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Identifying plant functional traits to assist ecological intervention in a drying landscape

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Identifying plant functional traits to assist ecological intervention in a drying landscape

This thesis is presented for the degree of

Doctor of Philosophy

Ana Luíza Muler

Edith Cowan University

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DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

1. *incorporate without acknowledgment, any material previously submitted for a degree or diploma in any institution of higher education;*
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Ana Luiza Muler

August 2016

ABSTRACT

Mediterranean-type ecosystems (*MTEs*) are among the most vulnerable to land use and climate change and many attempts are in place to restore these ecosystems. Therefore, it is necessary to assess differences in plants' ability to withstand water-stress, including biotic interactions. Such knowledge helps us understand community assembly, which is crucial for ecological intervention. This study involved: (1) reviewing the literature on traits that can differentiate functional types; (2) adapting the methodology to measure leaf water potential at turgor loss point (π_{tlp}) for small-leaved species; (3) using these traits to quickly identify water-use strategies of adult plants from Southwest Australia; (4) identifying the water-use strategies that juveniles have to survive first summer drought; and (5) determining whether there is facilitation between a deep-rooted species and seedlings through hydraulic redistribution.

The selection of functional response traits was based on their association with water-stress, with effect traits, and on methodologies that are easy, inexpensive and applicable for Mediterranean species. Relevant traits were identified from the literature, including: leaf carbon isotope composition, leaf nitrogen and phosphorus contents, leaf mass per area, π_{tlp}, and xylem vessel morphology. Measurements of π_{tlp} through osmometry of extracted sap and through Pressure-volume curves were compared. Selected traits were then measured for 15 species from different eco-hydrological habitats. Drought resistance of juveniles was assessed by measuring water relations, rooting depth/pattern and carbon reserves use of species from different eco-hydrological habitats. Lastly, seedlings were grown isolated or near donor plants. Water status and growth were measured, and stable isotopes were used to investigate water pathways within and between plants.

A strong correlation between the methodologies for measuring π_{tlp} was found. With analysis of these traits, it was possible to cluster adult species, from the Swan Coastal plain, into five functional groups that corresponded to their rooting depths. During drought, *Banksia* seedlings reduced stomatal conductance and appeared to use carbon reserves, whereas *Gompholobium tomentosum* seedlings tolerated higher water deficits, despite reduced stomatal conductance. Lastly, although seedlings were able to absorb hydraulically redistributed water, they grew, transpired and survived more when isolated from the deep-rooted plant.

In the literature review, theoretical analyses on functional traits and speculations on functional groups were made through a conceptual diagram. The osmometry technique is a suitable replacement for Pressure-volume curves since its estimations of π_{tlp} were accurate in small and large-leaved species. The functional traits approach can be transferable to other *MTEs* for application by restoration practitioners, as the traits selected were effective in determining functional groups, and were relatively easy and cost effective. The seedlings' responses to summer drought were consistent with their habitats and root-depth, which is an important factor for niche differentiating and community assembly. Competition between seedlings and deep-rooted plants supported the updated stress-gradient hypothesis. In conclusion, analyses of water-use strategies of Mediterranean species during summer allow predictions of differences in drought resistance. When this functional approach is applied for ecological intervention, restoration practitioners can select species with a better match to future environmental conditions of *MTEs*, particularly in large species sets.

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LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS

This thesis is presented as a series of manuscripts that are to be submitted for publication in international journals. I am the first author with a contribution of more than 50% to each publication. The thesis conforms to the "thesis with publication" style of Edith Cowan University.

I. Muler AL, Canham CA, van Etten EJB, Stock WD, Froend RH, *In prep.* Selection of key plant water deficit resistance traits for restoration: An overview for Mediterranean-type environments.

Journal targeted: Forest Ecology and Management

II. Muler AL, Canham CA, van Etten EJB, Stock WD, Froend RH, *In prep.*
Application of an osmometer technique to rapidly predict turgor loss point in small scherophyll leaves.

Journal targeted: Physiologia Plantarum

III. Muler AL, Canham CA, Melissa Karlinski, van Etten EJB, Stock WD, Froend RH, *In prep.* A functional ecological approach to identify plant resistance to water deficits in a Mediterranean-type ecosystem.

Journal targeted: Journal of Vegetation Science

IV. Muler AL, van Etten EJB, Stock WD, Froend RH, *In prep.* Responses to first summer drought of seedlings of four Southwestern Australian species.

Journal targeted: Australian Journal of Botany

V. Muler AL, van Etten EJB, Stock WD, Froend RH, *In prep.* Can hydraulically redistributed water assist survival and growth of surrounding seedlings during summer drought?

Journal targeted: Oecologia

CONTENTS

This thesis is dedicated to my two sisters and to my mother for always supporting me through this journey, far from home. Thanks girls!

CHAPTER ONE: GENERAL INTRODUCTION

1.1 INTRODUCTION

Worldwide, ecosystems have been disturbed on unprecedented scales, through processes such as extensive land use change, species invasion, climate change, and altered biogeochemical cycles (Dentener et al., 2006; Foster et al., 2003; McLauchlan, 2006). Under new conditions, the process of restoring a disturbed ecosystem to its previous state is challenging and, in many cases, impracticable (Harris et al., 2006; Suding, 2011). Thus, restoration projects should consider that current systems have already been transformed and may well continue changing in the future, due to climate and other global changes (Hobbs et al., 2006; Seastedt et al., 2008). It has been proposed that restoration projects should: (1) understand ecosystem dynamics; (2) diagnose damage; (3) set goals in a changing environment; and (4) incorporate socioeconomic aspects (Hobbs, 2007). Studies of ecosystem dynamics allow us to understand how ecosystem composition, structure and function respond to disturbances, including how they respond to restoration itself (Hobbs, 2007). Also, considering that ecosystems will continue changing, there may not be a unique and "correct" state to aim for in restoration. However, the predictions of future climate change and other abiotic conditions can be used to plan ecological intervention, in which it may be more appropriate to aim for intervention that re-establishes function and/or at least a subset of endemic species. This approach can lead to more successful outcomes than aiming for "complete" restoration of the ecosystem to an unsustainable original state. Therefore, understanding ecosystem dynamics and setting realistic restoration goals can successfully contribute to reinstating essential processes and establishing a ecosystem that is resilient and/or resistant to future changes (Hobbs, 2007). Moreover, understanding how natural communities function helps us to evaluate intervention success and guides intervention decisions. Therefore, the first step of ecological intervention projects should focus on understanding ecosystem dynamics, functionality and assembly.

An integrated view of community assembly proposes that ecological filters imposed by biotic and abiotic conditions determine which functional traits will be selected for a particular environment (Díaz et al., 1999; Hobbs and Norton, 2004; Leibold et al., 2004). Filters select the species whose traits are most suitable for a particular community. Understanding the interaction between these filters and the species pool is becoming extremely important for biodiversity conservation in an era of intense ecosystem fragmentation, habitat loss and climate change (Funk et al., 2008). Such knowledge would also be helpful in selecting appropriate species for ecological intervention projects (Sandel et al., 2011).

Water availability may function as an ecological filter in ecosystems that experience drought periods, selecting species with traits that enable them to survive times when water availability is low. In such communities, one of the factors that allows the co-existence of several plant species is a diversity of water-use strategies that results in niche differentiation and reduced competition (Walter, 1971). Plants can access water from different soil horizons (Meinzer et al., 2007; Walter, 1971), as well as use this resource in different ways (Cunningham, 2004; McDowell et al., 2008), including the ability to "avoid" or "tolerate" drought (Levitt, 1972). This is the case for Mediterranean-type ecosystems (*MTEs*), which experience hot, dry summers and mild, wet winters (Aschmann, 1973). Consequently, soil water availability during the dry summer represents a major environmental limitation under Mediterranean climate conditions (Castri, 1973). At the same time, *MTEs* are highly diverse (Cowling et al., 1996), containing plant species with quite different types of functional responses to assimilation of carbon and water use. There are species with different hydraulic architecture, specialized leaf physiology and morphology, efficient control of stomatal conductance, deep roots and specialized root morphology (Hernández et al., 2010; Mitchell et al., 2008; Vilagrosa et al., 2013; West et al., 2012).

Mediterranean ecosystems, however, are some of the most highly modified ecosystems on Earth (Hobbs et al., 1995) and are under risk of future droughts as a consequence of climate change (IPCC, 2014; Klausmeyer and Shaw, 2009). Enhanced seedling mortality due to water stress under Mediterranean conditions is already one of the main causes of low success in intervention projects, only 53% of success in sites of South Western Australia (Hallett et al., 2014; Lloret et al., 2005; Mendoza et al., 2009; Rokich, 2016; Vallejo et al., 2012). This scenario is likely to be aggravated under drier conditions predicted for *MTEs*. Therefore, we need to know more about strategies that plants employ to cope with the water stress that is already experienced in summer. This knowledge is relevant to identifying species whose traits enable them to survive in a drying environment, particularly in drying climates, as well as to detect important interactions that affect community assembly.

The characterization of plant responses to water stress, in terms of functional response types, is a promising approach for identifying the strategies important to summer drought survival (Gondard et al., 2003). Species can be classified into “hydraulic functional types” (HFTs), according to their specific hydrological functions within the ecosystem (Mitchell et al., 2008), and independently of taxonomic groups (Shugart and Woodward, 1997). In order to identify the HFTs in *MTEs*, analyses of functional traits of vast species sets are needed. Plant functional traits are attributes of organisms that are considered important for their responses to the environment, and can be used to define a species ecological role or function (Díaz and Cabido, 2001; Lavorel and Garnier, 2002; Wright et al., 2004).

The first step in screening plant functional types for tolerance of seasonal drought in *MTEs* is the selection of key physiological and anatomical traits related to water stress. A variety of reproductive, life- history and morphological traits have been used in ecological intervention projects (Ostertag et al., 2015; Pywell et al., 2003; Weiher et al., 1999). Some of these are linked to resistance to water deficits, and hence are used to identify HFTs (Mitchell et al., 2008). Furthermore, studies focusing on functional traits that can be measured efficiently, allow for multiple species to be assessed, which is important for diverse systems like *MTEs*.

Another important factor in ecological intervention of *MTEs* is that abiotic and competitive stress can be severe at the recruitment stage (Hallett et al., 2014; Lloret et al., 2005; Mendoza et al., 2009; Rokich, 2016; Vallejo et al., 2012). Therefore, the comparative ability of seedlings to cope with water deficits during establishment is also important for understanding community assembly and functioning (Pigott and Pigott, 1993; Zavala and Zea, 2004). Particularly during the first summer drought, as in Mediterranean ecosystems, the first exposure to prolonged drought represents a critical period for the establishment of seedlings (Cowling et al., 1987; Davis, 1991; Enright and Lamont, 1992a; Frazer and Davis, 1988). The ability to grow deep-roots, reduce stomata conductance, accumulate and use non-structural carbohydrates (NSC), and to tolerate high tensions in xylem vessels, are all strategies that, individually or in combination, can improve plant resistance to summer drought (Aroca, 2012; Chaves et al., 2003). Improving seedling establishment and survival, by selecting species appropriate for site hydrological conditions, has become particularly important for ecological intervention (Resco de Dios et al., 2006). Therefore, studies that include analyses of resistance to water deficits of adult, as well as of juveniles, will provide more complete information on how the community is functioning.

Seedlings of some species may survive the water deficits during their first summers by benefiting from interactions with already established plants. The rationale for this is that germination usually takes place in the first winter following fire, corresponding with the rainy season (Bond, 1984; Le Maitre and Midgely, 1992; Miller and Dixon, 2014). Thus, seedlings may have not yet developed the root systems that are capable of maintaining access to soil moisture during their first summer drought and/or may have not yet formed a robust hydraulic system able to tolerate high water deficits. Indeed, facilitation — positive interactions between individuals in which at least one benefits from the interaction (Stachowicz, 2001) — plays an important role in many plant communities (Callaway and Walker, 1997a). Facilitation acts to reduce the negative influences of a stressful environment, leading to multiple outcomes (Callaway, 1995). For instance, it can alter species distribution patterns, increase species coexistence and enhance diversity and productivity, all of which greatly influence ecosystem structure and function (Callaway, 1995).

Some species, whose roots are accessing water in deep layers, can facilitate access to water for neighbouring plants through “hydraulic redistribution” (HR: Caldwell et al., 1998; Pang et al., 2013; Yu and D’Odorico, 2015). Hydraulic lift is the passive movement of water upward from deep wet layers to shallow drier layers within plant root systems driven by a water potential gradient (Burgess et al., 1998; Richards and Caldwell, 1987). This is particularly relevant for systems where there is significant storage of water in deeper soil layers that can be potentially moved by hydraulic lift and redistribution. Some of this water if redistributed to the shallow soil layers may facilitate seedling growth and survivorship. Therefore, knowledge of the potential effects of facilitation on community assembly can help in identifying the species that require assistance during the first summer droughts, as well as in establishing the role of key species in restoration (Bruno et al., 2003).

1.2 AIMS

In order to assist with the conservation, management and ecological intervention of *MTEs* under increased risk of climatic drought, there is a need for novel and robust theory-based approaches. I propose the use of functional traits, and ultimately the classification of species into hydrological functional types, to assess responses of adult plants to water deficits in *MTEs*. Such knowledge can highly contribute to our understanding of community assembly and function, especially if associated to specific investigations on the strategies that juveniles use to survive first summer drought, including important interactions. This type of information will enable more accurate selection of plant species whose traits are more advantageous under changing environmental conditions. In a broad sense, the main question that inspired this thesis was: what water-use strategies enable Mediterranean plant species (adult and juveniles) to survive summer drought (Fig.1.1)? In order to address this question, I conducted five studies with species from southwest Australia. In three of these studies (Chapter 1, 2 and 3), I focused in identifying key functional traits, improving their methodology and measuring these traits in adult plants to assess their different strategies to survive summer drought. In the other two studies (Chapter 5 and 6), I focused in the strategies that juveniles have to survive summer drought, including important interactions (Fig.1.1). The specific aims were to:

1) **Review functional traits that are relevant to drought resistance under Mediterranean conditions.** In Chapter 2, a literature review was conducted in order to identify response traits that were associated with drought resistance, and that could be efficiently measured in a timely fashion and in a diverse of leaf shapes and sizes. These attributes are important since many plant species need to be assessed in diverse ecosystems such as *MTEs*.

2) **Improve and test the methodology for the measurement of one of the selected traits, allowing rapid, efficient and accurate measurements of small-leaved species.** In Chapter 3, I compared a standard methodology for measuring leaf water potential at turgor loss point (Pressure-volume curves) to an alternative methodology (osmometry of extracted sap). This alternative methodology has the potential to be more efficient

and suitable for small and large-leaved species, which is beneficial for assessment of diverse *MTEs* due to the high number of small-leaved species.

3) **Assess and compare the different strategies that adult plants use to survive summer drought in Southwest Australia**. In Chapter 4, I applied the trait-based framework proposed in Chapter 2, and measured the selected functional traits in species from contrasting eco-hydrological habitats within Southwest Australia.

4) **Determine the strategies that juveniles use to survive first summer drought in southwest Australia.** In Chapter 5, I focused in juveniles as they are under severe abiotic and competitive stress during their first summer drought. I conducted two greenhouse experiments and analysed water relations, accumulation and use of non-structural carbohydrates, and rooting depth/pattern of four species from two contrasting eco-hydrological habitats on the Swan Coastal Plain.

5) **Determine the importance of facilitation through hydraulic redistribution (HR) for the establishment of seedlings during their first summer drought in southwest Australia.** In chapter 6, I grew seedlings of two species in field conditions and controlled (+/-) their root interaction with a plant able to hydraulic lift water (referred to as a donor plant). I also simulated this experiment under glasshouse conditions. In both experiments, I monitored survival, growth and water relations of seedlings and donor plants, and used deuterium as a tracer of water movement within and between plants.

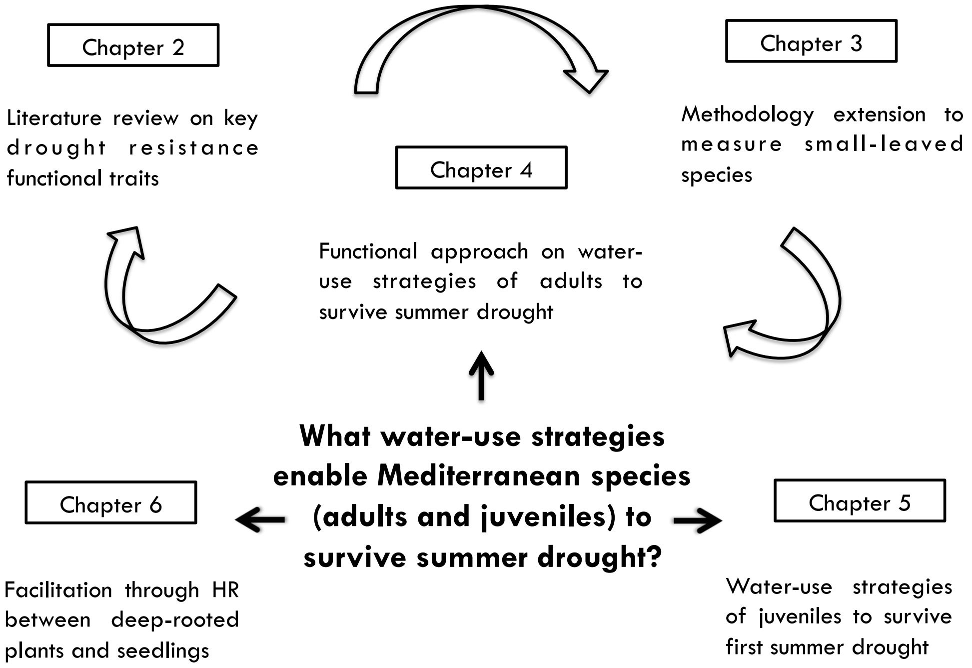

Figure 1.1 The structure of this thesis, with the main question in the centre surrounded by the three studies conducted to explore possible answers (Chapters 4, 5 and 6). These three chapters were designed during the propose stage and executed as planned. Chapters 2 and 3 were designed after the proposal stage as complementary studies that were needed to supported study 4. Blank arrows represent the relationship between chapter 2 that was first needed, followed by Chapter 3 that was also necessary in order to undertake the study in Chapter 4.

CHAPTER TWO: SELECTION OF KEY PLANT WATER DEFICIT RESISTANCE TRAITS FOR ECOLOGICAL INTERVENTION: AN OVERVIEW FOR MEDITERRANEAN-TYPE ENVIRONMENTS

2.1 ABSTRACT

Drought is likely to increase in intensity and frequency across most of the Mediterranean areas due to climate change. There is thus an urgent need to assess differences in the ability of plants to withstand water stress, especially when selecting appropriate species for ecological intervention. This study focused on Mediterranean-type ecosystems (*MTEs*) and reviewed and identified plant traits associated with drought resistance that are key in differentiating plant functional types, which is important when selecting species for ecological intervention in drying environments. Plant response traits were reviewed from the literature based on the following criteria – association with water stress, association with effect traits, and methodologies that are scalable, easy, inexpensive and applicable for Mediterranean species. I included carbon isotope composition of leaves ($\delta^{13}C$, as a proxy for water-use efficiency, *WUE*), leaf nutrient contents (*[N]* and *[P]*), leaf mass per area (*LMA*), water potential at leaf turgor loss point (π_{tlp}) and density and size of xylem vessels. In order to assist in the first step of this selection process, I put forward a Cartesian framework to classify species into functional types, where carbon assimilation is plotted against water deficits. Through this framework, analyses of these morphological and physiological traits allowed for theoretical identification of functional groups, when the traits were analysed in combination. The identification of functional groups in a community is the first step towards effective ecological intervention, as it allows for selection of species that will likely persist under drier conditions.

Key- words: drought resistance, ecological intervention, plant traits, water relations, plant functional types

2.2 INTRODUCTION

Mediterranean-type ecosystems (*MTEs*) experience seasonal drought characterized by hot, dry summers followed by mild, wet winters (Aschmann, 1973). Soil water availability during the dry summer is a major environmental limitation under Mediterranean conditions (Castri, 1973) and can lead to water deficits within leaf tissue and xylem vessels critically affecting physiological processes and overall plant performance and survival (Hsiao, 1973). *MTEs* also support a high diversity of uniquely-adapted plant species (Cowling et al., 1996) that present a wide range of functional responses to water use and carbon assimilation (Hernández et al., 2010; Mitchell et al., 2008a; Vilagrosa et al., 2013; West et al., 2012). Such responses include enhanced rooting depth, e.g. phreatophyte; efficient stomatal conductance, e.g. isohydric species; robust hydraulic architecture, e.g. species that can tolerate high xylem tension; and adaptations in leaf physiology and phenology, e.g. osmotic adjustment and leaf movements.

The high diversity of *MTEs*, however, is under threat as they represent some of the most highly altered ecosystems on the planet (Hobbs et al., 1995). In addition, climate change predictions suggest that temperature and rainfall variability will increase significantly, resulting in most *MTEs* becoming drier in the future (IPCC, 2014; Klausmeyer and Shaw, 2009). Within this context, there is a need to restore degraded and abandoned lands in order to maintain biodiversity and critical ecosystem functions. Ecological restoration has become an important and promising field of research (Dobson, 1997; Young, 2000) as its objective is to assist the recovery of degraded ecosystems with respect to their health, integrity and sustainability (Hobbs, 2007). In *MTEs*, however, water deficit stress is the principal cause of failure of many restoration projects (Mendoza et al., 2009; Vallejo et al., 2012), with seedling mortality being high especially over the first dry summer (Hallett et al., 2014; Lloret et al., 2005); and this scenario is expected to be exacerbated by the drier conditions projected for the future.

Designing and implementing ecological intervention plans based on purely historical information however can be inappropriate since species currently live under already modified environmental conditions with predictions of "no-analogue futures" due to climatic changes (Fox, 2007; Hobbs and Cramer, 2008; Seastedt et al., 2008). New approaches are required in ecological intervention to improve the success of future efforts within drying Mediterranean-type landscapes. To facilitate these new approaches, a better understanding of how plants function is needed, particularly the species traits likely to become critical for persistence in a drier future (Hobbs, 2007; Hobbs et al., 2011). Such knowledge is relevant in predicting change in ecosystem assembly and long-term success in *MTE* intervention projects subject to change in water availability.

Ecological filters of local biotic, e.g. plant and animal interactions, and abiotic variables, e.g. water and nutrient availability and climate, determine which functional species traits or trait values will facilitate survival under prevailing environmental conditions (Díaz et al., 1999; Temperton et al., 2004). Over time, changes in climate variables will alter ecological filters and consequently plant communities will be represented by species with physiological traits that enable them to persist and reproduce under the new conditions. Understanding the interactions between ecological filters, like water deficits, and the species pool is becoming extremely important for biodiversity conservation in the face of climate change (Funk et al., 2008; Myers and Harms, 2009, Figure 2.1). The selection of species with traits or traits values that provide an adaptive advantage under changing hydrological conditions should be an important goal for projects in *MTEs* under risk of climatic drought.

