric approach. Each pot was saturated with water and once water had drained completely, the weight of each pot was determined. Then, pots were reweighed after 24 hours (repeated over three days) to provide a proxy for maximal tree water use under well-watered conditions. Watering was reduced over four days from 80, 70, 60 to 50% of previous volume of water used. Once the 50% level was reached (day 4), this level was maintained for three weeks, until trees were destructively harvested. Soil water content was monitored with a Time Domain Reflectometry sensor (TDR; CS616, Campbell Scientific Australia, QLD, Australia) in each water-limited plant pot, plus one well-watered plant per provenance and [CO$_2$] treatment (48 TDRs in total).
3.2.4 Leaf water potential

Predawn and midday leaf water potentials (Ψ\text{pd} and Ψ\text{md}) of each tree were measured on the same day when leaf gas exchange measurements were collected (i.e. 12–13 May, before water treatment commenced; 11–12 June 2015, 21 days after water limitation started). One leaf per tree was collected at least 30 minutes before sunrise and during midday, placed into a moisturised plastic bag and kept cool in an ice box before taking it to the lab. Leaf water potential was measured using a pressure chamber (1505D, PMS Instruments Company, OR, USA) following Boyer (1967).

3.2.5 Leaf gas exchange

Leaf gas exchange on each tree was measured before initiation of the water treatment, as well as four weeks after initiation of the water limitation (referred to as day 25). Leaf light-saturated photosynthetic rates of CO\textsubscript{2} assimilation (A\text{sat}, μmol m\textsuperscript{-2} s\textsuperscript{-1}) and stomatal conductance of water vapour (g\text{w}, mol m\textsuperscript{-2} s\textsuperscript{-1}) of a newly developed, fully expanded leaf was measured on sunny days with LI-6400XT (Li-Cor Inc., NE, USA). Measurements were taken during photochemical active hours (9:30–15:30 hours) on all plants. A 2 × 3 cm chamber head with red/blue light source was used and [CO\textsubscript{2}] concentration set to the tree’s growing concentration. Conditions inside the cuvette were set to saturating light (1600 μmol m\textsuperscript{-2} s\textsuperscript{-1}), block temperature to midday room temperature (26°C) and mean ambient room CO\textsubscript{2} concentration (400 or 640 μmol mol\textsuperscript{-1}) for A\text{sat}. Flow rate inside the cuvette was set to 500 μmol s\textsuperscript{-1}. Leaf vapour pressure deficit was regulated by manipulating relative humidity inside the cuvette and was kept between 1.2–1.6 kPa. Leaves were allowed to equilibrate to conditions for at least five minutes. Gas analysers were matched every time before logging data. A minimum of five data points per plant were logged at 5 second intervals and later averaged.
3.2.6 Leaf $\delta^{13}$C

On the day of gas exchange measurements, one fully developed leaf per tree was collected for analyses of water use efficiency (WUE) over a longer time scale than spot measurements for $A$ and $g_s$. Leaves were taken during afternoon hours and snap-frozen in liquid nitrogen. Care was taken to not sample a leaf which was previously used for gas exchange measurements so that $\text{CO}_2$ sources of glasshouse room and LI-6400XT were not mixed. Leaves were dried at 60°C for three days and ground in a ball mill (MM400 Retsch GmbH, Germany). Samples were sent to the Australian National University to determine $\delta^{13}$C of the leaf bulk tissue ($\delta^{13}\text{C}_{\text{leaf}}$) using mass spectrometry. For these analyses, ground leaf tissue was oxidised under high temperatures and transformed into gases followed by separation in a gas chromatography column using helium as carrier gas and then cleaned in several steps to generate $\text{CO}_2$ and $\text{N}_2$. Gases were analysed for natural abundance of $^{12}\text{C}$, $^{13}\text{C}$, $^{14}\text{N}$ and $^{15}\text{N}$ using a continuous-flow stable isotope ratio mass spectrometer (Fison Isochrom CF-IRMS, former Fisons Instruments, UK).

Rubisco discriminates against the heavier $^{13}$C atom as the enzyme is fixing more of the fasterdiffusing lighter isotope resulting in depletion of $^{13}$C in photosynthates and plant material relative to the atmospheric abundance. The discrimination rate is dependent on the $\text{CO}_2$ concentration gradient between the intercellular leaf space ($c_i$) and the atmosphere ($c_a$). As the ratio of $c_i/c_a$ is regulated by $A$ and $g_s$, closing stomata leads to a low $c_i$ relative to $c_a$, resulting in Rubisco fixing a larger proportion of $^{13}$C and hence lower photosynthetic discrimination rate ($\Delta^{13}\text{C}$) which relates to water use efficiency. $\Delta^{13}\text{C}$ for the hours of 9:00–16:00 was calculated from leaf material ($\delta^{13}\text{C}_{\text{leaf}}$) and known atmospheric values ($\delta^{13}\text{C}_{\text{air}}$) and is expressed in ‰ after Farquhar et al. (1982):

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{leaf}}}{\delta^{13}\text{C}_{\text{leaf}}}$$

Equation 4

Since glasshouse air was artificially enriched with $^{13}$C depleted $\text{CO}_2$, the $\delta^{13}$C fraction of glasshouse rooms ($\delta^{13}\text{C}_{\text{air}}$) was also determined to calculate $\Delta^{13}\text{C}$. This was achieved by collecting measurements of air of all four glasshouse rooms separately.
using a Tuneable Diode Laser (TGA100; Campbell Scientific, UT, USA), repeated for each room several times during a diel cycle to account for diurnal variation of \( [\text{CO}_2] \). Mean \( \Delta^{13} \text{C} \) was calculated for each glasshouse room. With a known \( \Delta^{13} \text{C} \), WUE was calculated using the following formula:

\[
\text{WUE} = \frac{A}{g_s} = \frac{c_a}{1.6} \left( \frac{b' - \Delta^{13} \text{C}}{b' - a} \right)
\]

Equation 5

where \( g_s \) is stomatal conductance of water vapour, \( a \) is the average fractionation constant (4.4 ‰, O’Leary 1981) for the diffusion of gases through stomata and \( b' \) represents the carboxylation fractionation by Rubisco and PEP carboxylase (27‰, Farquhar & Richards 1984). The factor 1.6 accounts for the greater diffusivity of water vapour versus \( \text{CO}_2 \) in air. Thus, WUE is inversely proportional to \( \Delta^{13} \text{C} \).

### 3.2.7 Tree biomass and leaf area

After allowing trees to establish for two weeks, height (\( h \), cm) and diameter (\( d \), mm; measured 2 cm above soil surface) of stems was measured weekly throughout the duration of the experiment before and during water limitation. Volume of the main stem (\( v_{\text{stem}} \), cm\(^3\)) was estimated from \( h \) and stem radius (\( r \), in cm, calculated from \( d \)) assuming the shape of a cone and using the following formula:

\[
v_{\text{stem}} = \frac{1}{3} \pi r^2 h
\]

Equation 6

Relative growth rate (RGR) was calculated per day from the weekly measurements. Total leaf area (LA), leaf number, and dry weight of leaves, stem (including all branches), and root biomass (\( \text{DW}_{\text{leaf}}, \text{DW}_{\text{stem}}, \text{DW}_{\text{root}} \)) were recorded during destructive harvest of all 80 trees at the end of the experiment on 15 June 2015. Leaf area was measured using a Li-Cor 3100C leaf area meter (Li-Cor Inc., NE, USA). Total plant dry mass (\( \text{DW}_{\text{plant}} \)) was calculated by summing \( \text{DW}_{\text{leaf}}, \text{DW}_{\text{stem}} \) and \( \text{DW}_{\text{root}} \). Specific leaf area (SLA) was determined by dividing LA by \( \text{DW}_{\text{leaf}} \). Root-mass-fraction (RMF) was calculated by dividing \( \text{DW}_{\text{root}} \) by \( \text{DW}_{\text{plant}} \).
3.2.8 Non-structural carbohydrates

Analysis of non-structural carbohydrates (NSC) in subsamples of leaves, stems and roots was identical to the description provided in Chapter 2.

3.2.9 Calculation of percentage change

Percentage change of $A_{sat}$ and NSC concentrations between the mean of water-limited trees ($\bar{x}_{WL}$) and well-watered controls ($\bar{x}_{WW}$) as a measure of the plastic response were calculated as follows:

$$ \text{percentage change} = \frac{\bar{x}_{WL} - \bar{x}_{WW}}{\bar{x}_{WW}} \times 100 $$

Equation 7

Standard error of means (SE) for percentage change was calculated as:

$$ \text{SE} = \frac{\text{SD} \left( \frac{\bar{x}_{WL} - \bar{x}_{WW}}{\bar{x}_{WW}} \right)}{\sqrt{n}} $$

Equation 8

where SD is the standard deviation, $x_{WL}$ are the individual trait values of water-limited trees and $n$ is the number of observations per species and treatment group.

3.2.10 Statistical analysis

Data were analysed using R-3.3.3 software (R Core Team 2017). Two-way analysis of variance (ANOVA) was used to analyse effects of water and $[\text{CO}_2]$ treatment as well as provenance on plant traits like leaf water potentials, leaf gas exchange, growth related traits and NSC. Models were tested with all interactions between the three factors, detailed results can be found in the Supplementary Table S3.1. Stem volume two weeks since plant establishment was added as covariate to models for plant dry mass as well as root-mass-fraction since tree size varied between water treatment groups while trees were still well-watered. Effects were tested for data taken on the same days. ANOVA assumptions of data normality and equal variance were tested using the Shapiro-Wilk and Levene’s test as well as a quantile-quantile plot for visually assessment. The ‘car’ package (Fox & Weisberg 2011) was used for Anova() and leveneTest() functions. The Anova() function allows
to run ANOVAs with Type III sums of squares; `leveneTest()` facilitates Levene’s test for equal variance among test groups. The TukeyHSD() function from the base package was used to perform Tukey’s honest significant difference test when needed. Post-hoc tests were conducted when plant traits were significantly different across provenances. Values greater or smaller than two standard deviations of the mean were removed.

For physiological plant traits repeatedly measured on the same individuals before and during water limitation, the `lme()` function of the ‘nlme’ package (Pinheiro et al. 2017) was used for linear mixed-effects models with the different measuring campaigns (= phase) as a fixed factor. Relative growth rates of stem volume per day (RGR) were transformed logarithmically and then analysed for differences across provenances and [CO₂] treatment for the period before water limitation commenced (pre-treatment) as well during the water treatment with mean RGR of the pre-treatment as covariate.

All figures and regression analyses of midday leaf water potentials with photosynthesis and stomatal conductance were done using OriginPro 2017 (OriginLab Corporation, MA, USA). Coefficient of determination (R²) and p-values were calculated with the same program. Mean values are presented with one standard error. Correlations between \( \text{DW}_{\text{plant}} \) and \( \text{DW}_{\text{stem}} \), respectively and climate variables (MAT, MAP, ET/MAP) were analysed with Pearson’s correlation coefficient using the `cor.test()` function of the base package.

3.3 Results

3.3.1 Environmental conditions

In the glasshouse bay, RH ranged from 57–85% and throughout the experimental phase averaged 70 ± 1%. Vapour pressure deficit (VPD) fluctuated on a diurnal basis with MAT and RH, with minima during the night (0.2 kPa) and maxima during midday (1.8 kPa). Mean VPD at midday was 1.1 ± 0.01 kPa. During the experimental period from mid-March to mid-June 2015, photosynthetic photon flux density (PPFD) at midday (1200–1300 hours) at a nearby weather station averaged 948
µmol m\(^{-2}\) s\(^{-1}\). Furthermore, PPFD at midday ranged from 115 µmol m\(^{-2}\) s\(^{-1}\) on a cloudy day to 1904 µmol m\(^{-2}\) s\(^{-1}\) on the sunniest day. Temperatures during photoperiod hours averaged 26.9 ± 0.1°C. Mean [CO\(_2\)] inside the glasshouse bays was 401 ± 0.2 µmol mol\(^{-1}\) for the aCO\(_2\) treatment and 645 ± 0.1 µmol mol\(^{-1}\) for the eCO\(_2\) treatment during the experiment.

Average volumetric water content (VWC) in well-watered pots was 9.4 ± 0.02 %, regardless of provenance or [CO\(_2\)] treatment (Fig. 3.2). Reduction of water supplied to pots caused soil slowly to dry out and VWC to decline linearly from 9 to 5% during the first ten days, after which VWC declines were much slower. Soil of trees from Kilcoy Creek dried out faster compared to the other three provenances, which all followed a similar decline of VWC. Four weeks since commencement of water limitation treatment, VWC was 3.0 ± 0.01% and similar among provenances and [CO\(_2\)] treatments.

Figure 3.2: Time-course of volumetric water content (VWC) of soil before and during water limitation in *E. grandis*. Data are shown for pots kept under ambient (left) and elevated (right) [CO\(_2\)] conditions. Solid lines represent VWC of trees grown in well-watered conditions, dashed lines for those grown under water-limited conditions. Colours represent means of the four different provenances (dark blue: Mt. Lindsay; light blue: Kilcoy Creek; orange: Coffs Harbour; red: Woondum).
3.3.2 Leaf water potential

Leaf water potentials were significantly affected by water treatment ($p < 0.001$), but differences were very similar across provenances ($p > 0.20$). Before water treatment commenced, trees had a mean $\Psi_{pd}$ of $-0.5 \pm 0.02$ MPa. Following the establishment of 50% soil water use, $\Psi_{pd}$ was more negative in water-limited trees than in well-watered control trees ($p < 0.001$). Values were also significantly more negative in trees grown in eCO$_2$ than in aCO$_2$ ($p = 0.008$), regardless of their water status. Water-limited trees on day 25 had a mean $\Psi_{pd}$ of $-0.8 \pm 0.1$ MPa in aCO$_2$ and $-0.9 \pm 0.1$ MPa in eCO$_2$ compared to $\Psi_{pd}$ of well-watered trees averaging $-0.4 \pm 0.1$ MPa in aCO$_2$ and $-0.5 \pm 0.1$ MPa in eCO$_2$.

For midday conditions, [CO$_2$] was only an influencing factor resulting in $\Psi_{md}$ of well-watered trees to average at $-1.1 \pm 0.04$ MPa in aCO$_2$ and $-0.9 \pm 0.04$ MPa in eCO$_2$ before water treatment commenced ($p = 0.004$; Table 3.2). After 25 days of water limitation, $\Psi_{md}$ was more negative than in control trees ($p < 0.001$); water-limited trees in aCO$_2$ averaged $-1.6 \pm 0.04$ MPa water-limited trees in eCO$_2$ averaged $-1.7 \pm 0.04$ MPa.

Table 3.2: Mean predawn and midday leaf water potentials of *E. grandis* before and 25 days after water limitation commenced. Leaf water potentials at predawn ($\Psi_{pd}$, MPa) and midday ($\Psi_{md}$, MPa) were measured at ambient (aCO$_2$) and elevated (eCO$_2$) [CO$_2$] conditions on well-watered (WW) and water-limited (WL) trees. Water-limited trees grew in well-watered conditions (pre-treat.) before water was reduced to 50% of average volume of water used (day 25). One standard error of means ($n = 20$) are shown in brackets. Traits were not significantly different across provenances and hence values were pooled.

<table>
<thead>
<tr>
<th>phase</th>
<th>water treat.</th>
<th>CO$_2$</th>
<th>$\Psi_{pd}$</th>
<th>$\Psi_{md}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-treat. WW</td>
<td>aCO$_2$</td>
<td>$-0.4$ (0.03)</td>
<td>$-1.1$ (0.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>eCO$_2$</td>
<td>$-0.5$ (0.03)</td>
<td>$-0.9$ (0.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>aCO$_2$</td>
<td>$-0.3$ (0.03)</td>
<td>$-1.1$ (0.05)</td>
</tr>
<tr>
<td></td>
<td>eCO$_2$</td>
<td>$-0.4$ (0.02)</td>
<td>$-0.9$ (0.04)</td>
<td></td>
</tr>
<tr>
<td>day 25 WW</td>
<td>aCO$_2$</td>
<td>$-0.4$ (0.1)</td>
<td>$-1.1$ (0.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>eCO$_2$</td>
<td>$-0.5$ (0.05)</td>
<td>$-1.2$ (0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>aCO$_2$</td>
<td>$-0.7$ (0.1)</td>
<td>$-1.5$ (0.04)</td>
</tr>
<tr>
<td></td>
<td>eCO$_2$</td>
<td>$-0.9$ (0.1)</td>
<td>$-1.7$ (0.04)</td>
<td></td>
</tr>
</tbody>
</table>
3.3.3 Leaf gas exchange

*Photosynthesis*

$A_{sat}$ was significantly influenced by all three tested factors i.e. provenance, $[CO_2]$ and water treatment with interactions between provenance and water treatment. Before water treatment commenced, $A_{sat}$ of well-watered trees originating from the warmest climate (i.e. provenance Woondum) was 18% lower compared to the other provenances ($p = 0.007$; Fig. 3.3). Trees of provenances Mt. Lindsay, Kilcoy Creek and Coffs Harbour had similar mean $A_{sat}$ values averaging $22.1 \pm 0.6 \mu mol m^{-2}s^{-1}$ ($p > 0.77$), whereas trees from Woondum averaged only $18.2 \pm 1.4 \mu mol m^{-2}s^{-1}$ and differed from the other three provenances ($p = 0.007$, $p = 0.03$, $p = 0.09$). However, no significant correlation with climate parameters (MAT, MAP or ET/MAP) was found ($p > 0.26$).

Values for $A_{sat}$ of well-watered trees from Woondum were also the lowest in eCO$_2$. $A_{sat}$ was 26% lower compared to trees with the highest rates originating from Mt. Lindsay. Nevertheless, values of all well-watered trees were on average 23% greater in eCO$_2$ ($p < 0.001$) compared to aCO$_2$. $A_{sat}$ of well-watered trees was $19.0 \pm 0.6 \mu mol m^{-2}s^{-1}$ in aCO$_2$ and $23.3 \pm 0.9 \mu mol m^{-2}s^{-1}$ in eCO$_2$. However, well-watered trees from Mt. Lindsay had the greatest increase with 40% from aCO$_2$ to eCO$_2$ (Fig. 3.3).

Although water limitation negatively affected $A_{sat}$ on day 25 ($p < 0.001$), trees grown in eCO$_2$ still maintained greater rates than trees in aCO$_2$ ($p < 0.001$). $A_{sat}$ severely declined in all water-limited trees by four-fold in aCO$_2$ averaging $4.6 \pm 0.9 \mu mol m^{-2}s^{-1}$, but only two-fold in eCO$_2$ compared to well-watered trees averaging $9.9 \pm 1.3 \mu mol m^{-2}s^{-1}$. Nevertheless, while $A_{sat}$ was relatively similar across provenances in aCO$_2$, water-limited trees from the warmest climate (provenance Woondum) did not improve $A_{sat}$ rates in eCO$_2$ compared to the other three provenances ($p = 0.02$). Thus, in eCO$_2$ they were found to have the lowest assimilation rates averaging $5.2 \pm 1.7 \mu mol m^{-2}s^{-1}$, 50% lower than values found in trees from Mt. Lindsay averaging $11.2 \pm 1.7 \mu mol m^{-2}s^{-1}$.
Figure 3.3: Stomatal conductance \( (g_s; \text{panel A–B}) \), light-saturated photosynthesis \( (A_{sat}; \text{panel C–D}) \) and carbon isotope discrimination \( (\Delta^{13}C; \text{panel E–F}) \) of four \( E. \text{grandis} \) provenances. Trees grown in ambient \( [CO_2] \) \( (aCO_2; \text{left panels}) \) are shown as circles and trees grown in elevated \( [CO_2] \) \( (eCO_2; \text{right panels}) \) are shown as square symbols. Dashed lines and solid symbols represent well-watered trees; dotted lines and open symbols represent water-limited trees \( (n = 5 \text{ per provenance}) \). Data are shown for measurements taken before water treatment commenced \( (\text{pre-treat.}) \) and 25 days after drought was imposed \( (\text{day 25}) \). Error bars represent one standard error of mean. Colours represent provenances \( (\text{dark blue: Mt. Lindsay, light blue: Kilcoy Creek, orange: Coffs Harbour, red: Woondum}) \).

