Differential response to climate change among populations for woody plant species: An ecological and physiological approach

By

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Statement of Authentication

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

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A/C_i	Photosynthetic rates to intercellular [CO_2]
A/T_L	Photosynthetic rates to leaf temperature
A_{max}	CO_2-and light-saturated photosynthesis
A_{sat}	Light-saturated photosynthesis
A_{opt}	Light-saturated photosynthesis at thermal optimum
AGR	Absolute growth rate
[CO_2]	Atmospheric CO_2 concentration
C_A	Ambient [CO_2]
C_E	Elevated [CO_2]
DAP	Days after planting
E	Environment
G	Genotype
g_s	Stomatal conductance
J_{max}	Maximum rate of photosynthetic electron transport
LAR	Leaf area ratio
LMA	Leaf mass per area
LMF	Leaf mass fraction

MAP	Mean annual precipitation
NSC	Non-structural carbohydrates
PPFD	Photosynthetic photon flux density
PVC	Polyvinyl chloride
R_n	Leaf night respiration
RMF	Root mass fraction
Ss	Soluble sugar
St	Starch
SMF	Stem mass fraction
T_A	Ambient temperature
T_E	Elevated temperature
T_{opt}	Photosynthetic thermal optimum
TDR	Time domain reflectometer
TMF	Tuber mass fraction
V_{cmax}	Maximum rate of photosynthetic carboxylation
VPD	Vapour pressure deficit
VWC	Volumetric water content

Abstract

Changes in key climatic variables (e.g., atmospheric CO_2, air temperature and water availability) are occurring at unprecedented rates and having substantial impacts on functionality, biodiversity and productivity of terrestrial ecosystems. Because forests dominate terrestrial net primary production and play a prominent role in the global carbon cycle, understanding the capacity of woody species to cope with simultaneously changing climatic variables is critical for the management of natural resources and the conservation of biodiversity. One fundamental way that plants may respond to rapid climate change in the short-term is to adjust their growth and physiology via phenotypic plasticity – the ability of a genotype to express multiple phenotypes in response to environmental change, which is thought to be particularly important for woody species with long generation times. For any given species, plant populations originating from different environments usually differ in their responses to the same environmental change, as evidence of intraspecific variation in phenotypic plasticity. Although some progress has been made on intraspecific variation in woody plant response to climate change, no studies have looked into the interactive effects of concurrently changing climatic variables on their intraspecific variation in phenotypic plasticity. Therefore, my PhD thesis was designed to assess the impacts of key climatic variables (i.e., [CO_2], temperature, and water availability) on growth and physiology of woody plant populations originating from contrasting environments, with a focus on the intraspecific variation in their capacity to cope with climate change. Three Australian native woody species representing different taxa and functional groups were included in this research: *Telopea speciosissima* (Proteaceae; Shrub; open

woodland), *Eucalyptus grandis* (Myrtaceae; Tree; wet forest) and *Eucalyptus tereticornis* (Myrtaceae; Tree; dry forest), each of which consisted of two populations originating from climatically differentiated regions. Treatment levels (i.e., changes in [CO_2], temperature, and water availability) in this research were chosen based on predicted climatic conditions within this century. My goal was to use these woody species to generate improve understanding of woody plant growth and physiological responses under future climatic scenarios.

In the first experimental chapter, the main and interactive effects of elevated [CO_2] (C_E) and elevated temperature (T_E) on growth and physiology of the Coastal (warmer, less variable temperature environment) and the Upland (cooler, more variable temperature environment) genotypes of *T. speciosissima* were assessed. Seedlings were grown under two [CO_2] (400 µl l^{-1} and 640 µl l^{-1}) and two temperature (26/16 °C and 30/20 °C for day/night) treatments. Both genotypes were positively responsive to C_E (35% and 29% increase in whole-plant dry mass and leaf area, respectively), but only the Coastal genotype exhibited positive growth responses to T_E. It was observed that the Coastal genotype exhibited greater growth response to T_E (47% and 85% increase in whole-plant dry mass and leaf area, respectively) when compared with the Upland genotype (no change in dry mass or leaf area). No intraspecific variation in physiological plasticity was detected under C_E or T_E, and the interactive effects of C_E and T_E on intraspecific variation in phenotypic plasticity were also largely absent. Overall, T_E was a more effective climate factor than C_E in exposing genotypic variation in this woody species. Results from the chapter contradict the paradigm that genotypes from more variable climates will exhibit greater phenotypic plasticity in future climate regimes.

In the second experimental chapter, the main and interactive effects of elevated [CO_2] (C_E) and elevated temperature (T_E) on growth and physiological responses to drought of the Coastal (warmer and relatively wetter environment) and the Upland (cooler and relatively drier environment) genotypes of *T. speciosissima* were investigated. Seedlings were grown under two [CO_2] (400 μl l^{-1} and 640 μl l^{-1}) and two temperatures (26/16 °C and 30/20 °C for day/night). During the period of experiment, half of the seedlings were supplied with full watering (i.e., the *well-watered* treatment), while the other was subjected to controlled drought/recovery cycles (i.e., the *drought* treatment). The two genotypes showed similar declines in growth and photosynthesis under drought conditions across [CO_2] and temperature treatments, and did not exhibit differences in response to drought stress. Regardless of genotype, T_E negatively affected plant drought resistance by accelerating the process of drought seedlings becoming physiologically stressed, while C_E did not influence the capacity of plant drought resistance or alter the sensitivity of photosynthesis to declines in soil water content. Furthermore, C_E did not ameliorate the negative effects of T_E on drought response. Overall, these results suggest that woody plant populations originating from different environments may not necessarily show intraspecific variation in response to drought under current or predicted future climates. These findings also indicate that temperature is likely to be a stronger determinant than [CO_2] in affecting woody plant response to drought in the context of climate change.

The third experimental chapter aimed to examine the intraspecific variation in plant capacity to cope with simultaneously occurring climate extremes of two widely distributed *Eucalyptus* species (*E. grandis* and *E. tereticornis*). The main and interactive effects of warming (ambient + 3.5°C) and co-varying climate extremes (i.e., drought and heat waves) on growth and physiology of temperate (drier and cooler) and

tropical (wetter and warmer) provenances of each species were investigated. The two species in general did not show interspecific differentiation in response to the same environmental changes, but a significant intraspecific variation in plant growth response to warming and in photosynthetic response to heat waves was observed, both of which were correlated with taxon temperature of origin. Provenances of both species responded similarly in growth and physiology to single factor drought. It was also demonstrated that heat stress alone generally had little effect on plant growth and photosynthesis, but the synergism between drought and heat imposed significantly greater impact on plants than each applied separately. Furthermore, two distinct strategies (senescence of older mature leaves *vs.* complete closure of stomata) were observed, and both proved to be effective, in coping with combined drought and heat stress. Taken together, these results suggest that plant populations of widespread woody species may differ in their response to climate warming and heat waves depending on the climate of origin, but may not necessarily show difference in response to drought. These findings also indicate that drought is likely to be the dominant stressor during heat waves, while widespread woody species may possess different strategies to cope with the simultaneously occurring climatic extremes and show interspecific or even intraspecific variation.

In conclusion, my PhD research addressed the main and interactive effects of changes in multiple climatic variables (i.e., [CO_2], temperature, and water availability) on growth and physiology of three woody species representing different taxa and functional groups, with a focus on the intraspecific variation in their responses between populations originating from different environments. Results of this research were reported based on the treatment levels chosen for the experiments. Significant intraspecific variation in growth plasticity when responding to a constant mild

warming (T_E; ambient + 3.5–4.0 °C) was found in all three species, and intraspecific variation in photosynthetic responses to a short-term heat stress (ambient + 8 °C) was observed in the two *Eucalyptus* species. In contrast, populations did not differ in their growth or photosynthetic responses to elevated [CO_2] (C_E) or to sustained drought in most cases for all three species. These results together suggest that temperature would be more effective than [CO_2] or water availability in exposing intraspecific variation in phenotypic plasticity for woody plant populations under future climates. The relationships between phenotypic plasticity and source environment variability of plant populations differed among the three species. Results from the two *Eucalyptus* species confirmed the general prediction that greater levels of environmental variability will select for plants with greater phenotypic plasticity, while findings from *T. speciosissima* contradicted the paradigm, indicating that woody plant populations originating from more variable environments may not necessarily show greater phenotypic plasticity in response to climate change. In addition, T_E negatively affected plant resistance to drought and heat stress exacerbated the negative effects of drought on plant responses, suggesting that temperature may influence the responses of woody plants to drought under future climates.

Overall, my PhD work expands current knowledge regarding the interactive effects of simultaneously changing climatic variables on woody plant growth and physiology. More importantly, this research contributes valuable information on intraspecific variation in phenotypic plasticity of woody plant populations in response to changing climatic variables, as well as the association between phenotypic plasticity and source environment variability, which will assist in making robust predictions of the distribution and abundance of woody species under future climates.

Chapter 1

General introduction

1.1 Background

Increasing emissions of greenhouse gases from anthropogenic activities including rapid fossil fuel consumption and land use changes are contributing to the ongoing global climate change. Atmospheric carbon dioxide concentrations ([CO_2]) have been increasing from about 280 μl l^{-1} before the industrial revolution to over 400 μl l^{-1} nowadays and are projected to exceed 550–900 μl l^{-1} by the end of this century (Collins *et al.*, 2013). Rising [CO_2] is expected to cause a 0.3–4.8 °C increase in the global mean air temperature during same time period (Solomon *et al.*, 2009; Collins *et al.*, 2013). Embedded with this climatic warming trend, increases in the frequency and intensity of extreme climatic events such as drought and heat waves are also anticipated through this century according to current climate change models (Meehl & Tebaldi, 2004; Della-Marta *et al.*, 2007; Kharin *et al.*, 2007; Ballester *et al.*, 2010; Yao *et al.*, 2013). Similar predictions have been made for Australia in terms of climate change. By 2070, annual mean air temperatures in Australia are projected to increase by 1–6 °C, with summer temperatures exceeding 35 °C expected to occur over 10 times more frequently in the meantime (Pearce *et al.*, 2007). Annual precipitation is also predicted to decline in many parts of Australia in the coming decades (Pittock, 2003; Pearce *et al.*, 2007; Moise & Hudson, 2008).

Changes in these climatic variables are likely to substantially regulate plant growth, function and development, thereby affecting functionality, biodiversity and productivity of terrestrial ecosystems (Nemani *et al.*, 2003; Ciais *et al.*, 2005; Williams *et al.*, 2008; Allen *et al.*, 2010; Matesanz *et al.*, 2010; Barnosky *et al.*, 2012). On the global scale, forests cover about 30% of land surface and dominate terrestrial net primary production (up to *c.* 70%), playing a prominent role in the global carbon cycle (Schimel *et al.*, 2001; Karnosky, 2003; Norby *et al.*, 2005; FAO, 2006; Bonan, 2008; Beer *et al.*, 2010; Pan *et al.*, 2011). Therefore, quantifying and understanding the capacity of woody species to cope with simultaneously changing climatic variables is of particular importance for the management of natural resources and the conservation of biodiversity (Sala *et al.*, 2000; Mawdsley *et al.*, 2009).

To cope with the ongoing rapid anthropogenic climate change, species will have to rely on different approaches such as distinct ecological (e.g., habitat shifts and phenotypic plasticity) and evolutionary strategies (e.g., adaptation and gene flow), as well as in combination (Kawecki, 2008; Anderson *et al.*, 2012). One fundamental way that plant species may respond to changing climatic variables in the short-term is to adjust their growth and physiology via phenotypic plasticity – the ability of a genotype to express multiple phenotypes in response to environmental change (Bradshaw, 1965; Sultan, 2000; Nicotra *et al.*, 2010; Anderson *et al.*, 2012). For woody plant species with long generation times, phenotypic plasticity is thought to be particularly important for acting as a buffer against rapid climate change and providing growth advantages (Valladares *et al.*, 2007; Chevin *et al.*, 2010; Nicotra *et al.*, 2010), because their evolutionary responses by natural selection might be too slow to mitigate the effects of rapid environmental change.

For any given plant species, when genotypes show differentiated responses to the same environmental change, intraspecific variation in phenotypic plasticity exists, known as significant genotype (G) by environment (E) interactions (Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). Intraspecific variation in phenotypic plasticity would not only influence the habitat range occupied by plant species, but also affect the ecological and evolutionary responses of plant species to changing environments (Sultan, 2000; Van Kleunen & Fischer, 2005; Valladares *et al.*, 2007; Williams *et al.*, 2008; Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). For instance, genotypes with low phenotypic plasticity may tolerate and persist under extreme conditions to survive and maintain growth (Schlichting, 1986; Thompson, 1991), while genotypes with high phenotypic plasticity may be capable of rapid resource uptake and show increased growth when conditions are optimal (Grime & Mackey, 2002). Therefore, studies on intraspecific variation in phenotypic plasticity of woody plants in response to changing climates are essential for making robust predictions of woody species responses under global climate change, as well as identifying genotypes that exhibit the capacity to increase or maintain productivity under more extreme climatic conditions in the future (Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015; Moran *et al.*, 2016).

Although previous studies have demonstrated intraspecific variation in growth or physiological plasticity of woody plant species in response to elevated [CO_2] (e.g., Dickson *et al.*, 1998; Mohan *et al.*, 2004; Cseke *et al.*, 2009), or elevated temperature (e.g., Weston & Bauerle, 2007; Weston *et al.*, 2007; Drake *et al.*, 2015), or water deficit (e.g., Cregg & Zhang, 2001; Monclus *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Bansal *et al.*, 2015), the nature and basis of intraspecific variation in phenotypic plasticity within woody species under climate change remains largely unknown. To date, no study has looked into the interactive effects of concurrently changing climatic

variables on intraspecific variation in phenotypic plasticity of woody plant species. To better understand and predict how woody plants would respond to future climatic scenarios, manipulations of combinatorial experiments assessing the main and interactive effects of [CO_2], temperature and water availability on intraspecific variation of woody plant responses are necessary.

1.2 Review of literature

1.2.1 Intraspecific variation in phenotypic plasticity under climate change

1.2.1.1 Plastic phenotypic responses of plants to environmental changes

Phenotypes of individual plants are determined by genotypes that underlie quantitative traits, environmental conditions, and the interactions between genotype and environment (Howe *et al.*, 2003; Savolainen *et al.*, 2007; Wang *et al.*, 2010). It has been well acknowledged that the ability of plants to sense environmental changes and produce plastic responses is determined by a portion of the genetic variation, and that plastic phenotypic responses can both provide a buffer against rapid environmental changes and assist rapid adaptation (Jump et al., 2009, Lande, 2009, Chevin et al., 2010, Nicotra et al., 2010).

For any given plant species, different genotypes may respond similarly or differently to the same environmental changes, or show no response at all, resulting in the differential responses in phenotype (Fig. 1-1; also see Van Kleunen & Fischer, 2005; Valladares *et al.*, 2007; Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). Specifically, three primary patterns of response can be expected: (i) phenotype is

regulated by environment only through phenotypic plasticity and there is no genetic effect, in which phenotypic responses among genotypes will be similar (*Similar responses*; Fig. 1-1a); (ii) phenotypic responses under changing environments are controlled by genotypes that differ constitutively in traits and there is no environmentally induced change (*No environmental responses*; Fig. 1-1b); (iii) phenotype is mediated by environment (via phenotypic plasticity) and genotype (via genetic adaptation) as well as their interactions, in which phenotypic responses differ significantly under changing environmental conditions (*Differential responses*; Fig. 1-1c), as evidence of intraspecific variation in phenotypic plasticity (Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015).

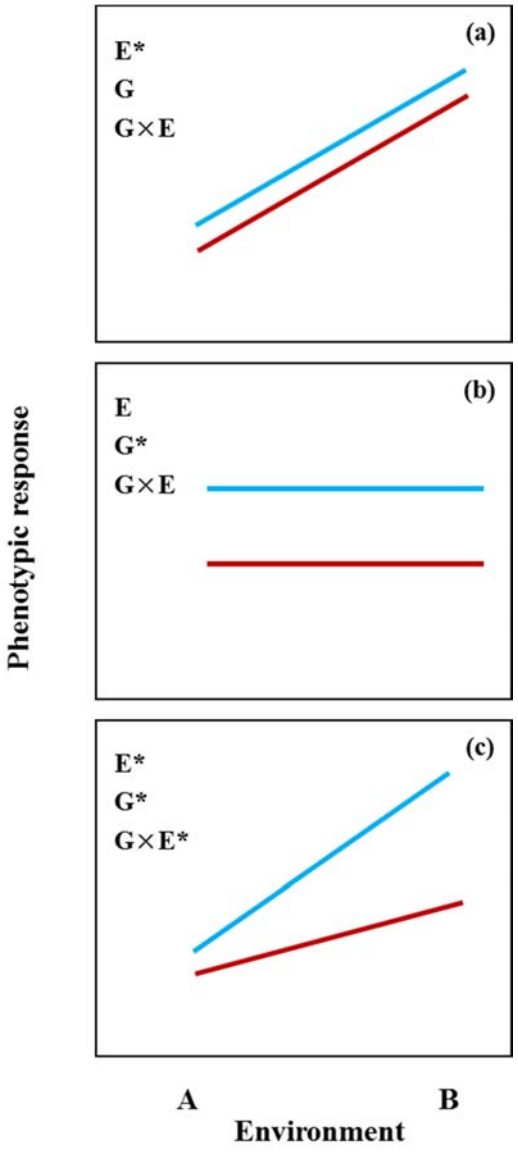

Figure 1-1 Reaction norms of different genotypes responding to a change from environment A to environment B (adapted from Nicotra *et al.*, 2010). The three patterns of response are: (a) similar responses between genotypes; (b) no environmental responses; and (c) differential responses between genotypes. The blue and red lines represent different genotypes; asterisks in the panel indicate whether there is a significant effect of environment (E) or genotype (G) and whether there is a significant genotype by environment interaction (G × E).

1.2.1.2 Intraspecific variation in woody species responses to changing climatic variables

Plant species and populations usually differ greatly in phenotypic plasticity (Weinig, 2000; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen & Fischer, 2005). It has been suggested that plant species may exhibit significant variation in functional traits among populations across environmental gradients responding to the same climate regime, because their populations are generally highly adapted to local conditions (Savolainen *et al.*, 2007; Hereford, 2009; Wang *et al.*, 2010; McLean *et al.*, 2014). Therefore, for a given woody species in response to changing climates, populations originating from different environments are likely to show differentiated plasticity in growth and physiological traits, as evidence of G × E interactions (Aspinwall *et al.*, 2015). In the following paragraphs of this section, a basic introduction of current knowledge about intraspecific variation in phenotypic plasticity of woody species is presented, with respect to the effects of changing key climatic variables (i.e., atmospheric CO_2, temperature and water availability) on their responses.

Elevated CO_2

Elevated [CO_2] (C_E) is generally reported to positively affect woody plant growth (see Ainsworth & Long, 2005; Seneweera & Norton, 2011; Wang *et al.*, 2012), but the effects may be genotype dependent. Although studies are limited, substantial intraspecific variation in woody plant responsiveness to C_E is usually observed. For example, in a series studies of *Populus tremuloides* (known as aspen) in response to long-term C_E at the Aspen free-air CO_2 enrichment site, significant difference in terms

of growth enhancement was found between two aspen genotypes (clones 216 and 271) (Isebrands *et al.*, 2001; Karnosky *et al.*, 2005; Kubiske *et al.*, 2007), despite the fact that these two clones showed similar increases in photosynthetic rates under C_E (Noormets *et al.*, 2001; Riikonen *et al.*, 2008; Taylor *et al.*, 2008). A further study on leaf-level transcriptomes also revealed significant intraspecific variation in expression patterns between these two aspen genotypes (Cseke *et al.*, 2009). Other studies on different hybrid poplar (*Populus*) clones (Ceulemans *et al.*, 1996; Dickson *et al.*, 1998) and different populations/provenances of red maple (*Acer rubrum*) (Mohan *et al.*, 2004) also found significant intraspecific variation in growth responses under C_E. However, this trend is not universal, because there are also cases showing limited intraspecific variation in woody plant responsiveness to C_E (Cantin *et al.*, 1997). Collectively, these studies suggest that the intraspecific variation in the response of woody species to C_E will be complicated and may be species specific.

Warming

For woody species, atmospheric warming can have a variable effect on plant growth and development, depending on the the taxon's climate of origin (Saxe *et al.*, 2001; Way & Oren, 2010; Drake *et al.*, 2015). Many studies suggest that a mild warming would be beneficial to the growth of woody plants from relatively cool regions at high latitudes or altitudes, where plant growth may be temperature-limited (e.g., Carter, 1996; Rehfeldt *et al.*, 1999; McKenzie *et al.*, 2001; Bunn *et al.*, 2005; Thomson *et al.*, 2009; Hanninen & Tanino, 2011). In contrast, warming is likely to negatively affect woody plants from tropical regions, where source temperatures are close to thermal optima such that further warming would be detrimental rather than

beneficial (Clark *et al.*, 2003; Feeley *et al.*, 2007; Doughty & Goulden, 2008; Clark *et al.*, 2010).

A commonly reported mechanism for the physiological responses of plants to warming is thermal acclimation (see Atkin & Tjoelker, 2003; Campbell *et al.*, 2007; Kattge & Knorr, 2007; Way & Oren, 2010). Numerous studies have investigated the intraspecific variation in thermal acclimation of photosynthesis or respiration in woody species, but the results are inconclusive. For instance, studies on two clonal genotypes of red maple (*Acer rubrum*) originating from thermally contrasting habitats demonstrated significant intraspecific variation in photosynthetic response to warming, in which the warm-origin genotype maintained higher photosynthetic rates and V_{cmax} under warmed conditions (Weston & Bauerle, 2007; Weston *et al.*, 2007). Another study on jack pine (*Pinus banksiana*) also observed that warm-origin populations showing a greater seasonal range in the base respiration rates and in the temperature sensitivity of respiration, when compared with cool-origin populations (Tjoelker *et al.*, 2009). However, there are also studies showing no intraspecific variation in thermal acclimation of photosynthesis or respiration between populations of loblolly pine (Teskey & Will, 1999), or populations of sugar maple (*Acer saccharum*) (Gunderson *et al.*, 2000).

Drought

Water is probably the most important factor limiting plant growth and function, by affecting almost all biochemical and physiological processes. Therefore, intraspecific variation in plant species response to drought and variable soil moisture has received more attention than any other climatic variable (Bohnert *et al.*, 1995;

Aspinwall *et al.*, 2015). For example, significant intraspecific variation in drought responses has been well documented in a wide range of woody species, including the genera *Eucalyptus*, *Pinus*, *Populus* and *Quercus* (e.g., Cregg & Zhang, 2001; Silva *et al.*, 2004; Monclus *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Bedon *et al.*, 2012; McLean *et al.*, 2014). Populations of woody species from different rainfall regions usually show evidence of local adaptation to climate to some extent (see McLean *et al.*, 2014), and therefore their capacity to cope with drought may differ. For instance, populations of woody species from more water-stressed environments often possess a suite of leaf-level traits (smaller, thicker leaves with higher water use efficiency) associated with greater water conservation, thereby showing less growth responsiveness but greater tolerance to drought (e.g., Gratani *et al.*, 2003; Baquedano *et al.*, 2008; Aranda *et al.*, 2010; Bansal *et al.*, 2015). By contrast, woody plant populations originating from more mesic regions are often found more susceptible to drought (Cregg & Zhang, 2001; Silva *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Dutkowski & Potts, 2012; Robson *et al.*, 2012).

1.2.1.3 Association between phenotypic plasticity and source environment variability of woody plant populations

Plant populations usually show intraspecific differentiation in phenotypic plasticity and the divergence among populations may be linked to the pattern of their source environmental variation. A long-standing hypothesis suggests that greater levels of environmental variability will select for genotypes that exhibit greater phenotypic plasticity (Galloway, 1995; Ackerly *et al.*, 2000; Weinig, 2000; Donohue *et al.*, 2001; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen

& Fischer, 2005). This hypothesis predicts that plant populations from habitats with more variable temperature conditions are likely to show greater growth and/or physiological responses to changes in temperature, relative to populations of the same species from less variable temperature climates. Although testing this hypothesis on woody plant species to date is extremely limited, there is at least one case study that supports the theory (Drake *et al.*, 2015). Specifically, this case study on two widespread eucalyptus species (*E. tereticornis* and *E. grandis*) showed that, for both species, provenances originating from cooler and more variable temperature climates exhibited higher plasticity in growth and photosynthetic capacity under warming, when compared with provenances from warmer and more uniform temperature climates (Drake *et al.*, 2015). Nevertheless, to validate this hypothesis, more studies on other woody species are necessary.

1.2.2 Effects of climatic variables on woody plant responses

1.2.2.1 Effects of elevated [CO_2] and elevated temperature

Many studies have investigated the main and interactive effects of elevated [CO_2] (C_E) and elevated temperature (T_E) on woody plant growth and photosynthesis (see Wang *et al.*, 2012). For woody species grown under non-water-limited conditions, C_E generally will stimulate biomass accumulation via the enhancement of carbon assimilation and increases in leaf area (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Seneweera & Norton, 2011; Wang *et al.*, 2012), despite frequently observed partial down-regulation of photosynthetic capacity (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Leakey *et al.*, 2009a). Increasing [CO_2] can also lead to accumulation of non-structural carbohydrates (NSC) (Stitt & Krapp, 1999; Nowak *et al.*, 2004; Robinson *et al.*, 2012), which mainly functions as carbon storage to reconcile temporal asynchrony between carbon demand (i.e., growth and metabolism) and carbon supply (i.e., photosynthesis) (Sala *et al.*, 2012). The effects of T_E on woody plants are more complicated and tend to differ, depending on whether warming exceeds their physiological thermal optima (Berry & Bjorkman, 1980; Sage & Kubien, 2007; Sage *et al.*, 2008). A mild increase in temperature (typically 3–5 °C higher than the ambient but still below the thermal optimum) is often reported to increase photosynthesis and dry mass production for woody plants not experiencing water limitation (Saxe *et al.*, 2001; Kattge & Knorr, 2007; Ghannoum *et al.*, 2010a, 2010b; Way & Oren, 2010).

The trend for the interactive effects of [CO_2] and temperature on woody plant species is not clear in the literature. Many studies show that T_E and C_E are likely to interact in a positive manner on woody plant growth and/or physiology (e.g., Callaway

et al., 1994; Peltola *et al.*, 2002; Ghannoum *et al.*, 2010a; Ayub *et al.*, 2011). For instance, more increases in woody plant net photosynthesis induced by C_E were found at higher temperatures when compared with non-warming treatments, according to results from a meta-analysis (Wang *et al.*, 2012). However, contrasting results also have been observed. For example, Wertin *et al.* (2011) reported that increases in air temperature resulted in a suppression of growth in trees grown near the southern limit (warmer temperatures) of the species distribution under C_E. In addition, many other studies found that the effects of T_E and C_E were additive rather than synergistic, suggesting no interaction between [CO_2] and temperature on woody plant responses (e.g., Morison & Lawlor, 1999; Lewis *et al.*, 2001, 2013; Lloyd & Farquhar, 2008; Gauthier *et al.*, 2014). Clearly, the interactive effects of T_E and C_E on woody species responses need to be further examined.

1.2.2.2 Effects of elevated [CO_2] and elevated temperature on drought response

Plants generally would close their stomata to reduce water usage when responding to drought or continuous water deficit, which subsequently result in drought-induced inhibition of photosynthesis and reductions in biomass accumulation, as well as reductions in carbohydrate reserves (Chaves, 1991; Flexas *et al.*, 2002; Chaves *et al.*, 2003; Muller *et al.*, 2011; Mitchell *et al.*, 2013). However, the effects of drought on plants are likely to be altered by changes in [CO_2] and temperature, both of which would influence the susceptibility of woody species in response to drought (Lewis *et al.*, 2013; Way, 2013).

Elevated [CO_2] (C_E) often reduces stomatal conductance (g_s) under non-water limiting conditions, as has been observed in most woody plants studied (see

Wullschleger *et al.*, 2002; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Wang *et al.*, 2012), despite that there are some exceptions as well (e.g., Saxe *et al.*, 1998; Ellsworth, 1999; Duan *et al.*, 2014, 2015). Reductions in g_s generally lead to reduced plant water usage, which may allow plants to maintain relatively more favourable water status during sustained drought and therefore ameliorate the negative impact of drought stress on plant physiology and growth (Morison, 1993; Poorter & Pérez-Soba, 2001; Wullschleger *et al.*, 2002; Ainsworth & Rogers, 2007; Duan *et al.*, 2013). However, the effects of C_E on woody species response to drought vary among studies. C_E was found to mitigate the negative effects of drought on plant performance in some studies (Ambebe & Dang, 2010; Wertin *et al.*, 2010; Ayub *et al.*, 2011; Duan *et al.*, 2013; Franks *et al.*, 2013; Lewis *et al.*, 2013), but not in others (e.g., Bobich *et al.*, 2010; Duursma *et al.*, 2011; Perry *et al.*, 2013; Duan *et al.*, 2014, 2015).

By contrast, the effects of elevated temperature (T_E) on plant drought responses tend to be fairly consistent. Generally, under drought conditions, rising temperatures will accelerate transpiration water loss for the need of larger evaporative cooling through the increase in vapour pressure deficits (VPD), which will in turn speed up the drawdown of soil water content and hence create a positive feedback loop to magnify or exacerbate the negative effects of drought (Larcher, 2003; Oishi *et al.*, 2010; De Boeck *et al.*, 2011; Will *et al.*, 2013; Teskey *et al.*, 2015). For example, the negative effects of T_E on plant drought responses have been observed in a wide range of woody species (e.g., Adams *et al.*, 2009; Allen *et al.*, 2010; Duan *et al.*, 2013, 2014, 2015; Will *et al.*, 2013; Zhao *et al.*, 2013)

Due to the contrasting effects of C_E and T_E regulating drought responses, their combined effects on woody species tolerance to water deficit may vary, possibly depending on the trade-offs between these two climatic factors (Duan *et al.*, 2013).

Some studies suggest that C_E and T_E can interact synergistically and affect physiological responses of woody plant seedlings to drought (Zeppel *et al.*, 2012), while other studies indicate that the effects of rising [CO_2] and warming on woody species under drought are simply additive (e.g., Ambebe & Dang, 2010; Duan *et al.*, 2013; Lewis *et al.*, 2013). Although the number of combinatorial experiments studying the interactive effects of [CO_2], temperature and water availability on woody species is growing recently (Ambebe & Dang, 2010; Wertin *et al.*, 2010, 2012; Zeppel *et al.*, 2012; Duan *et al.*, 2013, 2014, 2015; Lewis *et al.*, 2013; Gauthier *et al.*, 2014), to what degree C_E and T_E in combination will alter woody plant drought responses remains largely unknown.

1.2.2.3 Effects of climate extremes on woody plants

The short-term heat waves could trigger changes in processes from the molecular level to the whole plant, and the effects may vary among species and genotypes (Wahid *et al.*, 2007; Aspinwall *et al.*, 2015; Teskey *et al.*, 2015). The most commonly observed effects of heat waves on woody plants include reduction in biomass accumulation and leaf area development, inhibition of photosynthesis efficiency, and stimulation of mitochondrial respiration (Hamerlynck *et al.*, 2000; Ameye *et al.*, 2012; Bauweraerts *et al.*, 2013, 2014; Teskey *et al.*, 2015). However, effects of heatwaves on woody plants may vary a lot, depending on whether heat stress is coupled with drought stress.

It has been suggested that heat waves under well-watered conditions may only have small or transient effects on plants, because plants could continuously cool their leaves via transpiration to mitigate the heat stress, when there is sufficient water (De

Boeck *et al.*, 2010, 2011; Teskey *et al.*, 2015). In fact, woody plants under well-watered conditions can cope well with high temperatures (> 40 °C) over a short duration, in most circumstances (Cunningham & Read, 2006; Teskey *et al.*, 2015). For example, Ameye *et al.* (2012) reported that seedlings of *Pinus taeda* and *Quercus rubra* from a warm temperate region were capable of tolerating daytime temperatures exceeding 50 °C, without any sign of visible damage to leaves.

Given the fact that heat waves in the field typically occur in combination with periods of precipitation deficit (Vautard *et al.*, 2007; De Boeck *et al.*, 2010; Stefanon *et al.*, 2014), it is necessary to study the combined effects of co-occurring climate extremes on plant responses. In fact, it has been widely suggested that heat stress and drought in combination can impose significantly greater impacts on plants and ecosystems than each applied separately (Mittler, 2006; De Boeck *et al.*, 2011; Dreesen *et al.*, 2012; Bauweraerts *et al.*, 2013; Zinta *et al.*, 2014). During the simultaneously occurring climate extremes, the negative effects on plants induced by single factor drought are likely to be exacerbated by heat stress, suggesting that drought is the dominant stressor for plant species during heat waves (Reichstein *et al.*, 2007; De Boeck *et al.*, 2010, 2011; Bauweraerts *et al.*, 2014; Hoover *et al.*, 2014; Teskey *et al.*, 2015). However, to better understand the underlying mechanisms of woody plant responses to co-occurring climate extremes, more manipulative experiments investigating the impacts of heat stress and drought on woody species are needed.

1.3 Overview of my thesis

1.3.1 Thesis objectives

The overall objective of my PhD research was to assess the impacts of key climatic variables (i.e., [CO_2], temperature, and water availability) on growth and physiology of woody plant populations originating from contrasting environments, with a focus on the intraspecific variation in their capacity to cope with climate change. Three ecologically and economically important Australian native woody species representing different taxa and functional groups were included in this research: *Telopea speciosissima* (Proteaceae; Shrub; open woodland), *Eucalyptus grandis* (Myrtaceae; Tree; wet forest) and *Eucalyptus tereticornis* (Myrtaceae; Tree; dry forest), each of which consisted of two populations originating from climatically differentiated regions. The research was conducted in a state-of-the-art glasshouse facility located at the University of Western Sydney with pot-grown woody plant seedlings. The glasshouse was set to control [CO_2] (ambient and ambient + 240 $\mu l\ l^{-1}$) and temperature (ambient and ambient + 3.5–4.0 °C, or ambient + 8 °C) conditions for simulating current and future climatic scenarios within this century based on model predictions. These combinatorial studies on woody species representing varying taxa and functional attributes were aimed to improve understanding on intraspecific variation of woody plant growth and physiological responses to simultaneously changing climatic variables (i.e., [CO_2], temperature, and water availability). Specifically, my thesis sought to address the following questions:

(1) Do changes in climatic variables independently or interactively expose intraspecific variation in phenotypic plasticity of woody plant populations originating from different environments?

(2) If differentiated responses between woody plant populations exist, what are the relationships between phenotypic plasticity and their source environmental variability?

(3) How will climatic variables interactively affect growth and physiology of woody plants under future climates?

1.3.2 Outline of my thesis

Chapter 1 presented a general introduction for my PhD research.

Chapter 2 aimed to examine how genetically differentiated *T. speciosissima* populations originating from contrasting environments would respond to simultaneously changing [CO_2] and temperature under non-stressed conditions. The main and interactive effects of elevated [CO_2] (C_E) and elevated temperature (T_E) on growth and physiology of the Coastal (warmer, less variable temperature environment) and the Upland (cooler, more variable temperature environment) genotypes of *T. speciosissima* were assessed. Seedlings were grown under two [CO_2] (400 μl l^{-1} and 640 μl l^{-1}) and two temperature (26/16 °C and 30/20 °C for day/night) treatments. Both genotypes were positively responsive to C_E (35% and 29% increase in whole-plant dry mass and leaf area, respectively), but only the Coastal genotype exhibited positive growth responses to T_E. It was observed that the Coastal genotype exhibited greater growth response to T_E (47% and 85% increase in whole-plant dry mass and leaf area, respectively) when compared with the Upland genotype (no change in dry mass or leaf area). No intraspecific variation in physiological plasticity was detected under C_E or T_E, and the interactive effects of C_E and T_E on intraspecific variation in phenotypic plasticity were also largely absent. Overall, T_E was a more effective climate factor than

C_E in exposing genotypic variation in this woody species. Results from the chapter contradict the paradigm that genotypes from more variable climates will exhibit greater phenotypic plasticity in future climate regimes.

Chapter 3 investigated the main and interactive effects of elevated [CO_2] (C_E) and elevated temperature (T_E) on growth and physiological responses to drought of the Coastal (warmer and relatively wetter environment) and the Upland (cooler and relatively drier environment) genotypes of *T. speciosissima*. Seedlings were grown under two [CO_2] (400 μl l^{-1} and 640 μl l^{-1}) and two temperatures (26/16 °C and 30/20 °C for day/night). During the period of experiment, half of the seedlings were supplied with full watering (i.e., the *well-watered* treatment), while the other was subjected to controlled drought/recovery cycles (i.e., the *drought* treatment). The two genotypes showed similar declines in growth and photosynthesis under drought conditions across [CO_2] and temperature treatments, and did not exhibit differences in response to drought stress. Regardless of genotype, T_E negatively affected plant drought resistance by accelerating the process of drought seedlings becoming physiologically stressed, while C_E did not influence the capacity of plant drought resistance or alter the sensitivity of photosynthesis to declines in soil water content. Furthermore, C_E did not ameliorate the negative effects of T_E on drought response. Overall, these results suggest that woody plant populations originating from different environments may not necessarily show intraspecific variation in response to drought under current or predicted future climates. These findings also indicate that temperature is likely to be a stronger determinant than [CO_2] in affecting woody plant response to drought in the context of climate change.

Chapter 4 aimed to examine the intraspecific variation in plant capacity to cope with simultaneously occurring climate extremes of two widely distributed

Eucalyptus species (*E. grandis* and *E. tereticornis*). The main and interactive effects of warming (ambient + 3.5°C) and co-varying climate extremes (i.e., drought and heat waves) on growth and physiology of temperate (drier and cooler) and tropical (wetter and warmer) provenances of each species were investigated. The two species in general did not show interspecific differentiation in response to the same environmental changes, but a significant intraspecific variation in plant growth response to warming and in photosynthetic response to heat waves was observed, both of which were correlated with taxon temperature of origin. Provenances of both species responded similarly in growth and physiology to single factor drought. It was also demonstrated that heat stress alone generally had little effect on plant growth and photosynthesis, but the synergism between drought and heat imposed significantly greater impact on plants than each applied separately. Furthermore, two distinct strategies (senescence of older mature leaves *vs.* complete closure of stomata) were observed, and both proved to be effective, in coping with combined drought and heat stress. Taken together, these results suggest that plant populations of widespread woody species may differ in their response to climate warming and heat waves depending on the climate of origin, but may not necessarily show difference in response to drought. Drought is likely to be the dominant stressor during heat waves, while widespread woody species may possess different strategies to cope with the simultaneously occurring climatic extremes and show interspecific or even intraspecific variation.

Chapter 5 synthesized the major findings from my PhD research. Overall, significant intraspecific variation in growth plasticity when responding to a constant mild warming (T_E; ambient + 3.5–4.0 °C) was found in all three species, and intraspecific variation in photosynthetic responses to a short-term heat stress (ambient

+ 8 °C) was observed in the two *Eucalyptus* species. In contrast, populations did not differ in their growth or photosynthetic responses to elevated [CO_2] (C_E) or to sustained drought in most cases for all three species. These results together suggest that temperature would be more effective than [CO_2] or water availability in exposing intraspecific variation in phenotypic plasticity for woody plant populations under future climates. The relationships between phenotypic plasticity and source environment variability of plant populations differed among the three species. Results from the two *Eucalyptus* species confirmed the general prediction that greater levels of environmental variability will select for plants with greater phenotypic plasticity, while findings from *T. speciosissima* contradicted the paradigm, indicating that woody plant populations originating from more variable environments may not necessarily show greater phenotypic plasticity in response to climate change. In addition, T_E negatively affected plant resistance to drought and heat stress exacerbated the negative effects of drought on plant responses, suggesting that temperature may influence the responses of woody plants to drought under future climates.

Some results from my PhD research have been published in peer-reviewed journals:

Chapter 2: **Huang G**, Rymer PD, Duan H, Smith RA, Tissue DT (2015) Elevated temperature is more effective than elevated [CO_2] in exposing genotypic variation in *Telopea speciosissima* growth plasticity: implications for woody plant populations under climate change. *Global Change Biology*, **21**, 3800-3813.

I also participated in some other projects during my PhD candidature, and have been co-authored in the following peer-reviewed publications:

Duan H, O'Grady AP, Duursma RA, Choat B, **Huang G**, Smith RA, Jiang Y, Tissue DT (2015) Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated [CO_2] and temperature. *Tree Physiology*, **35**, 756-770.

Duan H, Duursma RA, **Huang G**, Smith RA, Choat B, O'Grady AP, Tissue DT (2014) Elevated [CO2] does not ameliorate the negative effects of elevated temperature on drought-induced mortality in *Eucalyptus radiata* seedlings. *Plant, Cell & Environment*, **37**, 1598-1613.