Plant functional traits are attributes relevant to the life- history and resource use of a species and can be used to define its ecological role or function (Díaz and Cabido, 2001; Lavorel and Garnier, 2002; Reich, 2014) and its response to environment change (Lavorel et al., 1997). Consequently, there is increasing interest in using traits (Cadotte et al., 2011; Gondard et al., 2003; Pywell et al., 2003; Sandel et al., 2011) as well as the genetic diversity and adaptability of species (Prober et al., 2015) to guide ecological intervention. For example, the potential for success of a intervention project can be increased by ensuring the maintenance or enhancement of diversity of functional types, particularly if there is uncertainty about future environmental states (Funk et al., 2008).

In *MTEs,* where ecological intervention is increasingly necessary due to the large amount of degraded and abandoned lands, and combined with a future scenario of severe climate changes, there is an urgent requirement to use plant functional traits as the basis for change analysis and species selection. A diverse field of reproductive, life-history and morphological traits have been used in ecological intervention (Ostertag et al., 2015; Pywell et al., 2003; Weiher et al., 1999); but, to my knowledge, there has been no synthesis of water stress-related physiological and anatomical traits associated with tolerance of seasonal drought in *MTEs*. Therefore, the aim of this study is to review the key physiological and anatomical functional traits that can be used for measuring and screening species for functional intervention in *MTEs* under threat of climate change.

2.3 FUNCTIONAL INTERVENTION

Restoration practitioners require novel, robust and theory-based approaches to maintain essential ecosystem processes and functions and to facilitate response to changing abiotic conditions, rather than restoration based on a historical assembly that may not persist in a rapidly changing world (Cramer et al., 2008; Seastedt et al., 2008). Plant functional traits vary across resource availability gradients in predictable ways, and hence can be linked to ecosystem properties and services (Lavorel, 2013). Consequently, functional traits are a valuable tool for exploring ecological strategies of species and overall community assembly and function (Reich, 2014).

Functional traits are classified as “response traits”, those that determine how organisms respond to environmental conditions; or “effect traits”, those that determine the effect of organisms on ecosystem functions (Laughlin, 2014). Response traits are often subjected to ecological filters and play an important role in community assembly and species interactions (Laughlin, 2014), whereas effect traits influence ecosystem processes through nutrient recycling and storage, modifications of soil water holding capacity, grazer efficiency, litter decomposition and primary production (Eviner and Chapin III, 2003). The community assembly comprising individuals with appropriate response traits, however, is what will determine effects on ecosystem properties (Grime, 2006).

Under the environmental filtering framework, ecological filters act at multiple scales to determine community composition, by filtering out species from the regional pool (Díaz et al., 1999). In *MTEs*, current land degradation and predicted changes in climate can alter physiological filters, removing species of the local community that lack trait values that confer a better match to changing environmental conditions. Species with appropriate attributes for the prevailing or future conditions, from both local and regional communites, can be identified and selected for ecological intervention, whereas mediation may be needed to maintain species that no longer possesses an advantage (Fig. 2.1).

Analyses of functional traits of species from both regional and local communities will then expand existing knowledge of current responses to drought that are present in the ecosystem. It will also allow for subsequent estimations of more advantageous strategies and trait values in line with future predictions of climate. Thus, finding such trait values will depend on the functional types present in both local and regional communities, on the type of alterations the ecosystem has had in the past, and on future predicted changes. In this way, different strategies to survive drought can be successful or not depending on the environmental changes. For example, in areas where the groundwater is declining, having deep roots to access groundwater may no longer be an advantage and species that have traits for tolerating prolonged water deficit may become more successful under the new conditions (Sommer and Froend, 2011).

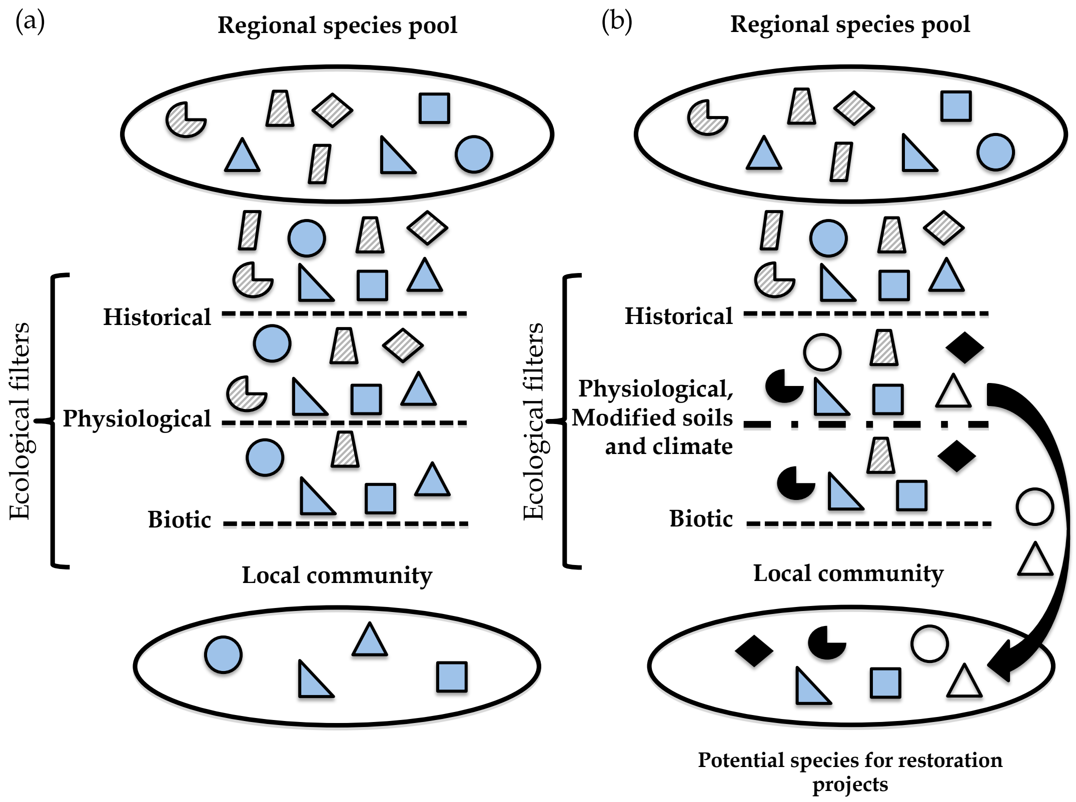

Figure 2.1 (a) Ecological filters act at multiple scales to determine community composition, by filtering out species from the regional pool that lack the appropriate traits (in dashed grey) and select the ones that possess such attributes (in blue). Historical, physiological and biotic filters are related to the arrival of species to a certain area as well as to their capacities to germinate, grow, survive, reproduce, compete and defend themselves. (b) Modifications of soil and climate will alter physiological filters, selecting only the species with the physiology that provides a better match to changing hydrological conditions (in blue). Thus, those that lack such trait values will be filtered out (grey). On the other hand, species from regional community that were filtered out previously may become compatible with the new conditions (in black). The species with the approppriate attributes can then be used for ecological intervention of equivalent areas (in blue and black), whereas mediation is needed to include species that lack such attributes (in white). This figure was modified from Funk et al., (2008).

2.4 RESISTANCE TO DROUGHT

Terrestrial plants experience a trade-off between carbon fixation and water loss since gas exchange and transpiration are processes mediated by stomatal behaviour. Therefore, plants have to cope with the fact that they must lose water in order to get a gain in carbon, as described in the Cowan-Farquhar model (Farquhar et al., 1980). However, it is soil water availability and the capacity of plants to absorb water and transport it from the roots to leaves that determines the magnitude to which transpiration leads to dehydration of leaf tissue.

Under drought, some plants reduce stomatal conductance to regulate the transpiration water losses as soil water potentials decrease and atmospheric conditions dry –these are known as isohydric plants (McDowell et al., 2008; Tardieu and Simonneau, 1998). Conversely, anisohydric plants do not reduce stomatal conductance under drought (McDowell et al., 2008; Tardieu and Simonneau, 1998). There is, however, a larger number of additional physiological and structural traits that may confer a resistance to water stress by either avoiding or tolerating it (Levitt, 1972).

Drought avoiders adopt strategies such as: increasing stomatal and cuticular resistance; changing the anatomy, surface area and orientation of leaves (Brodribb et al., 2003; Morgan, 1984); achieving a quiescent stage (dormant plants: Volaire and Norton, 2006; Volaire et al., 2001); having specialised water-storing tissues (succulent plants: Ogburn et al., 2010); growing deep roots to access deep water (phreatophytes: Meinzer, 1927); dying back to below ground parts or to seeds (annual species and seeders: Gutterman, 1994); and resprouting (Bond and Midgley, 2001; Keeley, 1986; Verdú, 2000). Drought tolerant plants are able to maintain hydraulic and stomata conductance, photosynthesis and growth during periods of low water availability (Sack et al., 2003). The understanding and identification of such strategies –that can be detected by functional traits– can assist in selecting species for intervention projects of Mediterranean ecosystems.

2.5 SELECTION OF PLANT TRAITS RELEVANT TO DROUGHT RESISTANCE IN *MTEs*

To be efficient, the use of response traits as a basis for planning and monitoring ecological intervention of biodiverse *MTEs* would necessitate a focus on a small number of traits. Selection of the principal response traits for *MTEs* vegetation, therefore should: 1) consider measured traits that reflect responses to the key factors, i.e. water deficit stress rather than other abiotic/ biotic factors; 2) include response traits that are linked (at least conceptually) to functional effects on ecosystem services; 3) offer efficacy of measurement across multiple species; and 4) use methodologies that can be applied to the wide range of leaf types, stem characteristics and plant habits of *MTEs* plant species (Mooney et al., 1973, Table 2.1). It should however be noted that a number of plant traits can be associated with drought adaptation, therefore it is crucial to select the ones that allow for uncomplicated and inexpensive measurement of multiple species.

Seven response traits were selected from the literature based on the criteria listed above. For instance, leaf $\delta^{13}C$ is a predictor of water-use efficiency (Farquhar et al., 1982; Farquhar and Richards, 1984) and can be used across species with a diverse array of leaf types to indicate their ability to respond to water deficits, without having to measure transpiration rates, which is time consuming. At the same time, leaf $\delta^{13}C$ is associated with carbon assimilation, primary productivity and efficient resource use and acquisition, all of which may positively affect intervention of ecosystems (Table 2.1). For example, species with high leaf $\delta^{13}C$ are using water more efficiently, thus less water is lost through transpiration and may be available for other organisms, or less water will be required if irrigation is needed during the intervention process; e.g. in a study about irrigation applied for ecological intervention of desert areas, the estimative is of 1-8 l of water per plant per month (Bainbridge, 2002). Similarly, leaf *[N]* and *[P], LMA,* π_{tlp} and wood density and anatomy are all associated with the ability of plants to resist water deficits and can be efficiently measured in species with a wide range of leaf types (Table 2.1).

Essentially, plants that had their stomata closed for long periods will likely present relatively higher leaf $\delta^{13}C$, lower concentrations of *[N]* and *[P]* and lower A_{max} compared to those that maintain stomata open. So, through the measurements of these leaf traits it is possible to identify plants' strategy to cope with water stress (Table 2.1 and Fig. 2.2). Similarly, plants that show more negative values of π_{tlp} and high hydraulic safety are able to maintain metabolism under conditions of low water availability. They can lower leaf π_{tlp} by accumulating solutes in their cells, and resist high tensions in their xylem vessels through greater mechanical support (Table 2.1 and Fig. 2.2). Leaf mass per area is a product of thickness and of density and, therefore, integrates both carbon and water-use capacity (Niinemets, 2001). High *LMA* is associated with hydraulic safety, low stomata conductance and leaf turgor maintenance (Table 2.1 and Fig. 2.2).

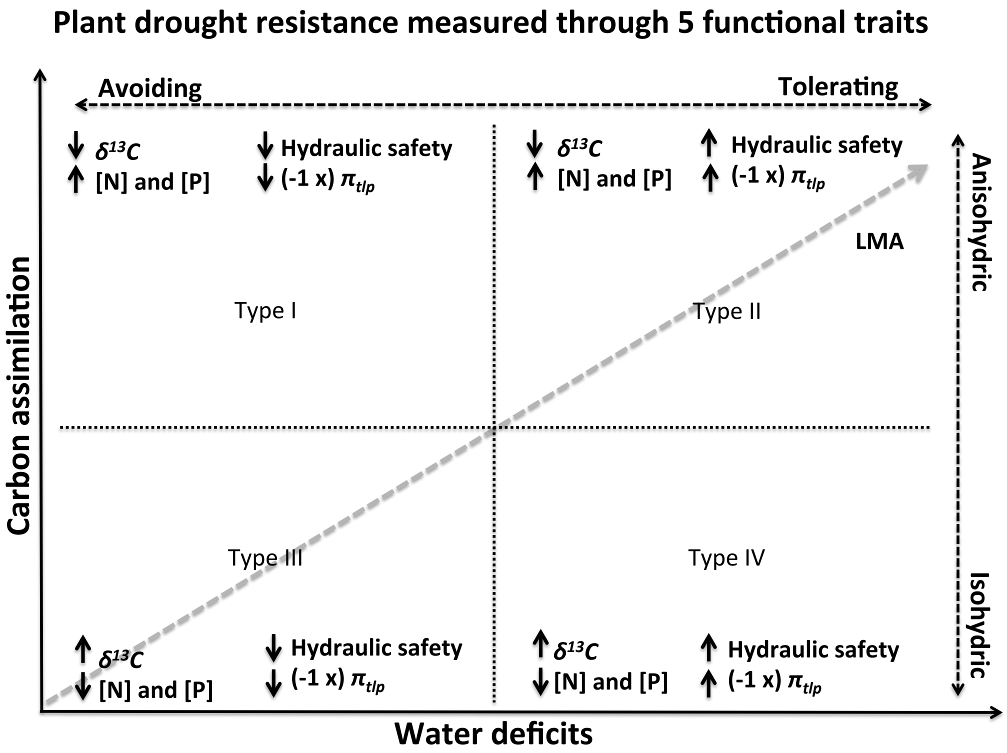

Figure 2.2 Conceptual graphic for plant drought resistance of Mediterranean-type ecosystems under the risk of climate drought, via a functional intervention approach. It includes a combination of seven key response traits that are related to resistance to drought and are valuable indicators of existing functional types, "strategies". Leaf water potential at turgor loss point, π_{tlp}, and hydraulic safety (combination of wood density and wood anatomy) are a proxy for "water deficits". Maximum photosynthesis rates and stomatal conductance measured as leaf *[P]* and *[N]* and $\delta^{13}C$ respectively are a proxy for "Carbon assimilation". Leaf mass per area (*LMA*) is related to both axes.

The conceptual diagram assembled in this study (Fig. 2.2) provides us with a broad screening of species' functional profiles in relation to water deficit resistance, where the x-axis represents increasing water deficits and the y-axis represents increasing levels of carbon assimilation, so that the different functional groups can be discriminated by the four hypothetical quadrants in the graphic, classified as "type I", "type II", "type III" and "type IV". The first quadrant ("type III") is characterised by low values for both axes, i.e. plants with low values of $-\pi_{tlp}$ (closer to zero, because π_{tlp} is represented as a negative value), low hydraulic safety, low leaf *[N]* and *[P]* and high $\delta^{13}C$. Drought deciduous species are an example of such a group as they lose leaves and enter dormancy under low water availability (Volaire et al., 2001).

At the other extreme, where both axes have high values ("type II"), species resist drought by having higher values of $-\pi_{tlp}$, high hydraulic safety, high leaf *[N]* and *[P]* and low $\delta^{13}C$. For example, some shallow rooted species, such as some Eucalyptus species from shallow soils from South Western Australia, are able to maintain gas exchange and possibly growth under low water availability. The combination of high values of water deficits and low values of carbon assimilation constitutes the quadrant composed of plants which maintain low $-\pi_{tlp}$, high hydraulic safety, low leaf *[N]* and *[P]*, and high $\delta^{13}C$, ("type IV"). Resurrection plants are an example of such group, as they maintain cell integrity during dehydration by accumulating specific carbohydrates in the leaves, and have narrow reticulate xylem vessels (Scott, 2000). Finally, in the top left corner of the diagram, high values of carbon assimilation and low water deficits result in plants that have high leaf *[N]* and *[P]*, low $\delta^{13}C$, higher values of $-\pi_{tlp}$ and low hydraulic safety, ("type I"). Phreatophytes are an example of this group, since they can access water from deep layers thus avoiding drought and maintaining photosynthetic gas exchange (Meinzer, 1927).

It is expected that many species present intermediary and more complex combinations of responses to drought than is represented here, nevertheless, the conceptual model presented (Fig 2.2) is a simple way to visualize and categorise key drought resistance strategies relevant to ecological intervention of *MTEs*. Where available, plant response trait values can contribute to and be ultimately sourced from global databases of plant traits e.g. "Plant Trait Database-TRY" project (Kattge et al., 2011) once sufficient records are available for *MTEs*. With this type of data available, restoration practitioners can use the approach proposed in this study by plotting the data as suggested in Fig. 2.2 to identify the functional groups present in the community. Once the functional strategies to survive summer drought is known, it is possible to: select species with the most appropriate traits to future climate conditions, to interfere when a species is not able to maintain itself without human intervention, or at least to understand why certain species may fail. This first examination of the community allow for more effective planning and can create more sustainable ecosystems, i.e. communities that will likely persist in face of future climate and other global changes.

Table 2.1 Brief descriptions of principal response traits– leaf carbon composition, leaf $\delta^{13}C$; foliar concentration of nitrogen and phosphorus, leaf *[N]* and *[P]*; leaf mass per area, *LMA*; water potential at turgor loss point, π_{tlp}; and hydraulic safety (density and diameter of xylem vessels)- as defined by criteria: "Response traits linked with functional effects", "Direct association with resistance to water deficits", "Efficacy of measurement" and "applicable to a wide range of leaf types"

Traits/ Criteria	Linked with functional effects	Associated with resistance to water deficits	Efficacy/ applicable to a wide range of leaf types	Trait range	References
Leaf $\delta^{13}C$	Carbon assimilation; Resource use and acquisition; primary productivity.	Assimilation of ^{13}C can be related with stomata conductance and water-use efficiency.	Oven-dry leaves, grind and measure with a spectrometer.	-16.2 – -29 (‰)	Farquhar et al., (1989 and 1982); Pérez-Harguindeguy et al., (2013); Sinclair et al., (2009); Smith and Griffiths, (1993)
Leaf [N] and [P]	Primary productivity, trophic transfer and nutrient cycling.	N and P are associated with the photosynthetic machinery. Fast- to- slow strategies.	Oven-dry leaves, grind and measure with: Acid-digestion and calorimetric, or combustion and spectrometry, or gas chromatography.	[N] = 6.3 – 18.1 [P] = 0.22 – 1.72 (mg g^{-1})	Bieleski, (1973); Ellsworth et al., (2014); Field and Mooney, (1986); Kattge et al., (2009); Pérez-Harguindeguy et al., (2013); Reich and Schoettle, (1988); Reich et al., (1997); Wright et al., (2004)
LMA	Productivity x environmental stress.	LMA is positively related to global solar radiation and to water limitation. Fast- to-slow strategies.	Measure area of a fresh leaf and its dry mass.	147 – 498 (g m^{-2})	Niinemets, 2001, 1999; Pérez-Harguindeguy et al., (2013); Reich et al., 1999; Witkowski and Lamont, 1991)
π_{tlp}	Primary productivity.	Ability to maintain leaf turgor and function through accumulation of active osmotic solutes.	Measure osmolality of extracted sap with an Osmometer.	-3.85 – 0.5 (MPa)	Baltzer et al., (2008); Bartlett et al., (2012a and b); Blackman et al., (2010); Kubiske and Abrams, (1990); Lenz et al., (2006); McDowell, (2011); Sack et al., (2010 and 2003)
Hydraulic safety (*HD*)	Carbon storage and primary productivity.	Stem with high Nº(density, D_s) of small (in diameter, Dv) xylem vessels with high wood density (WD) leads to high HD.	Dry mass/fresh volume of a stem section (WD) and prepare transversal stem sections for analysis of D_s and Dv with a microscope.	WD= 0.3 – 1.2 (g cm^{-3}) D_s= 100 – 1000 (Nº mm^{-2}) Dv= 10 – 57.8	Chave et al., (2009); Hacke et al., (2001); Pérez-Harguindeguy et al., (2013); Tyree et al., (1994)

2.6 CONCLUSION

Trait-based approaches enable a better understanding of linkages between biodiversity and ecosystem functioning. Within the response–effect framework (Lavorel and Garnier, 2002), trait-based studies are needed to predict how environmental changes influence ecosystem services which can be explored via identification of response traits that simultaneously determine community responses to environment and ecosystem function (Lavorel, 2013; Lavorel et al., 2011). This review focused on identifying single or groups of response traits that are efficient to measure and that will benefit ecological intervention in *MTEs* by enabling the selection of species assemblages that may persist under drier environments. In addition, the persistence of viable populations and assembly will also potentially maintain ecosystem services through links to effects traits. Finally, I suggest that this approach should be seen as a preliminary step that restoration practitioners could take towards prioritized intervention of *MTEs* under a changing climate followed by final decisions on species selection that consider other ecological aspects that influence ecological intervention success such as interactions with animals, fire resistance and seed/seedling costs.

CHAPTER THREE: APPLICATION OF AN OSMOMETER TECHNIQUE TO RAPIDLY PREDICT TURGOR LOSS POINT IN SMALL SCLEROPHYLL LEAVES

3.1 ABSTRACT

Leaf water potential at turgor loss point has been recognized as a key functional trait related to tolerance to water deficits. The methodologies developed to measure this trait, however, are either time consuming or impractical for small-leaved species. For Mediterranean-Type Environments (*MTEs*), where a large number of small-leaved species occur, there is an urgent need to find a suitable method to quantify leaf π_{tlp} that is efficient. I collected samples from 11 plant species (n=4), from a range of leaf sizes, and simultaneously measured them with two techniques: pressure-volume curves (*P-V curves*) and osmometry of extracted sap (*SE osmometry*). The first approach allows for the measurement of water potential at turgor loss point (π_{tlp}), water potential at full turgor (π_0), cell elasticity (ε) and apoplastic water (*af*); and the second is a potential alternative technique, which measures only π_0. Since *SE* osmometry is a more simple method and π_0 is known to be highly correlated with π_{tlp}, I investigated its application to small leaved plants. I also used two rehydration treatments (no rehydration and one hour rehydration) to investigate possible problems associated with *SE osmometry* technique. No differences between hydration treatments were found for *P-V* parameters and osmometer π_0 ($osm.\pi_0$). Values of $osm.\pi_0$ were correlated with $P\text{-}V\pi_0$ for non-rehydrated ($r^2 = 0.465$; $p = 0.018$) and rehydrated treatments; ($r^2 = 0.655$; $p = 0.002$) and highly correlated with π_{tlp} for non-rehydrated ($r^2 = 0.803$; $p = 2.75 \times 10^{-4}$) and rehydrated treatments ($r^2 = 0.719$; $p = 5.93 \times 10^{-4}$). I conclude that the *SE osmometry* technique is a suitable replacement for the time consuming *P-V curves* since it gave accurate estimations of π_{tlp} in both small and large leaved species. Although ε had support roles in the maintenance of low π_{tlp}, $P\text{-}V\pi_0$ alone was an excellent predictor of π_{tlp}. I finally suggest that field measurements of $osm.\pi_0$ be done without pre-hydration treatments and during contrasting periods of water availability.