**Stomatal conductance**

Stomatal conductance was impacted by water treatment and provenance including their interaction \( (p < 0.001) \), but was not significantly different between \( [CO_2] \).
treatments ($p = 0.22$). Well-watered trees from Woondum had lower $g_s$ compared to trees from Mt. Lindsay and Kilcoy Creek ($p < 0.001$). Hence, $g_s$ of trees originating from Woondum averaged $0.45 \pm 0.04$ mol m$^{-2}$ s$^{-1}$, whereas trees from Mt. Lindsay had the greatest $g_s$, reaching $0.73 \pm 0.05$ mol m$^{-2}$ s$^{-1}$. This represented a 60% difference in $g_s$ of trees from the coolest and wettest climate compared to trees that originated from Woondum with the warmest climate.

Stomatal conductance did also not differ between the two [CO$_2$] treatments during drought ($p = 0.74$), but was significantly reduced by water limitation ($p < 0.001$; Fig. 3.3). Water-limited trees had $g_s$ averaging zero compared to well-watered trees with $0.63 \pm 0.05$ mol m$^{-2}$ s$^{-1}$. Provenance-specific differences in $g_s$, as observed prior to water limitation, were not apparent once water was withheld.

**Water use efficiency**

While there were no detectable differences in discrimination against $^{13}$C isotopes in leaf dry matter among provenances, a greater WUE of trees grown in eCO$_2$ was apparent due to $\Delta^{13}$C of these trees being 9% lower compared to trees grown in aCO$_2$ ($p < 0.001$; Fig. 3.3).

Water reduction caused $\Delta^{13}$C values to decline by $-7\%$ compared to well-watered trees ($p < 0.001$). The effect of eCO$_2$ resulting in lower $\Delta^{13}$C remained evident during water limitation ($p = 0.003$). Values for $\Delta^{13}$C averaged $24.4 \pm 0.3\%$ in trees grown in aCO$_2$ and $23.1 \pm 0.2\%$ in eCO$_2$. Unexpectedly, $\Delta^{13}$C values in well-watered trees changed over time (Fig. 3.3), being significantly higher on day 25 compared to pre-treatment conditions ($p < 0.001$).

**Relative response of photosynthesis to water limitation**

Among provenances, the response of $A_{sat}$ to water limitation differed depending on [CO$_2$] treatment ($p = 0.007$), with trees from provenance Woondum not showing a difference ($p = 0.73$; Fig. 3.4). Separated for [CO$_2$] treatment, $A_{sat}$ had the strongest correlations with MAT and only minor correlations with MAT and ET/MAP. In aCO$_2$, $A_{sat}$ of water-limited trees from colder climates declined relative to well-watered
trees on day 25, whereas $A_{\text{sat}}$ of trees from the warmest climate (i.e. provenance Woondum) decreased less. Water-limited trees of provenances originating from the three coldest climates had a relative decrease of $-70$ to $82\%$ compared to $A_{\text{sat}}$ of well-watered trees in $aCO_2$. Rates of photosynthesis in water-limited trees from Woondum declined less ($-60\%$). Yet, responses changed under $eCO_2$, in particularly evident in trees from provenance Woondum (Fig. 3.4). Here, $A_{\text{sat}}$ of trees from the three cooler climates declined relatively less during water limitation ($-39$ to $47\%$) while water-limited trees originating from provenance Woondum declined the most ($-72\%$).

![Figure 3.4: Relative decrease of $A_{\text{sat}}$ in water-limited (WL) compared to well-watered (WW) trees on day 25, related to mean annual temperature (MAT). Trees were grown in ambient ($aCO_2$; panel A) and elevated [CO$_2$] ($eCO_2$; panel B). Five trees per group were used to calculate relative decrease of $A_{\text{sat}}$. Error bars represent one standard error. Colours represent different provenances (dark blue: Mt. Lindsay; light blue: Kilcoy Creek; orange: Coffs Harbour; red: Woondum).](image)

### Gas exchange relative to leaf water potentials

Both, $g_s$ and $A_{\text{sat}}$ declined with increasingly negative $\Psi_{\text{md}}$ during water limitation (Fig. 3.5). Yet, $g_s$ declined rapidly with declining $\Psi_{\text{md}}$ while $A_{\text{sat}}$ declined much slower. While the mitigating effect of $eCO_2$ on $A_{\text{sat}}$ under increasing water limitation was apparent, no such effect was detectable for $g_s$ (Fig 3.5). Once $\Psi_{\text{md}}$ exceeded $-1.5$ MPa, $g_s$ in water-limited trees was nearly zero, while rates of $A_{\text{sat}}$ varied between 0–15 $\mu$mol m$^{-2}$ s$^{-1}$. 
Correlations between $g_s$ and $A_{\text{sat}}$ on day 25 are shown in Fig. S3.1 (Supplementary Material). In eCO$_2$, the exponential fit had a steeper increase than in aCO$_2$, meaning that higher $A_{\text{sat}}$ was recorded in eCO$_2$ compared to similar $g_s$ in aCO$_2$.

### 3.3.4 Tree biomass and leaf area

**Biomass**

Total plant mass varied widely among individual seedlings ranging from 7.3–83.5 g, but was overall significantly different between [CO$_2$] treatments and provenances with no significant interactions between these factors. Well-watered seedlings of
trees from Woondum had the lowest total biomass (17.8 ± 2.7 g), whereas those from Mt. Lindsay had the greatest biomass (41.0 ± 4 g) followed by Kilcoy Creek at the end of the experiment. Provenances differed in mass of leaves (p = 0.006), stems (p = 0.002) and marginally in mass of roots (p = 0.08).

eCO₂ resulted in a significant increase in biomass of leaves (16%; p = 0.05), stems (25%; p < 0.001) and roots (31%; p = 0.001; Table 3.3) across water treatments. Furthermore, DWleaf and DWstem were not significantly affected by water treatment at the end of the experiment (p = 0.92 and p = 0.12). Yet, biomass of roots of trees grown under water-limited conditions were 55% greater compared to biomass of the well-watered seedlings (p = 0.001). After water limitation, rankings across provenances slightly changed and trees from Kilcoy Creek had the greatest stem mass in aCO₂ and the second greatest stem mass in eCO₂ after Coffs Harbour. Mean total plant mass (as well as DWstem) of well-watered trees but not water-limited trees was negatively correlated to mean annual temperature of seed origin in aCO₂ (Pearson’s r = −0.59, p = 0.006) and eCO₂ (Pearson’s r = −0.57, p = 0.009), however correlations for mean annual precipitation or ET/MAP were not significant (details can be found in Supplementary Table S3.2).
Table 3.3: Dry mass (g) of *E. grandis* at the end of experiment. Trees were grown in ambient (aCO$_2$) and elevated [CO$_2$] (eCO$_2$) atmospheres. Data is shown for well-watered (WW) and water-limited (WL) trees. Values are separated into biomass of leaf, stem, root tissue and total biomass and averaged per treatment group and provenance across four or five individuals. Standard errors of means are shown in brackets.

<table>
<thead>
<tr>
<th>provenance</th>
<th>Mt. Lindsay (L)</th>
<th>Kilcoy Creek (K)</th>
<th>Coffs Harbour (C)</th>
<th>Woondum (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water treatment</td>
<td>WW</td>
<td>WL</td>
<td>WW</td>
<td>WL</td>
</tr>
<tr>
<td>aCO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>15.3 (1.0)</td>
<td>10.6 (1.0)</td>
<td>14.1 (2.8)</td>
<td>13.5 (1.7)</td>
</tr>
<tr>
<td>Stem</td>
<td>17.6 (1.5)</td>
<td>13.3 (1.0)</td>
<td>15.7 (2.3)</td>
<td>20.0 (2.2)</td>
</tr>
<tr>
<td>Root</td>
<td>7.0 (1.0)</td>
<td>7.2 (1.1)</td>
<td>6.1 (1.1)</td>
<td>8.8 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td>39.8 (3.0)</td>
<td>31.2 (2.9)</td>
<td>35.9 (5.6)</td>
<td>42.3 (4.3)</td>
</tr>
<tr>
<td>eCO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>14.6 (2.9)</td>
<td>14.2 (2.4)</td>
<td>13.5 (1.3)</td>
<td>16.9 (2.8)</td>
</tr>
<tr>
<td>Stem</td>
<td>19.6 (3.6)</td>
<td>19.6 (2.4)</td>
<td>17.2 (1.8)</td>
<td>21.6 (1.4)</td>
</tr>
<tr>
<td>Root</td>
<td>8.0 (1.5)</td>
<td>9.6 (0.9)</td>
<td>8.6 (2.1)</td>
<td>10.5 (1.9)</td>
</tr>
<tr>
<td>Total</td>
<td>42.2 (7.9)</td>
<td>43.4 (5.0)</td>
<td>39.4 (4.7)</td>
<td>49.0 (5.9)</td>
</tr>
</tbody>
</table>
Root-mass-fraction (RMF) was mainly affected by water treatment, although marginally significant differences were also found for [CO₂] levels. In aCO₂, as well as in eCO₂, water limitation led to significant increases in the tree’s RMF by 28% (p < 0.001; Fig. 3.6), driven largely by increased investment in root tissues. Thus, whereas DWplant at harvest was not significantly different between water-limited and well-watered trees (p = 0.23), DWroot was 55% greater in trees of the water-limited treatment (p = 0.001). Furthermore, [CO₂] treatments positively affected RMF by 11% from aCO₂ to eCO₂ (p = 0.03; Fig. 3.6). Provenances differed marginally significant in their RMF (p = 0.07) with trees from Woondum having greater root to plant mass than trees from Coffs Harbour (p = 0.04).

![Figure 3.6](image_url)

**Figure 3.6:** Root-mass-fraction at harvest of *E. grandis* for well-watered (WW) and water-limited (WL) trees. Panel A shows means for trees grown in ambient CO₂ concentrations (aCO₂), panel B for elevated CO₂ concentrations (eCO₂; n = 20 per treatment). Greater values indicate a greater root relative to total plant mass. Error bars represent one standard error; *** indicates significant difference at p < 0.001.

**Leaf traits**

At final harvest, leaf area differed significantly among provenances (p < 0.001), but not between [CO₂] or water treatments (p > 0.12; Table 3.4). When averaged across treatments, leaf area was lowest for trees originating from Woondum (2074 ± 225 cm²). The greatest mean leaf area was found in trees of provenance Kilcoy Creek (3664 ± 275 cm²). Specific leaf area did not differ significantly among provenance (p = 0.59), but decreased in eCO₂ by −5% (p = 0.05) and with water limitation by −16% (p < 0.001). Thus, SLA of water-limited trees averaged 228 ± 5 cm² g⁻¹ compared to
well-watered control trees with 271 ± 5 cm² g⁻¹. Leaf size was only significantly different among provenances (p = 0.01), but not [CO₂] nor water treatments. Thus, trees from Mt. Lindsay and Kilcoy Creek had the largest leaves averaging 17.5 ± 0.9 cm² and 16.5 ± 1.1 cm², respectively.
Table 3.4: Leaf characteristics of *E. grandis* seedlings grown in ambient (aCO$_2$) and elevated [CO$_2$] (eCO$_2$). Data is shown for well-watered (WW) and water-limited (WL) trees. Leaf number, leaf area (LA, cm$^2$), specific leaf area (SLA, cm$^2$ g$^{-1}$), leaf size (cm$^2$) were averaged per treatment group and provenance across four or five individuals. Standard errors are shown in brackets.

<table>
<thead>
<tr>
<th>provenance</th>
<th>Mt. Lindsay (L)</th>
<th>Kilcoy Creek (K)</th>
<th>Coffs Harbour (C)</th>
<th>Woondum (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water treatment</td>
<td>WW</td>
<td>WL</td>
<td>WW</td>
<td>WL</td>
</tr>
<tr>
<td>aCO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf number</td>
<td>236 (24)</td>
<td>153 (19)</td>
<td>216 (40)</td>
<td>259 (42)</td>
</tr>
<tr>
<td></td>
<td>181 (48)</td>
<td>244 (17)</td>
<td>123 (21)</td>
<td>163 (24)</td>
</tr>
<tr>
<td>LA</td>
<td>4249 (143)</td>
<td>2415 (291)</td>
<td>3907 (644)</td>
<td>3444 (538)</td>
</tr>
<tr>
<td></td>
<td>2605 (646)</td>
<td>2581 (366)</td>
<td>1975 (582)</td>
<td>2284 (118)</td>
</tr>
<tr>
<td>SLA</td>
<td>281 (10)</td>
<td>227 (18)</td>
<td>275 (10)</td>
<td>252 (12)</td>
</tr>
<tr>
<td></td>
<td>271 (15)</td>
<td>233 (14)</td>
<td>276 (31)</td>
<td>230 (19)</td>
</tr>
<tr>
<td>leaf size</td>
<td>18.7 (1.8)</td>
<td>16.0 (1.4)</td>
<td>19.0 (3.0)</td>
<td>13.8 (2.1)</td>
</tr>
<tr>
<td></td>
<td>15.1 (1.9)</td>
<td>10.7 (1.7)</td>
<td>16.3 (3.3)</td>
<td>15.1 (1.8)</td>
</tr>
<tr>
<td>eCO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf number</td>
<td>238 (44)</td>
<td>168.2 (18)</td>
<td>231 (20)</td>
<td>209 (29)</td>
</tr>
<tr>
<td></td>
<td>256 (37)</td>
<td>288.4 (55)</td>
<td>134 (19)</td>
<td>168 (31)</td>
</tr>
<tr>
<td>LA</td>
<td>3939 (854)</td>
<td>3172 (512)</td>
<td>3636 (290)</td>
<td>3669 (786)</td>
</tr>
<tr>
<td></td>
<td>3678 (532)</td>
<td>3636 (364)</td>
<td>1902 (459)</td>
<td>2134 (617)</td>
</tr>
<tr>
<td>SLA</td>
<td>266 (11)</td>
<td>224 (13)</td>
<td>272 (13)</td>
<td>210 (16)</td>
</tr>
<tr>
<td></td>
<td>273 (12)</td>
<td>215 (8)</td>
<td>253 (14)</td>
<td>225 (17)</td>
</tr>
<tr>
<td>leaf size</td>
<td>16.2 (1.7)</td>
<td>19.2 (2.5)</td>
<td>16.4 (2.3)</td>
<td>16.9 (1.5)</td>
</tr>
<tr>
<td></td>
<td>14.4 (0.9)</td>
<td>13.4 (1.3)</td>
<td>13.6 (3.0)</td>
<td>12.0 (1.1)</td>
</tr>
</tbody>
</table>
Stem volume

Before water treatment commenced, log(RGR) of stem volume was not significantly different between [CO₂] treatment ($p = 0.53$) nor provenance ($p = 0.09$). However, differences among provenances were visible after trees were grown at 50% of previous water use (Fig. 3.7). Water limitation reduced log(RGR) ($p < 0.001$), yet water treatment by provenance interactions were detected ($p = 0.005$). Namely, trees originating from the warmest climate i.e. provenance Woondum maintained their log(RGR) during water limitation, having the greatest values averaging 0.004 cm$^3$ day$^{-1}$ 25 days after drought commenced which was close to means of well-watered trees averaging 0.005 cm$^3$ day$^{-1}$. Water-limited trees from Coffs Harbour and Mt. Lindsay had the lowest log(RGR) at this point, while trees from Kilcoy Creek had similar means to trees from Woondum.
Figure 3.7: Relative growth rate (log-transformed) of stem volume per day. Trees were grown in ambient (aCO$_2$; left panels) and elevated (eCO$_2$; right panels) [CO$_2$]. Solid lines represent well-watered trees (WW), dashed lines water-limited trees (WL). Shaded bands represent one standard error of means ($n = 3$–$5$). Water limitation phase commenced on the 18 May 2015, marked as grey background. Colours represent different provenances (dark blue: Mt. Lindsay; light blue: Kilcoy Creek; orange: Coffs Harbour; red: Woondum).
3.3.5 Non-structural carbohydrates

Carbohydrate concentrations were significantly affected by water treatment, but not \( \text{[CO}_2 \text{]} \). For this reason, data were pooled for \( \text{aCO}_2 \) and \( \text{eCO}_2 \) and only the effects of water limitation and provenance on NSC are presented. Among all treatments and provenances, concentrations were always higher in leaves than roots and stem. On average, the concentration of soluble sugars (Ss) plus starch (St) in well-watered trees was \( 12 \pm 0.2\% \), \( 6 \pm 0.1\% \) and \( 7 \pm 0.2\% \) in leaves, roots and stems, respectively. Mean values can be found in Supplementary Table S3.3.

At the end of the experiment, concentrations of Ss and St differed among the provenances in stem and root tissue \( (p < 0.05) \), but not leaves. The most significant differences were found in St concentration of roots \( (p = 0.002) \). However, differences were only apparent in water-limited trees \( (p = 0.09) \). Here, St content in water-limited trees from provenance Coffs Harbour and Kilcoy Creek was 10% greater compared to those found in trees from provenance Woondum and Mt Lindsay \( (p < 0.05) \).

Across all provenances, water limitation caused NSC, in particular Ss, to increase \( (p < 0.001; \text{Fig. 3.8}) \). In leaves, concentration of Ss in well-watered trees averaged \( 5.4 \pm 0.1\% \) whereas water-limited trees contained \( 7.2 \pm 0.2\% \). The greatest proportional change in Ss concentrations between water treatments were seen in stem and then root tissue \( (\text{Fig. 3.8}) \). Concentrations increased two- to four-fold in water-limited trees averaging \( 2.4 \pm 0.1\% \) in stem and \( 3.3 \pm 0.01\% \) in roots, respectively. Trees of provenance Mt. Lindsay and Kilcoy Creek had the greatest increase in Ss in stem tissue of 200%. Furthermore, St concentration also increased during water limitation in leaf and root tissue \( (p < 0.001) \), however the magnitude varied among provenances and was only a portion of the changes observed for Ss \( (\text{Fig. 3.8}) \). The greatest increase of St in leaves was found in water-limited trees of provenance Woondum.
Figure 3.8: Percentage change of non-structural carbohydrates (NSC) in three tissues of water-limited relative to well-watered *E. grandis*. Samples were collected at the final harvest. NSC are presented for two fractions: soluble sugars (Ss; left panels) and starch (St; right panels). Trees were pooled across [CO₂] treatments (n = 10 per provenance). Error bars represent one standard errors. Colours and abbreviations represent different provenances (dark blue: Mt. Lindsay (MT); light blue: Kilcoy Creek (KC); orange: Coffs Harbour (CH); red: Woondum (WD)). Asterisks present statistical significant differences to well-watered controls with * for \( p < 0.05 \), ** for \( p < 0.01 \) and *** for \( p < 0.001 \); n.s. marks non-significant results.

3.4 Discussion

This experiment was designed to test phenotypic plasticity of *Eucalyptus grandis* trees originating from different climate-of-origins to eCO₂ and mild water limitation. Testing how physiological functions and traits respond to environmental factors like eCO₂ or water limitation, produced different ‘winners’ and ‘losers’ among the
provenances of *E. grandis* trees compared to growing the same provenances under ambient and sufficient water conditions. This has important implications for the selection of species and provenances for future plantings in managed tree systems.