O'Carrigan A, Hinde E, Lu N, Xu XQ, Duan H, **Huang G**, Mak M, Bellotti B, Chen ZH (2014) Effects of light irradiance on stomatal regulation and growth of tomato. *Environmental and Experimental Botany*, **98**, 65-73.

Wu J, Liu Z, **Huang G**, Chen D, Zhang W, Shao Y, Wan S, Fu S (2014) Response of soil respiration and ecosystem carbon budget to vegetation removal in *Eucalyptus* plantations with contrasting ages. *Scientific Reports* **4**, 6262; doi: 10.1038/srep06262.

Chapter 2

Elevated temperature is more effective than elevated $[CO_2]$ in exposing genotypic variation in *Telopea speciosissima* growth plasticity

2.1 Introduction

Changes in atmospheric carbon dioxide concentrations ($[CO_2]$) and temperature are occurring at unprecedented rates, and are having substantial effects on biodiversity and primary production of terrestrial ecosystems (Nemani *et al.*, 2003; Williams *et al.*, 2008; Barnosky *et al.*, 2012). Atmospheric $[CO_2]$ and temperature have been rising over the past 150 years due to rapid fossil fuel consumption and land use change, and it is expected that atmospheric $[CO_2]$ will reach over 600 $\mu L\ L^{-1}$ within this century, accompanied by a 0.3–4.8 °C increase in the global mean air temperature (Collins *et al.*, 2013). Responses of woody species to elevated $[CO_2]$ (C_E) and elevated temperature (T_E) may be of particular importance because forests account for *c.* 70% of terrestrial net primary production and play a prominent role in the global carbon cycle (Melillo *et al.*, 1993; Schimel *et al.*, 2001; Karnosky, 2003; Norby *et al.*, 2005; Pan *et al.*, 2011).

One fundamental way that plant species may respond to increasing atmospheric [CO_2] and warming is to adjust their growth and physiology via phenotypic plasticity – the ability of a genotype to express multiple phenotypes in response to environmental change (Bradshaw, 1965; Sultan, 2000; Nicotra *et al.*, 2010; Anderson *et al.*, 2012). When genotypes of a given species respond differently to the same environmental change, genotypic variation in phenotypic plasticity exists (known as significant G × E interactions), which would not only influence the habitat range occupied by that species, but also affect the ecological and evolutionary responses of that species to changing environments (Sultan, 2000; Van Kleunen & Fischer, 2005; Valladares *et al.*, 2007; Williams *et al.*, 2008; Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). Genotypes with low phenotypic plasticity or stability may tolerate and persist under extreme conditions to survive and maintain growth (Schlichting, 1986; Thompson, 1991), while genotypes with high phenotypic plasticity may be capable of rapid resource uptake and show increased growth when conditions are optimal (Grime & Mackey, 2002). For woody plant species with long generation times, phenotypic plasticity is thought to be particularly important for acting as a buffer against rapid climate change and providing growth advantages (Valladares *et al.*, 2007; Chevin *et al.*, 2010; Nicotra *et al.*, 2010), because evolutionary response by natural selection might be too slow to mitigate the effects of rapid environmental change.

Plant populations usually show genetic differentiation in phenotypic plasticity and it is widely expected that more variable environments will select for genotypes that exhibit greater phenotypic plasticity (Donohue *et al.*, 2001; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen & Fischer, 2005). This theory suggests that, for a given woody species, populations originating from different environments are expected to show differential physiological and growth responses to

changing climate, as evidence of G × E interactions (Aspinwall *et al.*, 2015). For instance, research has predicted that the capacity of woody plants to cope with warming may vary among taxa, depending on the taxon's origin (Saxe *et al.*, 2001; Way & Oren, 2010; Drake *et al.*, 2015); studies on the red maple genotypes from thermally contrasting habitats to respond to warming have also confirmed the intraspecific divergence in plasticity of photosynthetic capacity (Weston & Bauerle, 2007; Weston *et al.*, 2007). Although previous studies have demonstrated intraspecific variation in growth or physiological plasticity of woody plant species under C_E (Ceulemans *et al.*, 1996; Dickson *et al.*, 1998; Isebrands *et al.*, 2001; Mohan *et al.*, 2004; Cseke *et al.*, 2009) or T_E (Weston & Bauerle, 2007; Weston *et al.*, 2007; Drake *et al.*, 2015), the nature and basis of intraspecific or genetic variation in phenotypic plasticity within woody species under climate change is still largely unknown. To my knowledge, no study has looked into the interactive effects of concurrently changing climatic variables such as [CO_2] and temperature on intraspecific variation in phenotypic plasticity of woody plants.

It is widely recognized that plants must achieve a balance between carbon assimilation, carbon storage, and growth (Smith & Stitt, 2007), all of which are directly or indirectly affected by the elements of climate change, such as C_E and T_E. Under C_E and non-limiting resource availability, whole-plant dry mass production of woody plants is generally enhanced via both higher photosynthetic rates per unit leaf and greater total leaf area (Ainsworth & Long, 2005; Ghannoum *et al.*, 2010a; Seneweera & Norton, 2011; Wang *et al.*, 2012), despite frequently observed partial down-regulation of photosynthetic capacity (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Leakey *et al.*, 2009a). Increasing [CO_2] can also lead to accumulation of non-structural carbohydrates (NSC) (Stitt & Krapp, 1999; Nowak *et al.*, 2004;

Robinson *et al.*, 2012), which mainly functions as carbon storage to reconcile temporal asynchrony between carbon demand (i.e., growth and metabolism) and carbon supply (i.e., photosynthesis) (Sala *et al.*, 2012). Plant response to T_E can be more complicated, depending on whether warming pushes various biochemical and physiological processes towards or away from their temperature optimum, as well as the thermal plasticity of temperature-sensitive processes (Berry & Bjorkman, 1980; Ghannoum *et al.*, 2010a). Elevating temperatures (typically 3–5 °C higher than the ambient) from those below the thermal optimum, are often reported to increase photosynthesis, plant size and dry mass production for woody plants (Saxe *et al.*, 2001; Kattge & Knorr, 2007; Ghannoum *et al.*, 2010a, 2010b; Way & Oren, 2010). Furthermore, C_E is likely to interact with T_E, synergistically affecting plant physiology and/or growth, as has been observed in a wide variety of woody species (Callaway *et al.*, 1994; Peltola *et al.*, 2002; Ghannoum *et al.*, 2010a; Ayub *et al.*, 2011; Wang *et al.*, 2012).

Telopea speciosissima R.Br. (Proteaceae), commonly known as the Waratah (Weston & Crisp, 1994), is an endemic woody species (and New South Wales floral emblem) in the Sydney Bioregion of Australia. This species occurs sporadically in small populations across a range of climatic and altitudinal zones, and generally flowers over a six-week period in spring (September – October in warmer areas, but later in cooler areas), followed by a vegetative flush of growth (Nixon, 1997). A previous study on morphology and population genetics of *T. speciosissima* has revealed three distinct gene pools (coastal, upland and southern) among natural populations; the coastal and upland gene pools mix at mid-elevations along an altitudinal gradient (Rossetto *et al.*, 2011). Distinction in climate between habitats of coastal and upland gene pools is mainly characterized by differences in air temperature and precipitation. The coastal region is warmer and wetter than the upland region, but

the latter experiences greater levels of temperature variability (Table 2-1). Thus, *T. speciosissima* is well suited for studying the association between phenotypic plasticity and source environment variability of genetically differentiated woody plant populations. Results from such studies will provide useful information on the importance of intraspecific variation in phenotypic plasticity in determining woody species growth and physiology under climate change.

To assess the capacity of *T. speciosissima* genotypes to cope with potential future climatic conditions, genetically differentiated natural populations of this species from coastal and upland regions (i.e., the Coastal genotype and the Upland genotype, respectively) were selected and grown under a factorial combination of CO_2 and temperature treatments. By measuring responses in growth, photosynthesis and carbohydrates, I examined the main and interactive effects of C_E and T_E on phenotypic plasticity of *T. speciosissima* genotypes. I hypothesized that: (1) the Upland genotype from more variable temperature environments will show greater growth and physiological plasticity in response to T_E; (2) the two genotypes will show similar plasticity in growth and physiology under C_E; and (3) the effect of T_E on growth and physiological plasticity will be enhanced by C_E.

Table 2-1 The 40-year (1971–2010) summary of precipitation and air temperature in the coastal (180 m altitude) and upland (1150 m altitude) regions, from which the *Telopea speciosissima* Coastal and Upland genotypes were sampled for this study

	Coastal region			**Upland region**		
	Mean	**Range**	**CV**	**Mean**	**Range**	**CV**
Precipitation (mm)						
Annual	1243	792–2044	0.266	856	393–1265	0.255
Summer	372	146–946	0.458	276	53–539	0.381
T_{max} *(°C)*						
Annual	22.8	21.9–23.8	0.159	18.5	17.0–20.0	0.296
Summer	**26.9**	24.1–29.8	0.043	**24.9**	20.8–29.1	0.069
T_{min} *(°C)*						
Annual	13.2	12.2–14.0	0.319	7.4	6.2–8.4	0.585
Summer	**18.1**	15.9–20.4	0.054	**12.6**	9.4–15.3	0.095

Range refers to the minimal and maximal values of annual/summer Means. CV, coefficient of variation, defined as the ratio of the standard deviation to the mean; T_{max}, maximum air temperature; T_{min}, minimum air temperature. CVs for precipitation were calculated based on the annual/summer means (n = 40); while CVs for temperature were first calculated based on the monthly means within each year (n = 12) or summer (n = 3), and then averaged across 40 years. Air temperatures selected as the reference for T_A in the experiment are shown in bold.

2.2 Materials and methods

2.2.1 Plant material and growth conditions

Two natural genotypes of *T. speciosissima* were included in this study, one originating from Patonga (33.53°S, 151.28°E, 180 m altitude, the coastal region), and the other from Newnes Forest (33.39°S, 150.21°E, 1150 m altitude, the upland region). The coastal region is characterized by more annual precipitation and higher average temperatures (but with lower temperature variation) when compared with the upland region (Table 2-1), according to climate records from a network of weather stations across Australia (i.e., SILO Climate Data) (Jeffrey *et al.*, 2001). As plant populations in these two regions have at least 90% identity specific to its corresponding gene pool, based on the seven simple sequence repeat loci in the previous report (Rossetto *et al.*, 2011), I defined them in this study as the Coastal genotype and the Upland genotype, respectively.

A total of 200 seeds were collected from 24 mother plants (12 for each genotype) and planted in forestry tubes filled with a homogenous peat and sand mixture (1:2). 25 seeds from each genotype were used as biological replicates and placed in one of four adjacent, naturally lit (direct sunlight attenuated by 10–15% due to the structure), [CO_2] and temperature controlled glasshouse compartments (3.0 m × 5.0 m × 3.5 m, width × length × height each), located at the University of Western Sydney, Richmond, NSW, Australia. Details of glasshouse design are described in Ghannoum *et al*. (2010a). Three months following seed germination (summer; January 2012), ten seedlings from each genotype (within each glasshouse compartment) were randomly selected and transplanted into PVC pots (15 cm diameter × 40 cm length) that contained about 10 kg of dry loamy-sand soil (86.5% sand and 9.5% clay).

A factorial [CO_2] and temperature design was applied to the four glasshouse compartments, with two [CO_2] (ambient (C_A) and elevated (C_E)) and two temperature (ambient (T_A) and elevated (T_E)) treatments. T_A was set at 26/16 °C for day/night while T_E was set to maintain a 4 °C increase in temperature above ambient (i.e., 30/20 °C for day/night). 26/16 °C was chosen for T_A because it approximates the mean of daily average temperatures in summer (i.e., the presumptive primary growing season for *T. speciosissima*) between the coastal and upland regions selected in this study. Based on the 40-yr historical climate data, summer daily average temperatures were about 26.9/18.1 °C and 24.9/12.6 °C in the coastal and upland regions, respectively, averaging at 25.9/15.4 °C (Table 2-1). Furthermore, over the 24-hour period, temperature in each compartment was changed five times to simulate a natural diel temperature cycle in the field. Within each temperature treatment, [CO_2] were maintained at 400 µl l^{-1} (C_A) and 640 µl l^{-1} (C_E). The rise in [CO_2] of 240 µl l^{-1} corresponded with the rise in temperature of 4 °C, reflecting predicted climatic conditions within this century (Solomon *et al.*, 2009; Collins *et al.*, 2013). Therefore, the four treatments in the study were: C_AT_A (400 µl l^{-1}, 26 °C), C_AT_E (400 µl l^{-1}, 30 °C), C_ET_A (640 µl l^{-1}, 26 °C) and C_ET_E (640 µl l^{-1}, 30 °C).

During the experimental period, mean relative humidity of the four glasshouse compartments was 65.3 ± 0.2 % (C_AT_A), 54.8 ± 0.2 % (C_AT_E), 65.5 ± 0.2 % (C_ET_A) and 52.0 ± 0.2 % (C_ET_E). Vapour pressure deficit (VPD) in the glasshouse compartments in T_A ranged from 0.1 to 2.9 kPa (averaged at 0.86 ± 0.01 kPa) and in T_E ranged from 0.2 to 4.3 kPa (averaged at 1.50 ± 0.01 kPa), but did not vary between [CO_2] treatments (Duan *et al.*, 2014). Seedlings were irrigated on a daily basis and rotated routinely within and between glasshouse compartments. Seedlings and treatments were rotated simultaneously, ensuring that seedlings in a given treatment

were cultivated under the same treatment conditions throughout the entire experimental period. On three occasions (30, 90 and 150 days after planting (DAP) into PVC pots), seedlings were fertilized with a commercial fertilizer (All Purpose, Brunnings, Victoria, Australia, N:P:K – 27:2:10).

2.2.2 Leaf gas exchange measurements

Leaf gas exchange measurements were conducted on attached, recently fully-expanded leaves using a Li-Cor 6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) supplying photosynthetic photon flux density (PPFD) using a red-blue light source (6400-02B). At 110 DAP, light-saturated photosynthesis (A_{sat}, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) were measured at saturating PPFD of 1200 μmol m^{-2} s^{-1}, growth [CO_2] (400 μl l^{-1} or 640 μl l^{-1}), mid-day growth temperature (26 °C or 30 °C), relative humidity of 55–65%, and leaf-to-air VPD between 1.0 and 2.0 kPa. CO_2- and light-saturated assimilation rates (A_{max}, μmol m^{-2} s^{-1}) were also determined immediately following measurement of A_{sat}, by adjusting measurement [CO_2] in the cuvette to 1800 μl l^{-1} but not changing other parameters. Each leaf was allowed 5–10 min to equilibrate before measurements were taken and five replicate seedlings were measured per genotype and treatment.

Photosynthetic assimilation rates to intercellular [CO_2] (A/C_i curves) were measured at PPFD of 1200 μmol m^{-2} s^{-1}, mid-day growth temperature (26 °C or 30 °C), relative humidity of 55–65%, and leaf-to-air VPD between 1.0 and 2.0 kPa, by raising cuvette [CO_2] in 11 steps (0, 50, 100, 150, 200, 300, 400, 640, 900, 1300 and 1800 μl l^{-1}). Five replicate seedlings were measured per genotype and treatment. The A/C_i curve fitting utility (version 0.4, updated in July 2007) developed by Sharkey *et al.*

(2007) was applied to estimate V_{cmax} (maximum rate of photosynthetic carboxylation, μmol m^{-2} s^{-1}) and J_{max} (maximum rate of photosynthetic electron transport, μmol m^{-2} s^{-1}) at measuring temperatures without constraining mesophyll conductance (i.e., not a fixed value). Both V_{cmax} and J_{max} were then corrected to a common temperature of 25 °C for comparisons between treatments.

At 80 DAP, the responses of photosynthetic assimilation rates to leaf temperature (A/T_L curves) were measured at PPFD of 1200 μmol m^{-2} s^{-1} and growth [CO_2] (400 μl l^{-1} or 640 μl l^{-1}), relative humidity of 55–65%, and leaf-to-air VPD between 1.0 and 2.0 kPa. The cuvette temperature was adjusted by raising the gas exchange chamber temperature in 6 steps (15, 20, 25, 30, 35 and 40 °C), as described in Ghannoum *et al.* (2010b). For each cuvette temperature level, the air temperature of the glasshouse room was raised to maintain leaves and whole plants at the same temperature for 30 min before measurements were taken. All seedlings (four replicates per genotype and treatment) were measured at the same temperature before the cuvette temperature was stepped up to the next level. Each leaf was allowed 5–10 min to equilibrate before measurements were made. All A/T_L curves were fitted using a polynomial function ($y = Ax^2 + Bx + C$), and then photosynthetic thermal optimum (T_{opt}, °C) and light-saturated photosynthesis at T_{opt} (A_{opt}, μmol m^{-2} s^{-1}) were estimated based on the fitted curves.

2.2.3 Growth measurements

At the end of the experimental period (about 270 DAP), all 80 seedlings (10 replicates per genotype and treatment) were destructively harvested and separated into leaves, stem, tuber and roots. Roots were washed free of soil. Total plant leaf area (cm^2)

was determined by a portable leaf area meter (LI-3100A, Li-Cor, Lincoln, NE, USA). All harvested samples were oven-dried at 70 °C for 72 h and then weighed for dry mass. For each seedling, leaf mass per area (LMA, total leaf dry mass / total leaf area, g m^{-2}) and leaf area ratio (LAR, total leaf area / total plant dry mass, m^2 kg^{-1}) were calculated. The fraction of total plant dry mass allocated to leaves (leaf mass fraction, LMF), stems (stem mass fraction, SMF), tubers (tuber mass fraction, TMF) and roots (root mass fraction, RMF) were also analyzed; Root/Shoot ratios were calculated as (TMF + RMF) / (LMF + SMF).

2.2.4 Carbohydrate analyses

Subsamples of oven-dried plant material were ground to a fine powder in a ball mill prior to determination of the concentration of total non-structural carbohydrates (NSC, sum of total soluble sugars and starch). Total soluble sugars were determined by the anthrone method and total starch was measured using the Megazyme total starch kit (Megazyme International Ireland, Wicklow, Ireland). Details of the NSC assay can be found in Mitchell *et al*. (2013). To calculate the soluble sugar (Ss) and starch (St) concentrations (mg g^{-1}), contents of the measured pool were standardized by dry weight of the sample. Whole-plant Ss, St and NSC were calculated by summing the weighted concentrations (concentration multiplied by the proportion of organ dry mass to total dry mass) of different plant organs (leaf, stem, tuber and root). All carbohydrate measurements were conducted on five replicate seedlings per genotype and treatment.

2.2.5 Statistical analysis

All data were analysed using a general linear model, factorial analysis of variance (ANOVA) with three main factors – genotype, growth [CO_2] and growth temperature, with two levels within each factor. The effect of ontology was also tested with the addition of plant dry mass as a covariate in the analyses, but overall there was no change from the original analyses. Tukey's HSD tests were used to compare means for both genotypes among the [CO_2] and temperature treatments (see Tables 2-3 and 2-4). Relationships between whole-plant dry mass and other parameters were analysed using linear regression analysis. Data were log-transformed when necessary to meet assumptions of homoscedasticity and normality. Results were considered significant in all cases if $P < 0.05$. All analyses were performed in R (version 3.1.0; R Foundation for Statistical Computing, Vienna, Austria).

2.3 Results

2.3.1 Plant growth and dry mass allocation

Whole-plant dry mass and leaf area varied significantly between genotypes and were both affected by growth [CO_2] and temperature (Fig. 2-1a and 1b; Table 2-2 and 2-3). Overall, the Coastal genotype was more productive and possessed higher leaf area, compared to the Upland genotype. Across genotypes, C_E increased whole-plant dry mass and leaf area by 35% and 29%, respectively. T_E also increased whole-plant dry mass and leaf area in the Coastal genotype by 47% and 85%, respectively, but did not significantly affect either trait in the Upland genotype, indicating genotypic variation in growth responses to temperature. LMA was higher under C_E and in the Upland genotype, but did not vary with temperature (Fig. 2-1c; Table 2-2 and 2-3). LAR did not show differences between genotypes or vary with [CO_2], but increased 24% under T_E (Fig. 2-1d; Table 2-2 and 2-3).

Dry mass allocation to different plant organs differed between genotypes, with allocation varying strongly with temperature (Fig. 2-2; Table 2-2 and 2-3). Across genotypes and [CO_2] treatments, T_E increased leaf and stem mass fractions, but decreased tuber and root mass fractions, thereby generating a 50% reduction in the mean Root/Shoot ratio. Compared to the Upland genotype, the Coastal genotype allocated more dry mass to below-ground organs (higher fraction of tuber and root mass; Fig. 2-2c and 2d), but less dry mass to above-ground organs (a lower fraction of leaf mass; Fig. 2-2a), resulting in significantly higher ratios of Root/Shoot (Fig. 2-2e). The fraction of stem mass did not vary between genotypes; the Coastal genotype showed a 43% increase in stem mass fraction under T_E, but no change occurred in the

Upland genotype, suggesting a significant genotype × temperature interaction (Fig. 2-2b and Table 2-2). C_E did not affect dry mass allocation.

2.3.2 Leaf gas exchange

In general, genotypes did not differ in leaf gas exchange parameters. However, C_E and T_E significantly affected all photosynthetic parameters, except g_s and A/T_L parameters (Table 2-2 and 2-3). Photosynthesis (A_{sat}) was 30% higher in C_ET_A and 19% higher in C_ET_E compared with the C_A treatments (Fig. 2-3a). Stomatal conductance (g_s) was not affected by C_E or T_E, although there was a significant interaction between genotype and temperature (Fig. 2-3b). Across temperature treatments, photosynthetic capacity traits (A_{max}, V_{cmax} and J_{max}) decreased by *c.* 20% under C_E (Fig. 2-3c, 3d and 3e). Growth temperature had little effect on A_{max} or $V_{cmax,}$ but J_{max} was significantly reduced by *c.* 16% under T_E; consequently, there was an 8% decline (on average) in J_{max}/V_{cmax} under T_E (Fig. 2-3f). C_E alone had no significant effect on J_{max}/V_{cmax}, but a 15% decrease in J_{max}/V_{cmax} was observed under C_ET_E, suggesting a significant interaction between [CO_2] and temperature (Table 2-2 and 2-3). Photosynthetic thermal optimum (T_{opt}) and light-saturated photosynthesis at thermal optimum (A_{opt}) did not differ between genotypes or vary between growth temperatures, but increased under C_E by an average of 8% and 26%, respectively (Fig. 2-4; Table 2-2 and 2-3). The average increase of T_{opt} was 2.4 °C for the Coastal genotype and 1.9 °C for the Upland genotype, respectively. The main and interactive effects of genotype, [CO_2], and temperature had little effect on A/T_L parameters, except for a marginally significant interaction between genotype and temperature on parameter C (Table 2-2).

Table 2-2 Main and interactive effects of genotype, [CO_2] and temperature on growth, photosynthetic and carbohydrate parameters of two *Telopea speciosissima* genotypes grown at two [CO_2] and two temperatures

	Main effects			**Interactions**			
Parameter	**Genotype**	**[CO_2]**	**Temperature**	**Genotype × [CO_2]**	**Genotype × Temperature**	**[CO_2] × Temperature**	**Genotype × [CO_2] × Temperature**
Growth							
Whole-plant DM (g)	**0.000**	**0.004**	**0.033**	0.452	**0.024**	0.406	0.511
Leaf Area (cm^2)	**0.000**	**0.013**	**0.000**	0.252	**0.008**	0.720	0.338
LMA ($g\ m^{-2}$)	**0.024**	**0.025**	0.346	0.190	0.467	0.391	0.128
LAR ($m^2\ kg^{-1}$)	0.129	0.658	**0.000**	0.330	0.364	0.353	0.622
Leaf mass fraction	**0.000**	0.268	**0.000**	0.638	0.693	0.650	0.780
Stem mass fraction	0.095	0.270	**0.000**	0.221	**0.014**	0.649	0.375
Tuber mass fraction	**0.000**	0.071	**0.000**	0.781	0.063	0.379	0.607
Root mass fraction	**0.012**	0.615	**0.000**	0.239	0.271	0.860	0.748
Root/Shoot ratio	**0.000**	0.507	**0.000**	0.408	0.401	0.580	0.985

Table 2-2 (continued)

	Main effects			Interactions			
Parameter	**Genotype**	**[CO_2]**	**Temperature**	**Genotype × [CO_2]**	**Genotype × Temperature**	**[CO_2] × Temperature**	**Genotype × [CO_2] × Temperature**
Leaf gas exchange							
A_{sat} (μmol m^{-2} s^{-1})	0.961	**0.000**	**0.021**	0.344	0.647	0.325	0.605
g_s (mol m^{-2} s^{-1})	0.247	0.836	0.304	0.941	**0.020**	0.367	0.081
A_{max} (μmol m^{-2} s^{-1})	0.374	**0.000**	0.106	0.363	0.394	0.101	0.628
V_{cmax} (μmol m^{-2} s^{-1})	0.175	**0.000**	0.080	0.897	0.446	0.319	0.740
J_{max} (μmol m^{-2} s^{-1})	0.177	**0.000**	**0.000**	0.440	0.301	0.884	0.688
J_{max}/V_{cmax}	0.614	0.059	**0.002**	0.583	0.808	**0.029**	0.099
A/T_L							
Parameter A	0.702	0.384	0.387	0.477	0.277	0.785	0.902
Parameter B	0.732	0.067	0.302	0.393	0.164	0.590	0.914
Parameter C	0.486	0.104	0.141	0.214	**0.043**	0.584	0.778
T_{opt} (°C)	0.837	**0.000**	0.380	0.666	0.252	0.194	0.677
A_{opt} (μmol m^{-2} s^{-1})	0.578	**0.000**	0.932	0.929	0.774	0.318	0.455

Table 2-2 (continued)

	Main effects			Interactions			
Parameter	**Genotype**	**[CO_2]**	**Temperature**	**Genotype × [CO_2]**	**Genotype × Temperature**	**[CO_2] × Temperature**	**Genotype × [CO_2] × Temperature**
Carbohydrates							
Whole-plant St (mg g^{-1})	**0.031**	**0.020**	**0.000**	0.891	0.507	0.542	0.385
Whole-plant Ss (mg g^{-1})	**0.004**	0.149	0.086	0.605	0.105	0.879	0.830
Whole-plant NSC (mg g^{-1})	**0.015**	**0.049**	**0.033**	0.570	0.399	0.504	0.584
Leaf St (mg g^{-1})	0.338	**0.002**	**0.000**	0.763	0.810	0.871	0.133
Stem St (mg g^{-1})	**0.002**	0.723	**0.000**	0.317	0.549	0.787	0.772
Tuber St (mg g^{-1})	**0.000**	0.234	**0.004**	0.052	0.597	0.675	0.677
Root St (mg g^{-1})	**0.014**	0.665	0.136	0.637	0.262	0.288	0.165
Leaf Ss (mg g^{-1})	**0.000**	0.702	0.540	0.406	0.164	0.115	0.293
Stem Ss (mg g^{-1})	0.273	**0.043**	0.173	0.640	0.743	0.074	0.420
Tuber Ss(mg g^{-1})	0.278	**0.002**	0.143	0.709	**0.013**	0.135	0.248
Root Ss (mg g^{-1})	**0.019**	**0.000**	**0.000**	0.373	0.290	**0.014**	**0.005**

DM, dry mass; LMA, leaf area per mass; LAR, leaf area ration; St, starch; Ss, soluble sugars; NSC, non-structural carbohydrates. *P*-values from the three-way ANOVA are presented, based on ten replicates ($n = 10$) for growth parameters and five replicates ($n = 5$) for the others. Significant values ($P < 0.05$) are shown in bold.

Table 2-3 Summary of means for growth, photosynthetic and carbohydrate parameters of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments, as described in the Materials and methods

		Treatment			
Parameter	**Genotype**	C_AT_A	C_AT_E	C_ET_A	C_ET_E
Growth					
Whole-plant DM (g)	Coastal	6.3 ± 0.9^{bcd}	9.2 ± 1.0^{ab}	8.0 ± 1.1^{abc}	11.7 ± 1.5^{a}
	Upland	4.5 ± 0.2^{cd}	4.3 ± 0.8^{d}	6.1 ± 0.8^{bcd}	6.5 ± 0.6^{abcd}
Leaf Area (cm^2)	Coastal	267 ± 46^{c}	524 ± 64^{ab}	322 ± 57^{bc}	559 ± 64^{a}
	Upland	205 ± 14^{c}	231 ± 44^{c}	283 ± 43^{c}	350 ± 31^{abc}
LMA ($g\ m^{-2}$)	Coastal	98.5 ± 3.8^{a}	92.3 ± 2.1^{a}	102.0 ± 4.9^{a}	109.0 ± 5.0^{a}
	Upland	102.5 ± 2.7^{a}	109.0 ± 4.6^{a}	106.9 ± 4.2^{a}	109.6 ± 1.8^{a}
LAR ($m^2\ kg^{-1}$)	Coastal	4.13 ± 0.37^{b}	5.64 ± 0.11^{a}	4.09 ± 0.35^{b}	4.98 ± 0.42^{ab}
	Upland	4.54 ± 0.21^{ab}	5.43 ± 0.28^{ab}	4.73 ± 0.37^{ab}	5.42 ± 0.18^{ab}
Leaf mass fraction (%)	Coastal	39.6 ± 2.7^{e}	52.1 ± 1.8^{abc}	40.6 ± 2.5^{de}	52.5 ± 2.2^{abc}
	Upland	46.2 ± 1.8^{cde}	58.3 ± 1.7^{ab}	49.4 ± 2.6^{bcd}	59.3 ± 1.6^{a}

Table 2-3 (continued)

		Treatment			
Parameter	**Genotype**	C_AT_A	C_AT_E	C_ET_A	C_ET_E
Stem mass fraction (%)	Coastal	11.6 ± 0.9^{cd}	17.2 ± 0.9^{a}	10.9 ± 0.8^{d}	15.0 ± 0.7^{abc}
	Upland	13.7 ± 0.6^{abcd}	15.4 ± 0.9^{ab}	13.5 ± 0.6^{bcd}	15.7 ± 0.9^{ab}
Tuber mass fraction (%)	Coastal	17.2 ± 2.5^{a}	3.7 ± 0.5^{cd}	13.8 ± 2.3^{ab}	3.8 ± 0.6^{cd}
	Upland	8.6 ± 1.2^{ab}	3.3 ± 0.5^{cd}	6.5 ± 0.9^{bc}	2.6 ± 0.2^{d}
Root mass fraction (%)	Coastal	31.5 ± 2.4^{ab}	27.0 ± 1.6^{abc}	34.7 ± 2.1^{a}	28.8 ± 2.1^{abc}
	Upland	31.5 ± 2.1^{ab}	23.0 ± 1.8^{bc}	30.6 ± 2.1^{abc}	22.4 ± 1.3^{c}
Root/Shoot ratio	Coastal	1.06 ± 0.18^{a}	0.45 ± 0.03^{bc}	1.02 ± 0.14^{a}	0.50 ± 0.05^{bc}
	Upland	0.69 ± 0.06^{ab}	0.36 ± 0.03^{c}	0.62 ± 0.07^{b}	0.34 ± 0.02^{c}
Leaf gas exchange					
A_{sat} (μmol m^{-2} s^{-1})	Coastal	9.6 ± 1.1^{ab}	8.8 ± 0.6^{b}	12.7 ± 0.2^{a}	11.3 ± 0.9^{ab}
	Upland	10.1 ± 0.7^{ab}	9.4 ± 0.9^{ab}	12.8 ± 0.5^{a}	10.3 ± 1.2^{ab}
g_s (mol m^{-2} s^{-1})	Coastal	0.17 ± 0.02^{a}	0.17 ± 0.02^{a}	0.16 ± 0.01^{a}	0.18 ± 0.03^{a}
	Upland	0.19 ± 0.01^{a}	0.18 ± 0.02^{a}	0.23 ± 0.01^{a}	0.14 ± 0.02^{a}
A_{max} (μmol m^{-2} s^{-1})	Coastal	19.6 ± 1.0^{a}	18.8 ± 0.9^{ab}	17.6 ± 0.4^{abc}	15.1 ± 1.2^{bc}
	Upland	18.7 ± 1.2^{abc}	19.8 ± 1.2^{a}	16.2 ± 0.8^{abc}	14.3 ± 1.1^{c}

Table 2-3 (continued)

		Treatment			
Parameter	**Genotype**	C_AT_A	C_AT_E	C_ET_A	C_ET_E
V_{cmax} (μmol m^{-2} s^{-1})	Coastal	60.6 ± 5.9[a]	52.5 ± 4.2[ab]	47.6 ± 1.0[ab]	43.0 ± 3.9[b]
	Upland	56.8 ± 3.5[ab]	50.7 ± 3.4[ab]	41.4 ± 2.3[b]	42.2 ± 2.0[b]
J_{max} (μmol m^{-2} s^{-1})	Coastal	81.5 ± 4.3[a]	66.7 ± 4.0[abcd]	70.5 ± 0.3[abc]	56.9 ± 4.5[cd]
	Upland	76.7 ± 4.4[ab]	69.4 ± 4.0[abcd]	64.1 ± 1.7[bcd]	53.9 ± 3.9[d]
J_{max}/V_{cmax}	Coastal	1.37 ± 0.07[ab]	1.28 ± 0.04[b]	1.48 ± 0.03[ab]	1.34 ± 0.06[ab]
	Upland	1.35 ± 0.02[ab]	1.38 ± 0.06[ab]	1.56 ± 0.05[a]	1.28 ± 0.06[b]
A/T_L					
Parameter A	Coastal	–0.019 ± 0.002[a]	–0.019 ± 0.001[a]	–0.022 ± 0.002[a]	–0.021 ± 0.001[a]
	Upland	–0.019 ± 0.001[a]	–0.023 ± 0.004[a]	–0.020 ± 0.002[a]	–0.023 ± 0.004[a]
Parameter B	Coastal	0.98 ± 0.09[a]	0.98 ± 0.07[a]	1.29 ± 0.12[a]	1.21 ± 0.07[a]
	Upland	0.96 ± 0.05[a]	1.25 ± 0.20[a]	1.12 ± 0.11[a]	1.29 ± 0.22[a]
Parameter C	Coastal	–4.04 ± 0.91[a]	–4.20 ± 0.75[a]	–7.71 ± 1.62[a]	–6.27 ± 0.40[a]
	Upland	–4.32 ± 0.15[a]	–7.91 ± 1.90[a]	–4.97 ± 1.31[a]	–8.03 ± 1.94[a]
T_{opt} (°C)	Coastal	25.8 ± 0.5[a]	26.1 ± 0.6[a]	28.7 ± 0.5[a]	28.0 ± 0.8[a]
	Upland	25.3 ± 0.1[a]	27.4 ± 1.1[a]	28.2 ± 1.2[a]	28.4 ± 0.6[a]

Table 2-3 (continued)

		Treatment			
Parameter	**Genotype**	C_AT_A	C_AT_E	C_ET_A	C_ET_E
A_{opt} (μmol m-2 s-1)	Coastal	8.6 ± 0.6^{a}	8.6 ± 0.6^{a}	10.9 ± 0.7^{a}	10.7 ± 0.8^{a}
	Upland	7.9 ± 0.7^{a}	8.9 ± 0.6^{a}	10.9 ± 0.4^{a}	10.1 ± 1.0^{a}
Carbohydrates					
Whole-plant St (mg g^{-1})	Coastal	16.2 ± 4.0^{ab}	10.1 ± 1.4^{bc}	25.7 ± 3.7^{a}	11.5± 1.1^{abc}
	Upland	15.8 ± 4.3^{abc}	6.4 ± 0.9^{c}	20.5 ± 5.0^{ab}	8.9 ± 1.0^{bc}
Whole-plant Ss (mg g^{-1})	Coastal	37.2 ± 2.3^{a}	43.1 ± 2.5^{a}	40.4 ± 3.5^{a}	46.2 ± 1.5^{a}
	Upland	35.8 ± 0.4^{a}	35.4 ± 1.8^{a}	36.7 ± 3.3^{a}	37.6 ± 2.2^{a}
Whole-plant NSC (mg g^{-1})	Coastal	53.4 ± 4.7^{ab}	53.3 ± 3.1^{ab}	66.1 ± 6.9^{a}	57.7 ± 1.9^{ab}
	Upland	51.6 ± 4.1^{ab}	41.8 ± 2.6^{b}	57.2 ± 7.4^{ab}	46.5 ± 3.0^{ab}

The four [CO_2] and temperature treatments are: C_AT_A (400 μl l^{-1}, 26 °C), C_AT_E (400 μl l^{-1}, 30 °C), C_ET_A (640 μl l^{-1}, 26 °C) and C_ET_E (640 μl l^{-1}, 30 °C). DM, dry mass; LMA, leaf area per mass; LAR, leaf area ration; St, starch; Ss, soluble sugars; NSC, non-structural carbohydrates. Values represent means ± 1 SE (n = 10 for growth parameters and n = 5 for the others). Within each parameter, different superscript letters indicate means that are significantly different at $P < 0.05$ based on Tukey's pair-wise comparisons.

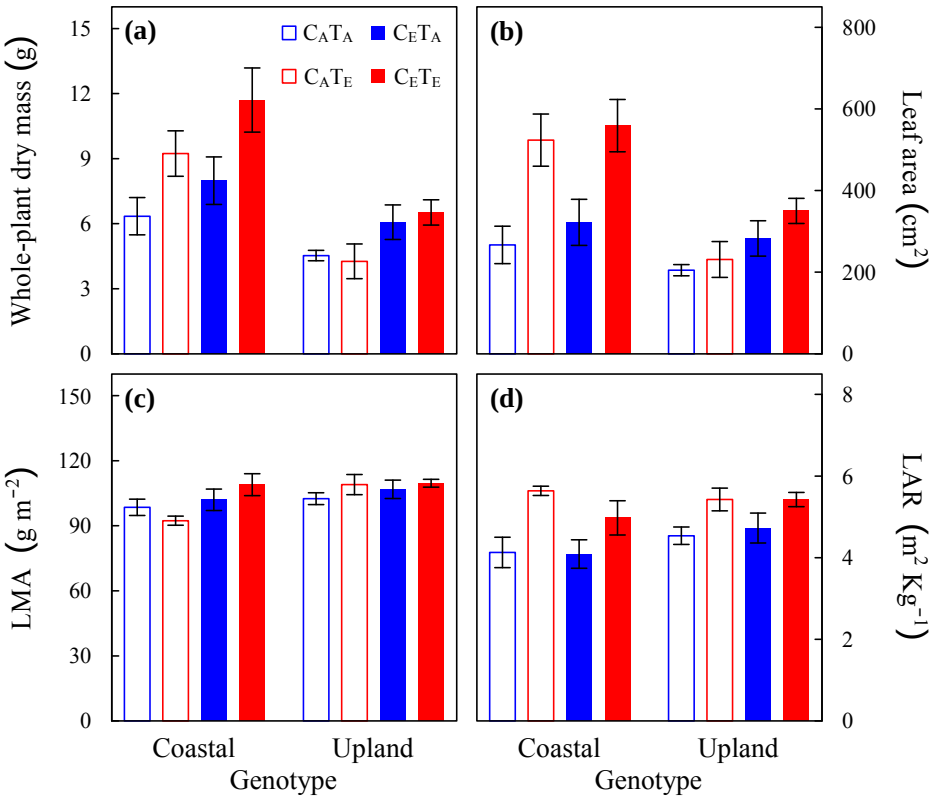

Figure 2-1 Whole-plant dry mass (a), leaf area (b), leaf mass per area (LMA) (c), and leaf area ratio (LAR) (d) of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments: C_AT_A (400 µl l^{-1}, 26 °C; open blue), C_AT_E (400 µl l^{-1}, 30 °C; open red), C_ET_A (640 µl l^{-1}, 26 °C; closed blue), and C_ET_E (640 µl l^{-1}, 30 °C; closed red). Values represent means ± 1 SE (n = 10).