Key-words: drought resistance, small leaved-plants, water relations, rehydration, plant functional traits

Abbreviations: *A*, leaf area; *AWF, apoplastic water fraction*; *D*, leaf density; ε, cell wall elasticity; *LDMC,* leaf dry mass content*;* *RWC*, relative water content = *RWD*, relative water deficit; *P-V* curves, pressure-volume curves; *P-V*π_0, water potential at full turgor measured through *P-V* curves; *osm.*π_0, water potential at full turgor measured through a osmometer; π_{tlp}, water potential at turgor loss point; *SLA*, specific leaf area; *SWF*, symplastic water fraction; *SE osmometry*, osmometry of sap extracted; *T*, leaf thickness.

3.2 INTRODUCTION

Plant functional traits are measurable chemical, physiological, morphological and phenological attributes of plants that respond to biotic and abiotic factors in the local environment (Dıaz and Cabido, 2001; Lavorel and Garnier, 2002). Studies of plant functional traits are important for ecosystem management and planning, and for designing and monitoring intervention (Gondard et al., 2003). Such knowledge is especially needed for intervention and management of Mediterranean-type environments (*MTEs*) since they are predicted to be at the highest risk of climate change, particularly water deficits (IPCC, 2014; Klausmeyer and Shaw, 2009; Rapacciuolo et al., 2014). Water potential at turgor loss point, π_{tlp}, has been recognized as a key functional trait related to tolerance to water deficits since cell turgor is essential for maintaining membrane integrity, transport of photosynthate, cell expansion and overall plant growth (Blackman and Overall, 2001; Cleland, 1971; Frensch and Hsiao, 1994; Roberts and Oparka, 2003). This measure gives a good indication of leaf and plant tolerance to water deficits by providing leaf water potential values at which leaves are able to maintain turgor and function (Lenz et al., 2006; Sack et al., 2003).

It has been suggested that there might be three ways through which plants lower π_{tlp} including: (i) through the accumulation of osmotically active compounds; (ii) by reallocating water from inside to outside cell walls to reduce symplastic water content; and (iii) by increasing cell wall elasticity. Relationships between low π_{tlp} and changes in elasticity of cell walls and in reallocation of water (Joly and Zaerr, 1987; Moore et al.,

2008) as well as in osmotic adjustment (Merchant et al., 2007; Morgan, 1984) have all been found in plants experiencing water deficits. Bartlett et al. (2012), however, suggested that differences within and across species in π_{tlp} are entirely attributable to osmotic potential at full turgor (π_o) and, therefore, measurements of π_o alone can provide accurate predictions of π_{tlp}.

The most common way to measure π_o and π_{tlp} is through the pressure-volume (*P-V*) curve technique, which requires the sequential measurement of leaf water potentials of dehydrating plant tissue (Tyree and Hammel, 1972). This method enables the estimation of other physiological parameters, such as cell elasticity, capacitance and cell apoplastic and symplastic fractions. However, the technique is time consuming with only 4-6 individuals analysed in a day (Bartlett et al., 2012a). Thus, other methodologies have been developed to determine π_o more quickly, including the use of a thermocouple psychrometer, also known as an osmometer. This technique measures osmotic potentials of extracts of freeze-thawed leaf discs, sap extracted from crushed tissues, and hot water extractions from fresh tissues (Callister et al., 2006; Kikuta and Richter, 1992; Turner, 1981). Of these, the freeze-thawed leaf discs methodology has been described as the most robust since dilution of the symplastic fraction with apoplastic water is minimized by excluding the primary and secondary veins when cutting discs from the leaves (Callister et al., 2006; Kikuta and Richter, 1992). Support for the technique has been provided by Bartlett et al., (2012a), who found that $osm.\pi_0$ measurements were strongly correlated to $P\text{-}V\pi_o$ ($r^2 = 0.80$) and to π_{tlp} ($r^2 = 0.86$).

The use of freeze-thawed leaf discs however poses a problem when used on species with sclerophyllous and/or very small leaves, cladodes and other “non-laminar shaped leaves”, since leaf discs cannot be obtained from such leaves. This limits the applicability of the technique when assessing certain ecosystems such as *MTEs* since these ecosystems have a wide range of leaf types and sizes, stem characteristics and plant habits (Pate et al., 1984). For instance, Mooney et al., (1973) and Orsham (2012) classified 429 common species, from an eco-region of Southwest Australia known as “kwongan”, by the frequency of leaf shape forms (terete, broad flat, long flat, dissected flat and minute scale leaves), size of leaves (within 9 categories from smaller than 0.10 to larger than 1640 cm^2), and consistency of leaves (malacophyll, sclerophyll, resinous succulence and water succulence). This diversity of leaf morphology results in 37%, 54% and 68% of species excluded from measurements of leaf π_{tlp} and π_o through the leaf disc technique because of leaf shape, size and consistency differences respectively.

Osmometer measurement of sap extracted from freeze-thawed leaves is more appropriate since it can accommodate a diversity of leaf types. Another advantage of this method, particularly for small-leaved plants, is the ability to press several leaves together in order to get sufficient sap for osmometer readings. Leaf π_{tlp} and π_o values measured through *SE osmometry* of leaves without their mid veins have been shown to be strongly correlated with those from *P-V* curves, and that such values are not dependent on the proportion of sap extracted from samples (Callister et al., 2006). Still, the determination of π_{tlp} and π_o by *SE osmometry* needs further testing for small leaves in which the separation of mid veins is impractical and several leaves need to be often crushed together so as to obtain enough sap for the osmometer.

The standard protocol for both the *P-V* curve and osmometer techniques includes an initial rehydration treatment to ensure that measurements of π_o are undertaken at full hydration. It is known, however, that prolonged rehydration can lead to hydration of airspaces thereby reducing the precision of π_o measurements, known as "plateau effect" (Kubiske and Abrams, 1991a, 1990). There is also evidence that results of rehydrated samples cannot be extrapolated to field studies, in which plants experience a broad range of tissue water potentials, and that non-rehydrated samples can be used (Kubiske and Abrams, 1990; Meinzer et al., 2014). Therefore, there is a need to investigate the accuracy of *SE osmometry* in response to rehydration treatments applied prior to measurements of π_{tlp} and π_o.

The main objective of this study was to identify a technique suitable for measuring leaf π_{tlp} and π_o that is quick and efficient across a range of small-leaved species characteristic of *MTEs*, producing results that are strongly correlated with those obtained from the widely accepted but time consuming *P-V* curve technique. Based on published evidence that supported the use of SE osmometry (Callister et al., 2006), I hypothesized that values of π_o determined from extracted sap measured with an osmometer (*osm.*π_0) will be strongly correlated with *P-V* curve measurements of π_o (*P-V*π_o) and π_{tlp} for species with large and small leaves, despite the inclusion of veins during the sap extraction process. To test this hypothesis, I constructed *P-V* curves and performed *SE osmometry* on non-rehydrated and rehydrated shoots of eleven species from the *MTE* of Australia.

3.3 MATERIALS AND METHODS

Study site and species selection

Plant material was collected from sites on the Gnangara Mound in Southwest Australia (Thackway and Cresswell, 1995). Gnangara Mound has a Mediterranean-type climate with mild, wet winters and hot, dry summers and contains several different aquifers (Thackway and Cresswell, 1995). Eleven species from four different taxonomic families were selected based on their high abundance. For each species, four individuals were used as replicates (Appendix 1).

Plant material collection and preparation

In September, which corresponds to the period of highest soil moisture, rainfall accumulated of approximately 430 mm from June to September of 2014, healthy looking branches (20-40 cm) were collected from each individual shortly before sunrise (predawn) and immediately sealed in humidified plastic bags to minimize water loss. Upon arrival at the laboratory, four smaller branches were excised from each replicate and split in two pairs; paired branches were either used in the original hydrated state or subjected to a rehydration treatment. For the rehydration treatment, branches were recut under water and the bases were kept in this water in dark cool place for one hour. Tests were undertaken to evaluate the time necessary for branches to reach full hydration without oversaturating them. Oversaturation was indicated by a gradual decline in $1/\psi$ as relative water deficit (*RWD*) increases during the P-V curves (Abrams and Kubiske, 1990; Dichio et al., 2003; Parker and Pallardy, 1987). To minimize the impact of oversaturation, the portions of stems that had been under water were also removed prior to determination of *P-V* curves.

From the two pairs of branches, one pair containing a non-rehydrated and a rehydrated branch was used to construct the *P-V* curves while the second pair was used for the osmometer readings. Samples were always kept in two humidified plastic bags inside a refrigerator at 4-5°C prior to analysis. Two replicates of each species were processed on the day of collection and the other two replicates on the following day due to time constrains.

Pressure-volume curves

P-V curves were constructed following the bench drying method described by Sack et al. (2003) in which a pressure chamber (Plant Moisture Stress Model 1000, Corvallis, Oregon) was used to determine water potential at regular intervals for material drying on the bench. Plots of fresh weight versus water potential for both treatments were undertaken to determine whether oversaturation has occurred. A correction was applied to samples where this phenomenon was detected, which involved eliminating the first points in which the plateau was evident (Sack et al., 2010). Plots of $1/\psi$ versus *RWD* were constantly updated during the *P-V* curve determination to ensure that three to five data points on the linear portion of the curve had been obtained. Osmotic potential at full turgor (MPa), osmotic potential at turgor loss point, relative water content at turgor loss point and modulus of elasticity at full turgor (ε, MPa) were all calculated for each sample following the methods of Sack et al., (2010). Finally, symplastic water fraction (*SWF*) was estimated from the extrapolation of the regression equation of the linear part of the *P-V* curve (-1/water potential x relative water content) to the x-axis. Apoplastic fraction (*AWF*) was calculated by 1- *SWF* (Tyree and Hammel, 1972).

Osmometer measurements

Leaves were rapidly picked from sample branches and folded in aluminium foil, placed in liquid nitrogen for two minutes and allowed to thaw for one hour before the extraction of leaf sap. A leaf press (Markhart leaf press LP-27, Wescor) specifically designed for vapor pressure osmometers was used for extracting cell sap straight to a filter paper disc that matches the diameter of the Wescor vapor pressure osmometer (VAPRO 5600) 10 µl chamber well. The saturated filter paper was then sealed in the osmometer chamber. The machine recorded measurements of leaf osmolality approximately every three to four minutes until equilibrium was indicated by an increase between successive measurements of < 5 mmol/kg. The osmometer was calibrated following the manufacturer instructions at the start of each day and after every 6-8 hours of intense use. Osmolality values were transformed into osmotic potential values (ψ) through a simplification of the Van't Hoff relation as given by the manufacturer's manual:

$$\psi(MPa) \sim -0.0025\ (m^3 \cdot MPa \cdot mol^{-1})\ x\ osmolality\ (mol\ m^{-3})$$

Leaf traits

All leaf traits measurements were performed on the non-rehydrated samples since the species selected were sclerophyllous plants, which are not expected to show much variation between different hydration treatments. A scanner consisting of a digital camera (Hitachi-KP-D40) and a computer program (Windias, Version 2.0) was used to measure leaf area, *A*, on ten to forty leaves, depending on their size, for each individual replicate. An electronic digital calliper (Absolute Digimatic, series 500) was used to measure leaf thickness, *T*, from the middle of six random leaves for each replicate. Leaves were then placed in a drying oven at 70°C for 72 hours prior to dry mass determination and calculation of leaf dry mass content, *LDMC,* (dry mass/fresh mass) and specific leaf area, *SLA*, (leaf area per dry mass, $cm^2\ g^{-1}$). Leaf density, *D*, was determined by 1/SLA*T ($g\ cm^{-3}$).

Data analysis

Linear mixed effect models, *lmer()* from package “lme4” (R, version 3.0.2), were performed to investigate if the hydration treatments (rehydration versus non rehydration) affected each of the ε, π_{tlp}, $P\text{-}V\pi_0$ and $osm.\pi_0$ individually. Comparisons between rehydrated and non-rehydrated samples were undertaken on the non-corrected data, so that the effect of the hydration treatments could be determined. For comparisons between osmometer and *P-V* curves, the *P-V* curves that presented the plateau effect were corrected according to Sack et al. (2010). Therefore, comparisons between *P-V* parameters and osmometer measurements included *P-V* values that were corrected for plateau effects.

Linear mixed modelling was applied to investigate whether the parameters $P\text{-}V\pi_0$ and ε could be used to predict π_{tlp} and to test whether $osm.\pi_0$ was a good predictor for $P\text{-}V\pi_0$ and π_{tlp}. Akaike information criterion corrected for low n (AICc) was used to select the most parsimonious models (the models with the lowest AICc) and differences >2 in AICc values are considered meaningful (Burnham and Anderson, 2004, 2002). I included species as a random factor and hydration treatments and leaf traits individually (*T*, A, *D*, *LDMC* and *SLA*) as fixed factors. Leaf traits were incorporated to explore the relationship between *P-V* parameters and to investigate whether the prediction of $P\text{-}V\pi_0$ and π_{tlp} by osmometer could be improved by taking into account differences in leaf structure. I subsequently performed liner regressions to explore relationships between

P-Vπ_0 and π_{tlp}, ε and π_{tlp} as well as between *osm.*π_0 and each of the *P-V* parameters, *P-V*π_0 and π_{tlp} for both non-rehydrated and rehydrated treatments. Finally, I explored the discrepancy between *osm.*π_0 and *P-V*π_0 by studying relationships between *AWF* and π_{tlp} and between ε and π_{tlp} using regression analysis.

3.4 RESULTS

The oversaturation phenomenon was detected in 43% of rehydrated samples, despite the short hydration period of 1 hour and the removal of sections of the stem that had been submerged. More surprisingly, 17% of the samples that were not rehydrated also showed a plateau effect. Despite these findings, no differences between the non-rehydrated and rehydrated treatments were found for the *P-V* parameters, *P-V*π_0 *(lmer, p = 0.22, Fig. 3.1a),* π_{tlp} *(lmer, p = 0.24, Fig. 3.1b), AWF (lmer, p = 0.22, Fig. 3.1c),* ε *(lmer, p = 0.38, Fig. 3.1e), with an exception of SWF (lmer, p = 0.02, Fig. 3.1d)*. The osmometer values of π_0 for non-rehydrated and rehydrated samples were also similar (lmer, p = 0.12, Fig. 3.1f, Appendix 2).

Among the models constructed to predict π_{tlp}, the best-fit model had both *P-V*π_0 and ε included (Table 3.1). Values of *P-V*π_0 were also well correlated with π_{tlp} for both non-rehydrated (linear regression, r^2 = 0.90, p = 0.0000181, Fig. 3.2) and rehydrated (linear regression, r^2 = 0.88, p = 0000116, Fig. 3.2) treatments. Values of ε were negatively related to π_{tlp} for non-rehydrated (linear regression, r^2 = 0.21, p = 0.09, Fig. 3.3) and for rehydrated (linear regression, r^2 = 0.66, p = 0.00152, Fig. 3.3) treatments.

The two best-fit models found to predict *P-*π_0 included only *osm.*π_0 and both leaf area (A) and *osm.*π_0 (Table 3.1). Values of *P-V*π_0 and *osm.*π_0 were correlated for non-rehydrated (linear regression; r^2 = 0.465, p = 0.018; Fig. 3.4) and rehydrated treatments (linear regression; r^2 = 0.655; p = 0.002, Fig. 3.4). For the prediction of π_{tlp} the best-fit model included only *osm.* π_0 (Table. 3.1) and both parameters were well correlated for non-rehydrated (linear regression; r^2 = 0.803; p = 0.000275, Fig. 3.5) and rehydrated treatments (linear regression, r^2 = 0.719, p = 0.000593, Fig. 3.5). Finally, AWF was positively correlated to π_{tlp} for non-rehydrated treatments when the outlier *Pultenaea reticulata* was removed (linear regression, r^2 = 0.44, p = 0.031) and for rehydrated treatments (linear regression, r^2 = 0.67, p = 0.001). For ε, there was no correlation with π_{tlp} for non-rehydrated treatments (linear regression, r^2 = 0.215, p = 0.099), but these parameters were correlated for rehydrated samples (linear regression, $r^2 = 0.66$, $p = 0.001$).The following general equation was generated from data from all species studied with a variety of leaf sizes and shapes and uses osmometer measurements of π_0 of non-rehydrated samples to predict π_{tlp} ($r^2 = 0.803$; $p = 0.000275$, Fig. 3.5): $\pi_{tlp} = 0.98\ osm.\pi_0 - 0.17$.

Table 3.1 Summary of the models created through the Akaike information criterion corrected for low n (AICc) to investigate whether osmotic potential at full turgor measured through an osmometer ($osm.\pi_0$) could predict the pressure-volume parameters: osmotic potential at full turgor ($P\text{-}V\pi_0$) and osmotic potential at turgor loss point ($P\text{-}V\pi_{tlp}$) alone or in combination with leaf traits and with the hydration treatment applied to samples prior to the analyses. A similar approach was used to test whether $P\text{-}V\pi_0$ was a good predictor of $P\text{-}V\pi_{tlp}$. The best models of each analysis plus the null model are represented in this table. Abbreviations as per text. "K" refers to degrees of freedom and AICc to Akaike information criterion for low sample size.

Models	K	AICc	ΔAICc	Weight	Cumulative Weight	Log-likelihood
Predicting $P\text{-}V\pi_0$ through osm.π_0						
Osm + A	**5**	**15.86**	**0.00**	**0.41**	**0.41**	**-0.93**
Osm	**4**	**16.25**	**0.39**	**0.33**	**0.74**	**-2.88**
Osm + LDMC	5	19.30	3.44	0.14	0.77	-2.65
Osm + D	5	19.47	3.61	0.12	0.75	-2.74
Osm + SLA	5	19.67	3.81	0.12	0.90	-2.83
Osm + T	5	19.69	3.83	0.12	0.90	-2.85
Null	3	29.14	13.28	0.00	1.00	-10.86
Predicting $P\text{-}V\pi_{tlp}$ through osm.π_0						
Osm	**4**	**7.35**	**0.00**	**0.53**	**0.53**	**1.58**
Osm + A	5	9.36	2.01	0.19	0.72	2.32
Osm + D	5	9.99	2.64	0.15	0.71	2.01
Osm + SLA	5	10.78	3.43	0.12	0.91	1.61
Osm + LDMC	5	10.84	3.49	0.11	0.90	1.58
Osm + T	5	10.85	3.50	0.11	0.91	1.58
Null	3	20.90	13.55	0.00	1.00	-6.75
Predicting $P\text{-}V\pi_{tlp}$ through $P\text{-}V\pi_0$						
PV + ε	**5**	**-22.73**	**0.00**	**0.53**	**0.53**	**18.36**
PV	4	-20.50	2.23	0.17	0.70	15.50
PV + A	5	-19.18	3.55	0.25	0.73	16.59
PV + T	5	-18.50	4.23	0.19	0.70	16.25
PV + D	5	-18.41	4.32	0.19	0.73	16.20
PV + LDMC	5	-17.55	5.18	0.14	0.90	15.77
PV + SLA	5	-17.26	5.47	0.12	0.90	15.63
Null	3	20.21	42.94	0.00	1.00	-6.40

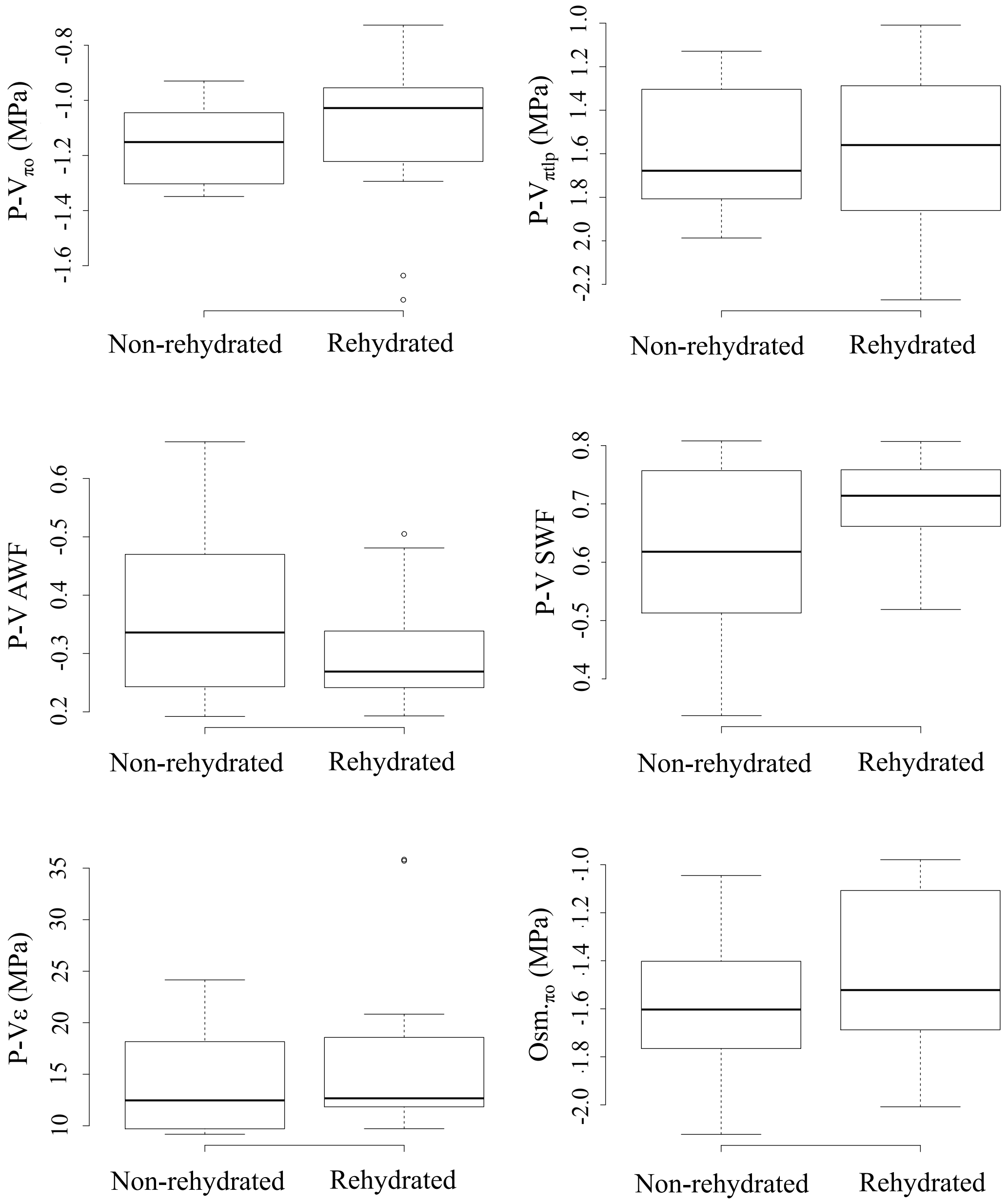

Figure 3.1 Differences in pressure-volume parameters between hydration treatments: **a)** osmotic potential at full turgor (P-Vπ_0, *lmer*, p= 0.22), **b)** osmotic potential at turgor loss point (P-Vπ_{tlp}, *lmer*, p=0.24), **c)** apoplastic water fraction (P-V AWF, *lmer*, p=0.22), **d)** symplastic water fraction (P-V SWF, *lmer*, p=0.02), **e)** cell elasticity (P-Vε, *lmer*, p=0.38), and **f)** osm.π_0 (*lmer*, p=0.12) measured in 11 plant species from Gnangara Mound in Southwest Australia (*lmer*, n=11, p = 0.24, p = 0.22, p = 0.38, p = 0.08, p = 0.04 and p = 0.12 respectively).