**General tree response to water limitation**

Not surprisingly, even the mild water limitation triggered declines of stomatal conductance (*g*s) and photosynthesis (*A*) as well as increases of water use efficiency (WUE). However, these adjustments of *g*s and WUE were not provenance-specific, indicating that this response to water limitation is likely to be genetically stable. It is widely acknowledged that reductions in *g*s with increasing water limitation are a mechanism to reduce water loss of a plant and associated xylem cavitation (Arndt *et al.* 2008; Duan *et al.* 2013a; Lewis *et al.* 2013; Liang & Maruyama 1995; Osakabe *et al.* 2014). As CO₂ influx into the intercellular space also declines with reduced stomatal conductance, photosynthesis rates decline with decreasing concentrations inside the leaf. With greater reduction of *g*s than *A* during water limitation, WUE increases, hence improving the water loss to carbon gain balance (Whitehead & Beadle 2004).

In addition, water limitation clearly reduced relative growth rates of stem volume potentially due to declines of cell turgor and thus cell division (Hsiao *et al.* 1976) in three of the four provenances. Specific leaf area also declined with water limitation, often suggesting that trees produced thicker leaves with more mesophyll cells per unit area (Searson *et al.* 2004). This morphological alteration may allow for greater rates of *A* and associated increases in carbon gain per unit leaf area produced. In concert with alteration of leaf morphology, root-mass-fraction of water-limited *E. grandis* increased as well. This is a well-documented mechanism that aims to increase the ratio of potential soil water uptake capacity to loss of water via transpiration from leaves (Kozlowski *et al.* 1992; Pritchard *et al.* 1999).

With effects of water limitation on carbon assimilation and growth, non-structural carbohydrates (NSC) and in particular soluble sugars (Ss) showed a great response to water deficit in *E. grandis* by increasing in all tissues in the order of 22–45% in
leaves to 107–210% in stem. Carbohydrates in form of starch (St) are thought to be the carbon reserve of plants that can be utilised when needed to maintain cell metabolism (Martínez-Vilalta et al. 2016). Several studies have shown that starch reserves have been converted to sugars during severe water stress (Duan et al. 2013a; Mitchell et al. 2013, Sevanto et al. 2014), hence St concentration may decline while Ss concentration increases. Increased Ss as seen in *E. grandis* is often used for osmotic adjustment as a mechanism to lower osmotic potential and hence increase water influx and cell turgor (Martínez-Vilalta et al. 2016). When analysing leaf sap of 3-year old *E. grandis* genotypes, NSC contributed to leaf osmotic potential at full turgor by more than 40% during summer drought (Callister et al. 2008). Furthermore, since relative growth rate (RGR) was declining in water-limited *E. grandis*, but *A* had not reached zero after 25 days of soil water deficit, lower sink activity when growth was reduced before photosynthesis can help explain the changes in NSC concentration in *E. grandis*. A study on five eucalypt species testing leaf metabolite change during a 2-month long drought came to the same conclusion and also pointed to a change in carbon partitioning, where the activity of enzymes producing the soluble sugar sucrose was increased (Warren et al. 2011a).

**General tree response to eCO₂**

Similar to previous studies (e.g. Saxe et al. 1998), eCO₂ had little to no effect on stomatal opening in *E. grandis*. Generally, in trees grown under eCO₂ (560–700 µmol mol⁻¹) maximum decline of *gₛ* may reach ~10 to 20% (Gunderson et al. 2002; Phillips et al. 2011; Rey & Jarvis 1998; Will & Ceulemans 1997). Well-watered and water-limited trees grown in eCO₂ also displayed greater WUE compared to their counterparts in aCO₂. However, neither did leaf area (LA) increase with eCO₂, nor did *gₛ* vary markedly. Both trait observations indicate that WUE of *E. grandis* growing under eCO₂ was higher due to increased rates of *A*, and not the result of diminished water use. Similarly, WUE on a leaf level basis was increased by about 30% from aCO₂ to eCO₂ (640 µmol mol⁻¹) in *Eucalyptus saligna*, yet greater leaf area
consequently increasing whole-tree water use indicated that morphological changes had a greater impact than physiological adjustments (Sherwin et al. 2013).

Although no differences were found for $g_s$, eCO$_2$ led to greater $A_{sat}$ (23%) in well-watered trees and doubled (50%) rates of $A_{sat}$ in water-limited trees. Numerous experiments have documented an upregulation of photosynthesis under eCO$_2$ (30–60%), yet the rates of upregulation differed substantially among functional groups and species (Ainsworth & Rogers 2007; Leakey et al. 2012; Resco de Dios et al. 2016). Stem biomass of *E. grandis* also increased by 25% in eCO$_2$, a response often seen across various trees species (Conroy et al. 1992; Ghannoum et al. 2010; Resco de Dios et al. 2016; Roden et al. 1999; Tingey et al. 1996). However, no $G \times E$ interaction for biomass traits related to [CO$_2$] was found. Well-watered species of *Eucalyptus* with similar [CO$_2$] treatments were reported to increase plant dry mass by 44% in fast-growing *E. saligna* and an even greater increase of 75% in slow-growing *E. sideroxylon* (Ghannoum et al. 2010). Yet, similar to results for *E. grandis*, RGR was not increased in eCO$_2$ which the authors relate to the reduced leaf area ratio while maintaining net assimilation rate (unit dry mass increase per unit leaf area). A responsiveness of carbon assimilation and growth in *E. grandis* to eCO$_2$ is thus confirmed, yet results show that provenances of *E. grandis* fall at the lower end of possible positive responsiveness to eCO$_2$ when soil water is sufficient. Regardless of photosynthetic enhancement, the pool of NSC in *E. grandis* did not increase substantially in eCO$_2$, contrary to what is generally reported in the literature and especially for leaves (Ayub et al. 2011; Centritto et al. 1999; Duan et al. 2013a; Körner & Miglietta 1994). Extra carbon available to *E. grandis* might have been used to increase in above-ground biomass, as seen by the 19% increase in total plant weight in eCO$_2$.

**Genotypic variation**

The first research question asked to what extent growth, photosynthesis, stomatal conductance, water use efficiency, and carbohydrate accumulation differed among well-watered trees from cooler and wetter climates compared to those originating from warmer and drier climates when grown under aCO$_2$. Results document that
indeed intra-specific differences among some of these traits of *E. grandis* exist. Trees from provenance Woondum – with the warmest and driest climate origin – had the slowest growth rates but slightly greater root-to-shoot ratios, as well as lower rates of $A_{\text{sat}}$ and $g_s$, however WUE was not different among provenances. Carbon assimilation rates of the remaining three provenances could not be separated according to climate-of-origin, and there was no strong correlation between $A_{\text{sat}}$ and biomass traits. However, total dry mass of well-watered trees was significantly and negatively correlated with MAT. Trees from Mt. Lindsay were the tallest individuals when well-watered (Table 3.4), confirming a genotype effect ($G$) for *E. grandis*.

Accordingly, widespread *Eucalyptus* species (e.g. *E. camaldulensis*, *E. tereticornis*) are known to vary greatly in growth when grown in a common environment due to their capacity to adapt to local growing conditions (Liang *et al.* 1994; Wang *et al.* 1994; Wenlong & Kunnan 1992; Wu *et al.* 1994). Biomass production of different *E. grandis* provenances grown in the tropical monsoon climate of China was correlated with climate-of-origin (Liang *et al.* 1994). Here, stem volume was negatively correlated with latitude of origin, and the largest trees originated from tropical North Queensland whereas the shortest trees came more from sub-tropical South Queensland or NSW. Significant differences in growth parameters were also found for other *Eucalyptus* species, although not always directly attributable to climate parameters (Wang *et al.* 1994; Wenlong & Kunnan 1992; Wu *et al.* 1994).

Leaf size was also related to climate-of-origin in *E. grandis*. Leaves of trees from Mt. Lindsay and Kilcoy Creek originating from the two coldest climates were 23% larger than from the other two provenances. Reduced leaf area can result in lower transpirational water loss, especially beneficial in xeric climates and leaf size of eucalypts has been shown to be indicative of soil water availability (Cernusak *et al.* 2011).

Overall, results for *E. grandis* in the current study do not point to selective pressure on leaf physiological traits for drought adaptation at seed origin. Yet, morphological traits like biomass or leaf size were lower in trees from warmer climates.
contributing to a beneficial water use strategy on a whole-tree basis. In fact, MAT rather than MAP might be the most important climatic driver for *E. grandis* since it is growing in the sub-tropics and tropics of North East Australia, where water is abundant through most of the year.

**Genotypic response to eCO₂**

The second research question asked if intra-specific differences in metabolic functioning observed under well-watered conditions in aCO₂ would persist in eCO₂. Indeed, *A*, WUE and biomass of stem and roots increased in eCO₂ compared to aCO₂. Trees from the coolest climate (Mt. Lindsay) had the greatest increase of *A*ₙₐ₅ by 40% from aCO₂ to eCO₂, resulting in an 11% increase of final stem dry weight. Additional supply of [CO₂] triggered enhancement of plant traits like photosynthesis and growth rates even during water limitation in three provenances having high performance, but not in trees coming from the warmest climate that already performed poorly under ambient conditions. Therefore, *A*ₙₐ₅ in trees from the warmer climate characterising the origin of the Woondum provenance increased by a mere 3% with no measurable effect on final stem dry weight. This indicates that eCO₂ does not increase *A* or growth of well-watered *E. grandis* from warmer climates as a result of different temperature optima for *A* when grown in the same environment. It has been shown for *Eucalyptus* spp. that provenances from warmer climate respond with declining *A* and growth when grown in temperatures 3.5°C above their summer temperatures while trees from cooler climates had a positive response to warming (Drake *et al.* 2015). Hence, provenances like Mt. Lindsay or Kilcoy Creek may have been grown in conditions beneficial for their physiological adaptation. In *Acer rubrum*, 23% of variation in growth response to eCO₂ could be explained with seed origin, indicating the existence of different local adaptations regarding carbon allocation strategies to climate regimes within a single tree species (Mohan *et al.* 2004). This result highlights the importance of selecting appropriate provenances to match tree stock with environmental conditions.
Chapter Three  
Intra-specific differences during eCO$_2$ and mild water limitation

*Genotypic response to water limitation and eCO$_2*

The third research question focused on the positive influence of eCO$_2$ on A and WUE during water limitation and whether the genotypic response observed under aCO$_2$ can be observed under these modified conditions. My results demonstrate that despite the negative effect of water limitation on A, $g_s$ and SLA, trees grown in eCO$_2$ had higher rates of A, improved WUE and greater final biomass compared to water-limited trees growing under aCO$_2$. It must be again noted that this positive effect on A and biomass was statistically significant only for trees that originated from cooler climates, not for provenance Woondum. At the end of the experiment, trees from Kilcoy Creek had consistently large biomass yield across water and [CO$_2$] treatments. While trees from Mt. Lindsay were the tallest in the well-watered treatment, they were less drought tolerant than trees from other climates. For example, they had a greater biomass reduction (38%) after 25 days of water limitation compared to trees from Kilcoy Creek (10%). Nevertheless, biomass of water-limited trees was not significantly linked to climate parameters like MAT or MAP contrary to correlations found for well-watered trees. Hence, testing tree productivity under different biotic stresses is essential given that $G \times E$ interactions can differ in response to water limitation and the resultant effects on growth and productivity may not match those observed under favourable conditions (Costa e Silva *et al.* 2006).

Faster growth is often related to lower drought tolerance due to shifts in carbon allocation strategies and adaptations of leaf gas exchange. Similar to findings in my study, trees of the genus *Populus, Pinus, Fagus* and *Eucalyptus* originating from wetter climates showed faster growth and developed a greater leaf area during well-watered conditions, yet had greater reductions of growth during soil water deficit compared to trees from more drought prone environments (Corcuera *et al.* 2010; 2011; Monclus *et al.* 2009; Osório & Pereira 1994; Thiel *et al.* 2014; Viger *et al.* 2016).
Chapter Three Intra-specific differences during eCO\textsubscript{2} and mild water limitation

*Winners and losers in future climate*

Long-term genetic adaptation at seed origin was most prominent in trees from provenance Woondum, which had the lowest or no decline in A and biomass during water limitation. Yet, these traits were already the lowest measured during well-watered conditions, relative to the other provenances. Whereas eCO\textsubscript{2} had only minor effects on tree physiology and growth, water limitation was the major driver for changes in A, g\textsubscript{s}, WUE, NSC concentrations, SLA, root mass and growth in young trees, leaving the tallest seedlings most vulnerable to drought. This study demonstrated that the four tested provenances of *E. grandis* followed two different strategies to balance carbon allocation for growth with the capacity to tolerate water limitation. Trees from the warmest climate with a potentially higher risk to experience water limitation had a conservative carbon and water use strategy defined by low gas exchange and slow growth, compared to provenances from colder climates with greater gas exchange rates and growth but hence more prodigal water use strategies. Other provenance trials have found similar results (Li 1999, Li *et al.* 2000; Mäkelä *et al.* 1996), demonstrating how physiological adaptation of different tree provenances to local environments can improve growth and survival in their natural habitat.

Plantations with *E. grandis* cover more than one million ha in Brazil and over 300,000 ha in South Africa, with numerous other countries also utilizing this plantation species (McMahon *et al.* 2010a). With increasing global demand for forest products (FAO 2014) and a changing climate, research is now focussing on how to improve biomass yield of this species. More recently, the role of potential genetic diversity within a single species has been suggested as a pathway to maximise productivity in future climates (Campoe *et al.* 2012 and references within). Thus, this study confirms that provenance by environment interactions exist which highlights the need for testing plantation trees and their productivity response not only in favourable conditions, but most importantly in future growing conditions to identify the best performing trees.
Chapter Three  
Intra-specific differences during eCO2 and mild water limitation

*Limitations of the study*

One limitation of this study was the tree age since drought and eCO2 response may vary with developmental status due to morphological and physiological alterations with age and size (Saxe et al. 1998). Despite ameliorating effects of eCO2 to drought on growth seen in young trees, there is evidence that this effect diminishes in mature trees (e.g. Hättenschwiler et al. 1997, Tognetti et al. 2000). Furthermore, the four tested provenances are only a small subsample within the wide distribution of *E. grandis*. Future studies will have to test more detailed gradients for MAT and MAP to verify results reported here.

Plants often encounter water shortage during summer associated with high irradiation and accumulation of ROS. Due to timing of the drought treatment in winter, trees may have experienced lower oxidative stress than normally seen during naturally occurring droughts.

**3.5 Conclusion**

Depending on climate-of-origin, *Eucalyptus grandis* followed two different performance strategies. Trees from cooler climates had relatively high rates of $A$ and $g_s$ resulting in greater final biomass and leaf area. However, being ‘programmed’ for high growth performance resulted in relative greater reductions of growth during water limitation. On the other hand, trees of provenance Woodnum originating from the warmest climate appeared to follow a more conservative growth strategy that resulted in lower leaf area and rates of $A$ and $g_s$, yet appeared beneficial when dealing with water limitation where relative growth rates will decline less. The greater ratios of root-to-shoot mass of this provenance occurring naturally in warmer climate further support the existence of this strategy. Thus, clear differences among provenances of *E. grandis* are apparent and can be harnessed to fit future growing conditions. Results from this study illustrate the importance of multi-factor experiments when assessing suitability of plantation trees since there might be a trade-off between growth under optimal and stress conditions.
Among the four studied provenances, this experiment has identified trees from provenance Kilcoy Creek to be the most suitable genotype for future plantings of *E. grandis*. Trees from Kilcoy Creek exhibited one of the highest biomass yield under water-limited conditions in either aCO$_2$ or eCO$_2$. Moreover, falling just short of biomass yield produced by trees originating from Mt. Lindsay, growth of the Kilcoy Creek trees remained strong when water availability was not limited. Given current climate forecasting, it is most likely that future plantings will be exposed to eCO$_2$ and an increase in periodic shortages of water. Hence, trees from Kilcoy Creek will yield sufficient quantities of biomass in ‘good years’ but more importantly remain superior in productivity in ‘bad years’.
Chapter Four: Drought-hardiness of *Eucalyptus melliodora* and *E. coolabah* is linked to leaf area and carbohydrate reserves

4.1 Introduction

Long-lived organisms, including trees, will encounter a range of abiotic stresses relatively frequent throughout their life span that vary in severity and intensity (Niinemets 2010). Thus, mild droughts can trigger acclimation processes or drought-hardiness, defined as an improved capacity to cope with water limitation due to exposure to previous water limitation that triggered physiological and morphological adjustments. Nonetheless, severe drought will likely lead to increased mortality rates generated by different mechanisms (Sperry & Love 2015). Furthermore, soil water deficit in the field usually develops slowly over weeks or months; hence, experimental studies in controlled environments with gradual establishment of repeated soil water deficit are needed to understand if, and how, trees will respond to water limitation in the future.

*Tree response to water limitation*

Water limitation due to long, severe and/or frequent drought events can result in mortality due to a combination of hydraulic failure, reduced carbon scarcity and potentially be aggravated by pest and pathogen infestation of stressed individuals (McDowell 2011; McDowell *et al.* 2008; Sala *et al.* 2010). Tree mortality occurs when water limitation becomes too frequent and/or too intense (Gutschick & BassiriRad 2003; Mitchell *et al.* 2016; van Mantgem *et al.* 2009). Survival of trees in response to water limitation includes a range of strategies that can be categorised under the concepts of avoidance, resistance and resilience i.e. recovery (De Boeck
et al. 2011). Resistance is described as a physiological state which is stable during environmental stress (Grimm & Wissel 1997). Acclimation to achieve resistance may include alterations in growth and physiological traits during changed environmental conditions (Kozlowski & Pallardy 2002).

Water limitation leading to soil water deficit triggers a variety of physiological responses in trees which can manifest within minutes, but can also lead to defoliation and growth reduction, if water stress is severe up to months later (Mitchell et al. 2013). There are important inter-specific differences in the strategies to tolerate soil water deficit that arise from distinct morphological, anatomical and physiological adjustments across environments, resulting in varying survival rates (McDowell et al. 2008). Thus, species originating from drier climates often show greater growth rates and greater resistance to xylem cavitation than trees from wetter environments during water limitation (Brodribb & Cochard 2009; McDowell et al. 2008). The time required to reach hydraulic failure varies between species making some species more vulnerable to water limitation than others (Bucci et al. 2012; McDowell et al. 2008). This can be directly regulated by different sapwood anatomy, but also impacted by stomatal sensitivity to cell turgor, root size and depth, as well as leaf area.

Furthermore, species within the genus Eucalyptus have distinct adaptations of their biochemical composition depending on their climate distribution to improve osmotic adjustment (Merchant et al. 2006, 2007; Warren et al. 2011a). Osmotic adjustment can support water uptake from the soil by lowering osmotic potential in cells, hence triggering water influx into the plant to maintain cell turgor pressure (Merchant et al. 2007; Warren et al. 2011a, 2012). The capacity for osmotic adjustment is species and population dependant, but may be an important mechanism for plant tolerance to drought (Kozlowski & Pallardy 2002). Osmotic adjustment can quickly be facilitated by either synthesising specific osmolytes or using stored soluble carbohydrates such as sucrose (Adams et al. 2013; Mitchell et al. 2013, 2014).
Recovery after water limitation

Recovery is defined here as the return to the pre-disturbance state classified as baseline after abiotic stress (Grimm & Wissel 1997). Similar to the initial response time to water limitation, the time necessary to recover from water limitation can be very short (e.g. opening of stomata), but depending on the trait of interest could also take years (e.g. replenishment of carbohydrate storage) (Lewis et al. 2013; Mitchell et al. 2016). Similar to drought tolerance, recovery rate may vary based on drought severity and different plant traits (Hodgson et al. 2015; Liang & Zhang 1999; Mitchell et al. 2016; Niinemets 2015).