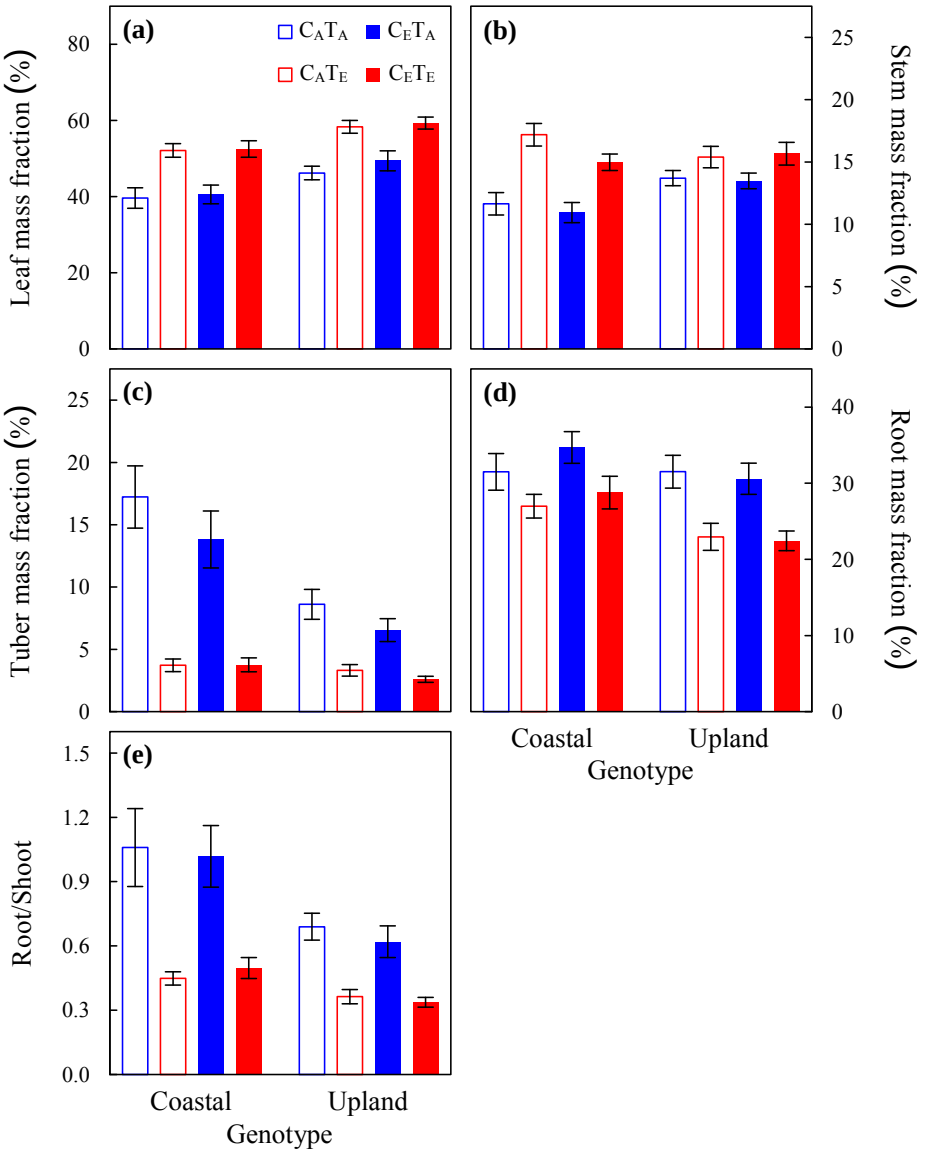

Figure 2-2 Plant dry mass allocation of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments, including leaf mass fraction (a), stem mass fraction (b), tuber mass fraction (c), root mass fraction (d), and the Root/Shoot ratio (e). Values represent means ± 1 SE (n = 10).

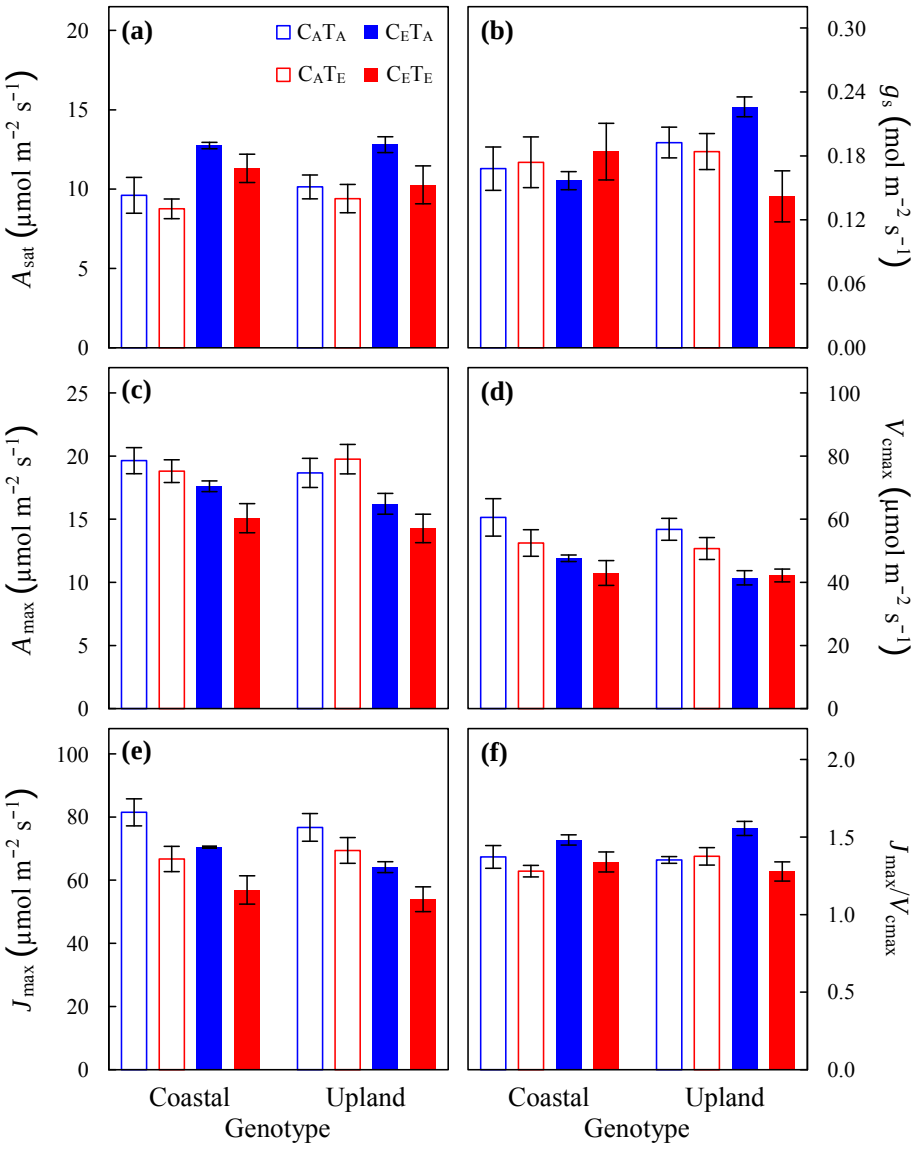

Figure 2-3 Light-saturated photosynthesis (A_{sat}) (a), stomatal conductance (g_s) (b), CO_2- and light-saturated assimilation rates (A_{max}) (c), maximum rate of photosynthetic carboxylation (V_{cmax}) (d), maximum rate of photosynthetic electron transport (J_{max}) (e), and the J_{max}/V_{cmax} ratio (f) of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments. Values represent means ± 1 SE (n = 5).

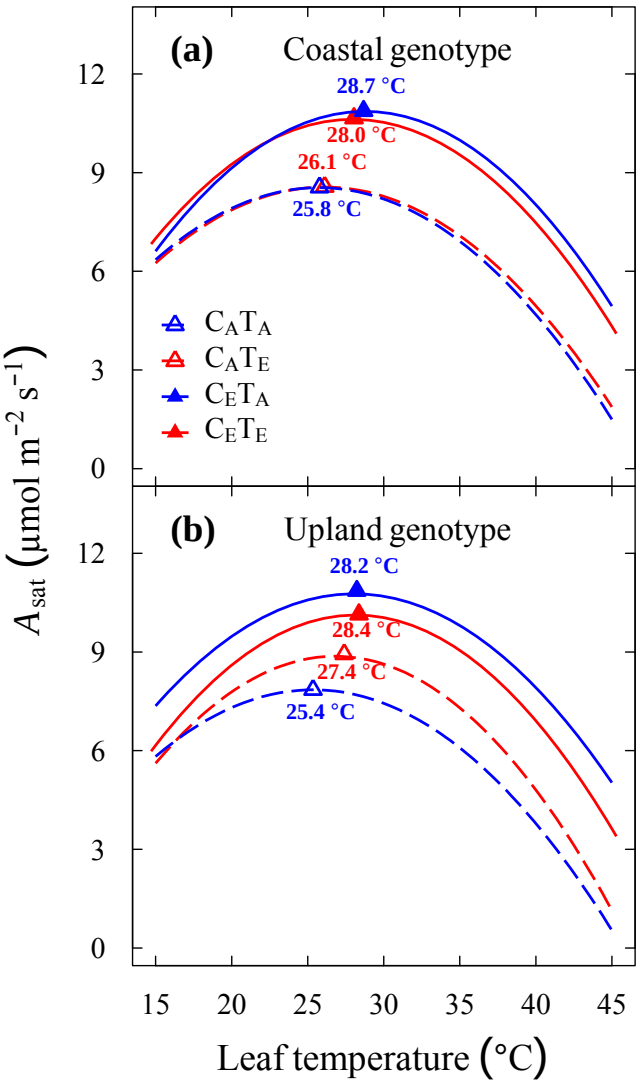

Figure 2-4 The simulated responses of CO_2 assimilation rates to leaf temperature (A/T_L) in *Telopea speciosissima* Coastal genotype (a) and Upland genotype (b) grown under the four [CO_2] and temperature treatments: C_AT_A (dashed blue), C_AT_E (dashed red), C_ET_A (solid blue), and C_ET_E (solid red). Curves represent the output of the averaged polynomial fits ($A_{sat} = A*T_L^2 + B*T_L + C$, where T_L is leaf temperature and A, B and C are the fitted parameters shown in Table 2-3) from 4 seedlings for each genotype per treatment. Coloured triangles and texts around the top of simulated curves indicate the photosynthetic thermal optimums (T_{opt}) under different treatments.

2.3.3 Non-structural carbohydrates (NSC)

Concentrations of whole-plant starch (St), soluble sugars (Ss), and non-structural carbohydrates (NSC) all varied between genotypes, but only St and NSC were significantly influenced by growth [CO_2] and temperature (Fig. 2-5; Table 2-2 and 2-3). Across treatments, the Coastal genotype had *c.* 25% and 15% higher whole-plant St and Ss, respectively, resulting in 18% higher (on average) NSC compared with the Upland genotype. C_E stimulated whole-plant St by 35%, while T_E reduced whole-plant St by 52%. No significant [CO_2] or temperature effect was found on whole-plant Ss. Consequently, C_E increased whole-plant NSC by 14%, but T_E decreased whole-plant NSC by 13% (Fig. 2-5c).

Across [CO_2] and temperature treatments, the Coastal genotype had higher stem, tuber and root St, but similar leaf St when compared with the Upland genotype (Tables 2-2 and 2-4). Regardless of genotype, C_E stimulated leaf St by *c.* 65% but did not change St in other organs. T_E decreased leaf, stem and tuber St by 65%, 54% and 52%, respectively, without affecting root St. Averaged across treatments, the Coastal genotype had 25% higher leaf Ss and 10% higher root Ss, compared with the Upland genotype (Tables 2-2 and 2-4). C_E reduced stem Ss by 13%, but increased tuber and root Ss by 24% and 35%, respectively. T_E decreased root Ss for both genotypes, but reduced tuber Ss for the Upland genotype only (significant genotype × temperature interaction). For the Upland genotype, the positive effect of C_E on root Ss was offset by T_E (significant [CO_2] × temperature interaction), resulting in a significant genotype × [CO_2] × temperature interaction (Tables 2-2 and 2-4).

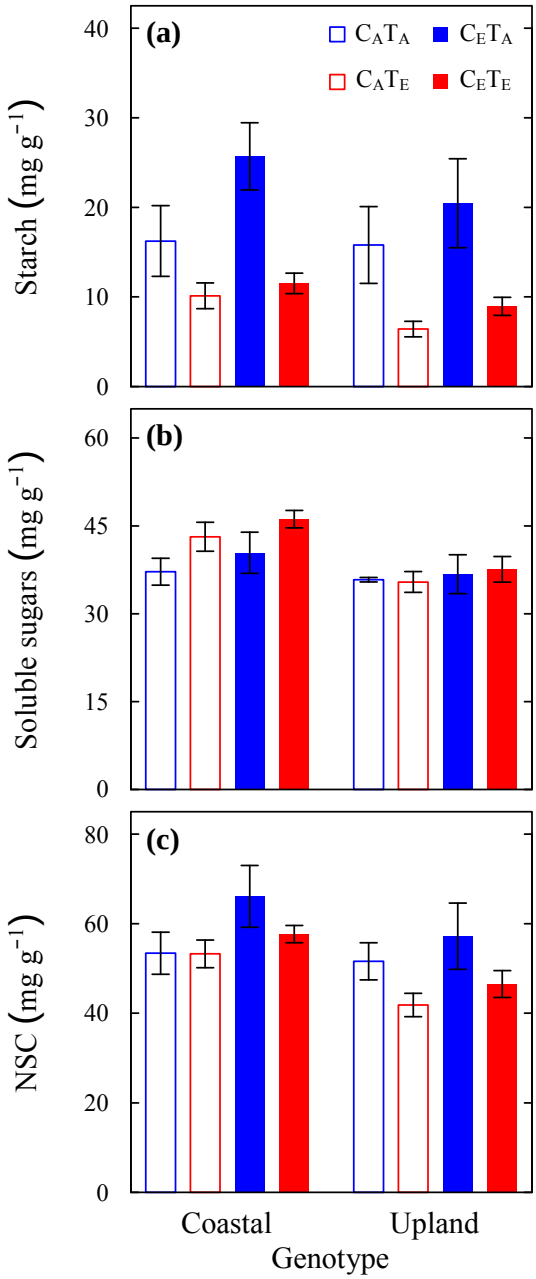

Figure 2-5 Whole-plant starch (a), soluble sugars (b), and non-structural carbohydrates (NSC) (c) of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments. Values represent means ± 1 SE (n = 5).

Table 2-4 Summary of means for starch and soluble sugar concentrations in different organs (leaf, stem, tuber and root) of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments, as described in the Materials and methods

		Treatment			
Parameter	**Genotype**	C_AT_A	C_AT_E	C_ET_A	C_ET_E
Starch					
Leaf (mg g^{-1})	Coastal	19.4 ± 4.4^{abc}	9.2 ± 2.4^{cd}	41.0 ± 7.4^{a}	11.3 ± 0.6^{bcd}
	Upland	25.3 ± 7.6^{abc}	6.3 ± 0.8^{d}	30.8 ± 6.9^{ab}	12.4 ± 2.1^{bcd}
Stem (mg g^{-1})	Coastal	6.2 ± 1.2^{a}	2.6 ± 0.6^{abc}	6.5 ± 1.2^{a}	4.1 ± 1.5^{abc}
	Upland	4.3 ± 0.5^{ab}	2.0 ± 0.6^{bc}	3.7 ± 0.7^{abc}	1.3 ± 0.1^{c}
Tuber (mg g^{-1})	Coastal	16.8 ± 4.8^{a}	7.2 ± 2.6^{ab}	16.9 ± 5.7^{a}	5.3 ± 1.5^{ab}
	Upland	2.8 ± 0.5^{ab}	1.9 ± 0.7^{b}	6.3 ± 1.1^{ab}	3.1 ± 0.1^{ab}
Root (mg g^{-1})	Coastal	15.6 ± 6.4^{a}	17.1 ± 1.7^{a}	10.9 ± 3.3^{a}	15.9 ± 2.2^{a}
	Upland	7.3 ± 1.7^{a}	11.5 ± 2.2^{a}	10.8 ± 2.4^{a}	7.4 ± 1.1^{a}

Table 2-4 (continued)

Parameter	Genotype	Treatment			
		C_AT_A	C_AT_E	C_ET_A	C_ET_E
Soluble sugar					
Leaf (mg g^{-1})	Coastal	61.6 ± 4.0^{ab}	65.9 ± 3.4^{ab}	63.5 ± 5.3^{ab}	70.7 ± 4.1^{a}
	Upland	57.9 ± 2.2^{ab}	48.1 ± 2.9^{b}	49.0 ± 4.8^{b}	54.4 ± 4.1^{ab}
Stem (mg g^{-1})	Coastal	22.8 ± 3.3^{a}	20.6 ± 1.7^{a}	15.8 ± 0.9^{a}	20.9 ± 1.3^{a}
	Upland	22.8 ± 3.1^{a}	22.2 ± 0.9^{a}	18.8 ± 1.8^{a}	21.3 ± 1.1^{a}
Tuber (mg g^{-1})	Coastal	24.0 ± 1.0^{b}	26.7 ± 1.2^{ab}	31.1 ± 1.6^{ab}	32.7 ± 2.1^{ab}
	Upland	25.3 ± 1.8^{ab}	22.8 ± 1.8^{b}	35.5 ± 4.7^{a}	23.6 ± 3.0^{b}
Root (mg g^{-1})	Coastal	16.8 ± 0.9^{abc}	13.6 ± 0.3^{bc}	20.0 ± 1.6^{ab}	17.3 ± 1.7^{abc}
	Upland	12.4 ± 0.8^{c}	12.7 ± 0.7^{c}	24.2 ± 3.8^{a}	12.5 ± 1.1^{c}

The four [CO_2] and temperature treatments are: C_AT_A (400 µl l^{-1}, 26 °C), C_AT_E (400 µl l^{-1}, 30 °C), C_ET_A (640 µl l^{-1}, 26 °C) and C_ET_E (640 µl l^{-1}, 30 °C). Values represent means ± 1 SE (n = 5). Within each parameter, different superscript letters indicate means that are significantly different at $P < 0.05$ based on Tukey's pair-wise comparisons.

2.3.4 Relationships between biomass and physiological parameters

To assess those factors that may have regulated plant biomass, I examined the relationships between whole-plant dry mass and physiological parameters (i.e., photosynthetic traits and carbohydrate variables). Whole-plant dry mass increased with increasing A_{sat}, whole-plant Ss and leaf Ss, but decreased with increasing stem Ss ($P < 0.05$ in all cases), without significant differences between within-treatment correlations. No other associations between whole-plant dry mass and physiological traits were observed. A_{sat} accounted for only 10% of the variation in whole-plant dry mass (Fig. 2-6a), while whole-plant, leaf and stem Ss accounted for 20%, 22% and 11% of the variation in whole-plant dry mass, respectively (Fig. 2-6b, 6c and 6d).

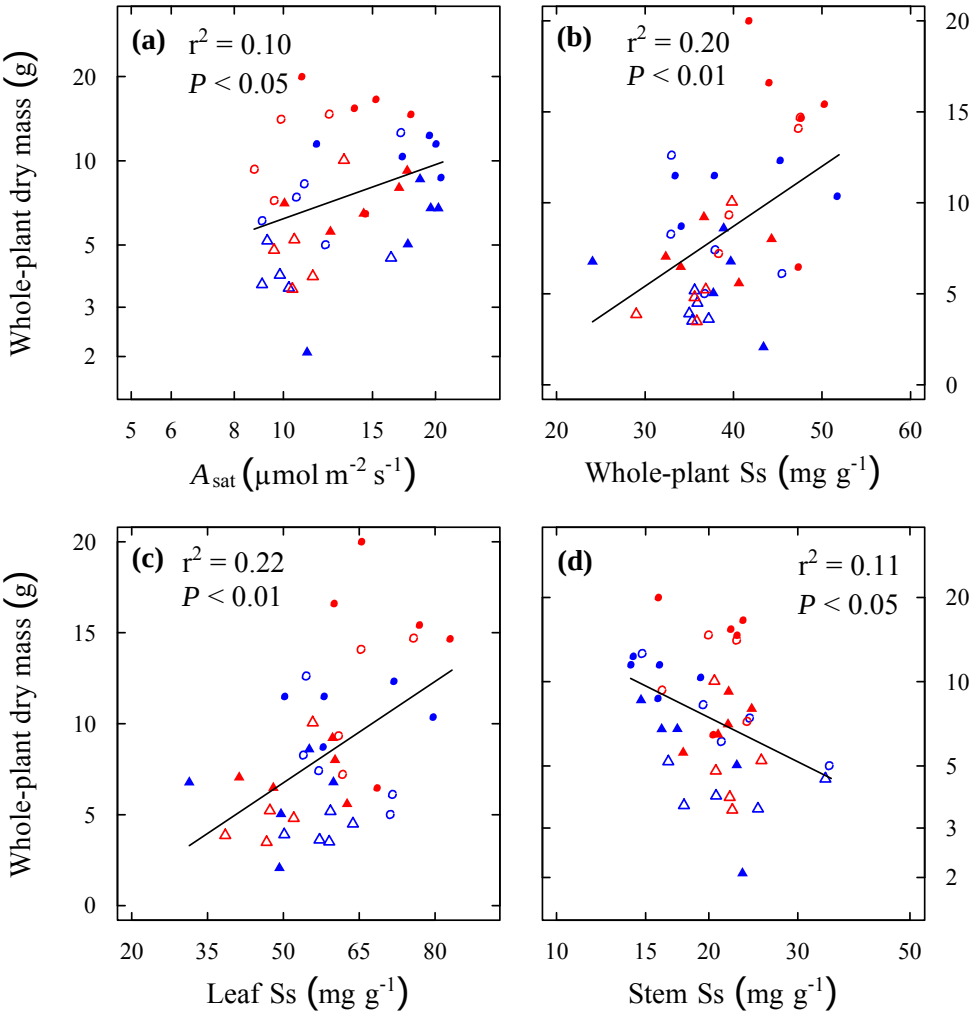

Figure 2-6 The relationships between whole-plant dry mass and A_{sat} (a), whole-plant soluble sugars (whole-plant Ss) (b), leaf soluble sugars (leaf Ss) (c) and stem soluble sugars (stem Ss) (d) of *Telopea speciosissima* Coastal (circles) and Upland (triangles) genotypes grown under the four [CO_2] and temperature treatments: C_AT_A (open blue), C_AT_E (open red), C_ET_A (closed blue) and C_ET_E (closed red). There were five replicates per treatment, and each data point represents a single observation. Data were fitted using a linear regression (solid line). Data points for A_{sat} and stem Ss were log-log transformed before fitting. The adjusted r^2 value and its significance for each fitting are shown.

2.4 Discussion

In contrast to the first hypothesis, the Coastal genotype of *T. speciosissima* from less variable temperature environments showed greater plasticity in growth with T_E, rather than the Upland genotype that experienced greater levels of temperature variability. In addition, genotypes did not vary in their responses in most physiological traits under T_E. The second hypothesis was fully supported as genotypes responded similarly in growth and physiology under C_E, indicating no genotypic variation in phenotypic plasticity in response to [CO_2]. The third hypothesis was not supported because the interactive effects of [CO_2] and temperature on growth and physiology were largely absent in this study, and the genotypic variation in growth response to temperature was not affected by [CO_2]. Overall, these results indicate that temperature may be more effective than [CO_2] in exposing intraspecific variation in growth plasticity for genetically differentiated woody plant populations under future climates. Results of this study also suggest that woody plant populations originating from more variable environments may not necessarily show greater phenotypic plasticity in response to changing climates.

2.4.1 Intraspecific variation in woody plant responses to warming and elevated [CO_2]

Significant intraspecific variation in growth plasticity between the two *T. speciosissima* genotypes was observed when responding to warming, with the Coastal genotype exhibiting greater increments in growth traits such as whole-plant dry mass, leaf area and SMF, compared to the Upland genotype. The differentiation between genotypes in growth response to warming reported here is consistent with the general

prediction that plant populations may exhibit genetic variation in phenotypic plasticity (Donohue *et al.*, 2001; Alpert & Simms, 2002; Van Kleunen & Fischer, 2005; Aspinwall *et al.*, 2015). It has been suggested that there is predictable intraspecific variation in the capacity of woody species to respond to T_E (Saxe *et al.*, 2001; Weston & Bauerle, 2007; Weston *et al.*, 2007; Way & Oren, 2010; Drake *et al.*, 2015). For example, Drake *et al.* (2015) studied 21 provenances of two widely distributed eucalyptus species (*Eucalyptus tereticornis* and *Eucalyptus grandis*) grown in conditions simulating ambient summer temperatures at seed origin and warmed temperatures (+ 3.5 °C), and found that the effect of warming on plant biomass and leaf area strongly interacted with the provenance's climate-of-origin. Similarly, I found that the growth capacity of woody plants in response to warming may vary among genotypes from contrasting climates.

Unlike other studies showing intraspecific variation in plasticity of physiological traits such as photosynthetic variables under T_E (Weston & Bauerle, 2007; Weston *et al.*, 2007; Drake *et al.*, 2015), differentiation in physiological plasticity of the two *T. speciosissima* genotypes in response to warming was largely absent in this study. T_E had similar effects on most physiological traits between the two genotypes, despite that there was significant genotype by temperature interaction on a few physiological traits including g_s, A/T_L parameter C and tuber Ss. This phenomenon suggests that the effect of warming on growth plasticity was not parallel with the effect of warming on physiological plasticity in this study. This pattern may be attributed to the difference in plant size between the two *T. speciosissima* genotypes. Under warming, both genotypes allocated more biomass to the above-ground for vegetative growth, as indicated by the reduced Root/Shoot ratio and the increased LAR, but the magnitudes of these changes did not differ between genotypes. However, the

Coastal seedlings were bigger than the Upland counterparts, and therefore the Coastal genotype allocated more mass in essence to leaves and stems under warming conditions. In such circumstances, when compared with the Upland genotype, the Coastal genotype not only had greater whole-plant leaf area, but also showed greater increase in leaf area to warming, which is possibly the primary cause of the intraspecific variation in growth (i.e., biomass) response to temperature between the two *T. speciosissima* genotypes in this study.

Despite the fact that many traits (including growth and physiology) measured in this study showed a significant response to C_E, no interaction between genotype and [CO_2] was found for any of the growth or physiological traits, indicating that the two *T. speciosissima* genotypes had similar phenotypic plasticity under C_E. Although most studies on intraspecific variation in woody species responsiveness to C_E demonstrate substantial intraspecific differentiation in the responses of plant growth and/or physiology to changing [CO_2] (Ceulemans *et al.*, 1996; Dickson *et al.*, 1998; Isebrands *et al.*, 2001; Mohan *et al.*, 2004; Cseke *et al.*, 2009), some studies show limited intraspecific variation in woody plant responsiveness to C_E (e.g., Cantin *et al.*, 1997), In this study, both genotypes of *T. speciosissima* were equally limited by carbon availability and therefore showed strong increases in leaf area (29%) and mass production (35%) when grown in C_E. Subsequently, rising [CO_2] is not likely to generate differential responses in genotypes of *T. speciosissima* in future climates.

I did not observe significant interaction between temperature and [CO_2] in most traits measured in this study, except the ratio of J_{max}/V_{cmax} and the root Ss, suggesting that the effects of T_E and C_E were generally independent in the two *T. speciosissima* genotypes. There is no clear trend in the literature for the interactive effects of temperature and [CO_2] on woody plant species. Many studies show that C_E is likely to

interact with T_E, synergistically affecting woody plant growth and/or physiology (Callaway *et al.*, 1994; Peltola *et al.*, 2002; Ghannoum *et al.*, 2010a; Ayub *et al.*, 2011; Wang *et al.*, 2012). However, findings from the present study are consistent with other studies indicating that the effects of increasing [CO_2] and warming are additive (Lewis *et al.*, 2001; Lloyd & Farquhar, 2008; Ghannoum *et al.*, 2010b; Lewis *et al.*, 2013). In addition, I also did not find significant interactive effects of temperature and [CO_2] on the genotypic variation in phenotypic plasticity for most traits, except in root Ss. Especially for traits that showed interaction between genotype and temperature (i.e., plant dry mass, leaf area, SMF, g_s, A/T_L parameter C and tuber Ss), genotypic variation in phenotypic plasticity under T_E was not affected by changes in [CO_2]. I suggest that the lack of interactive effects of temperature and [CO_2] on genotypic variation in phenotypic plasticity in this study may be partially due to the absence of interactive effects of temperature and [CO_2] on plant growth and physiology.

2.4.2 Association between phenotypic plasticity and source environment variability of woody plant populations

Plant populations usually show genetic differentiation in phenotypic plasticity and the divergence among populations may be influenced by the pattern of environmental variation. A long-standing hypothesis suggests that greater levels of environmental variability will select for genotypes with greater phenotypic plasticity (Galloway, 1995; Ackerly *et al.*, 2000; Weinig, 2000; Donohue *et al.*, 2001; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen & Fischer, 2005). Although testing this hypothesis on woody plant species is limited, there is at least one case study that supports the theory (Drake *et al.*, 2015). Specifically, this case study

on two widespread eucalyptus species (*E. tereticornis* and *E. grandis*) showed that, for both species, provenances originating from cooler and more variable temperature climates exhibited higher plasticity in growth and photosynthetic capacity under warming, when compared with provenances from warmer and more uniform temperature climates (Drake *et al.*, 2015).

Results from this study contradict the current paradigm. I observed that the Coastal genotype of *T. speciosissima* (warmer and less variable temperature environments) rather than the Upland genotype (cooler and more variable temperature environments), exhibited higher growth plasticity in response to T_E. The differentiation in phenotypic plasticity among plant populations may be associated with source environment variability and linked to the intrinsic difference in adaptation to distinct source environments. Plant populations usually are highly adapted to local conditions, showing the greatest fitness in their home environments (Savolainen *et al.*, 2007; Hereford, 2009; Wang *et al.*, 2010). The upland region in this study is *c.* 2–5 °C cooler than the coastal region (Table 2-1), and the temperature difference between these regions has been estimated to be larger during the Last Glacial Maximum (Barrows *et al.*, 2001; Hesse *et al.*, 2003). This long-term temperature differential may have shaped and maintained the genetic differences between the coastal and upland populations of *T. speciosissima* (Rossetto *et al.*, 2011). The Upland genotype that might have been adapted to cooler temperatures, may not have the capacity to fully utilise warmer temperatures in terms of plant growth, and therefore showed lower growth plasticity in response to T_E when compared with the warmer-origin Coastal genotype. However, to more rationally explain why the results in this study contradict the long-standing paradigm, further studies with a more specific and thorough design (e.g., with both

ecological and evolutionary aspects included) on *T. speciosissima* would be more informative.

In conclusion, I found that the Coastal genotype of *T. speciosissima*, which originated from warmer and less variable temperature environments, showed greater plasticity in growth with warming than the Upland genotype from cooler and more variable temperature environments. On the other hand, C_E did not expose genotypic variation in growth or physiological responses, either individually or interactively with T_E. These findings suggest that temperature will be more effective than [CO_2] in exposing intraspecific variation in growth plasticity for genetically differentiated woody plant populations under future climates. Overall, results from this study contradict the paradigm that genotypes from more variable climates will exhibit greater phenotypic plasticity in future climate regimes.

Chapter 3

Drought responses of two genetically differentiated *Telopea speciosissima* populations under elevated [CO_2] and temperature

3.1 Introduction

Increasing emissions of greenhouse gases from anthropogenic activities, such as fossil fuel consumption and land use changes, are contributing to ongoing climate change. By the end of the 21st century, atmospheric carbon dioxide concentrations ([CO_2]) are projected to exceed 550–900 $\mu l\ l^{-1}$, which would lead to an increase of 0.3–4.8 °C in the global mean air temperature (Solomon *et al.*, 2009; Collins *et al.*, 2013). Embedded with this climatic warming trend, increases in the frequency and intensity of extreme climatic events such as drought are also expected, because warming usually causes greater evaporation and thus surface drying (Kharin *et al.*, 2007; Trenberth, 2011; Coumou & Rahmstorf, 2012; Dai, 2013; Prudhomme *et al.*, 2014). Changes in [CO_2], temperature and water availability are likely to substantially regulate plant growth, function and development, thereby affecting functionality, biodiversity and productivity of terrestrial ecosystems (Cramer *et al.*, 2001; Nemani *et al.*, 2003; Ciais *et al.*, 2005; Allen *et al.*, 2010). Globally, forests cover c. 30% of land surface and

contribute more than half of terrestrial net primary production, and thereby play a dominant role in the terrestrial carbon cycle (Karnosky, 2003; FAO, 2006; Bonan, 2008; Pan *et al.*, 2011). Therefore, quantifying and understanding the capacity of woody species to cope with simultaneously changing climatic factors is of particular importance.

Water is essential for almost all biochemical and physiological processes occurring in plant organisms, and therefore is probably the most important determinant of plant growth and function (Boyer, 1982). Plants generally respond to drought or continuous water deficit by closing their stomata to reduce water usage, which results in drought-induced inhibition of photosynthesis and reductions in biomass accumulation, as well as reductions in carbohydrate reserves (Chaves, 1991; Flexas *et al.*, 2002; Chaves *et al.*, 2003; Muller *et al.*, 2011; Mitchell *et al.*, 2013). However, the effects of drought on plants may be altered by changes in [CO_2] and temperature, both of which would influence the susceptibility of woody species in response to drought (Lewis *et al.*, 2013; Way, 2013). Elevated [CO_2] (C_E) often leads to reduced stomatal conductance (g_s) and thereby reduced plant water usage, which allows plants to maintain relatively more favourable water status during sustained drought and therefore ameliorate the negative impact of drought stress on plant physiology and growth (Morison, 1993; Poorter & Pérez-Soba, 2001; Wullschleger *et al.*, 2002; Ainsworth & Rogers, 2007; Duan *et al.*, 2013). By contrast, elevated temperature (T_E) usually increases water loss due to higher air vapour pressure deficit (VPD) and the need for larger evaporative cooling, thereby exacerbating the drought stress on plants (Larcher, 2003; Oishi *et al.*, 2010; Will *et al.*, 2013).

Given the contrasting effects of C_E and T_E regulating drought responses, their combined effects on woody species tolerance to water deficit may vary, possibly

depending on the trade-offs between these two climatic factors (Duan *et al.*, 2013). Some studies suggest that C_E and T_E can interact synergistically and affect physiological responses of woody plant seedlings to drought (Zeppel *et al.*, 2012), while other studies indicate that the effects of rising [CO_2] and warming on woody species under drought are simply additive (e.g., Ambebe & Dang, 2010; Duan *et al.*, 2013; Lewis *et al.*, 2013). Although the number of combinatorial experiments studying the interactive effects of [CO_2], temperature and water availability on woody species is growing recently (Ambebe & Dang, 2010; Wertin *et al.*, 2010, 2012; Zeppel *et al.*, 2012; Duan *et al.*, 2013, 2014, 2015; Lewis *et al.*, 2013; Gauthier *et al.*, 2014), the degree to which both C_E and T_E will alter the responses of woody plants to drought remains largely unknown.

The capacity of woody plants to cope with climate change in the short term may critically depend on their phenotypic plasticity, which is the ability of a genotype to express multiple phenotypes in response to environmental change (Bradshaw, 1965; Sultan, 2000; Nicotra *et al.*, 2010; Anderson *et al.*, 2012). When genotypes of a given species respond differently to the same environmental change, there is genotypic variation in phenotypic plasticity, known as significant genotype × environment interactions (Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). Generally, genotypes demonstrating low phenotypic plasticity in growth may tolerate and persist under extreme conditions to survive (Schlichting, 1986; Thompson, 1991), while genotypes with high phenotypic plasticity may be capable of rapid resource uptake and show increased growth when conditions are optimal (Grime & Mackey, 2002). For a given woody species responding to the same climate regime, populations originating from contrasting environments are likely to show intraspecific variation in growth and physiological plasticity, because plant populations are generally highly adapted to their

original local conditions (Savolainen *et al.*, 2007; Hereford, 2009; Wang *et al.*, 2010; McLean *et al.*, 2014).

Intraspecific variation in plant response to drought and variable soil moisture has received much attention and been well documented in many woody species, such as *Eucalyptus*, *Pinus*, *Populus* and *Quercus* (e.g., Cregg & Zhang, 2001; Silva *et al.*, 2004; Monclus *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Bedon *et al.*, 2012; McLean *et al.*, 2014). For woody plant populations from different environments, the intraspecific differentiation in response to water deficit is usually associated with their source environmental conditions. For instance, populations originating from more mesic regions are usually more susceptible to drought (Cregg & Zhang, 2001; Silva *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Dutkowski & Potts, 2012; Robson *et al.*, 2012), while populations from more stressful environments tend to be less responsive to water stress (Gratani *et al.*, 2003; Baquedano *et al.*, 2008; Aranda *et al.*, 2010; Bansal *et al.*, 2015). However, whether these patterns would be altered by other climatic factors is still unknown. To my knowledge, few studies have investigated the interactive effects of concurrently changing climatic variables such as [CO_2] and temperature on the intraspecific variation of woody plants in response to drought.

The primary objective of this study was to assess the main and interactive effects of C_E and T_E on drought responses of *Telopea speciosissima* R.Br. (Proteaceae; commonly known as the Waratah) populations. Two natural populations were selected in this study, with one originating from the coastal region (warmer and relatively wetter environment) and the other one from the upland region (cooler and relatively drier environment). These two populations are also genetically differentiated according to a previous study on population genetics (Rossetto *et al.*, 2011), and therefore were defined here as the Coastal genotype and the Upland genotype,

respectively. In a related study assessing the effects of [CO_2] and temperature on growth and physiology of these two *T. speciosissima* genotypes grown under well-watered conditions (Huang *et al.*, 2015), I found that the relatively faster growing Coastal genotype showed higher growth plasticity in response to T_E, but growth of both genotypes responded similarly to C_E. However, both C_E and T_E, alone or interactively, did not expose intraspecific variation in physiological plasticity between the two genotypes.

In this study, I extended previous research by manipulating a third experimental factor (i.e., water availability) in addition to [CO_2] and temperature, evaluating the potential intraspecific variation of *T. speciosissima* genotypes in response to simultaneously changing climatic variables. The following hypotheses were tested: (i) the Upland genotype from drier environment would be more resistant to drought stress, and show less reduction in growth and physiology when compared with the Coastal genotype; (ii) regardless of genotypes, T_E would increase water loss, thereby accelerating the process of stomatal closure and consequently exacerbating the drought stress; (iii) for both genotypes, C_E would promote water use efficiency, thereby slowing down the stomatal closure and consequently ameliorating the drought stress; and (iv) C_E would also ameliorate the negative effects of T_E on plant responses to drought for both genotypes.

3.2 Materials and methods

3.2.1 Plant material and growth conditions

Two natural populations of *T. speciosissima* were selected for this study, each of which originated from the eastern (the coastal region) and the western (the upland region) edge of the species distribution, respectively. Specifically, the coastal population was chosen from Patonga (33.53°S, 151.28°E, 180 m altitude) and the upland population was selected from Newnes Forest (33.39°S, 150.21°E, 1150 m altitude). According to the 40-year (1971–2010) climate records from the SILO Climate Data (Jeffrey *et al.*, 2001), the coastal region has higher mean annual precipitation (1243 mm; range 792–2044 mm) than the upland region (856 mm; range 393–1265 mm). In addition, the coastal region is also characterised by warmer but less variable temperatures, when compared with the upland region. In summer days, the mean maximum temperature is 26.9 °C (range 24.1–29.8 °C) for the coastal region and 24.9 °C (range 20.8–29.1 °C) for the upland region, while the mean minimum temperatures are 18.1 °C (range 15.9–20.4 °C; the coastal region) and 12.6 °C (range 9.4–15.3 °C; the upland region), respectively.

It has been reported that *T. speciosissima* contains three distinct gene pools (coastal, upland and southern) throughout its natural distribution (Rossetto *et al.*, 2011). The coastal and the upland populations selected in this study were chosen based on their high gene identity specific to the corresponding gene pools (i.e., > 90% coastal and upland gene pools, respectively), and thus were defined as the Coastal genotype and the Upland genotype, respectively. For each genotype, 200 seeds from 12 mother plants (with 10–40 seeds per mother plant depending on its reproductive capacity) were collected and planted in forestry tubes filled with a peat and sand mixture (1:2)

for germination. Seeds of each genotype were divided into 4 groups, with each group (50 seeds) consisting of 5 mother plants and 10 seeds from each mother plant. The 4 seed groups were then randomly assigned into one of the four adjacent glasshouse compartments (each 3.0 m × 5.0 m × 3.5 m in width × length × height), with natural sunlight (direct light attenuated by 10–15% due to structure) and [CO_2]/temperature control, which are located at the campus of the University of Western Sydney (Richmond, NSW, Australia) (Huang *et al.*, 2015). Detailed description of the glasshouse design can be found in Ghannoum et al. (2010a).

A factorial [CO_2] and temperature design was applied to the four glasshouse compartments, with two [CO_2] (ambient (C_A) and elevated (C_E)) and two temperature (ambient (T_A) and elevated (T_E)) treatments. The C_A treatment was targeted at 400 µl l^{-1} while C_E was maintained at 640 µl l^{-1}. Two glasshouse compartments for T_A were set at 26/16 °C (day/night), approximating the mean (25.9/15.4 °C for day/night) of averaged daily temperatures in summer of the coastal and the upland regions selected in this study. Justification of this temperature setting can be found in a related study on *T. speciosissima* (Huang *et al.*, 2015). The other two compartments for T_E were designed to maintain a constant 4 °C increase in temperature relative to the ambient daily temperature cycle, which was 30/20 °C for day/night. In addition, temperature in each compartment was changed five times over the 24-hour period to simulate a natural diel temperature cycle in the field. The four treatments in this study were therefore termed as follows: C_AT_A (400 µl l^{-1}, 26 °C), C_AT_E (400 µl l^{-1}, 30 °C), C_ET_A (640 µl l^{-1}, 26 °C) and C_ET_E (640 µl l^{-1}, 30 °C).