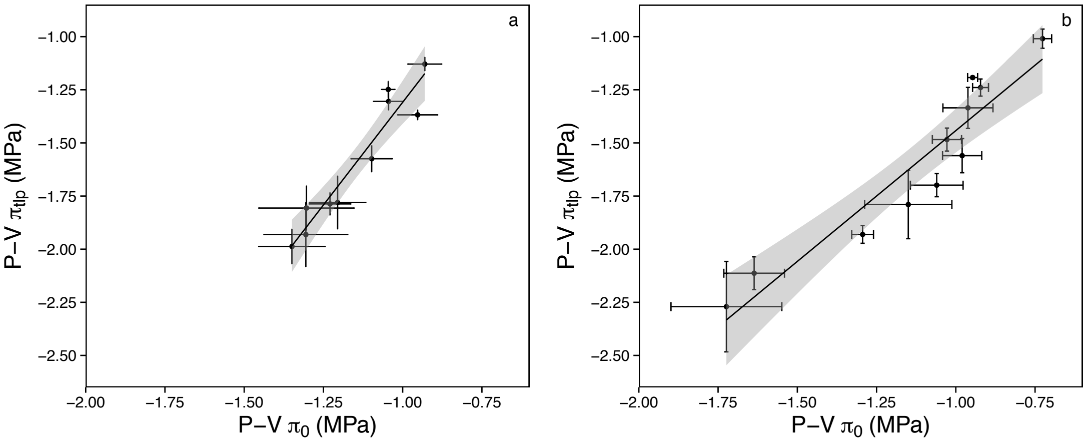

Figure 3.2 Relationship between osmotic potential at turgor loss point (*P-V*π_{tlp}) and osmotic potential at full turgor (*P-V*π_0) measured through pressure-volume curves, with 11 Southwestern Australian species for **(a)** non-rehydrated (n=10; r^2 = 0.90; p = 0.0000181) and **(b)** rehydrated (n=11; r^2 = 0.88; p = 0.0000116). Grey shade is 95% confidence intervals and error bars represent standard errors of the means.

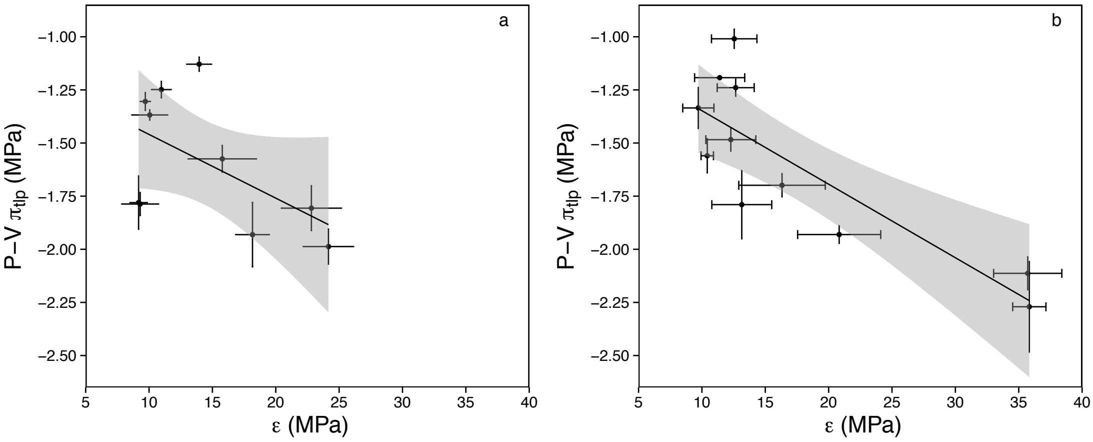

Figure 3.3 Relationship between osmotic potential at turgor loss point (*P-V*π_{tlp}) and cell elasticity (ε) measured through Pressure-volume curves, with 11 Southwestern Australian species for **(a)** non-rehydrated (n=10; r^2 = 0.21; p = 0.10) and **(b)** rehydrated (n=11; r^2 = 0.66; p = 0.00152). Grey shade is 95% confidence intervals and error bars represent standard errors of the means.

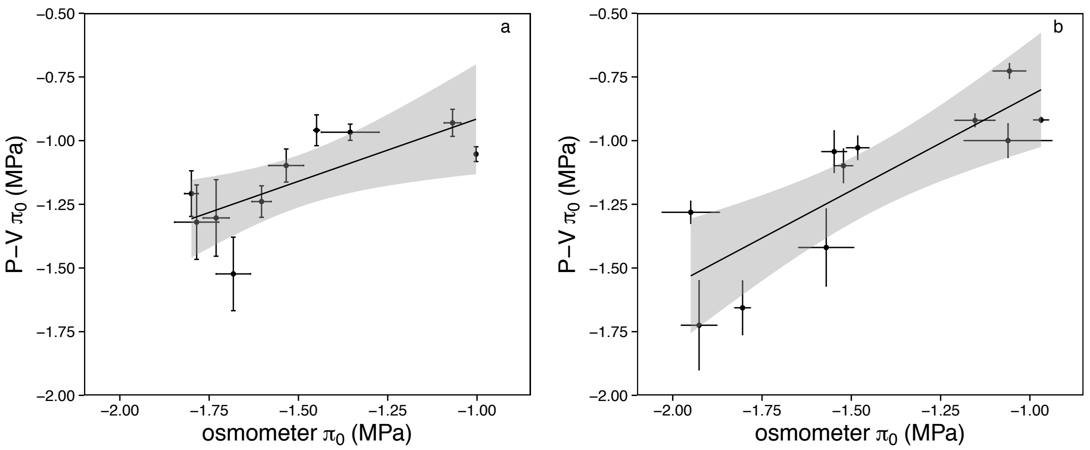

Figure 3.4 Relationship between osmotic potential at full turgor measured through pressure-volume curves ($P\text{-}V\pi_0$) and an osmometer π_0 ($osm.\pi_0$), with 11 Southwestern Australian species for (a) non-rehydrated (n=10; r^2 = 0.465; p = 0.018; $P\text{-}V\pi_0$= 0.49$osm.\pi_0$ -0.42) and (b) rehydrated (n=11; r^2 = 0.655; p = 0.002; $P\text{-}V\pi_0$= 0.74$osm.\pi_0$ -0.079). Grey shade is 95% confidence intervals and error bars represent standard errors of the means.

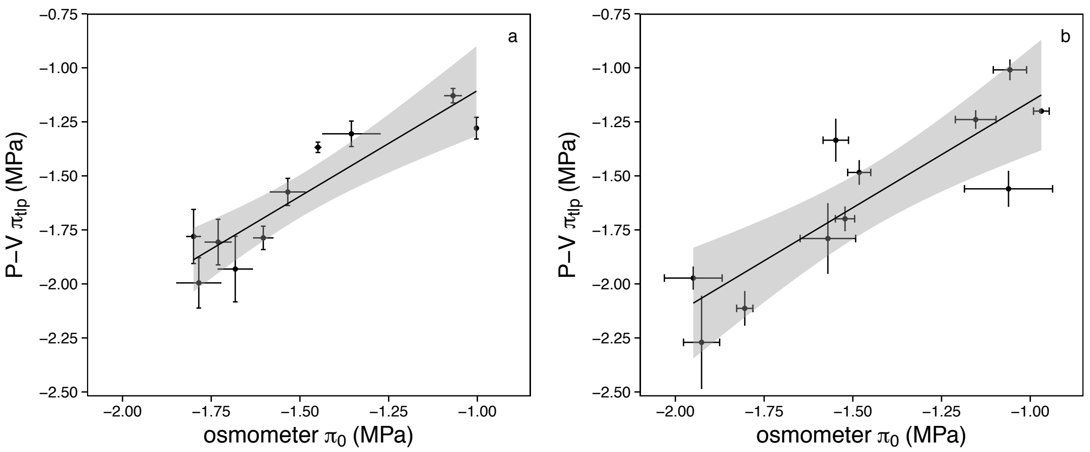

Figure 3.5 Relationship between turgor loss point measured through pressure-volume curves (π_{tlp}) and osmotic potential at full turgor measured through an osmometer ($osm.\pi_0$) with 11 Southwestern Australian species for (a) non-rehydrated (n=10; r^2 = 0.803; p = 0.000275; π_{tlp} = 0.98$osm.\pi_0$ -0.13) and (b) rehydrated (n=11; r^2 = 0.719; p = 0.000593; π_{tlp} = 0.98 $osm.\pi_0$ -0.17). Grey shade is 95% confidence intervals and error bars represent standard errors of the means.

3.5 DISCUSSION

Sap-extracted osmometry technique as an alternative method

The osmometry technique has advantages over the *P-V* curve method since it enables 30-40 rather than 6-8 samples to be analysed in a day. It also allows for species with leaves varying in size (approximately 5 to 150 mm) to be measured for π_0 and π_{tlp}. Values calculated using this osmometry technique are consistent with those derived from *P-V* curves as a strong linear relationship ($r^2 = 0.465$; $p = 0.018$) was found between the two suggesting that the time consuming *P-V* curve determination of π_0 and π_{tlp} can now be rapidly and accurately undertaken with *SE osmometry.* The leaf parameters π_0 and π_{tlp} are strongly associated with plant tolerance of water deficits (Bartlett et al., 2012b), and are thereby important for understanding current and potential future plant distributions in areas of significant climate change. Knowledge of leaf water relations' parameters such as π_0 and π_{tlp} would also assist in the selection of species used for management and planning of ecological intervention projects.

While values of *P-V*π_0 and *osm.*π_0 were correlated, yet they were not equal. There was a discrepancy from 1:1 between both methodologies, similar to what Bartlett et al. (2012a) found when comparing *osm.*π_0 readings of leaf discs with *P-V*π_0 of 30 species from different biomes. Values of *osm.*π_0 were at one extreme less negative for values closer to zero, and more negative for the other extreme. In Bartlett's study, this bias was explained by apoplastic dilution and cell wall dissolution, since species with higher osmotic concentrations tended to have greater cell wall investment. This means that the apoplastic dilution was weakly offset by the cell wall dissolution for values closer to zero and increasingly became offset by it with increasing osmotic concentration. The current study supports this finding, AWF was higher for species with less negative values of *P-V*π_{tlp} and ε was higher for species with more negative values of *P-V*π_{tlp}. Species with the lower *P-V*π_{tlp} also showed high ε and therefore higher cell wall investment. When samples were frozen in liquid nitrogen and subsequently crushed for the extraction of sap, symplastic water was likely influenced by both components: dilution by apoplastic water and concentration by cell wall dissolution. This relationship, however, only applies for rehydrated samples. Nevertheless, osmometer values of π_0 were well correlated to π_{tlp} regardless of the hydration treatment.

Determinants of water potential at turgor loss point

Values of $P\text{-}V\pi_0$ and π_{tlp} were also strongly correlated and this result is consistent with the meta-analysis of Bartlett et al. (2012b) in which osmotic potential at full turgor was described as the major driver of leaf water potential at turgor loss point. Values of ε were also correlated with π_0 and π_{tlp}. Indeed, the best-fit model for the prediction of π_{tlp} included both *P-V* parameters: $P\text{-}V\pi_0$ and *ε*. It is also known that very negative values of π_0 and π_{tlp} might result in very low values of relative water content at turgor loss point, $\mathrm{RWC_{tlp}}$, which can induce shrinkage and possibly interrupt cell metabolic processes (Bartlett et al., 2012b). To maintain constant $\mathrm{RWC_{tlp}}$, live cells can modify the elasticity of their cell walls in order to become more rigid and withstand turgor pressure, avoiding threshold shrinkage, known as the "cell water conservation hypothesis" (Cheung et al., 1975; Jones, 1992). Although ε clearly had a support role in the maintenance of π_{tlp} in this study, π_0 alone was enough to reliably predict π_{tlp} and thus show differences in tolerance to drought among species. This enables the use of SE osmometry to calculate plant tolerance to drought (speculated through π_{tlp} estimations) since the only leaf parameter that can be measured through SE osmometry is π_0, which in turn was highly correlated to π_{tlp} in this study.

Effects of hydration treatments

Samples from both hydration treatments had to be corrected for oversaturation, which suggests that this phenomenon can happen naturally when water is freely available to plants and oversaturation has previously been recorded for non-rehydrated samples (Evans et al., 1990; Kubiske and Abrams, 1991a; Parker and Pallardy, 1987). Other studies also found that rehydration treatments can lead to shifts in *P-V* curves resulting in incorrect calculations of π_{tlp}, $P\text{-}V\pi_0$, and ε parameters, due to the fact that solutes can leak from symplastic to apoplastic water in addition to air spaces within the leaf becoming filled with water (Kubiske and Abrams, 1991b; Meinzer et al., 2014; Parker and Pallardy, 1987). Although I found the plateau effect in some of the samples and therefore their *P-V* curves were shifted, I did not find differences between hydration treatments for π_{tlp}, $P\text{-}V\pi_0$, ε and $osm.\pi_0$. Therefore, the one-hour rehydration treatment did not markedly affect the values of *P-V* parameters and $osmo.\pi_0$ (Fig. 3.1). The apoplastic water fractions, on the other hand, were influenced by the rehydration treatment (Fig. 3.1). However, the calculation of symplastic and apoplastic parameters through *P-V* curves can be imprecise because it may require extrapolation beyond the range of the data (Andersen et al., 1991; Wardlaw, 2005).

It has been suggested that *P-V* curves should be constructed immediately after sampling, without any hydration treatment (Kubiske and Abrams, 1990; Yan et al., 2012) in samples comprising a range of hydration levels (Meinzer et al., 2014). In this study, samples were collected before dawn during spring, which corresponds with the wettest time of the year. Thus, a rehydration treatment was not necessary since the majority of samples were naturally fully hydrated. For *MTEs*, where summers are dry and winters wet, measurements of $osm.\pi_0$ and estimations of π_{tlp} can be obtained by sampling the wettest and driest periods of the year. In this way it is possible to investigate natural seasonal trajectories of water relations that are associated with leaf physiological functions without the confounding effect of rehydration treatments.

3.6 CONCLUSION

I suggest that SE osmometry of extracted sap is an appropriate technique for the measurement of leaf water potential at turgor loss point and at full turgor for a range of leaf types. This is significant for *MTEs* in which a large percentage of species have small, sclerophyllous leaves, which are not able to be measured using the leaf disc method. The more rapid SE osmometry of extracted sap is a suitable replacement for the time-consuming *P-V* curves to accurately and efficiently determine leaf water potential at turgor loss point and at full turgor. The application of SE osmometry without rehydration is preferable, particularly if plants are well hydrated in the field, such as in spring as no marked differences were found between treatments, with exception for SWF. Further seasonal work can be undertaken to fully understand the accuracy of this technique when leaf tissues will be naturally dehydrated. Finally, the use of SE osmometry allows for the rapid assessment of drought tolerance of plant species with a diverse of leaf shapes and sizes, making it useful across a range of ecosystem types within MTEs.

CHAPTER FOUR: A FUNCTIONAL ECOLOGICAL APPROACH TO IDENTIFY PLANT RESISTANCE TO WATER DEFICITS IN A MEDITERRANEAN-TYPE ECOSYSTEM

4.1 ABSTRACT

Mediterranean-type ecosystems (*MTEs*) have been highly modified and are predicted to become drier in the future. In this context, my objective was to develop an approach to identify plant water-use strategies through analysis of functional traits to assist intervention projects in selecting drought resistant species. I selected six areas across a gradient of water availability; including areas prone to waterlogging, areas with high depth to water table (>10 m), and areas with shallow soil and no water table. I then measured twelve functional traits associated with water use in fifteen plant species. Next, I applied multivariate analyses to examine how traits varied in relation to each other, grouping species based on these traits and investigating similarities within and between functional groups and sites. Functional trait correlations were consistent with the worldwide leaf and wood economic spectra, among the twelve traits measured, six appear to be most important to explain trait variation –maximum diameter of xylem vessels (D_{max}), number of xylem vessels per mm^{-2} (D_s), leaf mass per area (*LMA*), *WD* (stem density), foliar carbon isotope composition ($\delta^{13}C$), and leaf water potential at turgor loss point (*TLP*). Species were clustered into five different functional groups that corresponded to their different rooting depths. Differences within and between functional groups and sites are reported through their Euclidean distances. Analyses of functional traits provided insights into the water-use strategies of native plants, revealing those species with greatest potential to resist water deficits. It can also enable the formation of a more functionally diverse assembly of species bearing complementary traits, which in turn can be used to strengthen resistance to invasion in restored communities. This functional ecological approach is transferable to other *MTEs* and for application by restoration practitioners since the traits selected are relatively easy and cheap to measure and require only simple analytical approaches.

Key-words: resistance to water deficits, plant functional traits, water availability, plant functional types, Mediterranean-type ecosystem.

4.2 INTRODUCTION

A great many species live under modified environmental conditions due to climatic and other global changes (Fox, 2007; Hobbs and Cramer, 2008; Seastedt et al., 2008). In a world of constant and extensive change, there is a need for novel and robust theory-based approaches to ecological intervention aimed at maintaining essential ecosystem processes under changing abiotic conditions, rather than applying methods based on historical assembly that may not persist in a rapidly changing world (Cramer et al., 2008; Seastedt et al., 2008).

Plant functional traits are attributes relevant to the life history and resource use of a species, and can be used to help define its ecological role or function (Díaz and Cabido, 2001; Lavorel and Garnier, 2002; Reich, 2014). They are a valuable concept for identifying ecological strategies in an ecosystem, as well as understanding community dynamics and assembly (Reich, 2014). Indeed, there is increasing interest in using functional traits to guide intervention (Gondard et al., 2003; Laughlin, 2014; Pywell et al., 2003; Sandel et al., 2011), and to support agricultural (Wood et al., 2015) and ecosystem management (Cadotte et al., 2011; Kahmen et al., 2002).

Mediterranean-type ecosystems represent some of the most highly altered ecosystems on the planet (Hobbs et al., 1995) and are consequently under threat, requiring better management and intervention projects. Mediterranean ecosystems typically experience seasonal drought during the hot, dry summers followed by mild, wet winters (Aschmann, 1973) and therefore soil water availability during the dry summer represents a major environmental limitation (Castri, 1973). Summer drought can lead to water deficits in leaf tissues and within xylem vessels, critically affecting physiological processes and overall plant yield and survival (Hsiao, 1973). Climate change predictions suggest that temperature and rainfall variation will increase significantly, resulting in most *MTEs* becoming drier with potentially more regular and extensive periods with plant water deficits (IPCC, 2014; Klausmeyer and Shaw, 2009).

Many plants from *MTEs* possess a broad range of functional traits related to water use and carbon assimilation that includes deep roots, specialized root morphology, control of stomatal conductance, hydraulic architecture, and leaf physiology and phenological attributes (Hernández et al., 2010; Mitchell et al., 2008a; Vilagrosa et al., 2013; West et al., 2012). This diversity of strategies maintains efficient water use and niche partitioning, which is directly associated with community assembly (Hobbs and Norton, 2004; Weiher et al., 1998). Therefore, knowledge of water-use strategies of native plants has the potential to allow ecological intervention and management projects to design sustainable assemblies of species in a drying environment.

Species perform specific hydrological functions within an ecosystem and may hence be classified into hydraulic functional types (HFTs, Mitchell et al. 2008) independent of taxonomic groups (Shugart and Woodward, 1997). Such a functional classification may be relevant to screening species for planning *MTE* intervention in changing environments. There are several functional traits that have been linked to resistance to water deficits, as well as used to identify HFTs (Mitchell et al., 2008). Some of these traits can also be measured efficiently, enabling species datasets to be developed in a short period of time (Chapter 2 of this thesis). For instance, water potential at turgor loss point (π_{tlp}) has been recognized as a key functional trait, since it directly quantifies leaf and plant tolerance to water deficits by providing leaf water potential values at which leaves are able to maintain turgor and function (Bartlett et al., 2012b).

Functional traits associated with hydraulic architecture are also relevant as they represent potential for resistance to high tensions in xylem vessels generated by water deficits. Among these, wood density (*WD)*, xylem vessel density (D_s) and average and maximum vessel diameter (D_{ave} *and* D_{max}) have been linked to tolerance to water deficits (Chave et al., 2009; Hacke and Sperry, 2001; Tyree et al., 1994). Additionally, functional traits associated with carbon assimilation and photosynthesis capacity (A_{max}) also play an important role in resistance to water deficits. From these, carbon isotope composition of leaves ($\delta^{13}C$) can be used as a proxy for water-use efficiency (Farquhar et al., 1989, 1982; Sinclair et al., 2009; Smith and Griffiths, 1993), whereas concentrations of nitrogen (*[N]*) and phosphorus (*[P]*) in the leaves can be used as a proxy for A_{max} (Ellsworth et al., 2014; Kattge et al., 2009; Reich and Schoettle, 1988; Wright et al., 2004). Finally, leaf mass per area (*LMA*) is a functional trait that consists of two variables, leaf density and thickness, and is positively related to global solar radiation, from adaptive modifications in thickness to water limitations from increases in density (Niinemets, 2001, 1999; Reich et al., 1999; Witkowski and Lamont, 1991). Although measurements of leaf water potential at pre-dawn and mid-day require a significant time investment during data collection, they are valuable traits that can represent maximum and minimum leaf water status (Bhaskar and Ackerly, 2006). Nitrogen isotope composition of leaves ($\delta^{15}N$) can also be associated with soil water availability (Amundson et al., 2003; Craine et al., 2009; L. Handley et al., 1999). All of these functional traits are valuable indicators of plant resistance to water deficits and provide complementary information about plant strategies to cope with water deficits when analysed in combination (Chapter 2 of this thesis).

With low success in introducing plants due to drought stress during summer months (Davis, 1991; Vallejo et al., 2006, 2012), new approaches are required for ecological restoration and management in *MTEs*, since they are under increased risk of climatic drought. Studies comprising analysis of functional traits expand existing knowledge of current responses to water deficits and contribute to our understanding of community assembly. Such knowledge will also facilitate estimations of the most advantageous strategies and trait values under changing environmental conditions. Furthermore, studies focusing on functional traits that can be efficiently measured allow large species sets to be assessed, which is important for highly diverse ecosystems like *MTEs*. Therefore, the aims of this study were to: (1) investigate how the above mentioned functional traits covary across gradients of water availability in a *MTE; and* (2) to identify the principal traits associated with each HFT and their distribution along the water gradient.