Nevertheless, many eucalypt species are re-sprouters; for example, if trees survive water limitation and water becomes available again, they will quickly recover via formation of new shoots through epicormic buds even after complete defoliation (Zeppel et al. 2015). Recovery from drought, especially after defoliation, involves mobilisation of stored carbohydrates which are used to support growth of new leaves and xylem tissue (Bucci et al. 2003; Palacio et al. 2012). Given that more carbon can be stored in plants with greater biomass, carbon pools increase with tree age and size; hence, this may potentially extend resource utilisation during prolonged droughts (Niinemets 2010; Zeppel et al. 2015).

Acclimation

Since abiotic and biotic stress may occur frequently in a long-lived tree, long-term physiological acclimation may contribute substantially to survival (Anderegg et al. 2015; Mitchell et al. 2016; Ogle et al. 2015). The process of acclimation can involve morphological, physiological or biochemical modifications such as reduced growth (Anderegg et al. 2015; Ogle et al. 2015), stomatal conductance (Gallé et al. 2011; Martorell et al. 2014), osmotic adjustment (Merchant et al. 2006) and carbon and biomass allocation to roots (Hasibeder et al. 2015). The resultant phenotype can be beneficial to stress tolerance of trees once they experience the same stress in the future (Niinemets 2010; Ogle et al. 2015). Acclimation processes may include reduced rates of growth over several years following the environmental stress, due
to changes in allocation of carbon among sinks (Anderegg et al. 2015). Trees may also upregulate osmotic adjustment leading to greater rates of photosynthesis during the recovery phase following severe water limitation, as has been observed in some *Eucalyptus* species (Correia et al. 2014; Warren et al. 2012). Furthermore, the elasticity of cell walls of *Eucalyptus grandis* was adjusted following repeated water limitation events (Fan et al. 1994). Thus, various tree traits can be affected by these acclimation processes.

A ‘chronic stress’ hypothesis has been postulated that water deficit causes long-term xylem cavitation, which affects plant traits like photosynthesis, growth, leaf area and phloem transport, extending recovery time and increasing vulnerability to stress, eventually leading to mortality (see Sperry & Love 2015). Previous research has shown increased vulnerability to cavitation during repeated drought cycles (Anderegg et al. 2013) and slower recovery due to a reduction in root mass (Plaut et al. 2013). Furthermore, more negative water potentials during severe drought were associated with longer recovery times of photosynthesis (Resco et al. 2009).

Overall, more research is needed on tree response to repeated drought events, in particular for trees that represent different climates-of-origin.

*Research objectives*

Most trees experience several periods of water limitation during their life. Since short-term acclimation to drought events may lead to changes in tree function over time, multiple stress events should be applied when selecting tree species for improved long-term biomass yield. As the frequency of water limiting events is predicted to increase in south eastern Australia (CSIRO & Bureau of Meteorology 2015), it is important to better understand how repeated water limitation will impact physiological response and performance of trees during the selection of drought tolerant trees for superior biomass yield or survival rates under changing climate.

The present experiment examines the capacity of one provenance of *Eucalyptus coolibah* originating from a semi-arid climate and one provenance of *E. melliodora*
originating from a temperate climate to cope with different levels of water limitation. This study focused on whether trees varied in their capacity to acclimate to prolonged water limitation of two different severities (during ‘drought 1’) and whether acclimation during drought 1 affects tree physiological performance. Furthermore, I tested if and how water limitation altered carbon allocation and use, and if these effects differed between the two species originating from different climate ranges. For this purpose, I collected samples for carbohydrate analysis. Also, the recovery of these provenances after drought 1 was monitored for leaf gas exchange. Finally, a second and more severe water limitation (termed ‘drought 2’) was used to test for possible acclimation imposed on the provenances by experiencing drought 1.

As this research will be conducted over an extended experimental time, it will be possible to test effects of water limitation on larger trees that better reflect field conditions. Integrating the observed inter-specific differences to water limitation into a framework of whole-tree physiological functioning will help identify distinctive ecological strategies that have evolved over time in *Eucalyptus* species from a range of climates.

**Hypotheses**

1. Trees originating from semi-arid climates are more drought resistant (i.e. more water use efficient during prolonged water limitation) and will maintain greater stomatal conductance ($g_s$) and rates of net CO$_2$ assimilation ($A$) compared to trees originating from temperate climates.

2. Trees from semi-arid climates are better adapted to water limitation and will recover faster than trees from temperate climates.

3. The intensity of water limitation will affect recovery times after drought 1 and responses to drought 2. Mildly water-limited trees will recover quickly and acclimate to the next drought event by reducing $g_s$ and increasing WUE,
while severely water-limited trees will not fully recover leaf water potentials and $A$.

4.2 Material and Methods

4.2.1 Species

Two eucalypt species (*Eucalyptus melliodora* A. Cunn. Ex Schauer, common name: Yellow Box; *E. coolabah* Blakely & Jacobs, common name: Coolabah) were chosen for this experiment due to their contrasting distribution in eastern Australia. *Eucalyptus melliodora* is a drought tolerant tree that grows from western Victoria to southern Queensland along the western slopes and tablelands of the Great Dividing Range (Brooker *et al.* 2006). In contrast, *E. coolabah* is mostly found in semi-arid climate along riparian areas of the Darling River system (Northern New South Wales to Northern South Australia) as well as the Northern Territory and coastal and inland Queensland (Brooker *et al.* 2006). Both species grow on a variety of soil types.

Seeds were purchased from the Australian Tree Seed Centre (CSIRO Australia) as seedlots associated with an exact collection location. Seeds of both species were selected to represent contrasting climates according to the Köppen classification (Köppen 1936). Climate at seed origin of *E. coolabah* is classified as semi-arid, climate of *E. melliodora* seed origin as temperate climate with hot summers and dry winters (Table 4.1). Climate data were obtained from eMAST (www.emast.org.au) which is part of the Terrestrial Ecosystem Research Network (TERN). Monthly mean temperature (MAT), precipitation (MAP) and evapotranspiration (ET) were averaged for the periods of 1971–2010.
Table 4.1: Location and environmental characteristics of the selected tree populations of *E. coolabah* and *E. melliodora*. Mean minimum and maximum annual air temperatures: $T_{\text{min}}$, $T_{\text{max}}$ ($^\circ$C); mean annual precipitation: MAP (mm); ratio of evapotranspiration/precipitation: ET/MAP are shown for the two locations. Note that higher ET/MAP represents greater water limitation.

<table>
<thead>
<tr>
<th>species</th>
<th>seedlot</th>
<th>site coordinates</th>
<th>$T_{\text{min}}$</th>
<th>$T_{\text{max}}$</th>
<th>MAP</th>
<th>ET/MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coolabah</em></td>
<td>12816</td>
<td>31.45° S 143.11° E</td>
<td>12.7</td>
<td>27.0</td>
<td>262</td>
<td>6.09</td>
</tr>
<tr>
<td><em>E. melliodora</em></td>
<td>15338</td>
<td>36.29° S 146.41° E</td>
<td>8.1</td>
<td>21.9</td>
<td>659</td>
<td>1.84</td>
</tr>
</tbody>
</table>

4.2.2 Growth conditions

Seeds were sown in February 2015. After germination and seedling establishment, 30 seedlings of each species were selected and transplanted in pots at a plant nursery (Greening Australia, Richmond, NSW, Australia). After ten weeks (April 2015) the seedlings were planted into large woven potting bags (75 L, Garden City Plastics, NSW, Australia) in a poly tunnel facility. A sandy potting mix (Rocket Pot Mix, pH 5.5–6.0, Depco PTY LTD, NSW, Australia) with high organic matter of coir and pine bark was used as the growing medium. A 5 cm thick layer of gravel at the bottom of the pot facilitated faster water drainage. The trees were incorporated into the experimental design of a larger research project involving the same tree species and thus were dispersed randomly throughout the facility in Richmond, NSW at 33.61° S 150.75° E. Potting bags were placed on pallets (110 x 110 cm, Abbey Pallets PTY LTD, NSW, Australia), which allowed for sufficient drainage and prevented roots from growing into the ground (Fig. 4.1).
Figure 4.1: Schematic overview of the experimental design in the poly tunnel. 91 pallets were distributed throughout the tunnel accommodating five pots each (except for the last row of smaller pallets which held three pots each), accommodating a total of 308 trees for this project. Species times water treatments were subdivided into 96 irrigation zones controlled by one soil moisture sensor per zone. Crosses represent location of temperature/relative humidity sensors to monitor environmental conditions inside the poly tunnel. Symbol colours represent treatment groups: dark blue = well-watered controls \((n = 5)\); light blue = well-watered during drought 1, water-limited during drought 2 \((n = 5)\); orange = mild water limitation during drought 1 and 2 \((n = 10)\); red = severe water limitation during drought 1 and 2 \((n = 10)\) for *E. coolabah* (closed circles) and *E. melliodora* (open squares).

Pots were connected to an automated watering system (Acclima CS3500 controller, Landscape Technologies, NSW, Australia) with drip irrigation for each pot (supplied by Pacific Mist Watering Systems NSW Pty Ltd, NSW, Australia). Drip irrigation consisted of two pressure regulated rings placed around the base of each tree emitting ca. 18 L per hour (Fig. 4.2A). Trees were assigned to one of 96 watering zones of three to five trees spread out across three pallets and irrigation for each watering zone was triggered by a Time Domain Transmissometry sensor (Acclima Digital TDT sensor, Landscape Technologies, NSW, Australia) which was buried 10 cm below the soil in one of the zone’s pots at a 45° angle pointing to the plant’s base. Minimum and maximum thresholds for volumetric water content of the soil (VWC) were programmed into the system and controlled automatically.
Figure 4.2: Pot arrangement on pallets inside the poly tunnel. Image A depicts the irrigation system with drip line rings around recently planted seedlings. Image B shows trees after 8 months.

The facility did not have climate control and thus trees experienced ambient fluctuations of air temperature (T) and relative humidity (RH). These environmental conditions were recorded at two locations, T and RH with Rotronic HygroClip2 (HC2-S3, Rotronic Instruments Corp, NY, USA) and photosynthetic photon flux density (PPFD) with Apogee SQ-420 PPFD sensor (Apogee Instruments Inc., UT, USA). Data were logged every 15 minutes with a data logger (CR1000, Campbell Scientific, QLD, Australia; see Fig. 4.1).

All trees were grown under well-watered conditions (phase 1) until end of February 2016. At that time, watering treatments were begun that included two experimental phases of water limitation separated by a short re-watering to field capacity phase (see Fig. 4.3). At the beginning of the first drought (dry-down, phase 2), soil volumetric water content (VWC) was slowly reduced by gradually lowering the maximum thresholds for VWC to reach two discrete levels of water limitation. To account for different rates of soil drying in each pot, VWC of each pot was monitored regularly with a mobile soil moisture probe i.e. Time Domain Reflectometry (Acclima Digital TDR-315 sensor, Landscape Technologies, NSW, Australia) and water was added to pots. Each of the two water limitation treatments was applied to a group of 10 trees per species. A third group of well-watered trees (field capacity; n = 10 per species) was maintained throughout drought 1. Once VWC of 8% for the mildly water-limited group and 3% for the severely water-limited group were reached (drought 1, phase 3), values were
maintained for additional six weeks by keeping VWC relatively stable (Fig. 4.3). This phase was followed by a short recovery interval (phase 4; 15 July to 05 August 2016) where all trees were watered daily to saturation. Starting on 06 August 2016, recovery was followed by the second, more intense and rapid drought (drought 2, phase 5), which entailed no water for all trees that were previously subjected to either of the two levels of water limitation \((n = 20\) per species) and half of the trees that were well watered throughout drought 1 \((n = 5\) per species). The remaining five trees per species served as well-watered controls.

### 4.2.3 Leaf water potential

Predawn and midday leaf water potentials \((\Psi_{pd} \text{ and } \Psi_{md})\) were frequently measured on all 60 trees before and during water limitation on ten dates within the same week of gas exchange measurements. One leaf per tree was collected at least 30 minutes before sunrise and during midday, placed into a moisturised plastic bag and kept cool in an ice box before taking it to the lab. Leaf water potential was measured using a pressure chamber (1505D, PMS Instruments Company, OR, USA) following Boyer (1967). The difference between \(\Psi_{pd} \text{ and } \Psi_{md}\) was calculated and named \(\Delta \Psi_{l}\) to assess water potential regulation. The difference between \(\Psi_{md}\) of water-limited at stomatal closure and \(\Psi_{md}\) of well-watered trees was calculated for drought 1 and 2 and called \(\Delta \Psi_{l}\). This trait was used to assess drought tolerance of the two species, where more negative values indicate more anisohydric behaviour (Farrell et al. 2017).

### 4.2.4 Leaf gas exchange

Leaf gas exchange during day- (photosynthesis) and night-time (respiration) was measured on all 60 trees at ten different times throughout the experiment: once during the well-watered (phase 1) (17–18 January 2016); three times during the first dry-down (phase 2) (22 March, 26 April, 16 May 2016); three times during drought 1 (phase 3) (17 and 29 June, 12 July 2016); once at the end of the recovery phase (phase 4) (1 August 2016); and twice during the final drought 2 to leaf mortality (phase 5) (22 August and 5 September 2016).
Leaf net CO₂ assimilation (A, μmol m⁻² s⁻¹) and stomatal conductance of water vapour (gs, mol m⁻² s⁻¹) of a newly developed, fully expanded leaf was measured on sunny days during daylight hours (9:30–15:30 hours) using an infrared gas analyser (LI-6400XT, Li-Cor Inc., NE, USA) equipped with a 2 × 3 cm chamber head with red/blue LED light source. Conditions inside the cuvette were set to observed high light conditions in the poly tunnel (1200 μmol m⁻² s⁻¹), leaf temperature in the cuvette was set at 30°C for the first three measurement campaigns and then reduced to 28°C due to cooler ambient temperatures. CO₂ concentration inside the cuvette was set to 400 µmol mol⁻¹ and flow rate inside the cuvette was set to 500 µmol s⁻¹. Water vapour was scrubbed to maintain a leaf vapour pressure deficit (VPD) of around 1.3 kPa. Gas analysers were matched every time before logging data. A minimum of five data points per plant were logged at 5 second intervals and later averaged. Intrinsic water use efficiency (IWUE) was estimated by dividing A by gs.

Night-time respiration (Rdark) of leaves was measured with the same equipment on one newly fully expanded leaf per tree at least two hours after darkness. Rdark was measured after measurements of A and gs. Here, CO₂ concentration inside the cuvette was set to 400 µmol mol⁻¹, flow was 300 µmol s⁻¹ and leaf temperature was set to 22°C (early in the experiment) and lowered to 20°C (later in the experiment) to simulate natural growing conditions.

4.2.5 Leaf area and stem biomass measurements

Specific leaf area (SLA) was recorded for each tree at the end of phase 3 (17 June 2016). Twenty leaves from each of the 60 trees were harvested and their area (LA) was measured (Li-Cor 3100C, Li-Cor Inc., NE, USA). Leaves were dried in an oven at 60°C for three days before recording dry weight (DW). SLA was calculated as quotient of LA and DW. All leaves of each tree were counted three times during the experiment (8 April, 16 May and 17 June 2016) to estimate total LA of each tree by multiplication of LA of the subsample with total leaf number.
During the severe drought (drought 2, phase 5), water was withheld completely to initiate complete defoliation. Leaf death was defined as the point when no green leaf remained on a tree although it may still be attached to the stem. Once this point was reached, the above-ground tissue was harvested. The majority of dry leaves remained on the tree. As pots were distributed across the poly tunnel, leaves which abscised were easily identifiable for each tree and collected from the previously swept ground in a paper bag. Plant material was dried at 70°C for three days before recording dry weight of leaves (DW_{leaf}) and stems (DW_{stem}). LA of fresh leaves was estimated from DW_{leaf} by using SLA (Mitchell et al. 2014). Tree harvest commenced on 30 August 2016 and ended on 1 November 2016. All well-watered control trees were harvested on 13–14 October 2016.

### 4.2.6 Non-structural carbohydrates

Samples of branches and roots were collected for analyses of non-structural carbohydrates (NSC), specifically starch (St) and soluble sugars (Ss). These samples were collected from each tree at the end of drought 1 (phase 3; 12 July 2016) and during the tree harvest towards the end of drought 2 (phase 5; varying dates according to mortality of individual trees). A hand full of fine roots and coarser roots with up to 0.5 cm diameter were dug out of the pot and immediately microwaved for 10 seconds to stop metabolism, before being dried at 70°C for three days. Once dried, the entire sample was ground to a fine powder using a shaker mill (MM400 Retsch GmbH, Germany). Analyses of the mixed sample and calculation of St and Ss (mass-based) was identical to the description provided in Chapters 2 and 3.

### 4.2.7 Calculation of percentage change

Percentage change of $A$, $g_s$, $iWUE$, $R_{dark}$ and LA of water-limited trees ($\bar{x}_{WL}$) relative to well-watered controls ($\bar{x}_{WW}$) as a measure of the plastic response of the species was calculated according to Equation 7 with standard errors calculated as in Equation 8.
4.2.8 Statistical analysis

Data were analysed using R-3.3.3 software (R Core Team 2017). Repeated measures of leaf area as well as all tree physiological traits ($\Psi_{pd}$, $\Psi_{md}$, $A$, $g_s$, $R_{\text{dark}}$ and iWUE) were analysed using the lmer() function from the ‘lme4’ package (Bates et al. 2014). Here, water treatments and species including interactions, as well as the phase of the experiment, were fixed effects and tree number included as random effect. Since a different set of trees was measured before water limitation commenced until the dry down in March 2016, data generated before March were analysed separately. Here, two-way analysis of variance (ANOVA) was used to analyse effects of water treatments and species on plant traits as fixed factors. Effects were tested for data taken within the same experimental phase. Furthermore, a two-way ANOVA was used to test if individual physiological traits at the end of phase 3, drought 1 (12 July 2016), phase 4, recovery (1 August 2016) as well as towards the end of phase 5, drought 2 (5 September 2016) differed significantly ($p < 0.05$ for all analyses). Dry weight of leaves and stem, leaf area at harvest, specific leaf area during drought 1, and carbohydrate concentrations separately for each time point, were analysed with two-way ANOVA. Detailed results can be found in the Supplementary Table S4.1. The TukeyHSD() function from the base package was used for Tukey’s honest significant difference test when effects of water treatment were significant.

Statistical test assumptions of data normality and equal variance were tested using the Shapiro-Wilks and Levene’s test. The ‘car’ package (Fox & Weisberg 2011) was used for Anova() and leveneTest() functions. The Anova() function allows calculation of F-statistics with Type III sums of squares for both aov() and lmer() tests. Where needed, data were transformed using either logarithmic or square root transformations for physiological traits or logit transformation for carbohydrate concentrations to assume normality. For days until leaf death, data were non-transformable and hence a Kruskal-Wallis test using the kruskal.test() function was performed separately for effects of water treatments and species. Regression analyses of predawn leaf water potentials with leaf gas exchange traits, as well as correlations between $A$ and NSC, were done using OriginPro 2017.
(OriginLab Corporation, MA, USA). Coefficient of determination ($R^2$) and $p$-values in figures were calculated with the same program. Convex hulls in Fig. 4.7 were generated by connecting raw data points with the same program. Centroids for each hull were calculated by averaging the five boundary coordinates generated by OriginPro. Mean values are presented with one standard error.