All successfully germinated seedlings were allowed to grow in the forestry tubes for three months and then were transplanted into cylindrical polyvinyl chloride (PVC) pots (15 cm diameter × 40 cm length) containing dry loamy-sand soil (86.5%

sand and 9.5% clay, moderate fertility) in January (summer) 2012. Soil was collected from a local dry sclerophyllous forest (Menangle, NSW, Australia), with the following characteristics: pH = 5.0, organic carbon content = 1.4%, total Kjeldahl N = 1300 mg kg^{-1}, total P = 217 mg kg^{-1}, C : N : P = 65 : 6 : 1, Ca < 10 mg kg^{-1}, Mg < 10 mg kg^{-1}, Na = 20 mg kg^{-1}, K < 10 mg kg^{-1}, Al = 5560 mg kg^{-1}, Fe = 14800 mg kg^{-1} (ALS Laboratory Group, Analytical Chemistry and Testing Services, Smithfield, NSW, Australia). About 10 kg of dry soil was filled to each PVC pot. A PVC cap with four drainage holes covered with 2 mm mesh was placed at the bottom of each pot. Prior to the controlled watering (see the watering regime below), all transplanted seedlings were irrigated to field capacity on a daily basis and fertilized twice (4 weeks and 12 weeks after transplanting, respectively) with a commercial fertilizer (All Purpose, Brunnings, Victoria, Australia, N:P:K – 27:2:10). Seedlings were randomly rotated within and among glasshouse compartments routinely to minimize the potential effects of position on plant performance.

3.2.2 Watering regime

Following about three months (from January 2012 to March 2012) of additional growth under well-watered conditions, 4 sib seedlings (i.e., siblings) germinated from seeds of each mother plant (with main stem length and basal diameter mostly representing the mean values of siblings from the same maternal parent) were selected. These 4 siblings were paired based on similar growth parameters (i.e. stem length and basal diameter) and then randomly assigned into one of two groups. One group of siblings continued to receive full watering (hereafter '*well-watered*' treatment) throughout the experimental period, while the other group was subjected to

drought/recovery cycles (hereafter '*drought*' treatment). The use of mother plants and siblings was aimed at minimizing the potential maternal effects on seedling drought response. In total, this experiment consisted of 160 individual potted seedlings (2 genotypes × 2 [CO_2] × 2 temperature treatments × 2 watering treatments × 5 mother plants × 2 siblings). Every selected pot was weighed in the morning (between 09:00–10:00 hours) to determine water loss every second or third day. The *well-watered* seedlings were maintained at field capacity by supplying the same amount of water to the pot that was lost during each weighing interval. By contrast, the *drought* treatment was achieved by withholding water in the *drought* seedlings. Two drought events, plus a recovery phase (full watering) in the middle, were imposed on the *drought* seedlings to more realistically mimic natural field drought events that usually occur as multiple dry-wet cycles.

It was not feasible to use leaf water potential as the indicator of physiological drought stress because there was insufficient leaf material for multiple destructive leaf samplings for water potential measurements. Instead, I employed non-destructive sampling of stomatal conductance (g_s) to monitor the status of stress during the whole experimental period, given that g_s has been reported as an effective indicator of plant and leaf water stress (Ayub *et al.*, 2011). The threshold of g_s for defining whether a given seedling was physiologically stressed was set at 0.05 mol m^{-2} s^{-1} for both drought events, similar to previous drought studies on *Eucalyptus* species (Ayub *et al.*, 2011; Duan *et al.*, 2014). The recovery phase following the first drought event was achieved by rewatering *drought* seedlings to field capacity and keeping them well-watered for 2 weeks (the third fertilization was also applied during this period), allowing g_s of *drought* seedlings to be fully recovered. After that, water was withheld from seedlings for the second drought event.

Following the second drought event, I did not harvest the *drought* seedlings immediately, but instead allowed them to desiccate further until all seedlings exhibited zero photosynthesis. At that time, all seedlings (including *well-watered* and *drought*) were destructively harvested. Therefore, this experiment was implemented in the following stages: *pre drought* (Stage Pre), *first drought* (Stage D1), *recovery* (Stage R), *second drought* (Stage D2) and *final harvest* (Stage H). Soil volumetric water content (VWC; $m^3\ m^{-3}$) of each pot was assessed using a handheld TDR probe (20 cm in length; HydroSense II soil moisture measurements system; CS658, Campbell Scientific, Logan, UT, USA). To minimize the potential negative effects of SWC measurements on seedling root systems, SWC was only measured at the end of each stage (except for the *final harvest*, when the soil in drought pots was too dry), plus two more assessments during the first drought event. During the experimental period, vapour pressure deficit (VPD) in the four glasshouse compartments averaged 0.86 kPa (range 0.1–2.9 kPa) in T_A and 1.50 kPa (range 0.2–4.3 kPa) in T_E, but did not vary between [CO_2] treatments (Duan *et al.*, 2014).

3.2.3 Resistance to drought

The capacity of *T. speciosissima* seedlings in resisting drought stress was assessed by determining time periods for *drought* seedlings to become physiologically stressed. Specifically, I tracked the status of stomatal conductance in each individual *drought* seedling and recorded the date when g_s was lower than the defined threshold (i.e., < 0.05 mol $m^{-2}\ s^{-1}$). The time period (in weeks) for each *drought* seedling to become stressed was calculated as the difference between the recorded date and the start date of the *drought* treatment. For example, if a *drought* seedling showed signs

of becoming physiologically stressed at the fifth week after the onset of *drought*, its time period for resisting drought was determined to be 5 weeks. Because seedlings had different capacity to resist water deficit, showing a wide range of duration of the *drought* treatment, the assessment of seedling resistance to drought was conducted on an individual basis. In other words, although the *drought* seedlings in this study had the same onset date of *first drought*, the immediately followed *recovery* (a two-week period for each seedling) and *second drought* treatments were imposed on each individual *drought* seedling separately, at various dates.

3.2.4 Leaf gas exchange measurements

Leaf-level gas exchange measurements were taken on attached, recently fully-expanded leaves via a portable open path gas exchange system (Licor-6400XT, Li-Cor, Lincoln, NE, USA) supplying photosynthetic photon flux density (PPFD) by a red-blue light source (6400-02B). Light-saturated photosynthesis (A_{sat}, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) were measured at saturating PPFD of 1200 μmol m^{-2} s^{-1}, growth [CO_2] (400 μl l^{-1} or 640 μl l^{-1}), mid-day growth temperature (26 °C or 30 °C) , relative humidity of 45–65% and leaf-to-air VPD between 1.0 and 3.0 kPa. Each leaf was allowed 5–10 min to equilibrate before readings were taken. Measurements were conducted on 5 replicate seedlings per [CO_2], temperature and watering treatment combination of each genotype on a weekly basis. The 5 replicate seedlings consisted of one sibling from each of the five mother plants.

3.2.5 Growth measurements

Throughout the experiment, the main stem length (cm) and basal diameter (cm) of all seedlings were measured at the end of each defined stage. The stem volume (cm^3) of each seedling at each stage was calculated as the stem basal area multiplied by the stem length (i.e., assuming the seedling stem is cylindrical, volume = $\pi/4 \times \text{diameter}^2 \times \text{length}$), following the approach in Kubiske *et al.* (2006). If new sprouting from the lignotuber was observed in a seedling during the experiment, volumes of the newly sprouted stems were also calculated and added to the total stem volume of that seedling. This non-destructive method was reliable for estimating growth during the experiment, because there was a strong linear relationship between dry mass and estimated stem volume across all seedlings at the harvest (on log-log scales, adjusted $r^2 = 0.67$, $P < 0.0001$).

At the end of the experiment, all 160 seedlings (10 replicates per [CO_2], temperature and watering treatment combination of each genotype) were destructively harvested and separated into different organs (leaf, stem, tuber and root). The root system was washed free of soil. All harvested organs were oven-dried at 70 °C for 72 h and then weighed for dry mass (g). Total plant leaf area (cm^2) of *well-watered* seedlings was determined by a portable leaf area meter (Li-3100A; Li-Cor, Lincoln, NE, USA), and then used to calculate the relationships between leaf area and leaf dry mass. Both genotypes showed a strong relationship in linear regression between leaf dry mass and area (for the Coastal genotype, adjusted $r^2 = 0.91$, $P < 0.0001$; for the Upland genotype, adjusted $r^2 = 0.95$, $P < 0.0001$). Fitted parameters from the linear regressions were used to estimate total plant leaf area for *drought* seedlings.

3.2.6 Carbohydrate analyses

Oven-dried plant material from the harvest was ground to fine powder in a ball mill for the determination of non-structural carbohydrates (NSC). The NSC concentration was assayed as the sum of total starch (St) concentration and soluble sugar (Ss) concentration, following procedures described in Mitchell *et al*. (2013). Specifically, dried organ samples (about 20 mg) were weighed and then extracted with 5 ml of 80% aqueous ethanol (v/v). The mixture was boiled at 95 °C in a water bath for 30 min and then centrifuged at 3000 rpm for 5 min. The supernatant was collected for further use, while the pellet was re-extracted once with 5 mL of 80% aqueous ethanol (v/v) and once with 5 ml of distilled water, then boiled and centrifuged as before. All collected supernatants were pooled and evaporated to the last 1-3 ml in a rotational vacuum concentrator (RVC 2-25 CD; Christ, Germany) at 40°C. Total soluble sugars were determined on the supernatants by the anthrone method (Ebell, 1969), while total starch was analyzed on the pellets remaining after the ethanol and water extractions, and assayed enzymatically using a total starch assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Whole-plant Ss, St and NSC were calculated by summing the weighted concentrations (concentration multiplied by the proportion of the organ dry mass to the whole-plant dry mass) of the four plant organs (leaf, stem, tuber and root). All carbohydrate analyses were conducted on 5 replicate seedlings (consisting of one sibling from each of the five mother plants) per [CO_2], temperature and watering treatment combination of each genotype.

3.2.7 Statistical analysis

There were a total of 16 treatment combinations in this study: two genotypes × two [CO_2] treatments × two temperature treatments × two watering treatments. Time-series measured and calculated growth and physiological parameters during the experiment (i.e., stem volume, A_{sat} and g_s) were analysed using a four-way repeated measures analysis of variance (RMANOVA), with measuring time (4 levels for gas exchange parameters and 5 levels for growth parameters) as a fixed repeated factor. Values obtained from the *final harvest* (i.e., dry mass, leaf area, and carbohydrate traits) were analysed via four-way ANOVAs to account for genotype, [CO_2], temperature and watering treatments. Plant (n = 10 for growth parameters and n = 5 for physiological and carbohydrate parameters) was included as a random effect in all analyses. Tukey's HSD test was used to determine differences among treatments in each parameter. Logarithmic or square root transformations were applied when necessary to satisfy the assumptions of residual homoscedasticity and normality.

Generalized linear models (GLM) were applied to test the effects of fixed factors (i.e., genotype, [CO_2] and temperature) on plant resistance to drought (the number of weeks to show the sign of physiological stress, count data), with plant size (i.e. the calculated stem volume) prior to drought treatment as a covariate to eliminate the potential confounding effects of plant size on drought resistance. In addition, independent two-sample *t*-tests were applied to the physiological and growth parameters in the *pre drought* to confirm that there were no significant differences between seedlings assigned to the *well-watered* and *drought* treatments. All statistical tests were performed in R (version 3.2.0; R Foundation for Statistical Computing, Vienna, Austria) and results were considered significant in all cases if $P < 0.05$.

To assess whether C_E and T_E would affect the sensitivity of photosynthesis to declines in soil water content, gas exchange traits (i.e., A_{sat} and g_s) as a function of soil VWC were analysed within each [CO_2] and temperature treatment for each genotype. A_{sat} was fitted with three-parameter sigmoid regression: $y = y_{asym} / (1 + e^{(-(VWC - VWC_{mid}) / k)})$, where y_{asym} is the estimated asymptote for the sigmoid regression, VWC_{mid} is the inflection point of soil VWC (where $y = y_{asym} / 2$) and k is a scaling parameter. Because g_s could not be significantly fitted with the sigmoid function, it was fitted with two-parameter linear regression on log-log scales: $\log_{10}(y) = y_0 + m \times \log_{10}(VWC)$, where y_0 and m are the intercept and slope for the linear regression, respectively. The effects of [CO_2] and temperature on parameters for each curvilinear or linear regression were analysed using 95% confidence intervals.

3.3 Results

At the onset of the *first drought*, growth parameters (i.e., stem length, basal diameter, and stem volume) and physiological traits (i.e., A_{sat} and g_s) were all similar between seedlings assigned to the *well-watered* and *drought* treatments within each [CO_2] and temperature treatment combination of each genotype ($P \geq 0.15$ in all cases), suggesting no bias in the initial allocation of seedlings to different watering treatments.

3.3.1 Plant growth

Across [CO_2], temperature and watering treatments, the Coastal genotype had higher whole-plant dry mass and leaf area than the Upland genotype (Fig. 3-1; Table 3-1). Regardless of watering treatment, C_E had positive effects on growth performance for both genotypes, while the positive growth response to T_E was only found in the Coastal genotype (significant genotype × temperature interaction; Table 3-1). The *drought* treatment, on the other hand, negatively affected dry mass accumulation and leaf growth in both genotypes, causing a reduction of 15–39% in whole-plant dry mass and an 18–43% decline in leaf area, respectively. However, declines induced by *drought* did not vary between genotypes or show significant difference among [CO_2] and temperature treatment combinations, indicating no interaction between *drought* and other treatments (Fig. 3-1; Table 3-1).

Stem volume in both genotypes had a similar pattern with plant dry mass in the response to experimental treatments, showing substantial increase under C_E but significant decline in the *drought*, as well as differentiated responses to T_E (Fig. 3-2; Table 3-2). Although a significant decrease in stem volume was found in *drought* seedlings (averaged across the five stages; $P = 0.015$) in relative to their *well-watered*

counterparts, the effect of *drought* on stem volume was not significant until the *final harvest* (Fig. 3-2). For the other four stages (i.e., the *pre drought*, *first drought*, *recovery*, and *second drought*), there was no significant difference in stem volume between the two watering treatments. Changes in stem volume induced by *drought* at the *final harvest* were mainly attributed to declines in the main stem basal diameter, but not due to changes in the main stem length (Fig. A-1 and A-2; Table 3-2).

Table 3-1 Summary (*P* values) of four-way ANOVAs testing for the main and interactive effects of [CO_2] (C), temperature (T) and watering (W) treatments on growth and carbohydrate parameters of two *Telopea speciosissima* genotypes (G)

	Growth		**Carbohydrates**		
Effect	**Dry mass**	**Leaf area**	**St**	**Ss**	**NSC**
G	**< 0.001**	**< 0.001**	0.068	0.404	0.196
C	**< 0.001**	**< 0.001**	**0.005**	0.059	**0.013**
T	**0.012**	**< 0.001**	**0.001**	0.850	**0.048**
W	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.667
G × C	0.662	0.383	0.535	0.527	0.837
G × T	**0.009**	**0.005**	0.608	0.276	0.400
C × T	0.558	0.975	0.557	0.163	0.422
G × W	0.503	0.785	**0.018**	**0.049**	**0.026**
C × W	0.561	0.984	0.087	0.549	0.761
T × W	0.497	0.970	**< 0.001**	0.081	0.280
G × C × T	0.554	0.694	0.380	0.160	0.158
G × C × W	0.493	0.457	0.719	0.274	0.318
G × T × W	0.442	0.344	0.450	0.694	0.731
C × T × W	0.513	0.636	0.083	0.208	0.085
G × C × T × W	0.696	0.340	0.435	0.223	0.513

St, starch; Ss, soluble sugars; NSC, non-structural carbohydrates. Significant values ($P < 0.05$) are shown in bold. Analyses were run on data obtained from harvest samples, with ten replicates ($n = 10$) for growth and five replicates ($n = 5$) for carbohydrates.

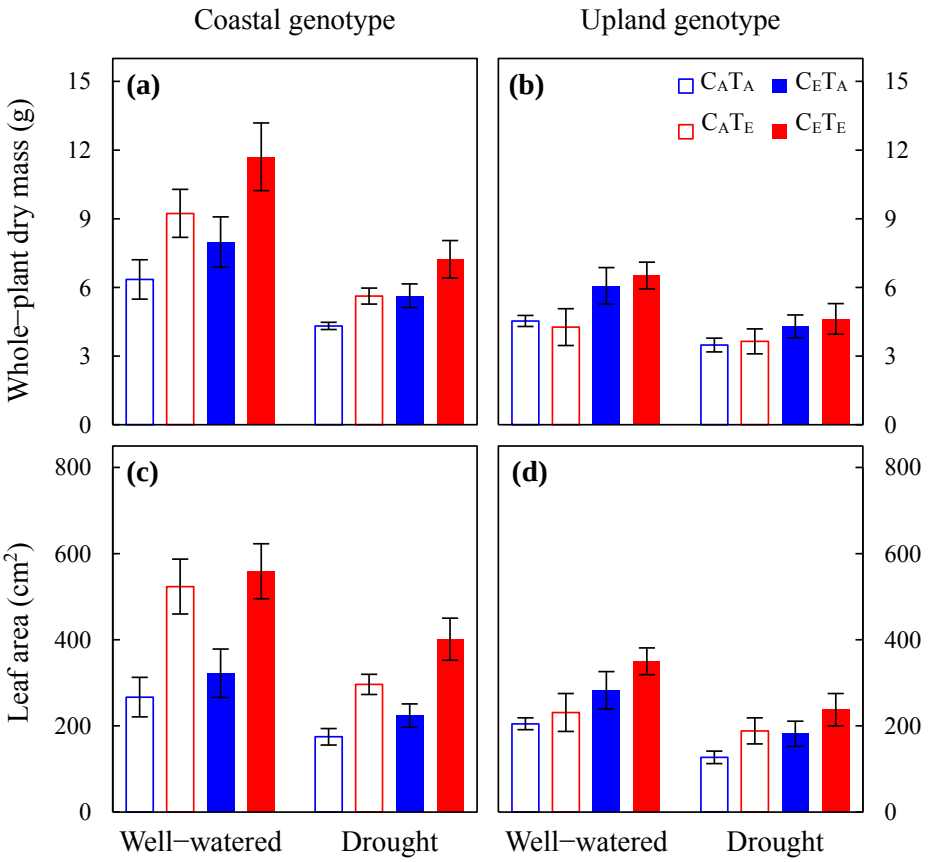

Figure 3-1 Whole-plant dry mass (a and b) and leaf area (c and d) of *Telopea speciosissima* Coastal (the left panel) and Upland (the right panel) genotypes in *well-watered* and *drought* conditions exposed to four [CO_2] and temperature treatment combinations: C_AT_A (400 µl l^{-1}, 26 °C; open blue), C_AT_E (400 µl l^{-1}, 30 °C; open red), C_ET_A (640 µl l^{-1}, 26 °C; closed blue), and C_ET_E (640 µl l^{-1}, 30 °C; closed red). Values represent means ± 1 SE (n = 10).

Table 3-2 Summary (*P* values) of four-way repeated measures ANOVAs testing for the main and interactive effects of [CO_2] (C), temperature (T) and watering (W) treatments on growth and gas exchange parameters of two *Telopea speciosissima* genotypes (G)

	Growth			**Gas exchange**	
Effect	**Length**	**Diameter**	**Volume**	A_{sat}	g_s
G	**< 0.001**	0.179	**< 0.001**	0.765	0.558
C	**0.002**	**0.002**	**< 0.001**	**< 0.001**	0.732
T	**< 0.001**	**0.045**	**< 0.001**	**< 0.001**	**< 0.001**
W	0.821	**0.030**	**0.015**	**< 0.001**	**< 0.001**
G × C	0.419	0.756	0.804	0.950	0.300
G × T	**< 0.001**	**0.021**	**< 0.001**	0.823	0.938
C × T	0.599	0.472	0.369	0.292	**< 0.001**
G × W	0.959	0.348	0.268	0.264	**0.013**
C × W	0.876	0.845	0.663	**0.003**	0.687
T × W	0.484	0.475	0.884	**0.014**	0.376
G × C × T	0.091	0.844	0.938	0.714	0.251
G × C × W	0.724	0.665	0.855	0.597	0.612
G × T × W	0.769	0.976	0.519	0.224	0.486
C × T × W	0.771	0.139	0.680	0.300	0.353
G × C × T × W	0.458	0.780	0.894	0.592	0.056

A_{sat}, light-saturated photosynthesis; g_s, stomatal conductance. Significant values ($P < 0.05$) are shown in bold. Analyses were run on data obtained during the experiment (multiple measurements), with ten replicates ($n = 10$) for growth parameters and five replicates ($n = 5$) for gas exchange traits.

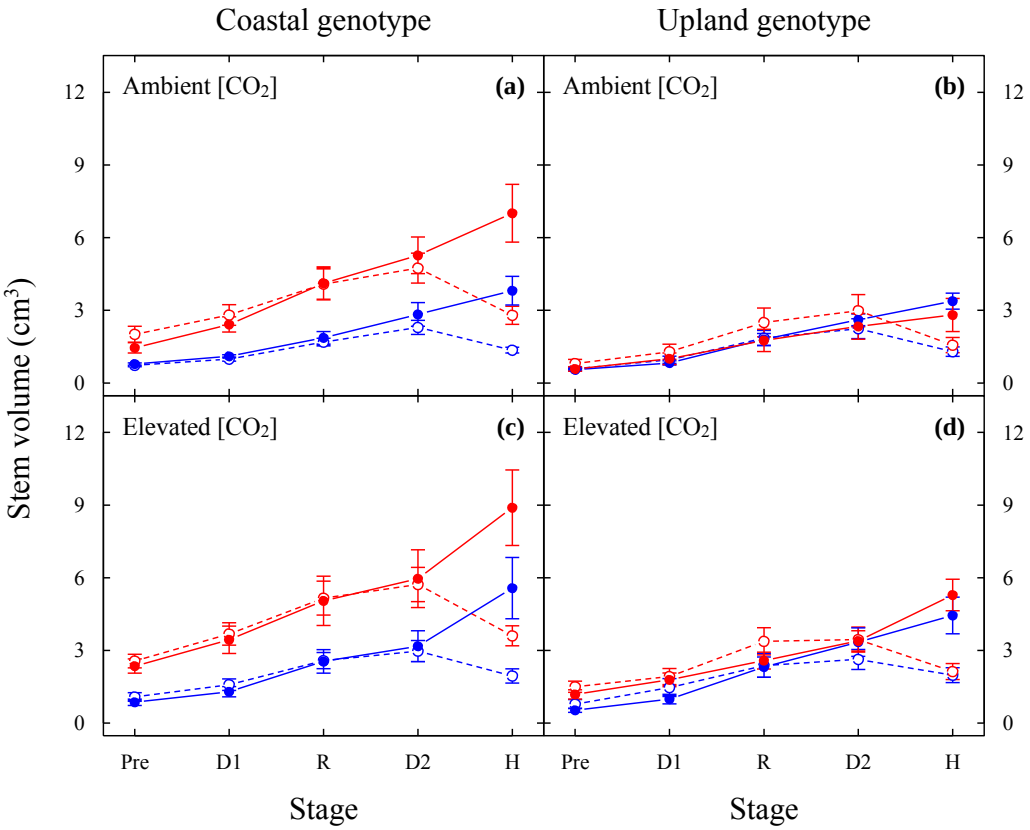

Figure 3-2 Progression of stem volume in *Telopea speciosissima* Coastal (the left panel) and Upland (the right panel) genotypes in *well-watered* (closed symbols) and *drought* (open symbols) conditions subjected to *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures and *ambient* (C_A; the top panel) and *elevated* (C_E; the bottom panel) [CO_2] during the experimental stages: *pre drought* (Stage Pre), *first drought* (Stage D1), *recovery* (Stage R), *second drought* (Stage D2), and *final harvest* (Stage H). Values represent means ± 1 SE (n = 10).

3.3.2 Non-structural carbohydrates

Regardless of watering treatment, both [CO_2] and temperature treatments had significant effects on the concentrations of whole-plant starch (St) and non-structural carbohydrates (NSC) in both genotypes, but did not significantly influence the concentrations of soluble sugars (Ss) (Fig. 3-3; Table 3-1). C_E stimulated whole-plant St by 22–32% in the two genotypes, while T_E decreased whole-plant St by 33–37%, consequently leading to a 10–14% increase in whole-plant NSC under C_E but an 8% (on average) decline in whole-plant NSC under T_E.

The *drought* treatment had contrasting effects on whole-plant St and Ss, and the effect size on each parameter varied between genotypes (significant genotype × watering interactions) (Fig. 3-3; Table 3-1). Averaged across genotypes, [CO_2] and temperature treatments, *drought* seedlings decreased whole-plant St by 56% but increased whole-plant Ss by 23%, when compared with *well-watered* seedlings. The negative effect of *drought* on whole-plant St was larger in the Coastal genotype (-61%) than in the Upland genotype (-49%). In contrast, the Coastal genotype showed a smaller increase (+12%) in whole-plant Ss under *drought* conditions, when compared with the increase in the Upland genotype (+35%). As a consequence of the opposite effects and the different effect sizes of *drought* on St and Ss, the *drought* treatment diminished the difference in whole-plant NSC between genotypes (significant genotype × watering interaction; Table 3-1). In addition, for both genotypes, the negative effect of *drought* on whole-plant St differed between temperature treatments (significant temperature × watering interaction; Fig. 3-3; Table 3-1). Averaged across genotypes and [CO_2] treatments, a larger decline in whole-plant St induced by *drought* was observed in T_A (-71%), when compared with the decrease in T_E (-22%).

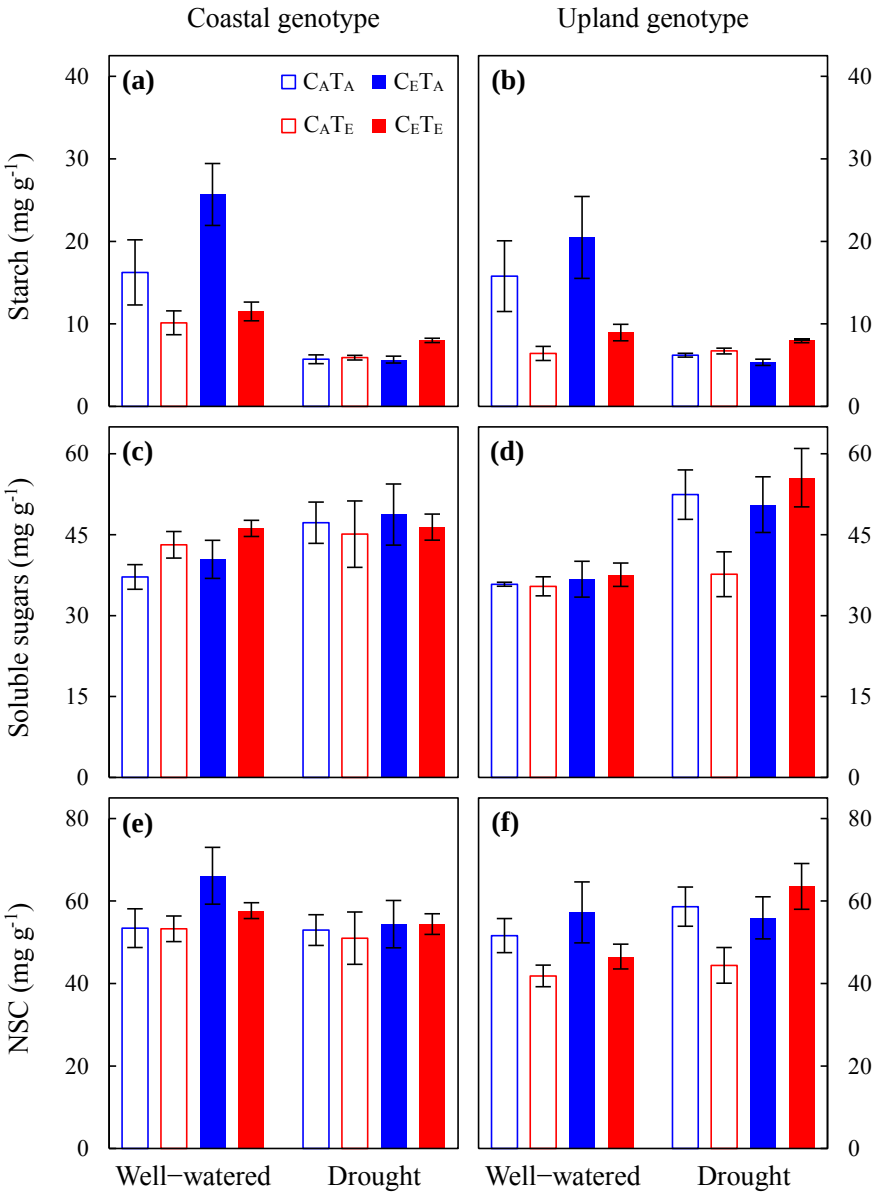

Figure 3-3 Whole-plant starch (a and b), soluble sugars (c and d), and non-structural carbohydrates (NSC) (e and f) of *Telopea speciosissima* Coastal and Upland genotypes in *well-watered* and *drought* conditions exposed to four [CO_2] and temperature treatment combinations. Values represent means ± 1 SE (n = 5). Other details are as described for Fig. 3-1.

3.3.3 Leaf gas exchange

During the experimental period, both photosynthetic rates (A_{sat}) and stomatal conductance (g_s) did not differ between the two genotypes, but were significantly affected by temperature or watering treatments (Fig. 3-4 and 3-5; Table 3-2). C_E stimulated A_{sat} of both genotypes, but the magnitudes of stimulation varied between watering treatments. Averaged across stages, genotypes and temperature treatments, a larger increase of A_{sat} under C_E was found in *well-watered* seedlings (+40%) when compared with *drought* seedlings (+28%), suggesting a significant genotype × watering interaction. In contrast, T_E overall tended to decrease A_{sat} of both genotypes at both [CO_2] treatments, but the negative effect was only significant for the *well-watered* treatment (significant temperature × watering interaction), leading to an average 17% decline in A_{sat} of *well-watered* seedlings (Fig. 3-4). The *drought* treatment substantially decreased A_{sat} and g_s for both genotypes (Fig. 3-4 and 3-5; Table 3-2). The decline of g_s under *drought* was larger in the Coastal genotype (-52%; averaged across stages, [CO_2] and temperature treatments) than in the Upland genotype (-39%), indicating a significant genotype × watering interaction. In addition, the decline in g_s induced by T_E was only significant under C_E (-31% on average across stages, genotypes and watering treatments; Fig. 3-5; Table 3-2).

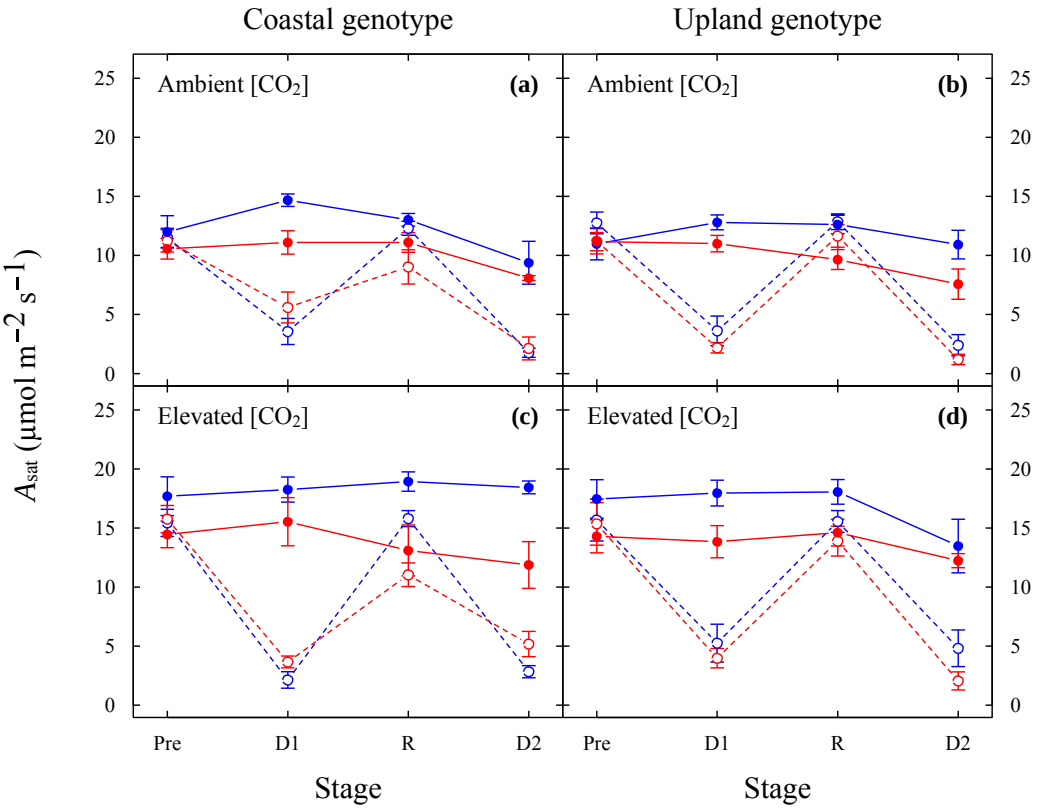

Figure 3-4 Progression of light-saturated photosynthesis (A_{sat}) in *Telopea speciosissima* Coastal (the left panel) and Upland (the right panel) genotypes in *well-watered* (closed symbols) and *drought* (open symbols) conditions subjected to *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures and *ambient* (C_A; the top panel) and *elevated* (C_E; the bottom panel) [CO_2] during the four experimental stages: *pre drought* (Stage Pre), *first drought* (Stage D1), *recovery* (Stage R). Values represent means ± 1 SE (n = 5).

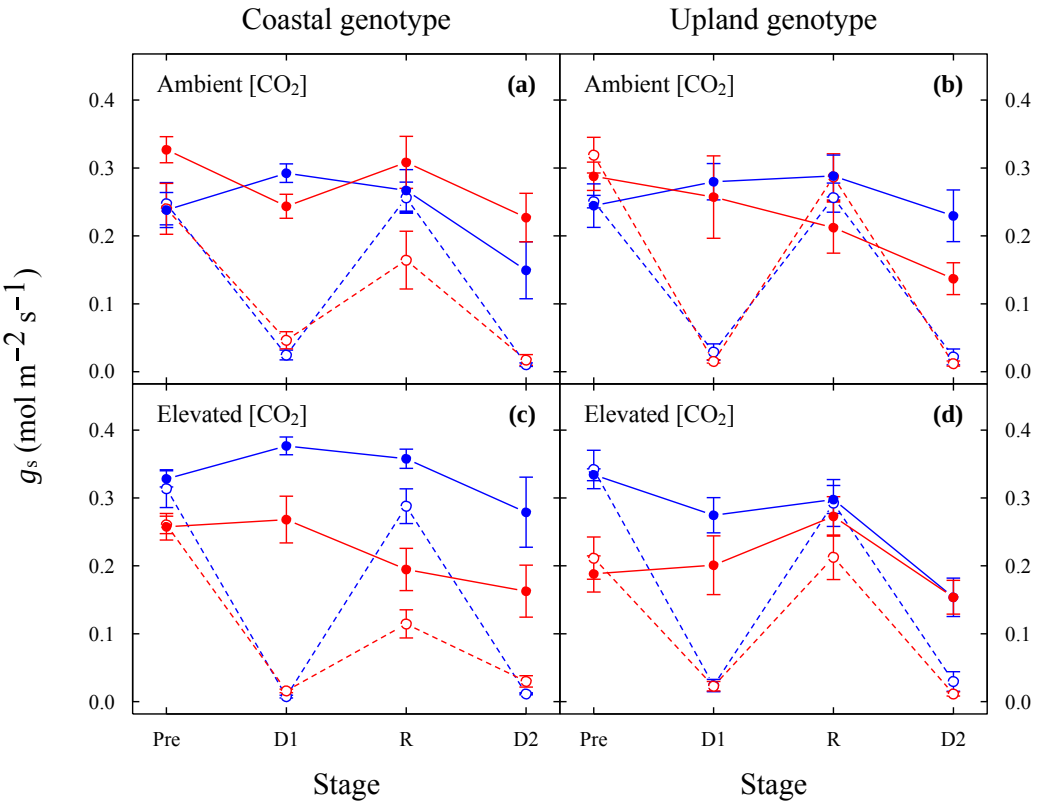

Figure 3-5 Progression of stomatal conductance (g_s) in *Telopea speciosissima* Coastal and Upland genotypes in *well-watered* and *drought* conditions subjected to four [CO_2] and temperature treatment combinations during the four experimental stages. Values represent means ± 1 SE (n = 5). Other details are as described for Fig. 3-4.

The relationships between A_{sat} and soil VWC were fitted with three-parameter sigmoid functions. Overall, no significant difference between the two genotypes was found in any of the three fitted parameters at any [CO_2] and temperature treatment combination, suggesting no intraspecific variation in the sensitivity of A_{sat} to declines in soil water content (Fig. 3-6; Table 3-3). Regardless of temperature treatment, when soil water was not limiting, the estimated asymptote for A_{sat} was higher under C_E than C_A by 36% in the Coastal genotype and by 20% in the Upland genotype, respectively. However, the estimated asymptote for A_{sat} did not differ between temperature treatments for both genotypes (Fig. 3-6a and 6b; Table 3-3). As drought stress intensified (i.e., soil water content decreased), A_{sat} of both genotypes converged among the four [CO_2] and temperature treatment combinations, thereby promoting 50% loss of A_{sat} at similar soil water content across [CO_2] and temperature treatments. In other words, the inflection point (VWC_{mid}) of each sigmoid regression did not differ among [CO_2] and temperature treatments for both genotypes (Fig. 3-6a and 6b; Table 3-3). The relationships between g_s and soil VWC were assessed by linear regressions (on log-log scales). The linear fitting parameters did not differ between genotypes or among [CO_2] and temperature treatments, suggesting that there was no intraspecific variation in the sensitivity of g_s to declines in soil water content, and that the sensitivity was not affected by either [CO_2] or temperature (Fig. 3-6c and 6d; Table 3-4).

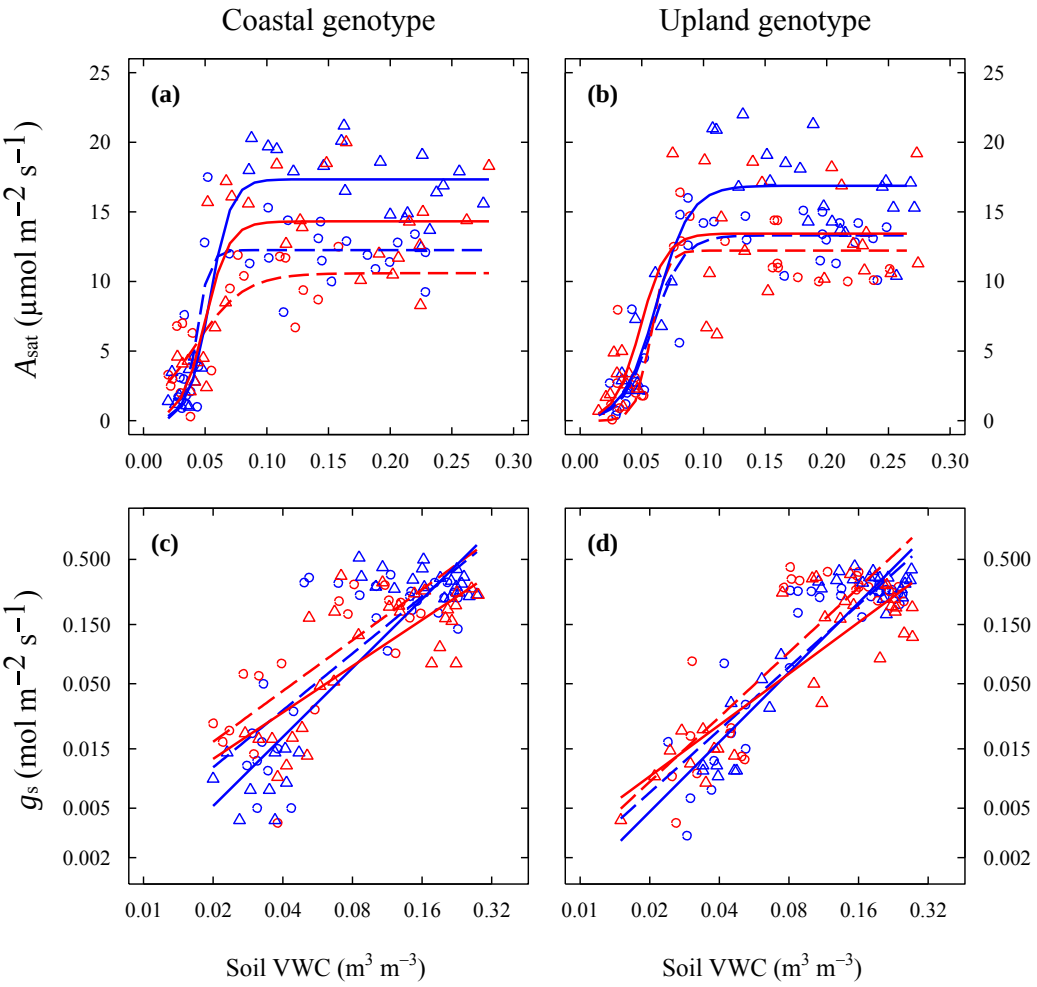

Figure 3-6 Light-saturated photosynthesis (A_{sat}; a and b) and stomatal conductance (g_s; c and d) of *Telopea speciosissima* Coastal and Upland genotypes in the *drought* treatment as a function of soil VWC exposed to *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures and *ambient* (C_A; circles) and *elevated* (C_E; triangles) [CO_2]. Data are fitted for each of the four [CO_2] and temperature treatment combinations: C_AT_A (the blue dash line), C_AT_E (the red dash line), C_ET_A (the blue solid line), and C_ET_E (the red solid line). Data for A_{sat} are fitted with a three-parameter sigmoid regression, and data for g_s are fitted with a linear regression on log-log scales. Fit parameters are shown in Table 3-3 and Table 3-4, respectively.

