WESTERN SYDNEY
UNIVERSITY

Hawkesbury Institute
for the Environment

Forest ecosystem water use: does species identity and ecosystem composition matter?

by

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A thesis submitted in fulfilment of requirements for the
award of the degree of Doctor of Philosophy

Hawkesbury Institute for the Environment

Western Sydney University

October 2015

For my family

Acknowledgements

This thesis would not have been possible without the guidance, encouragement and support of several people, each of whom contributed in different ways to the preparation and completion of the study.

First and foremost, I am extremely grateful to my supervisory panel, Professor David Ellsworth, Dr. Tony Haigh and Dr. Brendan Choat, for their advice, guidance, understanding and patience throughout my PhD candidature. I recognise that not all supervisors would be supportive of an engagement, marriage and new baby during a PhD candidature, and I am thankful that each of you continue to be so supportive and understanding. You are incredible scientists and wonderful people and I am so pleased I got to work closely with each of you.

It is with immense gratitude that I acknowledge Mr Burhan Amiji, whom I've had the pleasure of working alongside for the last 5 years. My many field experiments and the planting and maintenance of two plantations with over 2000 trees would not have been possible without your tireless efforts. Thank you for caring for the plantations, assisting with measurements, downloading data and being such a great support throughout my candidature.

I share the credit of my work with many colleagues who have been my mentors, who have provided invaluable advice and comments on my manuscripts and thesis and who have helped me to grow as a researcher and to think more broadly when trying to understand and present my work. I thank Tamara Amiji, Craig Barton, Catherine Beattie, Julia Cooke, Danielle Creek, Kristine Crous, Remko Duursma, Teresa Gimeno, Arnaud Héroult, Liz Kabanoff, Yan-Shih Lin, Nathan Linforth, Jeff Powell, Audrey Quentin, Anna Ósvaldsson, Stephanie Stewart

and Glenn Stone. My special thanks to a very dear friend, Sarah Beck, for her continual encouragement, support and friendship in life and study and for sharing many exciting life experiences with me along the way.

This study was supported by an Australian Research Council (LP0992238) Australian Postgraduate award, a University of Western Sydney APA top-up and a Hawkesbury Institute for the Environment APA top-up, all to Aimee E. Bourne.

Lastly, thank you to my family for their continual encouragement, support, love and guidance throughout my studies and my life; each of you had a part to play in making this possible and for that I will be forever grateful. Thank you to my parents for giving me a solid moral foundation, a love for learning and reading and a respect for and admiration of nature. Thank you to Sheardy for knowing and understanding when and how you're needed and for always being there at the right time. Thank you to Willow for bringing happiness, life, love, excitement and many smiles and cuddles into our family. Finally, thank you to Justin for reminding me what's important in life, for loving me at my lowest and highest points and for providing our family with everything we could ever hope for. I love you all.

Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except where 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.

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List of Abbreviations

$\delta^{13}C$	Leaf carbon isotopic ratio
ε	Bulk modulus of elasticity
Π_0	Osmotic potential at full turgor
Ψ_{leaf}	Leaf water potential
Ψ_{min}	Minimum leaf water potential
Ψ_{pd}	Pre-dawn leaf water potential
Ψ_{stem}	Stem water potential
Ψ_{CAV}	Water potential at point of cavitation
Ψ_{TLP}	Water potential at point of loss of turgor
A_{net}	Leaf-level photosynthetic rate
AGB	Aboveground biomass
AGBI	Aboveground biomass increment
AI	Aridity index
b	Reference canopy conductance (G_{Cref})
C	Competitive index
D	Vapour pressure deficit
D_{10}	Diameter at 10 cm from ground

D_{max}	Maximum monthly vapour pressure deficit
$\mathrm{D_h}$	Hydraulically weighted vessel diameter
DBH	Diameter at breast height, 1.3 m
$e^{\mathrm{b/m}}$	Vapour pressure deficit at point of stomatal closure
E	Whole canopy transpiration
E_{L}	Leaf level transpiration
EucFACE	*Eucalyptus* free air CO_2 enrichment site
g_{s}	Stomatal conductance
g_{s5}	5% of maximal stomatal conductance
g_{s50}	50% of maximal stomatal conductance
G'	Initial canopy conductance
G_{C}	Canopy conductance
G_{Cmax}	Maximal canopy conductance
G_{Cref}	Canopy conductance at a D of 1 kPa, reference canopy conductance
HFE	Hawkesbury Forest Experiment
HGR	Height growth rate
HPV	Heat pulse velocity
HRM	Heat ratio method

J_{S}	Sap flux density
J_{Smax}	Maximal sap flux density
K_{final}	Final hydraulic conductivity
K_{G}	Conductance coefficient
K_{h}	Hydraulic conductivity
K_{initial}	Initial hydraulic conductivity
K_{s}	Stem water transport capacity
$-m$	Slope of the G_{Cref} vs. D relationship
MAP	Mean annual precipitation
n	Sample size
P	Pressure
P_{x}	Point of x% loss of xylem conductivity
P_{12}	12% loss of xylem conductivity
P_{50}	50% loss of xylem conductivity
P_{88}	88% loss of xylem conductivity
PLC	Percentage loss of xylem conductivity
PV	Pressure volume
RGR	Relative growth rate
RWC	Relative water content of leaf

RWC_{TLP}	Relative water content at turgor loss point
T	Temperature
TDR	Time domain reflectometry
TWU	Tree water use
V_0	Molar volume of air
VI	Stem volume index
VIGR	Volume index growth rate
$WD_{\max}$	Monthly maximum water deficit
WUE	Water use efficiency

Publications

Chapter 2 was published as Bourne A.E., Haigh A.M. and Ellsworth D.S. (2015) Stomatal sensitivity to vapour pressure deficit relates to climate of origin in *Eucalyptus* species in *Tree Physiology* **35** (3): 266-278.

Chapter 3 has been submitted for publication as ‘Species climate range influences leaf water relations, hydraulic and stomatal traits in seven *Eucalyptus* species’ to *Functional Ecology*.

Chapter 4 will be submitted for publication as ‘Competition and complementarity influence species transpiration, growth and soil moisture in mixtures’ in the near future.

Abstract

Transpirational water use by trees has been long known to be regulated by evaporative demand and temperature, solar radiation, stomatal conductance and tree leaf area. More recently control of transpiration by plant hydraulic traits has been highlighted, and these as well as stomatal conductance and its response to air saturation vapour pressure deficit remain unstudied for the majority of Australian native tree species. To predict how forested ecosystem water use may change under future climates and enable better estimates of catchment water losses, we must understand stomatal and hydraulic behaviour of trees in the field under a range of conditions. In this study, I quantified traits describing stomatal and hydraulic behaviour for five *Eucalyptus* species from differing climates. Patterns in whole tree water use, stomatal sensitivity and responses to low water availability of these species in a common garden were correlated with species identity and with their characteristic climate of origin. I found that different *Eucalyptus* species employed different strategies to deal with water deficits which were linked to hydraulic, anatomical and leaf tissue water relations characteristics, and also with the original climatic range of the species. Tree water use, growth and tolerance of low water availability were enhanced in species mixtures compared to monocultures, an effect ascribed to asymmetric competition of component species in these mixtures. A basis for incorporating species stomatal and hydraulic parameters into forest stand-level water use models is provided. Ultimately, doing so will enhance predictions of water use, and enable estimates of stand water-use efficiency and productivity under current and future climate conditions. The findings are key to inform plantation and

land management decisions, and can assist in the identification of vulnerable species or ecosystems, and the conservation of catchment water supplies in a changing climate.

Chapter 1: Introduction

1.1 Overview

Transpiration by plants dominates the loss of water from vegetated land surfaces, with recent estimates indicating that tree water use accounts for 80-90% of all terrestrial evapotranspiration (Jasechko et al. 2013). Consequently, the water available to catchments and for other purposes is largely dependent on the transpiration of surrounding vegetation (Wilson et al. 2001). Forests account for approximately 31% of all vegetation, covering over 4 billion ha of land worldwide (FAO 2010), and are the dominant form of vegetation surrounding catchments where they have large influences on soil water and catchment water supplies (Whitehead and Beadle 2004; Eamus and Froend 2006). Significant risks to forests under drought and climate stress have been identified (Allen et al. 2010; Anderegg et al. 2013a; Anderegg et al. 2013b). Reliable estimates of forested ecosystem water use are therefore required to make informed land and catchment management decisions. However, many commonly used models of forested ecosystem transpiration (Williams et al. 1996; Zhang et al. 1996; Baron et al. 1998; Baldocchi et al. 2001; Samanta et al. 2007) are species-neutral, thus lacking accuracy and reliability for tree species with variations in physiological parameters, and for ecosystems with differing community compositions. Those models which do incorporate species specific parameters (e.g. SPA and MAESPA; Williams et al. 1996; Duursma and Medlyn 2012) require information about stand structure and water balance, and leaf physiology and plant hydraulic traits for component species, among other parameters specific for the study system. One forest growth model, 3-PG, is often used to predict the carbon balance of Australian ecosystems (Almeida et al. 2007; Almeida et al. 2010) and through using species-specific parameters has been shown to accurately predict the water balance of forests, including species mixtures (Forrester and Tang 2016). However, some studies have demonstrated limitations in 3-PG's ability to model soil and plant water balance accurately (Almeida and Sands 2016) and while economically important species like *Eucalyptus globulus* and *Eucalyptus grandis* have been parameterised for 3-PG (Sands and Landsberg 2002; Almeida et al. 2007; Rodríguez-Suárez et al. 2010; Almeida and Sands 2016), many species have not been measured for physiological traits and consequently the trait information required for input into such models is not always available.

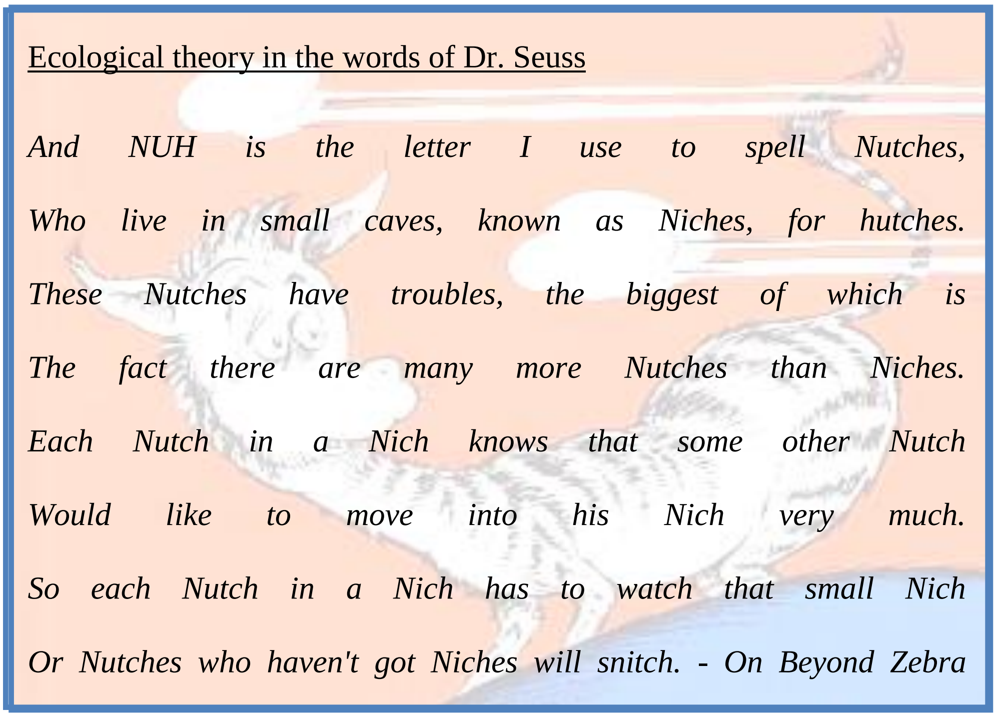
It is widely known that transpiration is regulated by evaporative demand, temperature, solar radiation and stomatal conductance (Jarvis and McNaughton 1986). Additionally, there are numerous reports that species identity influences water use (Pataki et al. 2000b; Monson et al. 2010; Zeppel et al. 2010), where species identity refers to the particular type/variety of species being studied. Additionally, the roles of species-specific hydraulic traits in constraining transpiration and species survival have recently been highlighted (Sperry et al. 1998; Hubbard et al. 2001; Comstock 2002; Meinzer et al. 2009). Transpiration and survival are likely to be influenced by trade-offs between hydraulic safety and efficiency (Jarvis and McNaughton 1986; Sperry et al. 2008). By examining interspecific differences in these trade-offs we can identify water-use efficient, drought-tolerant species which may be suitable for future plantations in environments with fluctuating water availability. Furthermore, these comparisons may enable correlations of physiological characteristics with climate descriptors for multiple species. These correlations may assist predictions of how a species will respond to different environmental conditions based on the climate characteristics of its provenance, and accordingly, may enable the use of these response parameters in models that utilise species-specific trait information (e.g. SPA and MAESPA; Williams et al. 1996; Duursma and Medlyn 2012).

The climate and tree species of Australia provide a useful system to make comparisons of plant physiological characteristics along aridity gradients. Coastal-to-inland rainfall gradients larger than 1000 mm per annum in less than 200 km across in south western and south eastern Australia (Givnish et al. 2014). Accordingly, studies have examined $\delta^{13}C$ and leaf attributes (Anderson et al. 1996; Schulze et al. 2006), stomatal sensitivity to *D* (Cunningham 2004), leaf tissue water relations (Merchant et al. 2007; Baltzer et al. 2008), photosynthetic parameters (Cernusak et al. 2011) and hydraulic traits (Gleason et al. 2013) along aridity gradients within Australia. While these studies provide significant contributions to understanding how plant physiological traits are influenced by species climate of origin, there are few studies that address these relationships by measuring plants within a common environment (Merchant et al. 2007; Baltzer et al. 2008) or that do so using trees in natural conditions (Anderson et al. 1996; Vander Willigen et al. 2000). Furthermore, there are no studies which constrain these measurements to species within a genus under common environmental conditions, which would allow for the consideration of genetic or species-level constraints on plant physiological traits.

Interspecific comparisons of plant physiological traits, such as stomatal sensitivity to *D*, hydraulic and anatomical characteristics, can enable the categorisation of species according to particular functional characteristics. By categorising species into different groups (e.g. Reich et al. 2001; McClenahan et al. 2004; Mitchell et al. 2008) according to particular functional characteristics, such as water-relations traits, crown architecture, tree heights and N-fixation, we can begin to determine how these species may interact in different species assemblages. Several studies have examined such interactions within different ecosystems (Callaway and Walker 1997; Maestre et al. 2009; del Rio et al. 2014; Grossiord et al. 2014b; Jucker et al. 2014; Wright et al. 2014), leading to reports that ecosystem processes vary with changes in ecosystem functional group richness (Johnson et al. 1996; Tilman et al. 1997; Fargione et al. 2007; Monson et al. 2010). However, studies examining interspecific interactions and their influence on soil moisture, and consequently component tree transpiration, growth, water-use efficiency (WUE) and response to water limitation, in mixed ecosystems are limited. Determining the effect of ecosystem species richness on water use and soil moisture is crucial to predicting how ecosystems will react to climate change, and is essential for evaluating catchment water availability under future climates.

In this thesis, I hypothesise that species identity affects individual tree water use due to interspecific variation in hydraulic characteristics and soil moisture access. Three questions are posed in relation to this hypothesis: (i) is the stomatal conductance sensitivity to climate related to their place of origin (Chapter 2), (ii) is interspecific variation in stomatal sensitivity to environment also explained by plant hydraulics, anatomical characteristics and leaf tissue water relations (Chapter 3) and (iii) is the water use and productivity of component species in stands influenced by competition within and between species in the ecosystem (Chapter 4)?

This thesis addresses critical gaps in current knowledge of how stomatal behaviour and hydraulic traits are related to species climatic range, and how multi-species ecosystems function in terms of water use and soil moisture depletion. I aim to understand how differences in plant function are related to species identity and how species geographic origin affects tree water use and hydraulic parameters. Additionally, I aim to understand how plot composition affects component tree water use and soil moisture acquisition through competition. Outcomes from this study can be used to ensure catchment water supplies are conserved in native vegetation situations and refine plantation management practices to reflect benefits/disadvantages of species mixtures in water-limited environments. However, this study compares a limited number of young trees and accordingly further information would be required to extrapolate these results to the broader context of conserving catchment water supplies in native vegetation situations. Additionally, the information from this study may be used to develop a broad framework of correlations between climate of origin and species stomatal, hydraulic and anatomical parameters, to enable prediction of physiological characteristics for a broad range of species based on climate of origin characteristics. Finally, this information can be used in current, species-specific models of forested ecosystem transpiration (Williams et al. 1996; Duursma and Medlyn 2012; e.g. 3-PG, SPA and MAESPA; Almeida and Sands 2016), enhancing accuracy and reliability of predictions from such models.



Ecological theory in the words of Dr. Seuss

And NUH is the letter I use to spell Nutches,
Who live in small caves, known as Niches, for hutches.
These Nutches have troubles, the biggest of which is
The fact there are many more Nutches than Niches.
Each Nutch in a Nich knows that some other Nutch
Would like to move into his Nich very much.
So each Nutch in a Nich has to watch that small Nich
Or Nutches who haven't got Niches will snitch. - On Beyond Zebra

1.2 Review of literature

1.2.1 The control of transpiration

The loss of water by vegetated ecosystems to the atmosphere is dominated by transpiration (E), the total flux of water moving through the stomata. Transpiration is an evaporative process whereby water is lost from the soil to the atmosphere via the plant's water conducting system (Jarvis and McNaughton 1986). This process allows for the intake of CO_2 for photosynthesis, leaf cooling, and passive flow of mineral nutrients and other dissolved substances in the xylem sap (Comstock and Sperry 2000). Transpiration is driven by vapour pressure deficit (D), a measure of evaporative demand, and controlled by changes in stomatal aperture which influence stomatal (g_s) and canopy (G_c) conductance (Jarvis and McNaughton 1986; O'Grady et al. 1999; Comstock and Sperry 2000; White et al. 2000). Stomatal closure occurs at high D, high soil water deficits, or in response to other signals such as changes in abscisic acid or light levels, in order to conserve the hydraulic pathway of the plant by minimising cavitation and subsequent hydraulic dysfunction (Jarvis and McNaughton 1986; Comstock 2002; Brodribb and Holbrook 2003; Brodribb et al. 2003; Martorell et al. 2014; McAdam and Brodribb 2014).

Plants must balance trade-offs between safety, the ability to maintain xylem tension within limits that do not cause extensive xylem cavitation, and efficiency, the ability to transport water at a rate that sustains CO_2 assimilation and cooling of the leaf, to survive in water limited environments (Nardini and Salleo 2000; Cochard et al. 2002; Johnson et al. 2011). Differences in hydraulic safety and efficiency form the basis for differences in transpiration and survival between species and subsequently influence how species will adapt under different climatic conditions.

The selective pressures that prevail within a species climate control how that species adapts and subsequently what characteristics it evolves and maintains in its natural environment (Givnish 1987; Ramirez-Valiente et al. 2010). Accordingly, previous studies have observed correlations between climate of species origin and plant physiological characteristics (White et al. 2003; Mitchell et al. 2008). For example, water availability of the species origin was strongly, positively correlated with vulnerability to cavitation (P_{50}) in a data synthesis including 226 species (Choat et al. 2012) and with leaf water potential at turgor loss point (Ψ_{TLP}) in a data synthesis involving 317 species (Bartlett et al. 2012).

While these and other studies provide valuable insights into linkages of climate with plant physiological characteristics, they compare species across vastly different environmental conditions, or across species from different genera, which does not allow consideration of the genetic or species-level constraints on species responses and their relation to climate. To determine how hydraulic and stomatal characteristics are related to climate and constrained genetically, species from a single genus with different climatic origins should be compared under the same environmental conditions (i.e. in a common garden). Understanding the genetic and species-level constraints on hydraulic and stomatal parameters is imperative to determining how species will adapt to future climatic conditions and subsequently how water use, productivity and survival of ecosystems will change in the future.

1.2.1.1 Species-specific influences on transpiration

Inter- and intra-specific variations in water use and stomatal sensitivity to D have been observed for many tree species in different settings and climates (Oren et al. 1999; White et al. 2000; Pataki and Oren 2003; Cunningham 2004; O'Grady et al. 2006; Mitchell et al. 2008; Monson et al. 2010; Héroult et al. 2013; Ocheltree et al.

2014). Studies comparing a single species across different ecotypes or provenances have shown that individuals from populations in areas of lower precipitation tend to have a more conservative water use strategy (Voltas et al. 2008), lower g_s (Woodward 1986; Gornall and Guy 2007) and lower transpiration (Li et al. 2000) compared to individuals from regions of higher precipitation. However, the relationship between stomatal response and climate could not be separated from the stomatal response to the prevailing environmental conditions in these studies because species were compared across multiple study sites with differing environmental conditions, and stomatal response was influenced by the climate in which the plants were studied (Jarvis and McNaughton 1986; O'Grady et al. 1999; Sperry 2000; Medlyn et al. 2011; Héroult et al. 2013).

Common garden plantings, in which different species are planted under the same environmental conditions, allow the separation of interspecific variation in stomatal responses to environmental conditions from interspecific variation in stomatal responses due to genetic constraints. Studies utilising common gardens have shown that species from regions with a lower precipitation tend to have a lower marginal water cost of carbon gain and higher tolerance of low water availabilities (Héroult et al. 2013) and, contrary to expectation, a lower WUE (Anderson et al. 1996; Sefton et al. 2002; Warren et al. 2006) compared to species originating in regions of higher precipitation (Table A-1). Despite its importance in predicting individual species and whole ecosystem water use, the influence of climate of origin on stomatal conductance and stomatal sensitivity to D is yet to be considered.

1.2.1.2 Limiting hydraulic dysfunction – safety vs. efficiency

Stomatal behaviour

Stomata act as pressure regulators, limiting pressure potential by controlling flow rate and thus reducing variation in leaf and consequently plant water potential (Ψ) with limiting soil moisture and D conditions (Sperry et al. 1998; Ewers et al. 2001b; Sperry et al. 2002). By closing stomata, plants restrict water loss and thus limit increases in xylem tension (O'Grady et al. 1999; White et al. 2000; Ewers et al. 2001b; Sperry et al. 2002), but by doing so they compromise photosynthetic activity and growth potential (Chaves et al. 2002) as well as driving increases in leaf temperature (Hamerlynck and Knapp 1994; Nicotra et al. 2008). To minimise increases in xylem tension, stomata must be capable of sensing changes in Ψ (Sperry et al. 2002), which can be explained by chemical and/or hydraulic (i.e. cavitation threshold) signalling to stomata (Jarvis and McNaughton 1986; Tardieu and Davies 1993; Jones 1998; Hubbard et al. 2001; Nardini et al. 2001; Cochard et al. 2002; Comstock 2002).

Several studies have examined the possible link between g_s and xylem cavitation (Jones and Sutherland 1991; Nardini and Salleo 2000; Salleo et al. 2000; Salleo et al. 2001; Cochard et al. 2002; Meinzer et al. 2009; Johnson et al. 2011) leading to the hypothesis that cavitation-induced decreases in hydraulic conductivity (K_h) of stems (Sperry et al. 1993), roots (Domec et al. 2006) and leaves (Salleo et al. 2000) may lead to stomatal closure. This response would prevent further increases in xylem tension which could result in increased cavitation and air-seeding (Tyree and Sperry 1988). While previous studies have reported a relationship between stem hydraulic traits, leaf tissue water relations and g_s in a broad range of angiosperm and conifer species (Brodribb and Holbrook 2003; Brodribb et al. 2003; Meinzer et al.

2009; Martorell et al. 2014), the effects of xylem cavitation on guard cell turgor and thus g_s are not well understood. Buckley (2005) reviewed the available research and reported that the idea that guard cells are mediated by their local water status explained most aspects of stomatal responses to changing leaf water balance. However, despite the importance of the relationship between xylem cavitation and stomatal behaviour for understanding how plants have adapted to conditions where water availability is highly variable (Brodribb and Holbrook 2004), there is uncertainty regarding how plants sense cavitation and subsequently signal to stomata.

It is likely that stomatal closure is associated with the maintenance of xylem integrity (Tyree and Sperry 1988), and thus that gas exchange and stomatal regulation have hydraulic constraints (Salleo et al. 2001). However, it is not yet known whether stomata prevent cavitation from occurring by closing prior to the onset of cavitation (Ψ_{CAV}), or whether they sense cavitation, thus closing to prevent further hydraulic dysfunction. To determine the critical point of stomatal closure, comparisons of g_s to increasing loss of conductivity (PLC and P_{50}) and Ψ are required (Salleo et al. 2000). Additionally, comparisons of stomatal sensitivity between species and its relation to the start of stomatal closure will assist in understanding how different species regulate g_s.

Hydraulic adaptations

Eco-physiological theory and several studies have established strong correspondence between species maximum g_s and stem water transport capacity (K_s) (Comstock and Sperry 2000; Sperry 2000; Hubbard et al. 2001; Taylor and Eamus 2008; Pangle et al. 2015). These two traits must be in balance for plants to regulate leaf water potential (Ψ_{leaf}) and thus maintain water transport while minimising the risk of hydraulic dysfunction. Species maximum g_s and K_s are also related to physiological traits including leaf desiccation tolerance (Brodribb and Holbrook 2003; Sack et al. 2003), wood density (Wright et al. 2006b; Lens et al. 2011), rooting depth (Jackson et al. 1999; Eberbach and Burrows 2006) and xylem structure (Hacke et al. 2001). These references and others support the relationship between stomatal behaviour and plant hydraulics in a body of research that has largely emerged since the key works of Tyree and Sperry (1988) and Zimmermann (1983).

During transpiration, xylem water comes under physical tension (Brodribb et al. 2003) which can result in air-seeding, the movement of air bubbles through pit membranes into the water column resulting in cavitation (Edwards and Jarvis 1982; Zimmermann 1983) of stems (Tyree and Sperry 1989; Meinzer et al. 2009) and roots (Domec et al. 2006). Cavitation results in decreased K_{h} and supply of water to downstream parts of the plant (Sperry and Ikeda 1997; Sparks and Black 1999) resulting in reduced transpiration (Ewers et al. 2001b), photosynthesis, and even plant mortality via carbon starvation, desiccation, or both (McDowell et al. 2008). While high cavitation resistance (hydraulic safety) is promoted by narrow, short, frequent xylem vessels or vessel elements (Lens et al. 2003; Lens et al. 2004) and thick, less porous pit membranes (Choat et al. 2008), these anatomical features result in decreases in K_{h} and consequently hydraulic efficiency, illustrating the trade-offs that species must negotiate (Table 1; Sperry et al. 2008). While some comparative studies of hydraulic architecture have been conducted (Hubbard et al. 1999; Prior and Eamus 2000; Brooks et al. 2002; McClenahan et al. 2004; Mitchell et al. 2008), we still lack a full understanding of the interaction between structural traits influencing hydraulic safety and efficiency and the subsequent influence on tree and ecosystem water use (Tyree and Ewers 1991b; Lens et al. 2011). It is important to determine how traits influencing hydraulic safety and efficiency vary interspecifically, and how they are linked with the species native climate, in order to predict how species survival, productivity and water use may change under future climates.

Tight correlations between species climate variables and leaf tissue water relations (White et al. 2000; White et al. 2003; Bartlett et al. 2012), vulnerability to embolism (Mitchell et al. 2008; Beikircher and Mayr 2009; Choat et al. 2012) and vessel anatomy (Villar-Salvador et al. 1997; Fisher et al. 2007; Medeiros and Pockman 2014) have been observed, however many of these studies compare species across different environments or along particular environmental gradients and consequently do not permit extrapolation of the climatic effects on hydraulic safety and efficiency parameters. Interspecific variation in physiological characteristics occur due to differences in the selective pressures within the species climate (Givnish 1987; Ramirez-Valiente et al. 2010), and thus the trade-offs between hydraulic safety and efficiency that a species must make are likely to reflect the species climate of origin. Despite their importance in predicting plant survival and distribution there are few studies comparing hydraulic, anatomical, stomatal or leaf tissue water relations across species from differing climates under the same environmental conditions (i.e. in a common garden).

One study which compared plant physiological traits with climate of origin reported positive correlations of Ψ_{TLP}, osmotic potential and symplastic water fraction, and a negative correlation of bulk modulus of elasticity, with water availability of origin for 24 species (Baltzer et al. 2008). However, this study was conducted on tree seedlings planted in pots in a shade house, and only considered tropical tree species, thus limiting its applicability to juvenile and mature trees growing in natural environments and for tree species from more arid regions respectively. Another study reported that xylem conduit dimensions, maximum K_s, pit pore sizes and vulnerability to cavitation were genetically determined in five evergreen, subtropical species including four angiosperms and one gymnosperm (Vander Willigen et al. 2000) but the linkage of these characteristics to the climate of species origin was not specifically examined. These studies provide insights into the mechanisms governing plant physiological responses to climate and environmental change, but they are not necessarily applicable to species from more arid regions, and are consequently unlikely to reflect the same species-specific responses as those observed in species from more arid regions. In addition to aiding predictions of transpiration and survival, observing interspecific variation in plant physiological parameters while keeping environmental conditions constant can enable classification of species into different hydraulic functional types (Reich et al. 2001; McClenahan et al. 2004; Mitchell et al. 2008). The classification of species into different hydraulic functional types can assist in segregating species regarding water use strategies and characteristics, allowing the development of experiments for examining how ecosystem composition affects physiological characteristics.

Table 1-1: Xylem anatomical characteristics resulting in enhanced hydraulic safety and efficiency, and the rationale for each trait.

Efficiency	**Safety**	**Rationale**
Thin, porous pit membranes	Thick, less porous pit membranes	Greater porosity and thinner pit membranes result in higher vulnerability and emboli spread (Choat et al. 2008; Lens et al. 2011).
Less frequent vessels	More frequent vessels	Higher conduit frequency generally correlates with narrower conduits (less vulnerable, see below) and enables a higher number of 'redundant' conduits (Sperry et al. 2008; Zanne et al. 2010).
Long vessels	Short vessels	Longer conduits result in a larger number of pit connections to other conduits enhancing the chances of air-seeding and embolism spread (Loepfe et al. 2007). Additionally, longer conduits tend to have wider diameters (Sperry et al. 2006; Pan et al. 2015) and wider conduits are more vulnerable to cavitation (below).
Wide vessels	Narrow vessels	Larger, more conductive conduits tend to be more vulnerable to cavitation both intra- (Tyree and Sperry 1989; Domec and Gartner 2001; Domec et al. 2008) and inter-specifically (Kavanagh et al. 1999; Martinez-Vilalta et al. 2002; Choat et al. 2005). This may be due to the 'rare-pit' hypothesis, that larger conduits have larger pit areas so a higher probability of having a pit large enough for seeding emboli (Wheeler et al. 2005; Christman et al. 2009).

1.2.2 Functional groups and competition

1.2.2.1 Competition

Species generally exhibit different combinations of characteristics, with some of these traits helping individuals to cope with water deficits depending on the biophysical constraints within their environment (McClenahan et al. 2004; Mitchell et al. 2008; Meinzer et al. 2009). Different species that share a set of functionally-related traits are considered to be in the same plant functional type (Wright et al. 2006b) or hydraulic functional type (Mitchell et al. 2008) and are accordingly expected to occupy a similar niche, fulfilling the same roles and utilising the same resources within the ecosystem (Tilman et al. 1997; Fargione et al. 2003). Previous classifications have elucidated the water use strategies employed by species within the study ecosystem, enabled the characterisation of water use in species-rich vegetation assemblages, and provided clues towards understanding water use partitioning in different soil types. By categorising species, we can begin to determine how these species may interact and compete in different species assemblages. Species with similarities in particular characteristics, such as P_{50} and wood density, are likely to have similarities in vulnerability to cavitation. Furthermore, species can vary widely in their g_s and K_s responses to environmental conditions, whereby two species may have similarities in their mean rates of these traits, but the overall response could differ greatly, giving us an indication of differences in transpiration and water use strategies employed by different species. In combination, these physiological traits can allow a broad categorisation of species responses based on shared functional traits, whereby species with similar characteristics are likely to experience greater competition when planted together than would species occupying different ecosystem niches.

The occurrence of multiple functional types within a community can be interpreted as a form of niche partitioning, since co-existence of multiple species with different resource utilisation strategies suggests that multiple versus few functional types can ‘fill in’ or maximise resource use within an ecosystem (Hatton et al. 1997). Since each set of species uses resources differently, greater species and functional group richness will result in more complete use of available resources and decreased competition (Kelty 1992; Vandermeer 1992; Reich et al. 2001). There is long-standing interest among ecologists in the significance of mixed species ecosystems in which co-existence through niche partitioning is promoted (MacArthur 1955; Hutchinson 1957). Such niche partitioning can occur in the form of soil moisture partitioning, whereby species occupying the same niche will compete for the same soil water resources. Interactions like this may result in one species out-competing and suppressing the other, both species experiencing reductions in resource availability, or one or both species searching for resources in a different niche, where resources are more available. These interactions are somewhat dependent on component species dominance and competitive ability, and will differ dependent on community composition and species identity. While these interactions are a key component to understanding ecosystem dynamics and interspecific interactions in species mixtures, studies examining resource partitioning and competition in species mixtures are limited, particularly when considering soil moisture partitioning in mixed species, forested ecosystems.

1.2.2.2 Ecosystem processes influenced by competition

Niche theory predicts that increases in ecosystem functional group richness will result in increases in the rates of various ecosystem processes and functions (Tilman et al. 1997; Loreau et al. 2001; Hooper et al. 2005). Accordingly, studies conducted in grass, herb and forb ecosystems have shown that enhanced functional group richness results in increases in ecosystem productivity (Johnson et al. 1996; Tilman et al. 1996; Tilman et al. 2001; Callaway et al. 2003; Fargione et al. 2007), reproductive capacity (Callaway et al. 2002) and stability (Tilman et al. 2006) among other ecosystem processes and functions. Though in practice there have been many forest mixture studies conducted, few of these studies have been published due to a lack of structure and composition by many more than 2-species. Accordingly, the majority of studies examining species mixtures focus on grassland ecosystems (e.g. Tilman et al. 1996; Reich et al. 2001; Tilman et al. 2006) limiting the conclusions that can be drawn for forested ecosystems despite forests accounting for approximately 30% of all vegetation globally (FAO 2010). Determining the effects of mixed plantings on component species and ecosystem function in forests are important as forested ecosystems have large effects on soil water availability and catchment water supplies (Whitehead and Beadle 2004; Eamus and Froend 2006) and the majority of native Australian forested ecosystems are composed of multiple species.

Those studies focussing on forested ecosystems have reported increases in the rates of various ecosystem functions in mixtures, such as productivity and transpiration, but such studies are limited in number and often have limiting study design factors. Forrester et al. (2010) reported an increase in productivity and WUE in 2-species mixtures containing *Eucalyptus globulus* and a N-fixing species, *Acacia mearnsii*. However, as N-fertilisation is likely to affect ecosystem function separately and potentially differently to increases in species richness, it is unclear if increases in productivity and WUE resulted from changes in N or changes in water in this study. Studies without N-fixers provide mixed reports, including no change in growth, water use or WUE (Grossiord et al. 2013), enhanced complementarity of soil water uptake (Schume et al. 2004; Meissner et al. 2012; Grossiord et al. 2014a; Schwendenmann et al. 2015), increases in soil moisture seepage (Sprenger et al. 2013), declines in soil moisture availability and increased WUE during dry years (Grossiord et al. 2014b; Grossiord et al. 2014c) in mixtures compared to monoculture plantations. However, few of these studies (Schume et al. 2004; Meissner et al. 2012; Sprenger et al. 2013; Schwendenmann et al. 2015) addressed the influence of species richness and competition on soil moisture partitioning. This is despite its importance for understanding resource partitioning and predicting water use, survival and growth of species mixtures under changing environmental conditions. Despite these varied reports of influences of competition on forest ecosystem function, a recent review (Forrester 2015) found that there was still a common response across all of the studies, such that whenever the species interactions resulted in increased growth in mixtures, the transpiration and often WUE of that species also increased in the mixture. However, given the lack of studies addressing the influences on soil moisture partitioning in species mixtures and the importance of understanding these relationships in order to predict water use, survival and growth of forest mixtures, it is important that further investigation into these aspects of ecosystem physiology be conducted in future.

1.2.3 Importance of the study

The *Eucalyptus* genus inhabits a wide array of climates within Australia and globally (DAFF 2011), thus providing a good system for examining species influences on water use and hydraulic parameters with regard to climate characteristics of the place of origin. *Eucalyptus*-dominated ecosystems transpire more than 80% of incoming precipitation in semi-arid zones of Western Australia (Wildy et al. 2004; Mitchell et al. 2009) and hence are key to their overall water balance and water yields. Additionally, *Eucalyptus* is an important genus economically, industrially and ecologically, covering more than 149 million hectares of land in Australia (DAFF 2011) and over 20 million hectares worldwide (Ball 1993; Iglesias-Trabado and Wilstermann 2009). Consequently, *Eucalyptus* species affect soil water availability and water yields in many regions of the world and they merit more detailed study of traits leading to their role in ecosystem water balance and promoting their growth in semi-arid climate conditions. Given the high diversity within the genus, further study of these traits is likely to lead to discoveries regarding how such traits enhance survivorship during extreme conditions and severe drought in particular.

In this study, multiple *Eucalyptus* species from contrasting climates of origin were planted in common gardens to enable extrapolation of the relationship between hydraulic and stomatal parameters and characteristics descriptive of the species climatic origin or range. Additionally, this study examines the influence of mixture ecosystems on component *Eucalyptus* species water use and productivity, and plot soil moisture acquisition. This study will examine the relationships between hydraulic and stomatal parameters and their relation to climatic origin or range and will provide insights into the implications when multiple species of *Eucalyptus* are maintained in plantation and natural ecosystems. This information is important for determining differences in water use strategies between differing species, predicting species responses to changing environmental conditions, providing information for input into forested ecosystem transpiration models (e.g. SPA, WAVES and MAESPA; Williams et al. 1996; Zhang et al. 1996; Duursma and Medlyn 2012 respectively) and enabling informed plantation and catchment management decisions globally.