4.3 Results

4.3.1 Environmental conditions

In summer 2015/2016 (December and January), mean temperatures inside the tunnel house reached 26.3 ± 0.04°C with 68.4 ± 0.2% mean relative humidity (RH) between 10:00–16:00 hours. Photosynthetic photon flux density (PPFD) at midday averaged 850 ± 13 µmol m$^{-2}$ s$^{-1}$; during this time, mean vapour pressure deficit (VPD) was 1.37 ± 0.03 kPa. Night-time temperatures averaged 18.8 ± 0.2 °C. In Winter 2016 (June/July), daytime temperatures reached 21.3 ± 0.03°C and mean RH of 62.3 ± 0.1%; PPFD at midday averaged 559 ± 5 µmol m$^{-2}$ s$^{-1}$. Night-time temperatures in the poly tunnel averaged 9.8 ± 0.03°C.

All trees were grown under well-watered conditions at 25% VWC until March 2016 (phase 1 in Fig. 4.3). In the following 7 months, trees were subjected to two experimental droughts (phases 2, 3 & 5 in Fig. 4.3). In drought 1, VWC was gradually reduced (phase 2 in Fig. 4.3) reaching 8% and 3% VWC after three months, and thereafter held relatively stable for six weeks (phase 3 in Fig. 4.3). Drought 1 was followed by a short recovery interval (15 July to 5 August 2016; phase 4 in Fig. 4.3) when all trees were watered daily to saturation. This was followed by drought 2 which dried out the soil in pots very rapidly over two months until VWC was close to zero (phase 5 in Fig. 4.3).
4.3.2 Overview of physiological traits

Tree physiological traits (leaf water potential and leaf gas exchange) were similar between species and significantly different between water treatments especially between well-watered and severely water-limited trees during drought 1 and drought 2 (Fig. 4.5). Details for each trait are discussed in the following text.
Figure 4.4: Physiological traits of *E. coolabah* (closed circles; *n* = 5 or 10) and *E. melliodora* (open squares; *n* = 5 or 10) during 2016. Shaded areas correspond with experimental phases described in Fig. 4.3. Traits shown: predawn leaf water potential (*Ψ*<sub>pd</sub>, A–B), midday leaf water potential (*Ψ*<sub>md</sub>, C–D), intrinsic water use efficiency (iWUE, E–F), stomatal conductance (*g*<sub>s</sub>, G–H), rate of net CO<sub>2</sub> assimilation (*A*, I–J), and leaf dark respiration (*R*<sub>dark</sub>, K–L). Data shown are means for trees exposed to different treatments. Symbol colours represent treatment groups: dark blue = well-watered controls (*n* = 5); light blue = well-watered during drought 1, water-limited during drought 2 (*n* = 5); orange = mild water limitation during drought 1 and 2 (*n* = 10); red = severe water limitation during drought 1 and 2 (*n* = 10). Error bars show one standard error of means.
4.3.3 Leaf water potential

Water potentials gradually declined during the dry-down (phase 2) and 18 weeks after water limitation commenced (phase 3, drought 1), $\Psi_{pd}$ of *E. coolabah* remained significantly more negative than *E. melliodora* ($p = 0.008$), while $\Psi_{md}$ were similar. At the same time, $\Psi_{pd}$ and $\Psi_{md}$ of trees under different watering regimes became increasingly and significantly separated ($p < 0.001$; Table 4.2). At the end of drought 1 (12 July 2016), $\Psi_{pd}$ of well-watered and mildly water-limited trees were similar. However, severely water-limited trees had $\Psi_{pd}$ of $-1.7 \pm 0.3$ MPa in *E. coolabah* and $-1.9 \pm 0.4$ MPa in *E. melliodora* that significantly differed from the well-watered controls. Accordingly, trees assigned to the different water treatments had different mean values for $\Psi_{md}$ with well-watered trees averaging $-1.4 \pm 0.1$ MPa, mildly water-limited trees $-2.1 \pm 0.2$ MPa and severely water-limited trees averaging $-3.2 \pm 0.2$ MPa (Table 4.2). $\Delta \Psi_l$, which is the difference between $\Psi_{pd}$ and $\Psi_{md}$, was significantly different between species at the end of drought 1 ($p = 0.008$; Table 4.2); however, post-hoc tests could not identify specific treatment groups which were different. Means of $\Delta \Psi_l$ for *E. coolabah* were $-1.1 \pm 0.2$ MPa for well-watered trees and $-1.3 \pm 0.2$ for severely water-limited trees, whereas means of $\Delta \Psi_l$ in *E. melliodora* were $-1.8 \pm 0.2$ MPa in mildly water-limited trees and $-1.3 \pm 0.1$ MPa in well-watered trees.

Table 4.2: Leaf water potential of *E. coolabah* and *E. melliodora* during the last days of drought 1. Means of predawn ($\Psi_{pd}$, MPa) and midday water potentials ($\Psi_{md}$, MPa) as well as the difference between the two leaf traits ($\Delta \Psi_l$) are shown separately for each species and water treatment (WW = well-watered controls; mild = mild water limitation; sev. = severe water limitation). Values in brackets represent one standard error of means. Letters behind symbols show statistical significance across all six watering treatments and both species separately for each physiological trait.

<table>
<thead>
<tr>
<th>species</th>
<th>treatment</th>
<th>$n$</th>
<th>$\Psi_{pd}$</th>
<th>$\Psi_{md}$</th>
<th>$\Delta \Psi_l$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coolabah</em></td>
<td>WW</td>
<td>5</td>
<td>$-0.4 (0.1)^a$</td>
<td>$-1.5 (0.2)^a$</td>
<td>$-1.1 (0.1)^a$</td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>11</td>
<td>$-0.7 (0.2)^a$</td>
<td>$-1.8 (0.2)^{ab}$</td>
<td>$-1.1 (0.1)^a$</td>
</tr>
<tr>
<td></td>
<td>sev.</td>
<td>10</td>
<td>$-1.7 (0.3)^b$</td>
<td>$-3.0 (0.3)^{cd}$</td>
<td>$-1.2 (0.2)^a$</td>
</tr>
<tr>
<td><em>E. melliodora</em></td>
<td>WW</td>
<td>5</td>
<td>$-0.1 (0.02)^c$</td>
<td>$-1.4 (0.1)^a$</td>
<td>$-1.3 (0.1)^a$</td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>10</td>
<td>$-0.5 (0.2)^a$</td>
<td>$-2.3 (0.2)^{bc}$</td>
<td>$-1.8 (0.2)^a$</td>
</tr>
<tr>
<td></td>
<td>sev.</td>
<td>9</td>
<td>$-1.9 (0.4)^b$</td>
<td>$-3.4 (0.2)^d$</td>
<td>$-1.5 (0.3)^a$</td>
</tr>
</tbody>
</table>
During phase 4 (recovery from drought 1), $\Psi_{pd}$ of both species and all water treatments recovered to levels indicating full hydration. Water potentials at midday however only recovered fully in one species ($p < 0.001$; Fig. 4.4). Values for *E. melliodora* averaged around $-0.5$ MPa across water treatments, yet $\Psi_{md}$ for *E. coolabah* was $-1.0 \pm 0.1$ MPa in formerly mildly water-limited trees compared to $-0.8 \pm 0.1$ MPa in well-watered control trees.

During drought 2 (phase 5), leaf water potentials of all trees that received zero water declined similarly. Watering regimes of drought 1 had no effect on patterns of dry-down and resulting $\Psi_{pd}$ and $\Psi_{md}$ during drought 2 (Fig. 4.4). Four weeks into drought 2, mean $\Psi_{pd}$ in water-limited trees of both species reached $-4.2 \pm 0.3$ MPa while mean $\Psi_{md}$ averaged $-4.6 \pm 0.2$ MPa.

At stomatal closure, $\Delta \Psi_{md}$ was more negative in *E. melliodora* than in *E. coolabah* indicating a more anisohydric behaviour of the former species. Severely water-limited trees during drought 2 showed the greatest differences during this study with $\Delta \Psi_{md}$ of *E. melliodora* averaging $-2.1$ MPa compared to $-1.1$ MPa in *E. coolabah* (see Table S4.2 in the Supplementary Material for more details).

### 4.3.4 Leaf gas exchange

Before water treatment commenced, $g_s$, $A$ and iWUE were similar among all trees of both species ($p > 0.53$; Fig. 4.4). In January 2016 (phase 1), $g_s$ was on average $0.62 \pm 0.05$ mol m$^{-2}$ s$^{-1}$, while $A$ averaged $20.91 \pm 0.57$ µmol m$^{-2}$ s$^{-1}$ with a mean iWUE of $43.06 \pm 4.25$ µmol mol$^{-1}$. At the same time, $R_{dark}$ did not differ significantly between trees of both species ($p = 0.31$), reaching on average $1.3 \pm 0.1$ µmol m$^{-2}$ s$^{-1}$ in *E. melliodora* and $1.18 \pm 0.13$ µmol m$^{-2}$ s$^{-1}$ in *E. coolabah*.

Both tree species responded similarly to the two water limitation treatments (Fig. 4.4), where severely water-limited trees showed significantly less physiological activity compared to mildly water-limited plants ($p < 0.001$; Fig. 4.5). At the end of drought 1, $g_s$ was $0.28 \pm 0.05$ mol m$^{-2}$ s$^{-1}$ in well-watered trees and declined to $0.09 \pm 0.02$ mol m$^{-2}$ s$^{-1}$ in mildly and $0.02 \pm 0.004$ mol m$^{-2}$ s$^{-1}$ in severely water-limited trees, representing a decline of $g_s$ by $-68\%$ and $-93\%$. Similarly, $A$ declined from
13.1 ± 0.8 µmol mol⁻¹ in well-watered trees to 8.7 ± 0.9 µmol mol⁻¹ (~34%) in mildly and to 2.0 ± 0.4 µmol mol⁻¹ (~85%) in severely water-limited trees. Pronounced variation in $g_s$ and $A$ of individual trees resulted in a vague relationship between water limitation and iWUE (Fig. 4.6). In contrast, severe water limitation clearly reduced $R_{dark}$ ($p = 0.002$), whereby $R_{dark}$ of *E. melliodora* was significantly lower (approx. ~50%) than that of *E. coolabah* (approx. ~40%) ($p = 0.05$; Figs. 4.5 and 4.6).

**Figure 4.5:** Relative changes in leaf physiological traits of water-limited *E. coolabah* and *E. melliodora* compared to well-watered controls at the end of drought 1. *E. coolabah* is shown as closed circles for each water treatment ($n = 10$) and *E. melliodora* as open squares ($n = 10$). Panel A: rate of net CO₂ assimilation ($A$); panel B: stomatal conductance ($g_s$); panel C: intrinsic water use efficiency (iWUE); panel D: dark respiration ($R_{dark}$). Colours represent the level of water limitation: orange = mild water limitation, red = severe water limitation. Error bars show one standard error. Asterisks indicate significant differences between water-limited and well-watered trees within each species separately (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; $p > 0.05$ was n.s. = not significant). Different letters indicate significant species differences between the mildly and severely water-limited trees examined separately ($p < 0.05$).

Clear and significant relationships of $A$ and $g_s$ with $\Psi_{pd}$ were apparent at the end of drought 1 (Fig. 4.6), documenting that increasing water limitation resulted in more
negative $\Psi_{pd}$ accompanied by exponential declines in $A$ and $g_s$ ($p < 0.001$). Less clear were the relationships between iWUE and $\Psi_{pd}$ due to the greater range of values in severely water-limited trees. However, increasing water limitation resulted in declining $\Psi_{pd}$, hence leading to declining $A$, $g_s$ and $R_{\text{dark}}$ (Fig. 4.6).

Figure 4.6: Predawn leaf water potential ($\Psi_{pd}$) related to leaf physiological traits of *E. coolabah* and *E. melliodora* at the end of drought 1. *E. coolabah* is shown as closed circles and *E. melliodora* as open squares. Panel A shows raw data relating $\Psi_{pd}$ and leaf stomatal conductance ($g_s$), panel B rate of net CO$_2$ assimilation, panel C intrinsic water use efficiency (iWUE) and panel D leaf dark respiration ($R_{\text{dark}}$). Symbol colours represent treatment groups: dark blue = well-watered controls ($n = 5$); light blue = well-watered during drought 1, water-limited during drought 2 ($n = 5$); orange = mild water limitation during drought 1 and 2 ($n = 10$); red = severe water limitation during drought 1 and 2 ($n = 10$). Lines in panels A, B and D show best-fit functions and related statistical information.

Recovery of $g_s$, $A$ and $R_{\text{dark}}$ from water limitation in phase 3 differed significantly between the two species (each trait $p < 0.001$). Generally, and irrespective of the water treatment during drought 1, variability of measured leaf gas exchange traits was greater in *E. coolabah* compared to *E. melliodora* (Fig. 4.7). Interestingly, $\Psi_{pd}$ in trees that experienced the greatest reductions in water availability were the least negative in both species. The range of $\Psi_{pd}$ of *E. coolabah* trees that were mildly
water-limited during drought 1 was similar to well-watered control trees, while that of the same cohort of *E. melliodora* trees showed less negative values compared to the well-watered trees of the same species (Fig. 4.7). Despite these indicators for high capacity of rehydration after drought, *g*ₚ of *E. melliodora* was just half (0.12 ± 0.03 mol m⁻² s⁻¹) and *A* was two thirds (8.3 ± 1.5 µmol m⁻² s⁻¹) that of well-watered control trees. However, this was not the case in *E. coolabah* where *A* and *g*ₚ had recovered to levels similar or even exceeded levels compared to trees that were always well-watered. In both species, and regardless of the previous water-limitation treatment, rates of *R*ₚdark were comparable to the well-watered control trees (*p* = 0.6).
Figure 4.7: Predawn leaf water potential ($\Psi_{pd}$) related to leaf physiological traits of *E. coolabah* (left panels) and *E. melliodora* (right panels) at the end of the recovery phase. Trees are compared under well-watered conditions during recovery from drought 1. Convex hulls with centroids were drawn around all data points to depict range differences and overlaps of well-watered control trees (blue) compared to severely (red) and mildly (orange) water-limited trees during drought 1. Panels A–B: stomatal conductance ($g_s$); panels C–D: rate of net CO$_2$ assimilation (A); panels E–F: dark respiration ($R_{dark}$).

Regardless of the watering treatment during drought 1, both species responded similarly to the severe water deficit imposed during drought 2 (Fig. 4.8). At the end of drought 2, $A$ had declined by $-90\%$ (18.2 ± 1.1 to 2.0 ± 0.6 $\mu$mol m$^{-2}$ s$^{-1}$) in water-limited trees relative to well-watered controls. The same observation was made for $g_s$ (0.44 ± 0.03 to 0.04 ± 0.02 mol m$^{-2}$ s$^{-1}$). Furthermore, $R_{dark}$ during drought 2 was
not different between the two water-limited treatments ($p = 0.79$). Means for all water-limited trees were significantly lower compared to well-watered controls, declining by about −30% after four weeks ($p = 0.01$; Fig. 4.8). Thus, $R_{\text{dark}}$ of water-limited *E. coolabah* trees averaged $0.69 \pm 0.06 \mu\text{mol m}^{-2} \text{s}^{-1}$ relative to well-watered controls with $0.97 \pm 0.09 \mu\text{mol m}^{-2} \text{s}^{-1}$ and water-limited *E. melliodora* trees had $0.53 \pm 0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to controls with $0.82 \pm 0.08 \mu\text{mol m}^{-2} \text{s}^{-1}$. Similar to responses to water limitation observed during drought 1, $g_s$, $A$ and $R_{\text{dark}}$ were significantly and negatively (curvilinear) related to $\Psi_{pd}$ (Fig. 4.8).
4.3.5 Leaf mortality

The number of days required for complete mortality of leaves at the end of drought 2 did not differ among water-limited trees, nor were species-specific differences observed. The watering treatments during drought 1 (i.e. well-watered, mild and
severe water limitation) had no effect on the time when leaf mortality was complete. In short, canopies of *E. coolabah* completely defoliated after 50 ± 3 days, whereas those of *E. melliodora* were completely defoliated after 53 ± 2 days (Table 4.3).

Table 4.3: Days until complete leaf mortality was reached during drought 2. Values are means for each cohort of trees that was exposed to different watering treatments during drought 1 (WW/mort.: well-watered during drought 1, no water during drought 2; mild = mild water limitation during drought 1, no water during drought 2; sev. = severe water limitation during drought 1, no water during drought 2). Values in parenthesis show one standard error of means. Superscript letters indicate statistical significance (*p* < 0.05) between water treatments and species.

<table>
<thead>
<tr>
<th>species</th>
<th>treatment</th>
<th>n</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coolabah</em></td>
<td>WW/mort.</td>
<td>5</td>
<td>49b (7.0)</td>
</tr>
<tr>
<td>mild</td>
<td></td>
<td>10</td>
<td>42b (3.2)</td>
</tr>
<tr>
<td>sev.</td>
<td></td>
<td>10</td>
<td>52b (4.1)</td>
</tr>
<tr>
<td><em>E. melliodora</em></td>
<td>WW/mort.</td>
<td>5</td>
<td>51b (7.7)</td>
</tr>
<tr>
<td>mild</td>
<td></td>
<td>10</td>
<td>52b (3.6)</td>
</tr>
<tr>
<td>sev.</td>
<td></td>
<td>9</td>
<td>47b (2.5)</td>
</tr>
</tbody>
</table>

4.3.6 Leaf area and stem biomass

Overall, LA of *E. coolabah* was −24% smaller (20746 ± 687 cm²) compared to *E. melliodora* (25727 ± 1269 cm²) at the beginning of drought 1; trees were about 3 m tall. Changes in LA during drought 1 and drought 2, however, showed some species-specific differences. Leaf area of *E. coolabah* progressively declined during drought 1 and even further during drought 2, while that of *E. melliodora* remained relatively stable (Fig. 4.9). Towards the end of drought 1, LA of mildly water-limited *E. coolabah* trees had declined −24%, while that of severely water-limited trees had declined by −48% compared to LA of well-watered trees. In contrast, LA of *E. melliodora* did not decline as a result of mild or severe water limitation.

Differences between species in LA were not detected for SLA (*p* = 0.07). In June 2016 (phase 3, drought 1), SLA in *E. coolabah* averaged 75 ± 2 cm g⁻¹ and 85 ± 2 cm g⁻¹ in *E. melliodora*. During drought 2, *E. coolabah* trees which were severely water-limited during drought 1 further reduced their LA to 8390 ± 1362 cm², compared to
a LA of 26724 ± 5981 cm² for trees of the same species that remained well-watered throughout drought 1 and 2 ($p < 0.001$). No such changes were observed for LA of *E. melliodora*.

![Figure 4.9](image)

Dry weight of stems ($DW_{stem}$) of trees that experienced water limitation at the end of drought 2 was on average ~20% lower compared to well-watered trees ($p = 0.002$; Table 4.4). However, neither species, nor the level of water limitation that trees were exposed to during drought 1, had a significant effect on $DW_{stem}$ at the end of the experiment. Values at harvest ranged from 282–1156 g (average: 808 ± 49 g) in trees that were previously well-watered during drought 1 and drought 2. At the end of the study, average $DW_{stem}$ of trees that experienced water limitation during drought 1 and/or drought 2 was less (637 ± 20 g), but the wide range of values potentially diminished detection of statistical significance. While stems of *E. melliodora* had developed lignotubers and those of *E. coolabah* had not, water treatments had no effect on the dry weight of these storage organs (Table 4.4).