Table 3-3 Summary of parameters in the fitted sigmoid regressions between light-saturated photosynthesis (A_{sat}) and soil VWC of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments

Genotype	Treatment	R^2	y_{asym}		k		VWC_{mid}	
			Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Coastal	C_AT_A	0.768	12.254b	10.919, 13.589	189.748	46.822, 332.673	0.043	0.038, 0.048
	C_AT_E	0.610	10.598b	7.977, 13.220	49.938	-4.310, 104.187	0.041	0.021, 0.062
	C_ET_A	0.921	17.340a	16.283, 18.396	117.169	-2.738, 237.077	0.054	0.040, 0.067
	C_ET_E	0.645	14.315b	12.527, 16.103	104.177	16.884, 191.469	0.050	0.042, 0.058
Upland	C_AT_A	0.830	13.315b	12.152, 14.479	80.814	34.022, 127.606	0.059	0.048, 0.069
	C_AT_E	0.854	12.225b	10.730, 13.721	165.537	-122.958, 454.032	0.057	0.042, 0.073
	C_ET_A	0.817	16.877a	15.521, 18.233	74.611	26.040, 123.182	0.062	0.052, 0.072
	C_ET_E	0.683	13.444b	11.672, 15.215	96.327	-45.675, 238.330	0.049	0.029, 0.069

The four [CO_2] and temperature treatments are: C_AT_A (400 µl l^{-1}, 26 °C), C_AT_E (400 µl l^{-1}, 30 °C), C_ET_A (640 µl l^{-1}, 26 °C) and C_ET_E (640 µl l^{-1}, 30 °C). The three-parameter sigmoid regressions were fitted as: $y = y_{asym} / (1 + e^{(-(VWC - VWCmid) / k)})$, where y_{asym} is the estimated asymptote for each regression, VWC_{mid} is the inflection point of soil VWC (where $y = y_{asym} / 2$) and k is a scaling parameter. Adjusted R^2 values ($P < 0.001$ in all cases) indicate the goodness-of-fit for regressions. Different letters indicate a significant difference among [CO_2] and temperature treatments for each parameter of each genotype based on the 95% confidence interval (i.e., 95% CI).

Table 3-4 Summary of parameters in the fitted linear regressions between stomatal conductance (g_s) and soil VWC of *Telopea speciosissima* Coastal and Upland genotypes grown under the four [CO_2] and temperature treatments

Genotype	Treatment	R^2	y_0		m	
			Estimate	**95% CI**	**Estimate**	**95% CI**
Coastal	C_AT_A	0.600	0.609	0.084, 1.134	1.520	1.052, 1.988
	C_AT_E	0.548	0.536	-0.255, 1.327	1.356	0.738, 1.974
	C_ET_A	0.792	0.846	0.463, 1.228	1.842	1.485, 2.199
	C_ET_E	0.560	0.199	-0.237, 0.634	1.238	0.826, 1.650
Upland	C_AT_A	0.759	0.669	0.297, 1.041	1.675	1.318, 2.032
	C_AT_E	0.687	0.848	0.289, 1.407	1.729	1.229, 2.230
	C_ET_A	0.833	0.833	0.524, 1.141	1.862	1.547, 2.178
	C_ET_E	0.734	0.284	-0.060, 0.628	1.372	1.059, 1.684

The four [CO_2] and temperature treatments are: C_AT_A (400 µl l^{-1}, 26 °C), C_AT_E (400 µl l^{-1}, 30 °C), C_ET_A (640 µl l^{-1}, 26 °C) and C_ET_E (640 µl l^{-1}, 30 °C). Linear regressions were fitted on log-log scales: $\log_{10}(y) = y_0 + m \times \log_{10}(\text{VWC})$, where y_0 and m are the intercept and slope for each regression, respectively. Adjusted R^2 values ($P < 0.001$ in all cases) indicate the goodness-of-fit for regressions. 95% CI stands for the 95% confidence interval.

3.3.4 Resistance to drought

The capacity of *T. speciosissima* seedlings in resisting drought stress declined with plant size (i.e., the covariate; stem volume in this case), and there was a significant effect of temperature treatment on the capacity after removing the variance accounted for by the plant size (Fig. 3-7). Compared with T_A, increase in temperature negatively affected the capacity of seedling resistance to drought, leading to a quicker closure of stomata as drought progressed. At any common plant size, T_E accelerated the rates of g_s decline under *drought*, advancing the time for *drought* seedlings to become physiologically stressed by 1.5 weeks on average (Fig. 3-7). Apart from the temperature effect, the capacity of seedling resistance to drought did not vary between genotypes or [CO_2] treatments, or among the treatment combinations.

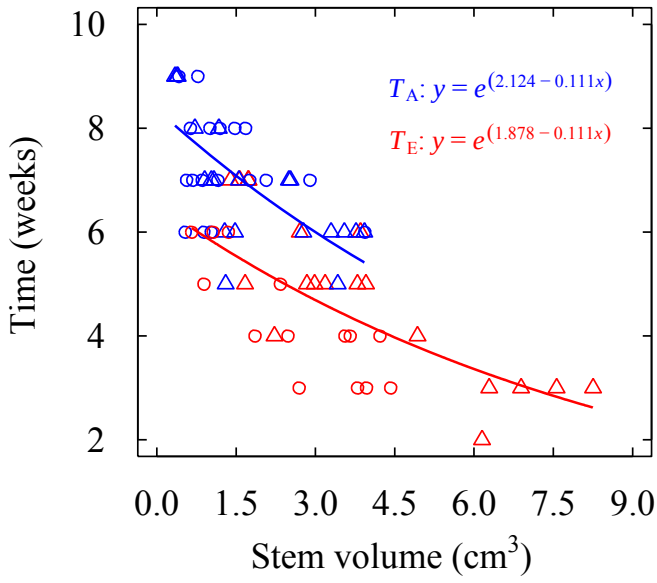

Figure 3-7 Drought resistance (time for a seedling becoming physiologically stressed) versus plant size (stem volume) of drought-treated *Telopea speciosissima* exposed to *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures and *ambient* (C_A; circles) and *elevated* (C_E; triangles) [CO_2]. Data are fitted with exponential regressions based on a generalized linear model ($P < 0.001$, $R^2 = 0.927$). Fittings for temperature treatments are shown in the plot.

3.4 Discussion

In contrast to the first hypothesis, the two *T. speciosissima* genotypes showed similar capacity in resisting drought stress, as well as similar reductions in growth and A_{sat} induced by drought across [CO_2] and temperature treatments. The second hypothesis was supported because T_E accelerated the process of stomatal closure by drying the soil more quickly, and thereby reduced the time for drought seedlings to become physiologically stressed (i.e., $g_s < 0.05$ mol m^{-2} s^{-1}). The third and the fourth hypotheses were both rejected, as C_E neither had impact on the capacity of plant drought resistance for both genotypes, nor ameliorated the negative effects of T_E on plant drought responses. Taken together, these findings indicate that genetically differentiated woody plant populations originating from different environments may not necessarily show intraspecific variation in response to drought stress under either current climates or predicted future climates. Furthermore, these results suggest that temperature would be a stronger determinant influencing the capacity of woody plants to resist drought than [CO_2].

3.4.1 No intraspecific variation in growth and photosynthetic responses to drought

Between the two *T. speciosissima* genotypes originating from contrasting environments with differentiated precipitation, differences in the declines of growth and photosynthesis induced by drought were largely absent across [CO_2] and temperature treatments. In addition, their sensitivity of photosynthetic traits (i.e., A_{sat} and g_s) to declines in soil water content and the capacity of plant drought resistance (measured by the time for drought treated seedlings to become physiologically stressed)

also did not differ between the two genotypes. These results collectively suggest that there is no intraspecific variation in the response to drought between the *T. speciosissima* genotypes in this study, contradicting observations on other woody species, in which plant populations from different precipitation regions usually showed differentiated responses under drought conditions (e.g., Ramirez-Valiente *et al.*, 2010; McLean *et al.*, 2014; Bansal *et al.*, 2015). Specifically, these studies indicated that woody plant populations originated from more mesic regions were usually more susceptible to drought (Cregg & Zhang, 2001; Silva *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Dutkowski & Potts, 2012; Robson *et al.*, 2012), while populations from more stressful environments tended to be less responsive to water stress (Gratani *et al.*, 2003; Baquedano *et al.*, 2008; Aranda *et al.*, 2010; Bansal *et al.*, 2015).

The lack of intraspecific variation in response to drought in this study could be attributed to the fact that there might be no inherent difference in the capacity to cope with drought between the two *T. speciosissima* genotypes. Although the Coastal and the Upland genotypes were sampled from regions with different precipitation, both regions can be characterized as high rainfall regions (more than 850 mm per year) with no difference in precipitation variability (see Huang *et al.*, 2015), suggesting the relative uniformity of precipitation conditions between the two regions. Therefore, these two genotypes might have been adapted to somewhat similar non-water-stressed environments and may not differ in their inherent capacity of coping with water deficit. Similar results were found in a drought manipulating study on provenances of two widely distributed *Eucalyptus* species, where provenances originating from contrasting environments (tropical *vs.* temperate) did not show intraspecific variation in most growth and physiological responses to drought (Huang *et al.*, unpublished data). In that study, provenances were also selected from regions with relatively

sufficient precipitation (all > 890 mm rainfalls per year) and similar precipitation variability, despite that there was significant difference in the mean annual precipitation (MAP) between them. In contrast, woody plant populations exhibiting intraspecific variation in the drought responses usually distribute across low (MAP < 400 mm), mid (MAP between 400 and 800 mm) and high (MAP > 800 mm) rainfall regions (Aranda *et al.*, 2010; Ramirez-Valiente *et al.*, 2010; McLean *et al.*, 2014; Bansal *et al.*, 2015), or at least two contrasting rainfall regions (Cregg & Zhang, 2001; Gratani *et al.*, 2003; Silva *et al.*, 2006; Robson *et al.*, 2012), suggesting that these populations may possess inherent difference in their capacity to cope with water stress due to local adaptation.

3.4.2 Effects of T_E and C_E on woody plants in response to drought

I observed a significant effect of temperature on the capacity of seedling resistance to drought after removing the variance accounted for by the plant size. For both *T. speciosissima* genotypes, T_E accelerated the rates of g_s decline under drought conditions and thereby reduced the time for drought seedlings to become physiologically stressed. Results from this study are consistent with the prevailing findings that an increase in air temperature usually exacerbates the negative impacts of water stress on woody plants (Adams *et al.*, 2009; Allen *et al.*, 2010; Duan *et al.*, 2013, 2014, 2015; Will *et al.*, 2013; Zhao *et al.*, 2013). However, the quicker closure of stomata under T_E did not reflect in the sensitivity of g_s as a function of soil VWC in this study. For both temperature treatments, g_s positively correlated with soil water content in a similar manner, suggesting that the nature of *T. speciosissima* stomata in response to declines in soil water content was not altered by changes in temperature.

Therefore, I hypothesize that the negative impacts of T_E on the drought resistance of *T. speciosissima* seedlings may be working as follows: under drought conditions, higher temperatures will accelerate transpiration water loss through the increase in vapour pressure deficits, which will in turn speed up the drawdown of soil water content and hence create a positive feedback loop to magnify or exacerbate the negative effects of drought (De Boeck *et al.*, 2011; Will *et al.*, 2013; Teskey *et al.*, 2015).

By contrast, an increase in [CO_2] neither impacted the capacity of plant drought resistance, nor altered the sensitivity of A_{sat} or g_s to declines in soil water content for both *T. speciosissima* genotypes in this study. In addition, C_E did not ameliorate the negative effects of T_E on drought resistance, suggesting that C_E may be a less strong determinant than T_E on regulating plant response to drought. Observations about the effects of C_E on woody plant drought response are considerably inconsistent in literature. Some studies indicate that C_E would lead to partial closure of stomata, thereby reducing transpiration water loss and mitigating the negative effects of drought on plant performance (Ambebe & Dang, 2010; Wertin *et al.*, 2010; Duan *et al.*, 2013; Lewis *et al.*, 2013); while other studies (Duan *et al.*, 2014, 2015) suggest that C_E may only have a negligible effect on woody plant response to drought, consistent with findings of this study. The absence of [CO_2] effects on *T. speciosissima* drought response may be explained by the fact that g_s in this study overall did not differ between [CO_2] treatments across all experimental stages, indicating that C_E did not significantly reduce g_s to improve plant water usage and therefore did not ameliorate the negative effects of drought. Although most woody plants show a significant decrease in g_s under C_E (Wullschleger *et al.*, 2002; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Wang *et al.*, 2012), there are some exceptions as well (Saxe *et al.*, 1998; Ellsworth,

1999; Lewis *et al.*, 2002; Ghannoum *et al.*, 2010a; Duan *et al.*, 2014, 2015). Given the inconsistence and complexity of [CO_2] effects on plant drought response (Wullschleger *et al.*, 2002; Franks *et al.*, 2013), further studies with a systematic manner are necessary for exploring mechanisms that underpin woody plant response to drought and C_E.

In conclusion, the two *T. speciosissima* genotypes neither showed difference in their capacity in resisting to drought stress, nor exhibited differentiated declines in growth and photosynthesis under drought conditions across [CO_2] and temperature treatments, suggesting that there might be no inherent difference in their capacity to cope with drought. Regardless of genotype, T_E imposed a negative effect on plant drought resistance, accelerating the process of drought seedlings becoming physiologically stressed. In contrast, C_E did not affect the capacity of plant drought resistance or alter the sensitivity of photosynthesis to declines in soil water content for both *T. speciosissima* genotypes. Furthermore, C_E did not ameliorate the negative effects of T_E on drought response. Collectively, these findings suggest that woody plant populations originating from differentiated environments may not necessarily show intraspecific variation in response to drought under current climates or future climates. These results also indicate that temperature is likely to be stronger determinant than [CO_2] affecting the capacity of woody plants in resisting to drought in the context of climate change.

Chapter 4

Intraspecific variation of two widely distributed eucalypts in response to drought and heat waves under ambient and future temperatures

4.1 Introduction

Global mean air temperature is expected to increase 0.3–4.8 °C by the end of the 21st century because of the rise in greenhouse gasses, and embedded with this climate warming, increased frequency of climate extreme events are also anticipated (Solomon *et al.*, 2009; Rahmstorf & Coumou, 2011; Collins *et al.*, 2013). Current climate change models predict alterations in the amount and variability of precipitation (e.g., drought events), as well as increases in the frequency and intensity of heat waves through this century (Meehl & Tebaldi, 2004; Della-Marta *et al.*, 2007; Kharin *et al.*, 2007; Ballester *et al.*, 2010; Yao *et al.*, 2013). Heat waves in fact have been contributing to the increase in global air temperature (Coumou & Robinson, 2013; Coumou *et al.*, 2013), and in the field they typically occur in combination with periods of precipitation deficit (Vautard *et al.*, 2007; De Boeck *et al.*, 2010; Stefanon *et al.*, 2014). Although there is no generally accepted way to delineate heat waves, they are commonly defined as periods of consecutive days during which air temperature is

excessively higher than normal and likely to have substantial impacts on the functions of organisms and ecosystems (Frich *et al.*, 2002; Tebaldi *et al.*, 2006; Smith, 2011; Perkins & Alexander, 2013; Reichstein *et al.*, 2013).

Plant response to warming can be very complicated because most biochemical and physiological processes in plants are simultaneously regulated by temperature towards or away from their temperature optimum (Berry & Bjorkman, 1980; Ghannoum *et al.*, 2010a). A constant mild increase in air temperature (typically 3–5 °C higher than the ambient) is generally expected to enhance the growth of cool-climate-origin tree species, but likely to have no effect or a negative effect on the growth of woody plants originated from warm climates (Saxe *et al.*, 2001; Way & Oren, 2010; Drake *et al.*, 2015). The short-term acute heat waves accompanied by drought, on the other hand, are posing significant negative impacts on plant performance and ecosystem function, leading to substantial reductions in ecosystem productivity and increased tree mortality (Ciais *et al.*, 2005; Reichstein *et al.*, 2007, 2013; Allen *et al.*, 2010; Zhao & Running, 2010; Bastos *et al.*, 2013; Teskey *et al.*, 2015). Because forests dominate terrestrial ecosystem production (up to *c.* 70%) and play a key role in the global carbon cycle (Schimel *et al.*, 2001; Norby *et al.*, 2005; Beer *et al.*, 2010; Pan *et al.*, 2011), understanding the capacity of woody species to cope with climate warming and co-varying climate extremes, is of particular importance. Although many studies have assessed the effects of constant mild warming on woody species (Way & Oren, 2010; Wang *et al.*, 2012), manipulative experiments investigating the impacts of simultaneously occurring climate extremes (e.g., heat waves and drought) on woody plants for more than a few hours, are scarce (Hamerlynck *et al.*, 2000; Bauweraerts *et al.*, 2013, 2014).

The capacity of plant species to cope with rapid climate change will be dependent on their phenotypic plasticity – the ability of a given genotype to express multiple phenotypes as a function of its environment (Bradshaw, 1965; Sultan, 2000; Nicotra *et al.*, 2010). Phenotypic plasticity is particularly important for long-lived woody species by acting as a buffer against rapid environmental changes and providing growth advantages in the short- and long-term (Valladares *et al.*, 2007; Chevin *et al.*, 2010; Nicotra *et al.*, 2010), because there might be a lag in the evolutionary response by natural selection to mitigate the effects of rapid climate change. When genotypes of a given species show differentiated responses to the same environmental change, there is intraspecific variation in phenotypic plasticity, known as significant genotype (G) by environment (E) interactions (Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). Investigations of intraspecific variation in phenotypic plasticity of woody plants in response to climate change are essential for making robust predictions of woody species responses to global change (Moran *et al.*, 2016), as well as identifying genotypes that exhibit the capacity to increase or maintain productivity under more extreme climatic conditions in the future (Aspinwall *et al.*, 2015).

Plant populations usually demonstrate differentiation in phenotypic plasticity and a long-standing hypothesis suggests that greater levels of environmental variability will select for plants with greater phenotypic plasticity (Galloway, 1995; Weinig, 2000; Donohue *et al.*, 2001; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen & Fischer, 2005). Widespread woody species may also exhibit variation in functional traits among populations across environmental gradients, because plant populations are generally highly adapted to local conditions (Savolainen *et al.*, 2007; Hereford, 2009; Wang *et al.*, 2010; McLean *et al.*, 2014). Thus, for a given woody species responding to the same climate regime, populations originating from

contrasting environments are likely to show differentiated plasticity in growth and physiological traits, as evidence of G × E interactions. For instance, warming can have variable effects on woody plant growth, depending on the taxon climate of origin (Saxe *et al.*, 2001; Way & Oren, 2010). However, only a limited number of studies have tested the responses of woody plant genotypes/populations from thermally differentiated habitats to warming, and demonstrated intraspecific variation in the plasticity of growth and/or photosynthetic traits (Weston & Bauerle, 2007; Weston *et al.*, 2007; Drake *et al.*, 2015; Huang *et al.*, 2015).

Intraspecific variation in plant response to drought and variable soil moisture has received much attention and been well documented in many species (Aspinwall *et al.*, 2015). For example, significant G × E interactions under variable soil water conditions have been reported in a wide range of woody plants, including *Eucalyptus*, *Pinus*, *Populus* and *Quercus* (Cregg & Zhang, 2001; Silva *et al.*, 2004; Monclus *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Bedon *et al.*, 2012; McLean *et al.*, 2014). The intraspecific differentiation of woody plant populations in response to water deficit has also been linked to their source environmental conditions, in which populations from more mesic regions are generally more susceptible to drought (Cregg & Zhang, 2001; Silva *et al.*, 2006; Ramirez-Valiente *et al.*, 2010; Dutkowski & Potts, 2012; Robson *et al.*, 2012), and populations from more stressful environments tend to be less responsive to water stress (Gratani *et al.*, 2003; Baquedano *et al.*, 2008; Aranda *et al.*, 2010; Bansal *et al.*, 2015). However, the mechanisms that underlie patterns of intraspecific variation in phenotypic plasticity of woody species to respond to both warming and drought remain largely unknown.

The short-term heat waves could trigger changes in processes from the molecular level to the whole plant, and the effects may vary among species and

genotypes (Wahid *et al.*, 2007; Aspinwall *et al.*, 2015; Teskey *et al.*, 2015). The most commonly observed effects of heat waves on woody plants include reduction in biomass accumulation and leaf area development, inhibition of photosynthesis efficiency, and stimulation of mitochondrial respiration (Hamerlynck *et al.*, 2000; Ameye *et al.*, 2012; Bauweraerts *et al.*, 2013, 2014; Teskey *et al.*, 2015). The simultaneous occurrence of heat waves and drought events is common, and together they can impose significantly greater impacts on plants and ecosystems than each applied separately (Mittler, 2006; De Boeck *et al.*, 2011; Dreesen *et al.*, 2012; Bauweraerts *et al.*, 2013; Zinta *et al.*, 2014). In the combination of climate extremes, heat waves as a single factor may only have small or transient effects on plants that have sufficient water to mitigate the heat stress through transpirational cooling, but negative effects induced by single factor drought are likely to be exacerbated by heat stress, suggesting that drought is the dominant stressor for plants during heat waves (Reichstein *et al.*, 2007; De Boeck *et al.*, 2010, 2011; Bauweraerts *et al.*, 2014; Hoover *et al.*, 2014; Teskey *et al.*, 2015). Nevertheless, intraspecific variation in the response of processes to co-occurring climate extremes has been rarely investigated in woody species, and the relationship between taxon origin and capacity to tolerate climate extremes, is still unclear.

Eucalypts are foundation tree species in Australian ecosystems, many of which are also economically important as an essential source of timber and pulpwood. Some *Eucalyptus* species are widely distributed from temperate to tropical regions, which vary in key climatic variables such as air temperature and rainfall patterns. Thus, widespread *Eucalyptus* species provide a useful model system to predict intraspecific variation in response to future climatic conditions among populations across environmental gradients, and to investigate the association between phenotypic

plasticity and source environment variability of populations. In a related study using provenances of widely distributed *Eucalyptus grandis* and *Eucalyptus tereticornis* originating along a latitudinal transect (from the temperate to the tropical regions) in eastern Australia, Drake *et al.* (2015) observed that the effect of +3.5 °C warming on plant growth and physiology under well-watered conditions strongly depended on taxon climate of origin for both species, suggesting predictable intraspecific variation in the capacity of the two *Eucalyptus* species in response to climate warming. Cool-origin provenances responded positively to warming with increases in growth and photosynthetic capacity, while warm-origin provenances, in contrast, showed reductions in growth and photosynthetic capacity under warming conditions.

In this study, I extended previous research by examining intraspecific variation of these two *Eucalyptus* species in response to warming and climate extremes (i.e., drought and/or heat waves), on a subset of provenances that originate from the edge of the species distribution ranges. For both species, the temperate provenances are obtained from drier and cooler (but more variable in temperatures) environments, when compared with the tropical provenances (Table 4-1). The following hypotheses were tested: (i) warming will be beneficial to cool-origin provenances, but have negative or non-significant effects on provenances of warm-origin; (ii) provenances from drier regions will be less responsive to drought stress than provenances from more mesic regions; (iii) warm-origin provenances will be more susceptible to heat waves because the heat stress may exceed their thermal optima, when compared with cool-origin provenances; and (iv) drought will be a more severe stressor than heat waves for plants under extreme climatic conditions.

Table 4-1 The 40-year (1973–2012) summary of precipitation and air temperature in the temperate and tropical locations, from which the *temperate* and the *tropical* provenances of each species (*E. grandis* and *E. tereticornis*, respectively) were selected for this study

	Temperate			**Tropical**		
	Mean	**Range**	**CV**	**Mean**	**Range**	**CV**
E. grandis						
Precipitation (mm)						
Annual	1238	599–1657	0.769	1615	677–3095	1.274
Nov–Feb	450	161–759	0.598	944	280–2305	0.817
T_{mean} *(°C)*						
Annual	18.1	17.4–18.7	0.216	23.8	23.0–24.7	0.106
Nov–Feb	22.1	20.9–22.9	0.072	26.4	25.6–27.0	0.024
T_{max} *(°C)*						
Annual	23.1	22.4–24.3	0.164	28.1	27.2–29.0	0.093
Nov–Feb	26.8	25.6–28.5	0.060	30.8	29.6–31.8	0.030
T_{min} *(°C)*						
Annual	13.2	12.2–14.3	0.318	19.5	18.4–20.9	0.134
Nov–Feb	17.3	16.0–18.5	0.099	21.9	21.4–22.7	0.035

Table 4-1 (continued)

	Temperate			Tropical		
	Mean	**Range**	**CV**	**Mean**	**Range**	**CV**
E. tereticornis						
Precipitation (mm)						
Annual	891	421–1549	0.962	1880	708–2996	1.247
Nov–Feb	329	59–877	0.684	1011	303–1793	0.797
T_{mean} *(°C)*						
Annual	13.9	13.3–14.8	0.287	24.7	24.0–25.5	0.093
Nov–Feb	17.9	16.5–19.3	0.100	26.9	26.2–27.7	0.020
T_{max} *(°C)*						
Annual	19.5	18.4–20.7	0.204	28.4	27.5–29.2	0.084
Nov–Feb	23.4	21.4–25.7	0.082	30.9	29.8–32.1	0.026
T_{min} *(°C)*						
Annual	8.3	7.1–9.4	0.491	20.9	19.8–22.2	0.111
Nov–Feb	12.4	11.4–13.4	0.146	23.0	22.4–23.5	0.022

CV, coefficient of variation, defined as the ratio of the standard deviation to the mean; T_{mean}, mean air temperature; T_{max}, maximum air temperature; T_{min}, minimum air temperature. CVs were first calculated based on the monthly means within each year (n = 12) or each Nov–Feb (n = 4), and then averaged across 40 years, for both precipitation and temperature.

4.2 Materials and methods

4.2.1 Plant material

Two widely distributed *Eucalyptus* species, *E. grandis* and *E. tereticornis*, were selected in this study because they are distributed along a common latitudinal gradient in eastern Australia, both ranging from the temperate region to the tropical region. However, unlike *E. tereticornis* that is distributed almost continuously across eastern Australia, *E. grandis* has a relatively disjunct distribution consisting of a core southern range and a smaller northern range, connected by a few sporadic occurrences between the two ranges (Drake *et al.*, 2015).

For each species, two natural populations from its distribution edges near the coast (i.e., one population from each edge of the latitudinal gradient) were included. Specifically, the two populations of *E. grandis* were selected from Bulahdelah State Forest, NSW (32.33 °S, 152.25 °E, 20 m altitude, the temperate region) and Mount Molloy, QLD (16.58 °S, 145.40 °E, 390 m altitude, the tropical region), respectively; while for *E. tereticornis*, one population originated from Yurammie State Forest, NSW (36.49 °S, 149.45 °E, 170 m altitude, the temperate region) and the other population was from West Normanby River, QLD (15.50 °S, 145.14 °E, 140 m altitude, the tropical region). These populations are of known geographic origin, so we referred to each of them as a 'provenance'. The four provenances were also a subset of provenances studied in Drake *et al.* (2015). To simplify, two provenances of each species selected in this study were defined as the *temperate* provenance and the *tropical* provenance, respectively.

Seeds of the four provenances (two for each species) were obtained from the Australian Tree Seed Centre (CSIRO, Canberra, ACT, Australia) and planted in small

pots for germination in a nursery on the University of Western Sydney campus (Richmond, NSW, Australia). Successfully germinated seedlings were allowed to grow for two months in the nursery before they were transplanted into the experimental growth conditions described below.

4.2.2 Growth conditions

The setup of growth temperatures for provenances in this study was based on Drake *et al.* (2015). The current study was a subsequent experiment of that research, with fewer provenances but more experimental factors involved. Overall, seedlings of each provenance were grown under two temperature regimes, one mimicking the ambient summer temperature of origin for each provenance (the *ambient* temperature treatment, hereafter 'T_A'), and the other one simulating a constant 3.5 °C increase in temperature above the ambient (the *elevated* temperature treatment, hereafter 'T_E').

Provenances were assigned to the *ambient* temperature conditions based on climate records from the SILO Climate Data (Jeffrey *et al.*, 2001). Specifically, the mean air temperature of each provenance during summer months of November to February over the past decades was calculated and applied as reference for the *ambient* growth temperature of that provenance. With this approach, a temperature of 18 °C was assigned to the two *temperate* provenances in this study as T_A, while T_A for the two *tropical* provenances was set at 28.5 °C. According to the 40-year (1973–2012) climate records (Table 4-1), these temperature settings were appropriate at some degree for the majority of the four provenances, except the *temperate* provenance of *E. grandis*, of which the target *ambient* temperature (18 °C) was about 4 °C lower than the mean summer temperature at the seed origin (22.1 °C). Nonetheless, the *temperate*

provenance of *E. grandis* was included in this study because one of the objectives was to compare species differences in response to stress under common temperatures. The decision was made based on the following justifications: (1) 18 °C matches perfectly with the mean annual temperature at the seed origin (18.1 °C); and (2) 18 °C is still within the historical daily temperature range during summer at the seed origin (from 16 °C to 28.5 °C). Both justifications indicate that the target *ambient* temperature for the *temperate* provenance of *E. grandis* is within the provenance's field thermal range.

The manipulation of growth temperatures was accomplished using four adjacent, naturally lit (direct sunlight attenuated by 10–15%), and temperature-controlled glasshouse bays (3.0 m × 5.0 m × 3.5 m, width × length × height each) located at the University of Western Sydney (Richmond, NSW, Australia), as described in Ghannoum *et al.* (2010a). In each bay, the air temperature was controlled at three set-points over the 24-hour period to simulate a natural diel temperature cycle. The average temperature range for the diel temperature cycle was about 8–9 °C, with a mid-day maximum temperature (between 10:00–16:00 hours), a night-time minimum temperature (between 20:00–06:00 hours), and moderate temperatures at other times (between 06:00–10:00 and 16:00–20:00 hours). During the course of the experiment, the mean observed air temperature within each bay was highly correlated with the target temperature (observed air temperature = 1.69 + 0.95 × target temperature, $r^2 = 0.99$, $P < 0.0001$), as described in the related study (Drake *et al.*, 2015).

After two-month growth in the nursery, seedlings were transplanted into polyvinyl chloride (PVC) pots (15 cm diameter × 40 cm length) containing about 10 kg of dry loamy-sand soil (86.5% sand and 9.5% clay, moderate fertility) in late spring 2012 (late October 2012). Soil was collected from a local dry sclerophyllous forest

(Menangle, NSW, Australia) and characteristics of the soil can be found in Drake *et al.* (2015). A PVC cap with four drainage holes covered with 2 mm mesh was placed at the bottom of each pot. For each species, sixty of the most uniform seedlings of each provenance were selected and paired into twins based on their stem length and basal diameter. For each pair, seedlings were randomly assigned into one of two groups. The two groups were then transplanted and placed into *ambient* and *elevated* temperature conditions, respectively. This approach ensured that there was no bias in the initial size of seedlings of the same provenance grown under different temperatures. Before the onset of the controlled watering (see the watering regime described below), all seedlings were irrigated to field capacity on a daily basis and randomly rotated within glasshouse bays fortnightly. In addition, seedlings were also fertilized every three weeks with a commercial liquid fertilizer (500 ml Aquasol at 1.6 g l^{-1}; 23% N, 4% P, 18% K, 0.15% Mn, 0.06% Fe, 0.06% Cu, 0.05% Zn, 0.011% B, 0.0013% Mo; Yates Australia, Padstow, NSW, Australia).

4.2.3 Watering regime and heat wave treatment

Following a three-month (from late-October 2012 to mid-January 2013) growth period under well-watered conditions, ten seedlings (with stem length and basal diameter most resembling the mean values of each provenance of each species in each bay) were selected. These ten seedlings were paired, based on similar growth (e.g., similar stem length and basal diameter), and then assigned randomly into one of two groups. One group of seedlings continued to receive full watering every day (i.e., the *well-watered* treatment, hereafter '*well-watered*'), while the other group of seedlings were exposed to drought/recovery cycles (i.e., the *drought* treatment,

hereafter '*drought*'). Therefore, this experiment consisted of 80 individual potted seedlings (2 species × 2 provenances × 2 temperature treatments × 2 watering treatments × 5 replicate seedlings). Every selected pot was weighed every day in the morning (between 09:00–10:00 hours) to determine water loss and then irrigated with specific amounts of water. The well-watered seedlings were supplied with the amount of water that was lost each day to maintain pots at field capacity, while the drought seedlings were watered in a controlled way (see the following paragraphs for detailed description). Two drought cycles (a drought cycle consisted of a drought event followed by a recovery) were applied to seedlings in the *drought* treatment to more realistically simulate natural field drought events, which usually consist of multiple dry-wet cycles.

To compensate for plant size differences and more slowly stress the seedlings, I applied standardized drought stress across species, provenances and temperature treatments, by controlling the water loss in each drought pot to the same maximum amount every day, until drought stress emerged. For instance, at the early stage of the drought event, if the maximum water loss was set as 200 g per day, I added water to pots that lost >200 g to maintain water loss at 200 g daily; while for pots that lost <200 g, no water was added to them. When drought seedlings started exhibiting wilting symptoms, watering was applied to avoid mortality but maintain stressed conditions; this was accomplished by adding small amounts of extra water to pots, in addition to replacing the actual water loss. The amount of extra water added during the drought events of this study was arbitrarily set as 0, 50 and 100 g per day, respectively, depending on the degree of seedling wilt. This standardized drought strategy was successfully established on previous studies of other *Eucalyptus* species grown in the glasshouse (Ayub *et al.*, 2011; Lewis *et al.*, 2013; Duan *et al.*, 2014).

Based on soil water status in drought pots and whether a heat stress was imposed, the experiment was implemented in the following stages: *pre drought* (Stage Pre), *first drought* (Stage D1), *first recovery* (Stage R1), *second drought* (Stage D2), *heat wave* (Stage HW; see the description below), and *final recovery* (Stage R2) (Fig. 4-1). The relative pot weight of each drought seedling, rather than the soil water content, was used as an indicator of drought/recovery progression in this study, due to the following reasons: (1) soil volumetric water content (VWC) assessment by handheld TDR probes on a daily basis would be substantially harmful to the roots of seedlings; (2) it was feasible to assess the soil VWC on all seedlings via TDR probes with cables. Therefore, I recorded the accumulative net water loss daily for each drought seedling, and calculated the relative pot weight (i.e., dividing the remaining pot weight at a specific time point by the corresponding *pre drought* pot weight) to demonstrate how the experiment was implemented (Fig. 4-1).

The *first drought* was imposed on 87 days after planting (hereafter 'DAP') into PVC pots and lasted for seven days (i.e., from 87 DAP to 93 DAP; see Fig. 4-1). During this stage, the daily maximum water loss was set on a gradually increasing trend at the first four days (i.e., 100, 100, 200 and 300 g per day for the four days, respectively), but then was reduced to 100 g per day for seedlings that were still not visibly wilting at the following three days to avoid severe drought stress. For seedlings started showing wilting symptoms, calculated watering amounts (i.e., the actual water loss plus a small amount of extra water) were applied to not only maintain the drought stress, but also avoid mortality. By the end of this stage (i.e., on 93 DAP), the accumulated water loss for each drought pot averaged *ca.* 700 g (Fig. 4-1). After all measurements (see the detailed description of measurements below) were taken, the *first recovery* stage was applied, during which all drought seedlings were rewatered to

field capacity and kept well-watered for seven days (i.e., from 94 DAP to 100 DAP) to allow for a full recovery. Fertilizer was also applied once in the middle of the *first recovery*. Following the *first recovery*, the *second drought* was conducted on 101 DAP, with a similar watering strategy but a longer time period and more accumulated water loss at maximum when compared to the *first drought* (Fig. 4-1). Consequently, on the day before the *heat wave* treatment (i.e., the 10th day of the *second drought*, 110 DAP), the mean accumulated water loss was *ca.* 900 g per drought pot. The detailed description of the *heat wave* treatment is shown in the following paragraph.

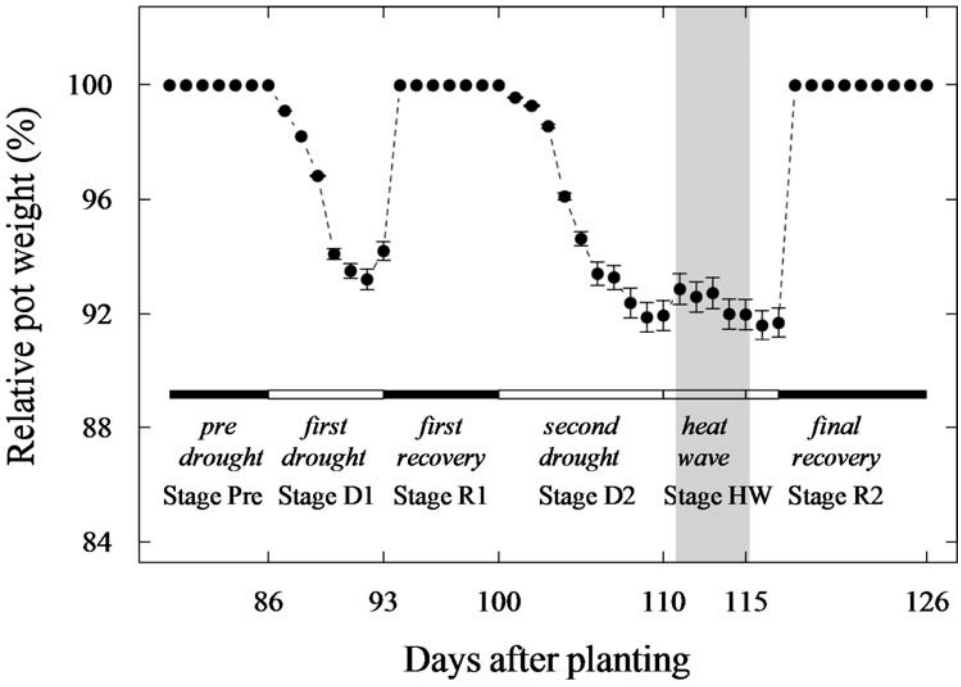

Figure 4-1 Progression of the relative pot weight of all *drought* seedlings across species, provenances and temperature treatments during the experimental stages: *pre drought* (Stage Pre), *first drought* (Stage D1), *first recovery* (Stage R1), *second drought* (Stage D2), *heat wave* (Stage HW) and *final recovery* (Stage R2). The scale below symbols indicates watering regimes in *drought* seedlings, i.e., the controlled drought (open) and the full watering (closed). Relative pot weights are calculated as dividing the pot weight at a specific time point by the corresponding *pre drought* pot weight. Labelled dates on the x-axis denote when all plants were measured for plant size and leaf gas exchange characteristics. Values represent means ± 1 SE (n = 40).

Ten days after the *second drought* was applied, a short-term heat stress was imposed on all seedlings regardless of watering treatment and defined as the *heat wave* treatment (Fig. 4-1). Specifically, on the 11th day of the *second drought* (i.e., 111 DAP), growth temperatures (including day- and night-time temperatures) for all provenances under both *well-watered* and *drought* treatments were elevated by 8 °C above the previous temperature settings for five days (i.e., from 111 DAP to 115 DAP), following the definition that a heat wave is a period of at least five consecutive days with temperature exceeding normal by at least 5 °C (Frich *et al.*, 2002; Tebaldi *et al.*, 2006). Although the *heat wave* in this study was actually nested in the second drought event, for more robust data interpretation and statistical analysis, they were treated as independent stages according to the definition of experimental stages (Fig. 4-1). After the *heat wave* treatment was ceased, drought seedlings were first maintained drought-stressed for two days (i.e., from 116 DAP to 117 DAP) and then rewatered to field capacity in the next nine days (i.e., the *final recovery* stage, from 118 DAP to 126 DAP) to allow a full recovery. After that, all seedlings were destructively harvested.