Findings regarding differences in tree functional groups and their stomatal behaviour and hydraulic characteristics can help inform land management practices. There is currently debate in Australia and overseas regarding forest management and the conversion of native stands to plantation monocultures. This type of management removes the functional group diversity present at a site and consequently limits the capture and exploitation of soil moisture and other resources within these ecosystems to the manner in which it is manifest in the dominant plantation species. Removal of species and/or functional groups thus very greatly narrows the available strategies on-site for coping with adverse climate conditions including drought. Since changes in ecosystem functional composition are likely to result in changes in ecosystem processes (Johnson et al. 1996; Tilman et al. 1997; Loreau et al. 2001), current and future plantation management and bush regeneration practices may need to be adapted to reflect the need to include functional groups that were formerly present on managed forest and woodland areas. Overall, this study will result in a greater understanding of the effects of species identity on component species and ecosystem water use. This is important in predicting species responses to environmental conditions and subsequent changes in water availability, as well as critiquing current plantation management and bush regeneration practices, to improve plantation efficiency, productivity and stability, and to ensure ecosystem processes are maintained.

There are forecasts of changes in rainfall frequency and intensity in future decades (BOM 2010) and it is therefore important that we can predict how water use, productivity, soil moisture acquisition and survival of individual species and ecosystems will be influenced. Determining the relationship between species climate of origin and stomatal and hydraulic characteristics holds the capacity to enable these predictions to be made across a broad range of species under different climatic contexts. In order to achieve this, a strong, consistent and universal relationship of species climate of origin or range with stomatal and hydraulic characteristics would need to be observed across a variety of species occurring in a broad range of climates. Once achieved, species-specific stomatal and hydraulic parameters could be extrapolated from the species climate of origin characteristics, enabling all species with climate data for the species provenance to be characterised. This would minimise the cost and resources required for determining physiological characteristics for different species which may be required for input into species-specific models such as SPA and MAESPA (Williams et al. 1996; Duursma and Medlyn 2012 respectively). Ultimately, this would enable a greater use of species-specific parameters in hydrological and physiological models of plant and ecosystem transpiration, consequently enabling predictions of growth, water use and survival for a variety of species from a broad range of climates.

Australia contains climates ranging from arid to humid in which the distribution of species is frequently limited by water availability. This diverse range of climates is inhabited by a range of different species, where individuals experience large differences in water availability and evaporative demand dependent on the climate of their natural distribution. Thus the Australian continent and species provide ideal systems for examining the influence of climate of origin on plant physiological characteristics, particularly along an aridity gradient. Results from this study will yield understanding of how different *Eucalyptus* species, and ecosystems composed of *Eucalyptus* species, respond to climate variability across multiple years. Understanding how stomatal conductance to water vapour is regulated will allow us to predict how native and plantation ecosystems respond to such changes, and accordingly how this will affect water availability with anticipated changes to rainfall patterns.

1.3 Research objectives

There are significant gaps in the literature regarding the influence of climate of species origin and range on stomatal and hydraulic characteristics and about the response of species mixtures on component tree water use and soil moisture. The following questions and hypotheses were formulated to examine these gaps and to determine whether species identity and ecosystem composition have effects on component species and ecosystem water use. In Chapter 2, I ask:

1) Does species identity influence G_c and stomatal sensitivity to D? I hypothesise that: (a) *Eucalyptus* species originating from wetter regions will have a higher water use and lower D at stomatal closure compared to those originating from drier regions, and (b) stomatal sensitivity to D will be higher in *Eucalyptus* species originating from wetter compared to drier regions. This study will lead to an understanding of how species identity influences water use and transpiration responses to environmental conditions, which is valuable in terms of catchment and plantation management, the conservation of our water supplies and obtaining the trait data required for input into models of tree water use.

Given that climatic constraints on stomatal behaviour are likely related to hydraulic characteristics and leaf tissue water relations, I expect that any interspecific differences observed in stomatal sensitivity to D are related to these traits. Thus in Chapter 3, I ask:

2) Are species differences in stomatal sensitivity and water use linked to hydraulic characteristics and thus are hydraulic characteristics linked to species climatic range? I hypothesise that: (a) xylem and leaf tissue water

relations traits will reflect the species climatic range, and (b) species from humid regions will have a tighter coordination between stomatal and hydraulic characteristics. This will further our understanding of hydraulic safety and efficiency trade-offs and the relationship between hydraulic characteristics, leaf tissue water relations and climate parameters. These observations will enable identification of water efficient, drought tolerant species for future plantings and will assist in obtaining the hydraulic parameters required for input into models of tree transpiration. Furthermore, the work presented in this chapter will enable the characterisation of species into different hydraulic functional types, which will assist in understanding how different species might behave under more variable ecosystem conditions.

Using observations from Chapters 2 and 3, different species functional types with regard to stomatal behaviour, hydraulic and leaf tissue water relation characteristics can be assessed. Consequently, in Chapter 4, I ask:

3) Does ecosystem composition affect component tree water use and soil moisture depletion for focal species? I hypothesise that (a) the inclusion of additional species in mixture will enable higher soil moisture exploitation than in monocultures, thus reducing competitive water stress in component species relative to monocultures, and (b) species complementarity will mean that species in mixtures will be less affected by periods of low soil moisture than species in monocultures. However, in regions where evapotranspiration greatly exceeds precipitation, or during periods of severe water limitation, competition for water may result in component species in mixtures being more influenced by low soil moisture than those in monocultures.

Additionally, poorer competitors may be more resource limited when planted in mixture with superior competitors, thus resulting in transpiration and growth suppression due to water limitation.

Overall, this thesis aims to examine tree species with differing water use strategies individually and in competition, enabling reconsideration of early plantation design and management practices towards the inclusion of species suited to certain environmental conditions, or the inclusion of multiple species in mixed plantations. By revealing the effects of species identity and increased functional group richness on tree and ecosystem water use we may be able to design plantations that use water more efficiently, are more drought tolerant and also more stable with variable water supplies. The intent would be to inform management strategies capable of achieving these with the same if not higher productivity for a given amount of water used. This may enable the conservation of water for industry, agriculture, native and plantation ecosystems and our own catchment water supplies. Furthermore, the information obtained from the research reported in this thesis will enable more accurate predictions of species-specific responses, and component species responses in mixtures, to differing environmental conditions, and will also provide additional data for use in multi-species tree models, such as MAESPA (Duursma and Medlyn 2012), that may enhance our capacity to predict water use under future climate scenarios.

This research was conducted on *Eucalyptus* species grown together in two separate plantations at the Hawkesbury Forest Experiment (HFE; Figure 1-1) site in Richmond, NSW (Barton et al. 2010). The Chapters of this thesis are linked hierarchically from stomata to stem to whole plant physiology (Figure 1-2). Research reported in Chapters 2 and 3 used a common garden of seven *Eucalyptus* species from contrasting climates (Figure 1-3 and Table 1-2). For these two chapters a common garden approach involving species from different distributional and geographical ranges (Figure 1-3 and Table 1-2) was used to ensure that environmental conditions were kept consistent across species. This approach can help in understanding how species physiological traits vary under the same environmental conditions, enabling evaluation of responses across species originating from different climates or with different climatic ranges and thus the extrapolation of genetic constraints on species responses to the environment. The *Eucalyptus* species in these chapters represent those from wetter to drier climatic origins, with narrow to broad geographical ranges (Figure 1-3) and were selected based on their differences in climatic characteristics and physiological traits.

Research reported in Chapter 4 involved the use of a mixture plantation containing two replicates each of two monocultures, two 2-species mixtures and one 8-species mixture plantation, involving two of the *Eucalyptus* species previously compared in work reported in Chapters 2 and 3 in addition to another six species. These two focal species, *E. crebra* and *E. tereticornis*, were selected based on differences in distributional range and climate characteristics (Figure 1-3), previously measured stomatal (Chapter 2), hydraulic and leaf tissue water relations parameters (Chapter 3), and their close proximity to one another in natural ecosystems near the study site. The remaining six species in the 8-species mixture plantations were selected based on previously reported differences in growth, distribution and leaf morphology, representing differences in functional type and potential differences in water use, and their presence in Cumberland Plain woodland, the dominant vegetation type near the study site.

The discussion presented in Chapter 5 examines the interrelation of these three separate experimental chapters and draws broad conclusions from the study. The results of these studies can assist in i) developing a broad framework of species responses using correlations of species physiology with climate of origin, ii) informing current models of forested ecosystem water use which incorporate species-specific physiological parameters, iii) enabling more reliable predictions of productivity, distribution and survival under changing climates and iv) providing a resource for the alteration of catchment and plantation management practices in order to enhance survival and productivity of plantations while maintaining catchment water resources.

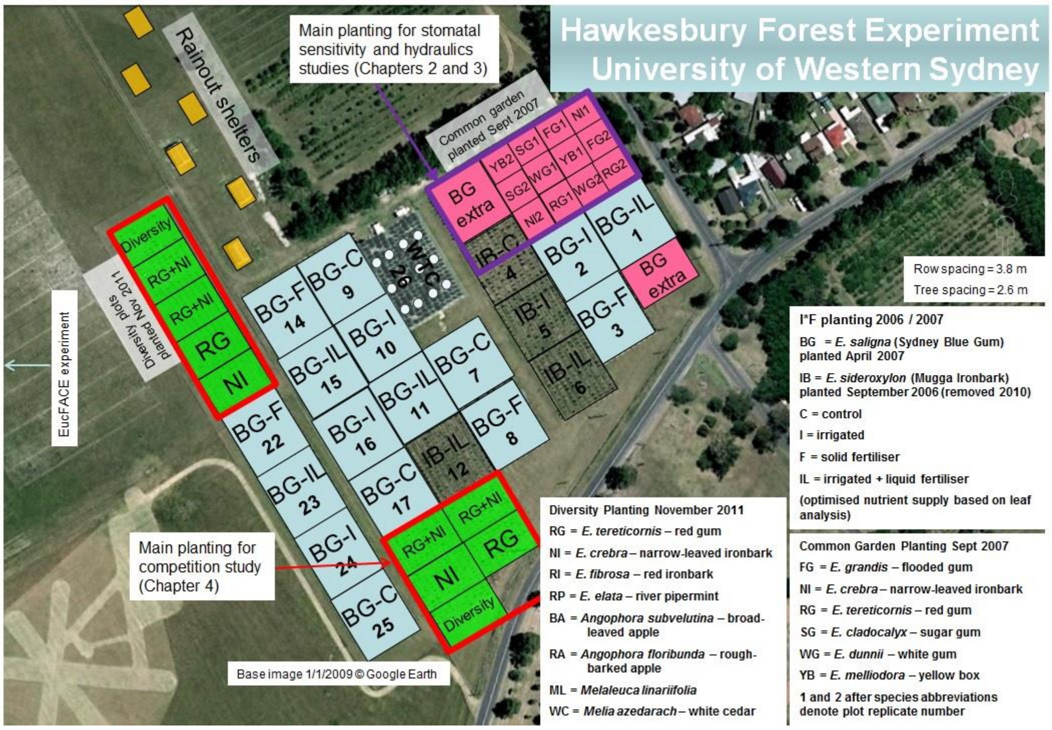


Figure 1-1: The HFE site in Richmond, NSW, outlining the position of the common garden (top right), diversity plantation (bottom left), HFE (centre) and *Eucalyptus* free air CO_2 enrichment (EucFACE) site with respect to the HFE site (left), and the plot arrangement for each of the plantations used in this thesis.

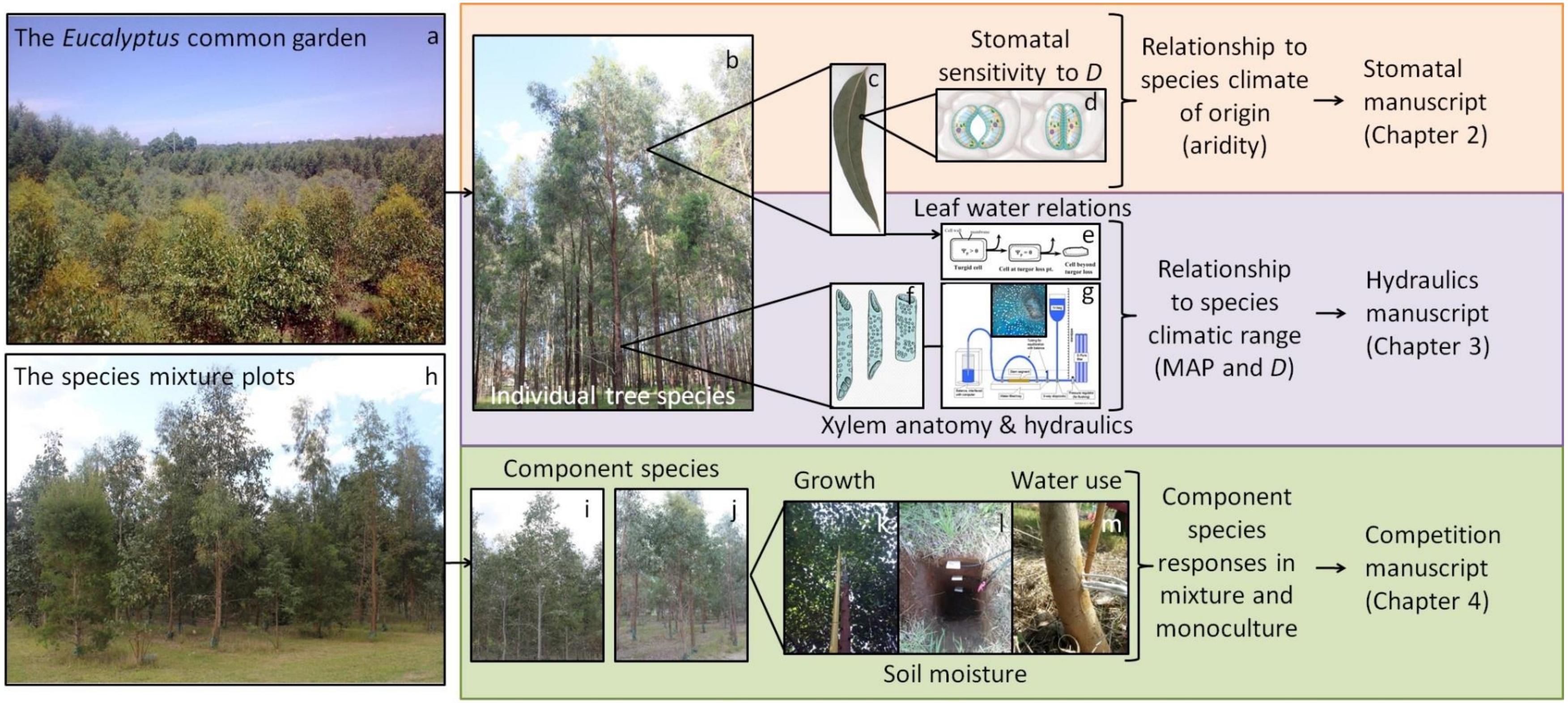


Figure 1-2: Mind map relating the separate Chapters of this thesis, designated by different coloured boxes, on a hierarchical level. Image sources are labelled with a letter in the top right corner of images as follows: a – David Ellsworth, photograph from February 2010; d – Campbell et al. (2009) Biology, 8th Edition; e – Ellsworth lab notes; f – Purves et al. (1995), Life: The Science of Biology, 4th Edition; g - Sperry Lab Methods, http://biologylabs.utah.edu/sperry/methods.html. All other images (b-c, h-m) were photographed by Aimee Bourne throughout her PhD candidature.

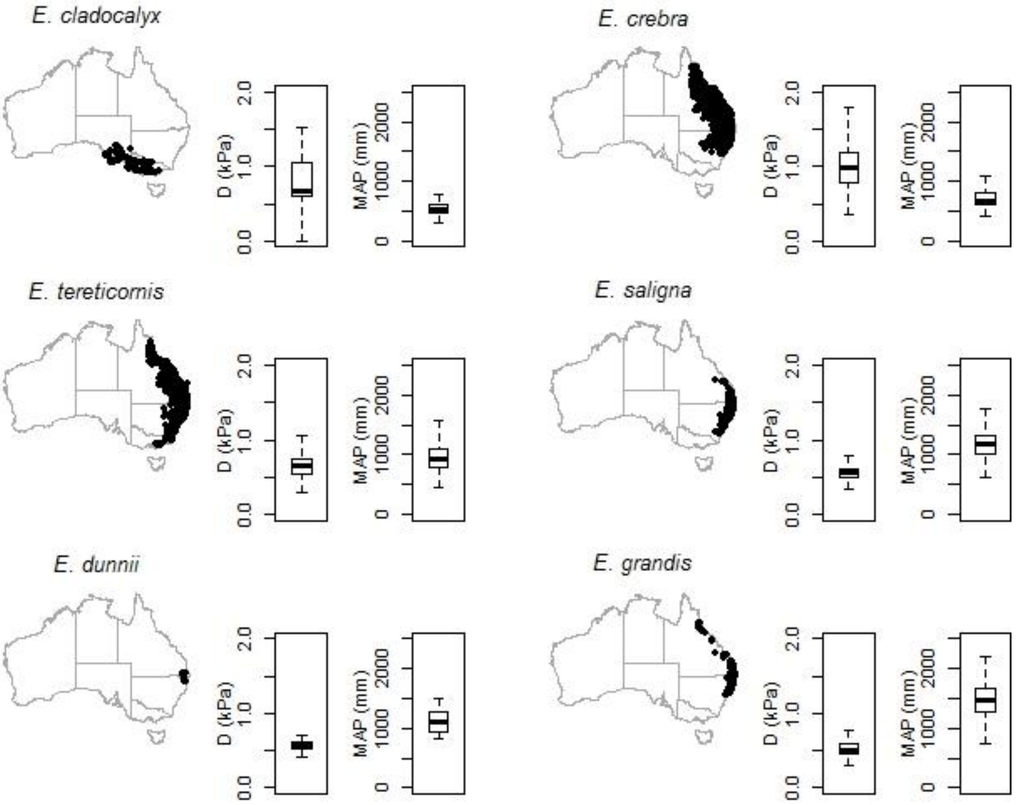


Figure 1-3: Distribution of 6 *Eucalyptus* species and climatic range for two climate variables related to water relations. Species distribution data was extracted from Atlas of Living Australia (ALA; http://www.ala.org.au; accessed 5th February 2015). In maps, black points represent the species distribution according to ALA. Boxplots indicate the maximum mean monthly *D* (left) and MAP (right) from the species range. The natural distribution for *E. cladocalyx* is actually more restricted than depicted here, but there is currently no reliable way to obtain this information.

Table 1-2: The native geographical extent, climate range, seed source, class, rainfall and volume index change for the seven *Eucalyptus* species studied. Some information here as presented by Héroult *et al*. (2013) and used for comparing and classifying species into different climates of origin. Mean annual rainfall and annual rainfall in species range were obtained from the Atlas of Living Australia (http://www.ala.org.au; accessed 22 July 2014) for all trees in the species range using the annual precipitation parameter. The volume index (DBH^2 x height) change per year is calculated for each species from 2010-2012 using measurements of 50 trees per species where the error shown is the standard error between two plots of each species. No standard error is given for *E. saligna* as measurements were obtained from a single, larger plot. Significant differences were determined at the 99% confidence level ($p < 0.01$; Tukey HSD) and are indicated by different letters.

Species	Class	Seed source	Latitudinal range	Mean annual rainfall (mm)	Annual rainfall in species range (mm)	Volume index change ($m^3 \cdot ha^{-1} \cdot yr^{-1}$)
E. cladocalyx	Sub-humid	Bundaleer, SH; 33.33ºS, 138.55ºE	32-35ºS	537.50	238-1072	17.2 ± 2.9^{ab}
E. crebra	Sub-humid	South Windsor, NSW; 33.61°S, 150.81°E	12.5-34°S	752.1	392-2419	29.7 ± 4.8^{ab}
E. dunnii	Humid	Sheepstation Creek, NSW; 28.41°S, 153.02°E	28-30°S	1112.8	778-1614	91.8 ± 15.5^{a}
E. grandis	Humid	South Africa, genetically improved; 30.55 ºS, 22.93 ºE	17-33ºS	1476.4	745-2311	83.0 ± 10.9^{a}
E. melliodora	Sub-humid	Wagga Wagga, NSW; 35.11°S, 147.37°E	24-38°S	692.6	317-1968	3.9 ± 0.1^{c}
E. saligna	Humid	Styx River State Forest, NSW; 30.37°S, 152.10°E	21-36°S	1194.7	609-2384	62.2^{ab}
E. tereticornis	Intermediate	Narellan, NSW; 34.04°S, 150.73°E	15-38°S	976.8	451-2638	38.2 ± 7.4^{b}

Chapter 2: Stomatal sensitivity to vapour pressure deficit relates to climate of origin in *Eucalyptus* species

2.1 Abstract

Selecting plantation species to balance water use and production requires accurate models for predicting how species will tolerate and respond to environmental conditions. Although interspecific variation in water use occurs, species specific parameters are rarely incorporated into physiologically based models because often the appropriate species parameters are lacking. To determine the physiological control over water use in *Eucalyptus*, five stands of *Eucalyptus* species growing in a common garden were measured for sap flux rates and their stomatal response to vapour pressure deficit (D) was assessed. Maximal canopy conductance and whole canopy stomatal sensitivity to D and reduced water availability were lower in species originating from more arid climates of origin than those from humid climates. Species from humid climates showed a larger decline in maximal sap flux density (J_{Smax}) with reduced water availability, and a lower D at which stomatal closure occurred than species from more arid climates, implying larger sensitivity to water availability and D in these species. I observed significant ($p < 0.05$) correlations of species climate of origin with mean vessel diameter ($R^2 = 0.90$), stomatal sensitivity to D ($R^2 = 0.83$) and the size of the decline in J_{Smax} to restricted water availability ($R^2 = 0.94$). Thus aridity of climate of origin appears to have a selective role in constraining water use response among the five *Eucalyptus*

plantation species. These relationships emphasise that within this congeneric group of species, climate aridity constrains water use. These relationships have implications for species choices for tree plantation success against drought induced losses and the ability to manage *Eucalyptus* plantations against projected changes in water availability and evaporation in the future.

2.2 Introduction

Forest plantation practices in water-limited regions are underlain in part by evaluating the hydrological impact of changing vegetation cover from existing grass or fallow land to tree plantations. This requires reliable physiological models of water loss rates and their environmental control for plantations, including regulation of water use by multiple climate factors (Aston 1984; Meinzer et al. 2001). Some of the common land surface hydrology models (Zhang et al. 1996; Baron et al. 1998; Samanta et al. 2007) have their theoretical basis in the Penman-Monteith equation (Penman 1948; Monteith et al. 1965), but in so doing they are strongly species-neutral (Jarvis and McNaughton 1986; Overgaard et al. 2006) and use generalised parameters and relationships to environmental drivers. Parameterisations of both the Penman-Monteith equation, and models of leaf-level stomatal conductance response to the environment for multiple species (e.g., Leuning 1995; Damour et al. 2010), would allow better predictions of how individual trees, species and forested ecosystems will respond to changing climate conditions. These model parameterisations would assist land managers in selecting appropriate plantation species, subsequently decreasing rates of plantation failure, improving survival under future climates and climate extremes (Gutschick and BassiriRad 2003) and improving productivity and water-use efficiency of these plantations.

Advances in plant hydraulic theory (Sperry et al. 1998; Katul et al. 2003) have been successfully combined with models of stomatal behaviour (Leuning et al. 1995; Gao et al. 2002; Damour et al. 2010), yielding a useful framework where species stomatal responses to vapour pressure deficit (D) can be compared and contrasted (Oren et al. 1999). Such information can inform land-use decision making and model formulations for scenario testing to evaluate alternative management options. Interspecific variation in forest tree transpiration responses to environmental conditions are usually attributed to differences in plant hydraulic and anatomical characteristics (Merchant et al. 2007; Mitchell et al. 2008; Johnson et al. 2011), stomatal behaviour (White et al. 2000; Héroult et al. 2013), leaf area (O'Grady et al. 1999; Schwinning and Ehleringer 2001; Zeppel and Eamus 2008), and rooting depth (Rice et al. 2004; Mitchell et al. 2009). However, these previous species comparisons have been conducted without regard to phylogenetic differences among species and have involved different species types that coincidentally co-occurred or occupied a site, including comparisons of evergreen conifers and deciduous angiosperms (Schwinning and Ehleringer 2001; Pataki and Oren 2003; Monson et al. 2010). By restricting interspecific comparisons to genotypes within a species (Silim et al. 2009) or species within a single genus (Héroult et al. 2013) we can obtain finer distinctions among species transpiration responses to the environment, which will help guide species selection in species-rich zones.

Interspecific differences in stomatal responses to the environment can be obtained through comparison of stomatal sensitivity to D, as D strongly regulates stomatal conductance (g_s). Stomatal sensitivity to D is an estimate of the magnitude of the reduction in g_s or canopy conductance (G_C; the entire plant canopy analogue for g_s) for a given increase in D, i.e. the slope of the G_C vs. D response, and tends to be greater in species with high G_C at low D (Oren et al. 1999). The G_C at a reference D of 1 kPa (G_{Cref}) computed from the slope of the G_C vs. D response provides an indication of how much stomata respond to D at a standardised set of conditions relevant for species comparisons, such as intra-genus comparisons. According to hydraulic theory (Oren et al. 1999; Sperry et al. 2002), as long as stomata regulate leaf water potential near a constant value (isohydric behaviour; Bucci et al. 2005; Ewers et al. 2007; McDowell et al. 2008), a stomatal sensitivity to D (G_C to D slope) of 0.5-0.6 should be expected (Oren et al. 1999; Katul et al. 2009).

Eucalyptus species are prominent in afforestation and reforestation efforts in subtropical and tropical regions worldwide, and there is an imperative to evaluate the water management implications of these actions in many regions (Whitehead and Beadle 2004). This means that comparison of water use of different *Eucalyptus* species may present a model system for evaluating species-level control of transpiration and stomatal responses to environmental conditions. Furthermore, the wide ecological range inhabited by *Eucalyptus* species (Boland et al. 2006), and their tolerance of different climatic conditions across many continents (Dye and Olbrich 1993; Eberbach and Burrows 2006; Almeida et al. 2007; Zeppel et al. 2008; Mitchell et al. 2009), makes this genus an ideal candidate for an interspecific comparison of stomatal responses. Common garden experiments with *Eucalyptus* species have been used to compare leaf traits (Sefton et al. 2002; Warren et al. 2006), carbon isotope discrimination (Anderson et al. 1996; Turner et al. 2010) and conductance and photosynthetic parameters (Lewis et al. 2011; Héroult et al. 2013; Lin et al. 2013). These studies have found that species originating from arid regions tend to have narrower, smaller leaves (Warren et al. 2006), lower marginal water cost of carbon gain and higher tolerance of low water availabilities (Héroult et al. 2013) and, contrary to expectations, lower water-use efficiency (Anderson et al. 1996; Sefton et al. 2002) compared with species originating from humid regions. From this information, hydraulic theory (Sperry et al. 2002), hydraulic characteristics (Merchant et al. 2007), and observations of interspecific differences in stomatal behaviour for *Eucalyptus* species (White et al. 2000; Ngugi et al. 2004; Héroult et al.

2013), I predict that species originating from humid regions would require stomata more sensitive to D to maintain hydraulic integrity and regulate leaf water potential within limits (Choat et al. 2012). Similarly, *Eucalyptus* species from arid regions are expected to have a lower stomatal sensitivity to D than species from more humid locations, relying on cavitation-resistant water conducting systems rather than sensitive stomata to maintain hydraulic integrity (Merchant et al. 2007; Johnson et al. 2011). Specifically I hypothesised: (a) that *Eucalyptus* species originating from more humid regions will have higher G_C and sap flux density (J_S) and a lower D at stomatal closure than species originating from more arid regions, when growing in a common location. I also hypothesised: (b) that stomatal sensitivity to D will be higher in *Eucalyptus* species originating from humid regions compared to those from more arid regions.

2.3 Materials and methods

2.3.1 Study site

The study was conducted in a plantation of different *Eucalyptus* species grouped on the same site and soil in Richmond, New South Wales, Australia (33º33'S, 150º44'E). The experimental plantations of different species were established in a paddock of pasture grasses but with weed control in September 2007, described previously in Héroult et al. (2013). The plantation includes two replicate plots, each containing 35 trees of *Eucalyptus crebra* F.Muell., *E. dunnii* Maiden, *E. melliodora* A.Cunn. ex Schauer and *E. tereticornis* Sm., and one plot containing 110 trees of *E. saligna* Sm., all planted at a density of 1000 trees·ha^{-1}. Seeds were sourced from wild seed stock (See Table 1-2 for seed sources) except in the case of *E. grandis*, which had been selectively bred. I considered the driest part of each species range as an indication of their ability to tolerate dry conditions. Using the Koppen-Geiger climate classification system, *E. melliodora* and *E. crebra* are considered semi-arid zone species (Bsh), here called sub-humid zone species, *E. tereticornis* is at the transition from semi-arid to humid climates (Bsh and Cfa) so I call it a sub-humid/humid intermediate species, and *E. dunnii* (Cfa) and *E. saligna* (Cfa/Cfb) are humid zone species (Peel et al. 2007). Seedlings were fertilised at planting, hand watered for the first four months of establishment, and replaced if mortality occurred within the first year.

The plantation occurs on alluvial floodplain near the Hawkesbury River at an elevation of 25 m a.s.l. on Richmond Formation soils (Bannerman and Hazelton 1990). Permanent ground water is at least 15 m below the soil surface. The climate is sub-humid temperate, being characterised by hot summers and winters with frost possible though infrequent. Mean annual temperature is 17 ºC with a mean maximum and minimum temperature of 30.1 ºC and 3.5 ºC respectively. Average annual rainfall is 801 mm with a mean maximum and minimum rainfall of 128.7 mm and 31.7 mm per month respectively (Australian Bureau of Meteorology; http://www.bom.gov.au; Barton et al. 2010). The annual precipitation to evapotranspiration ratio is approximately 0.6 (Barton et al. 2010).

2.3.2 Meteorological parameters

A custom meteorological station (Campbell Scientific Australia, Townsville, Qld., Australia) employing sensors for temperature and relative humidity (HMP45C, Vaisala) at 2 m elevation, and solar radiation (LI-200 pyranometer, LI-COR Biosciences, Lincoln, NE, USA), wind speed (014A-L Met One anemometer) and direction (035B-L Met One windset) at 2.4 m elevation, was used to measure meteorological variables in an open clearing adjacent to the study plantations. Rainfall was recorded automatically at half hourly intervals (TB4 0.2 mm tipping bucket, Hydrological Services, Sydney, NSW, Australia) and manually in a separate rain gauge at 9:00 am each morning. The equation of Buck (1981) using relative humidity and temperature was used to calculate *D*.

2.3.3 Tree selection and physiological measurements

I measured sap flow on between five and seven trees each per species. Trees were chosen to represent the diameter and height distribution of the whole plot. Measurements of diameter at breast height (DBH, diameter at 1.3 m from base), diameter at 8 cm from the ground, and tree height were conducted on all trees in each plot in March, May, and December 2010 and May 2011. Volume index was calculated as diameter^2 * height. During May 2010 and March 2013, I felled and harvested three trees of each species to obtain total leaf area (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, NE, USA), sapwood cross-sectional area, bark thickness, trunk vessel diameter and area, wounding diameters, and wood density (displacement method). Wood density measured using stem disks yielded similar wood densities (difference ~3%) to stem core measurements (Haglöf, Långsele, Sweden). Light microscopy and SEM were used to assess the wounding damage by sensor insertion after trees were harvested. Both approaches generated wounding diameters between 0.18 and 0.2 cm for all species, within the expected range (Swanson and Whitfield 1981; Barrett et al. 1995).

2.3.4 Estimating sap flux density and tree water use

Sensors using the heat ratio method (HRM; (HRM30, ICT International, Armidale, NSW Australia; Burgess et al. 2001) were used to measure sap flux on five to seven trees of each of *E. crebra*, *E. dunnii*, *E. melliodora,* *E. saligna* and *E. tereticornis*. I measured sap flux for 18 months from January 2010 following techniques described by Burgess and Dawson (2004) and Dawson et al. (2007). Probes were inserted into axially aligned insertion holes which were drilled 5 mm apart at breast height (1.3 m from base) on the south side of the tree using a drilling guide and a specialised drill bit (1.35 mm diameter). Probes were moved 10 cm higher and to the side at three to six monthly intervals, and radial comparisons of sap flux on selected trees of each species were undertaken at the end of the study period. The HRM loggers measured apparent heat pulse velocity (HPV) at two points in the sapwood of each tree (7.5 and 22.5 mm from probe tip) at 15 minute intervals.

HRM sensors required small voltage corrections to provide a zero-flow baseline when there was no transpiration (Burgess et al. 2001; Dawson et al. 2007).

These uncorrected, raw zero-flow voltages were < 2% of a maximal sap flow reading for each sensor. The maximum sap velocity resolved by HRM sensors at 50 J heating was 45 $\text{cm}\cdot\text{hr}^{-1}$ and therefore I replaced values above this with the maximum velocity observed for respective sensors. This correction underestimates actual water use, however on average only 0.9% of the 51,000 measurements per tree were affected comprising 2.1%, 1.5%, 0.01%, 0.3% and 0.3% of the data during the study period for *E. crebra*, *E. dunnii*, *E. melliodora*, *E. saligna* and *E. tereticornis*, respectively. Furthermore, analysis of inner sensors when outer sensors were maximal revealed that no actual plateau in sap velocity occurred, so a true plateau in this response (e.g., Pfautsch et al. 2011) was not overlooked for this depth. To validate the accuracy and reliability of the sap flux sensors using these corrections, I undertook a comparison of actual water use with HRM measured water use on a cut *E. saligna* tree over a seven day period. The sensors accounted for more than 95% of the variation in total water use and were strongly ($R^2 = 0.99$) and positively correlated to measurements of cumulative water use (data not shown).

Supplementary measurements of wood density, sapwood area, bark thickness and wound diameter from probe insertion were used to correct HPV measurements to estimates of sap velocity per unit sapwood area (sap flux density; J_S) using methods described by Swanson (1994), Barrett et al. (1995) and Burgess et al. (2001). The weighted mean method described by Hatton et al. (1990) was used to integrate the two estimates of sap velocity (inner and outer readings per probe) along with sapwood area to an estimate of whole tree water use (E, $\text{kg}\cdot\text{m}^{-2}$ $\text{sapwood}\cdot\text{s}^{-1}$).

2.3.5 *Canopy conductance measurements*

Canopy conductance (G_C, m·s^{-1}) was estimated from average tree transpiration (E, kg·m^{-2} sapwood·s^{-1}), vapour pressure deficit (D, kPa) and the conductance coefficient (K_G, kPa·m^{-3}·kg^{-1}, = 115.8 + 0.4236T) dependent on temperature (T, ºC) as derived by Ewers et al. (2001a):

$$G_C = \frac{E * K_G}{D} \tag{1}$$

Canopy conductance was converted to molar conductance units (G_C, mol·m^{-2}·s^{-1}) using the ideal gas law (Lide 2010):

$$G_C = G' * V_0 * (\frac{T_0}{T_{air}}) * (\frac{P_{air}}{P_0}) \tag{2}$$

where G_C is the molar canopy conductance (mol·m^{-2}·s^{-1}), G' is the initial canopy conductance (m·s^{-1}) and V_0 is the molar volume of air (44.6 mol·m^{-3}) at standard temperature (T_0 = 273.15 ºK) and pressure (P_0 = 101.3 kPa). This method assumes that sap flux densities are uniform throughout the sapwood area, D is close to the leaf-to-air vapour pressure deficit, there is no vertical gradient of D in the canopy, and stem hydraulic capacitance is low such that sap flux responds rapidly from water uptake. This last assumption was confirmed on analysis of changes in J_S upon sun-cloud-sun transitions (data not shown). Furthermore, as the species studied here had small leaves (< 50 cm^2) and open canopies they were considered closely coupled to the atmosphere, thus aerodynamic conductance was assumed to be near unity.

2.3.6 *Statistical analyses*

I used approaches based on the boundary-line analysis technique of Jarvis (1976) to compare the J_S and G_C responses to D among species, allowing interspecific comparison of responses under optimal, non-limited conditions. I conditionally sampled my data, excluding data collected during adverse conditions rather than subjecting the entire data set to analysis (e.g., Bréda et al. 1993; Cienciala et al. 1994; Oren et al. 1999). Favourable conditions were considered to be when solar radiation was non-limiting (Dai et al. 1992), in this case greater than 99 $W \cdot m^{-2}$ respectively. I also excluded data where $D < 0.3$ kPa. I binned species average sap flux measurements according to D with a bin size of 0.4 kPa, where each measurement binned was the average sap flux of five to seven trees. Bins with < 10 measurements in monthly data, and < 50 measurements in all other data, were eliminated from further analyses, resulting in a maximum D of 4 kPa being presented, despite D of up to 8 kPa being reached. I also fit the J_S-D relationship for each species to a nonlinear exponential saturation-type model using the raw, unbinned data, and compared the fit parameters among the different species. I compared the stomatal sensitivity to D among species through comparison of different parts of the canopy conductance to D relationship, namely the intercept and slope of the G_C to D response at a D of 1 kPa (Oren et al. 1999). The extrapolated D at stomatal closure ($e^{b/m}$; D when $g_s = 0$) was calculated and compared between species to determine whether this parameter differed interspecifically and whether it was related to climate of origin.

I conducted an ANOVA in Sigmaplot (v11.0, Systat, Inc., Chicago, IL, USA) to test for species differences in stomatal sensitivity to D and G_{Cref}, a Tukey's HSD in *JMP* (v. 5.0.1, SAS, Cary, NC, USA) to test for species differences in volume index change, and quantile regressions in *R* (R Development Core Team 2015) to determine if the G_C vs. D response differed by species. The comparison of quantile regressions was undertaken using a method outlined by Cade and Noon (2003) in which the quantiles for each species G_C response to D were obtained and response slopes were compared using two-tailed z-tests for population means ($n =$ five to seven trees per species) at different D values. To account for multiple pairwise comparisons a Bonferroni correction was applied to the alpha level. Bonferroni corrections have been shown to lead to a higher rate of Type II errors (Armstrong 2014). I therefore confirmed my statistical results with an independent analysis of $\ln(G_C)$ to $\ln(D)$ using non-parametric statistics (Kruskal Wallace test in R; R Development Core Team 2015) and compared individual species slopes using a Tukey's HSD in *R* (R Development Core Team 2015).