Leaf dry weight at harvest was significantly different between species ($p = 0.03$), but also varied across water treatments ($p < 0.001$) with significant interactions
between these two factors ($p = 0.01$; Table 4.4). In *E. coolabah*, $D_{W_{\text{leaf}}}$ were similar between water-limited trees averaging $143 \pm 12$ g ($p > 0.05$), but ~63% lower from well-watered trees averaging $382 \pm 86$ g ($p < 0.009$). Yet, no such difference was found in *E. melliodora* ($p > 0.05$) averaging $270 \pm 17$ g in well-watered controls and $212 \pm 11$ g in water-limited trees.
Table 4.4: Biomass and leaf area (LA) of *E. coolabah* and *E. melliodora* trees at the end of drought 2. Means are shown separately for dry weight of leaves and stems (DW\textsubscript{leaf} and DW\textsubscript{stem}) as well as lignotubers (DW\textsubscript{ligno}) of trees that had lignotubers. Leaf area on a fresh weight basis of defoliated trees was estimated from dry leaves remaining on the tree and scattered on the ground. Values are means for each cohort of trees that was exposed to different watering treatments (treat.) during drought 1 (WW/mort.: well-watered during drought 1, no water during drought 2; mild = mild water limitation during drought 1, no water during drought 2; sev. = severe water limitation during drought 1, no water during drought 2). Values in parenthesis show one standard error. Superscript letters indicate statistical significance ($p < 0.05$) between water treatments and species.

<table>
<thead>
<tr>
<th>spec. treat.</th>
<th>n</th>
<th>LA (cm\textsuperscript{2})</th>
<th>n</th>
<th>DW\textsubscript{leaf} (g)</th>
<th>n</th>
<th>DW\textsubscript{stem} (g)</th>
<th>n</th>
<th>DW\textsubscript{ligno} (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coo.</em> WW</td>
<td>5</td>
<td>26724\textsuperscript{a} (5981)</td>
<td>5</td>
<td>382.2\textsuperscript{a} (86.4)</td>
<td>5</td>
<td>866.6\textsuperscript{a} (89.4)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>WW/mort.</td>
<td>5</td>
<td>12502\textsuperscript{bcd} (867)</td>
<td>5</td>
<td>155.7\textsuperscript{bc} (15.1)</td>
<td>5</td>
<td>523.8\textsuperscript{b} (54.4)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>mild</td>
<td>11</td>
<td>12171\textsuperscript{cd} (1141)</td>
<td>11</td>
<td>161.1\textsuperscript{bc} (18.1)</td>
<td>11</td>
<td>645.4\textsuperscript{ab} (44.6)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8390\textsuperscript{d} (1362)</td>
<td>10</td>
<td>117.6\textsuperscript{c} (19.5)</td>
<td>10</td>
<td>576.5\textsuperscript{b} (32.9)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td><em>E. mel.</em> WW</td>
<td>5</td>
<td>22192\textsuperscript{ab} (1279)</td>
<td>5</td>
<td>270.4\textsuperscript{ab} (17.1)</td>
<td>5</td>
<td>748.8\textsuperscript{ab} (34.4)</td>
<td>5</td>
<td>124.3\textsuperscript{a} (22.9)</td>
</tr>
<tr>
<td>WW/mort.</td>
<td>5</td>
<td>16463\textsuperscript{abc} (2128)</td>
<td>5</td>
<td>197.1\textsuperscript{abc} (25.4)</td>
<td>5</td>
<td>697.0\textsuperscript{ab} (70.6)</td>
<td>4</td>
<td>194.4\textsuperscript{a} (140.3)</td>
</tr>
<tr>
<td>mild</td>
<td>10</td>
<td>18558\textsuperscript{abc} (1570)</td>
<td>10</td>
<td>219.1\textsuperscript{ab} (16.9)</td>
<td>10</td>
<td>691.9\textsuperscript{ab} (52.8)</td>
<td>8</td>
<td>76.1\textsuperscript{a} (14.0)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>17952\textsuperscript{abc} (1150)</td>
<td>10</td>
<td>212.5\textsuperscript{ab} (18.2)</td>
<td>10</td>
<td>659.3\textsuperscript{ab} (33.9)</td>
<td>7</td>
<td>87.0\textsuperscript{a} (19.0)</td>
</tr>
</tbody>
</table>
4.3.7 Non-structural carbohydrates

At the end of drought 1, concentration of Ss in branches was similar among treatments and species (Fig. 4.10). Yet, concentration of St in branches of *E. coolabah* was 9 ± 0.3% which was significantly greater than in *E. melliodora* where concentration of St was 7.7 ± 0.1% (*p* < 0.001). The concentration of Ss in roots of *E. coolabah* differed significantly among water treatments (*p* < 0.001). Trees which were mildly or severely water-limited displayed increased concentrations of Ss (mild water limitation: 10 ± 0.8%; severe water limitation: 10.7 ± 1.5%) compared to well-watered trees (7.2 ± 1.2%). In *E. melliodora* such effects of water limitation were absent, although overall concentrations of Ss in roots (22.8 ± 1.2%) were more than twice as high as roots of *E. coolabah*. Concentrations of St in roots of both species were similar at the end of drought 1 (Fig. 4.10).

At the end of drought 2, concentrations of Ss in branches of water-limited trees were significantly greater (102%) compared to well-watered control trees of both species (*p* < 0.001; Fig. 4.10). Contrastingly, concentration of St in branches had decreased in water-limited trees (−22%) compared to well-watered control trees (*p* < 0.001). Root Ss concentrations of well-watered *E. melliodora* (15.4 ± 2.1%) were significantly greater than in well-watered *E. coolabah* (2.3 ± 0.2%; *p* < 0.001).

Similarly, concentrations of St in roots were greater in well-watered *E. melliodora* averaging 22.1 ± 2.6% compared to St in roots of well-watered *E. coolabah* (*p* = 0.02). Changes in the concentration of Ss in roots of water-limited *E. coolabah* were similar to those observed during drought 1. The greatest changes in NSC were in roots of *E. melliodora* at the end of drought 2, where St was severely depleted by −50%, while concentrations of Ss had markedly doubled in water-limited, compared to well-watered trees (Fig 4.10).
Non-structural carbohydrate (NSC) concentrations in root tissue of *E. melliodora* at harvest were linearly correlated with $A$ measured 16 days since commencement of drought 2. While the relationship between $A$ and St was positive, that of $A$ and Ss was negative (Fig. 4.11). Interestingly, water-limited *E. melliodora* trees which had low rates of $A$ early during drought 2 had high concentrations of Ss in roots at time of leaf death, while at the same time concentrations of St in roots were low. No such pattern was observed for *E. coolabah* where water limitation had an effect on $A$ but not on concentrations of NSC in roots (Fig. 4.11).
Figure 4.11: Photosynthesis (A) related to concentrations of soluble sugars (Ss, left panel) and starch (St, right panel) in branches and roots. *E. coolabah* is shown as closed circles and *E. melliodora* as open squares. Measurements of A were collected 16 days after drought 2 had commenced, and concentrations of St and Ss represent conditions at the end of drought 2 when all leaves were dead. Symbol colours represent treatment groups: dark blue = well-watered controls (n = 5); light blue = well-watered during drought 1, water-limited during drought 2 (n = 5); orange = mild water limitation during drought 1 and 2 (n = 10); red = severe water limitation during drought 1 and 2 (n = 10). Lines show linear regressions (solid line = *E. coolabah*; dashed line = *E. melliodora*), grey shading indicates 95% confidence intervals with statistical information shown for each regression fit.

4.4 Discussion

The current study assessed whether *Eucalyptus coolabah* from a semi-arid climate and *E. melliodora* from a temperate climate differ in drought-hardiness when trees are exposed to two drought regimes of varying severity. Overall, physiological responses were very similar between the two species, especially when trees received very little water during drought 1 and 2. These results are similar to a meta-analysis on forest species, which found similar vulnerability to xylem embolism regardless of climate-of-origin (Choat et al. 2012). Some physiological
acclimation was detected with increased rates of $g_s$ and $A$ during recovery in *E. coolabah*. Nonetheless, species-specific differences to drought of traits not related to leaf gas exchange were detected and may be the result of adaptation to long-term climate at the home environment. There were differences in leaf area (LA) between the two species as well as observed changes in non-structural carbohydrates (NSC). While *E. melliodora* retained most of its LA during drought 1 and early stages of drought 2, *E. coolabah* progressively and significantly reduced LA according to the severity of water limitation during drought 1 and even more so during drought 2. At the same time, reserves of starch (St) in roots of *E. melliodora* were strongly depleted while the concentration of soluble sugars (Ss) markedly increased, indicating a pronounced mobilisation of carbon resources. In contrast, concentrations of St in roots of *E. coolabah* remained unchanged following droughts 1 and 2 and changes of Ss concentrations were of smaller magnitude compared to *E. melliodora*. The different strategies to cope with water limitation displayed by the species and their potential ecological advantages will be discussed in detail.

*Resistance to water limitation*

My first hypothesis stated that *E. coolabah* originating from a semi-arid climate will be more water use efficient than *E. melliodora* originating from a more mesic environment, and thus maintain greater $g_s$ and $A$ during water limitation. This hypothesis was rejected because no species-specific differences were apparent during drought 1, where $g_s$, $A$ and iWUE were similar in both species in the mild and severe water limitation treatments. Both species had similar sensitivity of $g_s$ to declining water potentials. Hence, both species followed similar water use strategies regarding leaf gas exchange under apparent water limitation, despite originating from different climate zones. This result stands in contrast to other studies of *Eucalyptus* species (*E. globulus*, *E. microtheca*, *E. saligna*, *E. sideroxylon*) exposed to severe soil water deficit (Granda *et al*. 2014; Lewis *et al*. 2013; Li & Wang 2003).
Stomata close with increasing water limitation to reduce transpirational water loss through the leaf and responses are very closely related to xylem water potentials (Martorell et al. 2014). In this study, mildly water-limited trees reduced $g_s$ by $-68\%$, and severely water-limited trees reduced $g_s$ by $-93\%$. During severe water limitation, $A$ may decline due to lower stomatal and mesophyll conductance, reduced CO$_2$ supply to Rubisco, inactivation of Rubisco or photosystem II, and damage to photosynthetic functions by oxidative stress compounds (Flexas et al. 2006; Gallé et al. 2007; Tognetti et al. 1995). Based on the observed similarity between species and water limitation treatments, photosynthetic biochemical functions appeared to be similar in $E. coolabah$ and $E. melliodora$ and did not increase the drought resistance of the species from semi-arid climate, as hypothesised. However, detailed correlations between intercellular [CO$_2$] ($c_i$) and $A$ were not measured and hence no further conclusions can be made.

A well described response of $Eucalyptus$ species during early stages of water limitation is the reduction of growth, while at the same time maintaining moderate rates of $A$ that lead to a slow decline of St under increasingly negative water potentials (Mitchell et al. 2013, 2014), as starch is a primary compound for storage of carbon (e.g. Martínez-Vilalta et al. 2016). If concentrations of NSC are measured at a single time point, the values may reflect the combination of increasing or decreasing rates of $A$, $R$, production of secondary metabolites for defence and/or osmotic adjustment, storage dynamics, and other metabolic processes (Adams et al. 2013; Hartmann & Trumbore 2016). Drought 1 did not result in changes in the concentration of St, either in roots or in branches of water-limited trees, suggesting that the water limitation treatments were not of sufficient intensity or duration to alter carbon storage as a consequence of lower $A$ and its impact on carbohydrate production or utilisation.

Reductions in $R_{dark}$ and LA are two additional processes that trees can use to acclimate to drought conditions. Both processes were observed during the present experiment, predominately in trees that experienced severe water limitation, which may reduce losses of carbon and water. Reduced respiratory activity is often triggered by the lack of available sugars as the result of decreased $A$ (Atkin &
Macherel 2009; Reich et al. 2009). However, respiration rates are also driven by ATP demand (e.g. for growth) which is also negatively affected by water deficit (Martínez-Vilalta et al. 2016). Mitochondrial respiration in leaves is often down-regulated during water limitation (Atkin & Macherel 2009), but may also remain unchanged or increased depending on the intensity and duration of water limitation (Atkin & Macherel 2009). In this study, a greater decline in $R_{\text{dark}}$ (leaf area basis) was seen in *E. melliodora* trees that were severely water-limited during drought 1, yet this species maintained a constant LA compared to severely water-limited *E. coolabah*. Thus, greater reductions of $R_{\text{dark}}$ in *E. melliodora* while maintaining the greater canopy size relative to *E. coolabah* may have achieved similar low rates for the whole tree.

In severe drought, $A$ declined up to $-90\%$, compared to $R_{\text{dark}}$ that declined up to $-40\%$, indicating greater sensitivity of $A$ compared to $R_{\text{dark}}$ in drought conditions (Duan et al. 2013a), as has been observed in other *Eucalyptus* species as a result of long-term or severe water limitation (Ayub et al. 2011; Crous et al. 2012). Carbohydrate concentrations were similar at the end of drought 1 in well-watered and droughted trees of *E. melliodora*, suggesting that reduction of $R_{\text{dark}}$ may have been due to changes in carbon allocation and metabolism since leaf shedding was not an adaptational trait of this species. The available data do not permit identification of the exact cause for the reduction of $R_{\text{dark}}$. Additional studies that further disentangle how prolonged water limitation affects catabolism of photoassimilates are necessary.

**Recovery from water limitation**

At the end of the recovery phase, leaf water potentials recovered in both species from the two water limitation treatments. However, leaf physiological traits, particularly $g_s$ and $A$ showed species-specific differences during recovery. While those traits fully recovered in *E. coolabah*, they were slow to recover in *E. melliodora*, which suggests that severe drought may have much longer lasting effects on *E. melliodora* potentially affecting long-term survival if drought return intervals become shorter in future climates. Generally, leaf gas exchange in woody
species recovers within a few days of re-watering if the drought is of low intensity and duration (Lewis et al. 2013; Liang & Zhang 1999), but recovery from severe water limitation may take weeks or longer (Duan et al. 2014; Gallé et al. 2007; Liang & Zhang 1999; Ruiz-Sánchez et al. 1997; Vomáčka & Pospíšilová 2003). However, depending on the severity of the stress, A, gs and photosystem II may not be fully recovered even after water potentials have recovered (Brodribb & Cochard 2009; Correia et al. 2014; Gallé et al. 2007; Jakob 2016; Martorell et al. 2014, Miyashita et al. 2005; Tognetti et al. 1995), suggesting faster recovery from hydraulic dysfunction and slower recovery from biochemical damage. In this regard, it was shown that a higher degree of lost hydraulic conductance during water stress will lead to slower or incomplete recovery of A in a woody legume (Resco et al. 2009). Hence, the incomplete recovery of A and gs in E. melliodora after nearly 3 weeks suggests that other factors than hydraulic dysfunction and its repair contributed to the incomplete recovery of these traits.

The degree of stress tolerance of a tree is related to the severity and duration of that stress, as well as the degree of acclimation. A tree species experiencing a mild stress event at regular intervals may develop tolerance against this stress compared to a species that experiences this stress less often (Anderegg et al. 2015; Gutschick & BassiriRad 2003; Mitchell et al. 2016). Thus, the faster recovery of leaf gas exchange in E. coolabah, originating from a semi-arid environment, after severe water limitation possibly reflects the natural environmental variation experienced by this species (Zeppel et al. 2015). Since rain is less common in the habitat of E. coolabah compared to that of E. melliodora, fast upregulation of leaf physiological function in response to improved availability of soil water is an advantage in arid environments compared to mesic environments. Other studies have shown delayed recovery of gs following severe drought in Quercus ilex (Gallé et al. 2011) and in Eucalyptus globulus trees, gs took one week to recover fully following severe drought (Correia et al. 2014).
Drought-hardiness

My third hypothesis proposed that water limitation during drought 1 would pre-adapt or acclimatise the physiological response to the subsequent drought 2. However, responses of leaf physiological traits \( (g_s, A, iWUE, R_{dark}) \) to the rapid and severe water limitation developed in drought 2 was similar for both species and all water limitation treatments, suggesting that drought-hardiness did not occur, as was similarly observed for other eucalypts (Duan et al. 2013a). However, other studies have observed adjustment of leaf physiology and morphology in *Eucalyptus* and other species following repeated drought cycles (Clemens & Jones 1978; Gallé *et al.* 2011; Guarnaschelli *et al.* 2003, 2006), suggesting that more empirical studies are required before general predictions about drought-hardiness after water limitation can be formulated.

*E. coolabah* displayed a very different survival strategy during water deficit compared to *E. melliodora* in terms of canopy size, despite having similar relationships between leaf water potential and stomatal conductance. Reducing canopy area is an effective mechanism to limit transpirational water loss through leaves relative to water uptake through roots, delaying the development of increasingly negative water potentials along the root-stem-leaf continuum. This strategy has been observed in many other *Eucalyptus* species (Gibson *et al.* 1995; Kozlowski & Pallardy 2002). Here, only *E. coolabah* was found to markedly reduce its leaf area (~60%) by the end of drought 2. This may have also resulted in less negative values of \( \Delta \Psi_{md} \) compared to *E. melliodora* during drought 2, suggesting a greater drought tolerance and isohydric strategy (Farrell *et al.* 2017). Considering that *E. coolabah* is native to a semi-arid climate of inland Australia where precipitation is historically low and sporadic, it suggests that this is an adaptation to frequent and severe soil moisture deficit (Rose *et al.* 2009). Such conditions are much rarer throughout the habitat of *E. melliodora* which originated from a more temperate climate. However, in the case of extreme water reduction (drought 2), greater leaf abscission of severely water-limited trees compared to mildly water-limited trees in drought 1 did not extend time to canopy defoliation in drought 2 for *E. coolabah*. It should be noted that in natural field settings, *E. coolabah* and
*E. melliodora* may have access to deeper soil water to stabilise water potentials (Farooq *et al*. 2009; Martínez-Vilalta *et al*. 2016).

Furthermore, *A* measured early during drought 2 was positively related to carbohydrate concentrations at the end of drought 2, suggesting that carbohydrate resources play a role in drought tolerance. While *E. coolabah* re-sprouts from epicormic buds, large lignotubers at the root base in *E. melliodora* store additional carbohydrates for recovery after disturbance, but also are bud stores for re-sprouting (Clarke *et al*. 2013). In *Pinus sylvestris* seedlings, survival was positively linked to carbon metabolism during an extreme drought that eventually resulted in tree mortality (Garcia-Forner *et al*. 2016). Nonetheless, neither lignotubers nor higher concentrations of *St* in *E. melliodora* compared to *E. coolabah* affected time-to-leaf-death; however, older trees may not be as dependant on these reserves.

At harvest, *Ss* concentrations were significantly increased in woody tissue of water-limited trees, perhaps reflecting reduction in *St* at the same time, yet water treatments in drought 1 did not affect the response of *E. coolabah* or *E. melliodora*. Conversion of *St* into *Ss* to utilise stored carbon in cells during severe water limitation has been observed in several other studies, including eucalypts (Duan *et al*. 2013a; Martínez-Vilalta *et al*. 2016; McDowell 2011; Mitchell *et al*. 2013; Quick *et al*. 1992). Reductions in *A* as a function of the drought treatments meant that root tissue was the main carbon source for maintaining metabolic cell function (Adams *et al*. 2013; Shvaleva *et al*. 2006). Transformation of *St* to *Ss* in root tissues was pronounced in *E. melliodora* but not in *E. coolabah*, suggesting different strategies to use stored carbon during water limitation. Since *A* was not different between water-limited *E. coolabah* and *E. melliodora*, daytime leaf physiology traits could not explain differences in NSC. Furthermore, *R_{dark}* was lower in water-limited *E. melliodora* relative to *E. coolabah* during drought 1 reducing the requirement to convert *St* to *Ss* for cell metabolism. In a study on severely water-limited *Ulmus minor*, more sensitive *g*ₚ and lower *A* as well as higher *R_{dark}* explained greater depletion of NSC reserves compared to *Quercus ilex* (Rodríguez-Calcerrada *et al*. 2017).
Ss can be used as osmotica to maintain cell turgor and support protein and membrane structures as well as hydraulic transport (Hartmann & Trumbore 2016; Jacob 2016; Sala et al. 2010, 2012), such that increasing concentrations of Ss in woody tissue of *E. melliodora* suggested that this may have been a mechanism to acclimate to the severe water limitation of drought. Osmotic adjustment has been shown in some *Eucalyptus* species (Guarnaschelli et al. 2003; Lemcoff et al. 2002; Merchant et al. 2007, 2009; Tuomela 1997), but not others (Mitchell et al. 2014; White et al. 1996); other species have modified cell wall elasticity to maintain cell turgor (Fan et al. 1994; Kozlowski & Pallardy 2002). The change in NSC in roots of *E. melliodora*’s suggests their use to support osmotic adjustment, which is a common defence mechanism against soil water deficit (Hsiao et al. 1976; Kozlowski & Pallardy 2002). Osmotic adjustment may reduce above-ground growth because carbon is diverted away from biomass allocation to shoots, but under mild water stress root growth may be continued to increase access to soil water (Chaves et al. 2003; Kozlowski & Pallardy 2002; Lemcoff et al. 2002).