4.2.4 Growth measurements

Two metrics of seedling growth were measured in this study: (1) stem length and basal diameter, and (2) final whole-plant dry mass and leaf area. The stem length (cm) and basal diameter (cm) of all seedlings were monitored throughout the experiment. Measurements were made on the final day of each defined stage, which was 86, 93, 100, 110, 115 and 126 DAP, respectively (Fig. 4-1). The main stem volume of each seedling was calculated from the stem length and basal diameter, and then used to estimate the absolute growth rate (AGR) of each stage during the experiment. I

simply assumed that the seedling main stem is cylindrical and obtained the stem volume as the stem basal area multiplied by the stem length (i.e., volume = $\pi/4 \times$ diameter$^2 \times$ length), following the approach in Kubiske *et al.* (2006). The AGR of stem volume ($cm^3\ day^{-1}$) in each stage was calculated as the total stem volume increment during the stage divided by the number of days of that stage. This non-destructive technique of estimating growth rates during the experiment was reliable for this study, because there were strong relationships between plant biomass and estimated stem volume for both *E. grandis* (adjusted $r^2 = 0.53$, $P < 0.0001$) and *E. tereticornis* (adjusted $r^2 = 0.79$, $P < 0.0001$).

At the end of the experiment, all 80 seedlings were destructively harvested and separated into leaf, stem, and root components. The whole-plant leaf and stem fresh mass (g) were recorded and the entire root system was washed free of soil. All harvested components were oven-dried later at 70 °C for 72 h and then weighed for dry mass (g). The whole-plant leaf area (cm^2) was calculated based on the relationships between leaf area and leaf fresh mass of representative subsamples. Specifically, ten leaves of each harvested seedlings were randomly selected as a subsample, on which the fresh mass and leaf area were determined (Li-3100C Area Meter, Li-Cor Inc., Lincoln, NE, USA) to calculate fitted parameters of the simple linear regression. Both species in this study, *E. grandis* (adjusted $r^2 = 0.97$, $P < 0.0001$) and *E. tereticornis* (adjusted $r^2 = 0.85$, $P < 0.0001$), showed very strong relationships between leaf fresh mass and leaf area. Other leaf area variables such as leaf mass per area (LMA, total leaf dry mass / total leaf area, $g\ m^{-2}$) and leaf area ratio (LAR, total leaf area / total plant dry mass, $m^{-2}\ kg^{-1}$) were also calculated.

4.2.5 Leaf gas exchange measurements

To quantify the physiological performance under different environmental treatments during the experiment, gas exchange measurements at the leaf level were taken on each defined stage, at the same day when stem length and basal diameter were determined (Fig. 4-1). Light-saturated photosynthesis (A_{sat}, μmol m^{-2} s^{-1}), light- and CO_2-saturated photosynthesis (A_{max}, μmol m^{-2} s^{-1}), and mitochondrial night respiration (R_n, μmol m^{-2} s^{-1}) were measured on all 80 seedlings using eight identical portable open path gas exchange systems (Li-6400 with Li-6400-02B red-blue light source; Li-Cor Inc., Lincoln, NE, USA).

Measurements of A_{sat} and A_{max} were conducted from mid-morning to early afternoon (10:00–14:00 hours) on the youngest fully expanded leaf of each individual seedling. A_{sat} was determined at saturating light (1800 μmol m^{-2} s^{-1}), ambient CO_2 concentration (400 μl l^{-1}), a flow rate of 500 μmol m^{-2} s^{-1}, and the mid-day temperature of the glasshouse bay. Because the average growth temperature difference between the *temperate* and *tropical* provenances in this study was 10.5 °C, along with the 8 °C temperature difference between stages with and without *heat wave* treatment, it was not feasible for me to maintain a common constant leaf vapour-pressure-deficit (VPD) between provenances during the course of the experiment. Instead, I defined various leaf VPD ranges for A_{sat} measurements in the study, which were 0.8–2.2 kPa (0.8–3.6 kPa during the *heatwave*) for the *temperate* provenance and 1.2–3.2 kPa (1.4–4.0 kPa during the *heatwave*) for the *tropical* provenance, respectively. Each leaf was allowed 5–10 min to equilibrate before measurements were taken. After recording A_{sat}, the concentration of CO_2 in the cuvette block was adjusted to 1800 μl l^{-1} for the measurement of A_{max}, without changing other parameters. Leaf R_n was measured at night-time (at least 2h after sunset; 22:00–02:00 hours), on the same set of leaves that

were used for the day-time gas exchange measurements. The cuvette block was set up with zero light, ambient CO_2 concentration (400 µl l^{-1}), a flow rate of 300 µmol m^{-2} s^{-1}, and the night-time temperature of the glasshouse bay for measuring the respiration.

4.2.6 Statistical analysis

For each species, measured and calculated physiological and growth parameters (A_{sat}, R_n, A_{max}, and the AGR of stem volume) during the course of the experiment were analysed using a mixed model analysis of variance (ANOVA), with provenance (*temperate* vs. *tropical*), temperature treatment (T_A vs. T_E), and watering treatment (*well-watered* vs. *drought*) as categorical fixed effects. Because there was no control treatment for the *heat wave* in this study, the analysis was split into two components. First, for measuring time prior to the *heat wave* treatment, stage (four levels for physiological parameters: *pre drought*, *first drought*, *first recovery*, and *second drought;* but only three levels for AGR: *first drought*, *first recovery*, and *second drought*) was included as a fourth fixed effect associated with watering regime. Second, for the *heat wave* treatment, measurements from the *second drought* were used as a baseline to compare with measurements during the *heat wave*. Seedling (n = 5) was included as a random effect in all analyses. All mixed model ANOVAs were performed using the 'lme4' and 'nlme' packages (Bates *et al.*, 2014; Pinheiro *et al.*, 2016) in R (version 3.2.0; R Foundation for Statistical Computing, Vienna, Austria) and r^2 values of the fitting models were calculated (Nakagawa & Schielzeth, 2013).

Whole-plant dry mass and leaf area variables obtained from harvest were analysed via three-way ANOVA for each species to account for provenance, temperature and watering treatments, followed by Tukey's HSD tests determining

differences among temperature and watering treatment combinations of each provenance in each parameter. In addition, independent two-sample *t*-tests were applied to the physiological and growth parameters in the *pre drought* to confirm that there were no significant differences between seedlings assigned to the *well-watered* and *drought* treatments. Logarithmic or square root transformations were applied when necessary to satisfy the assumptions of residual homoscedasticity and normality. Results were considered significant in all cases if $P < 0.05$.

4.3 Results

At the onset of the *first drought*, there were no significant differences in either growth traits (i.e., stem length, basal diameter, and stem volume) or physiological parameters (i.e., A_{sat}, R_n, and A_{max}) between seedlings assigned to the *well-watered* and *drought* treatments within each provenance and temperature treatment combination of each species ($P \geq 0.1$ in all cases). During the experiment, the two species showed similar responses in most growth and physiological traits to the same environmental changes. For both species at the *final recovery*, physiological parameters in all temperature and watering treatment combinations of both provenances were more or less identical to those at the *pre drought*, indicating that all *Eucalyptus* seedlings in this study fully recovered from stress at the end of the experiment.

4.3.1 Plant dry mass and leaf area variables

For both species, experimental warming (+3.5 °C) had contrasting effects on whole-plant dry mass between provenances (significant provenance × temperature interaction; Fig. 4-2; Table 4-2). In the *well-watered E. grandis*, warming increased dry mass in the *temperate* by 29% but decreased dry mass in the *tropical* by 33%. However, the significant interactive effects between provenance and temperature were diminished in the *drought*, with non-significant difference in dry mass between temperature treatments of both *E. grandis* provenances (significant provenance × temperature × watering interaction; Fig. 4-2; Table 4-2). In *E. tereticornis* across watering treatments, a 32% increase in whole plant dry mass occurred with warming in the *temperate* provenance, but there was no significant difference between temperature treatments in the *tropical*. Leaf area showed a similar pattern to whole

plant dry mass in the differential response to warming between provenances for both species (significant provenance × temperature interaction; Fig. 4-2; Table 4-2). However, warming had no effect on either leaf area per mass (LMA) or leaf area ratio (LAR) in any provenance of the two species when averaged across watering treatments (Fig. 4-3; Table 4-2).

The *drought* treatment substantially inhibited whole-plant dry mass accumulation and leaf area production for both species, and the negative effects did not vary between temperature treatments (Fig. 4-2; Table 4-2). Averaged across provenances and temperature treatments, a 29–37% reduction in whole-plant dry mass and a 38–42% decline in leaf area induced by *drought* were detected in the two species. In addition, the effects of *drought* on dry mass and leaf area also did not vary between provenances of both species, except that there was a significant provenance × watering interaction on the leaf area of *E. grandis*. Across temperature treatments, the *tropical* provenance of *E. grandis* in *drought* showed a larger decline (–58% on average when compared with *well-watered* treatments) in leaf area than the *temperate* provenance (–27%).

Table 4-2 Summary (*P* values) of three-way ANOVAs testing for the main and interactive effects of provenance (P), temperature (T) and watering (W) treatments on whole-plant dry mass and leaf area variables of *E. grandis* and *E. tereticornis* seedlings

	Growth			
Effect	**Dry mass**	**Leaf area**	**LMA**	**LAR**
E.grandis				
P	0.507	< **0.001**	< **0.001**	< **0.001**
T	0.157	0.278	0.336	0.139
W	< **0.001**	< **0.001**	0.441	**0.007**
P × T	< **0.001**	< **0.001**	0.955	0.709
P × W	0.778	**0.011**	0.445	< **0.001**
T × W	0.752	0.667	0.122	**0.039**
P × T × W	**0.044**	0.066	0.181	0.871
E.tereticornis				
P	< **0.001**	0.925	**0.011**	< **0.001**
T	0.145	0.328	0.192	0.301
W	< **0.001**	< **0.001**	< **0.001**	< **0.001**
P × T	**0.001**	**0.002**	0.553	0.733
P × W	0.780	0.761	**0.004**	0.538
T × W	0.891	0.242	0.076	**0.039**
P × T × W	0.397	0.300	0.949	0.855

LMA, leaf area per mass; LAR, leaf area ratio. Significant values ($P < 0.05$) are shown in bold.

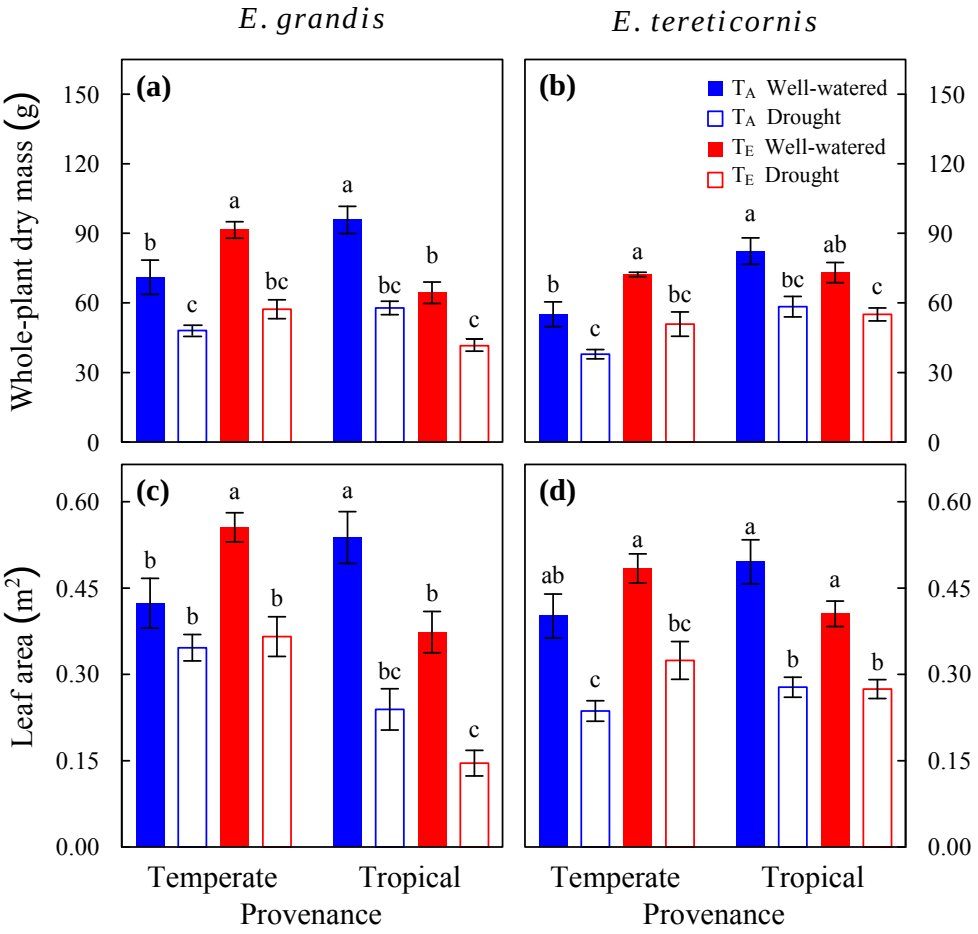

Figure 4-2 Whole-plant dry mass (a and b) and leaf area (c and d) of *E. grandis* and *E. tereticornis* seedlings from *temperate* and *tropical* provenances subjected to *well-watered* (closed bars) and *drought* (open bars) treatments under *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures. Different lowercase letters above the bars depict significant differences among temperature and watering treatment combinations of each provenance in each species ($P < 0.05$) determined by Tukey's HSD tests. Values represent means ± 1 SE ($n = 5$).

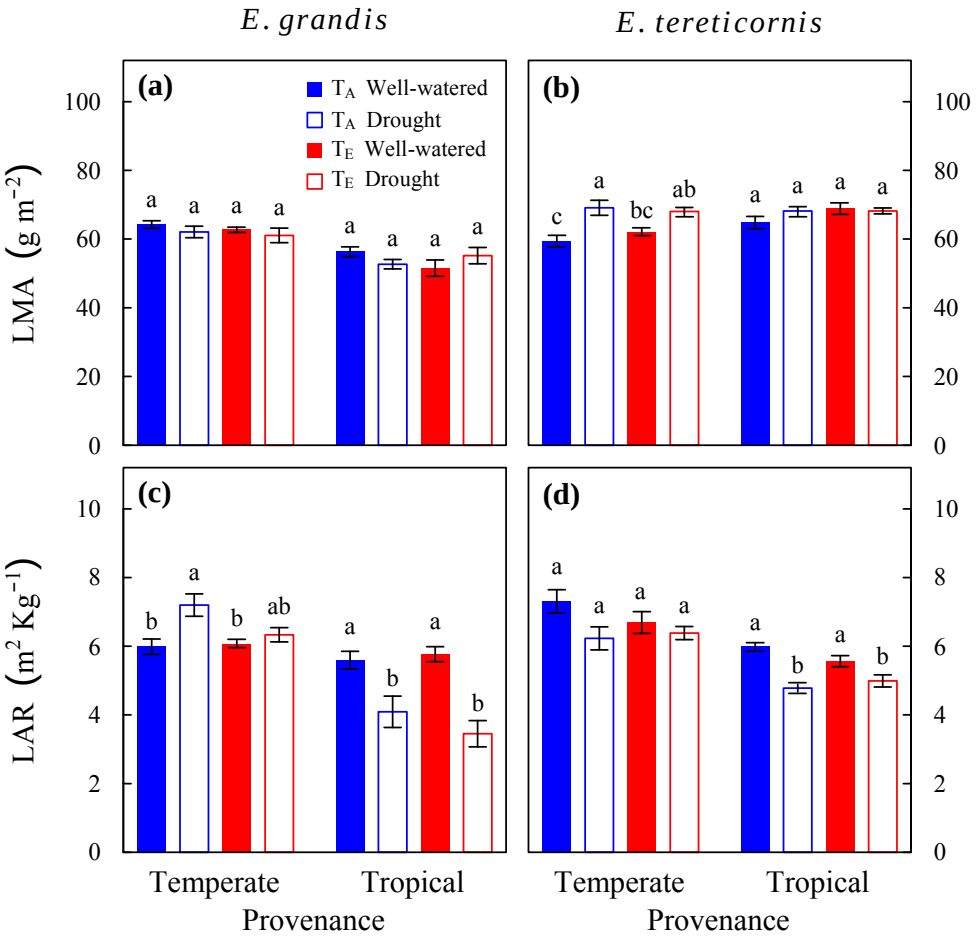

Figure 4-3 Leaf mass per area (LMA) (a and b) and leaf area ratio (LAR) (c and d) of *E. grandis* and *E. tereticornis* seedlings from *temperate* and *tropical* provenances subjected to *well-watered* and *drought* treatments under *ambient* and *elevated* temperatures. Values represent means ± 1 SE (n = 5). Other details are as described for Fig. 4-2.

The *drought* treatment did not alter LMA in *E. grandis*, but stimulated LMA by 7% (on average) in *E. tereticornis* (Fig. 4-3; Table 4-2). The stimulating effect of *drought* on LMA varied between provenances of *E. tereticornis* (significant provenance × watering interaction), with 13% increase in the *temperate* but no significant change in the *tropical*. Regardless of temperature treatment, *drought* had contrasting effects on LAR between provenances of *E. grandis*, increasing LAR by 12% in the *temperate* but decreasing it by 34% in the *tropical* (significant provenance × watering interaction; Fig. 4-3c; Table 4-2). The stimulating effect of *drought* on LAR was only significant in T_A (significant temperature × watering interaction) for the *temperate E. grandis*. Averaged across provenances of *E. tereticornis*, *drought* decreased LAR in T_A by 17%, but did not significantly affect LAR in T_E (significant temperature × watering interaction; Fig. 4-3d, Table 4-2).

4.3.2 Absolute growth rates of stem volume

Prior to the *heatwave* treatment, experimental warming (+3.5 °C) had contrasting effects on absolute growth rates (AGR) of stem volume between provenances for both species (significant provenance × temperature interaction; Fig. 4-4; Table 4-3). Averaged across the three stages before *heatwave* and watering treatments, warming stimulated AGR in *temperate* provenances (increases of 54% and 61% for *E. grandis* and *E. tereticornis*, respectively), but reduced AGR in *tropical* provenances (decreases of 37% and 29% for *E. grandis* and *E. tereticornis*, respectively). The *drought* treatment, on the other hand, significantly decreased AGR in all cases for both species, leading to an average of 36–38% decline across stages, provenances and temperature treatments (Fig. 4-4; Table 4-3). The magnitudes of

decline induced by *drought* differed between provenances of *E. grandis* (–49% in the *temperate vs.* –25% in the *tropical*; significant provenance × watering interaction), but was not different between provenances of *E. tereticornis*. In addition, for *E. tereticornis* seedlings under *drought* conditions, a smaller increase (in the *temperate*) or decrease (in the *tropical*) induced by warming was observed when compared with *well-watered* seedlings (significant provenance × temperature × watering interaction; Fig. 4-4b and 4d; Table 4-3).

The *heatwave* treatment (five consecutive days with temperature exceeding normal by 8 °C) significantly reduced AGR of stem volume in both provenances of both species when compared with the previous stage (i.e., the *second drought*), but the negative effect differed between watering treatments (Fig. 4-4; Table 4-4). Regardless of temperature treatment, the short-term acute heat stress did not cause changes in AGR of seedlings grown under *well-watered* conditions, but further reduced AGR of seedlings in the *drought* treatment (significant heatwave × watering interaction). In comparison with the *second drought*, an average of 61–67% decline in AGR (across provenances and temperature treatments) of the two species in *drought* was observed during *heatwave* (Fig. 4-4).

Table 4-3 Summary (*P* values) of mixed model ANOVAs testing for the main and interactive effects of provenance (P), temperature (T) and watering (W) treatments on growth and physiological traits of *E. grandis* and *E. tereticornis* seedlings before the *heatwave* treatment

	Growth	**Physiology**		
Effect	**AGR**	A_{sat}	R_n	A_{max}
E.grandis				
P	< **0.001**	0.165	< **0.001**	0.059
T	0.908	0.263	< **0.001**	0.776
W	< **0.001**	< **0.001**	< **0.001**	< **0.001**
P × T	< **0.001**	0.117	**0.004**	0.742
P × W	**0.006**	0.691	0.189	0.405
T × W	0.416	0.593	0.515	0.301
P × T × W	0.127	0.793	0.736	0.964
E.tereticornis				
P	**0.005**	0.269	**0.007**	0.617
T	0.283	0.208	**0.003**	0.326
W	< **0.001**	< **0.001**	0.387	< **0.001**
P × T	< **0.001**	0.590	0.268	0.616
P × W	0.477	0.250	0.523	0.126
T × W	0.823	0.913	0.288	0.414
P × T × W	**0.028**	0.279	0.184	0.165

AGR, absolute growth rate of stem volume; A_{sat}, light-saturated photosynthesis; R_n, night respiration; A_{max}, light- and CO_2-saturated photosynthesis. Significant values ($P < 0.05$) are shown in bold.

Table 4-4 Summary (*P* values) of mixed model ANOVAs testing for the effect of heatwave (H) and its interactions with provenance (P), temperature (T) and watering (W) treatments on growth and physiological traits of *E. grandis* and *E. tereticornis* seedlings

	Growth	**Physiology**		
Effect	**AGR**	A_{sat}	R_n	A_{max}
E.grandis				
H	**< 0.001**	**< 0.001**	**< 0.001**	**0.035**
H × P	0.944	**0.020**	0.496	**0.001**
H × T	0.280	0.909	0.487	0.709
H × W	**< 0.001**	0.602	0.508	0.379
H × P × T	0.418	0.757	**0.007**	**0.038**
H × P × W	0.297	**< 0.001**	0.207	**< 0.001**
H × T × W	0.639	0.123	0.695	**0.008**
H × P × T × W	0.177	0.278	0.867	0.653
E.tereticornis				
H	**< 0.001**	**< 0.001**	**< 0.001**	**0.013**
H × P	0.705	**0.013**	0.624	0.232
H × T	0.120	**< 0.001**	**0.008**	0.144
H × W	**< 0.001**	**0.018**	0.434	**0.003**
H × P × T	0.198	0.324	0.907	0.516
H × P × W	0.420	**0.016**	0.245	**0.037**
H × T × W	0.771	0.128	**0.016**	0.078
H × P × T × W	0.426	0.134	0.186	0.721

AGR, absolute growth rate of stem volume; A_{sat}, light-saturated photosynthesis; R_n, night respiration; A_{max}, light- and CO_2-saturated photosynthesis. Significant values ($P < 0.05$) are shown in bold.

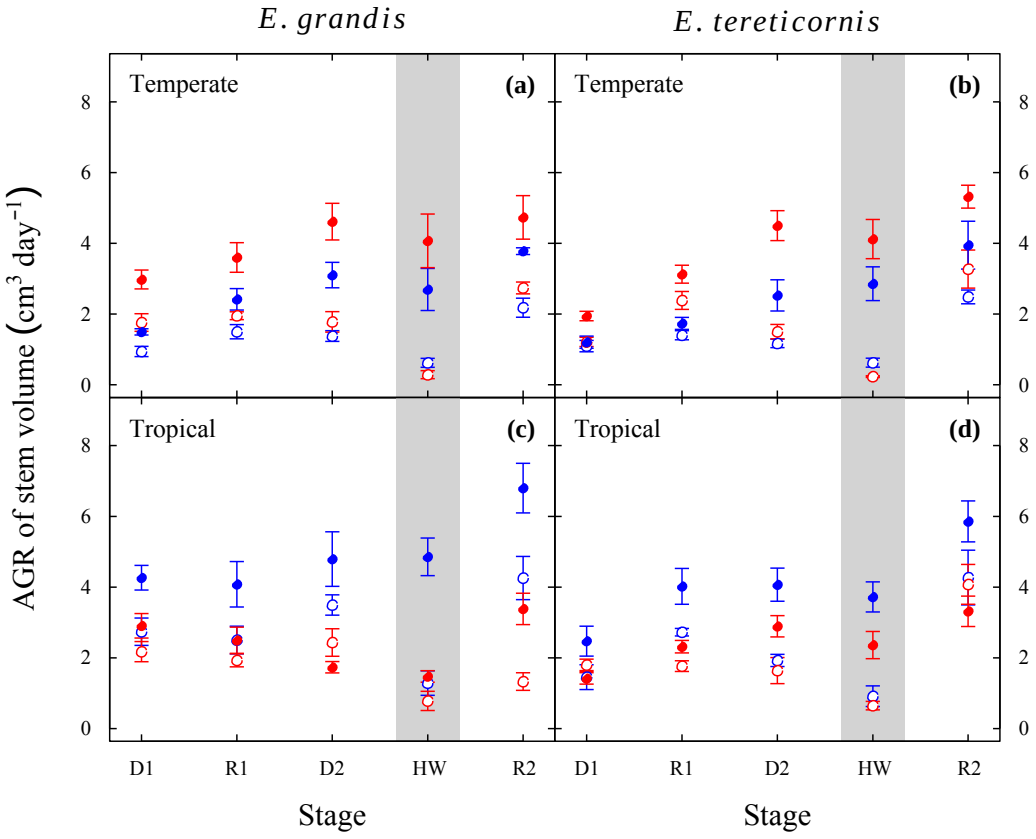

Figure 4-4 Progression of the absolute growth rate (AGR) of stem volume in *E. grandis* (the left panel) and *E. tereticornis* (the right panel) seedlings from *temperate* and *tropical* provenances subjected to *well-watered* (closed symbols) and *drought* (open symbols) treatments under *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures across all the experimental stages described in Fig. 4-1, except for the *pre drought*. The grey area indicates the period during which the heat wave (+8 °C) was applied. Values represent means ± 1 SE (n = 5).

4.3.3 Photosynthetic rates

During the four stages before *heatwave*, experimental warming (+3.5 °C) overall did not affect light-saturated photosynthetic rates (A_{sat}) in any provenance of any species, regardless of watering treatment (Fig. 4-5; Table 4-3). The *drought* treatment, on the other hand, significantly reduced A_{sat} in both provenances of both species (Fig. 4-5; Table 4-3). Specifically, during the *first drought*, A_{sat} was 63% (*E. grandis*) and 57% (*E. tereticornis*) lower in the *drought* than in the *well-watered*, averaged across provenances and temperature treatments. The magnitudes of negative drought effect on A_{sat} were generally similar between the two *drought* events (i.e., the *first drought* and the *second drought*; Fig. 4-5).

For both species, A_{sat} was significantly affected by the *heatwave* treatment, but the effects varied between provenances (significant heatwave × provenance interaction) and among provenance/watering combinations (significant heatwave × provenance × watering interaction) (Fig. 4-5; Table 4-4). In the *temperate E.grandis*, when compared with the *second drought*, the heat stress did not cause significant changes in A_{sat} of seedlings under *well-watered* conditions in both temperature treatments, but decreased A_{sat} by 81–87% for seedlings in *drought*. A contrasting pattern in the response to *heat wave* was observed in the *tropical E.grandis*, in which the heat stress reduced A_{sat} of *well-watered* seedlings by 36% (averaged across temperature treatments) but did not significantly affect A_{sat} in the *drought* treatment, when compared with the *second drought* (Fig. 4-5). However, the negative effect of heat stress on A_{sat} in the *well-watered tropical E.grandis* was only significant for seedlings in T_E (about 50% decline; Fig. 4-5c). In addition, A_{sat} of the *drought tropical E.grandis* tended to increase rather than further decline in *heatwave* (especially for seedlings under T_E) relative to the *second drought*, although the trend was not statistically significant (Fig. 4-5c).

The effects of *heatwave* on A_{sat} in *E. tereticornis* not only varied between provenances or among provenance/watering combinations, but also differed between temperature treatments (significant heatwave × temperature interaction) and between watering treatments (significant heatwave × watering interaction) (Fig. 4-5; Table 4-4). Averaged across provenances and watering treatments of *E. tereticornis*, the heat stress did not significantly affect A_{sat} in T_A but decreased A_{sat} in T_E by 44%, when compared with the *second drought*. Specifically, for the *well-watered E. tereticornis* seedlings, A_{sat} showed no response to *heatwave* in the *temperate* (regardless of temperature treatment) or in the *tropical* under T_A, but was decreased by 57% in the *tropical* under T_E. While for the *drought E. tereticornis* seedlings under *heatwave*, the decline of A_{sat} was not statistically significant in T_A regardless of provenance, but amounted to *c.* 75% in T_E for both provenances (Fig. 4-5).

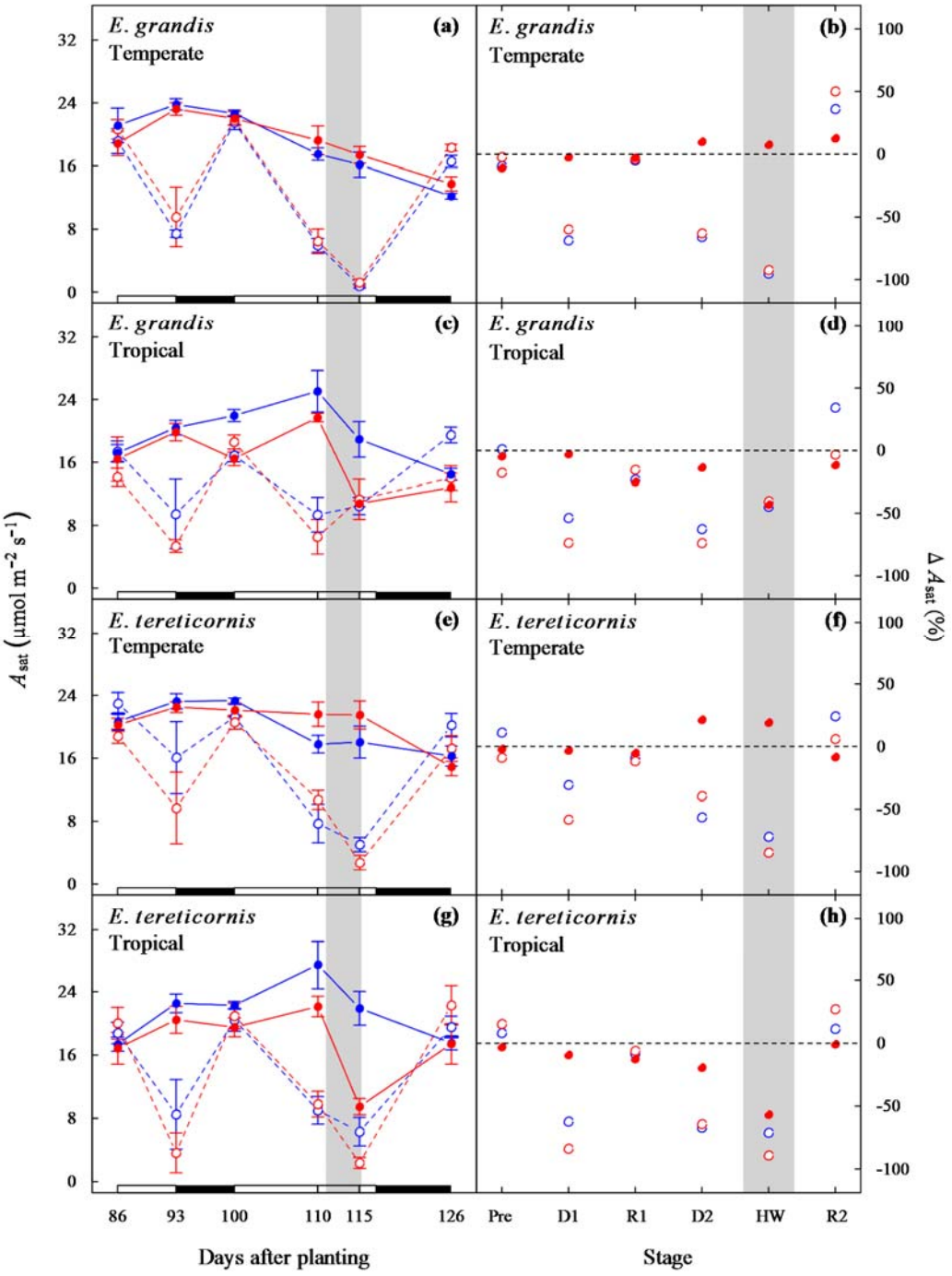

Figure 4-5 Progression of light-saturated photosynthesis (A_{sat}; the left panel) and percentage change in photosynthetic rates (Δ A_{sat}; the right panel) in *E. grandis* and *E. tereticornis* seedlings from *temperate* and *tropical* provenances subjected to *well-watered* (closed symbols) and *drought* (open symbols) treatments under *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures across the experimental stages described in

Fig. 4-1. The *x* axis scales in the left panel indicate watering regimes in *drought* seedlings, i.e., the controlled drought (open) and the full watering (closed). A_{sat} values represent means ± 1 SE (n = 5). Δ A_{sat} are calculated as follows: dividing the averaged A_{sat} of each temperature and watering treatment combination by the mean A_{sat} in the *ambient well-watered* treatment (shown as horizontal dashed lines at 0%, rather than coloured symbols) and then minus 100%. The grey area indicates the period during which the heat wave (+8 °C) was applied.

4.3.4 Night respiration

Prior to the *heatwave* treatment, experimental warming (+3.5 °C) stimulated night respiration (R_n) in both species, but varied between provenances of *E.grandis* (significant provenance × temperature interaction) (Fig. 4-6; Table 4-3). Averaged across the four stages before *heatwave* and watering treatments of *E.grandis*, warming increased R_n in the *temperate* by 31% but did not significantly affect R_n in the *tropical*. In addition, across stages and temperature treatments, the *drought* treatment overall significantly reduced R_n in *E.grandis*, but had no effect on R_n of *E. tereticornis* (Fig. 4-6; Table 4-3).

The *heatwave* treatment also stimulated R_n for both species (Fig. 4-6; Table 4-4). Across provenances, temperature and watering treatments, R_n in the two species were both increased by 28% when compared with the *second drought*. In *E.grandis*, the effect of *heatwave* on R_n varied among provenance/temperature combinations (significant heatwave × provenance × temperature interaction). Regardless of watering treatment, the heat stress increased R_n in T_A for both provenances of *E.grandis* (increases of 49% and 19% for the *temperate* and the *tropical*, respectively) and R_n in T_E for the *tropical* only (37% increase), but did not affect R_n in T_E for the *temperate* (Fig. 4-6; Table 4-4). For *E. tereticornis*, the stimulating effect of *heatwave* on R_n differed between temperature treatments (significant heatwave × temperature interaction) and among temperature/watering combinations (significant heatwave × temperature × watering interaction). Averaged across provenances, the heat stress increased R_n of *E. tereticornis* in T_A (increases of 66% and 27% in the *well-watered* and the *drought*, respectively, across watering treatments) and in T_E of the *drought* treatment (24% increase), but did significantly affect R_n in T_E of the *well-watered* seedlings (Fig. 4-6; Table 4-4).

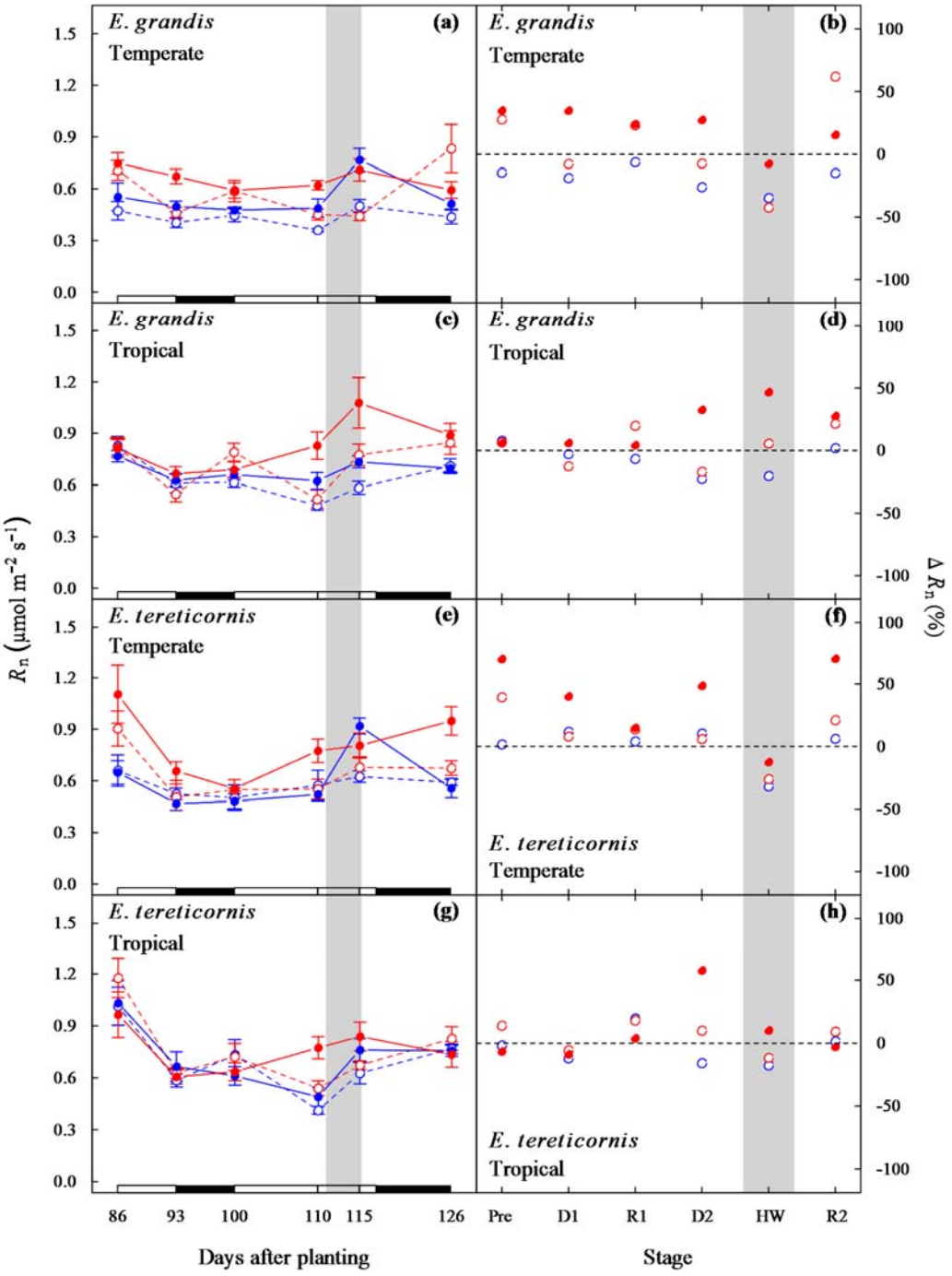

Figure 4-6 Progression of night respiration (R_n; the left panel) and percentage change in respiration (Δ R_n; the right panel) in *E. grandis* and *E. tereticornis* seedlings from *temperate* and *tropical* provenances subjected to *well-watered* and *drought* treatments under *ambient* and *elevated* temperatures across the experimental stages. Other details are as described for Fig. 4-5.

4.3.5 Photosynthetic capacity

For both species across the stages before *heatwave*, photosynthetic capacity (A_{max}) had a similar pattern with A_{sat} in the responses to experimental treatments, showing no change overall to warming (+3.5 °C) but significant decline in the *drought*, regardless of provenance (Fig. 4-7; Table 4-3). With the two *drought* events combined, A_{max} in the *drought* seedlings were 40% (*E. grandis*) and 34% (*E. tereticornis*) lower than in the *well-watered* seedlings, when averaged across provenances and temperature treatments.

The *heatwave* treatment significantly affected A_{max} of both species and the effects varied among provenance/watering combinations (significant heatwave × provenance × watering interaction) (Fig. 4-7; Table 4-4). In addition, the effects of *heatwave* on A_{max} of *E.grandis* also differed between provenances (significant heatwave × provenance interaction) and among provenance/temperature combinations (significant heatwave × provenance × temperature interaction) as well as temperature/watering combinations (significant heatwave × temperature × watering interaction) (Table 4-4). For the *temperate E.grandis*, when compared with the *second drought*, the heat stress increased A_{max} in the *well-watered* by 15% but decreased A_{max} of the *drought* seedlings by 72% across temperature treatments. While for the *tropical E.grandis*, a contrasting pattern in the response to *heat wave* was observed, in which the heat stress reduced A_{max} in the *well-watered* seedlings by 22% but increased A_{max} in the *drought* instead by 54%, averaged across temperature treatments (Fig. 4-7). Nevertheless, the decline of A_{max} in the *well-watered tropical E.grandis* was only significant for seedlings in T_E (decrease of 40%; Fig. 4-7c).

The effects of *heatwave* on A_{max} in *E. tereticornis* varied between watering treatments (significant heatwave × watering interaction) and among provenance/watering combinations (Fig. 4-7; Table 4-4). Averaged across temperature treatments of the *temperate E. tereticornis*, the heat stress increased A_{max} in the *well-watered* by 24% but decreased A_{max} of the *drought* seedlings by 37% when compared with the *second drought*. However, the heat stress did not cause significant changes to A_{max} in any temperature and watering treatment combination of the *tropical E. tereticornis* (Fig. 4-7).