Comparison of species responses and stomatal sensitivity to D in the context of climate of origin required a numerical descriptor of climate of origin. I used aridity index as a proxy for the climate aspect of the species geographical origin which was calculated by dividing the annual rainfall by the potential evapotranspiration (PET) at seed source. Monthly PET (Australian Bureau of Meteorology; http://www.bom.gov.au/watl/eto/; accessed 15 August 2014) and rainfall (Australian Bureau of Meteorology; http://www.bom.gov.au/climate/data; accessed 15 August 2014) was obtained for each species provenance from the meteorological station closest to species seed source. Additionally, I exported twelve climatic parameters relevant to hydrology from the spatial portal of the Atlas of Living Australia (http://spatial.ala.org.au/; accessed 22 July 2014) from plant locations within 30 km from the species seed source coordinates. I tested these inputs for pairwise correlations with main measures of the species physiological performance, specifically stomatal sensitivity to D, mean vessel diameter, absolute decline in maximal sap flux density (J_{Smax}) from moderate to low water availabilities and volume index growth, to address how species physiological responses related to climate of origin. The absolute decline in J_{Smax} was computed as the difference in mean J_{Smax} across a D of 3-3.8 kPa between moderate and low water availabilities. The percentage decline in J_{Smax} was calculated as the absolute decline in J_{Smax} as a percentage of maximal J_{Smax} (measured under moderate water availabilities) for each bin averaged across a D of 3-3.8 kPa. A subset of the correlations are displayed in supplemental material (Table A-2).

2.4 Results

2.4.1 Meteorological parameters and tree growth

During the study period (January 2010 – May 2011) rainfall, evaporative demand (*D*) and potential evapotranspiration (PET) followed expected seasonal patterns (Australian Bureau of Meteorology, http://www.bom.gov.au; unpublished data) with summer (December through February) having the highest and winter (June through August) having the lowest rainfall, *D* and PET respectively (Figure 2-1). The study period can be divided into low and moderate water availability periods. For the purposes of this study, dry (or low water availability) periods were classed as those times where maximal *D* exceeded 6 kPa or monthly rainfall was less than 5 mm (arrows in Figure 2-1), namely January and August 2010 and January and February, 2011. All other months were considered to have moderate water availability. Classifications of low and moderate water availabilities were supported by measurements of soil moisture and pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) water potentials (Héroult et al. 2013). Volume index change was lower for species originating from sub-humid compared to humid regions and was significantly different between *E. melliodora*, a sub-humid species, and the humid species *E. dunnii* and *E. saligna* (Table 1-2).

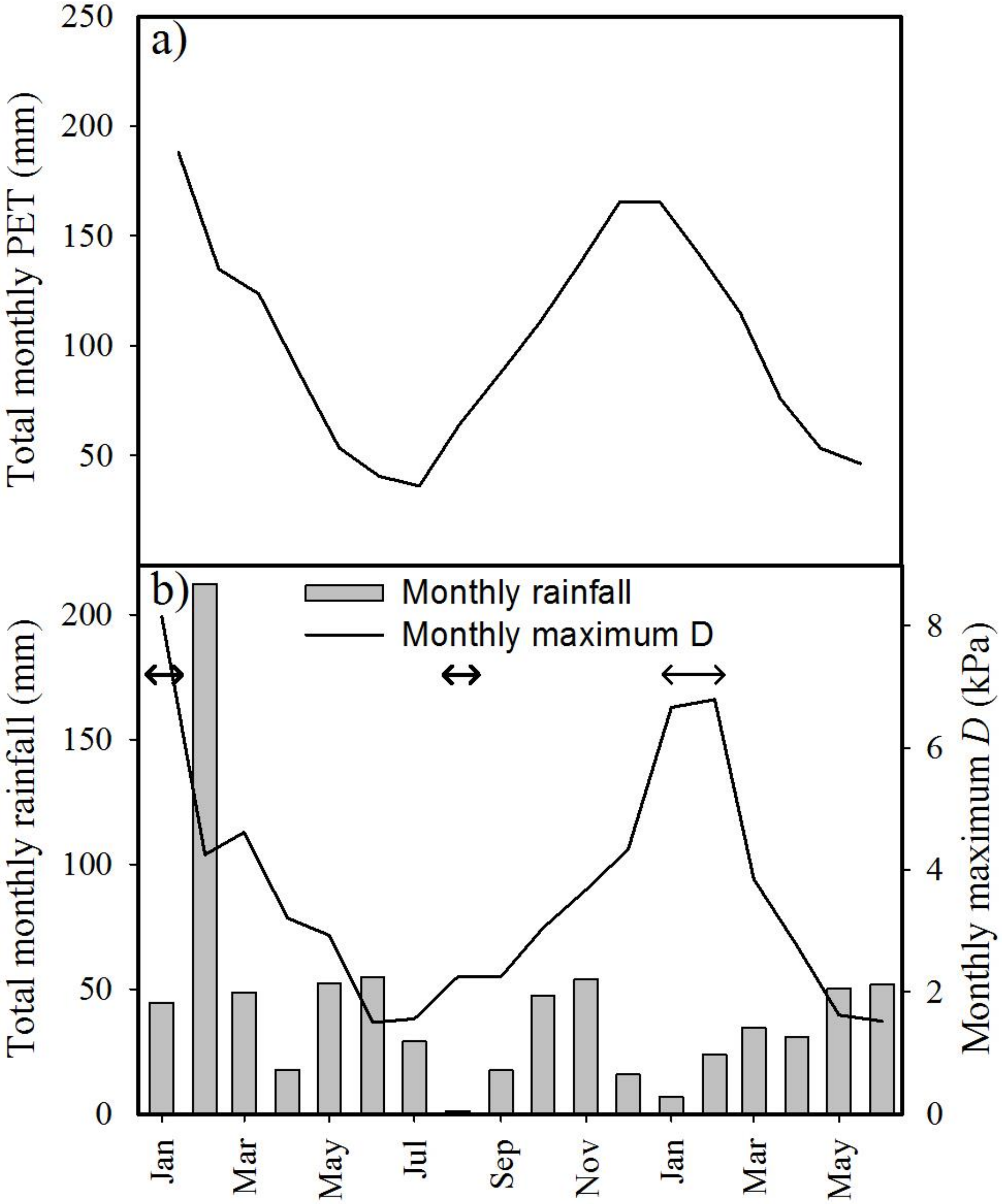


Figure 2-1: Total monthly a) potential evapotranspiration (PET) obtained from the Bureau of Meteorology records for Richmond and b) rainfall (columns) and maximal *D* (line) recorded at the study site over the study period of 1st January 2010 through to 31st June 2011. Arrows indicate dry periods and areas with no arrows are moderate water availability conditions as referred to in text.

2.4.2 Canopy conductance, sap flux density and stomatal sensitivity to D

All species showed a decline in G_C in response to increasing D. *E. melliodora*, a sub-humid species, had the highest normalised maximal G_C (G_C/G_{Cmax}) per unit leaf area compared to all other species measured (Figure 2-2a). At low D (< 1 kPa) there were significant differences between all species in the slope of the G_C-D response corresponding to humid vs. sub-humid species ($p < 0.01$; z-test). Humid species G_C declined significantly more for a given change in D (more negative slope) than sub-humid species at $D \sim 1$ kPa ($p < 0.05$). At $D = 0.9$ kPa, the slopes of the G_C-D response for the different species were *E. crebra* > *E. melliodora* > *E. saligna* = *E. tereticornis* > *E. dunnii* and at $D = 1.3$ kPa were *E. crebra* > *E. melliodora* = *E. tereticornis* > *E. dunnii* > *E. saligna* (Figure 2-2a, $p < 0.05$; z-test). There were significant differences between species in the ln(G_C) to ln(D) response such that humid species were significantly different from sub-humid species ($p = 0.001$; t-test), where *E. dunnii* and *E. saligna* were significantly different to *E. melliodora* ($p < 0.001$ and $p = 0.005$ respectively) and all other species were statistically similar ($p > 0.05$; Tukey's HSD). The differences I observed are not driven by species differences in G_{Cmax} as G_{Cmax} was similar amongst all species ($p > 0.05$; z-test). J_S increased with increasing D to a plateau for all species. Differences in both magnitude and curvature of the J_S to D response were observed. J_S was highest in humid species (*E. dunnii* and *E. saligna*) versus sub-humid species (*E. melliodora* and *E. crebra*). The intermediate species (*E. tereticornis*) was not different from any of the other species in this regard. In humid species the J_S declined at $D > 3$ kPa, whereas sub-humid species were able to maintain J_S at $D \geq 3$ kPa (Figure 2-2b).

Species with a higher reference G_C (G_{Cref}, G_C at $D = 1$ kPa) had a higher stomatal sensitivity to D (Figure 2-3a). *E. melliodora* had the lowest stomatal sensitivity to D and G_{Cref} and was significantly different to all other species while *E. dunnii* was significantly different to the species from sub-humid regions but not different to the other species from humid and intermediate regions (Figure 2-3a, $p < 0.05$; t-test). A linear relationship was observed between stomatal sensitivity to D and aridity index (Figure 2-3b, $R^2 = 0.77$, $p = 0.05$) and G_{Cref} and aridity index (Figure 2-3d, $R^2 = 0.77$, $p = 0.05$) where humid species (higher aridity index) had a higher stomatal sensitivity to D and a higher G_{Cref} than sub-humid species. The $e^{b/m}$ value, the extrapolated D at which complete stomatal closure occurs, was in most cases higher for my species than for Northern Hemisphere plants (Table 2-1).

The stomatal response to D under moderate and low water availabilities differed between species dependent on the climate of origin of the species (Figure 2-4). The majority of variation in the J_S response to D was observed at $D > 3$ kPa. Data up to a D of 8 kPa was measured, however the relationship at extreme D was unstable due to a small sample size (< 10 measurements per bin) and is thus not presented here. Sub-humid species had a lower maximal J_S which plateaued once D reached 2 kPa under low water availability (Figure 2-4a and c, filled symbols) whereas humid and intermediate species had a higher maximal J_S which plateaued then declined once this D was reached (Figure 2-4b and d-e, closed symbols). Additionally, there was a larger difference in the magnitude and curvature of the J_S to D response under differing water availabilities for humid compared to sub-humid and intermediate species (Figures 4 and 5a). The intermediate species, *E. tereticornis*, had a larger difference (12% decline, Figure 2-4e) between moderate and low water availability J_S than sub-humid species *E. crebra* (9% decline, Figure

2-4a) and *E. melliodora* (-8% decline, Figure 2-4c), and a smaller difference than humid species *E. dunnii* (32% decline, Figure 2-4b) and *E. saligna* (34% decline, Figure 2-4d). Species differences in the absolute decline in J_{Smax} under differing water availabilities were related to vessel diameter differences, where species with larger vessel diameters had a lower J_{Smax} under low water availability and a larger difference in J_{Smax} between low and moderate water availability than species with narrower vessels (Figure 2-5b). The same relationship was observed between J_{Smax} and vessel area, maximum vessel area and maximum vessel diameter (data not shown).

Table 2-1: Values (means) used for evaluating the dependency between the two parameters in the function: $G_C = -m \cdot \ln D + b$ using sap flux measurements. Data shown are from Table 2 in Oren et al. (1999) and summarized by regional class except *Liquidambar styraciflua* which was studied at multiple sites. All data from this study are as shown in Figure 2-3a. Northern temperate species include *Acer rubrum* L., *Fagus sylvatica* L., *Liriodendron tulipifera* L. and *Populus tremuloides* Michaux and tropical species include *Anacardium excelsum* L., *Goupia glabra* Aubl. and *Simarouba amara* Aubl.. Measurement parameters as follows: n is the number of trees measured per species (sample size), b is G_{Cref} in mmol·m^{-2}·s^{-1} ln(kPa)$^{-1}$ for leaf area measurements and mol·m^{-2}·s^{-1} ln(kPa)$^{-1}$ for sapwood area measurements and $e^{b/m}$ is the extrapolated D where stomata are completely closed. Letters denote significant differences between species ($p < 0.05$; Tukey's HSD).

Oren et al. (1999) data	***n***	***b***	***-m***	$e^{b/m}$
Northern temperate species	25	81	60.7	5.1
Tropical species	12	131.7	70.7	6.7
Liquidambar styraciflua	15	85	58.3	4.3
Tropical forest		53	30	5.8
Data from this study	Per unit leaf area			
E. crebra	6	84^{a}	41^{a}	7.9^{a}
E. dunnii	6	105^{a}	51^{a}	7.7^{a}
E. melliodora	7	163^{b}	82^{b}	7.4^{a}
E. saligna	7	99^{a}	49^{a}	7.6^{a}
E. tereticornis	5	123^{a}	62^{a}	7.2^{a}
	Per unit sapwood area			
E. crebra	6	43^{a}	21^{a}	7.6^{a}
E. dunnii	6	62^{b}	31^{b}	7.4^{b}
E. melliodora	7	24^{c}	11^{c}	8.9^{ab}
E. saligna	7	48^{ab}	24^{ab}	7.3^{b}
E. tereticornis	5	46^{ab}	23^{ab}	7.2^{b}

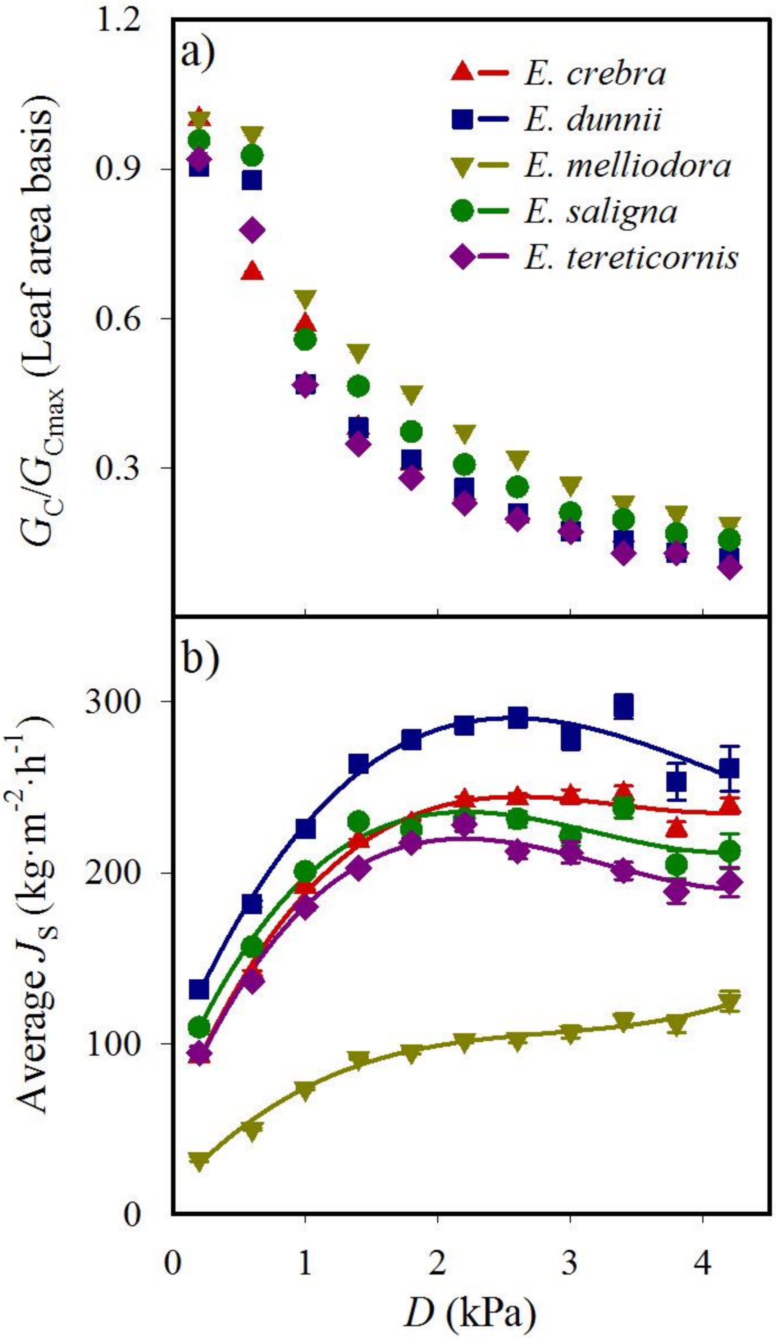


Figure 2-2: Responses of five *Eucalyptus* species under favourable conditions of a) maximal G_C/G_{Cmax} and b) average J_S to increasing D. Error bars represent the standard error of the mean across five to seven trees of each species. All data are binned using a 0.4 kPa bin size. Lines shown are splines as a guide.

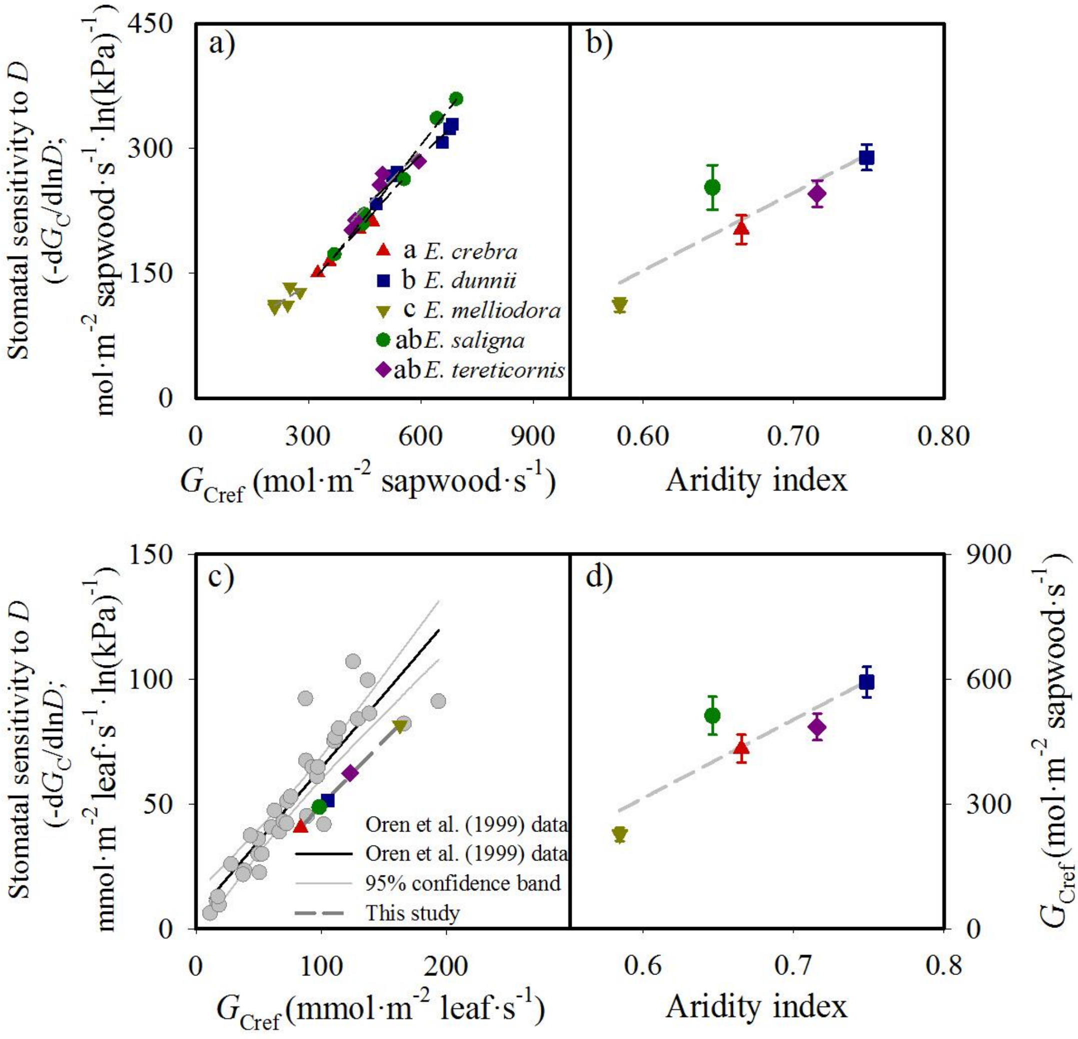


Figure 2-3: Relationships between stomatal sensitivity to D and a) G_{Cref}, b) aridity index for climate of origin and c) G_{Cref} and d) relationship between G_{Cref} and aridity index of five *Eucalyptus* species under favourable environmental conditions. Data in a), b) and d) is per unit sapwood area and in c) is per unit leaf area. Dashed lines in a) indicate the relationship between sensitivity to D and G_{Cref}, and in the case of *E. melliodora* excludes one outlier (slope with vs. without outlier is 0.468 and 0.481 respectively). Letters denote significant differences between species in sensitivity to D and G_{Cref} tested using a one-way ANOVA ($p < 0.05$). Each point in b) and d) is the average sensitivity to D for a species across each of five to seven trees. Aridity index is annual precipitation divided by PET for each species climate at seed source (Table 1-2). Dotted lines indicate the least-squares linear regressions for a) *E. crebra* ($R^2 = 0.98$), *E. dunnii* ($R^2 = 0.96$), *E. melliodora* ($R^2 = 0.55$), *E. saligna* ($R^2 = 0.99$) and *E. tereticornis* ($R^2 = 0.87$), b) all species stomatal sensitivity to D response to aridity index ($R^2 = 0.77$, $p < 0.05$), and d) all species G_{Cref} response to aridity index ($R^2 = 0.77$, $p < 0.05$). In c), Oren et al. (1999) data are from Figure 2-4b in Oren et al. (1999) and other points are averages of five to seven trees per species from this study. The slope of Oren et al. (1999) data is 0.59 and for my data, slope is 0.52 ($R^2 = 0.99$).

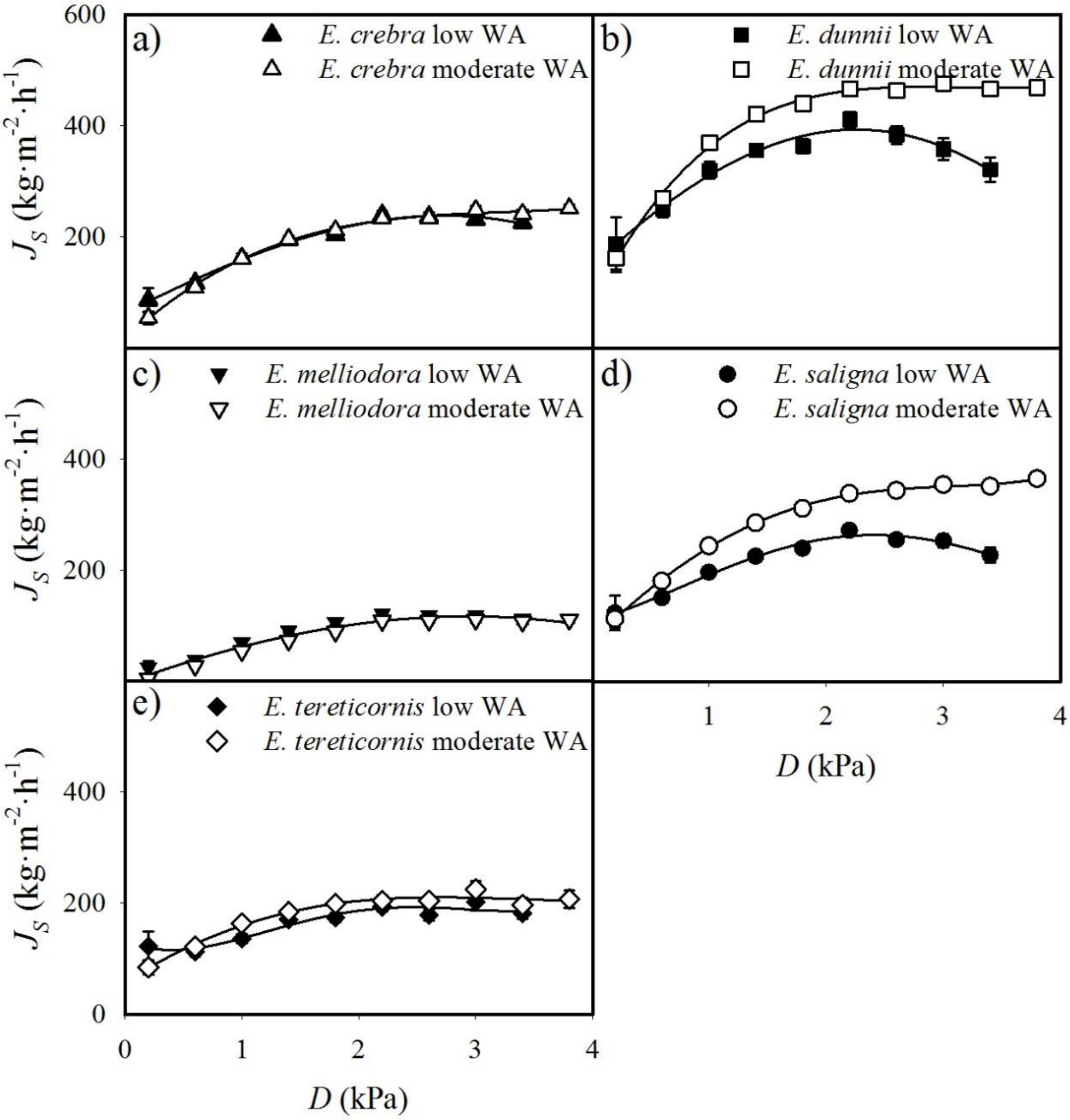


Figure 2-4: The relationship between sap flux density (J_S) and evaporative demand (D) of each species under low and moderate water availability (WA) under favourable conditions. a) and c) represent sub-humid zone, b) and d) humid zone and e) intermediate zone species. Error bars represent the standard error of the mean using a count of values per bin where all data are binned using a 0.4 kPa bin size. Lines shown are splines as a guide.

2.4.3 *Climate of origin and its relation to species physiological parameters*

Numerous significant correlations were observed between climatic parameters representing climate of origin and specific parameters chosen to represent the physiological performance of each species. Maximum monthly water deficit explained more than 90% of the variation between species observed in volume index growth, mean vessel diameter and absolute decline in J_{Smax} under low water availability ($R^2 \geq 0.90$, Table A-2, Figure 2-5a and c-d) and more than 80% of the variation observed in stomatal sensitivity to D ($R^2 = 0.83$, $p = 0.03$). Mean vessel diameter was highly and significantly correlated with absolute decline in J_{Smax} (Figure 2-5b, $R^2 = = 0.98$, $p = 0.001$) and volume index growth ($R^2 = 0.97$, $p = 0.004$, Table A-2), but not significantly correlated with stomatal sensitivity to D. Volume index growth was highly and significantly correlated with absolute decline in J_{Smax} ($R^2 = 0.96$, $p = 0.003$) and with stomatal sensitivity to D ($R^2 = 0.84$, $p = 0.03$). Increases in the maximum monthly water deficit at species origin resulted in smaller declines in J_{Smax} (Figure 2-5a), lower growth (Figure 2-5c) and narrower xylem vessels (Figure 2-5d). Wider vessels resulted in higher growth rates (Table A-2) and larger declines in J_{Smax} under low water availability (Figure 2-5b).

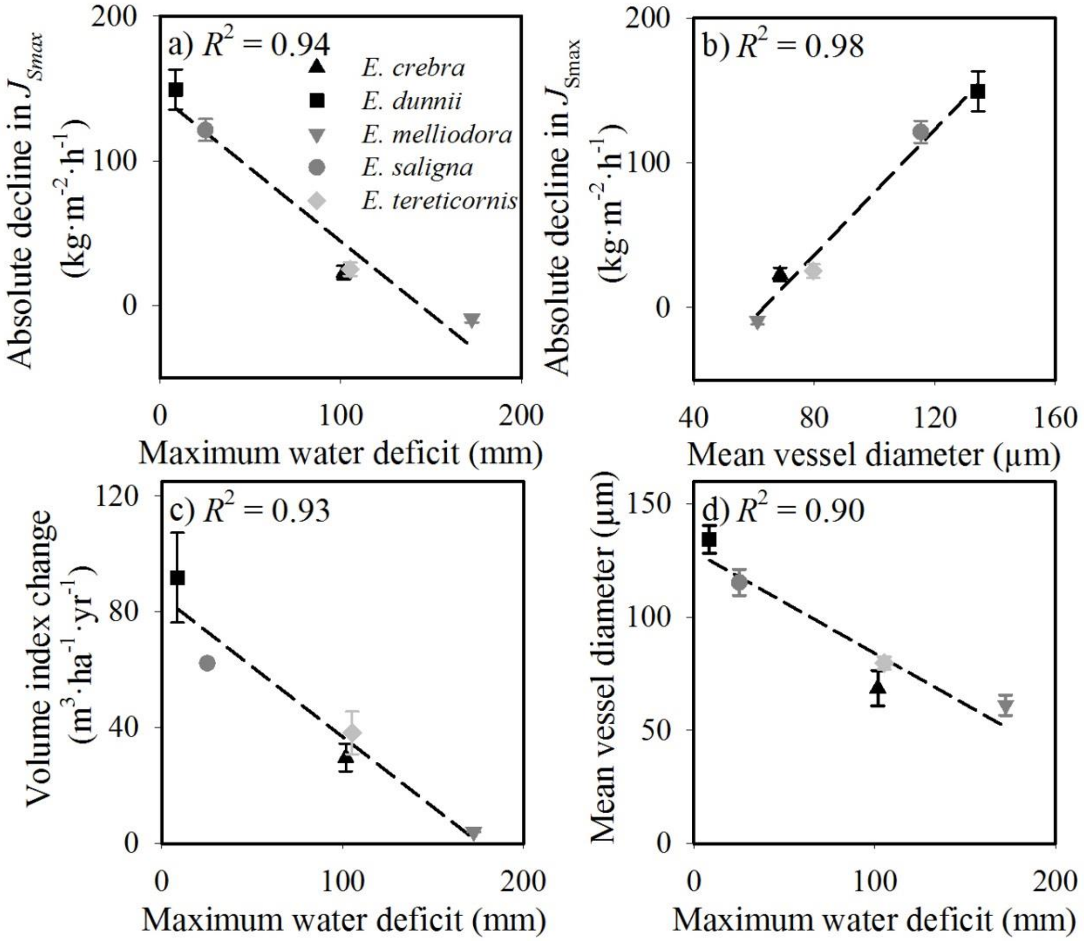


Figure 2-5: The relationship between (a) maximum monthly water deficit and absolute decline in J_{Smax} under favourable conditions, (c) volume index change and (d) mean vessel diameter and (b) the relationship between absolute decline in J_{Smax} and mean vessel diameter. Error bars represent the standard error of the mean of between five and seven trees each for decline in J_{Smax}, two plots for volume index change and five trees for mean vessel diameter. Maximum monthly water deficit was obtained from Atlas of Living Australia (www.ala.org.au, accessed 22nd July 2014). Pearson correlation coefficients are given in Table A-2. Dashed lines indicate the least-squares linear regression ($p < 0.05$).

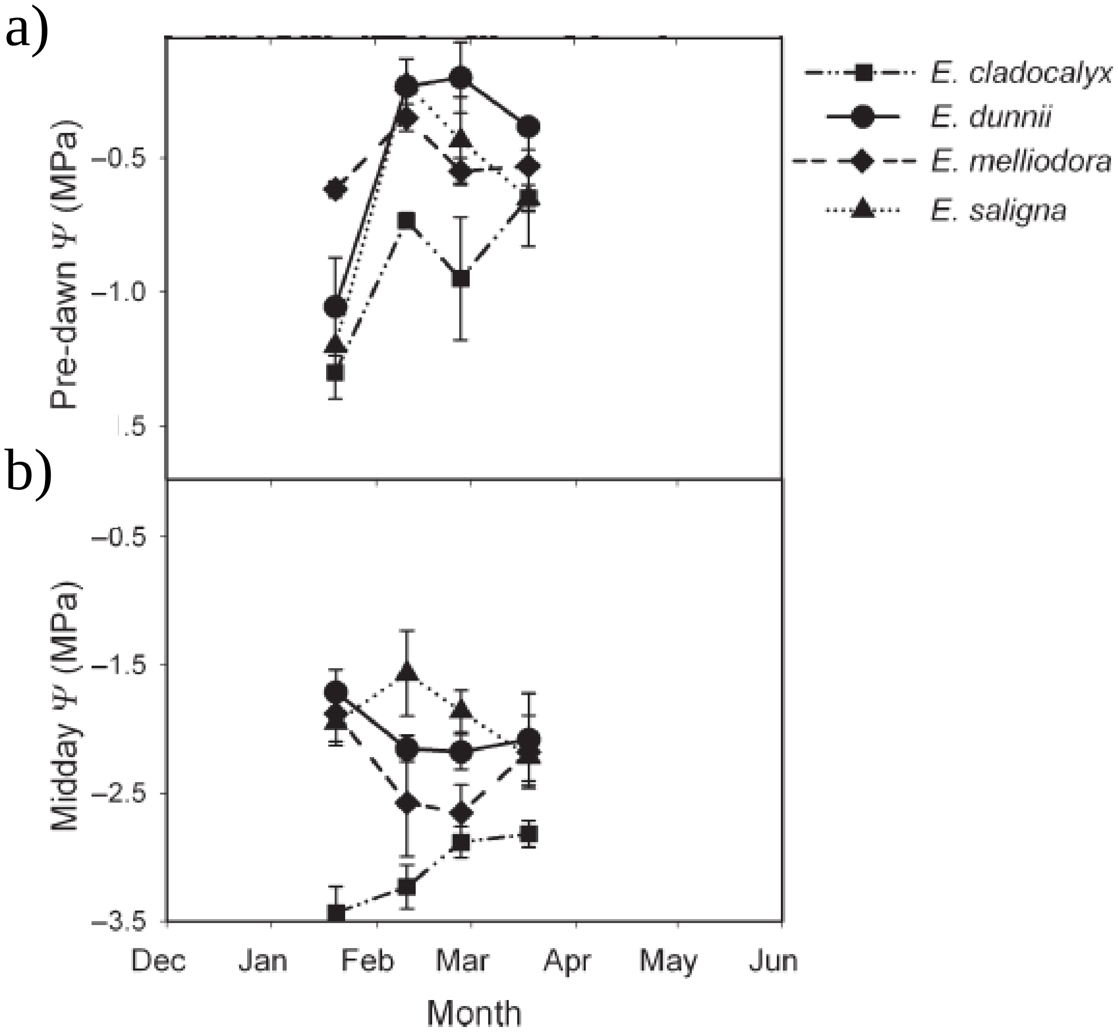


Figure 2-6: Pre-dawn (a) and midday (b) leaf water potentials of *E. cladocalyx*, *E. dunnii*, *E. melliodora* and *E. saligna* measured during the 2010 study period, as published in Héroult et al. (2013).

2.5 Discussion

Species differ in their mean and maximum sap flux density (Bush et al. 2008; Uddling et al. 2008) and canopy conductance traits across sites (Ewers et al. 2002; Pataki and Oren 2003). However, the source of this variation including species and provenance factors versus site factors has remained unclear. In my common garden study, I found that species differences in physiological parameters related well with climate of origin, subsequently constraining species water use and response to environmental conditions. How species from different climates regulate transpiration may provide insight into how these species tolerate current and future climates and climate extremes.

2.5.1 Interspecific differences in water use characteristics

I found evidence supporting my first hypothesis, that species originating from humid regions had a higher J_{Smax} and G_{C} on a sapwood area basis and a more rapid decline in J_{S} at lower D compared to species from sub-humid and intermediate regions. Responses of J_{S} and G_{C} to D followed relationships similar to previous studies (Morris et al. 1998; Pataki and Oren 2003; Hubbard et al. 2004; Bush et al. 2008; Uddling et al. 2008) and differed amongst species. I observed that the curvature of the G_{C} response to D and this relationship as a whole was significantly different between humid and sub-humid species (Figure 2-2a, $p = 0.001$; t-test). Interspecific differences in J_{S} and G_{C} have been attributed to differences in xylem type (Bush et al. 2008), local soil moisture (Pataki and Oren 2003), environmental variation or differences in sapwood area (Vertessy et al. 1995). While true for those studies, these traits were equalized in my study given that all species had diffuse porous xylem (Downes 1997), were grown in a common garden with the same environmental variables, soil type and moisture contents, and were compared via J_S calculated on a sapwood area basis. One explanation for the differences among species that I observed may be rooting depth (Brooks et al. 2002; Rice et al. 2004), yet this is improbable as all species likely had adequate access to soil water resources supported by pre-dawn water potential measurements taken during moderate and low water availability periods of this study (Figure 2‑5, Héroult et al. 2013). Rather, I observed that interspecific differences in the J_S and G_C response to D were likely due to other physiological restrictions, hydraulic architecture (Choat et al. 2003) and stomatal behaviour. Additionally, leaf traits for these species were reported to be tightly and significantly correlated with climate of species origin (Chapter 3, Figure 3-1 and Table A-3) which could also explain the interspecific differences in the J_S and G_C response to D for the species studied here.

2.5.2 Stomatal behaviour and sensitivity to D

The J_S response to D (Figure 2-2b), representing stomatal behaviour, and the extrapolated D at stomatal closure ($e^{b/m}$ in Table 2-1) were influenced by climate of origin. Humid species tended to have a higher maximal J_S at low D and a lower D at stomatal closure than sub-humid or intermediate species. The D at which stomatal closure occurred in the species studied here tended to be much higher than those previously reported for Northern Hemisphere species (Oren et al. 1999; Ewers et al. 2005; Ewers et al. 2007) as well as those for Southern Hemisphere temperate rainforest species (Cunningham 2004). Australian species originate from climates that are warmer and more arid than those of Northern Hemisphere temperate species. Thus the higher D at stomatal closure observed for my species is consistent with their climate of origin and also consistent with the idea that species from sub-humid climates are capable of tolerating a larger range of water potentials than those from humid climates (Maherali et al. 2004; Héroult et al. 2013). Given that the physiological characteristics enabling plants to inhabit different climates would influence species distributional ranges (Engelbrecht et al. 2007), and subsequently that more arid species are likely to have water conducting systems suited to coping with higher water deficits than humid species (Comstock 2002), I hypothesised that humid species would require stomata highly sensitive to changes in D to prevent cavitation. Stomatal sensitivity to D, calculated as the slope of the G_{C} vs. D response (Oren et al. 1999), and G_{Cref} differed interspecifically and were related to climate of origin (Figure 2-3b and 3d, $R^2 = 0.77$), where humid species required stomata more sensitive to changes in D compared to sub-humid species. The maximum conductance (G_{Cref}) is determined by the hydraulic properties of the system, which explains why it is related to the aridity index (Figure 2-3d).