Carbohydrates in young seedlings have been linked to plant survival (Garcia-Forner et al. 2016; Myers & Kitajima 2007; O’Brien et al. 2014; Poorter & Kitajima 2007). Thus, the greater osmotic adjustment in *E. melliodora*, together with retaining full leaf area could represent an alternative strategy to cope with apparent water limitation. In an environment that only consists of seasonal water shortage during an overall mesic climate, fast osmotic adjustment may be the more efficient strategy for *E. melliodora* to continue photosynthesis, cell metabolism and potentially growth after short-term drought (Chaves et al. 2003; Lemcoff et al. 2002; Tuomela 1997). In comparison, in semi-arid Australia where trees experience droughts potentially over months and osmotic adjustment would not be effective enough over a long period to prevent hydraulic failure, leaf shedding is more efficient for the reduction of water loss for *E. coolabah* (Chaves et al. 2003, Rodríguez-Calcerrada 2017). Yet, leaf shedding also results in delays of stem growth since new foliage becomes a carbon sink and needs to be developed quickly (Mitchell et al. 2016; Zeppel et al. 2015). Very similar results have been found for populations of *Quercus oleoides* when grown under well-watered and mild water-
limited conditions in a common environment (Ramírez-Valiente & Cavender-Bares 2017). Since carbon is lost during leaf abscission and other disturbances throughout the year, more carbon is needed to rebuild a new canopy, hence frequent events can severely reduce carbon reserves and risk tree mortality (see Whitehead & Beadle 2004 for review). Yet, leaf shedding and osmotic adjustment are not mutually exclusive in *Eucalyptus*, but how important each strategy is for plant survival is difficult to determine (Guarnaschelli et al. 2003).

**Limitations of this study**

Drought treatments in this study were implemented at the end of summer going into winter as well as early spring. Most drought events take place in summer when temperatures and irradiation are high. Thus, reported responses may not entirely correspond with responses seen under natural conditions where for instance greater water loss and accumulation of ROS represent further stress factors influencing tree physiology. Yet, climate models predict higher risks for droughts in South eastern Australia in winter (Cai et al. 2014) and hence it is likely that trees will encounter these conditions more often in natural settings.

**4.5 Conclusion**

This study investigated species adaptation to water limitation, including whether stress history will lead to acclimation that improves plant function in subsequent drought events. Physiological responses to water limitation were highly plastic and uniform between species with limited acclimation derived from previous drought events. However, different drought response strategies were observed in *E. coolabah* originating from a semi-arid climate and *E. melliodora* originating from a temperate climate. *E coolabah* utilised morphological adjustment (leaf shedding) whereas *E. melliodora* adjusted biochemistry (NSC) in response to drought. My results confirm inter-specific differences to water limitation and highlight the rigid response of trees to repeated stress due to natural selection at seed origin. The two observed mechanisms to water deficit did not change time-to-leaf death, hence making it unclear if either strategy will be beneficial during more frequent
droughts. However, leaf shedding in *E. coolabah* may not be a successful strategy if carbohydrate reserves are depleted to repeatedly form a new canopy if droughts become more frequent or severe in future climates while utilisation of NSC in *E. melliodora* can be rapidly adjusted to changing conditions. In conclusion, future research needs to focus more on carry-over effects of water limitations related to the different survival strategies of species to accurately predict tree performance in drier conditions.
Chapter Five: Synthesis and Conclusion

5.1 Thesis aim

My PhD research focused on the main and interactive effects of climate factors (eCO2, heat waves and water limitation) on growth and the physiological and morphological traits of different species and genotypes of *Eucalyptus*. More frequent extreme climate events, in association with rising [CO2], are predicted to have significant effects on tree function in future climates. In this thesis, I studied four ecologically and economically important *Eucalyptus* species native to Australia, originating from diverse climate regimes: *Eucalyptus camaldulensis* distributed in widespread habitats across the continent (Chapter 2); *E. grandis* in subtropical and tropical coastal Queensland (Chapter 3); *E. melliodora* in South East Australia, and *E. coolabah* occupying regions of semi-arid Australia (Chapter 4). Intra-specific differences in response to the main and interactive effects of eCO2, heat waves and drought were assessed in four to six provenances of *E. camaldulensis* and *E. grandis* to determine whether genotypic differences existed when exposed to different environments (*G x E*). Phenotypic plasticity in response to drying and wetting cycles, with an ultimate drought to mortality, was determined for two eucalypt species (*E. melliodora* and *E. coolabah*) originating from distinctly different climates. All of these experiments were designed to generate novel understanding about phenotypic plasticity in trees, originating from different climate regimes, to future climate conditions. This information could then be used to select species and genotypes most favourably suited for growth in forest plantations under current and future climate conditions. The following research questions were addressed:

1. Does intra-specific variation in phenotypic plasticity to environmental parameters (*G x E*) exist within selected trees species?
2. If phenotypic plasticity is present, can responses be related to climate-of-origin to identify superior trees for future climates?

3. Does acclimation occur when trees are exposed to variable climate conditions or are physiological and morphological traits genetically fixed and predictable for long-lived organisms like trees?

5.2 Intra-specific variation of traits in *E. camaldulensis* and *E. grandis* grown in aCO₂, ambient temperature and non-limiting water and nutrient conditions

Evolutionary pressure favours individuals with high growth and survival traits (Nicotra *et al.* 2010). Since environmental conditions can vary widely across geographical locations within the distributional range of a species, local adaptation of individual populations is subject to natural selection for greater fitness (i.e. greater growth and survival rates; Jump & Peñuelas 2004; Kawecki & Ebert 2004). Intra-specific differences in physiology and morphology exist for different genotypes, as well as populations, subsequently impacting survival and biomass of woody species (Aranda *et al.* 2010; Blackman *et al.* 2017; see Brown *et al.* 1994; Hancock & Hughes 2014; O’Brien *et al.* 2007; Robson *et al.* 2012). Yet, not all traits may be variable when trees are grown in the same environment. For example, leaf dark respiration (R<sub>dark</sub>) may be relatively similar across populations, as shown in *Pinus banksiana* (Tjoelker *et al.* 2008).

Phenotypic plasticity may be the key to improving tree performance in commercial forestry facing rapid climate change (Aspinwall *et al.* 2015; Nicotra *et al.* 2010). Yet, the extent of phenotypic plasticity of traits, when trees are grown in a common environment, is not fully understood. Accordingly, the first objective of this research was to assess the intra-specific variation in traits of *Eucalyptus camaldulensis* and *E. grandis* provenances, when grown in ambient environmental conditions.

My research found intra-specific differences in traits, when trees were grown in ambient environmental conditions, including photosynthesis (A), stomatal
conductance \((g_s)\) and biomass (Chapter 2 & 3). Growth was correlated with climate-of-origin in *E. grandis*, where trees from warmer MAT had lower biomass than trees from cooler MAT. In contrast, there was no statistical relationship for the six populations of *E. camaldulensis* regarding stem volume, but trees from the warmest MAT had 35\% greater total plant mass than trees from the cooler MAT due to differences in leaf and root dry weight.

In summary, there were significant differences in biomass between trees from different provenances of *E. camaldulensis* and *E. grandis* such that there is an opportunity to select high-yielding provenances for unique environments. The use of intra-specific variation for tree breeding in *Eucalyptus* is common (Booth 2013), but growth parameters and plant traits associated with carbon and water exchange may vary widely in their response to climatic gradients. Thus, large-scale and field-based provenance trials will be useful to select the appropriate genotypes for plantations. Moreover, phenotypic plasticity of growth in woody species in response to temperature and precipitation may be highly variable with regard to climate at seed origin (Hancock & Hughes 2014; O’Brien *et al.* 2007; Rose *et al.* 2009) and may involve more than climate variables as influencing factors (Correia *et al.* 2008; Hancock & Hughes 2014; Robson *et al.* 2012; Thiel *et al.* 2014). For example, soil nutrient and water availability as well as carbon allocation to sinks other than above-ground growth play an additional role in determining growth outcome.

### 5.3 Intra-specific variation of traits in *E. camaldulensis* and *E. grandis* grown in eCO\(_2\) \((G \times [CO_2])\) response

In general, eCO\(_2\) has a positive effect on \(A\), non-structural carbohydrates (NSC) and growth, yet the magnitude of the response may differ across genotypes (Ceulemans *et al.* 1996; Isebrands *et al.* 2001; Kubiske *et al.* 2007; Mohan *et al.* 2004). If \(G \times E\) occurs for eCO\(_2\) and growth enhancement of some genotypes is more positively responsive than others, then these genotypes could be selected for breeding programs. Thus, I designed two experiments to test responses of genotypes representing different *Eucalyptus* provenances to eCO\(_2\) (Chapter 2 & 3).
Overall, A, biomass and leaf area increased in *E. camaldulensis* and *E. grandis* when grown in eCO$_2$; however, some provenances were more responsive than others. In *E. grandis*, growth of small trees originating from warmer MAT minimally responded to eCO$_2$, whereas trees from cooler provenances increased stem mass up to 49%. Despite variable growth enhancement due to eCO$_2$, the relative rankings of tree biomass in the different provenances were mostly maintained in aCO$_2$ and eCO$_2$. The general lack of $G \times E$ interaction was similarly observed for genotypes of different tree species in a recent meta-analysis (Resco de Dios et al. 2016). In conclusion, currently fast-growing trees in aCO$_2$ will also be fast-growing trees in eCO$_2$ when no other abiotic factors are influencing growth; hence these trees can be selected for breeding programs for future eCO$_2$ environments.

### 5.4 Intra-specific differences of *Eucalyptus* to heat and drought ($G \times$ abiotic stress response)

Tolerance of extreme high temperatures or water limitation may also differ between genotypes due to morphological, physiological or biochemical alterations (Aspinwall et al. 2015). Trees with high phenotypic plasticity may be very productive under optimal temperatures and non-limiting water conditions, but also maintain growth and physiological function when environmental conditions are less favorable. Heat wave and drought events are predicted to become more common in future climates, so tree physiology, morphology and growth were assessed during heat wave and/or drought conditions for four *Eucalyptus* species (Chapter 2-4).

In my experiments, drought or drought plus heat decreased $A$, $g_s$, $R_{dark}$ and starch (St) concentrations, but increased water use efficiency (WUE) and soluble sugar (Ss) concentrations, to variable magnitudes across tree species and provenances. Interestingly, leaf water potentials were more negative during mild water limitation compared to well-watered conditions, but similar across provenances in *E. grandis* while being more negative in severely water-limited trees with larger canopies in *E. camaldulensis*. Drought severity had a greater impact on reductions of $A$, $g_s$ and $R_{dark}$ than intra- or inter-specific differences, which was evident when soil water was
severely reduced in *E. camaldulensis*, *E. melliodora* and *E. coolabah*. At the same time, Ss concentrations (especially in roots) after the stress event was strongly dependent on species (e.g. *E. melliodora* had greater Ss than *E. coolabah*). However, changes in concentration of Ss were also subject to intra-specific differences, where *E. camaldulensis* trees originating from warmer MAT had higher Ss after heat and drought than trees from colder MAT. These changes in Ss were independent from [CO$_2$] treatment or drought pre-conditioning, but clearly related to early rates of $A$ during the water limitation event in some species (*E. melliodora*). However, increased carbon storage for osmotic adjustment did not increase the time to leaf mortality during severe drought in *E. melliodora*, *E. coolabah* and *E. camaldulensis*, suggesting that the most important factor affecting plant function was water availability. Contrastingly, for several deciduous and conifer species, tree survival was positively related to NSC storage where carbon reserves can be used to support cell function (Canham *et al.* 1999; Garcia-Forner *et al.* 2016).

Although $G \times E$ was sometimes apparent, rankings across climate-of-origin remained relatively consistent in well-watered and drought conditions. Other studies have shown $G \times E$ interactions for $g_s$ (Viger *et al.* 2016), WUE (Corcuera *et al.* 2010; Ramírez-Valiente *et al.* 2010; Rose *et al.* 2009; Viger *et al.* 2016) and osmotic adjustment (Li 1998; Nguyen-Queyrens & Bouchet-Lannat 2003; Tschaplinski *et al.* 2006; Tuomela 1997). It has been shown that genotypes with greater osmotic adjustment during heat or water limitation can maintain higher $A$, $g_s$ and growth due to improved metabolic support of cells and ROS scavenging (Bedon *et al.* 2012; Iba 2002; Mittler 2002; Tschaplinski *et al.* 2006). Overall, water limitation was the factor primarily affecting tree physiology with great negative impact on physiology, biochemistry and growth. Minor changes due to phenotypic plasticity were observed, but could not overcome those effects, which have been shown for other species (Aranda *et al.* 2017; Gimeno *et al.* 2009). However, there is scope for improving biomass production by selecting trees that exhibit moderate growth reductions compared to other genotypes. Most likely, these trees will not be the largest during favourable growing conditions, yet be among the most
productive trees, while maintaining good biomass production during drier and hotter conditions.

While carbon allocation to roots and reduction of specific leaf area (SLA) was relatively similar across provenances during drought and heat events, reduction of above-ground growth and leaf area differed between *E. melliodora* and *E. coolabah* and across provenances in *E. camaldulensis* and *E. grandis*. Whereas the drought response of *E. coolabah* involved leaf abscission, *E. melliodora* exhibited greater osmotic adjustment, partially due to greater utilization of carbon reserves. On an intra-specific level, trees with low biomass production during favorable growing conditions were less impacted by water limitation than fast-growing genotypes of *E. grandis* and *E. camaldulensis*. In *E. grandis*, trees originating from cooler climates with high biomass production in well-watered conditions had significant reductions in growth when water was withheld. In contrast, slower growing trees from Kilcoy Creek in the well-watered treatment became the fastest growing trees in the drought treatment, indicating $G \times$ water interactions for *E. grandis*.

The tallest *E. camaldulensis* trees from Woondum had a growth set-back immediately after the heat and water treatment, yet managed to regain growth rates faster than trees from other provenances due to greater utilization of stored NSC. During recovery from the heat wave and drought treatment, greater NSC contributed to faster recovery of stem growth, as well as canopy establishment, in *E. camaldulensis* trees originating from warmer MAT. Greater tree size often affects drought tolerance negatively due to competition between carbon allocation to above-ground or below-ground sinks (O’Brien et al. 2007; Thiel et al. 2014). Trees from more xeric environments often exhibit specific adaptations to improve water conservation, including lower biomass, smaller but thicker leaves, and greater WUE than trees from mesic climates (Abrams 1994; Bansal et al. 2015; Corcuera et al. 2011; Cregg & Zhang 2001; Gratani et al. 2003; Li et al. 2000). Phenotypic differences across populations in response to water limitation in terms of growth and biomass allocation to roots (Costa e Silva et al. 2004; Cregg & Zhang 2001; Gibson et al. 1995; Hancock & Hughes 2014; Rose et al. 2009; Thiel et al. 2014;
Viger et al. 2016), leaf area (Gibson et al. 1995) and SLA (Bansal et al. 2015; Ramírez-Valiente et al. 2010) have been documented.

Despite differences in leaf gas exchange or carbon storage during abiotic stress found in E. camaldulensis and E. grandis, drought hardiness was not improved by previous exposure to water limitation in E. melliodora or E. coolabah. Thus, exposure to the first drought did not result in acclimation to the second drought (as reviewed for other woody species by Niinemets 2010) and observed differences in leaf area and NSC concentrations were the result of adaptation to long-term climate regimes at seed origin for the two species. In these two species, rapid climate change may increase mortality because they lack the capacity to quickly acclimate to extreme climate conditions, as has been observed in previous studies on trees including Eucalyptus (Dillon et al. 2015; Fan et al. 1994). At the same time, these findings proof that tree response to water limitation can remain stable and hence experiments only testing one stress event can already identify suitable candidates for future tree breeding. However, more research involving several drought-recovery cycles and different tree species are needed to confirm this pattern.

5.5 Intra-specific differences of Eucalyptus to a combination of environmental factors (G × [CO₂] × abiotic stress response)

Previous research has found ameliorating effects of eCO₂ on tree response to drought and heat events (Anderson & Tomlinson, 1998; Darbah et al. 2010; Hamerlynck et al. 2000; Kelly et al. 2016; Roden and Ball 1996; Taub et al. 2000). However, eCO₂ may also increase the negative effects of elevated temperature or reduced soil water availability by aggravating soil water deficit through greater water uptake and transpiration as the result of larger tree canopies produced in eCO₂ (McCarthy et al. 2010; Warren et al. 2011a). Yet, studies of the interactive effects of climate drivers on different genotypes and populations of Eucalyptus under simulated future climate conditions are rare (Booth et al. 2015). The core objective of this research was to test for variation in phenotypic plasticity to abiotic stress and eCO₂ in different genotypes of Eucalyptus (Chapter 2 & 3). During periods
of extreme temperature but sufficient water availability, eCO₂ improved $A$ and $g_s$ in *E. camaldulensis* trees and extended time-to-leaf-death of trees from the warmest MAT during soil water deficit compared to the provenances from cooler MAT. Stem biomass production in provenances of the second warmest MAT tested were equally superior when comparing water-limited *E. grandis* grown in aCO₂ to those grown in eCO₂ compared to other provenances. However, in *E. camaldulensis*, water-limited trees originating from the warmest MAT had the largest stem biomass in aCO₂, but trees originating from the second warmest MAT had the largest biomass when grown in eCO₂.

My research shows that physiological and growth responses will vary across provenances when trees are exposed to a combination of abiotic factors and eCO₂, yet these responses cannot always accurately be predicted by testing individual factors. For example, the ranking of growth responses in the different provenances, when subjected to eCO₂ and water limitation, are not necessarily predictable from growing trees in well-watered conditions and aCO₂. Environmental conditions at seed origin may trigger adaptive mechanisms of individual trees to increase fitness resulting in $G \times E$ interactions.