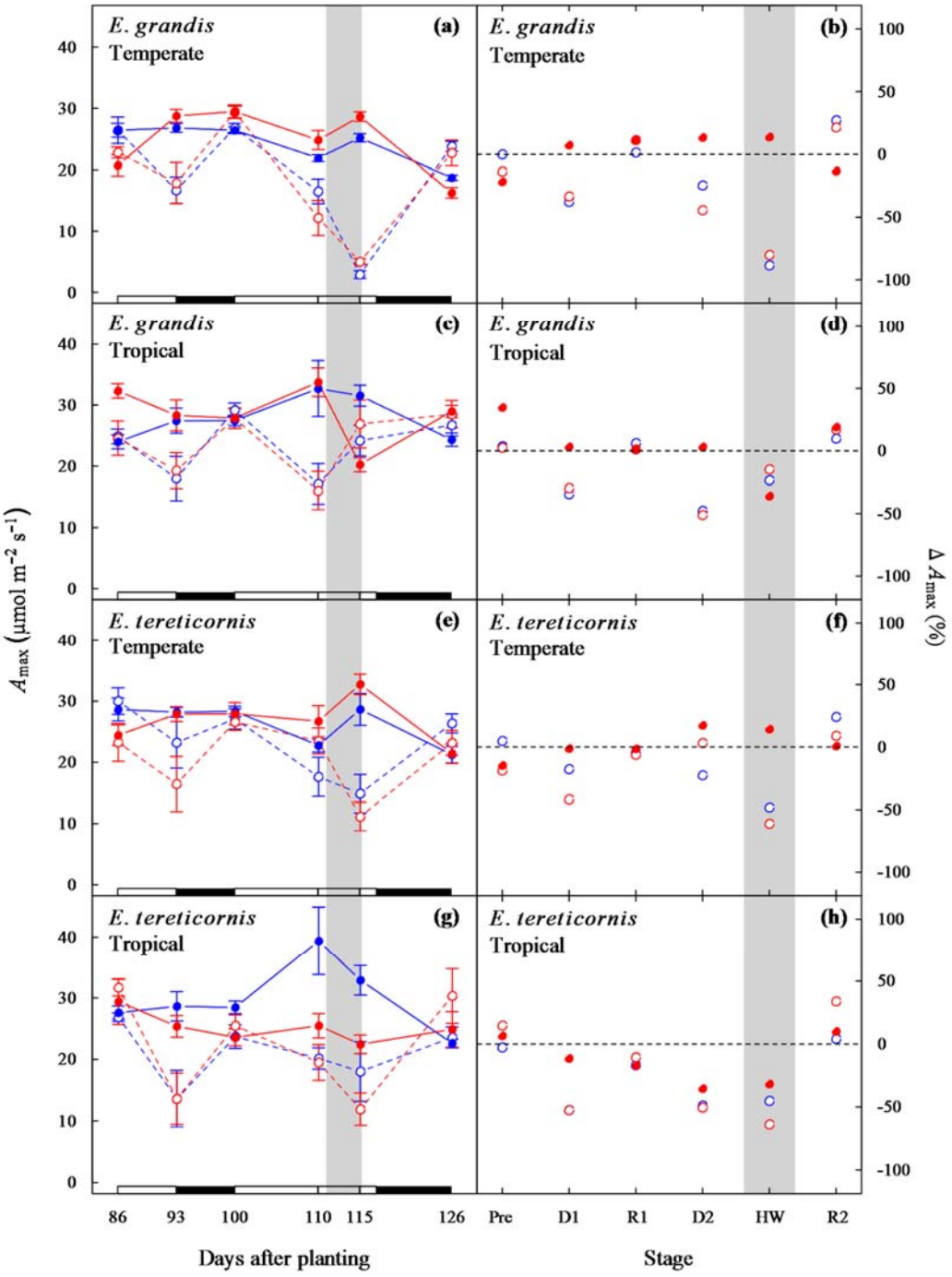

Figure 4-7 Progression of photosynthetic capacity (A_{max}; the left panel) and percentage change in the capacity (ΔA_{max}; the right panel) in *E. grandis* and *E. tereticornis* seedlings from *temperate* and *tropical* provenances subjected to *well-watered* and *drought* treatments under *ambient* and *elevated* temperatures across the experimental stages. Other details are as described for Fig. 4-5.

4.4 Discussion

Consistent with the first hypothesis, warming of +3.5 °C increased growth of cool-origin provenances for both species, but reduced or had no significant effect on growth of warm-origin provenances. In addition, intraspecific variation in the plasticity of AGR in response to warming was also observed between provenances and was associated with source environment variability. The second hypothesis was generally rejected as provenances of both species responded similarly in most traits under drought conditions, suggesting no intraspecific variation in phenotypic plasticity in response to drought. In partial support of the third hypothesis, photosynthetic traits of warm-origin provenances under well-watered conditions were reduced by heat waves to a greater degree in general, when compared with cool-origin provenances. The fourth hypothesis was largely supported because the negative effects of drought on growth and photosynthetic traits were exacerbated by heat waves in most provenances, but heat waves alone did not cause substantial changes in growth or physiology in most cases. Furthermore, two distinct strategies (senescence of older mature leaves *vs.* complete closure of stomata) were observed and both proved to be effective in coping with the combined drought and heat stress. Overall, these results suggest that (1) populations of widespread woody species originating from different environments may possess differentiated capacity to cope with climate warming and heat waves, related to the climate of origin, but may not necessarily show differentiation in response to drought; (2) drought is likely to be a more severe stressor than heat waves, dominating the plant responses to extreme climatic conditions; and (3) widespread woody species may utilize different strategies to cope with the co-occurring drought and heat waves.

4.4.1 Intraspecific variation in woody plant response to warming and climate extremes

For both *Eucalyptus* species in this study, provenances from contrasting temperature environments showed significant differentiation in growth response to the constant +3.5 °C warming, consistent with the prediction that the capacity of woody plants to cope with warming may vary depending on the taxon's climate of origin (Saxe *et al.*, 2001; Way & Oren, 2010; Drake *et al.*, 2015). Many studies suggest that a mild warming would be beneficial to the growth of trees from relatively cool regions at high latitudes or altitudes, where plant growth may be temperature-limited (e.g., Carter, 1996; Rehfeldt *et al.*, 1999; McKenzie *et al.*, 2001; Bunn *et al.*, 2005; Thomson *et al.*, 2009; Hanninen & Tanino, 2011). In contrast, warming is likely to negatively affect woody plants from tropical regions, where source temperatures are close to thermal optima such that further warming would be detrimental rather than beneficial (Clark *et al.*, 2003; Feeley *et al.*, 2007; Doughty & Goulden, 2008; Clark *et al.*, 2010). Results from this study show a pattern similar to the literature, with substantial increases in growth of cool-origin provenances but reductions or no change in growth of warm-origin provenances in response to warming. Combined with similar findings from the related study (i.e., Drake *et al.*, 2015), I suggest that the effects of future climate warming on plant growth are likely to vary among populations across the range of widely distributed woody species.

Significant intraspecific variation in the response of plant growth rates to warming in both *Eucalyptus* species was also observed. Regardless of response direction, provenances from cooler, more variable temperature environment experienced approximately twofold changes in AGR induced by warming, when compared to changes in provenances from warmer but less variable temperature

environment, suggesting that there is a relationship between the plasticity of AGR and the environmental variability of taxon origin. Plant populations usually exhibit intraspecific variation in phenotypic plasticity, and patterns of environmental variability would influence the differentiation in the plasticity of traits among populations. A long-standing hypothesis predicts that greater levels of environmental variability will select for plants with greater phenotypic plasticity (Galloway, 1995; Weinig, 2000; Donohue *et al.*, 2001; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen & Fischer, 2005), which is supported by results from the present study and the related study (Drake *et al.*, 2015). Although very few studies have tested this hypothesis on woody species, evidence from the existing literature is discordant. Results from a study on *Telopea speciosissima* contradict the current paradigm, with genotypes from less variable temperature environment showing greater growth plasticity to warming (Huang *et al.*, 2015). Therefore, to validate this hypothesis, more studies on other woody species are necessary.

For both *Eucalyptus* species, drought as a single factor affected most growth and physiological traits to a similar degree among provenances, suggesting that the intraspecific variation in phenotypic plasticity in response to drought was largely absent in this study. This phenomenon contradicts the prevailing observations from other woody species, in which populations from different precipitation regions usually show differentiation in response to drought (e.g., Ramirez-Valiente *et al.*, 2010; McLean *et al.*, 2014; Bansal *et al.*, 2015). I suggest that the absence of intraspecific variation in response to drought may be attributed to the relative uniformity of precipitation in source environment among the provenances in this study. The four *Eucalyptus* provenances are all from high rainfall coastal regions (above 890 mm per year; Table 4-1) with more or less similar monthly precipitation variability (especially

during the summer months), indicating that these provenances might have been adapted to similar non-water-stressed environments and their capacity to cope with drought may not differ within species. By comparison, plant populations showing intraspecific variation in the drought responses are generally distributed across low, mid and high rainfall regions (Aranda *et al.*, 2010; Ramirez-Valiente *et al.*, 2010; McLean *et al.*, 2014; Bansal *et al.*, 2015), or at least two of the three rainfall regions (Cregg & Zhang, 2001; Gratani *et al.*, 2003; Silva *et al.*, 2006; Robson *et al.*, 2012), indicating that there might be inherent differences in the capacity to cope with drought.

I observed significant intraspecific variation in photosynthetic responses during the heatwave treatment for both *Eucalyptus* species under well-watered conditions. Cool-origin provenances maintained A_{sat} and up-regulated A_{max} with the short-term heat stress, while warm-origin provenances down-regulated or maintained A_{sat} and A_{max}. This pattern may be attributed to the differentiated relationships between the heat stress and physiological thermal optima. If a heat stress exceeds photosynthetic temperature optimum, negative thermal impacts on photosynthesis usually occur (Sage & Kubien, 2007; Sage *et al.*, 2008). For tropical provenances during the heatwave in this study, averaged mid-day growth temperatures were targeted at 40.5 °C (T_A) and 44.0 °C (T_E), both higher than the absolute maximum temperature recorded in the field (37.5 °C for *E. grandis* and 39.5 °C for *E. tereticornis*; data obtained from the SILO Climate Data); target temperatures for temperate populations were 30.0 °C (T_A) and 33.5 °C (T_E), significantly lower than the field absolute maximum temperature records (≥ 40 °C). Therefore, the target mid-day temperatures for the tropical provenances during the heatwave (> 40 °C) were novel and probably supra-optimal for seedlings of warm-origin, leading to negative effects on C_3 photosynthesis at ambient CO_2 concentrations (Sage & Kubien, 2007; Sage *et*

al., 2008; Way & Sage, 2008); however, for the cool-origin provenances, the target mid-day temperatures were still within their thermal optima for photosynthesis.

4.4.2 The effects of climate extremes on plants

In this study, heat waves under well-watered conditions had little effect on plant growth rates (i.e., AGR) and photosynthetic rates (A_{sat}) in most cases, with the exception of significant declines in A_{sat} of warm-origin plants grown under warming conditions. The decline of A_{sat} observed here is a typical direct negative effect of high temperature on plants due to exceedance of thermal optima (Berry & Bjorkman, 1980; Sage & Kubien, 2007; Hozain *et al.*, 2010). The absence of marked effects induced by heat waves can often be attributed to the capacity for plants to continuously cool their leaves via transpiration to mitigate the heat stress, when there is sufficient water (De Boeck *et al.*, 2010, 2011; Teskey *et al.*, 2015). In fact, under well-watered conditions, woody plants can cope well with high temperatures (> 40 °C) over a short duration, in most circumstances (Cunningham & Read, 2006; Teskey *et al.*, 2015). For example, Ameye *et al.* (2012) reported that seedlings of *Pinus taeda* and *Quercus rubra* from a warm temperate region were capable of tolerating daytime temperatures exceeding 50 °C, without any sign of visible damage to leaves. Similarly, I did not observe leaf damage on the two *Eucalyptus* species growing under well-watered conditions during the heatwave, even for plants from the tropical regions.

The negative effects of single factor drought on plant growth and functioning generally were aggravated by the short-term acute heat stress in the study. Compared with declines induced by drought alone, larger decreases were generally found in growth and photosynthetic traits (e.g., AGR, A_{sat} and A_{max}) in response to combined

drought and heat waves for both *Eucalyptus* species, except for warm-origin *E. grandis* seedlings. This pattern was consistent with previous studies, in which the synergism between drought and heat waves imposed significantly greater impacts on plants, compared to each stress applied separately (De Boeck *et al.*, 2011; Dreesen *et al.*, 2012; Bauweraerts *et al.*, 2013, 2014; Zinta *et al.*, 2014). The impact of combined drought and heat stress may be hypothesised to occur as follows: during drought periods, high temperatures lead to increased vapour pressure deficits and consequently increased evapotranspiration, which will further dry the soil, and hence create a positive feedback loop to magnify or accelerate the effects of drought (De Boeck *et al.*, 2011; Teskey *et al.*, 2015). According to this hypothesis, the effect of heat on the two *Eucalyptus* species in this study worked mostly indirectly and mainly through drought, consistent with other studies (Reichstein *et al.*, 2007; De Boeck *et al.*, 2011). Taken together, these results are in agreement with the prediction that drought will be a more severe stressor than heat waves, dominating plant response to simultaneously occurring climate extremes (Reichstein *et al.*, 2007; De Boeck *et al.*, 2010, 2011; Bauweraerts *et al.*, 2014; Hoover *et al.*, 2014; Teskey *et al.*, 2015).

It is noted that the negative effects of drought on photosynthetic traits in warm-origin *E. grandis* were not exacerbated by the heat stress, contrasting the commonly observed pattern in cool-origin *E. grandis* and all provenances of *E. tereticornis*. Therefore, different strategies may be used in coping with combined heat and drought stress. The warm-origin *E. grandis* seedlings under drought conditions up-regulated or maintained A_{sat} and A_{max} during the heat wave by keeping their stomata open (i.e., relatively high stomatal conductance; see Fig. A-3 in the Appendix A), especially for seedlings grown under the +3.5 °C warmed conditions. With limited water, these processes were achieved through drought-induced senescence of older mature leaves

(G. Huang, personal observation; also see Fig. 4-3c), and maintenance of functionality in the remaining relatively young leaves. Transpiration water would be used to cool leaves and minimize potential damage of heat stress on both leaf and stem tissue (Kolb & Robberecht, 1996; Teskey *et al.*, 2015). This occurred at the expense of growth because the growth rates (i.e., AGR) did not benefit from the relatively high photosynthesis during the heat wave. By contrast, the other *Eucalyptus* seedlings (i.e., *E. tereticornis* and cool-origin *E. grandis*) did not show leaf senescence, but tended to close their stomata completely (i.e., stomatal conductance close to zero; see Fig. A-3) in response to heat and drought combined, consistent with findings from other woody species (Hamerlynck *et al.*, 2000; Zweifel *et al.*, 2006). This indicates that they may have used a different strategy rather than transpirational cooling to add protection against damage caused stresses, which is likely to be the accumulation of stress proteins, antioxidants and compatible solutes (Wang *et al.*, 2003; Ahuja *et al.*, 2010), evidenced by the significant higher LMA in these seedlings when compared with the warm-origin *E. grandis*. In this study, both strategies were successful in protecting plants against these multiple extreme abiotic stresses, as indicated by full recovery of photosynthetic traits in almost all seedlings after the stress was alleviated.

In conclusion, I demonstrated significant intraspecific variation in plant growth response to warming and in photosynthetic response to heat waves, both of which were correlated with taxon temperature of origin. However, the effects of single factor drought on plant growth and physiology did not show differentiation within species. The heat stress alone generally had little effect on plant growth and photosynthesis, but the synergism between drought and heat imposed significantly greater impact on plants than each applied separately. In addition, two distinct strategies (senescence of older mature leaves *vs.* complete closure of stomata) were observed and both proved

to be effective in coping with the combined drought and heat stress. Taken together, these results suggest that populations of widespread woody species originating from different environments may show differentiated capacity to cope with climate warming and heat waves, and they may utilize different strategies to cope with the co-occurring climatic extremes such as drought and heat stress. Drought is likely to be a more critical determinant than heat, dominating the plant responses under extreme climatic conditions in future.

Chapter 5

Synthesis and conclusions

5.1 Synthesis

Experiments presented in this thesis were designed to investigate the main and interactive effects of multiple climatic variables (i.e., [CO_2], temperature, and water availability) on growth and physiology of ecologically differentiated woody plant populations. Changes in climatic variables in this research were set to simulate the predicted climatic scenarios within this century based on model projections. Three Australian native woody species representing different taxa and functional groups were included: *Telopea speciosissima* (Proteaceae; Shrub; open woodland; Chapter 2 and 3), *Eucalyptus grandis* (Myrtaceae; Tree; wet forest; Chapter 4) and *Eucalyptus tereticornis* (Myrtaceae; Tree; dry forest; Chapter 4), each of which consisted of two populations originating from different environments. In particular, intraspecific variation in the capacity of each species to cope with changing climatic variables was assessed, in an effort to improve understanding of woody plant responses under future climatic scenarios. Specifically, this research sought to address the following questions:

(1) Do changes in climatic variables independently or interactively expose intraspecific variation in phenotypic plasticity of woody plant populations originating from different environments?

(2) If differentiated responses between woody plant populations exist, what are the relationships between phenotypic plasticity and their source environmental variability?

(3) How will climatic variables interactively affect growth and physiology of woody plants under future climates?

5.1.1 Intraspecific variation of woody plant response to [CO2] and temperature

One fundamental way that plant species may respond to changes in atmospheric [CO_2] and temperature is to adjust their growth and physiology via phenotypic plasticity (Sultan, 2000; Nicotra *et al.*, 2010). This mechanism is thought to be particularly important for woody species with long generation times, because their evolutionary response by natural selection might be too slow to cope with the rapid environmental changes. Although previous studies have demonstrated intraspecific variation in growth and/or physiological plasticity of woody plants in response to rising [CO_2] (Ceulemans *et al.*, 1996; Dickson *et al.*, 1998; Isebrands *et al.*, 2001; Mohan *et al.*, 2004; Cseke *et al.*, 2009) or increasing temperature (Weston & Bauerle, 2007; Weston *et al.*, 2007; Drake *et al.*, 2015), the nature and basis of intraspecific variation in phenotypic plasticity within woody species under climate change is still largely unknown. One of the objectives of this thesis was to assess whether there is intraspecific variation in response to elevated [CO_2] (C_E) and/or elevated temperature (T_E) in a number of woody species from different climates.

In this research, significant intraspecific variation in growth plasticity between plant populations responding to T_E (a constant mild warming; 3.5–4.0 °C above the ambient) was observed in all three studied woody species (Chapter 2–4). These findings are consistent with the general prediction that plant populations may exhibit intraspecific variation in phenotypic plasticity (Donohue *et al.*, 2001; Alpert & Simms, 2002; Van Kleunen & Fischer, 2005; Aspinwall *et al.*, 2015). However, differentiation in photosynthetic plasticity (e.g., A_{sat}, A_{max}) in response to T_E was largely absent in these species (Chapter 2–4), inconsistent with previous studies on other woody plants (Weston & Bauerle, 2007; Weston *et al.*, 2007; Drake *et al.*, 2015). This phenomenon suggests that there were no parallel effects of mild warming on growth and photosynthetic plasticity, which may be attributed to the lack of strong relationships between growth and photosynthetic traits in this research.

In the experiment on *T. speciosissima*, no interaction between population and [CO_2] was found for either growth or physiological traits, indicating that the two populations had similar phenotypic plasticity under C_E (Chapter 2 and 3). Although previous studies on woody species responsiveness to C_E usually demonstrate substantial intraspecific differences in the responses of plant growth and/or physiology to changing [CO_2] (Ceulemans *et al.*, 1996; Dickson *et al.*, 1998; Isebrands *et al.*, 2001; Mohan *et al.*, 2004; Cseke *et al.*, 2009), some studies show limited intraspecific variation in woody plant responsiveness to C_E (e.g., Cantin *et al.*, 1997), consistent with results from this study. Furthermore, interactive effects of C_E and T_E on the intraspecific variation of growth or physiological responses were not observed. The lack of [CO_2] effects on intraspecific variation of phenotypic plasticity suggest that populations of *T. speciosissima* might have been equally limited by carbon availability and may not differ in their inherent capacity to cope with changes in [CO_2].

Collectively, my research indicates that temperature would be more effective than [CO_2] in exposing intraspecific variation in growth plasticity for woody plant populations under future climates. However, given that whole-plant level performance (e.g., growth) is generally determined by many coordinated processes at molecular, biochemical and physiological levels (Leakey *et al.*, 2009b; Hacke *et al.*, 2012), further investigations combining multiple scales on more woody species are necessary for exploring the underlying mechanisms that drive woody plant response to changing [CO_2] and temperature.

5.1.2 Intraspecific variation of woody plant response to climate extremes

Two types of climate extremes were simulated in this research – drought (Chapter 3 and 4) and heat stress (Chapter 4). For all studied woody species, progressive drought imposed significant negative effects on growth and physiology, leading to reductions in biomass accumulation and leaf area development, as well as inhibition of photosynthesis. However, the *drought* treatment affected most growth and photosynthetic traits to a similar degree between populations of each studied species, suggesting that intraspecific variation in phenotypic plasticity in response to drought was largely absent in this research (Chapter 3 and 4). These findings contradict the prevailing observations from other woody species, in which populations from different precipitation regions usually show differentiation in response to drought (e.g., Ramirez-Valiente *et al.*, 2010; McLean *et al.*, 2014; Bansal *et al.*, 2015). The absence of intraspecific variation in response to drought in this research may be attributed to the relative uniformity of precipitation in source environment between populations of each species. For the three studied species, populations of each were both sampled

from high rainfall regions, indicating that they might have been adapted to similar non-water-stressed environments and therefore their inherent capacity to cope with drought may not differ between them.

Significant intraspecific variation in photosynthetic responses (i.e., A_{sat} and A_{max}) to a short-term heat stress (8 °C above the ambient source temperatures for five consecutive days) was observed for *Eucalyptus* species under well-watered conditions in this research (Chapter 4). This pattern may be attributed to the differentiated relationships between the heat stress and physiological thermal optima. Negative thermal impacts on C_3 photosynthesis at ambient [CO_2] usually occur when a temperature exceeds the photosynthetic temperature optimum (Sage & Kubien, 2007; Sage *et al.*, 2008; Way & Sage, 2008). For both *Eucalyptus* species in this study, target temperatures during the *heatwave* treatment were novel and probably supra-optimal for photosynthesis of warm-origin (the tropical) populations, but were still within thermal optima for photosynthesis of cool-origin (the temperate) populations. Nevertheless, growth responses (i.e., the absolute growth rates in stem volume) to heat stress did not differ between populations of any *Eucalyptus* species.

Taken together, this research indicates that woody plant populations originating from different environments may not necessarily show intraspecific variation in their responses to climate extremes, likely depending on how far the source environments have shaped their capacity to cope with a given stress through adaptation. In addition, to my knowledge, this is the first study observing significant intraspecific variation in woody plants responding to heat stress, which would provide some useful insights for future studies on woody species in response to thermal anomalies.

5.1.3 Association between phenotypic plasticity and source environment variability of woody plant populations

Plant populations usually exhibit intraspecific variation in phenotypic plasticity and the divergence among populations may be influenced by the patterns of environmental variability. A long-standing hypothesis predicts that greater levels of environmental variability will select for plants with greater phenotypic plasticity (Galloway, 1995; Weinig, 2000; Donohue *et al.*, 2001; Alpert & Simms, 2002; Gianoli & Gonzalez-Teuber, 2005; Van Kleunen & Fischer, 2005). Although significant intraspecific variation in growth plasticity in response to T_E (i.e., a constant mild warming) was found for all studied woody species in this research, relationships between phenotypic plasticity and source environment variability differed among the three species. Results from *Eucalyptus* species both support the long-standing hypothesis, in which populations originating from more variable temperature environments showed larger growth responses to T_E (Chapter 4). However, the coast-origin *T. speciosissima* from less variable temperature environments exhibited higher growth plasticity under T_E (Chapter 2 and 3), contradicting the current paradigm.

The discordant patterns in the relationship between phenotypic plasticity and source environment variability in this research suggest that woody plant populations originating from more variable environments may not necessarily show greater phenotypic plasticity in response to changing climates. I argue that differentiation in phenotypic plasticity among plant populations may be not only associated with source environment variability, but also linked to the intrinsic difference in adaptation to distinct source environments. Nevertheless, assessing the linkage between phenotypic plasticity and source environment variability on woody plant populations is extremely

limited to date. Therefore, to validate the long-standing hypothesis on woody plants, more studies on other species are required.

5.1.4 Interactions between climatic variables on woody plant responses

Climatic variables are generally predicted to change concurrently in the future (Solomon *et al.*, 2009; Rahmstorf & Coumou, 2011; Collins *et al.*, 2013). Therefore, to unravel the underlying mechanisms that drive woody plant responses to changing climates, investigating potential interactions between multiple climatic variables (e.g., [CO_2], temperature, and water availability) on plant growth and physiology is essential. In this research, at least two climatic factors were included for each experiment to assess: (i) the interactive effects of C_E and T_E on woody plants under non-stressed conditions (Chapter 2); (ii) the effects of C_E and/or T_E on woody plant drought responses (Chapter 3 and 4); (iii) the effects of multiple stresses (i.e., drought and heat stress) on woody plants (Chapter 4).

Although [CO_2] or temperature alone had significant effects on *T. speciosissima* growth and physiology, the interaction between C_E and T_E was absent on most traits measured, suggesting that the effects of C_E and T_E were generally independent in this study (Chapter 2). There is no clear trend in the literature for the interactive effects of [CO_2] and temperature on woody plant responses. Many studies suggest that C_E is likely to interact with T_E, thereby synergistically affecting woody plant growth and/or physiology (e.g., Callaway *et al.*, 1994; Peltola *et al.*, 2002; Ghannoum *et al.*, 2010a; Ayub *et al.*, 2011; Wang *et al.*, 2012). However, results from this research are consistent with other studies indicating that the effects of increasing

[CO_2] and warming are additive (e.g., Lewis *et al.*, 2001; Lloyd & Farquhar, 2008; Lewis *et al.*, 2013).

For all studied species, declines in growth induced by drought did not differ either between [CO_2] treatments or between temperature treatments in most cases, suggesting no effects of C_E or T_E on woody plant growth responses to drought in this research (Chapter 3 and 4). However, T_E was found to negatively affect the capacity of *T. speciosissima* resisting drought, accelerating the process of stomata closure induced by drought (Chapter 3). This phenomenon indicates that the net effects of T_E on drought responses may differ between the whole-plant level and the stomatal level, because the negative impacts of T_E on stomatal responses to drought may be offset by the beneficial effects of T_E on leaf area. On the other hand, C_E did not affect the capacity of *T. speciosissima* drought resistance, either individually or interactively with T_E, suggesting that temperature may be a stronger determinant than [CO_2] affecting the capacity of woody plants resisting to drought under future climates.

The simultaneous occurrence of heat stress and drought is common, and together they can impose significantly greater impacts on plant responses than each applied separately (Mittler, 2006; De Boeck *et al.*, 2011; Dreesen *et al.*, 2012; Bauweraerts *et al.*, 2013; Zinta *et al.*, 2014). Results from this research are generally in line with these findings. Specifically, the short-term heat stress under well-watered conditions had little effect on plant growth rates and photosynthesis in most cases. However, larger decreases were generally found in growth and photosynthetic traits (e.g., AGR, A_{sat} and A_{max}) in response to combined drought and heat stress for both *Eucalyptus* species, when compared with declines induced by drought alone (Chapter 4), indicating that heat stress would exacerbate the negative effects of drought on plant growth and functioning. These results are also in agreement with the prediction that

drought is likely to be a more critical determinant than heat stress, dominating plant response to simultaneously occurring climate extremes (Reichstein *et al.*, 2007; De Boeck *et al.*, 2010, 2011; Bauweraerts *et al.*, 2014; Hoover *et al.*, 2014; Teskey *et al.*, 2015).

Collectively, this research found that temperature and [CO_2] may not interactively affect woody plant growth and physiology, while temperature (either constant warming or short-term heat stress) may have significant impacts on woody plant responses to drought. Furthermore, drought tends to be the dominant stressor for woody plants when facing multiple climatic extremes.

5.1.5 Implications for woody plant response to changing climates

It has been well recognized that climate is not only affecting phenotypes via environmental effects on fitness, but also acting as a major selection force on genotypes (Savolainen *et al.*, 2007; Wang *et al.*, 2010). Therefore, when coping with changing climates, plant species may have to rely on both ecological and evolutionary strategies (Kawecki, 2008; Anderson *et al.*, 2012). Specifically, in the short-term, plants may adjust their growth and physiological performance via phenotypic plasticity; while in the long-term, plants may undergo evolutionary changes by genetic adaptation. For woody species with long generation times, phenotypic plasticity might be particularly important for acting as a buffer against rapid climate change and providing fitness advantages (Valladares *et al.*, 2007; Chevin *et al.*, 2010; Nicotra *et al.*, 2010), because their evolutionary responses by selection might be too slow to mitigate the effects of rapid environmental change.

Within species, plant populations/genotypes across environmental gradients are often highly adapted to local conditions (Savolainen *et al.*, 2007; Hereford, 2009; Wang *et al.*, 2010; McLean *et al.*, 2014), and therefore are likely to differ in their phenotypic responses to the same environmental change. Intraspecific variation in phenotypic plasticity would not only influence the habitat range occupied by plant species, but also affect the ecological and evolutionary responses of plant species to changing environments (Sultan, 2000; Van Kleunen & Fischer, 2005; Valladares *et al.*, 2007; Williams *et al.*, 2008; Nicotra *et al.*, 2010; Aspinwall *et al.*, 2015). For instance, genotypes with high phenotypic plasticity or broad niche breadth may be capable of rapid resource uptake and increase productivity under optimal conditions (Grime & Mackey, 2002; Banta *et al.*, 2012), which might benefit from the advantageous changes of some climatic variables (such as rising [CO_2] and/or a mild warming) and therefore be selected for under future climates. In contrast, genotypes with low phenotypic plasticity may tolerate and persist under unfavorable conditions to survive and maintain growth (Schlichting, 1986; Thompson, 1991), and therefore possibly be selected for under climate extremes.

In this research, although the Coastal genotype of *T. speciosissima* showed a greater growth plasticity responding to a mild warming than the Upland genotype (Chapter 2), these two genotypes did not differ in their responses to water deficit (Chapter 3), suggesting that they might have been adapted to somewhat similar non-water-stressed environments in the past. This speculation might be better supported with (i) a detailed characterization of the climatic variation in coastal and upland regions, and (ii) better plant fitness estimates incorporating growth, survival, and reproduction. The geological and vegetation records support the maintenance of *T. speciosissima* populations in both regions through the last glacial maxima, which

would have experience cooler and drier climatic conditions (Hesse *et al.*, 2003; Rossetto *et al.*, 2011). Has this resulted in more conservative growth strategy found in the Upland genotype? Plant size was the major determinant of the susceptibility to water limitation, and ultimately drought induced mortality. While the results presented here did not detect significant differences among genotypes in response to water limitations (with plant size as a covariate; see Chapter 3), there is evidence in the literature for larger plants and those with faster growth rates to be more susceptible to drought (see Cregg & Zhang, 2001; Lewis *et al.*, 2013; Aspinwall *et al.*, 2015). The long generation times of woody species make it unfeasible to evaluate reproductive output and therefore the fitness of plant genotypes in an evolutionary sense. Following this, the evolutionary value of phenotypic plasticity would be to maintain plant function and persistence in variable climates (Sultan, 2000; Valladares *et al.*, 2007; Chevin *et al.*, 2010; Nicotra *et al.*, 2010). While growth plasticity may be advantageous if it provides a competitive advantage leading to greater reproductive output, it may be a disadvantage in climatic regions that are unpredictable/variable where rapid growth may leave plant vulnerable to drought. For better understanding the complicated relationships between phenotypic plasticity and genetic adaptation for woody plant species, future studies with more thorough experimental designs (e.g., including both ecological and evolutionary aspects) and trait responses measured across hierarchical levels (e.g., from the whole-plant level to the molecular level) would be necessary.

5.2 Conclusions

This research addressed the main and interactive effects of changes in multiple climatic variables (i.e., [CO_2], temperature, and water availability) on growth and

physiology of three woody species representing different taxa and functional groups, with a focus on the intraspecific variation in their responses between populations originating from different environments. Findings of this research were reported based on the treatment levels chosen for the experiments. Significant intraspecific variation in growth plasticity when responding to a constant mild warming (T_E; ambient + 3.5–4.0 °C) was found in all three species, and intraspecific variation in photosynthetic responses to a short-term heat stress (ambient + 8 °C) was observed in the two *Eucalyptus* species. In contrast, populations did not differ in their growth or photosynthetic responses to elevated [CO_2] (C_E) or to sustained drought in most cases for all three species. These results together suggest that temperature would be more effective than [CO_2] or water availability in exposing intraspecific variation in phenotypic plasticity for woody plant populations under future climates. The relationships between phenotypic plasticity and source environment variability of plant populations differed among the three species. Results from the two *Eucalyptus* species confirmed the general prediction that greater levels of environmental variability will select for plants with greater phenotypic plasticity, while findings from *T. speciosissima* contradicted the paradigm, indicating that woody plant populations originating from more variable environments may not necessarily show greater phenotypic plasticity in response to climate change. In addition, T_E negatively affected plant resistance to drought and heat stress exacerbated the negative effects of drought on plant responses, suggesting that temperature may influence the responses of woody plants to drought under future climates.

In summary, my research expands current knowledge regarding the interactive effects of simultaneously changing climatic variables (i.e., [CO_2], temperature, and water availability) on woody plant growth and physiology. More importantly, this

work contributes valuable information on intraspecific variation in phenotypic plasticity of woody plant populations in response to changing climatic variables, as well as the association between phenotypic plasticity and source environment variability, which will assist in making robust predictions of the distribution and abundance of woody species under future climates. However, it should be noted that the magnitude of changes in climatic variables is likely to affect the magnitude of plant responses found in my experiments. Therefore, further studies with more thorough experimental designs (e.g., more treatment levels for each climatic factor, and more genotypes/provenances for each species) would be substantially helpful for validating the findings in this research.

Appendix A

Supplementary figures

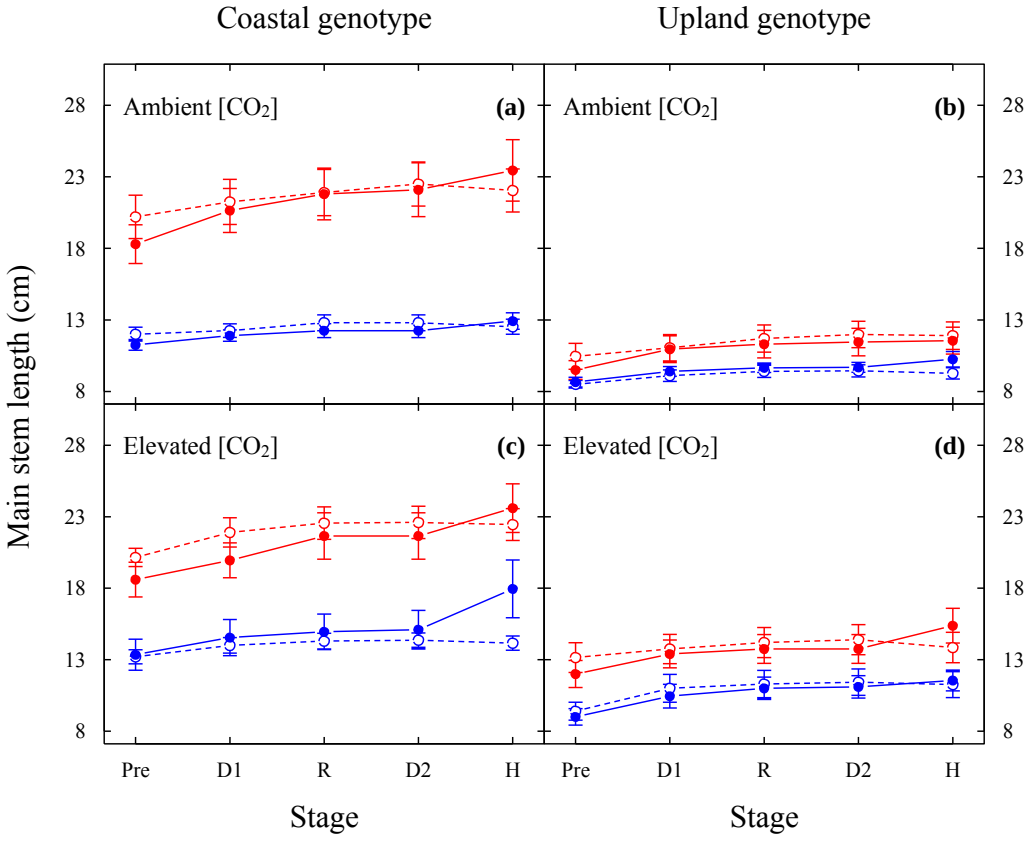

Figure A-1 Progression of main stem length in *Telopea speciosissima* Coastal (the left panel) and Upland (the right panel) genotypes in *well-watered* (closed symbols) and *drought* (open symbols) conditions subjected to *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures and *ambient* (C_A; the top panel) and *elevated* (C_E; the bottom panel) [CO_2] during the experimental stages: *pre drought* (Stage Pre), *first drought* (Stage D1), *recovery* (Stage R), *second drought* (Stage D2), and *final harvest* (Stage H). Values represent means ± 1 SE (n = 10).

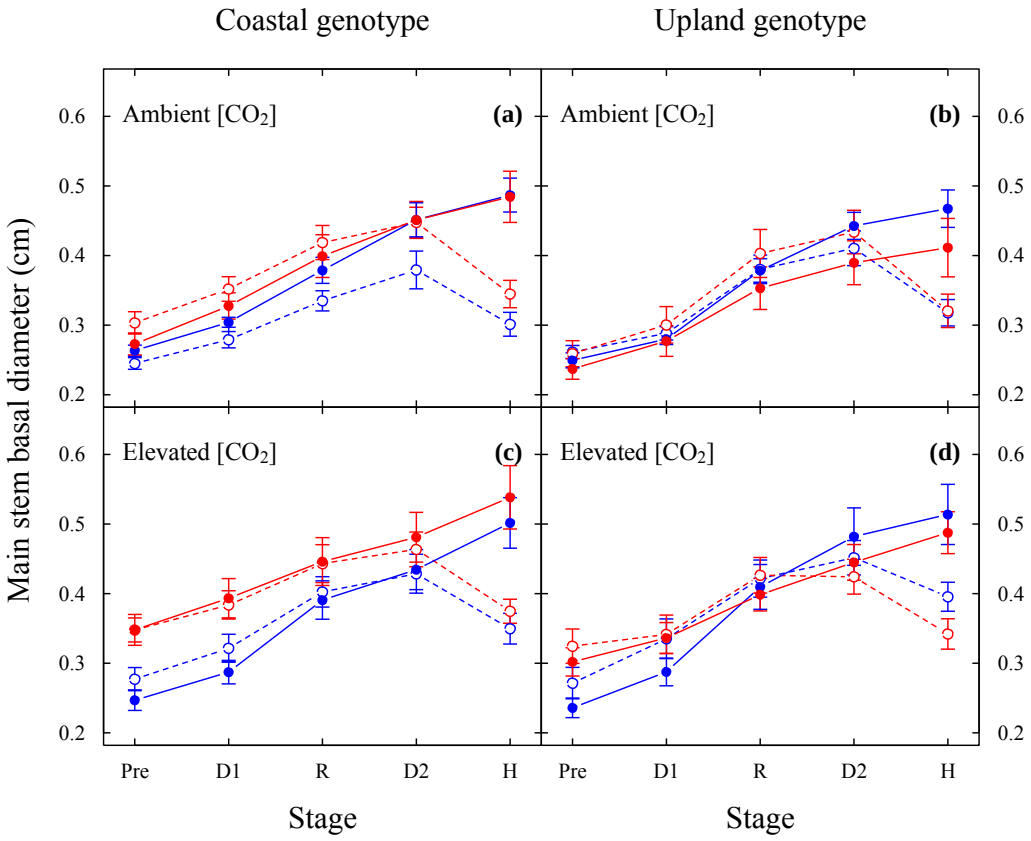

Figure A-2 Progression of main stem basal diameter in *Telopea speciosissima* Coastal and Upland genotypes in *well-watered* and *drought* conditions subjected to four [CO_2] and temperature treatment combinations during the experimental stages. Values represent means ± 1 SE (n = 10). Other details are as described for Fig. A-1.