4.3 MATERIALS AND METHODS

Study site and species selection

The study was conducted in Southwest Australia, a region characterized by a Mediterranean-type climate with mild, wet winters and hot, dry summers (Aschmann, 1973; Gentilli, 1972). Six sample sites were selected, with four located on the Swan Coastal Plain (SCP; annual average rainfall of 868/771 mm; Table 4.1) and two on the Eneabba Sandplain (ES; annual average rainfall of 495 mm; Table, 4.1). The SCP sites were situated approximately 38 km north of Perth city in an area on the Bassendean

Dune System; which consists primarily of quartz sand with negligible silt and clay, and thus the soils have a very low water-holding capacity (McArthur and Bttenay, 1960; McArthur, 1991; Table 1). There is also a wide range in depth (3 to 30m) to the water table (Thackway and Cresswell, 1995) across the dunal landscape. Mesic habitats with shallow depths to the water table are located lower in the landscape whereas drier habitats with no accessible water table are located on dune crests. Associated with this gradient in water availability are differences in species assembly and tolerance to water deficit (Beard, 1990; Froend and Sommer, 2010).

The two ES study sites were located near Eneabba, approximately 245 km north of Perth. None of these sites had accessible permanent water tables and, therefore, plants were dependent on retention of meteoric water in the unsaturated zone. Variation in depth of the unsaturated soil profile at Eneabba is suggested as the determinant of plant available water, with dune crest plants having greater available water due to increased storage capacity of the deep sandy profile (Lamont and Bergl, 1991). In contrast, inter-dunal swales of the ES are underlain by a shallow sandy horizon over an impermeable clay pan, which restricts root growth and available water during the dry summer months (Table 4.1).

At each study site, the five most dominant plant species (by percentage cover) were determined within 10 x 10 m quadrats and selected for sampling. A total of fifteen species from three different taxonomic families were selected, with five plants used as replicates for each species. The species selected were: *Adenanthos cygnorum* Diels., *Banksia attenuata* R.Br., *Banksia carlinoides* (Meisn.) A.R.Mast & K.R.Thiele, *Banksia hookeriana* Meisn., *Banksia ilicifolia* R.Br., *Banksia menziesii* R.Br., *Beaufortia elegans* Schauer, *Eremaea beaufortioides* Benth., *Eremaea pauciflora* (Endl.) Druce, *Hibbertia subvaginata* (Steud.) F.Muell., *Melaleuca leuropoma* Craven, *Regelia inops* (Schauer) Schauer., *Scholtzia laxiflora* Benth., *Scholtzia involucrata Endl* and *Verticordia nitens* (Lindl.) Endl. (Table 1). Species' root depths were from Froend R. H. and Sommer B. unpublished raw data. Samples were collected in March, which corresponds to the period of the year with the lowest available soil water.

Plant material collection and preparation

Branches (20-40 cm) were collected in the evening, between 1600-1700h, and immediately sealed in humidified plastic bags to minimize water loss. Upon arrival at the laboratory, branches were recut under water and kept in a dark cool place overnight. Samples were rehydrated following a standard method (Bartlett et al., 2012a; Sack et al., 2010, 2003), kept in humidified plastic bags and refrigerated at 4-5°C for a maximum of two days prior to analysis.

Table 4.1 Water availability at the five contrasting eco-hydrological habitats in relation to rainfall and depth to ground water.

Habitat	Mean Annual Rainfall	Depth to Ground Water	Water Availability	Site Name
Gnangara dampland and midslope	868[*]/771[#] (mm)	3-4.5[^] (m)	Perennially high water availability due to shallow water table.	GNSW GNMS
Gnangara midslope	868[*]/771[#] (mm)	12[^] (m)	Perennially high water availability due to shallow water table.	GNMD
Gnangara Dune Crest	868[*]/771[#] (mm)	30+[^] (m)	No access to water table. Reliant on water in unsaturated zone, which receives rainfall recharge in winter months, but little recharge over summer.	GNHD
Eneabba Dune Crest	495[&] (mm)	N/A. Soil profile is deep sands (approx. 15 m) over clay pan[±]	Deep sandy soil profile, reliant on water in unsaturated zone, which is recharged in winter, although there is less rainfall and higher evaporation compared with Gnangara sites.	ENHD
Eneabba Clay Pan Swale	495[&] (mm)	N/A. Soil profile is shallow sands (approx. 1 m) over clay pan[±]	Shallow sandy soil profile provides minimal soil water storage, therefore limited water availability, particularly in summer months with little rainfall and high rates of evaporation.	ENSW

[*] Represents long-term mean from 1876-1992 Perth Regional Office; [#], mean from 1944-2015 Perth Airport; [&], mean from 1972-2015 Eneabba station (Bureau of Meteorology 2015); [^] information used from Canham et al. (2009); and [±], from Canham et al (in prep.).

Osmometer measurements

For each branch, leaves were rapidly picked, wrapped in aluminium foil and placed in liquid nitrogen for two minutes. Samples were then allowed to thaw for one hour before the extraction of leaf sap. A leaf press (Markhart leaf press LP-27, Wescor), specifically designed for vapor pressure osmometers, was used to extract cell sap straight to a filter paper disc that matches the diameter of the Wescor vapor pressure osmometer (VAPRO 5600) 10µl chamber well. The saturated filter paper was then sealed in the osmometer chamber. Measurements of leaf osmolality were taken approximately every three to four minutes, with the chamber sealed, until equilibrium was indicated by an increase between measurements of < 5 mmol/kg. The osmometer was calibrated following the manufacturer instructions at the start of each measuring day and after every 6-8 hours of use. Osmolality values were transformed into osmotic potential values (ψ, *MPa*) through a simplification of the Van't Hoff relation:

$$\psi(MPa) \sim -0.0025\ (m^3 \cdot MPa \cdot mol^{-1})\ x\ osmolality\ (mol\ m^{-3})$$

Leaf water potential at turgor loss point (*TLP*) was estimated using values of osmotic potential at full turgor (*Ψ0)* through the equation developed in Chapter 3, as follow:

$$TLP = 0.98\Psi0 - 0.17$$

Leaf and stem traits

A scanner, a digital camera (Hitachi-KP-D40) and Windias v2.0 software were used to calculate leaf area (*A*) of ten leaves from each replicate. Leaves were then placed in a drying oven at 70°C for 72 hours to determine dry mass and leaf mass per area (*LMA* $g\ m^2$) was calculated. For each replicate, dry material was ground and analysed for foliar nitrogen and phosphorus (leaf *[N]* and *[P], mg* g^{-1}) as well as for foliar carbon and nitrogen isotope composition ($\delta^{13}C$ and $\delta^{15}N$). Leaf [P] was measured through nitric/perchloric digest followed by analysis on the inductively coupled plasma atomic emission spectroscopy, whereas $\delta^{13}C$ and $\delta^{15}N$ was measured through a continuous flow isotope ratio mass spectrometer (PDZ Europa 20-20, Sercon Ltd., Cheshire, UK), a procedure that also enabled measurements of leaf [N].

Fresh stem sections of approximately 5 cm long were excised from the base of the branches and their bark removed before their stem-specific densities (*WD*, $g\ cm^{-3}$) were measured using the water-displacement method (Pérez-Harguindeguy et al. 2013). For analyses of stem anatomy, standard methods were followed for embedding transverse sections of stem in paraffin wax using an Auto Tissue Processor and for sectioning in a microtome. Slides were assembled and stained following Ruzin (1999). Microphotographs were taken with an Olympus BX51 photomicroscope and analysed using the image analysis software ImageJ to count the number of xylem vessels per mm^2 (vessel density, $N^o\ mm^{-2}$, D_s) and to measure the average (D_{ave}) and maximum diameters (D_{max}) of 100 xylem vessels per replicate (µm), following Scholz et al. (2013). Leaf water potential of two leaves per replicate plant (five individual plants per species per site) was measured at predawn (*PD*, between 04:00h and 06:00h) and midday (*MD*, 11:00h and 13:00h). Measurements were made using a Scholander-type pressure chamber (PMS Instruments, Oregon, USA, Model 3005), with samples cut, bagged in a humidified plastic bag and measured in the field within 3 mins of sampling.

Data analysis

A number from one to six was attributed to each of the fifteen plant species used in this study, representing each of the six sites: Eneabba dune crest (ENHD), Eneabba clay pan swale (ENSW), Gnangara dune crest (GNHD), Gnangara dune crest and mid-slope (GNMD), Gnangara damp-land and mid-slope (GNMS), Gnangara damp-land (GNSW), respectively. I then transformed the values of *TLP*, *PD*, *MD* and $\delta^{13}C$ traits in positive by multiplying them by -1, applied natural log transformations on *WD*, *[P]*, *[N]*, *TLP*, *PD* and *MD*, and then standardized (scale to zero mean and unit variance). These transformations were necessary to make these values comparable between each other, as standardization best preserves distances between the variables; and traits with negative values were transformed in positive to facilitate interpretation. Pearson's correlations were used (R version 3.02, 2013) to test whether the coefficients of two-way trait relationships of the 15 plant species, were significantly different from 0, using 95% confidence intervals. Principal Component Analysis (PCA) was used to visualize similarity between species.

Primer-E (v6, Plymouth, UK) was used to perform a "Linktree" analysis, a non-parametric form of multivariate regression tree, to assess the degree of separation of groups in high-d space and determine which variables or traits are responsible for the greatest dissimilarity between groups created in each split (Clarke and Gorley, 2006). A one-way SIMPER routine was used to analyse variation between and within sites, as well as to explore the traits that most contributed to the variation. A similar approach was employed to investigate similarities and dissimilarities between and within functional groups previously determined through Linktree. Such analyses have been used in similar ecological studies and provided clear visualization of ecological groups and responses (Clarke et al., 2008; Mitchell et al., 2008). To visualize the functional and root-type groups within the first two principal components (based on site of occurrence for Eneabba and Gnangara), the R function "ordiellipse" was used to draw lines or polygons for dispersion ellipses using the standard error of the (weighed) average of scores, with the (weighed) correlation defining the direction of the ellipse principal axis.

4.4 RESULTS

Trait variation and correlations

Leaf mass per area was negatively correlated with leaf *[N]*, *[P]* and $\delta^{15}N$ (Pearson's r = -0.63, -0.65 and -0.69, respectively); *TLP* was positively correlated with *PD* and *MD* (Pearson's r = 0.69 and 0.77, respectively); and $\mathrm{D_s}$ was correlated with D_{max}, D_{ave} and *WD* (Pearson's r = -0.49, -0.50 and -0.48, respectively; Fig. 4.1, Fig. 4.2 and Table 4.2).

In the PCA analysis, the first three principal components accounted for 75.94% of the variation in traits. Based on the first two components (60.08% of trait variation), it was possible to visualize four main clusters of traits that were correlated to each other (Fig. 4.3): one corresponding to *LMA* and D_s; a second group comprising leaf *[N]* and *[P]*, and $\delta^{15}N$; a third group comprising D_{max} and D_{ave}; and a fourth group including *TLP*, *MD*, and *PD* (Fig. 4.2, Fig.4.3 and Table 4.2). Wood density, on the other hand, did not clearly group with any other trait, although it was weakly correlated with leaf *[N]*, $\delta^{15}N$, *PD*, *MD* and D_s (Fig. 4.2. Fig. 4.3 and Table 4.2). While *[P]*, *[N]*, $\delta^{15}N$, *PD*, *MD*, *TLP*, *LMA*, *WD* loaded strongly on the first two components, $\delta^{13}C$, D_{max} and D_{ave} were better represented in the third component.

Table 4.2 Pearson's correlation of all the twelve functional traits measured — average and maximum diameter of xylem vessel, number of xylem vessels per mm^2 (D_{ave} and D_{max} and D_s, respectively), leaf mass per area (*LMA*), wood density (*WD*), leaf concentration of phosphorus (*[P]*) and of nitrogen (*[N]*), leaf isotope composition of nitrogen (δ^{15}N) and of carbon (δ^{13}C), water potential at turgor loss point (*TLP*), pre-dawn (*PD*) and mid-day (*MD*) water potentials. The values of *TLP*, *PD*, *MD* and $\delta^{13}C$ traits were transformed in positive by multiplying them by -1. Significant correlations (p< 0.05) are indicated in bold.

	D_{ave}	D_{max}	D_s	*LMA*	*WD*	*[P]*	*[N]*	$\delta^{15}N$	$\delta^{13}C$	*TLP*	*PD*
D_{max}	**0.95**										
D_s	**-0.50**	**-0.49**									
LMA	-0.21	-0.10	**0.53**								
WD	-0.22	-0.23	**-0.48**	-0.29							
[P]	0.03	-0.02	-0.28	**-0.65**	0.20						
[N]	0.05	-0.02	-0.27	**-0.63**	**0.42**	**0.77**					
$\delta^{15}N$	0.04	-0.01	**-0.53**	**-0.69**	**0.48**	**0.52**	**0.53**				
$\delta^{13}C$	-0.19	-0.10	-0.05	-0.15	-0.04	0.10	0.00	-0.03			
TLP	**-0.38**	**-0.40**	**0.40**	0.36	0.08	**-0.40**	-0.37	-0.13	-0.24		
PD	-0.23	-0.31	-0.01	0.00	**0.56**	-0.06	0.08	0.29	**-0.45**	**0.69**	
MD	-0.28	**-0.37**	0.12	0.06	**0.44**	-0.10	0.07	0.17	-0.29	**0.77**	**0.94**

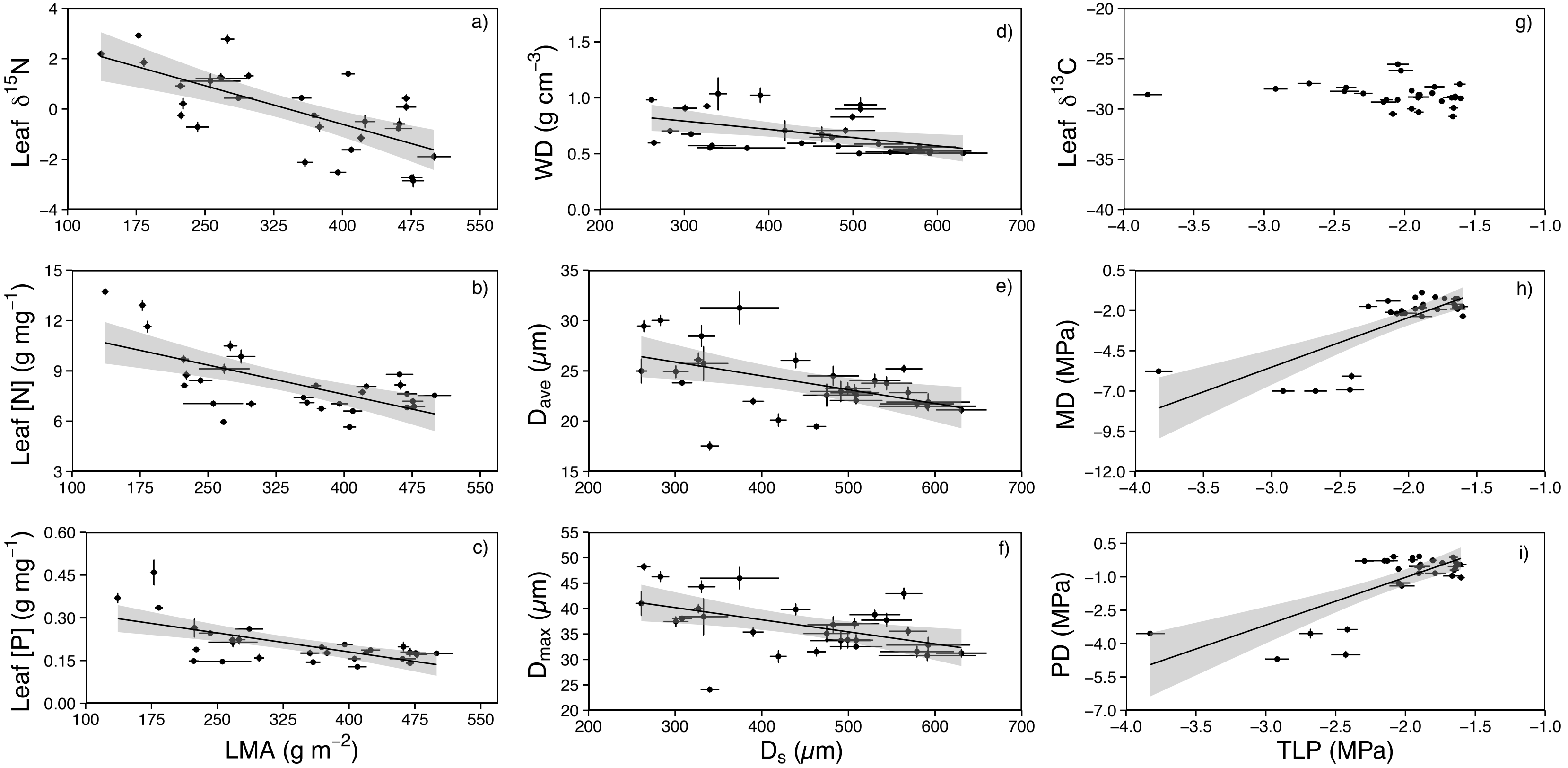

Figure 4.1 Scatter plots of selected functional traits based on their correlations. Leaf mass per area (*LMA*) was correlated with leaf isotope composition of nitrogen (δ^{15}N; Pearson's correlations; r = -0.69 and $p < 0.0000$), with leaf concentration of phosphorus (*[P];* r = -0.65 and $p < 0.0003$) and with nitrogen (*[N];* r = -0.63 and $p < 0.0001$). The number of xylem vessels per mm^2 (D_s) was reasonably correlated to wood density (WD; r = -0.48 and $p < 0.0089$), with maximum diameter of xylem vessel (D_{max}; r = -0.49 and $p < 0.0070$), and with average diameter of xylem vessels (D_{ave}; r = -0.50 and $p < 0.0063$). Water potential at turgor loss point (*TLP*) was not correlated to leaf isotope composition of carbon (δ^{13}C; r = -0.24 and $p < 0.2157$) but well correlated with both, pre-dawn (*PD*; r = 0.69 and $p < 0.0000$) and mid-day (*MD*; r = 0.77 and $p < 0.0000$) water potentials. Solid lines represent significant values of Pearson's correlation coefficients ($p<0.05$), grey shade is 95% confidence intervals and error bars represent standard errors.

Identification of hydraulic functional types

Linktree analysis separated the species into five different groups. The first group (group A, Fig. 4.2) comprised *H. subvaginata*, *R. inops* and *S. involucrata*, all occurring at the GNMS site, based on leaf *[N]*, leaf *[P]* or *LMA*. The second group comprised *M. leuropoma*, *B. carlinoides* and *B. elegans* (group B, Fig. 4.2), all occurring in ENSW and ENHD, based on *TLP*, *MD*, or *PD*. The third split used D_s to aggregate *A. cygnorum*, *E. beaufortioides*, *E. pauciflora*, *V. nitens*, *S. laxiflora*, *S. involucrata* (group C, Fig. 4.2) from all sites, with the exception of *S. involucrata,* which was from GNSW only. The next split grouped *B. attenuata* and *B. hookeriana* (group D, Fig. 4.2) occurring in ENHD, and *B. attenuata*, *B. menziesii* and *B. ilicifolia* from all different sites (group E, Fig. 4.2), based on $\delta^{13}C$, $\delta^{15}N$, *WD*, *PD* or D_s.

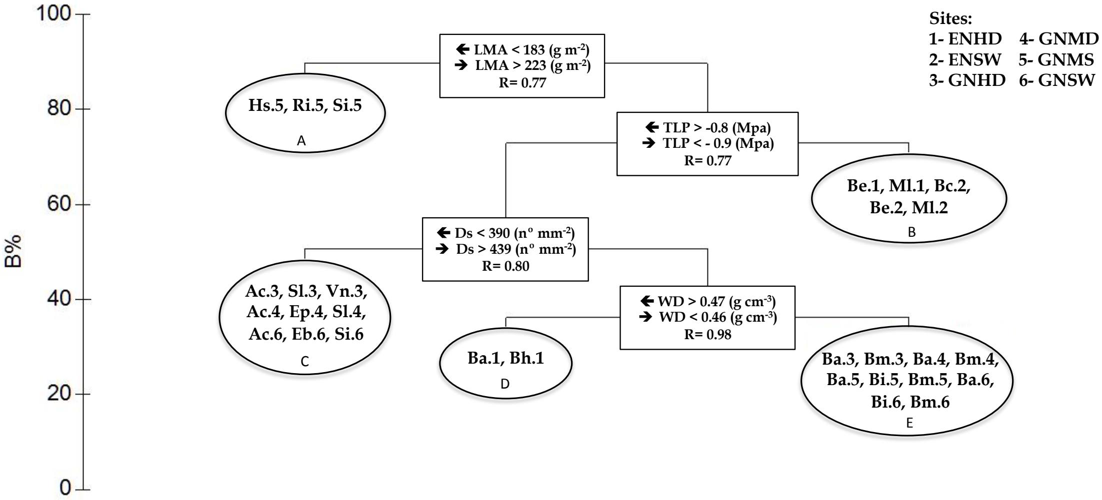

Figure 4.2 Linktree analysis using all 12 traits measured on the 15 plant species occurring in the 6 different sites. Species are written as *Adenanthos cygnorum* (Ac), *Banksia attenuata* (Ba), *Banksia carlinoides* (Bc), *Banksia hookeriana* (Bh), *Banksia ilicifolia* (Bi), *Banksia menziesii* (Bm), *Beaufortia elegans* (Be), *Eremaea beaufortioides* (Eb), *Eremaea pauciflora* (Ep), *Hibbertia subvaginata* (Hs), *Melaleuca leuropoma* (Ml), *Regelia inops* (Ri), *Scholtzia laxiflora* (Sl), Scholtzia involucrate (Si) and *Verticordia nitens* (Vn). Species' names are followed by a number corresponding to the occurrence site as per legend. First split occurred at 89.7% B value and was based on one of the three traits: leaf mass per area (*LMA*), leaf concentration of nitrogen *[N]*, and of phosphorus *[P]*. Second split occurred at 72.9% and was based on one of the three traits: water potential at turgor loss point (*TLP*), pre-dawn (*PD*), and mid-day (*MD*) water potentials. Third split occurred at 50.7% and was based on the density of xylem vessels per square millimeter (D_s) only. The last split falls under the 50% (36.2%) and therefore there is a high uncertainty about the separation. This split was based on one of the following traits: leaf carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition, wood density (*WD*), *PD*, and D_s. Traits shown in the figure were selected based on their efficiency of measurement.

Sites, groups and associated functional traits

Analysis of trait variation within sites showed that the sites ENHD, ENSW and GNMD, had the smallest values, i.e. these sites had greater within-site similarity of trait values among the 15 species sampled (SIMPER, Table 4.3). For site ENHD, *MD*, *LMA* and $\delta^{13}C$ accounted for 56.02% of the trait variation; for ENSW, *LMA*, *[N]* and *TLP* accounted for 70.85%; and for GNMD, D_s, *LMA*, $\delta^{15}N$, and *WD* accounted for 66.03% (Table 4.3). The Gnangara sites, GNHD and GNSW, presented intermediary values, indicating reasonable similarity between trait values among the 15 plant species measured (Table 4.3). For GNHD, D_s, *LMA*, D_{max}, $\delta^{15}N$, *WD* accounted for 79.59% of trait variation; and for GNSW, D_s, D_{ave}, $\delta^{15}N$ and *WD* accounted for 55.81%. The site GNMS had the highest value, indicating lowest similarity between trait values among the 15 species sampled. The traits- D_{ave}, D_{max}, *[N]* and *[P]*- accounted for 60.16% of GNMS trait variation (SIMPER, Table 4.3). The site ENSW displayed greater variation when compared to the Gnangara sites, followed by ENHD (SIMPER, Table 4.3). The two Eneabba sites were highly similar to each other; whereas Gnangara had sites that were also similar to one another (GNHD, GNSW and GNMD) and one site that was dissimilar to the other sites (GNMS when compared with the other sites, GNHD, GNMD and GNSW; Table 4.3).