On the basis of hydraulic theories described in Oren et al. (1999) and Sperry et al. (2002), all species should converge on a G_{Cref} stomatal sensitivity to D slope of 0.5-0.6 with variation around this value dependent on the range of D encountered by each individual plant (Oren et al. 1999; Katul et al. 2009). This slope has been observed numerous times on many different plant species (Oren and Pataki 2001; Mackay et al. 2003; Ewers et al. 2005; Ewers et al. 2007; Chen et al. 2012; Naithani et al. 2012; Ocheltree et al. 2014). My species followed a tight relationship between stomatal sensitivity to D and G_{Cref} (Figure 2-3c, $R^2 = 0.99$), however the slope of this relationship was significantly lower than the expected value of 0.6 reported by Oren et al. (1999), here at 0.52 (Figure 2-3c, $p < 0.001$; t-test) although not different from the 0.5-0.6 reported by Katul et al. (2009). While the lower slope in this study could be explained by the difference between leaf level and sap flow based measurements

(Oren et al. 1999), or the difference between sensor types used to measure sap flow (Uddling et al. 2009), these are unlikely because G_C derived from my sap flow based measurements is similar in magnitude to the leaf level g_s from Héroult et al. (2013). Furthermore, previous studies have found similar results to those presented by Oren et al. (1999) with different sap flow sensors (Ewers et al. 2005; Chen et al. 2012; Naithani et al. 2012) and while consistent under- or over-estimation of sap flux would change the G_{Cref} value it would not influence the slope of the G_{Cref} with respect to D. The species reported by Oren et al. (1999) which were most similar in stomatal sensitivity to D and G_{Cref} to those reported here were those species originating from more xeric environments, and the Australian species reported here can generally be considered xeric compared to Northern Hemisphere species. Consistent with my findings for sub-humid and humid species, the xeric species in Oren et al. (1999) had a lower slope of the G_{Cref} vs. D response, which is explained by hydraulic theory as the more xeric species would be exposed to a wider range in D resulting in a lower sensitivity to D. As all species in this study were grown and measured in a common climate, a wider D range does not explain differences in slope between species. Instead, the lower slope observed in my study supports the idea that more arid species exhibit less strict regulation of leaf water potential, instead relying on a safe water conducting system to restrict cavitation within the xylem (Sperry et al. 2008). A lower G_{Cref} vs. stomatal sensitivity to D slope has previously been attributed to plants with higher drought tolerance (Pataki et al. 2000a; Ogle and Reynolds 2002; Maherali et al. 2003) and a lower stomatal sensitivity to D has been hypothesised to be a general feature of plants with low maximum g_s due to a comparatively greater effect of cuticular conductance on gas exchange (Maherali et al. 2003). Following the idea that a lower slope is indicative of species with higher drought tolerance, species from sub-humid regions which exhibit less strict regulation of leaf water potential should be more drought-tolerant than species from humid regions which have a higher stomatal sensitivity to D, and this drought tolerance would possibly be due to differences in hydraulic characteristics.

2.5.3 Tolerance of changing water availability

Species tolerance of low water availability was linked to climate of origin, where humid species had a larger decline in J_{Smax} with declining water availability compared to sub-humid species (Figures 2-4 and 2-5a), and this is consistent with g_s observations on a similar set of species (Héroult et al. 2013). As reported by this study and Héroult et al. (2013), the sub-humid species *E. melliodora* exhibited less stomatal control than other species, maintaining J_S and g_s at extremely high D, whereas humid species showed large declines in J_S at high D. Given that the low water availability periods defined here are still relatively moist when compared to long-term climatic conditions (Table 5-2) I expect that under severe drought conditions the differences observed between species in these responses would be enhanced. These interspecific differences in tolerance of low water availability might be explained by differences in hydraulic architecture. Sub-humid species, adapted to regions of higher water deficit, had narrower vessels, potentially offering increased resistance to cavitation (Comstock and Sperry 2000) and maintenance of conductance at high D irrespective of water availability, whereas humid species had wider, potentially less cavitation resistant vessels, and larger declines in J_{Smax} with respect to decline in water availability. This correlation supports the idea that species originating from sub-humid areas develop hydraulic systems oriented towards cavitation resistance, meaning they are more capable of maintaining hydraulic integrity and conductance under high water deficits (Merchant et al. 2007; Johnson et al. 2011), whereas humid species rely on stomatal behaviour and sensitivity to *D* to maintain hydraulic integrity (Oren et al. 1999). While tight correlations between vessel diameter and absolute decline in J_{Smax} (Figure 2-5b) and aridity index (Figure 2-5d) were observed in this study, previous broad species comparisons have observed large variability in the relationship between climate and embolism resistance (Choat et al. 2012). As plants must negotiate trade-offs between xylem efficiency (i.e. the ability to transport large amounts of water) and safety (i.e. the ability to resist cavitation) it is consistent that a less cavitation resistant system would require stomatal closure at lower *D*, or using water from a capacitance pool, in order to protect the plant's hydraulic pathway. This information has implications for understanding how current plantations and forests will tolerate changes in climate and climate extremes, and can assist land managers in identifying vulnerable plantations and developing plantations that have higher survival, growth and productivity under these different climates.

Species with wide distribution ranges, e.g., *E. crebra*, *E. melliodora* and *E. tereticornis* (Figure 1-3 and Table 1-2), were more tolerant of water deficits and had narrower xylem vessels potentially offering more cavitation resistance than species with narrower distribution ranges, *E. dunnii* and *E. saligna* (Table A-2). With a large coast-to-inland aridity gradient in eastern Australia, traits such as cavitation resistance, stomatal sensitivity to *D* and tolerance of high water deficits may allow a larger distributional range for species, consistent with the idea that drought tolerance shapes plant distributions (Engelbrecht et al. 2007). It is important to note that within a species range large differences in physiology and morphology can be observed (Ladiges 1974), and accordingly further studies comparing these species across their respective species ranges would be informative. While *E. crebra* and *E. tereticornis* share a very similar seed source (< 60 km apart) and have significant overlap in their distributions, there are also large areas inland where these species do not co-occur (see Atlas of Living Australia, http://www.ala.org.au). I observed similarities in *E. crebra* and *E. tereticornis* in drought tolerance and J_S response to D, but differences in stomatal sensitivity to D, which could be attributed to plasticity (Roy and Mooney 1982; Nicotra and Davidson 2010) or potentially differences in the seed source exposure to local climate extremes (Gutschick and BassiriRad 2003). In order to ensure plantation survival, productivity and water-use efficiency, and subsequently conserve water resources, it is important that plantation managers consider species seed source, species tolerances to climate extremes, and the types of climate extremes the plants have adapted to when selecting species for certain climatic conditions, particularly given predictions of significant climatic changes in the future.

2.5.3 *Conclusions*

Climate of origin had an influence on characteristics defining the stomatal sensitivity to D, including G_C and J_S responses to D, and the D at stomatal closure ($e^{b/m}$), amongst *Eucalyptus* species. Species originating from more humid climates had a higher water use and also a higher stomatal sensitivity to D, and selection where these species have historically occurred would result in faster growing trees in favourable climate conditions and stomatal closure to maintain the hydraulic integrity of the plant when conditions were unfavourable. This is in contrast to sub-humid and intermediate species, which have been selected by their environment to maintain conductance despite high water deficits being reached, and seemed to rely on a reinforced hydraulic system (indicated by narrower vessels) rather than high stomatal sensitivity to *D*, to prevent cavitation from occurring. Stomatal and hydraulic characteristics are important in understanding how ecosystems will tolerate and respond to current and future climates, impacting survival, productivity and water-use efficiency, and emphasising the need for further comparison of species that avoid or tolerate large water deficits. The relationship between water use characteristics and climate of origin presents an opportunity for incorporating species specific parameters into Penman-Monteith models, which will improve predictions of survival and water use under current and future climates. The capacity to accurately predict survival and water use has implications for the selection of plantation species, identification of vulnerable ecosystems, conservation of catchment water supplies and enhancing plantation productivity under current and future climates.

The *Eucalyptus* common garden at 3 years old in February 2010 (top), measuring height with a height pole on an *E. saligna* tree (left) and an *E. crebra* wood disk taken during harvest in May 2010. Image source: David Ellsworth (top) and Aimee Bourne.

Chapter 3: Species climate range influences leaf water relations, xylem anatomy, hydraulic and stomatal traits in seven *Eucalyptus* species

3.1 Abstract

Plant hydraulic traits influence the capacity of species to grow and survive in water limited environments, but their comparative study at a common site has been limited. I examined whether selective pressure of species originating in drought prone environments constrains hydraulic traits among related species grown under common conditions. I measured leaf tissue water relations, xylem anatomy, stomatal behaviour and vulnerability to drought induced embolism of six *Eucalyptus* species growing in a common garden to determine whether these traits were related to current species climate range and to understand linkages between the traits. Hydraulically weighted xylem vessel diameter, leaf turgor loss point, osmotic pressure at full turgor, the water potential at stomatal closure and vulnerability to drought induced embolism were significantly ($p < 0.05$) correlated with climate parameters from the species range. There was a co-ordination between stem and leaf parameters as Ψ_{TLP}, P_{12} and g_{s50} were significantly correlated. The correlation of hydraulic, stomatal and anatomical traits with climate variables from the species' original ranges suggests that these traits are genetically constrained. The conservative nature of xylem traits in *Eucalyptus* trees has important implications for the limits of species responses to changing environmental conditions and thus for

species survival and distribution into the future, and yields new information for physiological models.

3.2 Introduction

Sensitivity to drought has been identified as an important factor shaping plant species distributions and community composition (BassiriRad et al. 1999; Engelbrecht et al. 2007; Kursar et al. 2009). The ability of plant species to grow and survive under different moisture regimes and to withstand drought is dependent on a number of physiological, anatomical and morphological traits including stomatal regulation of water loss, osmotic adjustment of leaf turgor loss point, rooting depth and xylem structure as well as whole-plant adjustments such as root and leaf mass fraction, leaf area ratio and root length (Anderson et al. 1996; Villar-Salvador et al. 1997; Schulze et al. 2006; Baltzer et al. 2008; Bourne et al. 2015; Pfautsch et al. 2016). Xylem cavitation resistance has emerged as a key variable in relation to plant drought resistance and the distribution of plants across gradients of water availability (Kursar et al. 2009; Choat et al. 2012; Schuldt et al. 2015; Anderegg et al. 2016). Low precipitation and high evaporative demand will drive high potential evapotranspiration and a decline in plant water potential and increase in xylem tension. Although declining water potentials can be delayed by stomatal closure and leaf shedding, prolonged drought will eventually result in extensive xylem cavitation, a sharp reduction in hydraulic conductivity, canopy die back and ultimately death of the whole plant (Choat 2013; Nardini et al. 2013).

Stomatal function is linked with hydraulic characteristics because (a) the plant hydraulic system controls the rate at which water can be transported to the canopy (Sperry et al. 2002; Brodribb and Holbrook 2003), and (b) stomatal regulation must maintain xylem water potentials within a range that limits cavitation to maintain hydraulic integrity (Delzon and Cochard 2014). Plants with high g_s and photosynthetic rates therefore generally require high K_h (Brodribb and Feild 2000; Santiago et al. 2004; Choat et al. 2011). Hydraulic conductivity is proportional to the sum of all conduit diameters to the fourth power (Tyree and Ewers 1991a), thus a small increase in vessel diameter results in large increases in K_h. However, xylem traits that promote high K_h may also increase the risk of cavitation and hydraulic dysfunction within the plant (Loepfe et al. 2007; Sperry et al. 2008). Subsequently, plants face trade-offs between hydraulic safety (e.g. high cavitation resistance) and hydraulic efficiency (high K_h) in order to survive and maintain productivity within the climate of their native distribution (Sperry et al. 2008; Pittermann et al. 2010; Johnson et al. 2011).

In the context of plant hydraulics, efficiency is defined as the amount of water transpired for a given pressure drop along the hydraulic pathway (Tyree et al. 1994); thus, a more efficient hydraulic system requires a smaller pressure drop to transport a given amount of water and is usually promoted by wider, longer xylem vessels. Conversely, hydraulic safety is promoted by narrower, shorter, more frequent xylem vessels and thicker, less porous pit membranes in the hydraulic system (Sperry et al. 2006; Loepfe et al. 2007; Sperry et al. 2008; Jansen et al. 2009). These adaptations enhance drought tolerance (Baas 1986; Choat et al. 2005), the degree to which a plant has adapted to maintain cell water status under dry or arid conditions (Oliver et al. 2010), by limiting the spread of embolism between xylem vessels via air-seeding (Loepfe et al. 2007; Choat et al. 2008). However these adaptations also compromise hydraulic efficiency, resulting in lower maximum K_h and therefore the rate at which water can be delivered to the transpiring leaf surface for a given pressure gradient (Sperry et al. 2008; Pittermann et al. 2010). This trade-off between safety and efficiency (Nardini and Salleo 2000; Sperry et al. 2008;

Johnson et al. 2011) forms the basis for the coordination of stem hydraulic traits and consequently their relation to stomatal traits previously reported (Brodribb et al. 2003; Meinzer et al. 2009). Studies examining the coordination between stomatal conductance and stem hydraulics have reported that the point of stomatal closure occurs prior to the onset of cavitation and is related to stem vulnerability and Ψ_{TLP} in different species (Brodribb and Holbrook 2003; Brodribb et al. 2003; Martorell et al. 2014). However, uncertainty remains about how these safety vs efficiency trade-offs are related to species climatic distribution, particularly when species are grown in a common climate. Understanding this relationship is an important precursor to predicting how species survival and productivity can be affected under current and future climatic conditions.

The selective pressures that prevail within a particular climate constrain species adaptations and subsequently which characteristics are maintained in future generations (Ramirez-Valiente et al. 2010). While species from arid regions may benefit from maintaining a low stomatal conductance, increasing water use efficiency by minimising transpiration, they also benefit from maintaining a hydraulic system that is resistant to cavitation and offers greater redundancy, i.e. narrow, short, frequent xylem vessels. Alternatively, a species originating from humid areas where water is readily available may benefit from anatomical characteristics oriented towards higher hydraulic conductance, assimilation rates and productivity (Fichot et al. 2009). Species oriented towards hydraulic efficiency typically exhibit stomatal closure at lower cavitation levels, allowing them high transport rates when water is available but minimising the risk of cavitation when water deficits develop (Klein et al. 2014; Manzoni 2014). In this way, while trait values favouring hydraulic safety may be selected for in species from more arid regions (e.g. Schuldt et al. 2015; Pfautsch et al. 2016), hydraulic efficiency and enhanced productivity through access to light, water and nutrient resources are more likely to drive selection in species from more humid regions. This is of particular significance in harsh climates where drought, high temperatures and high *D* are frequent, as is the case in many parts of Australia (Australian Bureau of Meteorology; http://www.bom.gov.au).

Previous studies have reported correlations between climate and stomatal behaviour (Héroult et al. 2013; Bourne et al. 2015), hydraulic characteristics (Maherali et al. 2004; Mitchell et al. 2008; Beikircher and Mayr 2009; Choat et al. 2012), vessel anatomy (Fisher et al. 2007; Medeiros and Pockman 2014; Pfautsch et al. 2016) and leaf tissue water relations (White et al. 2003; Bartlett et al. 2012). However, many of these studies compare different species along environmental gradients, which does not allow for determination of climatic effects on hydraulic, stomatal and leaf physiological traits of individual species. Common garden studies, which enable environmental conditions to be kept constant, can provide information on the influence of genotype on hydraulic traits (See Table A-1 for a summary of those on *Eucalyptus* species). Despite the importance of these traits in determining drought tolerance, survival and water use under different climatic conditions, relatively few studies have utilized common gardens to examine genotypic constraints on hydraulic traits. Vander Willigen *et al.* (2000) reported that leaf-specific and stem-specific conductivity were influenced by growth environment, while vulnerability to cavitation was genetically constrained. However, this was only considered in subtropical tree species and did not address how species climate range is related to these variables. Baltzer *et al.* (2008) reported that leaf water relations and lethal leaf water potentials were related to the species rainfall seasonality, however these were short-term experiments on seedlings in a glasshouse. They also did not consider xylem traits in their study which hold important implications for the water potential at which plants die (Kursar et al. 2009; Choat et al. 2012; Schuldt et al. 2015) as well as for leaf water relations outcomes (Wright et al. 2006a). To date, there are no studies that address the influence of species climate range on stomatal, hydraulic and anatomical traits in trees from sub-humid regions, or in species constrained to a single genus.

Using a common garden plantation of *Eucalyptus* species, I ask whether species climate range, specifically the mean annual precipitation and *D*, influences the leaf water relations, xylem anatomy, hydraulic and stomatal characteristics of a species. I expect that selective pressures from the species native climate constrain the genotypes maintained within a species and thus that the water use strategies employed by different species will be linked to the climate of species range. My specific hypotheses for this study are: (a) xylem anatomy, leaf tissue water relations, stomatal and hydraulic traits will correlate with precipitation and *D* of the native distribution of species, and (b) there is a co-ordination of stem hydraulics with leaf tissue water relations and the point of stomatal closure across species from different climatic ranges. Comparing multiple species from the same genus within a common garden, where confounding environmental and phylogenetic factors are minimised, allows extrapolation of genetic constraints on hydraulic, anatomical, stomatal and leaf tissue water relations traits.

3.3 Materials and methods

3.3.1 Study site

The study was conducted in a plantation of *Eucalyptus* species at Richmond, New South Wales, Australia (33º33'S, 150º44'E) near the Hawkesbury River on alluvial floodplain at an elevation of 25 m. Details of site, climate and soil characteristics for the site have been described previously (Section 2.3.1). Measurements were conducted on 4-5 year old trees of each species with average tree heights of 8.8, 8.3, 13.1, 15.5, 11.1 and 12.8 metres for *E. cladocalyx*, *E. crebra, E. dunnii, E. grandis, E. saligna* and *E. tereticornis* respectively. The driest part of each species range was used as an indication of their ability to tolerate dry conditions, and in accordance with the Koppen-Geiger climate classification system I classified *E. cladocalyx* and *E. crebra* as sub-humid climate zone species, *E. tereticornis* as a sub-humid/humid intermediate species, and *E. dunnii*, *E. grandis* and *E. saligna* as humid zone species. I used climate maps in Peel *et al.* (2007) overlain with species distributions from the Atlas of Living Australia (www.ala.org.au) for these classifications, consistent with Bourne *et al.* (2015).

3.3.2 Leaf tissue water relations

I conducted measurements of *PV* isotherms to measure leaf tissue water relations using techniques described by Tyree and Hammel (1972). A mature, sunlit leaf from six different trees of each of six species, namely *E. cladocalyx, E. crebra, E. dunnii, E. grandis, E. saligna* and *E. tereticornis*, was collected from the upper third of the tree crown. These measurements provided leaf tissue water relation traits as described by Koide *et al.* (2000) and Sack *et al.* (2003) and obtained using a spreadsheet available from Prometheus Wiki (Sack et al. 2011).

3.3.3 Anatomical characteristics

Estimates of vessel diameter and density were obtained for *E. cladocalyx*, *E. crebra*, *E. dunnii*, *E. grandis*, *E. saligna* and *E. tereticornis* trees. Sections of 20 µm thickness were taken from five separate branch segments from the upper third of the tree crown of different trees using a sliding microtome. Photographs of these sections, stained with Toluidine blue 0.05% (w/v), were taken using a digital camera (ProgRes® C14, JenOptik, Hallam, Australia) with the *Lumix Progress C4* software as seen at 40x (vessel counts) and 100x (vessel diameter) magnification under a microscope (Olympus BX60, Center Valley, PA, USA). I analysed these images and measured vessel diameter of all vessels >300 μm^2 throughout the sapwood of each section using *ImageJ* processing and analysis software (Abramoff et al. 2004). D_h, consistent with that measured by Sperry and Saliendra (1994), was calculated for interspecific comparisons of vessel anatomy and correlations with climate.

3.3.4 Stem hydraulics

Five to six branches from separate trees of *E. cladocalyx*, *E. crebra*, *E. dunnii*, *E. grandis*, *E. saligna* and *E. tereticornis*, with up to six 4-9 cm long subsamples per branch, were harvested using a boom lift (Snorkel MHP13/35 Trailer Mounted Lift, Snorkel Ltd, Meadowbrook, Qld, Australia) and measured for stem hydraulic characteristics. Xylem vulnerability curves were constructed using the bench dehydration method (Sperry et al. 1988) where branches were progressively dried down at 25 ºC for between 1 and 12 cumulative hours dependent on species selected, branch size and the desired water potential. Regardless of segment lengths considered, bench dehydration provides more accurate vulnerability estimates for long-vesseled species, prone to the ‘open vessel’ artefact, than either air injection or centrifuge methods (Choat et al. 2010; Cochard et al. 2013; Choat et al. 2016). As *Eucalyptus* species have long vessels (>1 m length) bench dehydration was used here.

Xylem tension was relaxed prior to measurements in order to avoid possible excision artefacts (Wheeler et al. 2013; Torres-Ruiz et al. 2015). Preliminary testing of xylem tension relaxation in these species revealed that rehydration for up to 2 hours followed by progressive cutting back underwater from upstream and downstream ends achieved best results. Accordingly, stems were harvested close to dawn and rehydrated for 2 hours in a walk in cold room set to 4 ºC. The first cut was made to the upstream end of the branch underwater, at least 30 cm from the desired segment, and left to rehydrate for 10 minutes. Successive cuts were made in two minute intervals, allowing rehydration between each cut, alternating between upstream and downstream ends of the segment. Water potentials on excised leaves were measured prior to cutting the branch using a Scholander type pressure chamber (Model 1505D Pressure Chamber Instrument, PMS Instrument Company, Albany, OR, USA) and K_{initial} and K_{final} were logged using a digital liquid flow meter (Liqui-Flow L10, Bronkhorst High-Tech BV, Ruurlo, Gelderland, Netherlands) and flow analysis programs FlowDDE (V. 4.69) and FlowPlot (Versions 4.69 and 3.34 respectively, Bronkhorst FlowWare, http://downloads.bronhorst.com).

3.3.5 Stomatal conductance and water relations measurements

Measurements of g_s and Ψ_{stem} were made on progressively dehydrated *E. cladocalyx*, *E. crebra*, *E. dunnii*, *E. grandis*, *E. saligna* and *E. tereticornis* branches over thirteen sunny days at times when trees were well hydrated post rainfall events and evaporative demand was not sufficiently high to cause rapid dry down of branches. Branches were collected from the upper third of five trees of each species using a boom lift, cut underwater and then transferred to water-filled buckets. Branches were kept in the sun and left to transpire for 30 minutes prior to measurements while leaves on secondary branches adjacent to g_s measurement leaves were bagged and foiled, ready for measurements of Ψ_{stem}. Four open-flow portable photosynthesis systems (Li6400, Li-Cor, Inc., Lincoln, NE, USA) and a pressure chamber were used to measure g_s and Ψ_{stem} respectively. Cuvette light levels and temperatures were adjusted to ambient conditions, as determined from a nearby weather station (Héroult et al. 2013; Bourne et al. 2015) and the portable photosynthesis systems. Chamber humidity was maintained as close as possible to ambient conditions at the beginning of measurements (~70% humidity) by removing a small amount of water vapour commensurate with that expected to be added by leaves during measurements. For each measurement, the CO_2 mole fraction within the chamber and at the leaf surface (C_a) was set at ambient levels (390 µmol·mol^{-1} CO_2) by mixing CO_2 from an internal source (CO_2 cartridge) with outside air. Stomatal ratios determined from microscopic observations of stomatal peels were used to correct stomatal ratios for each species. Three to eight fully expanded, healthy leaves on each branch were placed inside the cuvette and then logged five times for each g_s observation once the leaf reached equilibrium (coefficient of variation for A_{net} and g_s <1%). *E. tereticornis* and *E. saligna* only had overwintering leaves while all other species had spring leaves, yet stomatal conductance values were consistent with those conducted on spring leaves for *E. tereticornis* from an earlier measurement campaign. Measurement leaves and adjacent foiled and bagged leaves were then measured for Ψ_{leaf} and Ψ_{stem} respectively. Branches were then dried to more negative water potentials by removing them from the bucket and g_s and Ψ_{stem} were measured regularly until all available leaves were exhausted or g_s reached zero. An alternative measurement method involved connecting branches to a 2 mmol KCl reservoir and injecting india ink at the upstream end of the tubing, and while this method yielded the same results, it was more laborious and hence not used for all measurements.

3.3.6 Statistical analyses

Species climate range and distributional coordinates of the different species were mined from Atlas of Living Australia (http://spatial.ala.org.au/; accessed 12th December 2014) and synthesised and plotted using *R* (R Development Core Team 2015). A drawback of this approach is that it may include planted trees as well as those in their native distribution. The ALA species distributional data were trimmed to include only data between the 1st and 99th percentile as a small number of locations, such as those from botanical gardens and herbariums, were outside the natural distributional range of species. I conducted pairwise Pearson correlations of traits from species leaf tissue water relations measurements against specific climate parameters of the species range using *JMP* (v. 5.0.1, SAS, Cary, NC, USA). A large number of climate parameters averaged across the species range are inter-related, and I focussed analysis on a few of these as key climate indicators (mean annual precipitation, and D_{max}), with a wider set of climate parameters considered elsewhere (see Table S1). D_{max} was used in analyses in the form of $1/D_{max}$, consistent with stomatal models based on hydraulic theory and the negative exponential relationship between stomatal conductance and D (Tardieu and Davies 1993; Leuning 1995; Oren et al. 1999). The Ψ_{stem} at stomatal closure was assessed at two points, the beginning of stomatal closure (g_{s50}; when g_s reached 50% of g_{smax}) and complete stomatal closure (g_{s5}; when g_s reached 5% of g_{smax}) by considering the point at which g_s declined below a certain point of the maximum measured g_s using all g_s measurements. These parameters were used as indicators of stomatal behaviour as complete stomatal closure ($g_s = 0$) was not measured. Sigmaplot (v. 11.0, Systat Software, San Jose, CA, USA) was used for fitting g_s vs Ψ_{stem} curves and obtaining 95% confidence intervals using sigmoidal 3 parameter (*E. cladocalyx* and *E. crebra*), sigmoidal 4 parameter (*E. saligna* and *E. tereticornis*), chapman (*E. dunnii*) and quadratic (*E. grandis*) functions based on those that best represented the data. A Weibull function as re-parameterised by Ogle *et al.* (2009) was used for fitting vulnerability curves, from which P_{50} values and 95% confidence intervals were calculated in *R* (R Development Core Team 2015) using the fitplc package (Duursma 2014).

3.4 Results

3.4.1 Climate range is related to leaf tissue water relations, xylem anatomy, stomatal and hydraulic characteristics

All but one of the *Eucalyptus* species in my study were distributed along the eastern coastline of Australia with some species, such as *E. crebra*, distributed into more arid, inland regions of Australia (Figure 1-3). The exception to this was *E. cladocalyx*, which was distributed along the south-eastern coastline of Australia and was also distributed into more arid, inland regions (Figure 1-3). *Eucalyptus dunnii*, *E. cladocalyx*, *E. grandis* and *E. saligna* had narrower distributional ranges compared to the other species and *E. dunnii* had the most restricted distribution of all species (Figure 1-3), though the native range of *E. cladocalyx* is also very narrow (not shown) existing in few native pockets in the Flinders Ranges, Eyre Peninsula and on Kangaroo Island. The *D* and MAP of climate range differed across species with sub-humid species, *E. cladocalyx* and *E. crebra*, having a lower MAP and higher *D* compared to the humid species, *E. saligna*, *E. dunnii* and *E. grandis*, where *E. tereticornis* was intermediary between the two (Figure 1-3).

Species climate range was related to leaf tissue water relations and xylem anatomy of the six *Eucalyptus* species studied (Figures 3-1 and A-1 and Tables 3-1, 3-2 and A-2). Ψ_{TLP}, *VIGR*, Π_o, g_{s50}, $\mathrm{D_h}$ and P_{50} were correlated with MAP (R^2 = 0.76, 0.84, 0.66, 0.93, 0.75 and 0.53 respectively; Figures 3-1a, b, c, d, e and f, Table S1) such that species from more humid regions with a higher mean annual precipitation of species range had higher Ψ_{TLP}, *VIGR* and $\mathrm{D_h}$ and higher (less negative) P_{50} than species from sub-humid regions. These correlations were qualitatively similar for the inverse of maximal D (Table A-3). Mean annual aridity index and monthly maximum water deficit were also significantly correlated with leaf tissue water relations, anatomical, hydraulic and stomatal characteristics (Table A-3). Furthermore, a coordination and correlation between these stem and leaf characteristics was also observed (Figure 3-2, Table A-3) where Ψ_{TLP} was significantly positively correlated with $VIGR$ ($R^2 = 0.73$, $p = 0.03$) and negatively correlated with g_{s50} and P_{12} ($R^2 = 0.89$ and 0.64 respectively; Figure 3-2, Table A-3).

Table 3-1: Leaf tissue water relations, xylem anatomy and climate characteristics of 6 *Eucalyptus* species with standard errors of the mean across 7 trees of each species.

Species	Class	RWC_{TLP} (%)	ε (MPa)	VDens (mm^{-2})	VArea (μm^2)	VDiam (µm)	D_h (µm)	MAP (mm)	*AI*	$D_{\max}$ (kPa)	$WD_{\max}$ (mm)
E. cladocalyx	Sub-humid	84.1 ± 0.7	17.4 ± 0.6	113.01 ± 10.78	917.5 ± 58.0	33.1 ± 0.9	36.0 ± 1.3	537.49 ± 3.16	0.59 ± 0.004	0.78 ± 0.007	120.3 ± 1.0
E. crebra	Sub-humid	89.2 ± 1.5	26.4 ± 3.9	96.79 ± 5.89	1001.0 ± 130.3	34.3 ± 2.2	38.4 ± 2.0	743.37 ± 2.21	0.48 ± 0.001	1.00 ± 0.003	124.9 ± 0.4
E. tereticornis	Intermediate	90.0 ± 0.3	22.1 ± 0.9	78.36 ± 6.01	1195.1 ± 97.9	37.6 ± 1.6	41.2 ± 1.8	971.09 ± 3.13	0.70 ± 0.002	0.67 ± 0.002	87.6 ± 0.4
E. saligna	Humid	89.6 ± 0.6	22.6 ± 0.8	64.98 ± 3.85	1151.0 ± 96.0	31.4 ± 1.8	40.8 ± 1.7	1190.93 ± 3.98	0.93 ± 0.003	0.57 ± 0.001	51.2 ± 0.6
E. dunnii	Humid	87.6 ± 1.3	15.3 ± 1.8	95.23 ± 3.94	1154.2 ± 83.7	36.1 ± 1.3	39.6 ± 3.5	1109.58 ± 11.22	0.83 ± 0.009	0.57 ± 0.003	33.6 ± 1.2
E. grandis	Humid	88.9 ± 0.5	20.0 ± 0.8	97.52 ± 6.85	1171.3 ± 184.0	36.5 ± 2.4	41.4 ± 3.5	1476.37 ± 7.52	1.05 ± 0.006	0.53 ± 0.002	50.7 ± 0.7

Table 3-2: A comparison of xylem anatomical characteristics, leaf tissue water relations and hydraulic parameters of six *Eucalyptus* species.

Species	Ψ_{TLP} (MPa)	P_{50} (-MPa)	P_{12} (-MPa)	P_{88} (-MPa)	g_{s50} (-MPa)	g_{s5} (-MPa)	*VIGR* ($m^3 \cdot ha^{-1} \cdot yr^{-1}$)
E. cladocalyx	-3.13 ± 0.08	5.27	2.74	8.13	3.51	6.91	27 ± 5
		[6.23,4.57]	[3.97,1.22]	[2.30,1.32]	[5.07,1.94]		
E. crebra	-2.99 ± 0.11	4.90	3.26	6.41	3.30	6.35	52 ± 11
		[5.16,4.49]	[3.79,2.69]	[7.14,5.95]	[4.13,2.53]		
E. tereticornis	-2.47 ± 0.07	3.89	2.18	5.70	2.43	3.98	60 ± 4
		[4.30,3.51]	[2.75,1.60]	[6.99,4.87]	[3.30,1.68]	[4.74,3.27]	
E. saligna	-2.50 ± 0.04	3.40	1.81	5.16	2.34	4.57	133
		[3.78,3.08]	[3.60,0]	[6.72,4.34]	[3.23,1.73]		
E. dunnii	-2.27 ± 0.09	4.93	2.18	8.44	2.42	3.55	156 ± 31
		[5.46,4.49]	[2.76,1.56]	[11.12,7.12]	[2.86,1.84]	[4.74,2.37]	
E. grandis	-2.34 ± 0.04	3.83	2.14	5.63	1.95	2.28	165 ± 13
		[4.16,3.54]	[2.62,1.55]	[7.16,4.90]	[2.33,1.50]	[2.61,1.89]	

Errors presented are standard errors and numbers in square brackets are [lower, upper] 95% confidence intervals respectively.
No standard error is given for *E. saligna* VIGR as there was only one replicate plot.
In cases where upper and lower confidence intervals could not be estimated accurately they are not given.
g_{s5} and g_{s50} are the Ψ_{stem} at complete stomatal closure (5% of g_{smax}) and the beginning of stomatal closure (50% of g_{smax}) respectively.

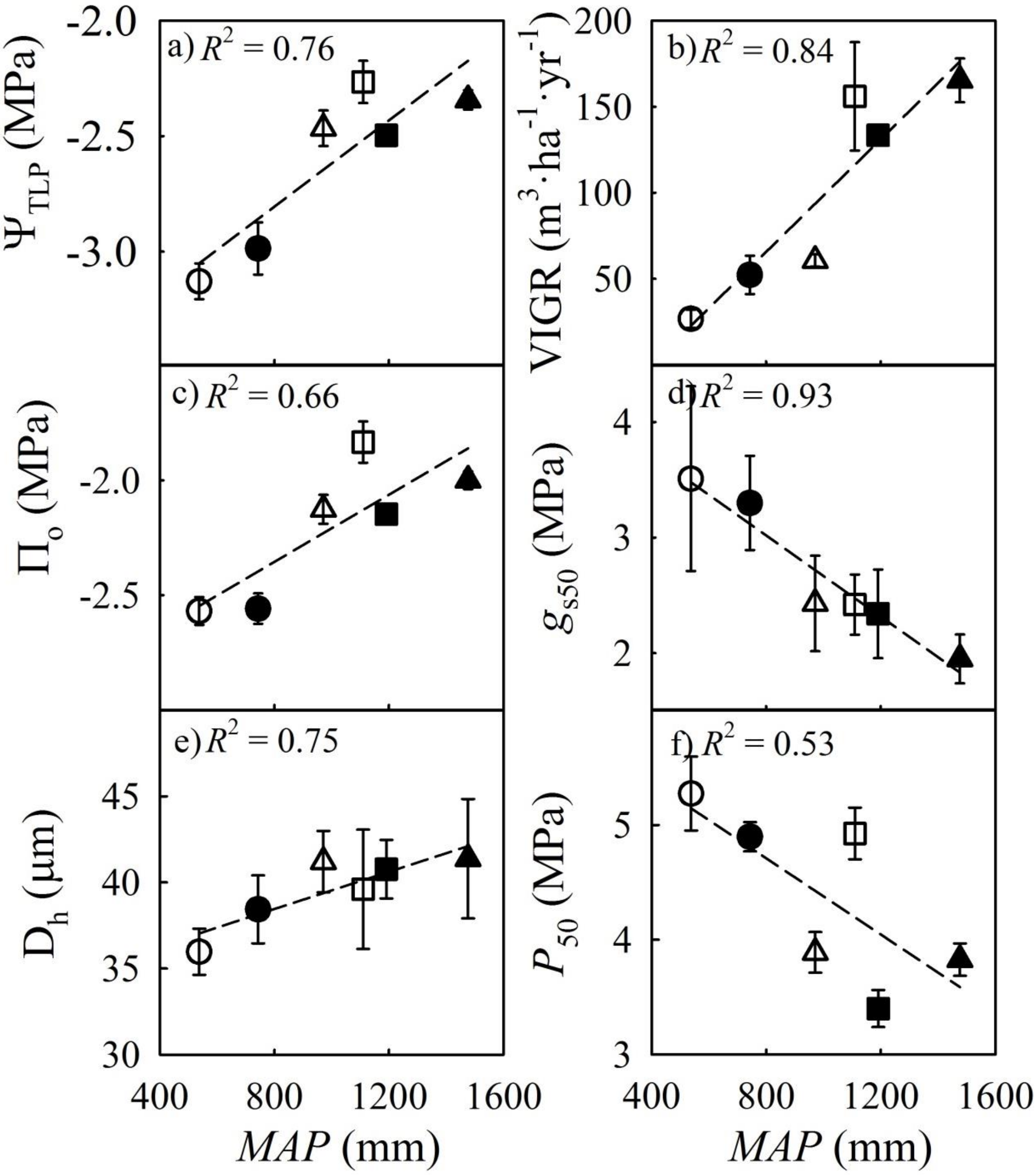


Figure 3-1: Correlations of leaf tissue water relations, xylem anatomy, growth and hydraulic characteristics with mean annual precipitation of the species range for six climatically contrasting *Eucalyptus* species. Symbols are as follows: *E. cladocalyx* (○), *E. crebra* (●), *E. tereticornis* (△), *E. saligna* (■), *E. dunnii* (□) and *E. grandis* (▲). Correlations and their significance are presented in supplemental material (Table S1). Dashed lines are shown as a guide. Error bars are standard errors of the mean for each species or climate parameter and are bidirectional.

3.4.2 Stomatal and hydraulic responses to Ψ_{stem}

Stomatal conductance declined as Ψ_{stem} became progressively more negative in all species (Figure 3-3). Species from humid regions tended to have higher maximal g_{s}, lower minimum g_{s} and a steeper decline in g_{s} for a given drop in Ψ_{stem} compared to species from sub-humid regions (Figure 3-3), though *E. saligna* had comparatively low g_{s} for a humid species (Figure 3-3d). Further, sub-humid species tended to maintain their g_{s}, still transpiring at Ψ_{stem} as low as -6 MPa (Table 3-2, Figure 3-3a-b), whereas humid species had a faster decline in g_{s}, completely closing stomata (g_{s5}) around or before -4 MPa (Table 3-2, Figure 3-3c-f). Stomatal closure (g_{s50}) started at Ψ_{stem} of -3.5 and -3.3 MPa in the sub-humid species *E. cladocalyx* and *E. crebra* (Table 3-2, Figure 3-3a-b) whereas in the intermediate and humid species *E. tereticornis*, *E. saligna*, *E. dunnii* and *E. grandis*, g_{s50} was at less negative Ψ_{stem} of -2.43, -2.34, -2.42 and -1.95 MPa respectively (Table 3-2, Figure 3-3c-f). E_{L} followed the same pattern as g_{s} for all species (data not shown).