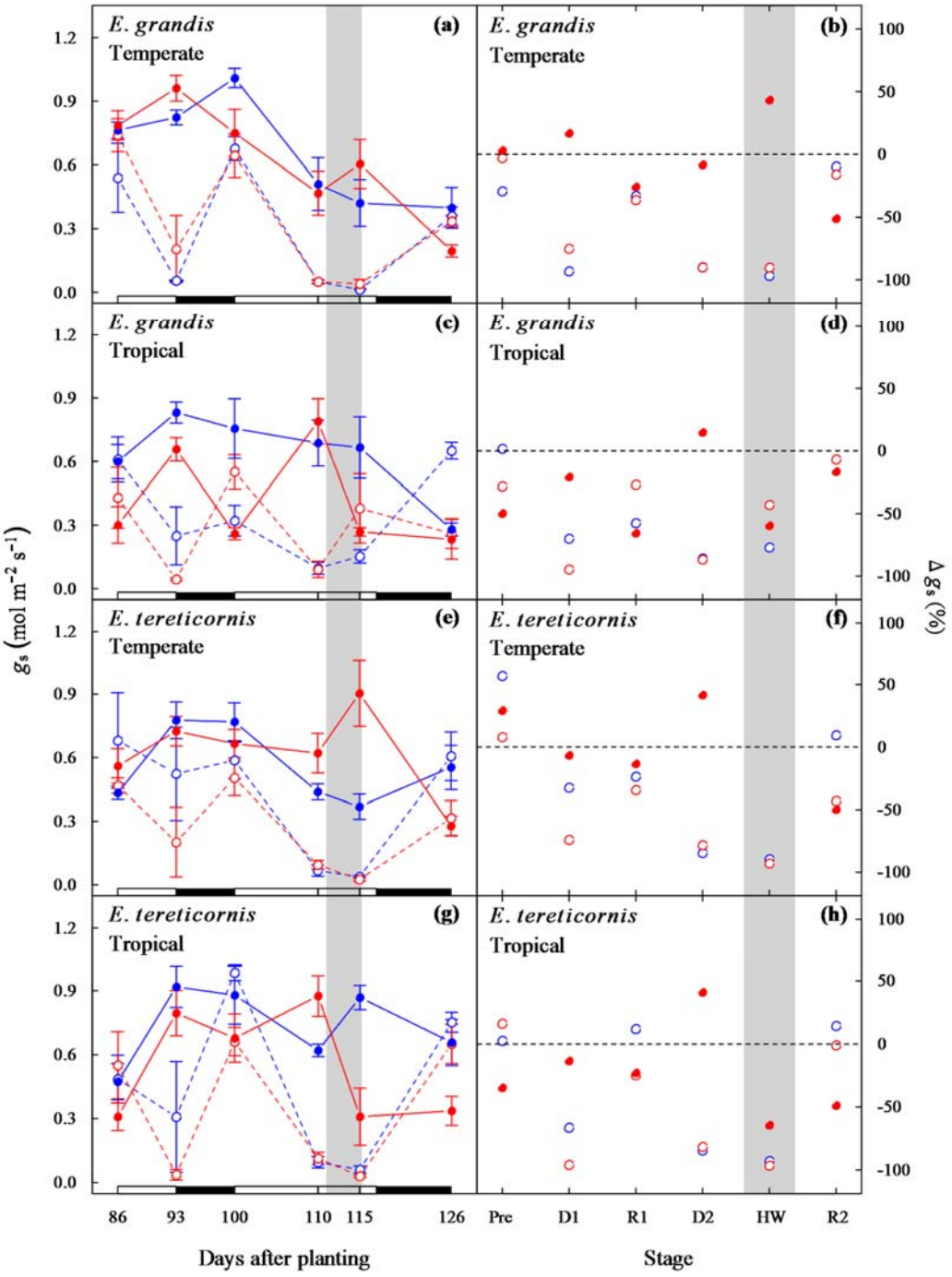

Figure A-3 Progression of stomatal conductance (g_s; the left panel) and percentage change in stomatal conductance (Δg_s; the right panel) in *E. grandis* and *E. tereticornis* seedlings from *temperate* and *tropical* provenances subjected to *well-watered* (closed symbols) and *drought* (open symbols) treatments under *ambient* (T_A; blue) and *elevated* (T_E; red) temperatures across the experimental stages described in the

Material and methods. The *x* axis scales in the left panel indicate watering regimes in *drought* seedlings, i.e., the controlled drought (open) and the full watering (closed). g_s values represent means ± 1 SE (n = 5). Δ g_s are calculated as follows: dividing the averaged g_s of each temperature and watering treatment combination by the mean g_s in the *ambient well-watered* treatment (shown as horizontal dashed lines at 0%, rather than coloured symbols) and then minus 100%. The grey area indicates the period during which the heat wave (+8 °C) was applied.

References

Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Linder CR, Sandquist DR, Geber MA, Evans AS, Dawson TE, Lachowicz MJ (2000) The evolution of plant ecophysiological traits: Recent advances and future directions. Bioscience, **50**, 979-995.

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, Villegas JC, Breshears DD, Zou CB, Troch PA, Huxman TE (2009) Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. Proceedings of the National Academy of Sciences, **106**, 7063-7066.

Ahuja I, De Vos RCH, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends in Plant Science, **15**, 664-674.

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist, **165**, 351-371.

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant, Cell & Environment, **30**, 258-270.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, Mcdowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A, Cobb N (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, **259**, 660-684.

Alpert P, Simms EL (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary Ecology, **16**, 285-297.

Ambebe TF, Dang QL (2010) Low moisture availability reduces the positive effect of increased soil temperature on biomass production of white birch (*Betula papyrifera*) seedlings in ambient and elevated carbon dioxide concentration. Nordic Journal of Botany, **28**, 104-111.

Ameye M, Wertin TM, Bauweraerts I, Mcguire MA, Teskey RO, Steppe K (2012) The effect of induced heat waves on *Pinus taeda* and *Quercus rubra* seedlings in ambient and elevated CO_2 atmospheres. New Phytologist, **196**, 448-461.

Anderson JT, Panetta AM, Mitchell-Olds T (2012) Evolutionary and Ecological Responses to Anthropogenic Climate Change. Plant Physiology, **160**, 1728-1740.

Aranda I, Alia R, Ortega U, Dantas AK, Majada J (2010) Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. Tree Genetics & Genomes, **6**, 169-178.

Aspinwall MJ, Loik ME, Resco De Dios V, Tjoelker MG, Payton PR, Tissue DT (2015) Utilizing intraspecific variation in phenotypic plasticity to bolster agricultural and forest productivity under climate change. Plant, Cell & Environment, **38**, 1752-1764.

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science, **8**, 343-351.

Ayub G, Smith RA, Tissue DT, Atkin OK (2011) Impacts of drought on leaf respiration in darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric CO_2 and growth temperature. New Phytologist, **190**, 1003-1018.

Ballester J, Rodo X, Giorgi F (2010) Future changes in Central Europe heat waves expected to mostly follow summer mean warming. Climate Dynamics, **35**, 1191-1205.

Bansal S, Harrington CA, Gould PJ, St Clair JB (2015) Climate-related genetic variation in drought-resistance of Douglas-fir (*Pseudotsuga menziesii*). Global Change Biology, **21**, 947-958.

Banta JA, Ehrenreich IM, Gerard S, Chou L, Wilczek A, Schmitt J, Kover PX, Purugganan MD (2012) Climate envelope modelling reveals intraspecific relationships among flowering phenology, niche breadth and potential range size in *Arabidopsis thaliana*. Ecology Letters, **15**, 769-777.

Baquedano FJ, Valladares F, Castillo FJ (2008) Phenotypic plasticity blurs ecotypic divergence in the response of *Quercus coccifera* and *Pinus halepensis* to water stress. European Journal of Forest Research, **127**, 495-506.

Barnosky AD, Hadly EA, Bascompte J, Berlow EL, Brown JH, Fortelius M, Getz WM, Harte J, Hastings A, Marquet PA, Martinez ND, Mooers A, Roopnarine P, Vermeij G, Williams JW, Gillespie R, Kitzes J, Marshall C, Matzke N, Mindell DP, Revilla E, Smith AB (2012) Approaching a state shift in Earth's biosphere. Nature, **486**, 52-58.

Barrows TT, Stone JO, Fifield LK, Cresswell RG (2001) Late Pleistocene glaciation of the Kosciuszko Massif, Snowy Mountains, Australia. Quaternary Research, **55**, 179-189.

Bastos A, Gouveia C, Trigo R, Running S (2013) Comparing the impacts of 2003 and 2010 heatwaves in NPP over Europe. Biogeosciences Discussions, **10**, 15879-15911.

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4, R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Bauweraerts I, Ameye M, Wertin TM, Mcguire MA, Teskey RO, Steppe K (2014) Water availability is the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves. Agricultural and Forest Meteorology, **189**, 19-29.

Bauweraerts I, Wertin TM, Ameye M, Mcguire MA, Teskey RO, Steppe K (2013) The effect of heat waves, elevated [CO_2] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. Global Change Biology, **19**, 517-528.

Bedon F, Villar E, Vincent D, Dupuy JW, Lomenech AM, Mabialangoma A, Chaumeil P, Barre A, Plomion C, Gion JM (2012) Proteomic plasticity of two *Eucalyptus* genotypes under contrasted water regimes in the field. Plant, Cell & Environment, **35**, 790-805.

Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carvalhais N, Rodenbeck C, Arain MA, Baldocchi D, Bonan GB, Bondeau A, Cescatti A, Lasslop G, Lindroth A, Lomas M, Luyssaert S, Margolis H, Oleson KW, Roupsard O, Veenendaal E, Viovy N, Williams C, Woodward FI, Papale D (2010) Terrestrial Gross Carbon Dioxide Uptake: Global Distribution and Covariation with Climate. Science, **329**, 834-838.

Berry J, Bjorkman O (1980) Photosynthetic Response and Adaptation to Temperature in Higher-Plants. Annual Review of Plant Physiology and Plant Molecular Biology, **31**, 491-543.

Bobich EG, Barron-Gafford GA, Rascher KG, Murthy R (2010) Effects of drought and changes in vapour pressure deficit on water relations of *Populus deltoides* growing in ambient and elevated CO_2. Tree Physiology, doi: 10.1093/treephys/tpq1036.

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to Environmental Stresses. The Plant Cell, **7**, 1099-1111.

Bonan GB (2008) Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. Science, **320**, 1444-1449.

Boyer JS (1982) Plant Productivity and Environment. Science, **218**, 443-448.

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics, **13**, 115-155.

Bunn AG, Graumlich LJ, Urban DL (2005) Trends in twentieth-century tree growth at high elevations in the Sierra Nevada and White Mountains, USA. Holocene, **15**, 481-488.

Callaway RM, Delucia EH, Thomas EM, Schlesinger WH (1994) Compensatory Responses of CO_2 Exchange and Biomass Allocation and Their Effects on the

Relative Growth-Rate of Ponderosa Pine in Different CO_2 and Temperature Regimes. Oecologia, **98**, 159-166.

Campbell C, Atkinson L, Zaragoza-Castells J, Lundmark M, Atkin O, Hurry V (2007) Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. New Phytologist, **176**, 375-389.

Cantin D, Tremblay MF, Lechowicz MJ, Potvin C (1997) Effects of CO_2 enrichment, elevated temperature, and nitrogen availability on the growth and gas exchange of different families of jack pine seedlings. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, **27**, 510-520.

Carter KK (1996) Provenance tests as indicators of growth response to climate change in 10 north temperate tree species. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, **26**, 1089-1095.

Ceulemans R, Shao BY, Jiang XN, Kalina J (1996) First- and second-year aboveground growth and productivity of two *Populus* hybrids grown at ambient and elevated CO_2. Tree Physiology, **16**, 61-68.

Chaves MM (1991) Effects of Water Deficits on Carbon Assimilation. Journal of Experimental Botany, **42**, 1-16.

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought - from genes to the whole plant. Functional Plant Biology, **30**, 239-264.

Chevin LM, Lande R, Mace GM (2010) Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. PLoS Biology, **8**, e1000357.

Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grunwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, **437**, 529-533.

Clark DA, Piper SC, Keeling CD, Clark DB (2003) Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984-2000. Proceedings of the National Academy of Sciences of the United States of America, **100**, 5852-5857.

Clark DB, Clark DA, Oberbauer SF (2010) Annual wood production in a tropical rain forest in NE Costa Rica linked to climatic variation but not to increasing CO_2. Global Change Biology, **16**, 747-759.

Collins M, Knutti R, Arblaster J, Dufresne J-L, Fichefet T, Friedlingstein P, Gao X, Gutowski WJ, Johns T, Krinner G, Shongwe M, Tebaldi C, Weaver AJ, Wehner M (2013) Long-term Climate Change: Projections, Commitments and Irreversibility. In: *Climate Change 2013: The Physical Science Basis.*

Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM) pp 1029-1136. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Coumou D, Rahmstorf S (2012) A decade of weather extremes. Nature Climate Change, **2**, 491-496.

Coumou D, Robinson A (2013) Historic and future increase in the global land area affected by monthly heat extremes. Environmental Research Letters, **8**, 034018.

Coumou D, Robinson A, Rahmstorf S (2013) Global increase in record-breaking monthly-mean temperatures. Climatic Change, **118**, 771-782.

Cramer W, Bondeau A, Woodward FI, Prentice IC, Betts RA, Brovkin V, Cox PM, Fisher V, Foley JA, Friend AD, Kucharik C, Lomas MR, Ramankutty N, Sitch S, Smith B, White A, Young-Molling C (2001) Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology, **7**, 357-373.

Cregg BM, Zhang JW (2001) Physiology and morphology of *Pinus sylvestris* seedlings from diverse sources under cyclic drought stress. Forest Ecology and Management, **154**, 131-139.

Cseke LJ, Tsai CJ, Rogers A, Nelsen MP, White HL, Karnosky DF, Podila GK (2009) Transcriptomic comparison in the leaves of two aspen genotypes having similar carbon assimilation rates but different partitioning patterns under elevated [CO_2]. New Phytologist, **182**, 891-911.

Cunningham SC, Read J (2006) Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia. Tree Physiology, **26**, 1435-1443.

Dai A (2013) Increasing drought under global warming in observations and models. Nature Climate Change, **3**, 52-58.

De Boeck HJ, Dreesen FE, Janssens IA, Nijs I (2010) Climatic characteristics of heat waves and their simulation in plant experiments. Global Change Biology, **16**, 1992-2000.

De Boeck HJ, Dreesen FE, Janssens IA, Nijs I (2011) Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist, **189**, 806-817.

Della-Marta PM, Haylock MR, Luterbacher J, Wanner H (2007) Doubled length of western European summer heat waves since 1880. Journal of Geophysical Research: Atmospheres, **112**, D15103, doi: 10.11029/12007JD008510.

Dickson RE, Coleman MD, Riemenschneider DE, Isebrands JG, Hogan GD, Karnosky DF (1998) Growth of five hybrid poplar genotypes exposed to interacting elevated CO_2 and O_3. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, **28**, 1706-1716.

Donohue K, Pyle EH, Messiqua D, Heschel MS, Schmitt J (2001) Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. Evolution, **55**, 692-702.

Doughty CE, Goulden ML (2008) Are tropical forests near a high temperature threshold? Journal of Geophysical Research: Biogeosciences, **113**, G00B07, doi:10.1029/2007JG000632.

Drake JE, Aspinwall MJ, Pfautsch S, Rymer PD, Reich PB, Smith RA, Crous KY, Tissue DT, Ghannoum O, Tjoelker MG (2015) The capacity to cope with climate warming declines from temperate to tropical latitudes in two widely distributed *Eucalyptus* species. Global Change Biology, **21**, 459-472.

Dreesen FE, De Boeck HJ, Janssens IA, Nijs I (2012) Summer heat and drought extremes trigger unexpected changes in productivity of a temperate annual/biannual plant community. Environmental and Experimental Botany, **79**, 21-30.

Duan H, Duursma RA, Huang G, Smith RA, Choat B, O'Grady AP, Tissue DT (2014) Elevated [CO_2] does not ameliorate the negative effects of elevated temperature on drought-induced mortality in *Eucalyptus radiata* seedlings. Plant, Cell & Environment, **37**, 1598-1613.

Duan H, O'Grady AP, Duursma RA, Choat B, Huang G, Smith RA, Jiang Y, Tissue DT (2015) Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated [CO_2] and temperature. Tree Physiology, **35**, 756-770.

Duan HL, Amthor JS, Duursma RA, O'Grady AP, Choat B, Tissue DT (2013) Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO_2] and elevated temperature. Tree Physiology, **33**, 779-792.

Dutkowski GW, Potts BM (2012) Genetic variation in the susceptibility of *Eucalyptus globulus* to drought damage. Tree Genetics & Genomes, **8**, 757-773.

Duursma RA, Barton CVM, Eamus D, Medlyn BE, Ellsworth DS, Forster MA, Tissue DT, Linder S, Mcmurtrie RE (2011) Rooting depth explains [CO_2] × drought interaction in *Eucalyptus saligna*. Tree Physiology, **31**, 922-931.

Ebell LF (1969) Variation in total soluble sugars of conifer tissues with method of analysis. Phytochemistry, **8**, 227-233.

Ellsworth DS (1999) CO_2 enrichment in a maturing pine forest: are CO_2 exchange and water status in the canopy affected? Plant, Cell & Environment, **22**, 461-472.

Fao (2006) Global forest resources assessment 2005: progress towards sustainable forest management. FAO Forestry Paper No. 147. Rome.

Feeley KJ, Wright SJ, Supardi MNN, Kassim AR, Davies SJ (2007) Decelerating growth in tropical forest trees. Ecology Letters, **10**, 461-469.

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Functional Plant Biology, **29**, 461-471.

Franks PJ, Adams MA, Amthor JS, Barbour MM, Berry JA, Ellsworth DS, Farquhar GD, Ghannoum O, Lloyd J, Mcdowell N, Norby RJ, Tissue DT, Von Caemmerer S (2013) Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. New Phytologist, **197**, 1077-1094.

Frich P, Alexander LV, Della-Marta P, Gleason B, Haylock M, Tank AMGK, Peterson T (2002) Observed coherent changes in climatic extremes during the second half of the twentieth century. Climate Research, **19**, 193-212.

Galloway LF (1995) Response to natural environmental heterogeneity: Maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. Evolution, **49**, 1095-1107.

Gauthier PP, Crous KY, Ayub G, Duan H, Weerasinghe LK, Ellsworth DS, Tjoelker MG, Evans JR, Tissue DT, Atkin OK (2014) Drought increases heat tolerance of leaf respiration in *Eucalyptus globulus* saplings grown under both ambient and elevated atmospheric [CO_2] and temperature. Journal of Experimental Biology, **65**, 6471-6485.

Ghannoum O, Phillips NG, Conroy JP, Smith RA, Attard RD, Woodfield R, Logan BA, Lewis JD, Tissue DT (2010a) Exposure to preindustrial, current and future atmospheric CO_2 and temperature differentially affects growth and photosynthesis in *Eucalyptus*. Global Change Biology, **16**, 303-319.

Ghannoum O, Phillips NG, Sears MA, Logan BA, Lewis JD, Conroy JP, Tissue DT (2010b) Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric [CO_2] and temperature. Plant, Cell & Environment, **33**, 1671-1681.

Gianoli E, Gonzalez-Teuber M (2005) Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). Evolutionary Ecology, **19**, 603-613.

Gratani L, Meneghini M, Pesoli P, Crescente MF (2003) Structural and functional plasticity of *Quercus ilex* seedlings of different provenances in Italy. Trees-Structure and Function, **17**, 515-521.

Grime JP, Mackey JML (2002) The role of plasticity in resource capture by plants. Evolutionary Ecology, **16**, 299-307.

Gunderson CA, Norby RJ, Wullschleger SD (2000) Acclimation of photosynthesis and respiration to simulated climatic warming in northern and southern populations of *Acer saccharum*: laboratory and field evidence. Tree Physiology, **20**, 87-96.

Hacke UG, Jacobsen AL, Brandon Pratt R, Maurel C, Lachenbruch B, Zwiazek J (2012) New research on plant-water relations examines the molecular, structural, and physiological mechanisms of plant responses to their environment. New Phytologist, **196**, 345-348.

Hamerlynck EP, Huxman TE, Loik ME, Smith SD (2000) Effects of extreme high temperature, drought and elevated CO_2 on photosynthesis of the Mojave Desert evergreen shrub, *Larrea tridentata*. Plant Ecology, **148**, 183-193.

Hanninen H, Tanino K (2011) Tree seasonality in a warming climate. Trends in Plant Science, **16**, 412-416.

Hereford J (2009) A Quantitative Survey of Local Adaptation and Fitness Trade-Offs. American Naturalist, **173**, 579-588.

Hesse PP, Humphreys GS, Selkirk PM, Adamson DA, Gore DB, Nobes DC, Price DM, Schwenninger JL, Smith B, Tulau M, Hemmings F (2003) Late Quaternary aeolian dunes on the presently humid Blue Mountains, eastern Australia. Quaternary International, **108**, 13-32.

Hoover DL, Knapp AK, Smith MD (2014) Resistance and resilience of a grassland ecosystem to climate extremes. Ecology, **95**, 2646-2656.

Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen TH (2003) From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Canadian Journal of Botany, **81**, 1247-1266.

Hozain MI, Salvucci ME, Fokar M, Holaday AS (2010) The differential response of photosynthesis to high temperature for a boreal and temperate *Populus* species relates to differences in Rubisco activation and Rubisco activase properties. Tree Physiology, **30**, 32-44.

Huang G, Rymer PD, Duan H, Smith RA, Tissue DT (2015) Elevated temperature is more effective than elevated [CO_2] in exposing genotypic variation in *Telopea speciosissima* growth plasticity: implications for woody plant populations under climate change. Global Change Biology, **21**, 3800-3813.

Isebrands JG, Mcdonald EP, Kruger E, Hendrey G, Percy K, Pregitzer K, Sober J, Karnosky DF (2001) Growth responses of *Populus tremuloides* clones to interacting elevated carbon dioxide and tropospheric ozone. Environmental Pollution, **115**, 359-371.

Jeffrey SJ, Carter JO, Moodie KB, Beswick AR (2001) Using spatial interpolation to construct a comprehensive archive of Australian climate data. Environmental Modelling & Software, **16**, 309-330.

Karnosky DF (2003) Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. Environment International, **29**, 161-169.

Karnosky DF, Pregitzer KS, Zak DR, Kubiske ME, Hendrey GR, Weinstein D, Nosal M, Percy KE (2005) Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant, Cell & Environment, **28**, 965-981.

Kattge J, Knorr W (2007) Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. Plant, Cell & Environment, **30**, 1176-1190.

Kawecki TJ (2008) Adaptation to Marginal Habitats. Annual Review of Ecology Evolution and Systematics, **39**, 321-342.

Kharin VV, Zwiers FW, Zhang XB, Hegerl GC (2007) Changes in temperature and precipitation extremes in the IPCC ensemble of global coupled model simulations. Journal of Climate, **20**, 1419-1444.

Kolb PF, Robberecht R (1996) High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. Tree Physiology, **16**, 665-672.

Kubiske ME, Quinn VS, Heilman WE, Mcdonald EP, Marquardt PE, Teclaw RM, Friend AL, Karnosky DF (2006) Interannual climatic variation mediates elevated CO_2 and O_3 effects on forest growth. Global Change Biology, **12**, 1054-1068.

Kubiske ME, Quinn VS, Marquardt PE, Karnosky DF (2007) Effects of elevated atmospheric CO_2 and/or O_3 on intra- and interspecific competitive ability of aspen. Plant Biology, **9**, 342-355.

Larcher W (2003) *Physiological plant ecology: ecophysiology and stress physiology of functional groups*, 4th edn. Springer Science & Business Media.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009a) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany, **60**, 2859-2876.

Leakey ADB, Ainsworth EA, Bernard SM, Markelz RJC, Ort DR, Placella SA, Rogers A, Smith MD, Sudderth EA, Weston DJ, Wullschleger SD, Yuan SH (2009b) Gene expression profiling: opening the black box of plant ecosystem responses to global change. Global Change Biology, **15**, 1201-1213.

Lewis JD, Lucash M, Olszyk D, Tingey DT (2001) Seasonal patterns of photosynthesis in Douglas fir seedlings during the third and fourth year of exposure to elevated CO_2 and temperature. Plant, Cell & Environment, **24**, 539-548.

Lewis JD, Lucash M, Olszyk DM, Tingey DT (2002) Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the

third and fourth years of exposure. Plant, Cell & Environment, **25**, 1411-1421.

Lewis JD, Smith RA, Ghannoum O, Logan BA, Phillips NG, Tissue DT (2013) Industrial-age changes in atmospheric [CO_2] and temperature differentially alter responses of faster- and slower-growing *Eucalyptus* seedlings to short-term drought. Tree Physiology, **33**, 475-488.

Lloyd J, Farquhar GD (2008) Effects of rising temperatures and [CO_2] on the physiology of tropical forest trees. Philosophical Transactions of the Royal Society B-Biological Sciences, **363**, 1811-1817.

Matesanz S, Gianoli E, Valladares F (2010) Global change and the evolution of phenotypic plasticity in plants. Year in Evolutionary Biology, **1206**, 35-55.

Mawdsley JR, O'malley R, Ojima DS (2009) A Review of Climate-Change Adaptation Strategies for Wildlife Management and Biodiversity Conservation. Conservation Biology, **23**, 1080-1089.

Mckenzie D, Hessl AE, Peterson DL (2001) Recent growth of conifer species of western North America: assessing spatial patterns of radial growth trends. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, **31**, 526-538.

Mclean EH, Prober SM, Stock WD, Steane DA, Potts BM, Vaillancourt RE, Byrne M (2014) Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. Plant, Cell & Environment, **37**, 1440-1451.

Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. Science, **305**, 994-997.

Melillo JM, Mcguire AD, Kicklighter DW, Moore B, Vorosmarty CJ, Schloss AL (1993) Global Climate-Change and Terrestrial Net Primary Production. Nature, **363**, 234-240.

Mitchell PJ, O'Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytologist, **197**, 862-872.

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends in Plant Science, **11**, 15-19.

Mohan JE, Clark JS, Schlesinger WH (2004) Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO_2. Global Change Biology, **10**, 233-247.

Moise AF, Hudson DA (2008) Probabilistic predictions of climate change for Australia and southern Africa using the reliability ensemble average of IPCCCMIP3 model simulations. Journal of Geophysical Research: Atmospheres, **113**, D15113, doi: 10.11029/12007JD009250.

Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Thiec D, Brechet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* x *Populus nigra*. New Phytologist, **169**, 765-777.

Moran EV, Hartig F, Bell DM (2016) Intraspecific trait variation across scales: implications for understanding global change responses. Global Change Biology, **22**, 137-150.

Morison JIL (1993) Response of plants to CO_2 under water limited conditions. Vegetatio, **104-105**, 193-209.

Morison JIL, Lawlor DW (1999) Interactions between increasing CO_2 concentration and temperature on plant growth. Plant, Cell & Environment, **22**, 659-682.

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y (2011) Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. Journal of Experimental Botany, **62**, 1715-1729.

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution, **4**, 133-142.

Nemani RR, Keeling CD, Hashimoto H, Jolly WM, Piper SC, Tucker CJ, Myneni RB, Running SW (2003) Climate-driven increases in global terrestrial net primary production from 1982 to 1999. Science, **300**, 1560-1563.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, Van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends in Plant Science, **15**, 684-692.

Nixon P (1997) *The Waratah*, 2nd edn. Kangaroo Press, Sydney.

Noormets A, Mcdonald E, Dickson R, Kruger E, Sôber A, Isebrands J, Karnosky D (2001) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. Trees, **15**, 262-270.

Norby RJ, Delucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, Mccarthy HR, Moore DJP, Ceulemans R, De Angelis P, Finzi AC, Karnosky DF, Kubiske ME, Lukac M, Pregitzer KS, Scarascia-Mugnozza GE, Schlesinger WH, Oren R (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. Proceedings of the National Academy of Sciences of the United States of America, **102**, 18052-18056.

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2 - do photosynthetic and productivity data from FACE experiments support early predictions? New Phytologist, **162**, 253-280.

Oishi AC, Oren R, Novick K, Palmroth S, Katul G (2010) Interannual Invariability of Forest Evapotranspiration and Its Consequence to Water Flow Downstream. Ecosystems, **13**, 421-436.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, Ciais P, Jackson RB, Pacala SW, Mcguire AD, Piao S, Rautiainen A, Sitch S, Hayes D (2011) A large and persistent carbon sink in the world's forests. Science, **333**, 988-993.

Pearce K, Holper PN, Hopkins M, Bouma WJ, Whetton P, Hennessy KJ, Power SB (2007) *Climate Change in Australia: technical report 2007*, CSIRO Marine and Atmospheric Research.

Peltola H, Kilpelainen A, Kellomaki S (2002) Diameter growth of Scots pine (*Pinus sylvestris*) trees grown at elevated temperature and carbon dioxide concentration under boreal conditions. Tree Physiology, **22**, 963-972.

Perkins SE, Alexander LV (2013) On the Measurement of Heat Waves. Journal of Climate, **26**, 4500-4517.

Perry LG, Shafroth PB, Blumenthal DM, Morgan JA, Lecain DR (2013) Elevated CO_2 does not offset greater water stress predicted under climate change for native and exotic riparian plants. New Phytologist, **197**, 532-543.

Pinheiro J, Bates D, Debroy S, Sarkar D, R Core Team (2016) nlme: Linear and Nonlinear Mixed Effects Models, R Package version 3.1-126, http://CRAN.R-project.org/package=nlme.

Pittock AB (2003) *Climate change: an Australian guide to the science and potential impacts*, Australian Greenhouse Office Canberra.

Poorter H, Pérez-Soba M (2001) The growth response of plants to elevated CO_2 under non-optimal environmental conditions. Oecologia, **129**, 1-20.

Prudhomme C, Giuntoli I, Robinson EL, Clark DB, Arnell NW, Dankers R, Fekete BM, Franssen W, Gerten D, Gosling SN, Hagemann S, Hannah DM, Kim H, Masaki Y, Satoh Y, Stacke T, Wada Y, Wisser D (2014) Hydrological droughts in the 21st century, hotspots and uncertainties from a global multimodel ensemble experiment. Proceedings of the National Academy of Sciences, **111**, 3262-3267.

Rahmstorf S, Coumou D (2011) Increase of extreme events in a warming world. Proceedings of the National Academy of Sciences of the United States of America, **108**, 17905-17909.

Ramirez-Valiente JA, Sanchez-Gomez D, Aranda I, Valladares F (2010) Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. Tree Physiology, **30**, 618-627.

Rehfeldt GE, Ying CC, Spittlehouse DL, Hamilton DA (1999) Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. Ecological Monographs, **69**, 375-407.

Reichstein M, Bahn M, Ciais P, Frank D, Mahecha MD, Seneviratne SI, Zscheischler J, Beer C, Buchmann N, Frank DC, Papale D, Rammig A, Smith P, Thonicke K, Van Der Velde M, Vicca S, Walz A, Wattenbach M (2013) Climate extremes and the carbon cycle. Nature, **500**, 287-295.

Reichstein M, Ciais P, Papale D, Valentini R, Running S, Viovy N, Cramer W, Granier A, Ogee J, Allard V, Aubinet M, Bernhofer C, Buchmann N, Carrara A, Grunwald T, Heimann M, Heinesch B, Knohl A, Kutsch W, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Pilegaard K, Pumpanen J, Rambal S, Schaphoff S, Seufert G, Soussana JF, Sanz MJ, Vesala T, Zhao M (2007) Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. Global Change Biology, **13**, 634-651.

Riikonen J, Kets K, Darbah J, Oksanen E, Sober A, Vapaavuori E, Kubiske ME, Nelson N, Karnosky DF (2008) Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO_2 and O_3. Tree Physiology, **28**, 243-254.

Robinson EA, Ryan GD, Newman JA (2012) A meta-analytical review of the effects of elevated CO_2 on plant–arthropod interactions highlights the importance of interacting environmental and biological variables. New Phytologist, **194**, 321-336.

Robson TM, Sanchez-Gomez D, Cano FJ, Aranda I (2012) Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. Tree Genetics & Genomes, **8**, 1111-1121.

Rossetto M, Thurlby K, Offord C, Allen C, Weston P (2011) The impact of distance and a shifting temperature gradient on genetic connectivity across a heterogeneous landscape. BMC Evolutionary Biology, **11**, 1-11.

Sage RF, Kubien DS (2007) The temperature response of C_3 and C_4 photosynthesis. Plant, Cell & Environment, **30**, 1086-1106.

Sage RF, Way DA, Kubien DS (2008) Rubisco, Rubisco activase, and global climate change. Journal of Experimental Botany, **59**, 1581-1595.

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiology, **32**, 764-775.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Biodiversity - Global biodiversity scenarios for the year 2100. Science, **287**, 1770-1774.

Savolainen O, Pyhajarvi T, Knurr T (2007) Gene flow and local adaptation in trees. Annual Review of Ecology Evolution and Systematics, **38**, 595-619.

Saxe H, Cannell MGR, Johnsen B, Ryan MG, Vourlitis G (2001) Tree and forest functioning in response to global warming. New Phytologist, **149**, 369-399.

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytologist, **139**, 395-436.

Schimel DS, House JI, Hibbard KA, Bousquet P, Ciais P, Peylin P, Braswell BH, Apps MJ, Baker D, Bondeau A, Canadell J, Churkina G, Cramer W, Denning AS, Field CB, Friedlingstein P, Goodale C, Heimann M, Houghton RA, Melillo JM, Moore B, 3rd, Murdiyarso D, Noble I, Pacala SW, Prentice IC, Raupach MR, Rayner PJ, Scholes RJ, Steffen WL, Wirth C (2001) Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature, **414**, 169-172.

Schlichting CD (1986) The Evolution of Phenotypic Plasticity in Plants. Annual Review of Ecology and Systematics, **17**, 667-693.

Seneweera S, Norton RM (2011) Plant Responses to Increased Carbon Dioxide. In: *Crop Adaptation to Climate Change.* (eds Yadav SS, Redden RJ, Hatfield JL, Lotze-Campen H, Hall AE) pp 198-217. Oxford, United Kingdom, Wiley-Blackwell.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant, Cell & Environment, **30**, 1035-1040.

Silva FCE, Shvaleva A, Maroco JP, Almeida MH, Chaves MM, Pereira JS (2004) Responses to water stress in two *Eucalyptus globulus* clones differing in drought tolerance. Tree Physiology, **24**, 1165-1172.

Silva JCE, Potts BM, Dutkowski GW (2006) Genotype by environment interaction for growth of *Eucalyptus globulus* in Australia. Tree Genetics & Genomes, **2**, 61-75.

Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. Plant, Cell & Environment, **30**, 1126-1149.

Smith MD (2011) An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. Journal of Ecology, **99**, 656-663.

Solomon S, Plattner GK, Knutti R, Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Sciences of the United States of America, **106**, 1704-1709.

Stefanon M, Drobinski P, D'andrea F, Lebeaupin-Brossier C, Bastin S (2014) Soil moisture-temperature feedbacks at meso-scale during summer heat waves over Western Europe. Climate Dynamics, **42**, 1309-1324.

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant, Cell & Environment, **22**, 583-621.

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends in Plant Science, **5**, 537-542.

Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Gioli B, Calfapietra C, Gielen B, Kubiske ME, Scarascia-Mugnozza GE, Kets K, Long SP, Karnosky DF (2008) Future atmospheric CO_2 leads to delayed autumnal senescence. Global Change Biology, **14**, 264-275.

Tebaldi C, Hayhoe K, Arblaster JM, Meehl GA (2006) Going to the extremes. Climatic Change, **79**, 185-211.

Teskey R, Wertin T, Bauweraerts I, Ameye M, Mcguire MA, Steppe K (2015) Responses of tree species to heat waves and extreme heat events. Plant, Cell & Environment, **38**, 1699-1712.

Teskey RO, Will RE (1999) Acclimation of loblolly pine (*Pinus taeda*) seedlings to high temperatures. Tree Physiology, **19**, 519-525.

Thompson JD (1991) Phenotypic Plasticity as a Component of Evolutionary Change. Trends in Ecology & Evolution, **6**, 246-249.

Thomson AM, Riddell CL, Parker WH (2009) Boreal forest provenance tests used to predict optimal growth and response to climate change: 2. Black spruce. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, **39**, 143-153.

Tjoelker MG, Oleksyn J, Lorenc-Plucinska G, Reich PB (2009) Acclimation of respiratory temperature responses in northern and southern populations of *Pinus banksiana*. New Phytologist, **181**, 218-229.

Trenberth KE (2011) Changes in precipitation with climate change. Climate Research, **47**, 123-138.

Valladares F, Gianoli E, Gomez JM (2007) Ecological limits to plant phenotypic plasticity. New Phytologist, **176**, 749-763.

Van Kleunen M, Fischer M (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. New Phytologist, **166**, 49-60.

Vautard R, Yiou P, D'andrea F, De Noblet N, Viovy N, Cassou C, Polcher J, Ciais P, Kageyama M, Fan Y (2007) Summertime European heat and drought waves induced by wintertime Mediterranean rainfall deficit. Geophysical Research Letters, **34**, L07711, doi: 10.01029/02006GL028001.

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: An overview. Environmental and Experimental Botany, **61**, 199-223.

Wang D, Heckathorn SA, Wang XZ, Philpott SM (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. Oecologia, **169**, 1-13.

Wang TL, O'neill GA, Aitken SN (2010) Integrating environmental and genetic effects to predict responses of tree populations to climate. Ecological Applications, **20**, 153-163.

Wang WX, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, **218**, 1-14.

Way DA (2013) Will rising CO_2 and temperatures exacerbate the vulnerability of trees to drought? Tree Physiology, **33**, 775-778.

Way DA, Oren R (2010) Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. Tree Physiology, **30**, 669-688.

Way DA, Sage RF (2008) Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) BSP]. Plant, Cell & Environment, **31**, 1250-1262.

Weinig C (2000) Plasticity versus canalization: Population differences in the timing of shade-avoidance responses. Evolution, **54**, 441-451.

Wertin TM, Mcguire MA, Teskey RO (2010) The influence of elevated temperature, elevated atmospheric CO_2 concentration and water stress on net photosynthesis of loblolly pine (*Pinus taeda* L.) at northern, central and southern sites in its native range. Global Change Biology, **16**, 2089-2103.

Wertin TM, Mcguire MA, Teskey RO (2011) Higher growth temperatures decreased net carbon assimilation and biomass accumulation of northern red oak seedlings near the southern limit of the species range. Tree Physiology, **31**, 1277-1288.

Wertin TM, Mcguire MA, Teskey RO (2012) Effects of predicted future and current atmospheric temperature and [CO_2] and high and low soil moisture on gas exchange and growth of *Pinus taeda* seedlings at cool and warm sites in the species range. Tree Physiology, **32**, 847-858.

Weston DJ, Bauerle WL (2007) Inhibition and acclimation of C_3 photosynthesis to moderate heat: a perspective from thermally contrasting genotypes of *Acer rabrum* (red maple). Tree Physiology, **27**, 1083-1092.

Weston DJ, Bauerle WL, Swire-Clark GA, Moore BD, Baird WMV (2007) Characterization of Rubisco activase from thermally contrasting genotypes of *Acer rubrum* (Aceraceae). American Journal of Botany, **94**, 926-934.

Weston P, Crisp M (1994) Cladistic biogeography of waratahs (Proteaceae, Embothrieae) and their allies across the pacific. Australian Systematic Botany, **7**, 225-249.

Will RE, Wilson SM, Zou CB, Hennessey TC (2013) Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest–grassland ecotone. New Phytologist, **200**, 366-374.

Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G (2008) Towards an Integrated Framework for Assessing the Vulnerability of Species to Climate Change. PLoS Biology, **6**, 2621-2626.

Wullschleger SD, Tschaplinski TJ, Norby RJ (2002) Plant water relations at elevated CO_2 - implications for water-limited environments. Plant, Cell & Environment, **25**, 319-331.

Yao Y, Luo Y, Huang JB, Zhao ZC (2013) Comparison of Monthly Temperature Extremes Simulated by CMIP3 and CMIP5 Models. Journal of Climate, **26**, 7692-7707.

Zeppel MJB, Lewis JD, Chaszar B, Smith RA, Medlyn BE, Huxman TE, Tissue DT (2012) Nocturnal stomatal conductance responses to rising [CO_2], temperature and drought. New Phytologist, **193**, 929-938.

Zhao J, Hartmann H, Trumbore S, Ziegler W, Zhang Y (2013) High temperature causes negative whole-plant carbon balance under mild drought. New Phytologist, **200**, 330-339.

Zhao MS, Running SW (2010) Drought-Induced Reduction in Global Terrestrial Net Primary Production from 2000 Through 2009. Science, **329**, 940-943.

Zinta G, Abdelgawad H, Domagalska MA, Vergauwen L, Knapen D, Nijs I, Janssens IA, Beemster GTS, Asard H (2014) Physiological, biochemical, and genome-wide transcriptional analysis reveals that elevated CO_2 mitigates the impact of combined heat wave and drought stress in Arabidopsis thaliana at multiple organizational levels. Global Change Biology, **20**, 3670-3685.

Zweifel R, Zimmermann L, Zeugin F, Newbery DM (2006) Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. Journal of Experimental Botany, **57**, 1445-1459.