Table 4.3 One-way SIMPER routine used to investigate the best-associated traits (higher contribution) with each site and functional group. Also, the differences within and between sites and within and between groups, in which numbers represent averaged square distances based on Euclidean distances.

	Trait contribution to site (%)							Trait contribution to group (%)				
	ENHD	ENSW	GNHD	GNMD	GNMS	GNSW		A	B	C	D	E
D_s	0.90	4.22	23.27	20.28	6.45	15.45	D_s	2.23	2.60	2.26	2.49	6.07
D_{ave}	4.12	3.56	9.09	7.28	16.44	11.64	D_{ave}	39.83	3.73	13.00	30.2	7.95
$\delta^{13}C$	14.69	1.31	2.90	1.11	3.40	5.68	$\delta^{13}C$	7.64	3.10	10.75	7.80	17.06
LMA	18.46	18.37	11.39	15.85	9.45	9.57	*LMA*	0.40	25.72	7.92	27.6	8.38
D_{max}	4.42	1.71	14.11	5.58	13.20	9.15	D_{max}	29.01	1.49	11.21	18.2	20.57
MD	22.87	0.99	0.87	2.04	1.82	0.93	*MD*	0.46	0.82	1.97	0.00	7.15
[N]	8.15	25.84	3.94	6.38	13.37	8.69	*[N]*	1.08	22.60	12.44	3.72	4.69
$\delta^{15}N$	2.58	4.03	13.15	16.71	6.96	14.95	$\delta^{15}N$	0.83	6.42	9.24	0.00	14.10
[P]	2.21	3.18	0.72	3.56	17.15	5.55	*[P]*	4.30	3.31	7.92	7.11	4.45
PD	8.76	1.15	1.02	1.44	1.64	1.05	*PD*	0.79	1.37	2.17	0.28	2.51
TLP	7.42	26.64	1.90	6.56	1.34	3.58	*TLP*	0.05	21.00	1.98	0.06	5.52
WD	5.44	9.00	17.64	13.19	8.77	13.77	*WD*	13.38	7.86	19.14	2.44	1.49
	Euclidean distances within and between sites							Euclidean distances within and between groups				
	ENHD	ENSW	GNHD	GNMD	GNMS	GNSW		A	B	C	D	E
ENHD	6.25						---	---	---	---	---	---
ENSW	15.68	5.02					A	14.30				
GNHD	22.01	34.71	8.69				B	49.07	4.35			
GNMD	21.37	32.91	13.90	6.57			C	30.95	33.63	5.66		
GNMS	31.47	43.21	28.28	23.72	14.35		D	44.40	20.22	26.38	2.04	
GNSW	26.08	26.08	16.89	14.90	25.49	8.91	E	44.57	28.98	20.99	15.9	2.51

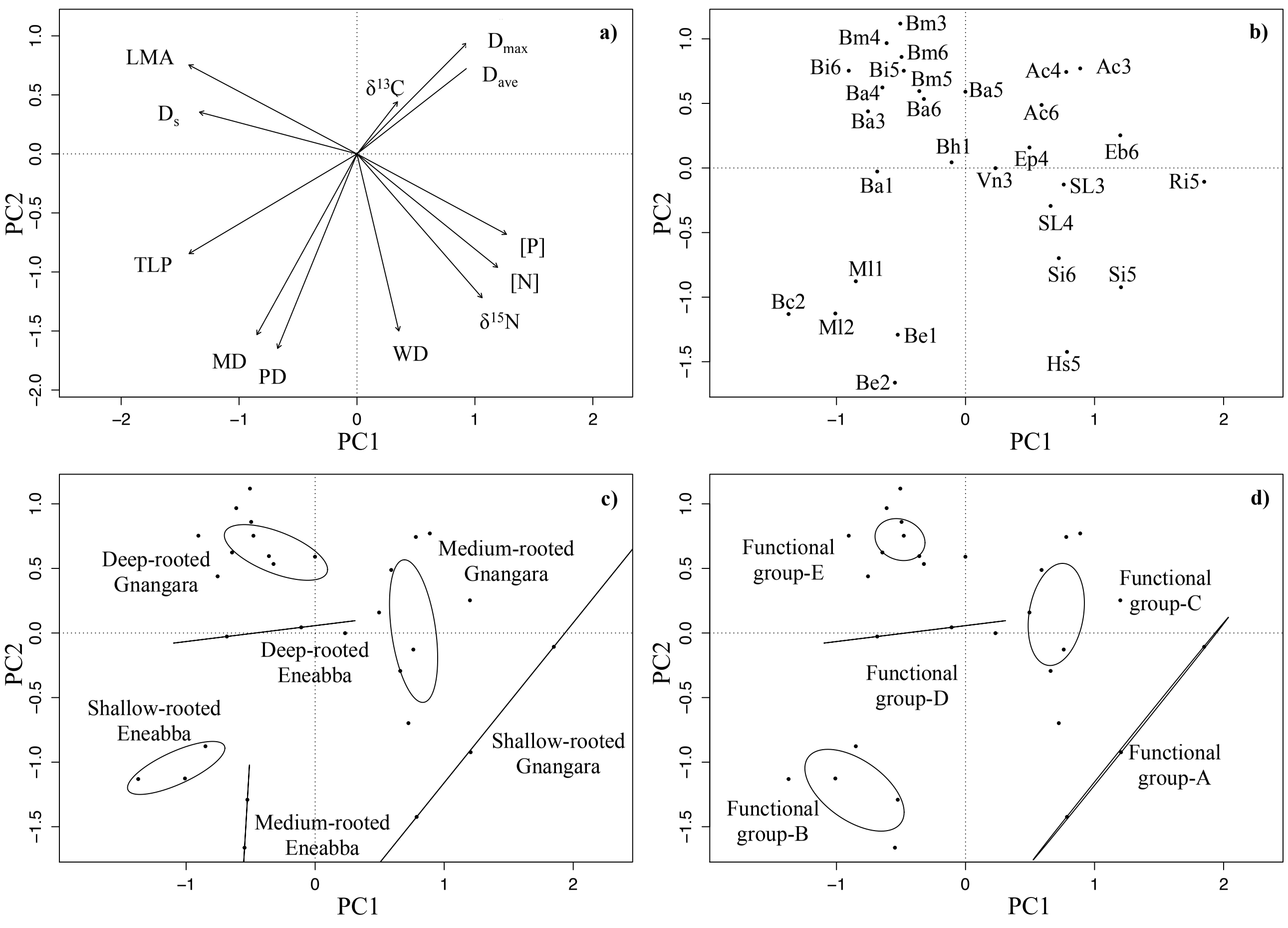

Figure 4.3 First two principal component analysis using **a)** the twelve functional traits measured: average, and maximum diameter of xylem vessel, number of xylem vessels per mm^2 (D_{ave}, D_{max} and D_s, respectively), leaf mass per area (*LMA*), wood density (*WD*), leaf concentration of phosphorus (*[P]*), and of nitrogen (*[N]*), leaf isotope composition of nitrogen ($\delta^{15}N$), and of carbon ($\delta^{13}C$), water potential at turgor loss point (*TLP*), pre-dawn (*PD*) and mid-day (*MD*) water potentials; and **b)** the species distribution in relation to the site of occurrence, letters represent abbreviations for species names and adjacent numbers represent the site of occurrence 1 - ENHD, 2 - ENSW, 3 - GNHD, 4 - GNMD, 5 - GNMS, and 6 - GNSW. First two components represent 60.08% of trait variation. The values of *TLP*, *PD*, *MD* and $\delta^{13}C$ traits were transformed in positive. **c)** Function "ordiellipse" used to draw standard error lines and ellipses in relation to species root type for Gnangara and Eneabba sites. **d)** Function "ordiellipse" used to draw standard deviation lines and ellipses in relation to functional groups.

Among the functional groups, group A had the lowest similarity within group, i.e., comprised of species whose trait values were more different to each other. The functional traits D_{ave}, D_{max} and *WD* accounted for 82.22% of trait variation within group A (Table 4.3). On the other hand, groups D and E had the highest values within group similarity. For group D, the functional traits D_{ave}, *LMA* and D_{max} accounted for 76.01% of trait variation, whereas for group E, $\delta^{13}C$, D_{max} and $\delta^{15}N$ accounted for 51.79% (Table 4.3). Groups B and C presented intermediate values, indicating that the species had reasonably similar trait values. For group B, the leaf traits *LMA*, *[N]* and *TLP* accounted for 69.32% of trait variation, whereas for group C, the leaf traits D_{max}, $\delta^{13}C$, D_{ave}, *[N]* and *WD* accounted for 66.54% of variation (Table 4.3). In regards to differences among sites, group A was the most distinct group, while group D was more similar to the other three groups (B, C and E); i.e. the relative distances between group A and the other groups were the highest, whereas distances between group D and groups B, C and E were the lowest (Table 4.3). Group B was also distinctly different from groups E and C, which in turn were reasonably similar to each other (Table 4.3). Species were also grouped in relation to root depth through the "ordiellipse" function, as: "shallow-rooted", "medium-rooted" and "deep-rooted" from Gnangara and Eneabba sites, thus corresponding with six root-depth groups (Fig. 4.3).

4.5 DISCUSSION

Associations between functional traits

The negative correlation of *LMA* and *[N]*, *[P]* and $\delta^{15}N$ (Table 4.2, Fig. 4.1 and Fig. 4.3) has been previously described under the "leaf economics spectrum" concept (Wright et al., 2004), in which plants can present a "fast-growing" or "slow-growing" strategy, reflecting adaptations to environmental gradients at small and broader scales (Reich et al., 1999, 1997; Westoby and Wright, 2006; Wright et al., 2004). These relationships can also be associated with drought resistance (Niinemets, 2001, 1999). The rationale for this is that *LMA* consists of two variables: leaf density (*D*; mass per unit volume) and thickness (Witkowski and Lamont, 1991). It is known that photosynthetic capacity scales negatively with *D* (Reich et al., 1999; Syvertsen et al., 1995), which in turn increases with increasing soil water limitation (Maksimov and Yapp, 1929; Shields, 1950). Foliar *[N]* and *[P]* are indirectly associated with photosynthetic capacity. This is because the majority of nitrogen in the leaves is associated with the RUBISCO enzyme as well as other components of the photosynthetic machinery (Field and Mooney, 1986; Lambers et al., 2008), while phosphorus is a major component of nucleic acids, sugar phosphates, ATP and phospholipids, all of which play important roles in photosynthesis (Bieleski, 1973). Thus, photosynthetic capacity is commonly highest in leaves with high nitrogen and phosphorus concentrations, at least for species with similar *LMA* (Ellsworth et al., 2014; Kattge et al., 2009; Reich et al., 2009, 1998). Lastly, leaf $\delta^{15}N$ is shown to be positively correlated to leaf *[N]* (Craine et al., 2009; Stock and Evans, 2006) and negatively with rainfall (Austin and Sala, 1999; Craine et al., 2009; Handley et al., 1999; Schulze et al., 1998).

The relationships found between D_s, D_{max}, D_{ave} and *WD* (Table 4.2, Fig. 4.1 and Fig. 4.3), although not strongly related, are also in agreement with those reported in the literature. The "wood economics spectrum" (Chave et al., 2009) is based on the relationships of wood density with mechanical and hydraulic properties. In a similar way, fast-growing species form an inexpensive hydraulic system with low wood density and large vessels, all of which results in a large hydraulic capacity. On the other extreme, slow-growing species form structurally expensive vessels with high wood density and small vessels, which leads to great safety and hence high survival, because the hydraulic system is robust and can resist pathogens, herbivores, cavitation and physical damage (Chave et al., 2009). Indeed, studies have shown a correlation between wood density and stem hydraulic properties (Chave et al., 2009; Markesteijn et al., 2011; Meinzer et al., 2008; Meinzer et al., 2008; Poorter et al., 2010; Russo et al., 2010; Zanne et al., 2010).

The balance between soil water availability and atmospheric evaporative demand determines plant water status, which is indicated by either leaf or xylem water potentials. Thus, the minimum water potential, a measure of plant water potential at the driest period, is a good proxy for plant water status since it reflects the maximum xylem tension that a plant must tolerate to maintain function (Bhaskar and Ackerly, 2006). Water potential at turgor loss point in turn represents the water potential value at which leaf cells lose turgor and function (Blackman et al., 2010; Brodribb and Holbrook, 2003; Cheung et al., 1975) and it is directly determined by osmotic potential at full turgor (Bartlett et al., 2012b). Therefore, plants may lower leaf water potential through osmotic adjustments under dry conditions to maintain adequate leaf water status. The relationship of plant water status and leaf osmotic adjustment is supported in my findings, since water potential at *TLP*, *PD* and *MD* were all well correlated (Table 4.2, Fig. 4.1 and Fig. 4.3).

The six leaf traits measured — D_{max}, D_s, *LMA*, *WD*, $\delta^{13}C$, and *TLP* — appear to be key traits underlying plant strategies in relation to water use, as they were relevant for explaining trait variation. These six traits were correlated to other important traits, i.e. D_{max} was well correlated with D_{ave}, *LMA* with leaf $\delta^{15}N$, *[N]* and *[P]*, and *TLP* with *PD* and *MD*, and therefore can be used as substitutes; D_s, *WD* and $\delta^{13}C$ together were reasonably correlated with *LMA*, leaf *[N]*, leaf $\delta^{15}N$, *TLP*, *PD*, *MD*, $\mathrm{D_{ave}}$, $\mathrm{D_{max}}$, (Table 4.2 and Fig. 4.1 and Fig. 4.3).

Plant water-use strategies and HFT identification

The Linktree analysis was consistent with the Pearson's correlation results and the PCA also supported the findings that at least the traits D_s, *LMA*, *WD*, and *TLP* are important parameters underlying plant water relations. The groups created were based on similarities in relation to these traits, rather than on taxonomic relationships. Interestingly, one species (*B. attenuata*) presented different strategies depending on the site, i.e. in the site with shallow soils, wood density was higher than when growing in soils where deep water was available (Fig. 4.2). Although plasticity has been shown to influence species drought responses (Lázaro-Nogal et al., 2015; Martorell et al., 2015; Mclean et al., 2014), measurements of these functional traits can provide a reasonable simplification for characterizing resistance to water deficits for many species or communities (Albert et al., 2010; Bartlett et al., 2014; Garnier et al., 2001; Rose et al., 2013). Still, there is a need to better understand the roles that plasticity may be playing in plant resistance to water deficits (Shipley et al., 2015) in order to make predictions of species responses to climate change more precise.

Linktree group E included the three *Banksia* species, *B. attenuata*, *B. menziesii* and *B. ilicifolia* (all of which are able to form deep-roots; Groom, 2004) across all sites. Thus, it is likely that they were all accessing deep water and hence presented similar responses, including hydraulic properties less associated with safety, such as high D_s and low *WD*. At the same time, these species presented high *LMA*, $\delta^{15}N$ and low *[N]* and *[P],* which can be associated with "slow-growing" strategies possibly due to adaptations to low availability of nutrients in the soils (Laliberté et al., 2013). However, *B. attenuata* and *B. hookeriana* occurring at the ENHD site (group D) presented a different strategy, with intermediate trait values. Although *B. attenuata* and *B. hookeriana* are able to access deep water through the formation of deep-roots, the site did not have an accessible water table and therefore these plants were likely experiencing higher water deficits when compared with plants from Gnangara system. Indeed, leaf water potentials at pre-dawn measured in *B. attenuata* and *B. hookeriana* occurring at the ENHD site were more negative than those of *Banksias* from Gnangara system (Appendix 3).

Group B contained the shallow-rooted species occurring at the Eneabba sites. These species experienced the highest xylem tensions, indicated by more negative values of *PD* and *MD,* and presented a hydraulic system that is associated with safety — lower values of D_{max} and D_{ave} — while maintaining more negative values of *TLP*. These species can be interpreted as "drought tolerators", as under low water availability plants with more negative TLP values and a robust hydraulic system are able to sustain stomatal and hydraulic conductance, photosynthetic gas exchange and growth.

Group A was composed of species that presented lower values of *LMA*, $\delta^{15}N$, and D_s and high values of *[P]*, *[N]* and *WD,* and are known to form shallow-roots (Groom, 2004). Interestingly, leaves of some individuals of *H. subvaginata* and *S. involucrata* turned yellow/brown at the end of summer, which may be related to tolerance of leaf dehydration and damaging radiation while stomatal conductance is low. These pigments enable leaves to persist over dry periods and to subsequently recover function when water availability increases over winter and spring (George, 2002; Lambers et al., 2014). Group C included species with medium depth roots from the Gnangara sites that had intermediate values of D_{max}, D_{ave}, $\delta^{13}C$, *[P]*, *[N]* and *WD* and low values of $\delta^{15}N$, *LMA*, D_s, *TLP*, *MD* and *PD*.

Changes to groundwater accessibility is known to significantly impact *Banksia* woodland vegetation, characteristic of the Swan Coastal Plain (SCP) as it is an important water source to both overstorey and understorey components of the vegetation (Groom et al., 2000; Zencich et al., 2002). Species from SCP differ in their abilities to access groundwater due to differences in their rooting pattern (Groom et al., 2000; Zencich et al., 2002). Not surprisingly, the response traits when analysed in combination revealed the important role that root depth plays in this community. This was clear because functional groups, clustered due to trait similarities, were analogous to root-depth groups, which in turn were clustered due to similarities in root-depth. Thus, in Gnangara and Eneabba systems, root depth is a suitable surrogate for water availability and consequently it is strongly related to functional traits that are associated with plant resistance to water deficit (Groom, 2004).

Species selection for ecological intervention projects

These different trait combinations represent alternative ecological strategies for balancing the costs that a plant incurs for the construction of a leaf and a hydraulic system versus the benefits in terms of carbon fixation via photosynthesis and water transport and safety (i.e. rapid resource acquisition vs. greater resource conservation). In this way, functional traits can be linked to ecosystem properties and services as they commonly vary across gradients of resource availability in predictable ways (Lavorel, 2013). Thus, these traits are a valuable tool for exploring individual ecological strategies of species and overall community assembly and functioning (Reich, 2014). However, concerns have been raised regarding these assumptions of trait-based ecology, which calls for more testing is needed to: i) reinforce that functional traits accurately indicate predictable relationships in measurable gradients of resource availability; ii) reinforce that they can be associated with individual fitness; and iii) establish when intraspecific variation can be disregarded (Shipley et al., 2016). Despite these caveats, studies that use a functional ecology approach for ecological intervention projects could succeed in meeting a number of intervention objectives, such as increasing survival or seedling recruitment of native species (Ostertag et al., 2015), predicting the success of particular restoration practices (Sandel et al., 2011), predicting the likely performance of species in restored vegetation (Pywell et al., 2003), and providing an indication of the potential for success of the completed restoration project (Engst et al., 2016).

Results of this study have demonstrated the range of plant water-use strategies found in a summer arid Mediterranean ecosystem in Southwest Australia through measurements of few functional traits — *[P]*, *[N]*, $\delta^{15}N$, $\delta^{13}C$, *PD*, *MD*, *TLP*, *LMA*,

WD, D_{max}, D_{ave} and D_s. The traits D_{max}, D_s, LMA, WD, $\delta^{13}C$, and TLP are sufficient to determine functional types as they explained well trait variation. An understanding of the role of the different groups of functional traits should enable the selection of species suitable for ecological intervention projects, which account for potential future climate change. The results of this study also showed how similar (redundant) or dissimilar (complementary) the different functional groups were through the application of a simple Principle component analysis.

Knowledge of the different functional groups is also important for increasing invasion resistance, since the selection of native species whose traits are more dissimilar could fill the different niches available and prevent the establishment of invasive species (Funk et al., 2008). For this to happen, it would also be necessary to define the weed functional traits to then align to the native aspect. Finally, restoration practitioners can apply this approach with relatively little effort, since the analyses of these functional traits provides an early screening of a species' functional profile in relation to drought resistance. Final species choices for any intervention project will have to account for complex issues and practical concerns beyond just water deficit resistance such as fire resistance, pollination, fruit/seed dispersal, culturally important species, plant establishment cost, and seed availability. Thus, species selected following this trait based approach will likely persist under drier conditions expected for *MTEs*.

4.6 CONCLUSION

In conclusion, this functional trait approach has provided valuable information about ecological strategies that plants employ to survive summer drought. It allowed the identification of functional groups in which traits associated with each group could be demonstrated. With the information provided from a small suite of traits and reasonably simple analyses undertaken in this study, it was possible to show that the functional trait approach is highly suitable for ecological intervention in a changing world, since it provides a rigorous approach for the selection of species that will enhance the success of intervention projects.

CHAPTER FIVE: SEEDLING RESPONSES TO FIRST SUMMER DROUGHT OF FOUR SOUTHWESTERN AUSTRALIAN SPECIES

5.1 ABSTRACT

Mediterranean summer drought can severely affect the recruitment stage of plants. The different responses that seedlings employ to survive summer drought may be a key factor for community assembly and ultimately for assisting management and intervention of ecosystems. I investigated the water-use strategies that four Mediterranean species presented during their first summer. I conducted two greenhouse experiments with Southwestern Australian species, in which seedlings grew in treatments that varied in water availability: with a shallow water table; with addition of rainwater; and without both, water table and rainwater. The first experiment focused on the ability of seedlings to grow deep roots, whereas the second experiment, on the accumulation and use of non-structural carbohydrates (NSC). I found two contrasting strategies for drought resistance in this study. The first strategy was associated with the reduction of stomatal conductance rates, accumulation and use of NSC, and growth of deep roots (*Banksia* seedlings). The second strategy was associated with the tolerance to higher water deficits and reduction of stomatal conductance rates (*G. tomentosum* seedlings). The information found can assist in determining seedlings' requirements to surviving drought, which is relevant for ecological intervention. Still, more studies are needed to investigate more specifically such mechanisms to drought resistance as well as the possibility for generalizations of drought response for similar species.