Vulnerability to drought induced embolism varied between the six species (Figure 3-4). Species from sub-humid regions tended to have a shallower increase in *PLC* for a given drop in Ψ_{stem} (Figure 3-4a-b) compared to humid zone and intermediate species (Figure 3-4c-f). The sub-humid species, *E. cladocalyx* and *E. crebra*, maintained *PLC* of <50% until Ψ_{stem} -5.3 and -4.9 MPa respectively (Table 3-2, Figure 3-4a-b) whereas the intermediate and humid species, *E. tereticornis*, *E. saligna*, *E. dunnii* and *E. grandis*, reached P_{50} at -3.9, -3.4, -4.9 and -3.8 MPa respectively (Table 3-2, Figure 3-4c-f). For all but *E. dunnii*, sub-humid species P_{50} was significantly more negative than humid species P_{50}, based on 95% CI (Figure 3-4). Ψ_{TLP} was more negative in sub-humid than humid species, negatively correlated with g_{s50} and P_{12} ($R^2 = 0.89$ and 0.64 respectively; Figure 3-3b-c) and in all species was similar to, and within the confidence limits of, the Ψ_{stem} at P_{12} and g_{s50} (Table 3-2, Figure 3-4). The difference between the lower 95% CI of P_{50} and upper 95% CI of g_{s50} for *E. cladocalyx*, *E. crebra*, *E. dunnii*, *E. saligna*, *E. tereticornis* and *E. grandis* was -0.50, 0.36, 1.63, -0.15, 0.21 and 1.21 respectively (Table 3-2). Accordingly, species from humid regions tended to have no overlap between the Ψ_{stem} at P_{50} and g_{s50}, starting to close stomata on average 0.72 MPa before P_{50} was reached, whereas in sub-humid species g_{s50} tended to be within the 95% CI's of P_{50} (Table 3-2).

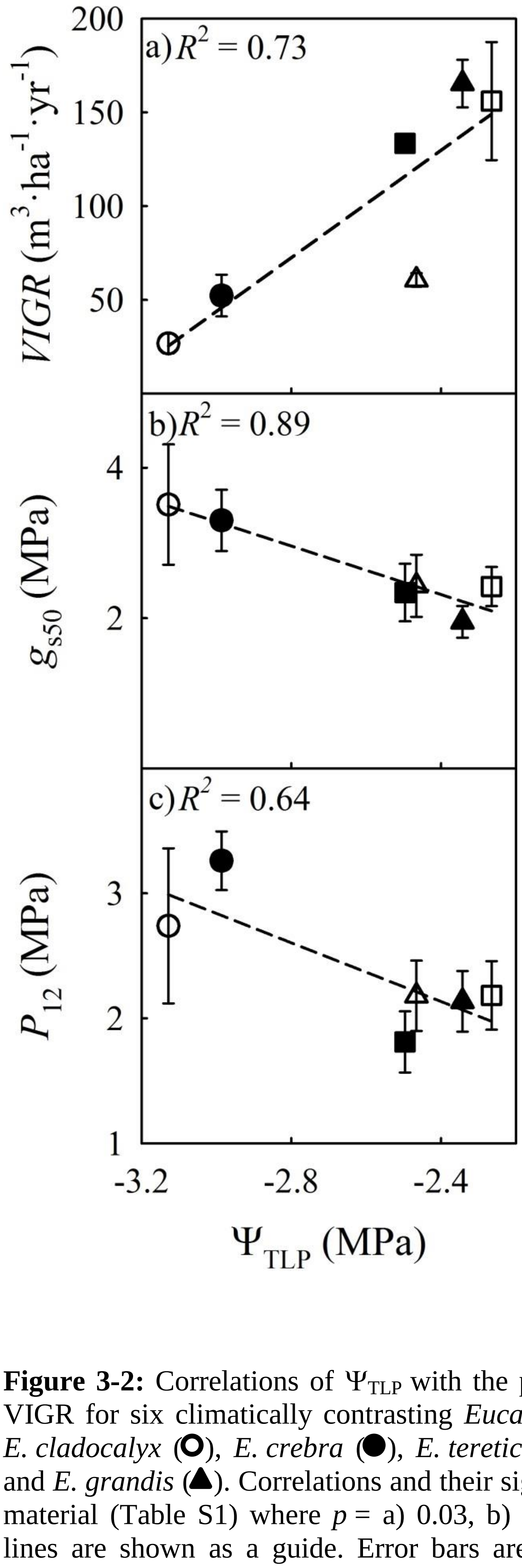


Figure 3-2: Correlations of Ψ_{TLP} with the point of stomatal closure (g_{s50}), P_{50} and VIGR for six climatically contrasting *Eucalyptus* species. Symbols are as follows: *E. cladocalyx* (○), *E. crebra* (●), *E. tereticornis* (△), *E. saligna* (■), *E. dunnii* (□) and *E. grandis* (▲). Correlations and their significance are presented in supplemental material (Table S1) where p = a) 0.03, b) 0.004 and c) 0.05 respectively. Dashed lines are shown as a guide. Error bars are standard errors of the mean for each species or climate parameter and are bidirectional.

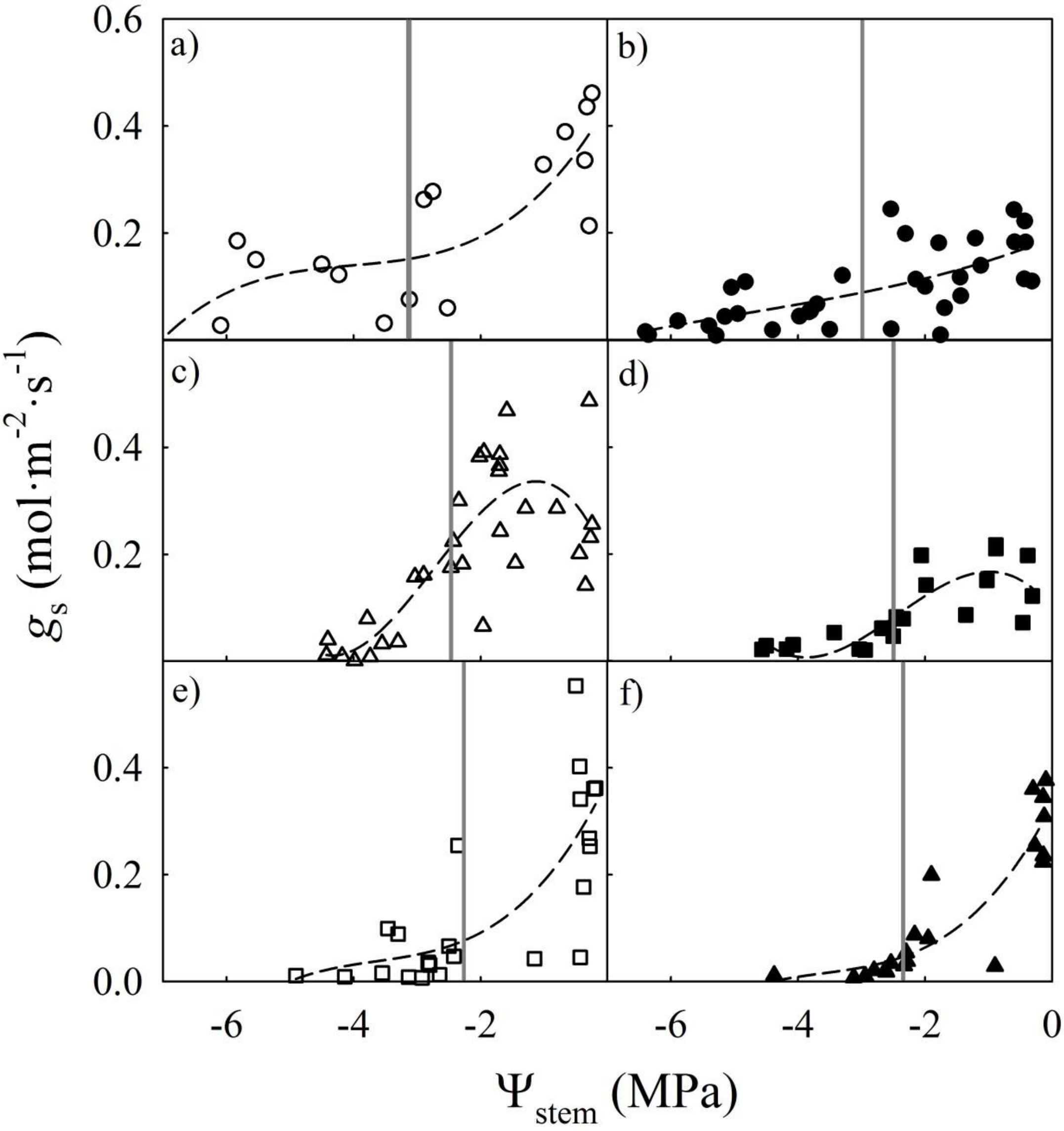


Figure 3-3: The response of g_s to declining Ψ_{stem} of six climatically contrasting *Eucalyptus* species, *E. cladocalyx* (○), *E. crebra* (●), *E. tereticornis* (△), *E. saligna* (■), *E. dunnii* (□) and *E. grandis* (▲). Black dashed line is a spline to highlight the relationship only. Vertical, grey lines represent Ψ_{TLP}.

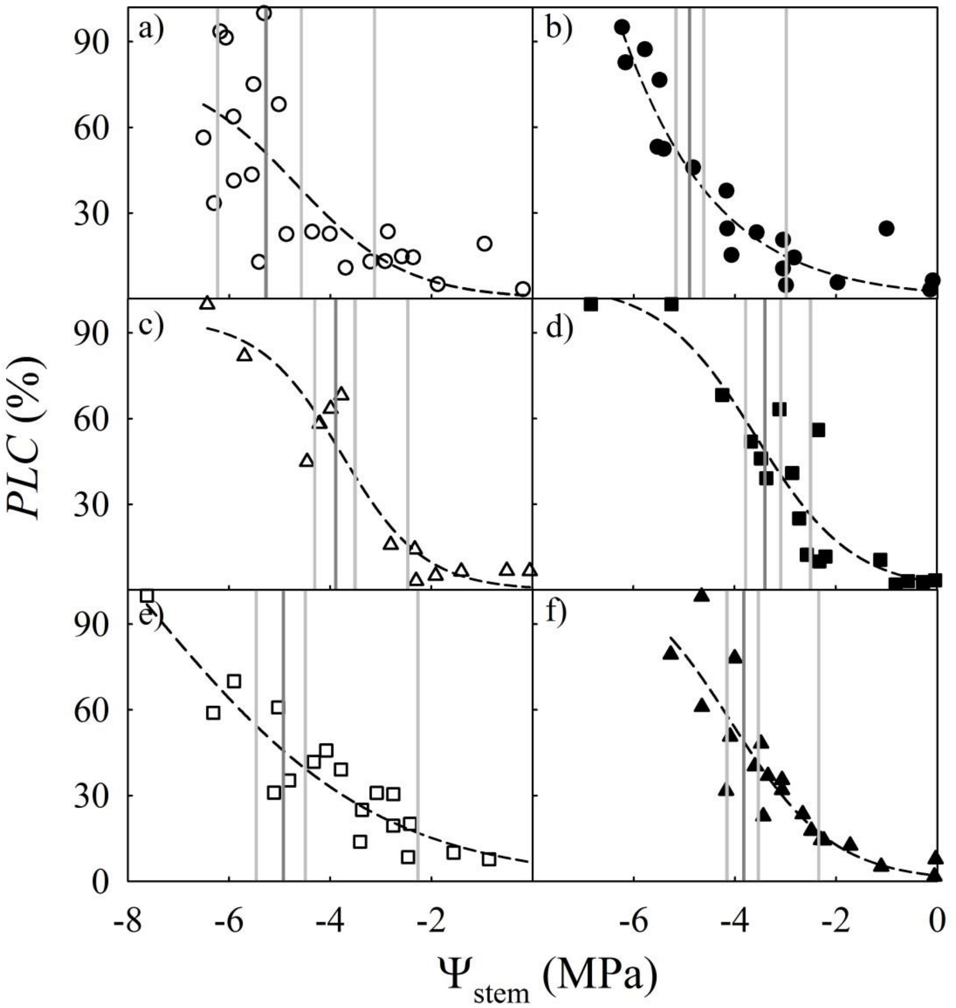


Figure 3-4: The response of *PLC* to declining Ψ_{stem} of six climatically contrasting *Eucalyptus* species, *E. cladocalyx* (○), *E. crebra* (●), *E. tereticornis* (△), *E. saligna* (■), *E. dunnii* (□) and *E. grandis* (▲). Dashed line represents the relationship for all *PLC* vs Ψ_{stem} data. Grouped vertical lines indicate P_{50} (dark grey centre line) and 95% CI (light grey adjacent lines) around the mean as obtained through *R* (R Development Core Team 2015) using the fitplc package (Duursma 2014). Separate grey vertical line indicates the Ψ_{TLP}, as in Table 3-2 and Figure 3-3.

3.5 Discussion

3.5.1 Climate range related to leaf tissue water relations and anatomical characteristics

I found evidence supporting my first hypothesis, that xylem anatomy, leaf tissue water relations, stomatal and hydraulic characteristics would correlate with the MAP and *D* of a species range when species are grown under common environmental conditions. I observed that species from more arid regions, indicated by a lower $1/D_{\max}$ and MAP, had lower Ψ_{TLP}, Π_0, *VIGR* and D_h and lower (more negative) P_{50} and g_{s50} than species from more humid regions (Figure 3-1). At the leaf level, a more negative Π_0 increases the cells capacity to maintain turgor at lower leaf water status (White et al. 2003; Bartlett et al. 2012); more negative Π_0 and Ψ_{TLP} also allow stomata to remain open at lower water potentials. Consequently, these characteristics facilitate stomatal closure at lower water potentials (more negative g_{s50}) and promote survival in sub-humid environments. My results suggest that a hydraulic system oriented towards high cavitation resistance is favoured in regions where droughts are frequent; however, these adaptations constrain the efficiency of water transport, subsequently limiting growth and disadvantaging the plant in areas of high water availability. Consistent with this, Pfautsch et al. (2016) reported that *Eucalyptus* species growing in arid environments have narrower xylem vessels than those growing in wet environments. The available data therefore strongly support the hypothesis that hydraulic traits of *Eucalyptus* species are adapted to the water availability of their native environment.

The correlations between hydraulic traits and species climate range provide evidence that these traits are genetically constrained in *Eucalyptus*. I observed that sub-humid species with distributions extending further inland (*E. cladocalyx* and *E. crebra*) had a higher drought tolerance than humid species with coastal distributions (*E. tereticornis*, *E. saligna*, *E. dunnii* and *E. grandis*), when considering drought tolerance to be a function of leaf tissue water relations (Figure 3-1 and Table 3-2), xylem anatomy (Figures 3-2 and A-1), stomatal behaviour (Figure 3-3) and vulnerability to cavitation (Figure 3-4). A study examining variation in xylem anatomical traits of *Eucalyptus* species growing under different rainfall regimes reported low intraspecific variation between populations, an observation consistent with low plasticity in these traits (Pfautsch et al. 2016). Similarly, previous work has suggested that phenotypic plasticity in cavitation resistance is limited (Martinez-Vilalta et al. 2009; Lamy et al. 2014), although there is some evidence of plasticity in this trait from other studies (Lopez et al. 2013; Schuldt et al. 2015). Consistent with the results of the present study, other common garden experiments have shown that differences in cavitation resistance are maintained when water availability is held constant (Sparks and Black 1999; Vander Willigen et al. 2000), providing further evidence that this trait is under strong genetic control.

3.5.2 *Coordination of stem hydraulics with stomatal behaviour*

In order to test my second hypothesis, that there is a co-ordination of stem hydraulics with leaf tissue water relations and the point of stomatal closure across species, I compared these characteristics across six climatically contrasting *Eucalyptus* species. I observed tight correlations of Ψ_{TLP} with the point of stomatal closure ($g_{\mathrm{s}50}$) and P_{12} (Figure 3-2), and a tight correlation between $g_{\mathrm{s}50}$ and P_{12}

($R^2 = 0.68$, $p = 0.04$; Table A-3) across all species, indicating a coordination of stem hydraulics with leaf tissue water relations and the point of stomatal closure. A correlation between Ψ_{TLP} and g_{s50} has previously been reported (Brodribb et al. 2003) and can be expected as stomata will close when guard cells lose turgor (Brodribb et al. 2003; Martorell et al. 2014). Interestingly, I observed that *E. cladocalyx*, a species from sub-humid regions, had statistically similar Ψ_{stem} at g_{s50} and P_{50} (overlap in 95% CI's; Table 3-2) while those from humid regions tended to be significantly different, with an average of 0.73 MPa difference in the 95% CI's of these parameters (Table 3-2, Figures 3-4 and 3-5). Significant differences between Ψ_{stem} at P_{50} and g_{s50} with similarities in Ψ_{stem} at P_{12} and g_{s50} have been observed previously for multiple different species (Brodribb et al. 2003). The results presented here suggest that *Eucalyptus* species from humid regions have a more conservative approach to stomatal closure, protecting the hydraulic system by closing stomata prior to significant cavitation (P_{50}), while species from more arid regions maintain low conductance even once the Ψ_{stem} at P_{50} is reached. This indicates some degree of coordination of leaf and stem hydraulics with stomatal behaviour in the species considered here.

Coordination of leaf and stem hydraulics with stomatal behaviour allows the plant to work within narrow safety margins (Choat et al. 2012), ensuring minimal cavitation (safety) while still enhancing transpiration (efficiency) and subsequently productivity, thus benefiting the plant regardless of the environmental conditions it encounters. Consistent with this, I observed a tight relationship between volume growth rates (*VIGR*) and physiological traits such as leaf water relations (Ψ_{TLP}, Π_0) and the point of stomatal closure (g_{s50}, g_{s12} and g_{s5}). This confirms that variation in the hydraulic strategies of these species is translated into differences in productivity and growth at the whole plant level. Overall, my results support the existence of a fast-slow spectrum of functional traits in *Eucalyptus* from different climate ranges (Reich 2014), with species from more arid climates occupying the slow (conservative) end of this trait spectrum.

3.5.3 Conclusions

Species climate range was tightly correlated to leaf tissue water relations, xylem anatomy, the Ψ_{stem} at stomatal closure (g_{s50}) and hydraulic characteristics of six different *Eucalyptus* species. Additionally, I observed coordination between stem hydraulics, leaf tissue water relations and the point of stomatal closure, which has been hypothesised but infrequently tested, and a difference in water use strategy linked to species climatic range. I observed that species from more humid regions exhibited characteristics of an efficient hydraulic system, preventing hydraulic dysfunction through a conservative approach to stomatal closure. In contrast, sub-humid species with ranges extending into more arid regions maintained a safe hydraulic system, limiting g_{s} and cavitation with narrower xylem vessels, and maintained leaf tissue characteristics tolerant of water limitation, enabling continual conductance even at very negative water potentials. By conducting this study on species from a single genus and measuring all species in a common garden I eliminated much of the variation that could be attributed to different climatic or environmental characteristics. Despite minimising this variation, I still observed a significant correlation of climate with leaf tissue water relations, xylem anatomy, stomatal behaviour and hydraulic characteristics between climatically contrasting *Eucalyptus* species. Additionally, I observed a tighter coordination of stem hydraulics with leaf tissue water relations and the point of stomatal closure in humid compared to sub-humid species, indicating a difference between species in the water use strategy employed. These observations indicate that the species climatic range has a large influence on shaping species responses to the environment, supporting the theory that genetic factors influence the expression of leaf tissue water relations, xylem anatomy, stem hydraulics and stomatal behaviour. In these species, it is likely that genetics control plant structural traits in particular, since they pertain to xylem anatomy and thus are not easily modified when growing in different climates. These findings hold important implications for species survival and adaptations to changes in climate, and may assist plantation and land management decisions to ensure survival of native and planted ecosystems under future climate scenarios.

The Li6400 open-flow portable photosynthesis system used for measuring gas exchange (top left) while progressively blocking vessels with india ink (top right) while connected to a KCl reservoir (bottom). Image source: Aimee Bourne, May 2012.

Chapter 4: Competition and complementarity influence species transpiration, growth and soil moisture in mixtures

4.1 Abstract

Despite the key role that competition for water plays in water-limited systems, there are few studies that specifically examine the water relations implications of competitive interactions amongst trees in forest plantations, and fewer still that examine both inter- and intra-specific competition despite their pertinence for biodiversity plantations. To determine how competition between individuals in monoculture and mixture plantations influences soil moisture exploitation, water use, growth and response to changing water availability, I compared these parameters in two focal *Eucalyptus* species across plots with different species composition. I found that competition for soil moisture was asymmetric for within- versus among-species situations. This resulted in the suppression of *E. crebra* aboveground biomass growth (-96.1%) and water use (-52.9%) when in mixture with a faster-growing species, while individuals from the faster growing *E. tereticornis* experienced enhanced growth (+67.7%) and tree water use (+83.8%) in 2-species mixture compared to its monoculture. Water use of component individuals in mixture were less influenced by reductions in water availability than those in monoculture of both focal species, whereby maximal sap flux was reduced by 52% in *E. crebra* and 42% in *E. tereticornis* during periods of

low soil moisture. Although growth and water use responses were species dependent, my findings support the use of mixture plantings for enhancing plot-level and component species growth, and reducing the influence of low water availability, for the species considered here. These observations are important for the development and adaptations of plantation and catchment practices, particularly in regions of water limitation.

4.2 Introduction

Forest plantations have been established rapidly over the last two decades to help support demands for wood and timber products, accounting for 264 million ha of land worldwide (FAO 2010). A central objective of such plantations is to maximise wood production for as little resource use as possible and consequently, recent research has focussed on options for enhancing productivity of these ecosystems (Whitehead and Beadle 2004; Erskine et al. 2006). Recent reports have shown that plantations comprising a mixture of species may be more productive than monocultures (Forrester et al. 2010; Kunert et al. 2012). This is a well-recognised phenomenon (over-yielding) that has been established for herbaceous plants (Loreau and Hector 2001; Hooper and Dukes 2004) and more recently analysed in forested ecosystems (Forrester 2014). This over-yielding phenomenon arises from complementarity of species resource use patterns and species facilitation, two known mechanisms of beneficial species interactions (Harper 1977; Loreau 1998). Over-yielding by species in forest stands may provide an option for land and plantation management practices to increase production whilst also sustaining productivity during years that might be unfavourable to one component species (Hooper and Dukes 2004; Sapijanskas et al. 2014). However, as water is critical for tree growth (Binkley et al. 2004), over-yielding might also mean increased competition for water in component tree species growing in mixtures, or increased overall plot water use, thus making this alternative a useable option only in high-rainfall regions.

To date, complementarity of tree species growth strategies specifically leading to increased aboveground production in forest ecosystems are less well studied (but see

Forrester and Smith 2012; Grossiord et al. 2013; Forrester 2014; Grossiord et al. 2014b) compared to facilitation, particularly during species establishment (Forrester et al. 2006; Cavard et al. 2011; Lei et al. 2012; Wright et al. 2014). If two different functional groups or guilds of species are planted in combination, the differential resource requirements of the two can result in one species out-competing the other for growth resources, potentially resulting in suppression of the other species. This asymmetric competition (Hutchinson 1957; Vandermeer 1972; Forrester et al. 2006), is often attributed to asymmetry in tree size or height, leading to canopy stratification and suppression via competition for light resources (Forrester et al. 2004; Hunt et al. 2006). Another situation that can develop in multi-species plantations applies to the stratification of root systems for accessing soil moisture and nutrients (Neave and Florence 1994; Schulze et al. 1994; Khanna 1997). However, while root stratification is linked with increased production in herbaceous species mixtures (Trenbath 1975) this has not been reported for tree stands, despite studies reporting increased productivity in mixed forests (Forrester et al. 2010; Kunert et al. 2012; Jucker et al. 2014). Additionally, while some studies have examined soil moisture seepage (Sprenger et al. 2013), changes in soil water uptake depth (Grossiord et al. 2014a) and root stratification or soil moisture partitioning (Meissner et al. 2012; Grossiord et al. 2014c) in mixed species forests, few studies have considered their effect on plot soil moisture and subsequent tree transpiration (but see Forrester et al. 2010; Schwendenmann et al. 2015). Given that water availability is an important factor structuring ecosystems (Engelbrecht et al. 2007) it is important that soil moisture acquisition and its effect on component species transpiration is evaluated in mixed species forests. This information is particularly important in the selection of complementary species for mixture plantations and the improvement of land and plantation management practices.

Soil water resources and below-ground competition for water are important drivers of tree growth and water use, particularly in *Eucalyptus* plantations as they are grown in places where seasonal evapotranspiration usually exceeds incoming rainfall in at least one quarter of the year (Whitehead and Beadle 2004). Interspecific interactions and the competitive exploitation of soil moisture depend on species physiology and rooting characteristics as well as the environment and resource limitations of the ecosystem (Bertness and Callaway 1994; Callaway and Walker 1997; Forrester et al. 2006). These interspecific interactions can shift from facilitation during periods of resource limitation to competition when resources are non-limiting (Wright et al. 2014). Elucidating the effect of interspecific interactions on soil moisture acquisition enables better understanding of how transpiration of native forests, and consequently catchment water supplies, will be influenced by changes in climate. Additionally, determining how species responses vary between monoculture and mixture, and in response to differing environmental conditions, can assist in predicting how the survival of mixed ecosystems may react to changing climatic conditions.

The ability to tolerate changing water availability is an important factor shaping plant species distributions and community compositions (BassiriRad et al. 1999; Engelbrecht et al. 2007; Kursar et al. 2009), yet few studies have addressed how ecosystem composition influences this response. If a species mixture is composed of species with contrasting functional traits, then complementarity and facilitation in resource use (Loreau et al. 2001) should enable a reduction in competitive water stress within that ecosystem. Conversely, if species have similar functional traits (i.e.

functional redundancy) then the overlap in resource use may lead to enhanced competition and water stress for component species. Enhanced survival and resistance to low water availability has been reported for forbs, succulents, grasses and mixed marsh communities (Nagase and Dunnett 2010; Steudel et al. 2011), but these studies are inapplicable to forested ecosystems, and the few studies that have compared mixed forests are inconsistent. Grossiord and others (2014b) reported increased water stress and competition in species mixtures, supported by reports of lower soil moisture in mixtures due to enhanced competition (Grossiord et al. 2014c). Other studies have reported enhanced resistance to low water availability in mixtures (Lebourgeois et al. 2013; Pretzsch et al. 2013) although this has been reported to occur in some regions but not in others (Grossiord et al. 2014d). The discrepancies between these studies can be explained by differences in climatic, edaphic and developmental conditions, or species selection, but some general patterns still emerge such that if interactions between species improve water availability or uptake then they have a greater influence on sites where water is limited (Forrester 2014). However, given the large influence that water availability has on structuring ecosystems, and the variability observed in previous mixed species studies, it is important that the response to water availability in component species in mixtures is characterised. By determining how interactions in mixtures influence component species responses to changing water availability, we can begin to understand how species and ecosystem composition and survival might be influenced by changing climate conditions.

There are few studies addressing the outcomes of competition on soil moisture acquisition in multi-species forests versus in monocultures (Meissner et al. 2012; Sprenger et al. 2013; Grossiord et al. 2014c) despite its importance in predicting how ecosystem transpiration may be affected by changes in climate. A few studies have examined water use patterns for competing tree species growing in mixtures, however they were limited by indirect measures of tree water use (Meissner et al. 2012; Grossiord et al. 2014c), were confounded through the use of a N-fixing species (Forrester et al. 2010; Forrester 2015), considered only in deciduous species (Grossiord et al. 2014b) or under high rainfall conditions (Kunert et al. 2012; Sprenger et al. 2013) and often did not address soil moisture partitioning. All of these studies are limited in some way in the applicability of the findings to broad ranges of climates and native ecosystems, though some generalisations can be made regarding specific species, sites and conditions. Studies that have addressed soil moisture acquisition or partitioning have reported that interspecific interactions in species mixtures can result in reduced stand soil moisture availability during dry years (Grossiord et al. 2014c), reduced soil seepage rates (Sprenger et al. 2013) and increased soil moisture partitioning through root stratification (Forrester et al. 2010; Kunert et al. 2012; Meissner et al. 2012), consistent with the theory of niche partitioning of resource use (Vandermeer 1972). From this information, I expect that species mixtures will have increased soil moisture exploitation, root stratification and resource use compared to monocultures, enabling higher component species transpiration and growth. Specifically, I hypothesise that i) mixture plantations will more completely exploit soil water, thus reducing signs of competition in component species relative to when in monoculture, and ii) species in mixtures will be less affected by periods of low soil moisture than species in monocultures, due to complementarity of water access within species mixtures. I chose *Eucalyptus* species as a focus for this study as they are important plantation species globally (Forrester et al. 2006; FAO 2009), but are also known to have large effects on soil water supplies (Calder et al. 1997; Whitehead and Beadle 2004; Eamus and Froend 2006). I expected that the outcomes from a study with *Eucalyptus* mixtures would help inform alternative plantation options, especially involving changing rainfall regimes and other environmental variation.

4.3 Materials and methods

4.3.1 Study site

The study was conducted in a plantation containing plots with different species assemblages grouped on the same site and soil in Richmond, New South Wales, Australia (33°33’S, 150°44’E). The experimental plots were established in November 2011 on recently cleared *Eucalyptus sideroxylon* A.Cunn. ex Woolls plantations. The plantation contains two monoculture plots each of *Eucalyptus crebra* F.Muell. and *Eucalyptus tereticornis* Sm., four 2-species mixture plots of these two species in combination, and two 8-species mixture plots containing these two species and a set of locally native species. The other species in the 8-species mixtures were *Angophora floribunda* Sm., *Angophora subvelutina* F.Muell., *Eucalyptus elata* Dehnh., *Eucalyptus fibrosa* F.Muell., *Melia azedarach* L. and *Melaleuca linariifolia* Sm. All plots contained 160 trees planted at a density of 1622 trees·ha^{-1}, which resulted in 160 trees per species in monoculture plots, 80 trees per species in 2-species mixtures and 20 trees per species in 8-species mixtures. Trees were planted in a staggered arrangement to allow canopy closure and interaction between species to be observed in a short time period. Seedlings were fertilised one week after planting with a tablet of Initiator Systemic Plant Insecticide and Fertiliser (Bayer Australia Ltd, Pymble, NSW, Australia) placed 10 cm from the base of each tree. They were then watered as required for the first year of establishment (November 2011-February 2012 and August-November 2012), and replaced if mortality occurred within the first year. Species were chosen based on their diversity in stomatal sensitivity, tolerance of low water availability (Bourne et al. 2015), hydraulic characteristics and water use strategies (Chapter 3). I considered the driest part of each species range as an indication of their ability to tolerate dry conditions and of their water use strategy. Climate and soil characteristics of the experimental plots have been previously described (Section 2.3.1).

4.3.2 Meteorological parameters and soil moisture measurements

A customised meteorological station, as described previously (section 2.3.2), was used to measure meteorological parameters. Shallow soil moisture beneath each plot was recorded at half hourly intervals at depths of 5-35, 35-65 and 65-95 cm below the soil surface using time-domain reflectometry (TDR; CS616, Campbell Scientific Australia, Townsville, Qld., Australia). Soil temperature was recorded with CS107-L sensors (Campbell Scientific Australia, Townsville, Qld., Australia) placed alongside the TDR probes and was recorded at half hourly intervals in both monoculture plantations and one mixture plantation. Soil moisture at depth was measured using a neutron hydroprobe (503DR, CPN Corporation, Concord, California, USA) with a radiation source of 1.85 GBq (50 mCi) Americium-241: Beryllium. These measurements of soil moisture were taken monthly and surrounding major rainfall events from October 2013, in one set of replicate plots. Neutron probe and TDR measurements were tightly correlated with correlations averaged for 35 cm and 65 cm depths resulting in $r = 0.98$, 0.93, 0.96 and 0.82 for *E. crebra* monoculture, *E. tereticornis* monoculture, 2-species mixture and 8-species mixture plots respectively. Soil texture observations conducted during installation of neutron probe tubes, along with analysis of raw neutron probe counts, revealed a change in soil texture and water holding capacity at 35 cm and again at 125-150 cm depth in all plots. Accordingly, calibrations accounting for the change in soil texture and type throughout the soil profile were used to calculate soil matric potential, referred to as soil water potential, so that soil moisture could be compared across plots regardless of changes in soil texture.

4.3.3 Physiological measurements and allometric calculations

I measured diameter at 8 cm from ground, diameter at breast height (DBH; diameter 1.3 m from base), and tree height on all trees in each plot every two months for the first six months, every three months for the next year and biannually thereafter. Volume index (VI) for each tree was calculated as diameter^2 * height. In November 2013 two trees of *E. crebra*, *E. tereticornis*, *E. elata*, *E. fibrosa*, *A. floribunda* and *M. azedarach* were harvested from a mixture plot to obtain estimates of total aboveground biomass (AGB) and wood density by displacement at both 8 cm height and 1.3m height (DBH). Additional measurements of wood density using stem cores (Haglöf, Långsele, Sweden) were conducted in October and December 2014, which have been shown to yield similar results to those obtained from disks for these species (Bourne et al. 2015). AGB was estimated by applying allometric equations from Paul et al. (2013) to individual trees based on DBH measured in December 2014 for all species except *M. linariifolia*, for which the diameter at 10 cm (D10) was used due to branches below breast height. Species specific allometric equations were used for *E. crebra* and *E. tereticornis*, and the 'Generic *Eucalyptus* tree' allometric equation was used for *E. elata, E. fibrosa*, *A. floribunda* and *A. subvelutina*, while the '*Melaleuca* sp.' allometric equation was used for *M. linariifolia* (Paul et al. 2013). A generic, 'universal tree' allometric equation was used for *M. azedarach*, consistent with Paul et al. (2013). The total tree AGB measured on harvested trees was compared to allometrically estimated AGB for that individual tree to determine which allometric equation best fit the available data. *A. floribunda* and *A. subvelutina* biomass were calculated using the 'Generic *Eucalyptus* tree' allometric equation because *Angophora* and *Eucalyptus* species occur in the same family (Myrtaceae), are recognised to be morphologically similar (Brooker 2000) and because total AGB obtained from tree harvests were similar to those estimated using the 'Generic *Eucalyptus* tree' allometric equation. Given that allometric equations were chosen for the species based on best fit, estimates were tested against measured AGB on harvested trees within this study, and Paul et al. (2013) specifically addressed bias and precision through analysis of residuals within their study, with up to 3352 individuals used for the creation of generic allometric equations, I am confident that the use of generic allometric equations in certain instances would not have resulted in biased estimates of AGB for the species compared here. AGB increment (AGBI), height growth rate (HGR) and VI growth rate (VIGR) over one year were determined using the relative growth rate (RGR) equation of Fisher (1921):

$$RGR = \frac{W_2 - W_1}{T_2 - T_1} \tag{3}$$

where W_1 and W_2 were the initial and final AGB, VI or height respectively and T_1 and T_2 were the original and final census dates respectively. Relative growth rates were used here instead of basic growth measurements in order to more directly compare the growth of individuals in mixtures respective to those grown in monocultures over specified time periods, as is consistent with previous forest diversity studies (Forrester et al. 2006; Forrester 2014).

4.3.4 *Sap flux and tree water use*

Heat pulse velocity (HPV) probes (Tranzflo NZ Ltd., Palmerston North, New Zealand; James et al. 2002) were used to measure sap flux on three to four trees each of *E. crebra* and *E. tereticornis* in all plots. Trees were selected based on their diameter at 8 cm from ground at time of installation. I measured sap flux for 25 months from 1st December, 2012 until 31st December, 2014 following techniques described by Burgess and Dawson (2004) and Dawson et al. (2007). I installed probes into axially aligned insertion holes drilled 5 mm apart on the south side of the tree using a drilling guide and a specialised drill bit (1.8 mm diameter). Probes were initially installed 8 cm from ground level and then were moved 10 cm higher and to the side at three to six monthly intervals. Radial comparisons of sap flux on two trees of each species were undertaken during the study period. The HPV probes measured HPV at two points in the sapwood of each tree (5 and 15 mm from probe tip) at half hourly intervals. Heat pulse methods provide accurate measurements of sap flow in plant stems when reliable calibration procedures are used to relate the measured HPV to actual sap flow (Green et al. 2003). HPV sensors required small voltage corrections to provide a zero-flow baseline when there was no transpiration (Burgess et al. 2001; Dawson et al. 2007). Supplementary measurements of wood density, sapwood area, bark thickness and wound diameter from probe insertion were used in the correction of HPV measurements to obtain estimates of sap flux density (J_S) using methods described by Swanson (1994), Barrett et al. (1995) and Burgess et al. (2001). Wounding damage from sap flow probes was assessed using light microscopy observations and generated wounding diameters of 0.25 cm for both species, which were within the expected range (Swanson and Whitfield 1981; Barrett et al. 1995). The weighted mean method (Hatton et al. 1990) was used to integrate inner and outer estimates of sap velocity along with sapwood area to an estimate of whole tree water use (E, kg·m^{-2} sapwood·s^{-1}).

4.3.5 Leaf gas exchange and leaf properties

Diurnal measurements of leaf gas exchange were conducted over four sunny days during summer and autumn as prior work indicated that processes during this period have the strongest influence on intrinsic water-use efficiency and differentiate species (Anderson et al. 1996). Leaf gas exchange parameters (g_s and A_{net}) were measured every two hours on two recently fully expanded leaves of three replicate trees for each species in each monoculture and one 2-species mixture plot. Gas exchange measurements were conducted according to the protocol described by Héroult et al. (2013) using two open-flow portable photosynthesis systems (Li6400, Li-Cor, Inc., Lincoln, NE, USA) on leaves in the upper third of the tree crown. Prior to measurements, cuvette light levels and temperatures were adjusted to ambient conditions, as determined from a nearby weather station (Barton et al. 2010; Héroult et al. 2013 and described above) and the two portable photosynthesis systems. I measured pre-dawn water potential (Ψ_{pd}) prior to diurnal measurements, and leaf water potential (Ψ_{leaf}) immediately after gas exchange measurements, on all trees using a scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA). Half hourly shallow (<100 cm) soil moisture and meteorological data were measured on all dates.