### 5.6 Conclusion

My research expands the currently scarce knowledge regarding intra-specific variation in response to eCO₂, high temperature and water limitation in *Eucalyptus* species. The results of my experiments demonstrate that eCO₂ can have positive effects on well-watered and water-limited or heat stressed trees. Limited $G \times E$ interactions were observed in net CO₂ uptake of trees through photosynthesis, in the dynamics of non-structural carbohydrates and more broadly, in growth between aCO₂ and eCO₂. The main driver for variable physiological responses and biomass accumulation was water availability. Mechanisms to cope with water shortage were related to long-term adaptation of *Eucalyptus* species to their climate regime at seed origin, but not to drought pre-conditioning, which suggests limited responsiveness to rapid changes in climate. Correlations of growth and physiological performance to climate-of-origin when *E. camaldulensis* provenances
were grown in aCO₂ and well-watered conditions was very similar to correlations for trees grown in eCO₂ and heat or water stress. Yet, some minor ranking changes were observed due to water stress in *E. grandis*. These important findings show that phenotypic plasticity can be harnessed to maintain or even improve productivity of eucalypt plantations in the future. Targeted research, similar to the type of experiments reported in this thesis, will help to align provenances with the appropriate environmental conditions. As has been shown throughout this thesis research, careful consideration must guide the selection process because responses of trees to co-varying environmental conditions (e.g. changes in [CO₂], water availability and temperature) are unlikely to be additive and predictable from the main effects in isolation.
Bibliography


FSC, Forest Stewardship Council (2012). Strategic review on the future of forest plantations.


IPCC, Intergovernmental Panel on Climate Change (2013). Climate Change 2013: the physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.


Appendix I – Chapter Two Supplementary Material

Table S2.1: Statistical results with $p$-values for Analysis of Variance (ANOVA) for *Eucalyptus camaldulensis*. Plant traits were analysed for significant effects of $[\text{CO}_2]$ levels (C), water treatment (W) and provenance (P) before heat and drought treatment (pre-treat.), at the end of the treatment (heat and drought) and during recovery. Traits measured were midday leaf water potential ($\Psi_{\text{md}}$), leaf moisture content (MC$_{\text{leaf}}$), leaf osmotic potential ($\Psi_{\text{n}}$), light-saturated photosynthesis at growing $\text{CO}_2$ concentration ($A_{\text{sat}}$) and saturating $\text{CO}_2$ ($A_{\text{max}}$) as well as stomatal conductance ($g_s$ and $g_{\text{max}}$), intrinsic water use efficiency (iWUE), plant dry weight (DW$_{\text{plant}}$), leaf area (LA), root-mass-fraction (RMF), concentration of soluble sugars in stem (Ss$_{\text{stem}}$) and roots (Ss$_{\text{roots}}$) as well as starch concentrations (St) and days until leaf death (days). Results for $p$-values below the significance level of 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th>Trait</th>
<th>pre-treat.</th>
<th>heat and drought</th>
<th>recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P C P×C C P C W P×C P×W C×W P C W P×C P×W C×W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Psi_{\text{md}}$</td>
<td>0.08 0.05 0.44 0.04 0.25 $&lt; 0.001$ 0.07 0.4 0.27 - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC$_{\text{leaf}}$</td>
<td>0.52 0.45 0.06 0.93 0.28 $&lt; 0.001$ 0.83 0.74 0.98 0.02 0.54 $&lt; 0.001$ 0.12 0.65 0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Psi_{\text{n}}$</td>
<td>0.26 0.71 0.7 0.31 0.27 $&lt; 0.001$ 0.71 0.7 0.46 0.48 0.35 $&lt; 0.001$ 0.06 0.02 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{\text{sat}}$</td>
<td>0.29 $&lt; 0.001$ 0.38 0.29 $&lt; 0.001$ $&lt; 0.001$ 0.77 0.34 0.3 - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g_s$</td>
<td>0.75 0.007 0.46 0.2 $&lt; 0.001$ $&lt; 0.001$ 0.02 0.04 $&lt; 0.001$ - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iWUE</td>
<td>0.67 $&lt; 0.001$ 0.54 0.9 $&lt; 0.001$ 0.05 0.93 0.97 $&lt; 0.001$ - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{\text{max}}$</td>
<td>0.32 0.11 0.44 0.74 $&lt; 0.001$ 0.04 $&lt; 0.001$ 0.87 0.7 0.59 - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g_{\text{max}}$</td>
<td>0.92 0.009 0.61 0.07 $&lt; 0.001$ $&lt; 0.001$ 0.02 0.02 $&lt; 0.001$ - - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DW$_{\text{plant}}$</td>
<td>0.03 $&lt; 0.001$ 0.89 - - - - - - - 0.84 0.1 $&lt; 0.001$ 0.86 0.26 0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.04 $&lt; 0.001$ 0.81 - - - - - - - 0.11 0.12 $&lt; 0.001$ 0.7 0.98 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMF</td>
<td>0.03 0.15 0.27 - - - - - - - 0.73 0.05 0.38 0.41 0.87 0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ss$_{\text{stem}}$</td>
<td>- - 0.08 0.51 - 0.71 - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St$_{\text{stem}}$</td>
<td>- - $&lt; 0.001$ 0.1 - 0.02 - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ss$_{\text{roots}}$</td>
<td>- - 0.04 0.47 - 0.43 - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St$_{\text{roots}}$</td>
<td>- - 0.23 0.1 - 0.55 - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>days</td>
<td>- - 0.14 0.09 - 0.61 - - - - -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table S2.2: Pearson’s correlation coefficient (Pearson’s r) between concentrations (NSC<sub>conc.</sub>) and total non-structural carbohydrates (NSC<sub>total</sub>), respectively and climate variables of seed origin of <i>E. camaldulensis</i>: mean annual temperature (MAT), mean annual precipitation (MAP) and evapotranspiration/MAP (ET/MAP). NSC were separated for starch (St) and soluble sugars (Ss) in stem and root tissue. Test was conducted on water-limited <i>E. camaldulensis</i> after heat and drought stress and pooled across [CO<sub>2</sub>] conditions. Results for p-values below the significance level of 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th>trait</th>
<th>MAT</th>
<th>MAP</th>
<th>ET/MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>NSC&lt;sub&gt;conc.&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St&lt;sub&gt;stem&lt;/sub&gt;</td>
<td>0.29</td>
<td>0.19</td>
<td>−0.33</td>
</tr>
<tr>
<td>Ss&lt;sub&gt;stem&lt;/sub&gt;</td>
<td>0.67</td>
<td>&lt;0.001</td>
<td>0.63</td>
</tr>
<tr>
<td>St&lt;sub&gt;root&lt;/sub&gt;</td>
<td>0.41</td>
<td>0.06</td>
<td>−0.45</td>
</tr>
<tr>
<td>Ss&lt;sub&gt;root&lt;/sub&gt;</td>
<td>0.70</td>
<td>&lt;0.001</td>
<td>−0.66</td>
</tr>
<tr>
<td>NSC&lt;sub&gt;total&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St&lt;sub&gt;stem&lt;/sub&gt;</td>
<td>−0.04</td>
<td>0.86</td>
<td>0.05</td>
</tr>
<tr>
<td>Ss&lt;sub&gt;stem&lt;/sub&gt;</td>
<td>0.36</td>
<td>0.09</td>
<td>−0.32</td>
</tr>
<tr>
<td>St&lt;sub&gt;root&lt;/sub&gt;</td>
<td>0.09</td>
<td>0.69</td>
<td>0.14</td>
</tr>
<tr>
<td>Ss&lt;sub&gt;root&lt;/sub&gt;</td>
<td>0.35</td>
<td>0.11</td>
<td>−0.37</td>
</tr>
</tbody>
</table>
Table S2.3: Dry weight (DW) of leaves, stem and roots of *E. camaldulensis* after heat and water limitation (water treat.) for well-watered (WW) and water-limited trees (WL). Means (*n* = 2-4) are given with ± one standard error for each provenance (prov.) and [CO$_2$] level (aCO$_2$ for ambient and eCO$_2$ for elevated [CO$_2$]).

<table>
<thead>
<tr>
<th>prov.</th>
<th>water treat.</th>
<th>DW$_{\text{leaf}}$</th>
<th></th>
<th>DW$_{\text{stem}}$</th>
<th></th>
<th>DW$_{\text{root}}$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>aCO$_2$</td>
<td>eCO$_2$</td>
<td>aCO$_2$</td>
<td>eCO$_2$</td>
<td>aCO$_2$</td>
<td>eCO$_2$</td>
</tr>
<tr>
<td>YR</td>
<td>WW</td>
<td>31.3 (12.2)</td>
<td>59.0 (5.4)</td>
<td>71.5 (20.8)</td>
<td>70.3 (11.2)</td>
<td>64.3 (39.0)</td>
<td>86.9 (40.3)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>10.5 (5.1)</td>
<td>11.5 (2.7)</td>
<td>29.6 (8.0)</td>
<td>30.8 (8.0)</td>
<td>23.6 (9.5)</td>
<td>25.7 (15.5)</td>
</tr>
<tr>
<td>CW</td>
<td>WW</td>
<td>35.4 (14.2)</td>
<td>67.7 (7.5)</td>
<td>64.4 (25.0)</td>
<td>72.9 (9.4)</td>
<td>36.8 (16.8)</td>
<td>127.2 (8.5)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>12.3 (3.8)</td>
<td>6.7 (3.1)</td>
<td>25.2 (5.8)</td>
<td>16.2 (0.4)</td>
<td>12.4 (3.5)</td>
<td>14.1 (7.7)</td>
</tr>
<tr>
<td>AN</td>
<td>WW</td>
<td>46.8 (4.1)</td>
<td>51.1 (10.2)</td>
<td>57.4 (7.6)</td>
<td>68.5 (4.6)</td>
<td>31.9 (17.0)</td>
<td>71.0 (10.9)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>13.8 (2.5)</td>
<td>20.1 (12.2)</td>
<td>31.7 (3.7)</td>
<td>39.8 (6.6)</td>
<td>15.4 (2.7)</td>
<td>32.3 (8.3)</td>
</tr>
<tr>
<td>ND</td>
<td>WW</td>
<td>55.3 (2.9)</td>
<td>64.0 (9.5)</td>
<td>62.7 (1.7)</td>
<td>79.8 (5.5)</td>
<td>76.6 (0.3)</td>
<td>64.8 (13.6)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>15.8 (4.2)</td>
<td>12.1 (8.9)</td>
<td>32.6 (5.3)</td>
<td>28.2 (12.9)</td>
<td>20.2 (8.9)</td>
<td>26.1 (19.2)</td>
</tr>
<tr>
<td>LC</td>
<td>WW</td>
<td>61.1 (6.2)</td>
<td>65.0 (23.9)</td>
<td>64.6 (10.2)</td>
<td>69.3 (19.3)</td>
<td>56.5 (22.9)</td>
<td>69.6 (57.0)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>17.4 (4.8)</td>
<td>35.5 (1.8)</td>
<td>38.0 (11.4)</td>
<td>45.2 (8.9)</td>
<td>19.5 (4.9)</td>
<td>39.1 (11.0)</td>
</tr>
<tr>
<td>WC</td>
<td>WW</td>
<td>45.5 (16.6)</td>
<td>71.2</td>
<td>60.6 (12.4)</td>
<td>76.2 (2.2)</td>
<td>40.9 (7.3)</td>
<td>63.5 (1.7)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>22.9 (3.9)</td>
<td>19.1 (5.8)</td>
<td>41.8 (4.4)</td>
<td>40.1 (3.8)</td>
<td>34.9 (14.6)</td>
<td>28.9 (6.2)</td>
</tr>
</tbody>
</table>
Appendix II – Chapter Three Supplementary Material

Figure S3.1: Correlation between stomatal conductance ($g_s$) and photosynthesis rate at saturating light ($A_{sat}$) of water-limited (open symbols) and well-watered (closed symbols) *Eucalyptus grandis* on day 25. Trees were grown in ambient (aCO$_2$; left panel) and elevated [CO$_2$] (eCO$_2$; right panel). Colours represent different provenances (dark blue: Mt. Lindsay; light blue: Kilcoy Creek; orange: Coffs Harbour; red: Woondum).
Table S3.1: Statistical results with p-values for Analysis of Variance (ANOVA) for *Eucalyptus grandis*. Plant traits were analysed for significant effects of [CO$_2$] levels (C), water treatment (W) and provenance (P) before drought treatment (pre-treat.) and at the end of the treatment (day 25 and harvest). Traits tested were predawn leaf water potential ($\Psi_{pd}$), midday leaf water potential ($\Psi_{md}$), light-saturated photosynthesis rate ($A_{sat}$), stomatal conductance ($g_s$), water use efficiency ($\Delta^{13}$C), plant dry weight (DW$_{plant}$), leaf area (LA), specific leaf area (SLA), root-mass-fraction (RMF), concentration of soluble sugars in stem (Ss$_{stem}$) and roots (Ss$_{roots}$) as well as starch concentrations (St). Results for p-values below the significance level of 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>pre-treat.</th>
<th>day 25 and harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>C</td>
</tr>
<tr>
<td>$\Psi_{pd}$</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>$\Psi_{md}$</td>
<td>0.23</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>$A_{sat}$</td>
<td><strong>0.007</strong></td>
<td>&lt; <strong>0.001</strong></td>
</tr>
<tr>
<td>$g_s$</td>
<td>&lt; <strong>0.001</strong></td>
<td>0.74</td>
</tr>
<tr>
<td>$\Delta^{13}$C</td>
<td>0.42</td>
<td>&lt; <strong>0.001</strong></td>
</tr>
<tr>
<td>DW$_{plant}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SLA</td>
<td>0.59</td>
<td>0.05</td>
</tr>
<tr>
<td>RMF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ss$_{stem}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>St$_{stem}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ss$_{roots}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>St$_{roots}$</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table S3.2: Pearson’s correlation coefficient (Pearson’s r) between plant dry weight (DW_{plant}) and stem dry weight (DW_{stem}), respectively and climate variables of seed origin: mean annual temperature (MAT, °C), mean annual precipitation (MAP, mm) and evapotranspiration/MAP (ET/MAP). Tests were conducted separately for well-watered (WW) and water-limited (WL) trees as well as tree grown in ambient (aCO₂) and elevated (eCO₂) [CO₂] conditions. Results for p-values below the significance level of 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th>trait</th>
<th>treat.</th>
<th>CO₂</th>
<th>MAT</th>
<th>MAP</th>
<th>ET/MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>DW_{plant}</td>
<td>WW</td>
<td>aCO₂</td>
<td>-0.59</td>
<td>0.006</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eCO₂</td>
<td>-0.57</td>
<td>0.009</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>aCO₂</td>
<td>0.07</td>
<td>0.78</td>
<td>-0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eCO₂</td>
<td>-0.19</td>
<td>0.43</td>
<td>0.11</td>
</tr>
<tr>
<td>DW_{stem}</td>
<td>WW</td>
<td>aCO₂</td>
<td>-0.62</td>
<td>0.004</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eCO₂</td>
<td>-0.61</td>
<td>0.10</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>aCO₂</td>
<td>0.10</td>
<td>0.58</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eCO₂</td>
<td>-0.13</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Table S3.3: Concentration of non-structural carbohydrates at harvest of E. grandis. Means (± one standard error in brackets) are separated between leaf, stem and root tissue, starch (St) and soluble sugars (Ss), provenances (prov.) and water treatment (WW for well-watered controls and WL for water-limited trees). Means were pooled across [CO₂] levels, n = 9–10.

<table>
<thead>
<tr>
<th>prov.</th>
<th>water treat.</th>
<th>leaf</th>
<th>stem</th>
<th>root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>St</td>
<td>Ss</td>
<td>St</td>
<td>Ss</td>
</tr>
<tr>
<td>MT</td>
<td>WW</td>
<td>6.7 (0.3)</td>
<td>5.6 (0.2)</td>
<td>4.7 (0.1)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>7.8 (0.2)</td>
<td>7.5 (0.3)</td>
<td>4.6 (0.1)</td>
</tr>
<tr>
<td>KC</td>
<td>WW</td>
<td>7.0 (0.2)</td>
<td>5.2 (0.2)</td>
<td>4.9 (0.1)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>7.7 (0.3)</td>
<td>6.8 (0.4)</td>
<td>4.6 (0.1)</td>
</tr>
<tr>
<td>CH</td>
<td>WW</td>
<td>6.7 (0.2)</td>
<td>5.0 (0.3)</td>
<td>4.4 (0.2)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>6.9 (0.1)</td>
<td>7.2 (0.5)</td>
<td>4.7 (0.1)</td>
</tr>
<tr>
<td>WD</td>
<td>WW</td>
<td>6.1 (0.2)</td>
<td>6.0 (0.3)</td>
<td>4.5 (0.1)</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>7.8 (0.2)</td>
<td>7.4 (0.4)</td>
<td>4.7 (0.1)</td>
</tr>
</tbody>
</table>
Appendix III – Chapter Four Supplementary Material

Table S4.1: Statistical results of linear mixed effect models for tree physiology traits between the 1st drought, recovery and 2nd drought as well as ANOVA results for two time points before the water limitation of *Eucalyptus coolabah* and *E. melliodora*. Physiological response variables were analysed for significant differences between species (spec.) and water treatment (treat.). Traits tested were predawn leaf water potential ($\Psi_{pd}$), midday leaf water potential ($\Psi_{md}$), stomatal conductance ($g_s$), photosynthesis rate ($A$), intrinsic water use efficiency (iWUE) and leaf dark respiration ($R_{dark}$). Results for $p$-values below the significance level of 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th>trait</th>
<th>species</th>
<th>treat.</th>
<th>phase</th>
<th>spec.*treat.</th>
<th>spec.*phase</th>
<th>treat.*phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>$\Psi_{pd}$</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\Psi_{md}$</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$g_s$</td>
<td>0.53</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$A$</td>
<td>0.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>iWUE</td>
<td>0.93</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$R_{dark}$</td>
<td>0.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>dry-down</td>
<td>$\Psi_{pd}$</td>
<td>0.79</td>
<td>0.66</td>
<td>-</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\Psi_{md}$</td>
<td>0.008</td>
<td>0.09</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$g_s$</td>
<td>0.01</td>
<td>0.002</td>
<td>-</td>
<td>0.42</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$A$</td>
<td>0.17</td>
<td>0.07</td>
<td>-</td>
<td>0.59</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>iWUE</td>
<td>0.006</td>
<td>0.002</td>
<td>-</td>
<td>0.81</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$R_{dark}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1st drought-recovery-2nd drought</td>
<td>$\Psi_{pd}$</td>
<td>0.28</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>$\Psi_{md}$</td>
<td>0.73</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.16</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>$g_s$</td>
<td>0.06</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.41</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>$A$</td>
<td>0.001</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.81</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>iWUE</td>
<td>0.07</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.16</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>$R_{dark}$</td>
<td>0.001</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
<td>0.36</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table S4.2: Difference between mean midday leaf water potential ($\Psi_{md}$, MPa) of water-limited trees at stomatal closure during the 1st drought and 2nd drought and mean midday leaf water potential ($\Psi_{md}$) of well-watered trees ($\Delta\Psi_{md}$) as an assessment of drought tolerance. More negative $\Delta\Psi_{md}$ indicates more anisohydric behaviour. Data were separated between *Eucalyptus coolabah* and *E. melliodora* and water treatments (WW/mort. = well-watered during drought 1, water-limited during drought 2, mild = mild water limitation during drought 1 and 2, severe = severe water limitation during drought 1 and 2).

<table>
<thead>
<tr>
<th>species</th>
<th>phase</th>
<th>WW/mort.</th>
<th>mild</th>
<th>severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coolabah</em></td>
<td>drought 1</td>
<td>na</td>
<td>−0.3</td>
<td>−1.0</td>
</tr>
<tr>
<td><em>E. melliodora</em></td>
<td></td>
<td>na</td>
<td>−0.5</td>
<td>−1.3</td>
</tr>
<tr>
<td><em>E. coolabah</em></td>
<td>drought 2</td>
<td>−1.9</td>
<td>−2.6</td>
<td>−1.1</td>
</tr>
<tr>
<td><em>E. melliodora</em></td>
<td></td>
<td>−2.3</td>
<td>−2.3</td>
<td>−2.1</td>
</tr>
</tbody>
</table>