Key words: Root depth, non-structural carbohydrates, stomatal conductance rates, groundwater, and Mediterranean ecosystems

5.2 INTRODUCTION

The differing abilities of seedlings to cope with water deficits during the establishment stage is important for community assembly, dynamics and functioning, especially in ecosystems prone to drought periods (Pigott and Pigott, 1993; Zavala and Zea, 2004). Mediterranean-type ecosystems (*MTEs*) are characterized by having mild, wet winters and hot, dry summers (Aschmann, 1973), and thus water availability during summer drought represents a major environmental limitation under Mediterranean conditions (Castri, 1973). Indeed, seedling mortality is extensive over the first summer in rehabilitated communities in *MTEs* (Hallett et al., 2014; Lloret et al., 2005; Rokich, 2016; Standish et al., 2012) and water stress is one of the main causes of low success of intervention projects in *MTEs* (Mendoza et al., 2009; Vallejo et al., 2012). Furthermore, climate change is predicted to severely impact *MTEs*, with predictions of temperature increasing of 1 to greater than 2ºC and rainfall variability increasing significantly causing drier conditions, which will potentially aggravate the current scenario (IPCC, 2014). Therefore, there is a need to better understand current species distributions, where they occur as well as associated traits that allow for their survival in such areas, both currently and under future scenarios, which will assist in selecting the most appropriate species for intervention of ecosystems that have greater potential to persist under drier conditions. To achieve this goal, the identification of seedling functional responses and implied trade-offs across gradients of resource availability is thus critical (Körner et al., 2005; Lloret et al., 2004).

Mediterranean-type ecosystems sustain a high diversity of uniquely-adapted plant species (Cowling et al., 1996) that present a wide range of functional responses to water use and carbon assimilation (Hernández et al., 2010; Mitchell et al., 2008; Skelton et al., 2015; Vilagrosa et al., 2013; West et al., 2012). For instance, some plants can grow deep roots to access deep water (Meinzer, 1927), some of which rely on permanent access to deep water, called obligate phreatophytes (Eamus et al., 2006; Le Maitre et al., 2000), whereas others access groundwater only when it is available, known as facultative phreatophytes (Zencich et al., 2002). Under Mediterranean climate conditions, rapid early growth of deep roots is thus critical for survival of seedlings of deep-rooted species (Groom, 2004; Padilla and Pugnaire, 2007).

Increasing stomatal resistance is another strategy that some plants employ to cope with drought conditions (Brodribb et al., 2003). Although in reality there is a continuum of stomatal regulation in relation to water status, plants are commonly classified into one of two categories: isohydric or anisohydric (McDowell et al., 2008; Tardieu and Simonneau, 1998). Under low soil water potentials and dry atmospheric conditions, isohydric plants are able to reduce stomatal conductance, maintaining relatively constant midday water potentials regardless of drought conditions. Anisohydric species, on the other hand, keep stomatal conductance relatively high, allowing midday water potentials to decline under drought conditions (McDowell et al., 2008).

Drought-tolerant plants adopt anisohydric regulation and are able to maintain hydraulic and stomatal conductance, photosynthesis and growth by having high solute concentrations in their cells during periods of low water availability (Abrams and Kubiske, 1990; Baltzer et al., 2008; Sack et al., 2003). These plants commonly have a robust hydraulic system that can tolerate high tensions in their xylem vessels (Hacke et al., 2006; Jacobsen et al., 2007; Tyree and Sperry, 1989). They are however at risk of hydraulic failure when extensive cavitation takes place in their xylem vessels such that desiccation can no longer be reversed (McDowell et al., 2008).

At the other extreme, photosynthesis rates can drop to near zero as a consequence of stomatal conductance reduction in plants with isohydric regulation. These plants can eventually face carbon starvation or attacks from biotic agents, such as insect herbivory. The reduced ability to defend against herbivores and pathogens is a consequence of the depletion of carbohydrates reserves used to sustain primary metabolism while photosynthesis rates were low (McDowell et al., 2008). Recent studies have documented a decrease in the carbon reserves of some species during the summer period in Mediterranean climate regions, suggesting that such reserves might play a key role for survival under drought conditions (Adams et al., 2013; Galiano et al., 2012; Maguire and Kobe, 2015; Sanz-Pérez et al., 2009).

One proposed drought mortality mechanism suggests that an increase in Non-structural carbohydrates (NSC) can only be detected if growth is reduced faster than photosynthesis (McDowell, 2011; Muller et al., 2011; Tardieu et al., 2011). Prolonged drought, however, will eventually deplete the reserves for the maintenance of respiration and metabolism under inhibited photosynthesis, as found in recent studies (Adams et al., 2013; Galiano et al., 2011; Mitchell et al., 2013). Carbohydrates are necessary for phloem transport, turgor maintenance as well as for refilling embolized xylem vessels (McDowell, 2011). Water deficits and carbon limitation will reduce hydraulic conductance and together will decrease defensive capacity (McDowell et al., 2011). For species that present isohydric regulation of their stomata, accumulation and use of carbon reserves may be crucial for survival during first summer drought whereas for anisohydric seedlings, the ability to tolerate higher tensions in their xylem vessels might be more appropriate. In the case of phreatophytes, the growth of deep-roots to access deep water is probably the key for survival. These are three possible strategies for seedlings to survive summer drought in *MTEs*, but it is well known that many species employ a combination of strategies (Mitchell et al., 2013, 2008).

Studies that investigate deep root growth, non-structural carbohydrate dynamics and associated water relations over summer may reveal water-use strategies employed by seedlings to survive drought in Southwest Australia (*SWA*). These factors have been investigated but there are no studies that have examined these strategies in combination. Thus, this project focussed on the combinations of water-use strategies of seedlings of four *SWA* species, known to occur in two different eco-hydrological habitats, one area with shallow water table and another with deep water table, during their first summer drought. The study aimed to determine the ability of these species to grow deep roots, to control stomatal conductance and to seasonally use carbon reserves, both individually and in combination. More specifically, it focused on the responses of seedlings to drought conditions as in *MTEs* abiotic and competitive stress can be most severe at the seedling or juvenile stages. Therefore, niche differentiation determined by different responses to drought can be found at these young stages. This type of information is essential to understand the assembly and functioning of communities, which in turn is critical for intervention and management of more sustainable ecosystems that are under risk of future climate drought.

5.3 MATERIALS AND METHODS

Study species

Seeds of *Banksia attenuata* (R.Br.) and *Banksia littoralis* (R.Br.) from the Proteaceae family and of *Gompholobium tomentosum* (Labill.) and *Pultenaea reticulata* (Sm.

Benth.) from the Fabaceae family were bought from Nindethana Seed Company, Western Australia. These species were selected because they occur abundantly in the South–West Botanical Province of Western Australia and particularly on the Swan Coastal Plain (SCP, "FloraBase—the Western Australian Flora," 1998). They were also selected because they are known to occur in two different eco-hydrological habitats on the SCP. *B. attenuatta* and *G. tomentosum* occur across a gradient of water availability, except areas prone to waterlogging, but are able to survive in drier habitats; whereas *B. littoralis* and *P. reticulata* have a more restricted distribution, occurring mainly down-slope of the water availability gradient, in winter water-logged habitats ("FloraBase—the Western Australian Flora," 1998 and Groom, 2004). The experiments were conducted in a shade-house at the Edith Cowan University, Perth. Temperature and humidity data were acquired from Bureau of meteorology website ("Bureau of Meteorology," 2016) of two adjacent stations within 35 km (Hillarys Boat Harbor, station 009265) and 12 km (Wanneroo, station 009105) of the study site.

Experiment 1– deep roots and water relations

Experiment set up and treatments

The first experiment focused on the seedlings' ability to develop deep roots during early development until their first summer drought, as well as associated leaf morphological and physiological response traits. In August 2013, four plastic linings (of approximately 90 mm in diameter and 2 m in length) made of soft plastic with a weed mat bottom were placed inside of four 90 mm PVC pipes of 2 m long. The four pipes were then assembled inside a 190 l plastic drum and each plastic lining was filled with white-washed sand (Fig. 5.1). The top 10cm of sand from each pipe was replaced with topsoil collected from Bassendean dune system of the SCP, where the study species occur abundantly. There were 36 drums, so 12 of them could be assigned to a "shallow" water table treatment, in which drums were kept full (water column of approximately 80cm from the ground). Twelve were assigned to the "dry" treatment, in which drums were kept dry by previously drilling holes in their base to let water out. The last 12 blocks also had holes in the base of the drums, but were assigned to the "wet" treatment, in which pipes received 200 ml of previously collected rainwater three times a week to keep soil hydrated throughout the experiment. This experiment was set in September 2013 in a way that blocks, constituted of four pipes, had all the four species, each planted into one of the four pipes (Fig. 5.1). I used four seeds of each of the four species to increase the chances of having at least one seedling per pipe, in case germination was low.

All blocks were kept wet until the end of October by automatic irrigation set to three times a week, as well as any incipient rainfall. In November, I removed extra seedlings that germinated, allowing only two seedlings to grow in each pipe and started the water table treatments. The "shallow" and "dry" treatments were entirely dependent on rainfall, which is extremely low during the Perth summer as it is for all Mediterranean regions (Fig. 5.2; Aschmann, 1973; Gentilli, 1972). The "wet" treatment received 200 ml of water three times a week.

Plant measurements

In December, January and February, seedling growth (length) was measured with a ruler and stomatal conductance rates were measured from sunrise to sunset every two hours with a Porometer (Ap4 Delta-T devices, UK). In March 2014, pre-dawn (from 0400 to 0530 h) and mid-day (from 1100 to 1230 h) water potential measurements were taken from leaves of all the four species, with the exception for *G. tomentosum*, as there were not enough replicates for midday measurements, with a Scholander-type pressure chamber (PMS Instruments, Oregon, USA, Model 3005). After the measurement of water potentials all leaf and stem material was harvested, weighed for determination of fresh mass, and then placed in a drying oven at 70°C for 72 hours and weighed again for dry mass measurements. The pipes were removed from the glasshouse, placed horizontally on a bench and plastic linings were removed by carefully cutting it open without disturbing the roots. Root length was measured with a ruler and roots were collected at three different depths (0–60cm, 61–120cm and 121–180cm). Cluster-roots of *Banksias* were measured independently as they are more related to the acquisition of nutrients (Shane and Lambers, 2005a). Finally, soil water content was measured at the surface and then at every 20 cm depth intervals using a gravimetric method.

Experiment 2– non-structural carbohydrates and water relations

Experiment set up and treatments

The second experiment focused on the ability of seedlings to accumulate carbon reserves and some associated leaf morphological and physiological response traits, during early development until their first summer drought. In June 2014, the same pipes were cut to 50 cm long and filled with a shorter version of the soft plastic linings. Barrels were also cut to 40 cm in height and each of them received eight pipes. Plastic tubes inside the pipes were filled with white-washed sand and their top 10cm with native topsoil collected from the same area used in the previous experiment. This block was replicated 20 times, so that: i) ten could be assigned to a "dry" water table treatment, in which barrels had holes; and ii) ten to a "shallow" treatment, in which barrels were kept full (water column of approximately 20 cm from the ground; Fig. 5.1). Next, I used similar design of Experiment 1 for planting seeds, but this time each block had 8 pipes, so that two pipes were used for each species (Fig. 5.1). For the "dry" treatment, no surface water was provided and plants relied entirely on rainfall during winter, spring and summer, which was above 150 mm for each winter month and less than 10 mm for each summer month (Fig. 5.2). For the "shallow" treatment, seedlings relied on rainfall and on a water table.

Plant measurements

At the end of November, I performed the first set of measurements of growth and stomatal conductance rates from sunrise to sunset every two hours with a Porometer (Ap4, Delta-T devices, UK) on one seedling per pipe, with exception for *G. tomentosum*, whose measurements were done in two seedlings due to their small size. At the beginning of December, I removed the extra seedlings that germinated from all pipes. I also measured pre-dawn (from 0400 to 0530 h) and mid-day (from 1100 to 1230 h) water potentials from leaves of *Banksia* seedlings and from shoots of *P. reticulata* and of *G. tomentosum* with a Scholander-type pressure chamber (PMS Instruments, Oregon, USA, Model 3005). Next, i harvested four to six pipes containing each species from each treatment and determined their shoot and root length, fresh and dry mass. Cluster-roots of *Banksias* were measured separately. Leaf material was ground for analysis of carbon reserves. Growth and stomatal conductance rates were also measured in January and April so that changes in these parameters could be monitored over the driest part of the year.

In April, a scanner, a digital camera (Hitachi-KP-D40) and the Windias v2.0 software were used to calculate leaf area of 6-10 leaves from each individual plant. Leaves were then placed in a drying oven at 70°C for 72 hours to determine dry mass and calculate leaf mass per area (LMA; $g\ m^2$). Fresh stem sections of approximately 5 cm long were excised from the base of the plants and their bark removed before using the water-displacement method to determine their stem-specific density ($g\ cm^{-3}$; Pérez-Harguindeguy et al. 2013). Fresh and dry mass was determined for all remaining plant material, including roots. Three to four replicates from each of all species and treatments harvested in December and in April were oven dried for 2-3 days at 70ºC, finely ground and analysed at Cumberland Valley Analytical Services (Pennsylvania, US) for starch and water-soluble sugars. The analysis consisted of three steps: in the first, samples were extracted with water; in the second, phenol and sulphuric acid were added for digestion; and, in the third, measurements of photon absorbance were taken with a spectrophotometer. ACS grade sucrose was used as a standard. I also collected and analysed seedlings that died during the experiment (harvested when seedlings presented signs of death, this was when they were brown and dry looking) to investigate whether these seedlings had consumed all their carbon reserves.

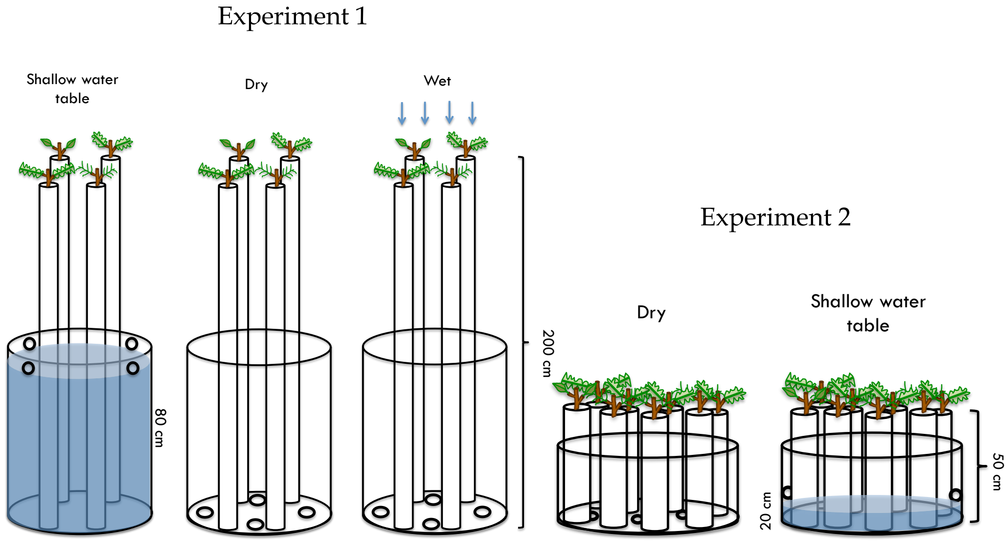

Figure 5.1 Experimental design of "Experiment 1", in which seedlings of four species (*Banksia attenuata*, *Banksia littoralis*, *Gompholobium tomentosum* and *Pultenaea reticulata*) were growing in each of the four pipes subjected to three different treatments. Treatment "shallow" had a water table at approximately 110-120 cm from the soil surface; treatment "dry" had no water table and relied entirely on rainfall during summer months; treatment "wet" was kept moist throughout the experiment. "Experiment 2" was a shorter version of "Experiment 1", in which seedlings from the same four species were subjected to two treatments. Treatment "shallow" had a water table at approximately 30 cm from the soil surface and treatment "dry" had no water table and relied entirely on rainfall during summer months. While "Experiment 1" focused in the growth of deep roots, "Experiment 2" focused in the storage and use of non-structural carbohydrates during summer months.

Weather conditions

Most of the rainfall occurred during the period from July to September 2013, with a total of 513 mm falling, which corresponded to approximately 89.5% of rain during the period that Experiment 1 was conducted (August 2013 to March 2014; Fig. 5.2). Winter temperatures ranged from 10ºC to 19ºC. The rainfall was extremely low for the period from December 2013 to March 2014 (total of 2.02 mm), which corresponded to only 2.3% of the rainfall received in Experiment 1. Summer temperatures ranged from 18ºC to 30ºC (Fig. 5.2). In Experiment 2 (June 2014 to April 2015), the period from June to September had a total rainfall of 394.1 mm, which corresponded to 66.8% of the rainfall received. From December 2014 to April 2015 rainfall was 125.2 mm, corresponding to 21.2% of the rainfall during Experiment 2. Temperatures ranged from 11 to 20 ºC during the winter months and from 16 to 29ºC for the summer months (Fig. 5.2).

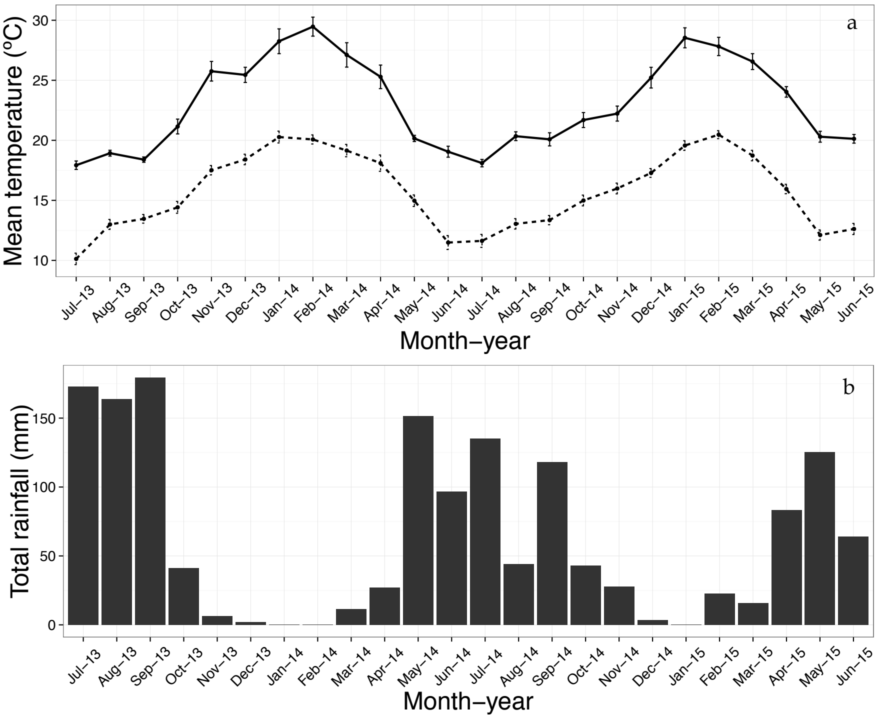

Figure 5.2 a) Mean of maximum and minimum temperature from July 2013 to June 2015, period when the two experiments were conducted, error bars are shown. Data was obtained from Hillarys Boat Harbor (station 009265). **(b)** Data of total rainfall from July 2013 to June 2015 was obtained from Wanneroo (station 009105).

Data analyses

Experiments “1” and “2” were analysed using R (version 3.0.2) with linear mixed effects modelling, function *lmer*() from the package ‘*lme4’* (Bates et al., 2015). For all analyses, “treatment” refers to the different treatments seedlings were submitted: “dry”, “shallow” and “wet” (Experiment 1; Fig. 5.1a) and “dry” and “shallow” (Experiment 2; Fig. 5.1b). “Month” refers to the three periods when the shoot length and stomatal conductance measurements were taken (December, January and February-April). “Time” refers to the time of the day when stomatal conductance rates were measured (5:30-7:30, 7:30-9:30, 9:30-11:30, 11:30-13:30, 13:30-15:30, 15:30-17:30 and 17:30-19:30). “Period” refers to the time of the day that water potential measurements were made (pre-dawn and mid-day). “Species” refers to the four species used, *B. attenuata*, *B. littoralis*, *G.tomentosum* and *P. reticulata* (“BA”, “BL”, “GT” and “PR”). In addition the identities of the individual seedlings measured and the barrel were included as random factors. Including “barrel” as a random factor was necessary because the experiments were set up as blocks, so the analysis could account for which block seedlings were growing in. On the other hand, “individual” was included as random factor when individual plants were measured more than once, which means that these observations were not independent. For all linear mixed models, the Akaike information criterion corrected for low n (AICc) was used to select the most parsimonious models (being the model with the lowest AICc), and differences >2 in AICc values are considered meaningful (Burnham and Anderson, 2004, 2002).

For “Experiment 1”, I used the function *lmer()* to investigate differences between the treatments in growth (seedling length) and stomatal conductance rates over three periods of the year, for each species independently. In this analysis, I included “month”, “treatment” and “time” (“time” being only applied for stomatal conductance analysis) as fixed factors. Then, I compared stomatal conductance rates between species with a subset of the data that included only data from the “dry” treatment of February. This subset was necessary because it allowed to specifically investigate differences between species under the driest conditions. Next, I used linear mixed modelling approach again to investigate differences in soil water content, shoot dry mass, root dry mass, root depth, and water potential, in which “treatment”, “species” and “period” were used as fixed factors for water potential analysis whereas “treatment” and “species” for all the other analyses. For *G. tomentosum*, water potential measurements were only taken at pre-dawn due to low number of replicates and because shoots were very small. Lastly, pair-wise comparisons were made to investigate differences between species for water potentials, stomatal conductance rates and root depth. For all pair-wise comparisons, I used the function *glht*() from the package '*multcomp'* (Hothorn et al., 2008).

For "Experiment 2", shoot dry mass, stomatal conductance and NSC were analysed using the function *lmer()* for each species individually, in which "treatment" and "month" (here referring to December and April measurements) were used as fixed factors. Stomatal conductance data was averaged for daily stomatal conductance, rather than using the extended version that included measurements for every two hours from sunrise to sunset. This was necessary to facilitate comparisons with shoot dry mass and non-structural carbohydrate data. Water potentials were analysed for each species individually with "month", "period" and "treatment" as fixed factors.

5.4 RESULTS

Experiment 1

The model that best explained soil water content included: treatment, species, depth to ground and the interaction between depth to groundwater and treatment (Appendix 4; Fig. 5.3). In the dry treatment, soil water contents for the depths 0-80, 81-180 and 181-200 cm were of approximately 0,71%, 2,48% and 10,8% respectively for B. *attenuata*; 0,80%, 2,58% and 11,0%, for *B. littoralis*; 0,86%, 2,87% and 8,19% for *G. tomentosum*; 1,18%, 2,79% and 9,32% for *P. reticulata*; and of 2,11%, 3,21% and 10,1% for pipes without any plant (Appendix 4; Fig. 5.3). In the shallow treatment, soil water contents for the depths 0-80 and 81-120 cm were of approximately 0,87% and 2,34% respectively for B. *attenuata*; 0,77% and 3,34% for *B. littoralis*; 1,22% and 3,58% for *G. tomentosum*; 0,90% and 3,76% for *P. reticulata*; and of 2,00% and 3,39% for pipes without any plant (Appendix 4; Fig. 5.3). In the wet treatment, soil water contents for the depths 0-180 and 181-200 cm were of approximately 3,32% and 8,32% respectively for B. *attenuata*; 3,35% and 10,41% for *B. littoralis*; 3,33% and 9,47% for *G. tomentosum*; 3,25% and 13,05% for *P. reticulata*; and of 3,75% and 8,95% for pipes without any plant (Appendix 4; Fig. 5.3). At 200cm of pipes that were subjected to the wet treatment, water accumulated a little at the bottom, despite the holes made for drainage and hence soil water content was very high.