Leaves were collected after Ψ_{leaf} was measured for determination of leaf fresh and dry mass (drying oven at 70 ºC for >48 hours), thickness (digital calipers, resolution 10 µm; Mitutoyo Handtools, South Melbourne, Vic., Australia) and leaf area (scanner and optical planimetry software; WinRhizo, Regent Instruments, Quebec, Canada). Leaf counts of all leaves on each gas exchange measurement tree were conducted in December 2013 in the week prior to diurnal gas exchange measurements whereby total canopy leaf area was calculated by multiplying the average leaf area for that specific tree against the total number of leaves counted. Carbon isotopic ratios ($\delta^{13}C$) were measured at the West Australian Biogeochemistry Centre (WABC, University of Western Australia, WA, Australia) using an Automated Nitrogen Carbon Analyser system consisting of a Sercon 20-22 mass spectrometer and an elemental analyser (Sercon limited, Crewe, Cheshire, UK). Two leaves from each of three trees in all plots in May 2013 and 2014 were analysed for $\delta^{13}C$ according to protocols outlined in Coplen et al. (2006) and Skrzypek (2013).

4.3.6 Competition indices

To compare the performance of species in mixtures to that in monoculture wI calculated a competitive index (C) for component trees, different to that commonly used in a forestry context (Biging and Dobbertin 1992; Vanclay 2006) but consistent with that described by Rewald and Leuschner (2009a) and referred to as 'relative yield' or 'relative productivity' in other diversity studies (Forrester et al. 2006; Forrester 2014; Forrester and Pretzsch 2015). The competitive index relates species RGR (equation 1 and Fisher 1921) in any parameter, in this case AGB, VI, HG and tree water use, in mixture to that in monoculture as follows:

$$C = \frac{RGR_{mix} - RGR_{mono}}{RGR_{mono}} \tag{4}$$

where C is the competitive index and RGR_{mix} and RGR_{mono} are the RGR of component trees in mixture and monoculture plots respectively. C was then multiplied by 100 to give a percentage change in the given parameter respective to monoculture.

4.3.7 Statistical analyses

Meteorological data and soil moisture data obtained from TDR and neutron probes was averaged to daily and monthly values (R Development Core Team 2015). Data obtained using the neutron probe was calibrated to give volumetric water content using data from moisture release curves specific to the soil in the area. The difference in soil moisture between dates was calculated and integrated across depths for each plot to obtain a value of plot soil moisture change per month and year using *R* (R Development Core Team 2015). Integrated soil water potentials for the upper 1.5 m of soil and below 1.5 m soil depths were calculated by obtaining the soil water potential for each of the separately measured soil depths and then averaging across all depths within the respective category (i.e. upper 1.5 m or below 1.5 m). Measurements of sap flow were scaled to J_S per unit sapwood or ground area using *R*. The J_S response to D was then evaluated during low and moderate soil moisture periods by filtering data for specific months and then binning data into 0.5 kPa bins, excluding bins containing <50 measurements. Low soil moisture was classed as months which had <5 mm rainfall or a maximal monthly $D \geq 6$ kPa (as in Bourne et al. 2015) and soil moisture <40% of that recorded at saturation (‘maximal’ soil moisture), namely October and November 2014. J_S was also filtered for sunny days, classed as days where total summed radiation was >6000 $W \cdot m^{-2}$ (approximately 800 $W \cdot m^{-2}$ for 7 hours a day), and then compared across plots and species within each plot using *R*. Average J_S was calculated across trees within each species within each plot. Unfiltered daily J_S was scaled to total tree water use per plot and summed to give a plot water use per month and per year using *R* (R Development Core Team 2015). Monthly values of soil moisture change and tree water use in each plot were summed and compared with annual precipitation to determine whether outgoing water use equalled incoming precipitation. The yearly tree water use was divided by AGB in December 2014 to calculate a yearly water-use efficiency (WUE) for each plot. Sigmaplot (v11.0, Systat, Inc., Chicago, IL, USA) was used to test for significant differences between plots and species measurements using t-test, Holm-Sidak test and ANOVA where indicated, and was also used to create figures.

4.4 Results

4.4.1 Meteorological conditions and soil water

Soil water potential was greater at deeper depths and steadily declined between rainfall events (Figure 4-1, arrows specify rainfall events). After 200 mm rainfall in December 2014, soil reached saturation at all depths at which point all plots had similar soil water potentials (Figure 4-1). Shallow soil water potential was similar in magnitude amongst different plots with the same sharp declines and increases corresponding with rainfall events throughout the study (Figure 4-1a, arrows). At 125 cm depth, soil water potential was similar in magnitude and response between 2-species mixture and *E. crebra* monoculture, and lower for *E. tereticornis* monoculture and the 8-species mixture (Figure 4-1b). While soil water potential was generally lower in monoculture plots than in the 2-species mixture at 125 cm depth, when grouped by plot type, monocultures were statistically similar to mixtures during all measurement months ($P = 0.36$, t-test). However, soil water potential at depth (>1.5 m) was significantly lower in monoculture than mixture plots during periods of water stress (October and November 2014, $P = 0.038$ and 0.046 respectively, t-test). Soil water potential was less variable temporally at depths below 125 cm (Figure 4-1c-d) being lower for *E. tereticornis* but similar across other plots at 225 cm depth (Figure 4-1c) and similar across all plots at 325 cm depth (Figure 4-1d). While other plots had similar soil water potential at 125 and 225 cm depths, *E. tereticornis* monoculture soil water potential was ~50% lower for these depths than all other plots at the beginning of the study period (Figure 4-1b-c), and declined more during 2014 at lower soil layers (225 and 325 cm depths) than in other plots (Figures 4-1c-d and A-2). With increasing soil depth, the

E. tereticornis monoculture reached minimum soil water potentials for respective depths at later dates (Figure 4-1b-d). The decline in soil moisture over time, providing an estimate of moisture depletion, varied between plots and at different depths. This decline was largest in *E. tereticornis* monoculture and 2-species mixture followed by the 8-species mixture then *E. crebra* monoculture (Figure A-2), however yearly soil moisture change was statistically similar between monoculture and mixture plot types (Figure 4-1; $P = 0.73$, t-test).

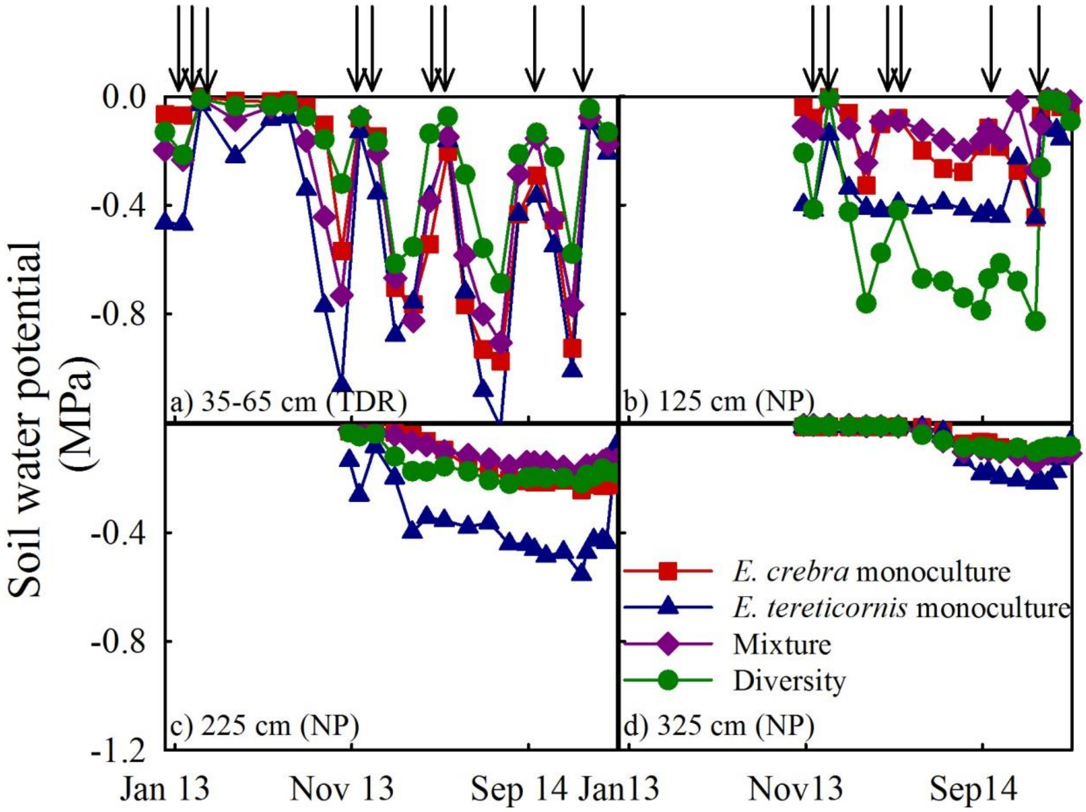


Figure 4-1: Monthly soil water potential at four depths using TDR (panel a) and neutron probe (NP; panels b-d) measurements for *E. crebra* monoculture (red square), *E. tereticornis* monoculture (blue triangle), 2-species mixture (purple diamond) and 8-species mixture (green circle) plots. The discussed measurement period for all panels is from 1st December 2012 until 31st January 2015, however NP measurements were only conducted from 1st November 2013 until 31st December 2014 and are spaced unevenly dependent on when measurements could be conducted. TDR measurements are the monthly average soil moisture and are presented monthly except for March and May 2013 which were omitted due to equipment malfunction. Arrows indicate dates where at least 100 mm rainfall was received in the 28 days prior, namely January-March and November-December 2013 and March, April, September and December 2014.

Evaporative demand (*D*) followed expected seasonal patterns (Australian Bureau of Meteorology, http://www.bom.gov.au) during the study period (December 2012 – December 2014) with summer (December through February) having the highest and winter (June through August) having the lowest *D* (Figure 4-2). Rainfall tended to be highest during the summer months and lowest during winter months in 2013, rising to a maximum in November 2013 and December 2014 (Figure 4-2). The fluctuations in soil moisture, rainfall and *D* were used to categorise months of moderate and low moisture conditions for comparisons of J_S between species across plot types. Months where maximal $D \geq 6$ kPa or monthly rainfall was <5 mm (Figure 4-2, arrows) and soil moisture was <40% of that recorded at saturation (October and November 2014) were considered periods of low water availability.

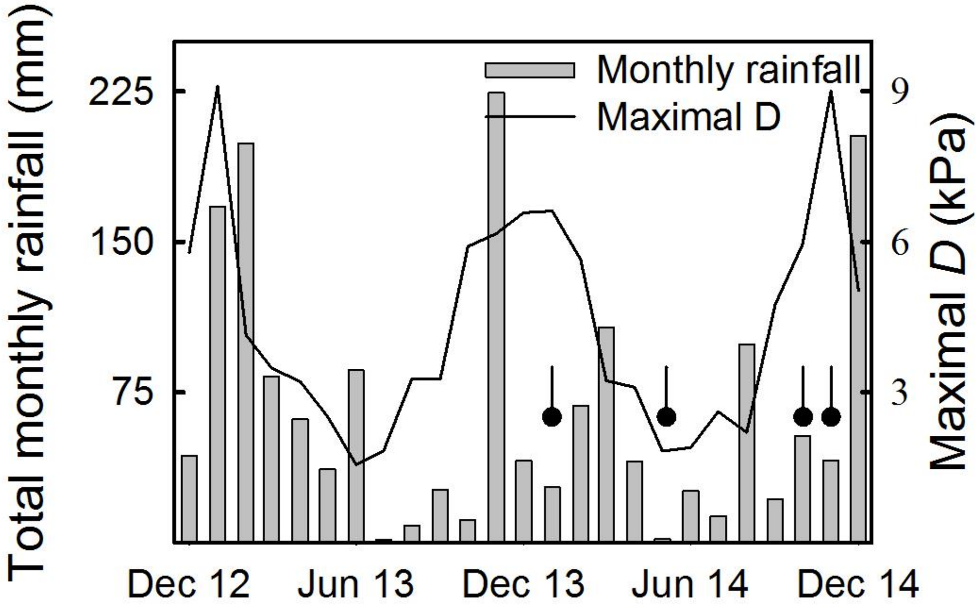


Figure 4-2: Total monthly rainfall (columns) and maximal *D* (line) recorded at the study site with a meteorological station over the study period of 1st December 2012 through to 31st December 2014. Dotted lines indicate months during the year of 2014 that were considered low water availability, i.e., months with <5 mm rainfall or maximal monthly *D* >6 kPa.

4.4.2 Sap flux density

J_S per unit sapwood area increased with increasing driving force (*D*) in all species and species-mixture combinations when soil water availability was favourable (Figures 4-3a and c). During low soil moisture conditions, J_S increased with increasing *D* in monocultures, but remained relatively constant across a range of *D* values for both species in mixtures (Figures 4-3b and d). J_S per unit sapwood area was similar across all plots for *E. tereticornis* but differed across plots for *E. crebra* during favourable moisture conditions (Figures 4-3a and c). Accordingly, during favourable conditions, maximal J_S was significantly different in monoculture compared to in 2-species mixture: 28% higher for *E. crebra* (Figure 4-3c; $P = 0.033$, Holm-Sidak test), and 19% lower in *E. tereticornis*. During low soil moisture conditions, J_S response to *D* was lower in *E. tereticornis* individuals in monoculture compared to mixture plots (Figure 4-3b) whereas *E. crebra* individuals were similar in J_S-D response across all plot types (Figure 4-3d). Low soil moisture resulted in significantly lower maximal J_S by 52% in monoculture for *E. crebra* ($P < 0.001$, Holm-Sidak test), and 42% lower maximal J_S, though not significantly lower, for *E. tereticornis* ($P = 0.076$, Holm-Sidak test). Maximal J_S was statistically similar across all other plots within a species regardless of soil moisture conditions (Figure 4-3; $P = 0.97$, ANOVA).

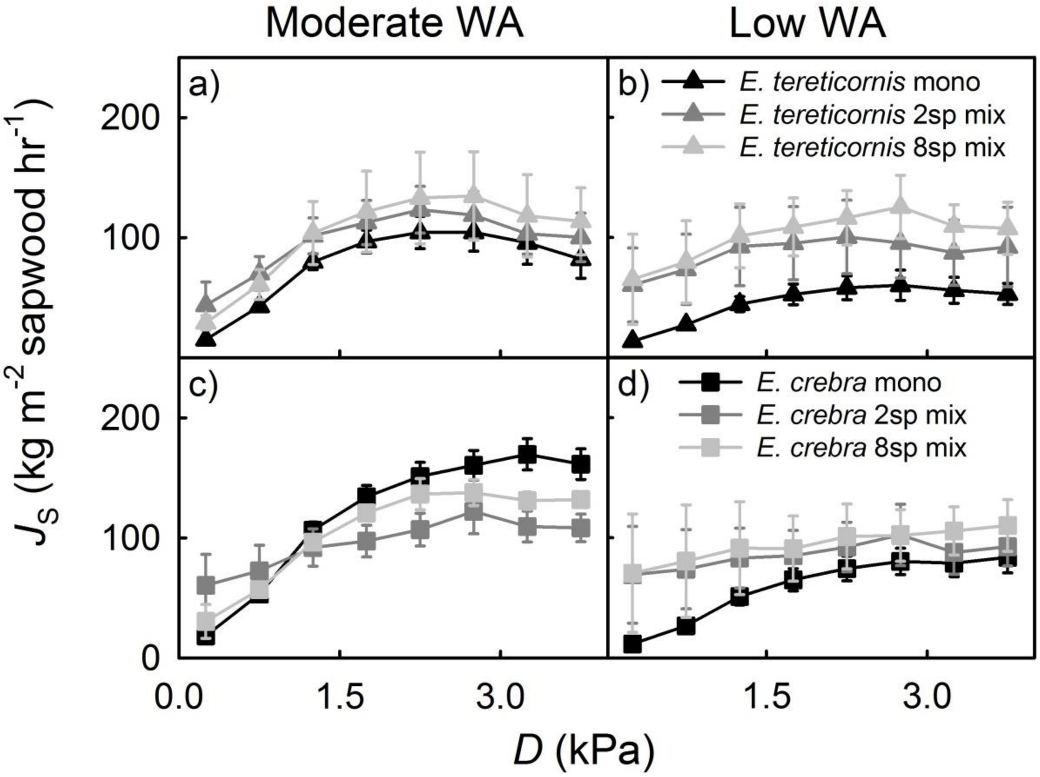


Figure 4-3: Component tree J_S (mean ± SE) during moderate (open symbols) and low (closed symbols) soil moisture periods for *E. tereticornis* (triangle) and *E. crebra* (square) trees in a-b) monoculture, c-d) 2-species mixture and e-f) 8-species mixture plots. J_S data was subsampled for months of low water availability according to meteorological and soil moisture measurements. Low soil moisture months were October and November 2014, and moderate water availability months were all other months in 2014 except winter months, June, July and August. Error bars show the standard error of the mean across 4-6 trees of each species in each plot type. Lines shown are as a guide.

4.4.3 Plot water use, soil moisture and growth

Plot water use, calculated as the summed tree water use of component species scaled to the plot level, was similar for 2-species mixtures and monocultures throughout the year except during September-November 2014, when the 2-species mixture used more water than either monoculture plot (Figure 4-4a). Low plot water use (Figure 4-4a) corresponded with low soil moisture (Figures 4-3, 4-4b and 4-4c) and declines in soil moisture (Figure 4-4d). Soil water potential in the upper 1.5 m of the soil profile (Figure 4-4b) and soil moisture integrated through the soil profile (Figure 4-4d) followed a similar yearly cycle to that of precipitation (Figure 4-4e) and were statistically similar across monoculture and mixture plot types ($P = 0.26$ and 0.49 respectively, t-test). Alternatively, soil water potential below 1.5 m steadily declined throughout the study, reaching a minimum in December 2014, and was similar across all plots except lower in the *E. tereticornis* monoculture (Figure 4-4c). When averaged for shallow (0-150 cm) and deep (150-325 cm) soil depths, soil water potential was statistically similar during all months for shallow soil layers (Figure 4-4b), but significantly higher in mixtures compared to monocultures at deeper depths during low moisture conditions in October ($P = 0.038$, t-test) and November 2014 ($P = 0.046$, t-test).

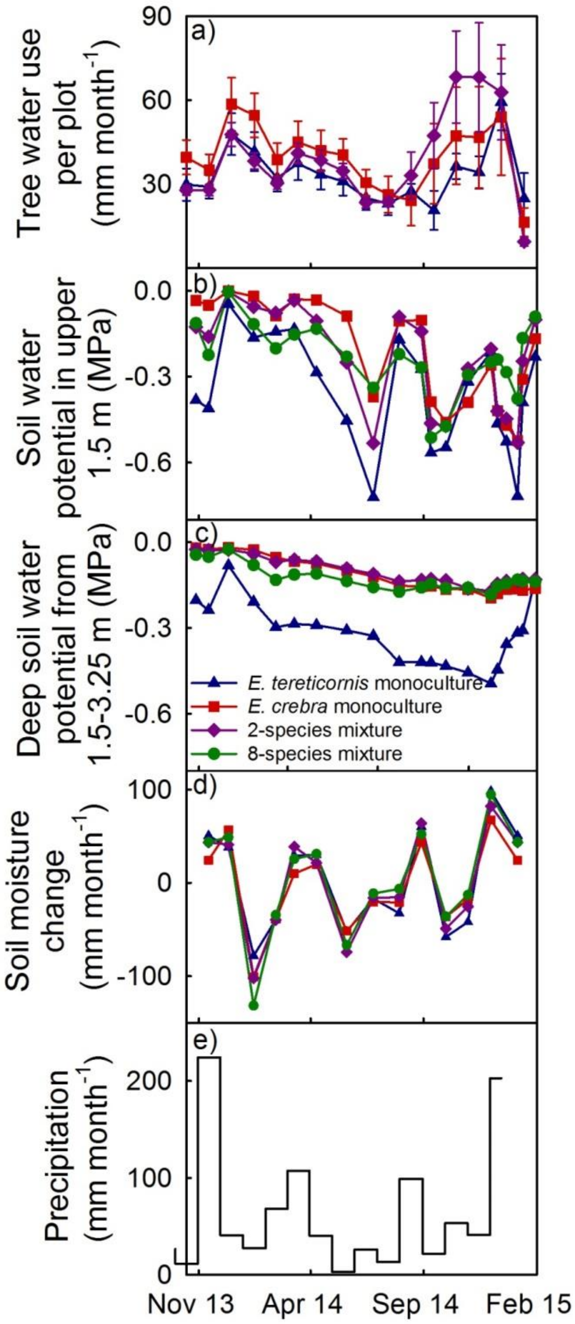


Figure 4-4: Mean monthly plot level a) tree water use, b) soil water potential in the upper 150 cm of soil, c) soil water potential in the 150-325 cm region, d) change in soil moisture from 25-325 cm depth using neutron probe data and e) monthly precipitation over the same time period from 1st December 2013 through to 31st December 2014. In a) error bars represent the standard error of the mean across 4-6 trees of each species. 2-species mixture plot water use is the average tree water use of *E. crebra* and *E. tereticornis* individuals in combination. Only two species (*E. crebra* and *E. tereticornis*) were measured in the 8-species mixture, so plot level tree water use could not be calculated. No error bars are presented in b) or c) as soil moisture was measured in only one location in each plot.

Despite monthly variation between plots, total yearly tree water use and soil moisture change were statistically similar across monocultures and mixtures (*P* = 0.39 and *P* = 0.49 respectively, t-test). Water use of component species scaled to the plot level and soil moisture accounted for 83.8%, 107.1% and 105% of the incoming yearly precipitation in *E. tereticornis* monoculture, *E. crebra* monoculture and 2-species mixture respectively (Figure 4-5). 8-species mixture plot level water use could not be calculated as only two of the eight component species were measured for tree water use. Plot level growth followed a similar trend to that of yearly water use, whereby plot VI growth was statistically similar across all plots (Table 4-1; *P* = 0.069, ANOVA) although highest in *E. tereticornis* and lowest in *E. crebra* monocultures respectively (Table 4-1). Plot AGB was significantly lower in *E. crebra* monoculture compared to all other plot types in November 2013 and December 2014 (*P* = 0.007 and 0.003 respectively, ANOVA) and was significantly higher in 8-species than 2-species mixture in December 2014 (Table 4-1; *P* = 0.017, Holm-Sidak test). Similarly, yearly AGBI in 2014 was significantly lower in *E. crebra* monoculture compared to all other plots (*P* < 0.05, Holm-Sidak test) and significantly higher in 8-species compared to 2-species mixture (*P* = 0.016, Holm-Sidak test).

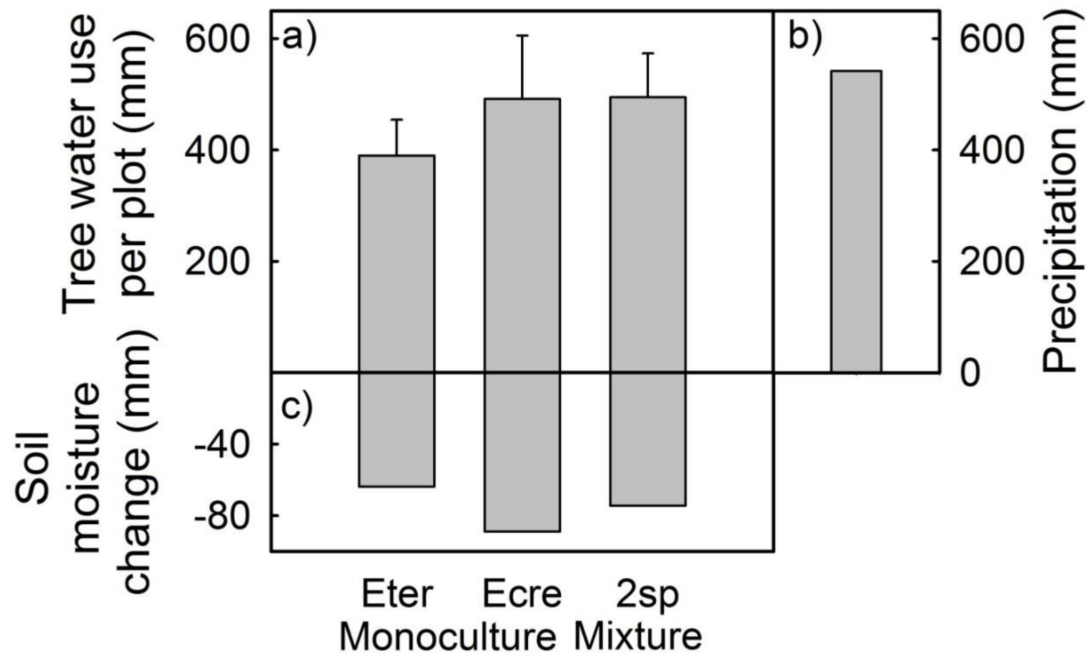


Figure 4-5: Yearly plot water balance showing a) tree water use per unit ground area (mean ± SE), b) change in soil moisture from 25-325 cm depth using neutron probe measurements and c) annual precipitation from 1st December 2013 until 1st December 2014. In a) each bar is the average yearly water use of 4-6 trees of each species in each plot type. Mixtures are presented as a total plot water use averaged across 4-6 trees of each species in each plot summed together to a total plot water use. 2-species mixture plot water use is the sum of *E. crebra* and *E. tereticornis* individuals in combination. Only two species were measured in the 8-species mixture so no tree water use was considered, and for consistency, soil moisture was also omitted from this figure.

Table 4-1: Plot level growth and water use parameters of three plot types. Aboveground biomass (AGB) is the sum of all tree biomass in each plot and was calculated using allometric equations (Paul et al. 2013) for growth data from December 2014, as described in methods. Standard errors indicate the difference between replicate plots except in the case of tree water use, in which case they are the difference between 4-5 trees of each species in each plot type. Letters indicate significant differences ($P < 0.05$) between plots as tested using the Holm-Sidak test and ANOVA in Sigmaplot.

	E. crebra monoculture	*E. tereticornis* monoculture	2-species mixture	8-species mixture
Plot volume index growth ($m^3 \cdot yr^{-1} \cdot ha^{-1}$)	11.0 ± 2.5^a	30.5 ± 3.3^a	27.0 ± 6.9^a	29.3 ± 1.6^a
Aboveground plot biomass 2013 ($g \cdot m^{-2}$)	159.8 ± 92.1^a	1341.2 ± 71.2^b	1041.2 ± 271.6^b	1499.5 ± 25.5^b
Aboveground plot biomass 2014 ($g \cdot m^{-2}$)	965.8 ± 172.6^a	2945.0 ± 111.7^{bc}	2552.0 ± 482.0^c	3765.5 ± 46.4^b
Plot AGB increment ($kg \cdot m^{-2} \cdot yr^{-1}$)	0.90 ± 0.16^a	2.75 ± 0.10^{bc}	2.38 ± 0.26^b	3.52 ± 0.04^c

4.4.4 Component species growth, water use, WUE and photosynthesis

Component species growth, represented by HGR, VIGR and AGBI, was lower for *E. crebra* in mixtures than in monoculture, and conversely for *E. tereticornis*, was greater in mixtures compared to monoculture (Table 4-2). *E. crebra* HGR and AGBI declined significantly by 33% and 96% ($P = 0.033$ and 0.039, Holm-Sidak test) in 2-species mixture compared to monoculture, and both parameters declined but were statistically similar between 8-species mixture and monoculture (Figure 4-6a and Table 4-2; $P = 0.69$ and 0.65, Holm-Sidak test). Likewise, *E. crebra* VIGR declined by 76% and 12% from monoculture to 2- and 8-species mixtures, although these plots were not significantly different to monocultures (Table 4-2; $P = 0.38$ and 0.88 respectively, Holm-Sidak test). *E. tereticornis* HGR and VIGR increased by 2% and 60% from monoculture to 2-species mixture, and increased by 8% and 45% from monoculture to 8-species mixture, although neither parameter was significantly different across plots (Table 4-2; $P > 0.05$, Holm-Sidak test). However, AGBI increased significantly by 68% and 99% for *E. tereticornis* in 2-species and 8-species mixtures compared to monoculture (Figure 4-6a and Table 4-2; $P = 0.002$ and 0.002 respectively, Holm-Sidak test). Thus *E. crebra* growth ranked monoculture < 8-species mixture < 2-species mixture whereas *E. tereticornis* growth ranked 2-species mixture < 8-species mixture < monoculture (Table 4-2). Component species VI growth in the diversity plot ranked as *M. azedarach* < *M. linariifolia* < *E. crebra* < *E. fibrosa* < *E. tereticornis* < *A. floribunda* < *A. subvelutina* < *E. elata* (Table 4-3) resulting in focal species having competitors both slower and faster growing than themselves in the 8-species mixture plot.

Component tree water use followed similar trends to that observed for component species growth rate (Table 4-2). *E. crebra* yearly tree water use was significantly higher by 52.9% in monoculture than in 2-species mixture (Table 4-2; *P* = 0.05, Holm-Sidak test), and 28.4% higher, though not significantly different, in monoculture compared to 8-species mixture (Figure 4-6b, Table 4-2; *P* = 0.33, Holm-Sidak test). Conversely, *E. tereticornis* yearly tree water use was significantly lower by 83.8% when in monoculture compared to 2-species mixture (*P* = 0.01, Holm-Sidak test), and 53.9% lower, though not significantly different, when in monoculture compared to in 8-species mixture (Figure 4-6b, Table 4-2, *P* = 0.12, Holm-Sidak test). Component species water use on a sapwood area basis was statistically similar across all species and plot combinations (*P* = 0.55, ANOVA), although in *E. tereticornis* was 50% and 68.4% lower in monoculture than 2- and 8-species mixtures respectively (Figure 4-6c).

From tree level growth rate and tree water use I calculated average tree level WUE for component species across different plot types. Tree WUE declined significantly by 29% in *E. crebra* when planted in 2-species mixture compared to in 8-species mixture (*P* = 0.049, t-test). Within each species, individuals in all other plots had an increase in tree WUE respective to when in monoculture, however none of these responses were significant (Figure 4-6d and Table 4-2; *P* = 0.062, ANOVA). For carbon isotope discrimination (δ^{13}C) as an indicator of leaf-level WUE, δ^{13}C was statistically similar in all species by plot combinations (*P* = 0.117, ANOVA) in 2014 but was greatest in *E. tereticornis* individuals when in monoculture followed by 2- and then 8-species mixture, and in *E. crebra* individuals when in 8- followed by 2-species mixture and then monoculture (Table 4-4). In 2013, leaf δ^{13}C was significantly lower in *E. crebra* when in 2-species mixture, and was significantly higher in *E. tereticornis* when in 8-species mixture ($P < 0.05$, Holm-Sidak test) than all other plots, while all other species by plot combinations were statistically similar ($P > 0.05$, Holm-Sidak test). Total canopy leaf area of an 8 cm tree and the leaf area:sapwood area ratio were statistically similar across all species in monoculture and 2-species mixtures (Table 4-4; $P = 0.16$ and 0.16 respectively, ANOVA). However, *E. tereticornis* trees were significantly larger than *E. crebra* trees during the study with a significantly higher growth rate (Tables 4-2 and 4-3), resulting in a higher total canopy leaf area per tree in *E. tereticornis* compared to *E. crebra*. Leaf level maximal photosynthetic rate (A_{net}) was statistically similar across all species and plot combinations (Table A-4; $P = 0.869$, ANOVA) however the higher leaf area in *E. tereticornis* individuals likely facilitated a higher tree-level photosynthetic rate and consequently enabled a higher growth rate.

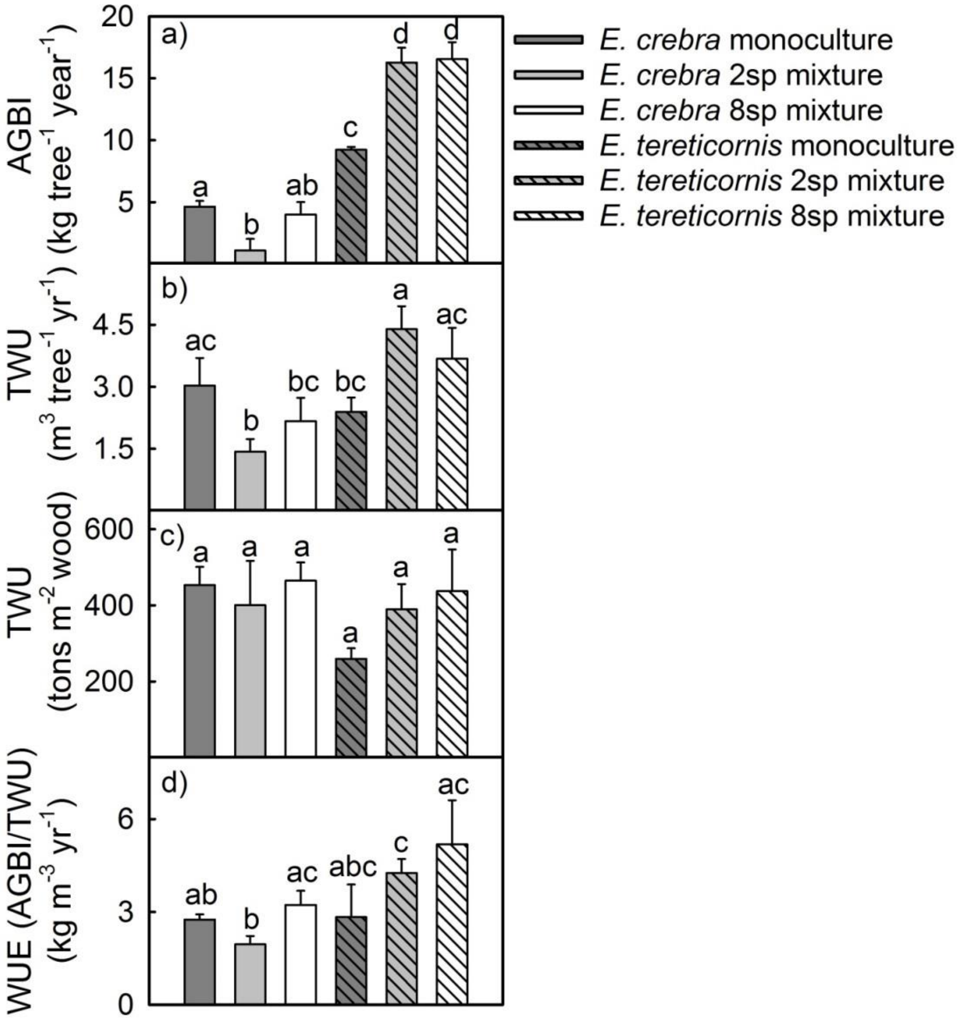


Figure 4-6: The a) growth in AGB (AGBI), b) tree water use (TWU) on a single tree basis, c) TWU on a sapwood area basis and d) WUE of component trees of *E. crebra* (unhatched) and *E. tereticornis* (hatched) in each plot type over one year from 1st December 2013 until 1st December 2014. WUE was calculated as AGBI/yearly TWU for component species, so is the annual water cost for carbon gained per tree. Error bars are the standard error of the mean of a) two plots per plot type and b-d) 4-5 trees per plot. Letters denote significant differences between species ($P < 0.05$; Holm-Sidak test).

Table 4-2: Growth and water use parameters of 2 *Eucalyptus* species from three plot types. HGR, VIGR and AGBI are the average growth per tree in these parameters over one year, determined using Fisher (1921). AGB and the AGBI were extrapolated with allometric equations (Paul et al. 2013) for growth data from December 2014. Letters denote significant differences ($P < 0.05$) within species between plots tested using Holm-Sidak test and ANOVA.

	E. crebra			*E. tereticornis*		
	Monoculture	2-sp mixture	8-sp mixture	Monoculture	2-sp mixture	8-sp mixture
Tree height growth rate (HGR; cm·yr^{-1}·tree^{-1})	171.5 ± 5.1^{a}	114.9 ± 0.8^{b}	162.9 ± 12.9^{ab}	194.1 ± 14.8^{a}	197.2 ± 19.0^{a}	210.5 ± 22.4^{a}
Tree HGR respective to monoculture (%)	0	-33.0	-5.1	0	+1.6	+8.4
Tree VIGR for component species (cm^{3}·yr^{-1}·tree^{-1})	8761 ± 2052^{ac}	2099 ± 12^{a}	7663 ± 2919^{a}	25260 ± 3013^{bc}	40372 ± 10254^{b}	36658 ± 4305^{b}
VIGR change respective to monoculture (%)	0	-76.1	-12.5	0	+59.8	+45.1
VIGR for component species per ground area (m^{3}·yr^{-1}·ha^{-1})	11.0 ± 2.5^{a}	4.8 ± 1.6^{a}	9.6 ± 3.6^{a}	30.5 ± 3.3^{b}	49.1 ± 12.1^{b}	34.7 ± 0.2^{a}
Proportion of VIGR accounted for by species per plot (%)	100	8.98	4.10	100	91.02	14.77
Tree AGBI for component species (kg·tree^{-1}·yr^{-1})	4.6 ± 0.5^{a}	1.1 ± 0.9^{b}	4.0 ± 1.0^{ab}	9.2 ± 0.2^{c}	16.3 ± 1.2^{d}	16.5 ± 1.4^{d}
AGBI change respective to monoculture (%)	0	-96.1	-28.2	0	+67.7	+99.2
Yearly component species tree water use (m^{3}·tree^{-1}·yr^{-1})	3.03 ± 0.67^{ac}	1.42 ± 0.31^{b}	2.17 ± 0.56^{bc}	2.39 ± 0.35^{bc}	4.40 ± 0.55^{a}	3.68 ± 0.74^{ac}
Yearly change in TWU respective to monoculture (%)	0	-52.9	-28.4	0	+83.8	+53.9
Component species WUE per tree (kg wood·m^{-3} water·yr^{-1})	2.7 ± 0.2^{ab}	1.9 ± 0.3^{b}	3.2 ± 0.4^{ac}	2.8 ± 1.0^{abc}	4.3 ± 0.4^{c}	5.2 ± 1.4^{ac}
WUE change respective to monoculture (%)	0	-29.1	+17.2	0	+50.2	+83.0

Table 4-3: The average VIGR, height and proportion of VIG of a tree of each component species in the 8-species mixture. Height was measured in December 2014 while VI growth and proportion growth are calculated over one year from November 2013 until December 2014. Errors are the standard errors of the mean growth in volume index between two replicate plots.

Species	VIGR of individual species ($m^3 \cdot yr^{-1} \cdot ha^{-1}$)	Average tree height (cm)	Percentage of plot VIG accounted for by species (%)
A. floribunda	50.8 ± 5.5^{a}	608.3 ± 30.0^{ab}	22%
A. subvelutina	59.9 ± 5.6^{a}	603.5 ± 36.2^{ab}	25%
E. crebra	9.6 ± 3.6^{b}	463.4 ± 37.8^{bc}	4%
E. elata	62.1 ± 0.3^{a}	855.2 ± 45.1^{d}	26%
E. fibrosa	10.6 ± 4.9^{b}	386.3 ± 40.0^{ce}	4%
E. tereticornis	34.7 ± 0.2^{c}	706.2 ± 43.7^{a}	15%
M. azedarach	1.9 ± 0.3^{b}	351.2 ± 25.9^{ce}	1%
M. linariifolia	4.0 ± 3.5^{b}	263.4 ± 29.7^{e}	2%

Table 4-4: Leaf parameters of 2 *Eucalyptus* species from three plot types. For δ^{13}C, standard errors indicate the difference between replicate plots whereas for leaf area, standard errors are the difference between 3 replicate trees in each plot type. Letters denote significant differences ($P < 0.05$) tested using Holm-Sidak test and ANOVA.

	E. crebra			*E. tereticornis*		
	Monoculture	2-sp mixture	8-sp mixture	Monoculture	2-sp mixture	8-sp mixture
Leaf δ^{13}C 2013 (‰ VPDB)	-30.2 ± 0.1^{ac}	-31.1 ± 0.1^{b}	-30.4 ± 0.1^{a}	-29.6 ± 0.4^{cd}	-30.3 ± 0.1^{a}	-29.3 ± 0.4^{d}
Leaf δ^{13}C 2014 (‰ VPDB)	-28.4 ± 0.2^{a}	-29.2 ± 0.3^{a}	-29.3 ± 0.2^{a}	-28.9 ± 1.2^{a}	-28.7 ± 0.2^{a}	-28.2 ± 0.1^{a}
Total canopy leaf area (cm^2) of an 8 cm diameter tree	1817.5 ± 566.6^{a}	2015.1 ± 356.2^{a}	-	2698.9 ± 418.6^{a}	3323.5 ± 451.4^{a}	-
Leaf area per unit sapwood area (cm^2·cm^{-2}) per tree	3615.7 ± 1127.3^{a}	4009.0 ± 708.7^{a}	-	5369.3 ± 832.8^{a}	6611.9 ± 898.0^{a}	-

4.5 Discussion

Competition for water plays a key role in structuring plant species distributions and community compositions (BassiriRad et al. 1999; Engelbrecht et al. 2007; Kursar et al. 2009), yet few studies have examined the water relations implications of competitive interactions amongst trees in forest plantations. In this study I observed both intra- and inter-specific competition between two *Eucalyptus* species within monoculture and mixture stands, addressing the impact of competition on soil moisture, tree growth and tree water use. Determining how competitive interactions influence soil moisture and tree water use may provide insight into how species mixtures will tolerate current and future changes in environmental conditions.

4.5.1 Competition for soil moisture

I hypothesised that differential use of soil moisture through root stratification would promote higher soil moisture exploitation in mixtures compared to monocultures, measured as lower soil moisture (Tilman et al. 1997; Reich et al. 2001; Fargione et al. 2003). Contrary to expectations however, I observed lower soil water potential in *E. tereticornis* monoculture compared to other plots (Figure 1-3) and significantly lower water potential at depth in monocultures than mixtures. *E. tereticornis* monoculture had the lowest soil moisture, with slightly more moisture uptake at the deepest measurement depths (225-325 cm depths) than other plots, while *E. crebra* had the highest soil moisture of all plots at these depths. Root water uptake, inferred from vertical profiles of soil moisture, can provide an indication of rooting depth (Rambal 1984; Dye 1996; Calder et al. 1997; Duursma et al. 2011). Following this approach, my observations of soil water potential suggest that individuals of *E. tereticornis* likely had more roots at depth than those of *E. crebra* when in monoculture (Figures 1 and A1), an observation that is consistent with previous reports of a shallow root system in *E. crebra* (Rice et al. 2004). These interspecific differences in rooting depth would have provided an opportunity for root stratification (Neave and Florence 1994; Schulze et al. 1994; Khanna 1997) and thus higher exploitation of soil moisture through the entire soil profile in mixtures, consistent with my hypothesis, yet this did not result in the outcome of lower soil moisture that I expected. One mechanism which may explain the higher soil moisture in observed mixtures compared to monocultures is hydraulic redistribution (Burgess et al. 1998; Bleby et al. 2010; Brooksbank et al. 2011) however I found no evidence of this during my study.

Rather, I expect that the higher soil moisture observed in mixtures over monocultures was achieved through suppression of inferior competitors in mixtures (e.g. Soares and Tome 1996; del Rio et al. 2014; Ledo et al. 2014), resulting in trees in mixture plots using fewer resources at the plot-level than they otherwise would in monoculture. The combination of deep (*E. tereticornis*) and shallow (*E. crebra*) rooted species resulted in slightly lower soil moisture in the upper 1.5 m of soil in 2-species mixture compared to *E. crebra* monoculture (Figures 4-1a and 4-4b) but higher soil moisture at deeper depths (Figures 4-1c-d and 4-4c). I hypothesise that this may have been due to the suppression of the shallow-rooted *E. crebra* and consequently reduced competition for moisture resources in *E. tereticornis* individuals which, based on soil moisture and root water uptake in *E. tereticornis* monoculture (Figures 4-1 and A-2), could obtain the required soil moisture from all measured soil layers. While the lower soil moisture observed in *E. tereticornis* monoculture compared to other plots could be attributed to microsite differences previously reported for this site (Figure 1 from Duursma et al. 2011), soil moisture reached similar saturation levels across all plots after heavy rain (Figure 4-1b-d), indicating that plots were capable of holding similar amounts of water. Instead, I expect that the lower soil moisture and exploitation of water at depth was due to the progressive depletion of soil moisture in the plantation establishment years, resulting in lower shallow soil moisture in early 2013 in *E. tereticornis* monoculture compared to other plots (Figure 4-1a).

4.5.2 Asymmetric competition in mixtures

While *E. crebra* and *E. tereticornis* individuals in monoculture had similar monthly (Figure 4-4a) and yearly (Figures 4-5a and 4-6b) water use rates, *E. tereticornis* had a significantly higher AGBI (Figure 4-6a-b; $P = 0.017$, ANOVA) enabling it to be the more dominant of the two focal species. This higher growth rate in *E. tereticornis* was likely due to a higher tree-level photosynthetic rate, facilitated by higher total canopy leaf area (Table 4-3). *E. tereticornis* individuals had lower growth and tree water use, and soil moisture was lowest, in monoculture compared to mixture plots (Figure 4-6, Table 4-2). Accordingly, I hypothesised that the inclusion of additional species in plots would reduce signs of competitive water stress in component species in mixtures compared to monocultures, thus facilitating enhanced tree water use, growth and WUE. Consistent with this, competition indices indicated decreased competition experienced by *E. tereticornis* individuals in species mixtures compared to monocultures for AGBI, HGR, VIGR, tree water use and WUE (Figure 4-6, Table 4-2). This suggests a lower inter- than intra-specific competition (Forrester et al. 2006; Beyer et al. 2013; del Rio et al. 2014; Hajek et al. 2014) experienced by *E. tereticornis* individuals. Conversely however, *E. crebra* individuals experienced higher inter-specific competition in mixtures compared to the intra-specific competition in monocultures for these same parameters (Figure 4-6, Table 4-2). These responses, coupled with the soil moisture data discussed above, demonstrate asymmetric competition where the dominant species (*E. tereticornis*) out-competed the inferior competitor (*E. crebra*) for resources, resulting in the eventual suppression of *E. crebra* growth and water use in 2-species mixture.

Asymmetric competition for soil resources has been observed in tree species mixtures previously (Soares and Tome 1996; Rewald and Leuschner 2009b; Lei et al. 2012; Beyer et al. 2013; del Rio et al. 2014; Hajek et al. 2014; Ledo et al. 2014), in which both above and below-ground influences and outcomes of asymmetric competition have been reported. Lei et al. (2012) examined competition in a four species tree mixture, and reported that dominant species benefited more from being planted in mixtures with other species than did inferior species, consistent with my observations. In this study, the greatest influence on growth and water use parameters for component trees of each species respective to monoculture was usually observed in 2-species mixture (Figure 4-6, Table 4-2) where the density of the two focal species, and thus likelihood of an *E. crebra* individual encountering an *E. tereticornis* individual (and vice versa), was greatest. The 8-species mixture contained additional species with differing functional traits compared to those in 2-species mixture, including species with significantly higher and lower growth rates compared to each of the focal species (Table 4-3). Accordingly, the increase in functional types present, and the reduced density of focal species, enabled greater access to resources and thus enhanced growth and water use for *E. crebra* individuals in 8- versus 2-species plots because of the greater probability of the proximity of a similar or slower growing species. Oppositely, this resulted in reduced resources, growth and water use for *E. tereticornis* individuals in 8-species compared to 2-species mixtures (Figure 4-6, Table 4-2).

Despite the suppression of *E. crebra* in species mixtures, plot productivity was enhanced in mixtures compared to monocultures by the higher yield of the more dominant, productive *E. tereticornis* (Table 4-2) and other component species (Table 4-3), consistent with ‘over-yielding’ (Harper 1977; Hooper and Dukes 2004). This over-yielding also resulted in enhanced plot water use in mixtures compared to monocultures temporally (Figure 4-4a). Accordingly, the species combinations and plot compositions presented here may only be beneficial in regions where water is readily available and enhanced growth and water use can be permitted. Previous studies have reported shifts in interspecific competitive interactions with increases in stand age (Soares and Tome 1996; Filipescu and Comeau 2007; Wright et al. 2014) and during this study I observed progressive increases in soil moisture and water use differences between plots over time (e.g. Figures 4-1a-c and 4-4a). Accordingly, I expect that the intra- and inter-specific interactions observed here will become more apparent as these stands age and root and canopy closure becomes more complete.

Due to resource limitations, sap flow was only measured in two focal species in 8-species mixtures, and sap flow and soil moisture were only measured in one replicate of each plot type. Accordingly, only component tree sap flow and plot water use of monocultures and 2-species mixtures could be considered here due to lack of replication at the plot-level. While soil moisture was only considered in one replicate of each plot type, both NP and TDR measurements were conducted in each plot, and these measurements reported similar temporal and spatial variation and were tightly correlated ($r = 0.98$, 0.93, 0.96 and 0.82 for *E. crebra* monoculture, *E. tereticornis* monoculture, 2-species mixture and 8-species mixture plots respectively). Replicate plots of mixtures were statistically similar in component species AGBI (P = 0.305 and 0.138, t-test), VIGR (P = 0.94 and 0.081, t-test), HGR (P = 0.058 and 0.12, t-test), diameter at 8 cm (P = 0.424 and 0.434, t-test) and DBH (P = 0.436 and 0.090, t-test) for *E. crebra* and *E. tereticornis* across replicate sites and were planted in the same study site within 100 m of one another. Given that tree growth rate was statistically similar across replicate plots, all plots were planted in the same site on the same soils, two separate measurements of soil moisture were in agreement and incoming precipitation was equalled by outgoing evapotranspiration measured with sap flow and soil moisture, I believe that soil moisture measurements were accurate and that soil moisture was likely similar across plot replicates.

4.5.3 Mixture individuals less influenced by low soil moisture

Previous studies conducted in forested ecosystems have indicated that mixture plantings experience increased water stress and competition between component species (Grossiord et al. 2014b) which, during low water availability conditions, results in a limitation of the productivity enhancement that is often observed in mixtures (Jucker et al. 2014). Conversely, it has been theorised that partitioning of soil water in mixed forests will result in enhanced complementarity during dry periods (Forrester and Smith 2012; Meissner et al. 2012). Consistent with the latter and in support of my hypothesis, I observed significantly higher maximal J_S and differences in the J_S response to D within species between plots during different soil moisture periods (Figure 4-3). This is evidence that component trees were less influenced by changing water availability when in mixture than monoculture. Additional support for this hypothesis was provided by a significantly higher component species, plot-level water use in mixtures compared to monocultures during two low soil moisture periods, October (P = 0.038, ANOVA) and November

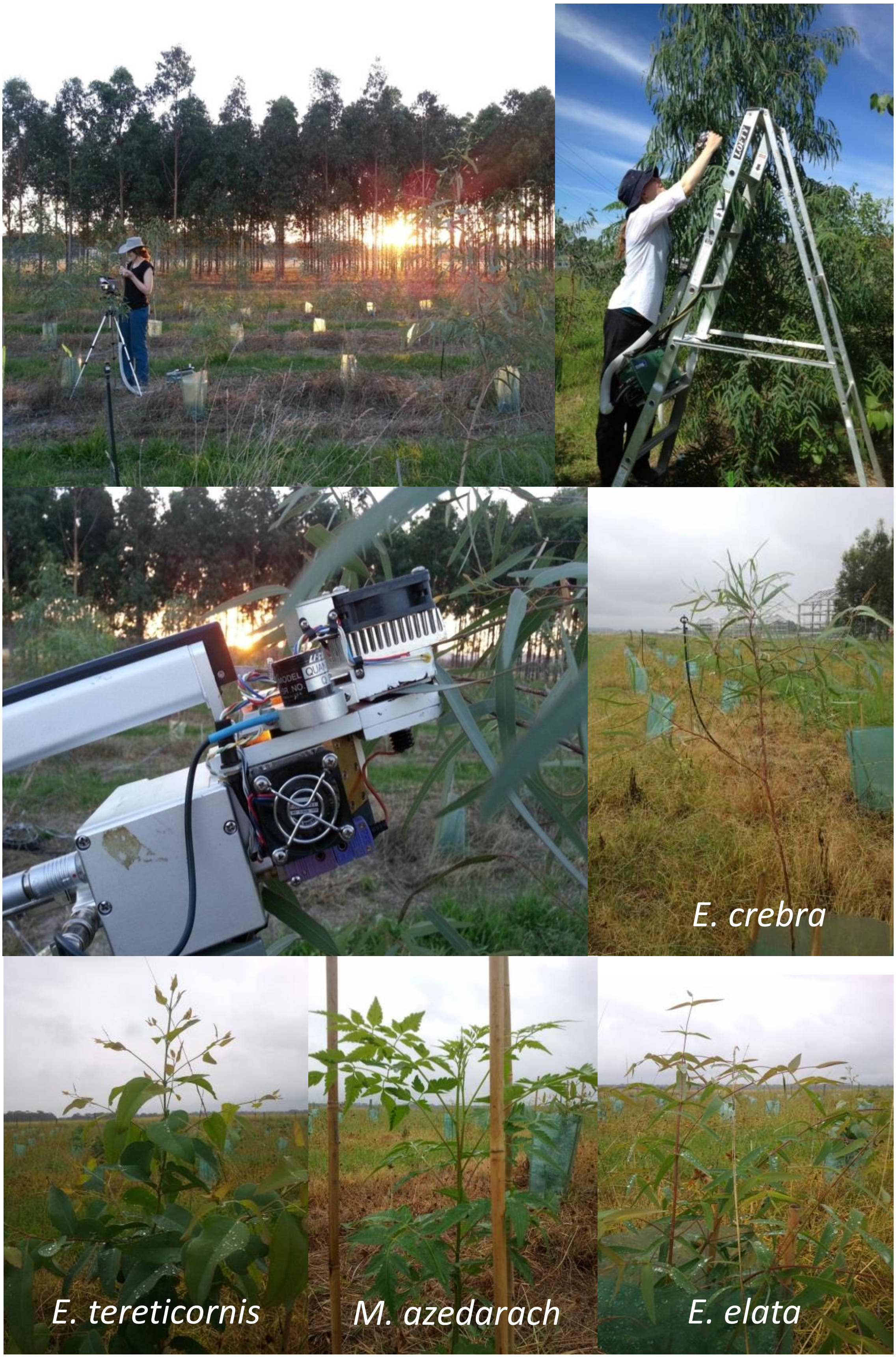
2014 (Figure 4-4a; $P = 0.046$, ANOVA). These observations suggest that during low water availability conditions, competitive water stress was alleviated in mixtures, facilitating similar water use responses to D as under non-limiting water availability conditions, which I attribute to higher soil moisture in mixtures compared to monocultures. Previous studies have observed that facilitative interactions are more likely to dominate competitive interactions under resource limited or stressful conditions (Bertness and Callaway 1994; Callaway and Walker 1997; Brooker and Callaghan 1998; Ledo et al. 2014; Wright et al. 2014). These previous studies, in combination with the observations reported here, provide support for the stress-gradient hypothesis which predicts that facilitative interactions will be more common in conditions of high abiotic stress relative to more benign abiotic conditions (Brooker et al. 2008; Maestre et al. 2009).

I observed that J_S response to D and maximal J_S were influenced less by low soil moisture in mixtures compared to monocultures, consistent with previous studies which have reported less influence on productivity over changing water availability conditions in mixed marsh (Steudel et al. 2011) and forest communities (Pretzsch et al. 2013) compared to monocultures of the same species. One study observed that species interactions in mixture stands led to lower soil moisture in boreal forests when compared to monoculture stands (Grossiord et al. 2014c), yet this only occurred in certain regions (Grossiord et al. 2014d). However, I observed a significantly lower soil moisture for monocultures than mixed plantings during these low moisture conditions (Figures 4-1 and 4-4b-c), and when comparing low and moderate soil moisture periods I observed no differences in soil moisture change integrated throughout the soil profile (Figure 4-4d). The discrepancy between my and previous studies may be due to plantation age or a difference in species and functional types used in these studies. Though generalisations can be made across the different species mixture studies conducted, the lack of studies reporting species responses to water limitation in mixtures compared to monocultures highlights the need for further research in this area.

4.5.4 Conclusions

I found support for my hypothesis that component species in mixture would have enhanced water use and growth compared to when planted in monoculture, although this was not attributed to differential soil moisture use or root stratification in these systems. I concluded that these enhanced responses were based on asymmetric competition between focal species for soil moisture resources, where the dominant *E. tereticornis* out-competed and eventually suppressed the growth of the slower growing *E. crebra* in mixture, resulting in similar soil moisture in *E. crebra* monoculture and 2-species mixture but significantly higher growth and water use for *E. tereticornis* individuals in mixtures compared to monoculture. I found support for my hypothesis that individuals in species mixtures would be less influenced by changing water availability based on a lower J_S to D and maximal J_S of individuals during low moisture conditions in monocultures but not in mixtures. This was likely facilitated by higher soil moisture in species mixtures. Component tree growth, water use and response to water availability depended on species identity and ecosystem composition, highlighting the importance of species selection for enhancing productivity and WUE in mixture plantations. My findings support the use of species mixtures for the promotion of component species growth and water use, and reduced influence of changing water availability, in selected *Eucalyptus* species. While further research into the effect of mixture plantings on ecosystem function is necessary, a conservative approach to maintaining species richness in native ecosystems and in plantation and land management practices may promote growth, WUE and survival in these ecosystems.

Mixture plantation at planting in November 2011 (top) and sap flow probes in *E. tereticornis* (left) and *E. crebra* (right) at the beginning of the study in November 2012. Image source: Aimee Bourne.



Gas exchange measurements in mixture plots (top and middle left) and the focal and two other species in the mixture plot as seedlings, in December 2012 (bottom and middle right). Image source: Aimee Bourne.

Chapter 5: Synthesis and conclusions

5.1 Synthesis

5.1.1 Overview of linkages between chapters

Many recent studies have reported species-specific influences on transpiration and its response to environmental conditions (Pataki et al. 2000b; Monson et al. 2010; Zeppel et al. 2010). These responses are important physiological traits input into species-specific models of tree transpiration (e.g. SPA and MAESPA; Williams et al. 1996; Duursma and Medlyn 2012), and are consequently important for ecohydrological modelling and informing plantation and catchment management practices globally. Differences in plant hydraulic and anatomical characteristics (Merchant et al. 2007; Mitchell et al. 2008; Johnson et al. 2011), stomatal behaviour (White et al. 2000; Héroult et al. 2013), leaf area (O'Grady et al. 1999; Schwinning and Ehleringer 2001; Zeppel and Eamus 2008), and rooting depth (Rice et al. 2004; Mitchell et al. 2009) have been implicated as the cause for such interspecific variation in forest tree transpiration. These characteristics are often related to the climate in which a species originates, providing an opportunity for categorising stomatal and hydraulic characteristics, and their response to environmental conditions. While the vast majority of species have not been measured for species-specific influences on transpiration, it may be possible to identify relationships between plant physiological parameters and climate variables enabling us to characterise and predict these responses for a broad range of species. These types of physiological studies can provide classification into ‘guilds’ or groups of species with shared or similar physiological parameter values, as has previously been conducted (e.g. Reich et al. 2001; McClenahan et al. 2004; Mitchell et al. 2008). The Biomass and Allometry Database for woody plants (BAAD; Falster et al. 2015) provides the option of downloading biomass and allometric records for particular genus' or species which provides useful information when considering such structural/hydraulic guild classifications. Climatic data is readily available for the majority of vegetated land surfaces, providing an ideal dataset to utilise in order to characterise species responses to environmental conditions across a broad range of climates. For models which enable the use of species-specific parameters, correlations of plant physiological characteristics with climate could enable predictions of species and ecosystem responses to differing climatic conditions to be made for a broad range of species, without having to measure each individual species' physiological characteristics.

The majority of interspecific comparisons of transpiration compare species under different climatic conditions, or different species that co-occur on a site. These comparisons consequently do not account for phylogenetic differences between species in transpiration responses to the environment or its relation to the climate of species origin. By comparing species from different climatic zones when planted under the same environmental conditions, and by restricting these comparisons to species within a genus, we can obtain finer distinctions among species transpiration responses to the environment. Consequently, we can relate these transpiration responses to the climate of origin of the species to identify and distinguish species-specific characteristics, many of which may be useful for input into models of forested ecosystem transpiration. Accordingly, I compared five *Eucalyptus* species from contrasting climates in a common garden environment (Chapter 2). I asked whether species climate of origin influenced J_S and G_C responses to D, and the stomatal sensitivity to *D*. Species climate of origin was significantly correlated with stomatal sensitivity to *D*, mean vessel diameter and the size of the decline in maximal sap flux density to restricted water availability in the five species studied. Consequently, I concluded that aridity constrains water use within the five *Eucalyptus* species studied, appearing to have a selective role in constraining transpiration responses to the environment.

Aridity is a major factor limiting species’ distributions in Australia, and thus drought sensitivity is an important factor shaping plant species survival and distribution (BassiriRad et al. 1999; Engelbrecht et al. 2007; Kursar et al. 2009). Selection for traits that allow tolerance of the native climatic range of a species will influence the genes passed on to future generations and consequently, species from more arid climates are likely to conserve traits conferring tolerance of drought and low stomatal sensitivity to *D*, as has been reported previously (Villar-Salvador et al. 1997; Cunningham 2004; Baltzer et al. 2008; Kursar et al. 2009; Bartlett et al. 2012; Choat et al. 2012). The drought tolerance of a species or population is influenced by trade-offs between hydraulic safety and efficiency and will be reflected in stomatal behaviour, xylem anatomy, leaf tissue water relations and hydraulic characteristics of the species (See Chapter 1 for explanation; Jarvis and McNaughton 1986; Sperry et al. 2008). I observed correlations of stomatal behaviour and xylem anatomy with climate aridity (Chapter 2) and hypothesised that these characteristics would be related to and influenced by hydraulic and leaf tissue water relations of the species.

While previous studies have reported correlations of species climatic range with stem hydraulics (Mitchell et al. 2008; Beikircher and Mayr 2009; Choat et al. 2012), xylem anatomy (Villar-Salvador et al. 1997; Fisher et al. 2007; Medeiros and Pockman 2014) and leaf tissue water relations (White et al. 2000; White et al. 2003;

Bartlett et al. 2012), these studies compared species along environmental gradients (e.g. Stewart et al. 1995) which do not allow the determination of climate effects on physiological and anatomical traits across species. I compared leaf physiological traits and hydraulic, anatomical and stomatal characteristics across seven *Eucalyptus* species from contrasting climates to determine whether these traits were related to the climate of species range (Chapter 3). I observed that the hydraulically weighted vessel diameter, Ψ_{TLP} and Π_0 were significantly negatively correlated with the inverse of monthly maximum vapour pressure deficit and positively correlated with MAP of the species range. Furthermore, I found that the water use strategy, encapsulating vulnerability to embolism (P_{50}) and stomatal behaviour, employed by a species was reflective of the species climate range. Therefore, climate aridity constrains leaf tissue water relations and hydraulic, stomatal and anatomical characteristics (Chapter 3) in addition to stomatal sensitivity to D and response to restricted water availability (Chapter 2) in the *Eucalyptus* species studied.

Ecosystem species richness or functional diversity also influences species distribution, survival, productivity and water use (Johnson et al. 1996; Loreau et al. 2001; Hooper et al. 2005; Thompson et al. 2014) and consequently should be considered when predicting how these ecosystem processes and functions might adjust under future climate scenarios. Natural forested ecosystems are often mixtures of species from different functional types and account for ~40 million hectares of land globally (FAO 2005). As many commonly used ecosystem water use models overlook species composition (e.g. Penman 1948; Monteith 1965; Priestley and Taylor 1972; McNaughton and Black 1973; Shuttleworth and Wallace 1985; Schymanski et al. 2007; Schymanski et al. 2008) this limits our ability to accurately predict the water use, productivity and survival of more complex, mixed-species ecosystems. Yet, these are the systems which hold significant catchment, plantation and land management implications. In extreme environments, with extremes of temperature and/or seasonality of rainfall, there is a limited range of parameter space that species can occupy to survive, and accordingly convergence in species traits can occur. However, in the species studied here, which occupy a range of different climates, though not as widely distributed as those occupied by woody species collectively, I observed significant differences in transpiration, growth potentials, hydraulic and stomatal characteristics. Differences in hydraulic, anatomical and stomatal characteristics observed between species in previous chapters (Chapters 2 and 3) enabled the segregation of species into different hydraulic functional types, facilitating the comparison of component species responses when in monocultures compared to when in mixture with other hydraulic functional types (Chapter 4).

The description of a functional group or type is a collection of species which occupy a similar niche, utilise similar resources and provide similar services to an ecosystem (Tilman et al. 1997; Fargione et al. 2003; Wright et al. 2006b; Mitchell et al. 2008). Previous work reported that increases in the functional types present in an ecosystem result in increases in ecosystem function and processes (Johnson et al. 1996; Loreau 2001; Tilman et al. 2001; Callaway et al. 2003; Jactel et al. 2005; Pautasso et al. 2005; Tilman et al. 2006; Forrester et al. 2010; Grossiord et al. 2013; Grossiord et al. 2014b). However, few of these studies examined these relationships in forested ecosystems, many were limited in the number of functional types compared and others contained confounding factors like N-fixing species, or were specific to particular climates, limiting their applicability to other ecosystem types and climates. The relationship between species richness and ecosystem function is important to understand in order to be able to accurately predict and model water use, survival and productivity of native ecosystems and plantations under current and future climates.

Based on niche theory (Vandermeer 1972; Schoener 1974; Hatton et al. 1997) I hypothesised that the inclusion of species occupying different ecosystem niches in mixture would enable higher soil moisture exploitation than in monoculture, thus reducing competitive water stress in component species relative to monoculture (Chapter 4). I observed that component species in mixture had higher water use, growth and tolerance of low water availability compared to when in monoculture. While mixture plantings enhanced ecosystem processes for a dominant species (*E. tereticornis*) the inferior competitor (*E. crebra*) experienced declines in productivity and water use when planted in mixture with the more dominant species. I concluded that these observations were due to asymmetric competition between the focal species, as *E. tereticornis* showed a positive response due to the reduction of *intra*specific competition, while *E. crebra* experienced a negative influence due to enhanced *inter*specific competition, when planted in mixture compared to monoculture. Accordingly, component species responses to being planted in combination with other functional types differed across species with differences in stomatal behaviour, hydraulic characteristics, growth and water use strategy. These findings hold important implications for understanding how mixed plantations and natural ecosystem mixtures respond to changes in water availability and interact based on component species interactions. Furthermore, these observations can help us to predict how mixed ecosystems may transpire and grow under varying environmental conditions, based on species-specific physiological characteristics and dominance of component species. Ultimately, this information can provide important insight into water availability fluctuations within ecosystems and consequently for catchments, enabling alterations to land and plantation management strategies based on species selection and community composition.

5.1.2 Future directions

My research provides important information about the stomatal sensitivity to *D*, drought response, anatomical, physiological and hydraulic characteristics and diversity-function relationships within and between different *Eucalyptus* species from contrasting climates. However, each of the research studies presented here has a few limitations and there are many avenues and options for further research to be undertaken on these and other *Eucalyptus* plantations in the future.

Chapters 2 and 3 reported correlations of climate aridity and range with stomatal sensitivity to *D*, water use under restricted water availabilities and leaf tissue water relations, hydraulic, stomatal and anatomical characteristics respectively. Given five and six *Eucalyptus* species were compared in these studies respectively, the conclusions could be strengthened and furthered to encompass a broader range of species and climates in future. Reassuringly though, I observed tight correlations of climate aridity with stomatal sensitivity to *D* (R^2 = 0.77, Figure 2-3a and b), Ψ_{TLP}, *VIGR*, Π_o, g_{s50}, D_h and P_{50} (R^2 = 0.76, 0.84, 0.66, 0.93, 0.75 and 0.53 respectively; Figures 2a, b, c, d, e and f, Table S1) despite these species occurring in the same genus and being planted in a common garden which would minimise much interspecific variation in these relationships. It is possible that particular relationships, such as those between climate of origin and water use under restricted water availabilities and leaf tissue water relations, would be influenced by changes in tree age due to differences in access to groundwater and other soil moisture resources. Additionally, provenance and identity of the species compared would likely influence many of these parameters, as was supported by the tight correlations of climate of origin with stomatal sensitivity, leaf tissue water relations, hydraulic and stomatal characteristics, yet differences between *E. tereticornis* and *E. crebra* individuals despite these species being obtained from very similar provenances. Given all of the species compared here were grown under common environmental conditions, yet maintained correlations with the climate of species origin or range, I expect that site would not have a large impact on the results observed here. However, based on these limitations, comparisons of older and larger trees across different sites and environmental conditions, including additional species and provenances, should be conducted in future.

While I included species from humid and sub-humid regions with differences in their distributional range, the species studied here do not encompass the broad aridity range available in Australia (BOM 2010). Accordingly, future studies should compare a larger number of species, species from climate extremes and the species studied here at the extremities of their climatic range to determine whether the correlations observed here are consistent across a broader range of environmental conditions. Furthermore, modelling the responses of individual species, and parameterising existing models, would further the field towards more accurate predictions of water use, productivity and survival of these and other species. Importantly, this study provides a basis for understanding the relationship between species climate of origin and plant physiological characteristics, and thus provides a promising starting point for broad categorisation of species responses based on climate of origin.

Chapters 2 and 3 were conducted in a common garden of monoculture plots of seven different *Eucalyptus* species (Figure 1-1, purple box). While this common garden was used to compare the response of different *Eucalyptus* species in monoculture, it also provides an existing competitive system with a gradient of interactions from entirely intra- to inter-specific. Preliminary comparisons of the VIGR of individuals in the centre and outskirts of monoculture plots revealed similar results to those reported in Chapter 4, where *E. tereticornis* individuals had higher HGR and VIGR when in mixture with *E. crebra* than in monoculture, and vice versa for *E. crebra* (Table 5-1). These competitive interactions increased over time, with tree HGR and VIGR respective to monoculture increasing by a further 30% and 59% in *E. tereticornis*, and decreasing by a further 32% and 25% in *E. crebra*, from 2010 to 2012 (Table 5-1). Based on the consistency between competition effects observed for growth, water use and soil moisture in Chapter 4, I expect that sap flow and soil moisture values would follow these preliminary VIGR observations. Accordingly, growth, water use and soil moisture could be measured and compared along the intra- to inter-specific gradient in this common garden to examine whether competition is dependent on species identity, whether it leads to suppression of inferior competitors and whether these interactions increase or decrease over time.

Chapter 4 reported increases in component species water use, growth and tolerance of low water availability across plots differing in species richness. This study was limited in the number of focal species measured, the functional types present and the replication of plot types due to resource and time constraints. Future studies could consider measuring both replicate plots of each plot type and all species in each plot so that estimates of plot-level water use and WUE can be evaluated. Despite my measurements being conducted early on in ecosystem development, I observed very clear differences in growth and water use of component species in mixtures compared to monocultures, which I expect would increase over time consistent with observations of VIGR change for common garden individuals (Table 5-1). Furthermore, the species selected here are all native to the Cumberland Plain Woodland, the dominant vegetation in the area in which these plantations were established. Accordingly, these plantations provide a model system for examining interactions between local species over time, particularly given data is available from the onset of these interactions.

Future studies should also consider the use of a higher diversity (i.e. >9 species; Balvanera et al. 2006) plot and a longer duration study, encompassing a broader array of environmental conditions including severe drought, compared to those encountered during this study (Table 5-2). The characterisation of grass, herb and shrub evapotranspiration and its measurement would be an informative facet to future studies, and would more closely reflect a natural ecosystem.

The observations reported in Chapters 2 and 4 focus on individual tree and plot water use. Although plot-level soil moisture is presented in Chapter 4, this data is restricted in the conclusions that can be made about individual trees and species in different plot types. A previous study on the same study site reported soil moisture uptake from depths below 100 cm (Duursma et al. 2011). Accordingly, further studies would benefit from examining soil moisture continuously at deeper depths, both within plots but also localised to individual trees. A combination of more detailed soil moisture data with a root harvest, the use of δ^{13}C labelling to determine rooting depth, and examinations of δ^{18}O isotopes of both soil and xylem sap, would provide insight into the interactions between component trees in different plot types throughout different conditions, and would allow characterisation of any soil moisture partitioning or root stratification occurring in more diverse plots. These in-depth soil moisture measurements, in addition with measurements of localised soil moisture, incoming precipitation, outgoing evaporation and canopy interception, would enable closure of the water balance for trees and plots and would assist the parameterisation of hydrological models for forested ecosystems. Future work should consider modelling transpiration under differing climatic conditions with currently used models (e.g. Penman-Monteith, 3-PG, WAVES, SPA and MAESPA) to determine whether employing species-specific parameters results in improvements in model estimates compared to when using generic parameters, and to determine what the associated error in modelled estimates using each option are. I consider the study periods presented here to have captured moderate to high water availability for this region, with some low water availability conditions considered in Chapter 2. Observing the responses of these species under drought conditions is important for determining how the dynamics of interspecific competition are altered. During moderate to high water availability conditions, *E. tereticornis* was the superior competitor and suppressed *E. crebra* growth and water use when in 2- and 8-species mixtures. However, based on its growth, stomatal behaviour (Chapter 2), hydraulic characteristics, leaf water relations and native distribution (Chapter 3), I hypothesise that *E. crebra* would be more capable of enduring periods of low water availability and would consequently be a better competitor in more arid regions, or in regions with sporadic rainfall. I observed that the competition between these species declined from 2- to 8-species mixture, and I would expect a similar pattern in droughted ecosystems, with species adapted to more arid conditions suppressing those adapted to wetter regions during drought. Additionally, the combination of these with other species, or different species combinations, would likely yield different results and accordingly further studies addressing these possibilities should be conducted in the future. The studies reported here provide a promising starting point, and the foundation of resources, for further research into climate of origin relationships with plant physiological traits, and interspecific competitive interactions in species mixtures. Ultimately, understanding how component trees in more diverse ecosystems interact is important to predictions of forest ecosystem water use and survival, and is thus important for conservation of our species, ecosystems and biological diversity as well as our catchment water supplies.

Table 5-1: The average VIGR, HGR and proportion GR of border trees and average trees in common garden monoculture plots from December 2010 until December 2011 and April 2012 until April 2013.

	Species	Tree HGR (cm·yr^{-1}·ha^{-1})	Tree VIGR (cm^{3}·yr^{-1}·ha^{-1})
	E. crebra		
2010-2011 December	Border	149.9 ± 32.8	28135.4 ± 8876.2
	Mean	150.3 ± 7.8	49116.9 ± 5093.5
	Border relative to mean (%)	-0.3	-42.7
2012-2013 April	Border	1.25 ± 0.03	23491.5 ± 7242.7
	Mean	1.86 ± 0.14	23491.5 ± 7242.7
	Border relative to mean (%)	-32.7	-67.5
Response change from 2010-2012 (%)		-32.4	-24.8
	E. tereticornis		
2010-2011 December	Border	206.8 ± 39.3	74153.4 ± 23116.6
	Mean	169.71 ± 20.41	42875.3 ± 6224.4
	Border relative to mean (%)	+21.8	+72.9
2012-2013 April	Border	1.53 ± 0.45	120892.1 ± 3891.9
	Mean	1.01 ± 0.18	52115.3 ± 8711.9
	Border relative to mean (%)	+51.8	+132.0
Response change from 2010-2012 (%)		+30.0	+59.0

Table 5-2: The environmental conditions experienced during this study and average environmental conditions from 1881-2015 in Richmond, NSW. Data was obtained from the bureau of meteorology website (http://www.bom.gov.au/climate/; accessed 24/09/2015) using the average of Richmond – UWS Hawkesbury (site 067021, 1881-2015) and Richmond RAAF (site 067033, 1928-1994 and site 067105, 1993-2015) observations. For BOM data *D* was calculated for the 3pm measurement of RH and temperature for each month and then averaged to get a monthly average maximum *D*. Superscripts are as follows: [a] – Long term rainfall data from UWS Hawkesbury (site 067021) where no other data was available, [*] *D* could only be calculated for 1939-2015 as 3pm temperature and relative humidity data was only available for these years.

Year	Mean yearly precipitation (mm)	Monthly average maximum *D* (kPa)	Monthly average minimum temperature (ºC)	Monthly average maximum temperature (ºC)
2010-2011 (Chapter 2)	516	3.68	5.3	31.6
2012 (Chapter 3)	876	3.53	4.6	31.8
2013-2014 (Chapter 4)	826	4.46	6.0	33.8
1907-2015	790	1.44[*]	10.9	23.9
1881-2015[a]	801	-	-	-

5.1.3 Implications of my research

I observed that climate aridity constrains stomatal sensitivity to D and response to restricted water availability (Chapter 2) and leaf tissue water relations, hydraulic, stomatal and anatomical characteristics (Chapter 3) in the *Eucalyptus* species studied. The correlations observed here of climate of origin with stomatal sensitivity to D ($R^2 = 0.77$, Figure 2-3d), decline in J_{Smax} ($R^2 = 0.94$, Figure 2-5a), growth ($R^2 = 0.93$, Figure 2-5c), trunk vessel diameter ($R^2 = 0.90$, Figure 2-5d), Ψ_{TLP} and Π_0 ($R^2 = 0.73$ and 0.69, Figure 3-1a and b) and $\mathrm{D_h}$ ($R^2 = 0.77$, Figure 3-1c) were significant and strong, and serve as a good starting point for a broader categorisation of species responses to climate. A broad correlation of species responses to climate would enable the input of physiological traits into species-specific transpiration models, and the categorisation of species responses to changing climates, according to species climate of origin characteristics.

Given that changes in climate and ecological responses are expected in the future (Walther et al. 2002; Parmesan and Yohe 2003; Parmesan 2006) it is becoming increasingly important to be able to assess and predict species responses to changing environmental conditions, and to determine how distribution and survival of species and ecosystems might be affected. Based on my observations, species physiological traits are somewhat constrained by the species climate of origin or range (Chapters 2 and 3), likely due to long-term genotypic adaptations to selective pressures within the species native climate. Previous studies have considered the rates of both genotypic and phenotypic change in plants whereby phenotypic plasticity enables the most accelerated changes in plant physiological traits (Aitken et al. 2008; Meier and Leuschner 2008; Turner et al. 2010; Anderson et al. 2012a;

Anderson et al. 2012b). However, there is little evidence that observed genetic shifts are of the type or magnitude to prevent climate change effects on species distribution or survival, and thus the species extinctions predicted for the future (Parmesan 2006). Determining how long such genotypic and phenotypic alterations would take to occur are important for predicting shifts in species distribution and survival under future climatic conditions, with changes in temperature, atmospheric CO_2 and water availability.

A large debate in the literature concerns drought-induced mortality (Allen et al. 2010; McDowell and Allen 2015), which has influenced every vegetated continent of the globe and is influenced by species identity and tree age (Fensham and Holman 1999; Anderegg et al. 2013a). Flow-on effects of drought-induced mortality are numerous, resulting in changes in community composition, vegetation-type and species distribution (Allen and Breshears 1998; Fensham et al. 2009; Collins et al. 2011), which influence nutrient cycling (Hanson and Weltzin 2000; Adams et al. 2012) and radiation, water and carbon fluxes (Royer et al. 2011; Adams et al. 2012) within ecosystems. Accordingly, drought-induced mortality is a key component to predicting how water availability and consequent transpiration of forested ecosystems will change under future climates, and how species distributions, ecosystem composition and vegetation types will respond. However, we currently lack the ability to predict mortality of tree species and forest types, in part due to uncertainty behind the mechanisms driving drought-induced mortality and the physiological thresholds of tree death (Allen et al. 2010).

Generally, larger and older trees are the most susceptible to drought stress and consequently drought-induced mortality, although seedlings are also vulnerable when near larger, more dominant competitors (Mueller et al. 2005; Nepstad et al.

2007; McDowell et al. 2008; Floyd et al. 2009). Recent advances in the understanding of mechanisms of tree mortality suggest that hydraulic failure, carbon starvation and a combination of these are the primary causes of drought-induced mortality (Sevanto et al. 2014; Camarero et al. 2015). However, as these mechanisms are species specific and reflect how individuals cope with drought stress, more research is required to be able to address how specific species and accordingly ecosystems might respond to drought. In my study, I observed differences in *Eucalyptus* species water use responses to changing water availability (Figure 2-4) which were significantly correlated with xylem vessel diameter ($R^2 = 0.98$, Figure 2-5), and a correlation of species vessel anatomy with climate of species origin ($R^2 = 0.77$, Figure 3-1b). Given hydraulic failure is one of the primary causes of drought-induced mortality (Sevanto et al. 2014), and that cavitation resistance is somewhat dependent on vessel characteristics (Lens et al. 2003; Lens et al. 2004; Choat et al. 2008), it can be hypothesised that a species likelihood of drought-induced mortality could be predicted based on the species climate of origin. However, studies of climate of origin influences on species responses would need to occur over many years, potentially multiple decades (Camarero et al. 2015), to observe enough severe or prolonged drought events to be able to determine any influences on drought-induced mortality.

At the site of a nearby (i.e. within 1 km of study site) climate change facility (EucFACE), examining a natural forested ecosystem, the woodland is composed of multiple species in mixture, with *E. tereticornis* as the dominant species and *E. crebra* as one of the inferior competitors in this system. This 80 year old ecosystem provides evidence of the outcomes of long-term interspecific interactions between these species. Accordingly, it provides support for my observation that

E. tereticornis is a dominant competitor, and that interspecific interactions can result in the suppression of the inferior competitor (*E. crebra*) over time within this climate (Chapter 4). Studies of the water use of individuals in the EucFACE are currently being conducted, and I expect that the water use per unit sapwood area is likely higher in EucFACE individuals than that reported for the trees measured here (Chapters 2 and 4) because the EucFACE trees are substantially larger, but also likely have access to groundwater, thus enabling higher water accessibility. Consistent with this theory, I observed an almost two-fold increase in J_S per unit sapwood area of 3 year old trees in the common garden plantation ($J_S \sim 200$ kg·m^{-2}·s^{-1}, Figure 2-4c) compared to 1 year old trees in the diversity plantations ($J_S \sim 100$ kg·m^{-2}·s^{-1}, Figure 4-3), suggesting that older, larger trees use more water. However, it is likely that tree size has less of an influence of water use per sapwood area once access to groundwater has been established, and once the transition from seedling to juvenile or mature tree has occurred. Measurements of groundwater access were not conducted in this study, so it is possible that the 3 year old trees in the common garden may have access to groundwater. If this is the case, it may explain any similarities that might be observed in water use rates between common garden and EucFACE trees when water use is considered on a sapwood area basis.

In my study, I identified competition for soil moisture resources as the major factor influencing the growth and water use of competing species (Chapter 4, specifically Figures 4-3 and 4-4). Similarly, in plantations located within the HFE study site and nearby (Figure 1-1), studies have reported large influences of water availability on evapotranspiration and J_S (Zeppel et al. 2011), leaf area via insect frass production (Gherlenda et al. 2015) and leaf respiration (Crous et al. 2011). However, these characteristics may fluctuate dependent on tree age. Duursma et al.

(2011) observed minimal influences of changing water availability in 2 year old *E. saligna* trees and attributed this to individuals accessing water at depth. Similarly, reports on 80 year old *E. tereticornis* trees at the nearby EucFACE site show no influence of water availability on stomatal behaviour (Gimeno et al. 2015), likely because individuals are accessing water from groundwater resources. Finally, in a study measuring 10 *Eucalyptus* species across different sites, Hatton et al. (1998) concluded that leaf water efficiency did not vary significantly among mature *Eucalyptus* trees. These reports differed from my observations in a common garden, where I found large differences in stomatal sensitivity to D, G_C, J_S, J_S response to water availability and tree VIGR reflecting the species climate of origin across five *Eucalyptus* species (Chapter 2, Figures 2-2 – 2-5). This may be due to a difference in juvenile (my) compared to mature (Hatton et al. 1998) trees, differences in access to groundwater resources or perhaps because the relationship hypothesised by Hatton et al. (1998) does not hold for all *Eucalyptus* species in all climatic conditions. Determining whether these relationships are consistent across a range of species, and in mixed ecosystems, is important for obtaining the necessary trait data to parameterise models in a range of environments.

The in-depth analysis of a range of physiological characteristics (Chapters 2 and 3) for particular species provides species-specific parameters necessary for input into forest ecosystem transpiration models (e.g. SPA, WAVES and MAESPA; Williams et al. 1996; Zhang et al. 1996; Duursma and Medlyn 2012 respectively). Species-specific observations can assist in the parameterisation of ecohydrological models, the selection of desirable species to ensure productivity and survival, the conservation of catchment water supplies and informing plantation and catchment management practices. By examining relationships between hydraulic characteristics, leaf water relations and stomatal behaviour, we gain more insight into the mechanisms by which a species can adapt to changing environmental conditions. This is important for predicting how individual species may respond to climate change, and how their water use, productivity and distributions may change with changes in selective pressures in their natural climatic range, allowing conservation of biological diversity and species assemblages in the future.

Collectively, the observations reported in this thesis indicate that *Eucalyptus* species are physiologically diverse with a close coordination between climate aridity and stomatal, hydraulic and anatomical characteristics. Importantly, the species studied were capable of maintaining this physiological diversity despite being planted and grown in the same environment, which holds important implications for predicting water use, growth and survival of species under differing climate scenarios. The *Eucalyptus* species considered here were strong competitors in species mixtures, enhancing productivity, water use and tolerance of low water availability compared to when planted in monoculture. My findings support the informed selection of species for plantation and catchment management, with particular species being more suited for particular environments under certain situations, as indicated above. Additionally, my findings support the use of species mixtures when a) the productivity and water use of a dominant species is preferred, b) a productivity and water use to that obtained in monoculture of a less dominant species, with the added productivity of another species, is favoured, or c) enhanced tolerance of low water availability is required. Natural ecosystems in Australia are generally composed of a diverse range of tree species with the addition of grasses and shrubs. Based on the observations reported here, this combination confers enhanced plot-level growth and water use, and tolerance of low water availability in component species, over ecosystems where only one species is present. Accordingly, a conservative approach to maintaining the biological diversity of natural ecosystems, as well as promoting mixture plantations where possible, may help ensure that these ecosystem services are maintained in a range of different ecosystems.

5.2 Conclusions

An understanding of the relationships of plant physiological and anatomical characteristics to climate of species origin and range is important for predicting how species water use and productivity may be affected by changing environmental conditions. Nevertheless, few studies have addressed these aspects of plant physiology, and those that have (Anderson et al. 1996; Vander Willigen et al. 2000; Cunningham 2004; Schulze et al. 2006; Merchant et al. 2007; Baltzer et al. 2008; Gleason et al. 2013) are limited in the range of species they compare, are compared under contrasting environmental conditions and do not address the genotypic constraints between physiology and climate. The effect of mixtures on component species responses is central to determining how natural, multi-species ecosystems transpire. However, studies on these relationships and their influence on water use, WUE and soil moisture are limited and are usually constrained in the number of species they compare. In this thesis, I addressed the following: (i) the relationship of climate of species origin and range with stomatal sensitivity to D, leaf tissue water relations and hydraulic, stomatal and anatomical characteristics, and (ii) the influence of species mixtures on component species growth, water use, tolerance of low water availability and soil moisture depletion in a set of *Eucalyptus* species growing in the field across multiple seasons. The goal was to contribute to the knowledge of the relationships of climate with plant stomatal and hydraulic information, and species mixtures with ecosystem function by (i) comparing physiological, anatomical, stomatal and hydraulic characteristics across different *Eucalyptus* species from contrasting climates under common garden conditions, and (ii) evaluating the mixture effect on component species and plantation growth, water use and soil moisture characteristics. The main hypotheses were that (i) climate of species origin and range constrains and is correlated with stomatal, hydraulic and anatomical parameters, and (ii) species mixtures would promote component species growth, water use and tolerance of low water availability due to enhanced soil moisture availability via root stratification.

This dissertation study demonstrated that there is a significantly negative correlation of aridity of climate of origin with stomatal sensitivity to *D* and decline in maximal sap flux density under low water availabilities for five *Eucalyptus* species from contrasting climates (Figure 2-3b and d). It also demonstrated that climate of species range is significantly correlated with leaf tissue water relations, xylem anatomy and hydraulic characteristics (Figures 3-2 and 3-3), and that water use strategy is constrained by the species climatic range in *Eucalyptus* (Chapter 3). Finally, it also demonstrated that mixtures positively influenced tolerance of low water availability (Figure 4-3), water use (Figure 4-5), WUE and growth (Figure 4-6) in *Eucalyptus* species plantations. Based on these studies, I conclude that transpiration is influenced by species identity and ecosystem composition, and that differences between species are influenced by hydraulic characteristics and leaf physiological relations which are correlated with species climate range. This holds significant implications for forestry, plantation, land and catchment management, and the results need to be considered in the key management decisions within such industries.

In summary, through detailed studies on the relationship of climate with plant physiology and mixture influences on component species responses, this thesis provides valuable information on how climate of origin constrains water use, hydraulic and anatomical characteristics of tree species, and how ecosystem composition can influence water use and productivity of component species and stands. The results obtained here may be used to further advance models of individual tree and multi-species stand water use (MAESPA; Duursma and Medlyn 2012). These observations are important for parameterisation of models of transpiration for individual trees and ecosystems. Additionally, the results obtained here can be used to inform land, plantation and catchment management practices towards more productive, water-use efficient and sustainable plantations that sustain water availability in catchments experiencing declining rainfall inputs. Most importantly, this information provides indications of how *Eucalyptus* species are constrained by species climate of origin and range, which holds important information for determining how species distribution, survival, productivity and water use may change under differing environmental conditions in the future.

Appendix A: Supplementary tables and figures

I. Chapter 1: Introduction

Table A-1: A summary of common garden studies comparing *Eucalyptus* species and provenances with regard to physiological characteristics.

Study	Species	Site location/characteristics	Experimental design	Plant age	Results
Anderson et al. (1996)	*E. camaldulensis*, *E. dives*, *E. melliodora*, *E. maculata*, *E. pilularis*, *E. botryoides*, *E. saligna*, *E. dunnii*, *E. paliformis*, *E. globulus*, *E. grandis*, *E. occidentalis*, *E. camaldulensis*, *E. viminalis* and *E. elata*	Wagga wagga, NSW. MAP 570 mm. Evaporation 1860 mm.	4.5 ha irrigated plantations. 6 seedlings of each species/provenance planted in 4 m by 5 m cells in 3 replicate blocks.	δ^{13}C and LMA - 7 months	Δ was positively correlated with MAP, December-march precipitation, annual soil moisture and precipitation seasonality and negatively correlated with potential evaporation during summer months. Variation in Δ and LMA reflect genetic adaptations to native habitats.
Héroult et al.	*E. cladocalyx*, *E. dunnii*,	Richmond, NSW.	See methodology for	2.5 years	Species from regions with lower

(2013)	*E. melliodora* and *E. saligna*	See methods for Chapter 2, section 2.3.1 of this thesis. In summary: MAP - 801 mm Min/max monthly rain – 31.7/128.7 mm Mean temp. - 17 ºC, Min/max temp - 3.5/30.1 ºC. Annual precip:evapotranspiration 0.6.	Chapters 2 and 3 of this thesis. Essentially, a common garden of 7 *Eucalyptus* species in replicated monoculture blocks of 35 trees each, planted at 1000 trees·ha^{-1} under natural climate conditions.		precipitation have lower marginal water cost of carbon gain and higher tolerance of low water availabilities. A relationship between marginal water cost of carbon gain and wood density across *Eucalyptus* species from differing climates.
Lewis et al. (2011)	*E. argophloia*, *E. camaldulensis*, *E. dunnii*, *E. globulus*, *E. sideroxylon* and *E. tereticornis*	Richmond, NSW. See methods for Chapter 2, section 2.3.1 of this thesis. In summary: MAP - 801 mm Min/max monthly rain – 31.7/128.7 mm Mean temp. - 17 ºC, Min/max	1.3 ha forest plantation with 20 individuals of 8 species planted in 10*16 m plots in one block.	7 years	Photosynthesis and night respiration were higher in xeric than mesic species, covaried and correlated with LMA, leaf N per unit area and daytime soluble sugar accumulation. Photosynthesis covaried with g_S. Differences in relationships between mesic and

		temp - 3.5/30.1 ºC. Annual precip:evapotranspiration 0.6.			xeric species in native habitats may be largely driven by environment rather than inter-specific genetic variation.
Li (1999)	*Eucalyptus microtheca* from different provenances	Helsinki, Finland Temperatures - 12-32 ºC RH – 40-70% Photon flux range – 800-100 μmol·m^{-2}·s^{-1}	Naturally lit greenhouse, 3 populations * 3 watering regimes with drought stress treatments.	Seedlings	Negative correlation between WUE and biomass productivity under one drought treatment with a positive correlation under another. Results suggest different water-use strategfies among populations with SE populations with lower WUE and NW and central populations with higher WUE (i.e. WUE dependent on population provenance).
Li et al. (2000)	*Eucalyptus microtheca* from different provenances	Helsinki, Finland Temperatures - 12-32 ºC RH – 40-70%	Naturally lit greenhouse, 3 populations * 3 watering regimes with	Seedlings	Significant variation in total biomass, height, root mass/foliage area ratio, foliage area/stem cross

		Photon flux range – 800-100 $\mu mol \cdot m^{-2} \cdot s^{-1}$	drought stress treatments.		sectional area ratio, specific leaf area (SLA), WUE and $\delta^{13}C$ among provenances. Significant positive correlations of mean driest quarter rainfall with total biomass, height, transpiration and SLA and negative correlations with root mass/foliage area ratio, foliage area/stem cross sectional area ratio, WUE and $\delta^{13}C$. Trees grown under drought stress had more foliage per stem area ratio.
Li and Wang (2003)	*Eucalyptus microtheca* from different provenances, West Kimberleys, Central Australia and Walgett.	Helsinki, Finland Temperatures - 12-32 ºC RH – 40-70% Photon flux range – 800-100 $\mu mol \cdot m^{-2} \cdot s^{-1}$	Naturally lit greenhouse, 3 populations * 3 watering regimes with drought stress treatments.	Seedlings	Populations from drier regions had lower biomass production and higher root/shoot ratio, foliage area/stem cross-sectional area ratio and specific leaf area density when under water deficit. Different populations may employ different

					survival strategies under drought stress.
Lin et al. (2013)	*E. crebra*, *E. tereticornis*, *E. saligna*, *E. melliodora*, *E. cladocalyx* and *E. dunnii*	Richmond, NSW. See methods for Chapter 2, section 2.3.1 of this thesis. In summary: MAP – 801 mm Min/max monthly rain – 31.7/128.7 mm Mean temp. – 17 ºC, Min/max temp – 3.5/30.1 ºC. Annual precip:evapotranspiration 0.6.	See methodology for Chapters 2 and 3 of this thesis. Essentially, a common garden of 7 *Eucalyptus* species in replicated monoculture blocks of 35 trees each, planted at 1000 trees·ha^{-1} under natural climate conditions.	1-2 years	Significant interspecific differences in the maximum rate of rubisco carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) but no difference in temperature responses of these parameters. Significant seasonal changes in V_{cmax} but not J_{max}, but no seasonal effect on temperature responses of either parameter. Growth environment had a larger effect on temperature responses than climate of origin for these species. Variation in photosynthetic temperature responses cannot be captured in one simple relationship with

					growth temperature.
McLean et al. (2014)	*E. tricarpa*	Two common garden plantings (Huntly and Lake Tyers) and nine natural forest sites with a rainfall gradient of 460-1040 mm	5 trees per row at a spacing of 4 * 1.8 m. Measured morphological and physiological traits reflecting climate responses in 9 provenances in natural habitat and in common gardens at each end of a rainfall gradient.	12 years	Local adaptation was evident in functional traits and differential growth rates in the common gardens. *E. tricarpa* individuals from provenances with lower rainfall were more plastic in leaf thickness whereas leaf size was more plastic in provenances with higher rainfall. Leaf density and stomatal physiology were highly and uniformly plastic.
Phillips et al. (2010)	*E. argophloia*, *E. camaldulensis*, *E. dunnii*, *E. globulus*, *E. grandis*, *E. occidentalis*, *E. sideroxylon* and *E. tereticornis*	Richmond, NSW. See methods for Chapter 2, section 2.3.1 of this thesis. In summary: MAP - 801 mm Min/max monthly rain –	1.3 ha forest plantation with 20 individuals of 8 species planted in 10*16 m plots in one block.	7-8 years	Nocturnal sap flux ($J_{S,n}$) contributed 5-7% of total J_S over 1 year. Main environmental driver of $J_{S,n}$ was D and wind speed. Higher foliage trees had greater $J_{S,n}$ and nocturnal g_s. *Eucalyptus* species

		31.7/128.7 mm Mean temp. - 17 ºC, Min/max temp - 3.5/30.1 ºC. Annual precip:evapotranspiration 0.6.			exerted strong nocturnal stomatal control over water loss.
Schulze et al. (2006)	422 species of *Eucalyptus*	Currency Creek, South Australia. MAP – 427 mm	Two separate sites with plantings in a 5 m grid pattern of *Eucalyptus* and *Corymbia* trees.	7-30 years	Large degree of variation in δ^{13}C, SLA and N concentration at any level of rainfall. SLA and leaf N concentration were similar along the aridity transect and in the common garden, indicating that these characteristics are inherent in the species and vary minimally with environment. Variation in leaf δ^{13}C was high, but not correlated with those values along the transect.

Sefton et al. (2002)	*E. occidentalis*, *E. grandis* and *E. camaldulensis*	Sydney, NSW. Environmental variables controlled in glasshouse environment: Temperature - 25/18 ºC day/night RH 48% on average. Radiation – natural.	Common garden with seedlings in pots inside glasshouse. Irrigated via drip irrigation, fertilised at planting	Gas exchange - 10 week seedlings Harvest – fortnightly until 12 weeks Leaf anatomy – 8, 10 and 12 weeks	Significant interspecific variation in specific leaf area (SLA) correlated with anatomy and photosynthesis. Leaf morphology affected N, water and CO_2 use. Differences in leaf structure reflect the most limiting resource experienced in the climate of origin.
Searson et al. (2004)	*E. grandis*, *E. sideroxylon* and *E. occidentalis*	Richmond, NSW. See methods for Chapter 2, section 2.3.1 of this thesis. In summary: MAP - 801 mm	Potted plants in a randomised block, common garden design in a glasshouse with two watering treatments.	Gas exchange – 10 and 13 weeks Harvests – 4	Photosynthetic capacity of *E. sideroxylon* and *E. occidentalis* was greater than *E. grandis* and stomatal conductance and WUE were negatively correlated.

		Min/max monthly rain – 31.7/128.7 mm Mean temp. - 17 ºC, Min/max temp - 3.5/30.1 ºC. Annual precip:evapotranspiration 0.6.		weeks and 13 weeks	*E. sideroxylon* and *E. occidentalis* had lower WUE than *E. grandis* under well-watered and water-limited conditions. There was no difference in N-use efficiency between species.
Turner et al. (2010)	60 *Eucalyptus* and *Corymbia* species including: *E. yilgarnensis*, *E. loxophleba*, *E. jutsonii*, *E. ravida*, *E. celastroides*, *E. calycogona*, *E. lesouefii*, *E. youngiana*, *E. intertexta*, *E. kingsmillii*, *E. urna*, *E. leptopoda*, *E. ewartiana* and *E. transcontinentalis*	Currency Creek, South Australia and Coolgardie, Western Australia. MAP – 427 mm, Currency Creek MAP – 216 mm, Coolgardie	Two separate sites with plantings in a 5 m grid pattern of *Eucalyptus* and *Corymbia* trees.	8-31 years	Significant differences in $\delta^{13}C$ among species with more negative $\delta^{13}C$ observed at wetter sites than drier sites. Specific leaf area (SLA) and leaf nitrogen content per unit leaf area differed significantly among species, but no common relationship between $\delta^{13}C$ and SLA or nitrogen content was observed. Strong species by environment interactions resulted in phenotypic plasticity in $\delta^{13}C$ being observed.

Warren (2006)	*E. nitens*, *E. viminalis*, *E. globulus*, *E. botryoides*, *E. saligna* and *E. grandis*	Two separate common garden sites: Mt Worth, Victoria MAP – 1220 mm PE – 1010 mm MAP – PE = +220 mm and Glencoe, NSW MAP – 600 mm PE – 1220 mm MAP – PE = -620 mm	88 seedlots representing 33 *Eucalyptus* species planted in a complete block design as 12-tree plots with 5 replicate plots.	16 years	Plant preference for N forms didn’t vary between sites despite large differences in relative abundances of N forms. Little evidence for acclimation of *Eucalyptus* species to differences in the relative availability of N forms. Suggests the possibility for considerable organic N uptake in the field when previous studies have only examined inorganic N.
Warren et al. (2005)	*E. sideroxylon* individuals from 15 populations with MAP range of 500 to 1500 mm.	Central Victoria, Australia. Mean temperature from 3.5-28.9 ºC. MAP - 550 mm PE – 1400-1600 mm.	Seeds planted in rip lines on 20 cm mounds with 5 trees per row at a spacing of 4 * 1.8 m.	3 years	Large difference among and within populations in all measured variables. Most relationships with seed-source rainfall were not significant, however there were weak positive correlations of specific leaf area (SLA),

					photosynthesis and stomatal conductance with seed-source rainfall. Populations collected from sites of similar rainfall often differed greatly in leaf morphology and physiology. There was significant genetic variation in leaf morphology and physiology, but for most traits, this variation was unrelated to rainfall at the site of seed collection.
Warren et al. (2006)	29 *Eucalyptus* species, namely: *E. cameronii*, *E. muellerana*, *E. regnans*, *E. delegatensis*, *E. dendromorpha*, *E. fastigata*, *E. fraxinoides*,	Two separate common garden sites: Mt Worth, Victoria MAP – 1220 mm PE – 1010 mm MAP – PE = +220 mm and	88 seedlots representing 33 *Eucalyptus* species planted in a complete block design as 12-tree plots with 5 replicate plots. Two separate common garden sites.	16 years	Site had a large effect on absolute values of leaf traits and relationships between pairs of traits. Leaf area and leaf width/length were the only traits related to rainfall of origin and there was no evidence that N allocation to Rubisco or thylakoid

	E. obliqua, *E. oreades*, *E. sieberi*, *E. triflora*, *E. elata*, *E. melliodora*, *E. bosistoana*, *E. paninculata*, *E. tereticornis*, *E. globulus*, *E. badgensis*, *E. benthamii*, *E. brookerana*, *E. cypellocarpa*, *E. dunnii*, *E. quadrangulata*, *E. smithii*, *E. viminalis*, *E. nitens*, *E. botryoides*, *E. grandis* and *E. saligna*.	Glencoe, NSW MAP – 600 mm PE – 1220 mm MAP – PE = -620 mm		proteins was related to rainfall of origin, or that WUE was greater in ecotypes from dry regions.

II. Chapter 2: Stomatal sensitivity to vapour pressure deficit relates to climate of origin in *Eucalyptus* species

Table A-2: Correlation matrix for species parameters with variables related to climate of origin where climate of origin variables used were means calculated across species points within a 30km radius of seed source from Atlas of Living Australia (http://www.ala.org.au; climate variables are annual means for years 2007-2010). Stomatal sensitivity to D is per unit sapwood area and the aridity index was calculated for climate at seed source using PET and precipitation estimates from BOM, as described in methods. ** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level and ^{ns} Correlation is not statistically significant.

	Volume index ($m^3 \cdot ha^{-1} \cdot yr^{-1}$)	**Stomatal sensitivity ($mol \cdot m^{-2} \cdot s^{-1}$)**	**Mean vessel diameter (µm)**	**Decline in J_{Smax} ($kg \cdot m^{-2} \cdot s^{-1}$)**	**Evaporation ($mm \cdot year^{-1}$)**	**Precipitation ($mm \cdot year^{-1}$)**	**Maximum water deficit ($mm \cdot month^{-1}$)**
Volume index growth	-						
Stomatal sensitivity	0.92^{*}	-					
Mean vessel diameter	0.97^{**}	0.83^{ns}	-				
Decline in J_{Smax}	0.96^{**}	0.82^{ns}	0.99^{**}	-			
Evaporation	-0.87^{ns}	-0.93^{*}	-0.79^{ns}	-0.83^{ns}	-		
Precipitation	0.81^{ns}	0.60^{ns}	0.78^{ns}	0.74^{ns}	-0.46^{ns}	-	
Water deficit	-0.97^{**}	-0.91^{*}	-0.95^{*}	-0.97^{**}	0.94^{*}	-0.66^{ns}	-
Aridity index	0.77^{ns}	0.87^{ns}	0.62^{ns}	0.57^{ns}	-0.69^{ns}	0.68^{ns}	-0.66^{ns}

III. Chapter 3: Species climate range influences leaf water relations, hydraulic and stomatal traits in seven *Eucalyptus* species

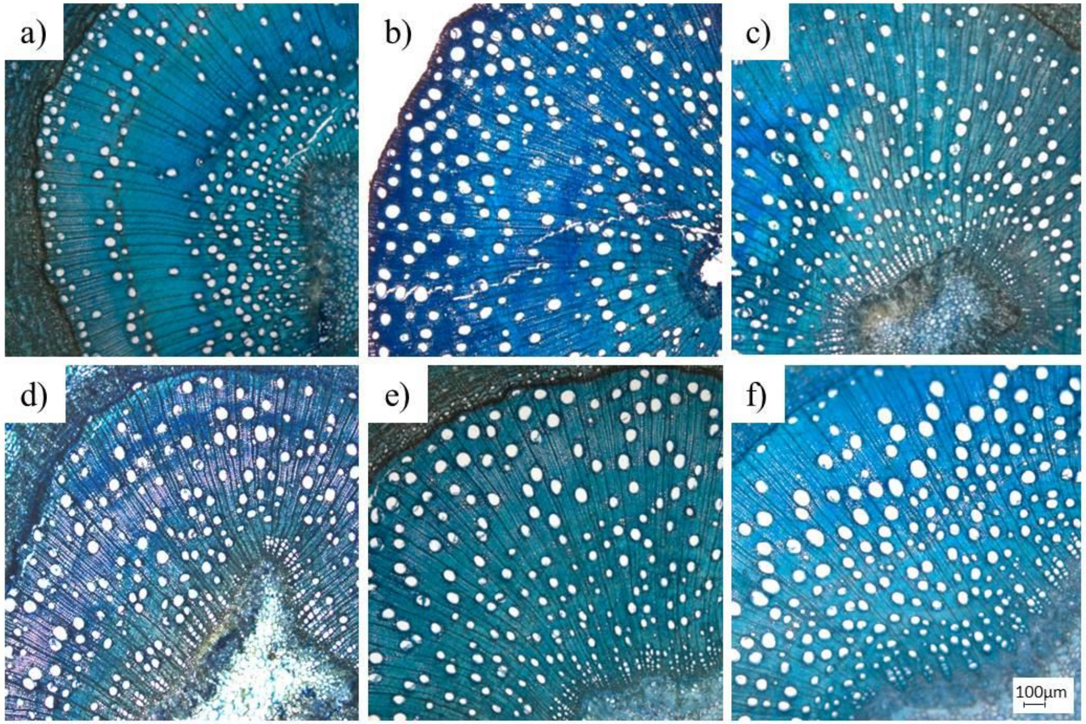


Figure A-1: Transverse sections of branch xylem tissue imaged with bright-field microscopy at 40X magnification for a) *E. cladocalyx*, b) *E. crebra*, c) *E. tereticornis*, d) *E. saligna*, e) *E. dunnii*, and f) *E. grandis*. Sections were stained with Toluidine blue, 0.05% (w/v). The scale is shown at bottom right and is consistent across all images.

Table A-3: Pairwise correlation matrix for hydraulic and anatomical parameters with variables related to the species climate range.

	Πo	**Ψ_{TLP}**	**VArea**	**D_h**	**VIGR**	**P_{12}**	**P_{50}**	**g_{s50}**	**g_{s12}**	**g_{s5}**	***AI***	**MAP**	**WD_{max}**
Ψ_{TLP} (MPa)	0.96***												
Vessel area (Varea; µm^2)	0.77*	0.90**											
D_h (µm)	-	0.73*	0.92**										
VIGR (m^3·ha^{-1}·yr^{-1})	0.74*	0.73*	-0.71*	-									
P_{12} (MPa)	-	-0.64*	-	-	-								
P_{50} (MPa)	-	-	0.70*	-0.74*	-	-							
g_{s50} (MPa)	-0.79*	-0.89**	-0.88**	-0.85**	-0.73*	0.68*	-						
g_{s12} (MPa)	-0.83*	-0.89**	-0.8*	-0.73*	-0.71*	-	-	0.93**					
g_{s5} (MPa)	-0.89**	-0.95**	-0.87**	-0.76*	-0.71*	-	-	-0.94**	0.98***				
AI	-	0.64*	-	-	0.8*	-0.72*	-	-0.81*	-0.73*	-0.69*			
MAP (mm)	0.66*	0.76*	0.70*	0.75*	0.84**	-	-	-0.93**	-0.88**	-0.84**	0.86*		
*WD*max	-0.90**	-0.85*	-	-	-0.89**	0.71*	-	0.75*	0.71*	0.75*	-0.77*	-0.72*	
$1/D_{max}$ (kPa^{-1})	0.76*	0.74*	-	-	0.76*	-0.84**	-	-0.79*	-0.73*	-0.73*	0.93**	0.74*	-0.87**

Values presented are R^2 values obtained using *JMP* (v 5.0.1, SAS Institute Inc., Cary, NC, USA).
Climate variables used were means calculated across all species points from Atlas of Living Australia (http://www.ala.org.au; accessed 12th December 2014; climate variables are annual means for years 2007-2010).
Abbreviations listed in the table of abbreviations within manuscript.
Asterisks indicate significant differences at ***0.001, **0.01 and *0.05 levels.
Where correlation was not statistically significant data was omitted (-).

III. Chapter 4: Competition and complementarity influence species transpiration, productivity and soil moisture in mixtures

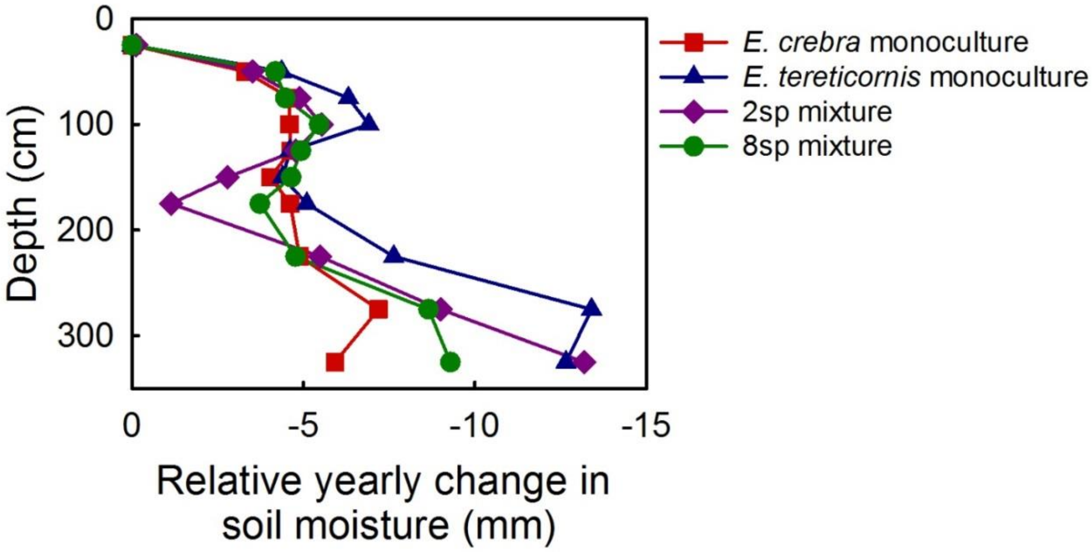


Figure A-2: Total soil moisture extraction relative to the maximal soil moisture measured for *E. crebra* monoculture (red square), *E. tereticornis* monoculture (blue triangle), 2-species mixture (purple diamond) and 8-species mixture (green circle) plots over the year 1st December 2013 to 1st December 2014, as measured using the neutron probe.

Table A-4: Maximal photosynthetic rate of two species in monoculture and 2-species mixture plots. Maximal A_{net} was the maximum measured over three days in three different months (December 2012, February 2013 and May 2013) averaged across two leaves in each of three trees per species per plot type. Standard errors indicate the difference between 3 replicate trees in each plot type. All species and plot combinations were statistically similar ($P = 0.87$, ANOVA).

Species	Plot type	Maximum A_{net} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)
E. crebra	Monoculture	28.76 ± 2.36^{ns}
	2-species mixture	27.14 ± 2.50^{ns}
E. tereticornis	Monoculture	24.56 ± 1.70^{ns}
	2-species mixture	27.81 ± 1.90^{ns}

Table A-5: Characteristics obtained from harvest for two trees of each of *E. crebra* and *E. tereticornis*. Wood density was measured during harvest in October 2013 and measured again in October and December 2014 (marked *). Root characteristics were measured separately from harvest in December 2013. Standard errors indicate the difference between replicate trees and *P*-values were determined using t-tests in sigmaplot. The taproot and lateral cross-sectional areas were divided by the sapwood area at 10 cm from ground to get sapwood specific cross-sectional areas in each case. Each tree of each species came from a different plot.

Species	*E. crebra*	*E. tereticornis*	*P*-values
Dry wood density (g·cm^{-2})*	0.66 ± 0.08	0.54 ± 0.02	<0.001
Rooting depth	>100 cm	>100 cm	-
Sapwood area at breast height (cm^{2})	8.20 ± 5.65	27.11 ± 0.23	0.42
Sapwood area at 10 cm (cm^{2})*	23.89 ± 9.36	71.62 ± 7.73	0.53
Tree height (cm)	291 ± 49	562 ± 29	0.48
Taproot cross-sectional area (cm^{2}) at 10 cm depth	11.6 ± 3.5	64.2 ± 3.6	0.009
Taproot cross-sectional area per sapwood area (cm^{2}·cm^{-2}) at 10 cm depth	0.51 ± 0.05	0.91 ± 0.15	0.27
Taproot cross-sectional area (cm^{2}) at 100 cm depth	7.3 ± 6.1	44.3 ± 17.1	0.19
Taproot cross-sectional area per sapwood area (cm^{2}·cm^{-2}) at 100 cm depth	0.24 ± 0.16	0.65 ± 0.31	0.075
Lateral root cross-sectional area (cm^{2})	9.3 ± 6.5	27.4 ± 12.9	0.083
Taproot cross-sectional area per sapwood area (cm^{2}·cm^{-2}) at 100 cm depth	0.33 ± 0.14	0.41 ± 0.22	0.058

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