B. attenuata seedlings formed roots that were deeper than roots of *G. tomentosum* and *P. reticulata*, whereas *B. littoralis* formed roots that were similar in length to all species (Appendix 4; Fig. 5.3)The most parsimonious model that best explained growth of deep roots included species only, and there were no marked differences in root length between treatments. The model that included both treatment and species, however, was equally good (Appendix 4; Fig. 5.3).

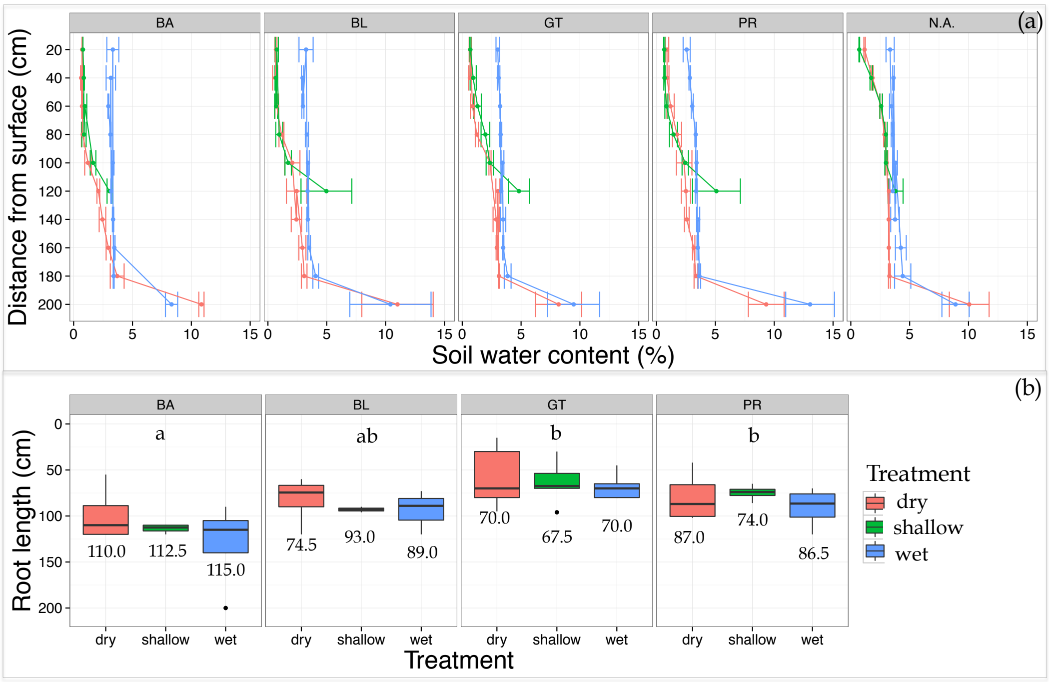

Figure 5.3 (a) Gravimetric water content of the soil profile for each of the four species, *Banksia attenuata* (BA), *Banksia littoralis* (BL), *Gompholobium tomentosum* (GT), and *Pultenaea reticulata* (PR); from each treatment, no water table and no rainfall addition (dry), shallow water table provided (shallow), and rainfall addition (wet). n= 3–6, with the exception for PR in the "shallow" treatment, which n=2. "N.A." was data collected from three pipes of each treatment that had no plants growing. **(b)** Root length of each species from each treatment. Lower case letters represent pair-wise comparisons between species. For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

Stomatal conductance of all species was influenced by treatment, month and time of the day that the measurement was taken, as well as by interactions between time and month and between time and treatment (Appendix 4, Fig. 5.4). For *B. attenuata* seedlings, however, the best model included month, time, treatment and the interaction between month and treatment only (Appendix 4, Fig. 5.4). Banksia seedlings presented similar behaviour, in December, stomatal conductance rates were relatively high in all three treatments, averages of 512.55 mmol m^{-2} s^{-1} for *B. attenuata* and 266.92 mmol m^{-2} s^{-1} for *B. littoralis*; with greater values from 0930 to 1130 am, which varied from 277.40 to 545.00 mmol m^{-2} s^{-1}. In January, stomatal conductance rates greatly declined in the "dry" and "shallow" treatments, reaching average values of 78.83 mmol m^{-2} s^{-1} for *B. attenuata* and of 97.69 mmol m^{-2} s^{-1} for *B. littoralis*. In February, averages of stomatal conductance rates for *B. attenuata* and *B. littoralis* (respectively) were higher in the "wet" treatment, 226.88 and 248.29 mmol m^{-2} s^{-1}; followed by the "shallow", with averages of 155.11 and 104.58 mmol m^{-2} s^{-1}; and the "dry", with values of 33.25 and 67.06 mmol m^{-2} s^{-1} (Fig. 5.4). In December, seedlings of *G. tomentosum* and *P. reticulata* from all treatments had similar stomatal conductance rates, being in average of 151.52 mmol m^{-2} s^{-1} and 127.35 mmol m^{-2} s^{-1} respectively. While in January and February, seedlings from the "wet" treatment presented higher stomatal conductance rates than seedlings from "shallow" and "dry" treatments. Values for *G. tomentosum* and *P. reticulata* (respectively) were in average of: 144.76 and 84.40 mmol m^{-2} s^{-1} for the "wet" treatment, 67.03 and 36.77 mmol m^{-2} s^{-1} for the "shallow" treatment, and 65.68 and 34.46 mmol m^{-2} s^{-1} for the "dry" (Fig. 5.4).

The best model for the subset of the stomatal conductance data (with "dry" treatment in February only) included "species" and "time" (Appendix 4, Fig. 5.4). Values were lowest for seedlings of *P. reticulata* and increased for *B. attenuata*, *G. tomentosum* and *B. littoralis* respectively, PR ≤ BA ≤ GT ≤ BL (*glht();* pairwise comparisons, Table 5.1).

Table 5.1: Pairwise comparisons (function "*glht ()*", from the package '*multcomp',* R version 3.0.2) of a subset of the stomatal conductance rates data. It includes only measurements taken on seedlings of the four species from the "dry" treatment measured during February.

Comparisons	Estimate	Standard Error	Z-value	p-value
BL X BA	30.896	11.460	2.696	**0.035**
GT X BA	21.762	10.509	2.071	0.161
PR X BA	-5.196	9.372	-0.554	0.945
GT X BL	-9.133	12.095	-0.755	0.874
PR X BL	-36.091	11.122	-3.245	**0.006**
PR X GT	-26.958	10.139	-2.659	**0.039**

Banksia seedlings grew more over January than over February, (Appendix 4, Fig. 5.5). Shoot length of *G. tomentosum* and *P. reticulata* were influenced by month, treatment and their interaction (Appendix 4, Fig. 5.5). They also produced most of their growth during January, with the exception of seedlings subjected to the "wet" treatment that showed similar growth throughout the summer months. Overall, seedlings from these two species had lowest shoot growth in the "dry" treatment, displayed intermediate growth in the "shallow" treatment and highest growth in the "wet" treatment (Fig. 5.5).

Seedlings of *B. attenuata* had higher shoot dry mass than the other three species and both *Banksia* seedlings grew more in the "wet" treatment, followed by the "shallow" and the "dry" (Appendix 4, Fig. 5.5). Seedlings of *G. tomentosum* and *P. reticulata* grew more in the "wet" treatment, whereas growth in shoot dry mass was similar in "shallow" and "dry" treatments (Appendix 4, Fig. 5.5). The model that best explained variation in water potential included species, treatment and the period of the day that measurements were taken (Appendix 4 and Table 5.2). The "dry" treatment was different from treatment "wet" (*glht*, Table 5.2 and 5.3) and "shallow" was similar to both "wet" and "dry" treatments (*glht*, Table 5.2 and 5.3). Pre-dawn values were different from mid-day values (*glht*, Table 5.2 and 5.3). Water potential values of *B. attenuata*, *B. littoralis* and *P. reticulata* were similar (*glht*, Table 5.2 and 5.3). However, water potential values of *G. tomentosum* were different from all the other species (*glht*, Table 5.2 and 5.3).

Table 5.2: Leaf water potential measurements made at pre-dawn and mid-day for both experiments in all four species. The four species used were: *Banksia attenuata* (BA), *Banksia littoralis* (BL), *Gompholobium tomentosum* (GT), and *Pultenaea reticulata* (PR). Treatments were: no water table and no rainfall addition (dry), shallow water table provided (shallow), and rainfall addition (wet). Averages and standard errors are shown (n=3–12).

Experiment 1			
Species	**Treatment**	**Pre-dawn**	**Mid-day**
BA	Dry	-0.77 ± 0.16	-1.27 ± 0.39
BL	Dry	-0.75 ± 0.05	-1.72 ± 0.98
GT	Dry	-1.99 ± 0.50	
PR	Dry	-1.23 ± 0.25	-1.20 ± 0.36
BA	Shallow	-0.47 ± 0.04	-1.18 ± 0.37
BL	Shallow	-0.53 ± 0.48	-1.33 ± 0.68
GT	Shallow	-1.45 ± 0.36	
PR	Shallow	-1.30 ±0.33	
BA	Wet	-0.45 ± 0.07	-1.25 ± 0.21
BL	Wet	-0.53 ± 0.16	-1.35 ± 0.40
GT	Wet	-0.74 ± 0.02	
PR	Wet	-0.42 ± 0.07	-1.05 ± 0.09
Experiment 2- December			
Species	**Treatment**	**Pre-dawn**	**Mid-day**
BA	Dry	-0.30 ± 0.05	-1.00 ± 0.28
BL	Dry	-0.29 ± 0.02	-1.46 ± 0.12
GT	Dry	-0.63 ± 0.07	-1.38 ± 0.14
PR	Dry	-0.32 ± 0.05	-0.99 ± 0.04
BA	Shallow	-0.35 ± 0.05	-1.38 ± 0.34
BL	Shallow	-0.40 ± 0.02	-1.55 ± 0.04
GT	Shallow	-0.48 ± 0.05	-0.98 ± 0.09
PR	Shallow	-0.28 ± 0.03	-0.76 ± 0.08
Experiment 2- April			
BA	Dry	-0.28 ± 0.04	-1.23 ± 0.13
BL	Dry	-0.28 ± 0.03	
GT	Dry	-0.31 ± 0.04	-1.14 ± 0.06
PR	Dry	-0.20 ± 0.00	-1.10 ± 0.10
BA	Shallow	-0.24 ± 0.01	-1.38 ± 0.20
BL	Shallow	-0.29 ± 0.04	-1.78 ± 0.12
GT	Shallow	-0.30 ± 0.03	-1.34 ± 0.07
PR	Shallow	-0.23 ±0.03	-1.00 ± 0.06

Table 5.3: Pairwise comparisons (function "*glht ()*", R version 3.0.2) of the leaf water potential data between treatments, periods and species, for data collected in Experiment 1.

	Factors	Estimate	Std. Error	Z-value	p-value
Treatment	shallow X dry	0.147	0.178	0.827	0.686
	wet X dry	0.518	0.168	3.088	**0.006**
	dry X shallow	0.371	0.171	2.174	0.076
Period	mid-day X pre-dawn	-0.599	0.130	-4.598	**0.000**
Species	BL X BA	-0.123	0.205	-0.603	0.931
	GT X BA	-0.770	0.209	-3.680	**0.001**
	PR X BA	-0.229	0.185	-1.241	0.600
	GT X BL	-0.647	0.221	-2.921	**0.018**
	PR X BL	-0.106	0.199	-0.531	0.951
	PR X GT	0.541	0.201	2.694	**0.035**

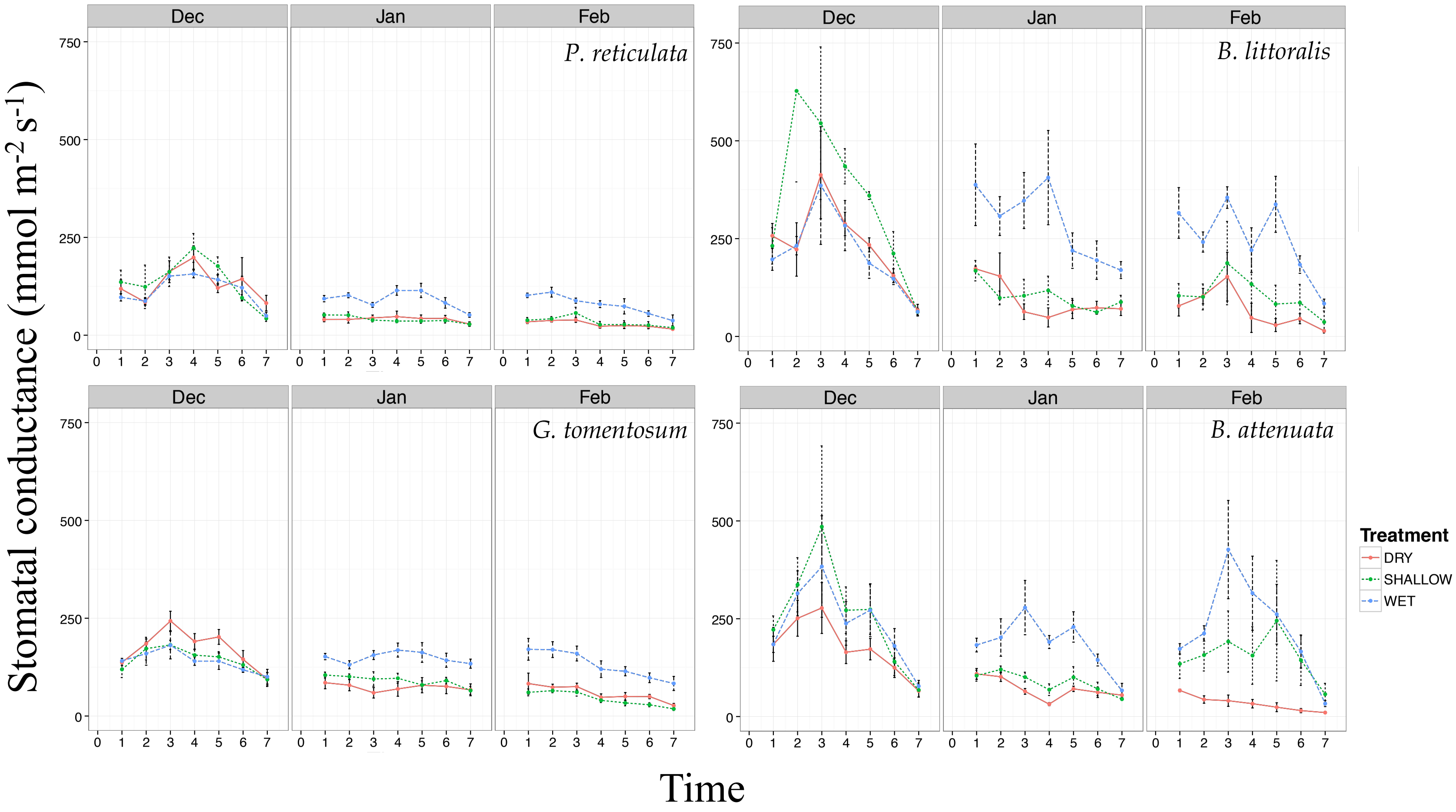

Figure 5.4 “Experiment 1”: Means of stomatal conductance of each of the four species from each of the three treatments, no water table and no rainfall addition (DRY), shallow water table provided (SHALLOW), and rainfall addition (WET). Data was collected in December, January and February, from sunrise (0530 h) to sunset (1930 h) every two hours (represented with “1, 2, 3, 4, 5, 6 and 7). Average and error bars are shown.

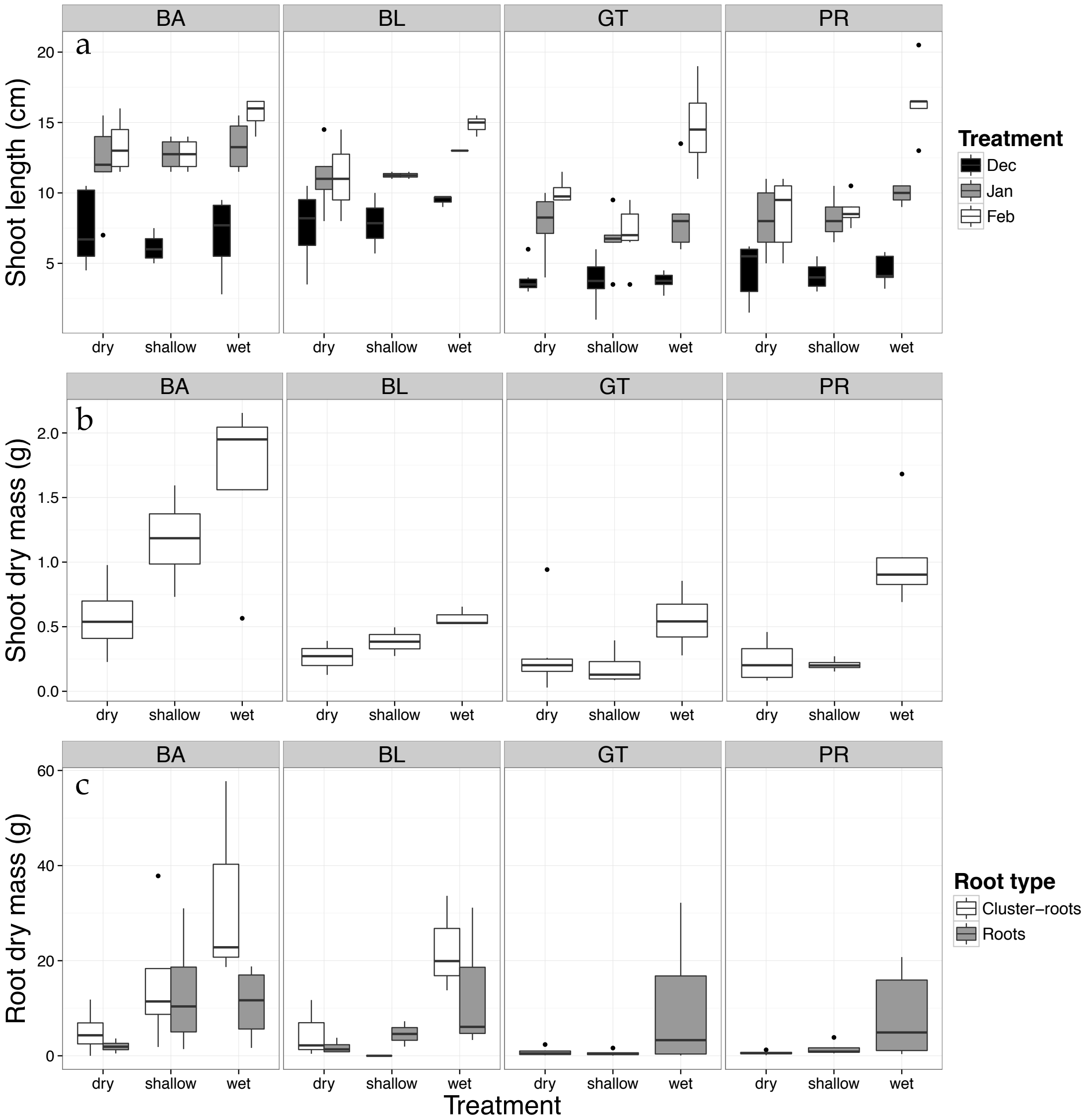

Figure 5.5 Data collected from "Experiment 1" for each of the four species (*Banksia attenuata "BA"*, *Banksia littoralis "BL"*, *Gompholobium tomentosum* "GT" and *Pultenaea reticulata "PR"*) from each of the three treatments. Treatment "shallow" had water table at approximately 110-120 cm from the surface, "dry" had no water table present and relied entirely in rainfall, and "wet" was kept moist throughout the experiment. **(a)** Shoot length measured in December, January and February. **(b)** Shoot dry mass measured in February. **(c)** Root dry mass measured in February. For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

Experiment 2

Seedlings of *B. attenuata* had low stomatal conductance rates when subjected to the "dry" treatment and higher when subjected to the "shallow" treatment in December. In April, this pattern repeated but with much higher values for seedlings from the "shallow" treatment; the model that best explained this variation included month and treatment (Appendix 5, Fig. 5.6). For *B. littoralis* seedlings, the best model included only treatment; stomatal conductance rates were lower for seedlings from the "dry" treatment and were equally high for seedlings from the "shallow" treatment (Appendix 5, Fig. 5.6). Seedlings of *G. tomentosum* had low stomatal conductance rates for both treatments during both months; the best model was the null, demonstrating no appreciable effects of treatment and month of measurement (Appendix 5, Fig. 5.6). Seedlings of *P. reticulata* had highest stomatal conductance rates in April. Although the best model included only month (Appendix 5), seedlings from the "shallow" treatment had marginally higher stomatal conductance rates than seedlings from the 'dry" treatment in April (Fig. 5.6).

The model that best explained the shoot dry mass of *B. attenuata* seedlings included month, treatment and the interaction of both (Appendix 5, Fig. 5.6). Their dry mass was equal between treatments in December and higher in April, especially for seedlings from the "shallow" treatment (Appendix 5, Fig. 5.6). Seedlings of *B. littoralis* and of *G. tomentosum* visually followed a similar pattern to *B. attenuata* seedlings but with lower values; the most parsimonious model, however, was the null for both species (Appendix 5, Fig. 5.6). Seedlings of *P. reticulata* grew more in April than in December, and, although the best model included only month (Appendix 5), seedlings from the "shallow" treatment had marginally higher values of dry mass than seedlings from the "dry" treatment (Fig. 5.6).

The model that included "month" and the null model were equally good at explaining the percentage of NSC in shoot dry mass of *B. attenuata* seedlings (Appendix 5, Fig. 5.6). However, the percentage of NSC decreased in seedlings subjected to the "dry" treatment from December to April, but it did not change for the seedlings from the "shallow" treatment (Fig. 5.6). For *B. littoralis*, the model that best explained the percentage of NSC was the one that included month, treatment and the interaction of the two. The percentage of NSC for *B. littoralis* decreased from December to April for seedlings subjected to the "dry" treatment. Similarly, seedlings from the “shallow” treatment presented a decrease from December to April, but in a smaller scale (Appendix 5, Fig. 5.6). Seedlings of *G. tomentosum* increased the amount of NSC in their tissues in both treatments from December to April, but there were no differences between treatments –the best model included only month (Appendix 5, Fig. 5.6). *P. reticulata* seedlings did not change the percentage of non-carbohydrate in their tissues –the best model was the null (Appendix 5, Fig. 5.6).

Lastly, measurements of water potential on *B. attenuata* seedlings were different between pre-dawn and mid-day and, mid-day values were slightly more negative for April (Fig. 5.6); the best model, however, included period only (Appendix 5 and Table 5.2). In contrast, *B. littoralis* water potential values were influenced by month, treatment and the interaction of the two (Appendix 5 and Table 5.2). Water potentials measured in seedlings of *G. tomentosum* were influenced by time, month, treatment and the interaction of month and time and of month and treat. Mid-day values were slightly more negative in April, whereas pre-dawn values were slightly more negative in December (Appendix 5 and Table 5.2). Similarly, time, month, treatment and the interaction of month and time influenced water potential measurements of *P. reticulata* (Appendix 5 and Table 5.2).

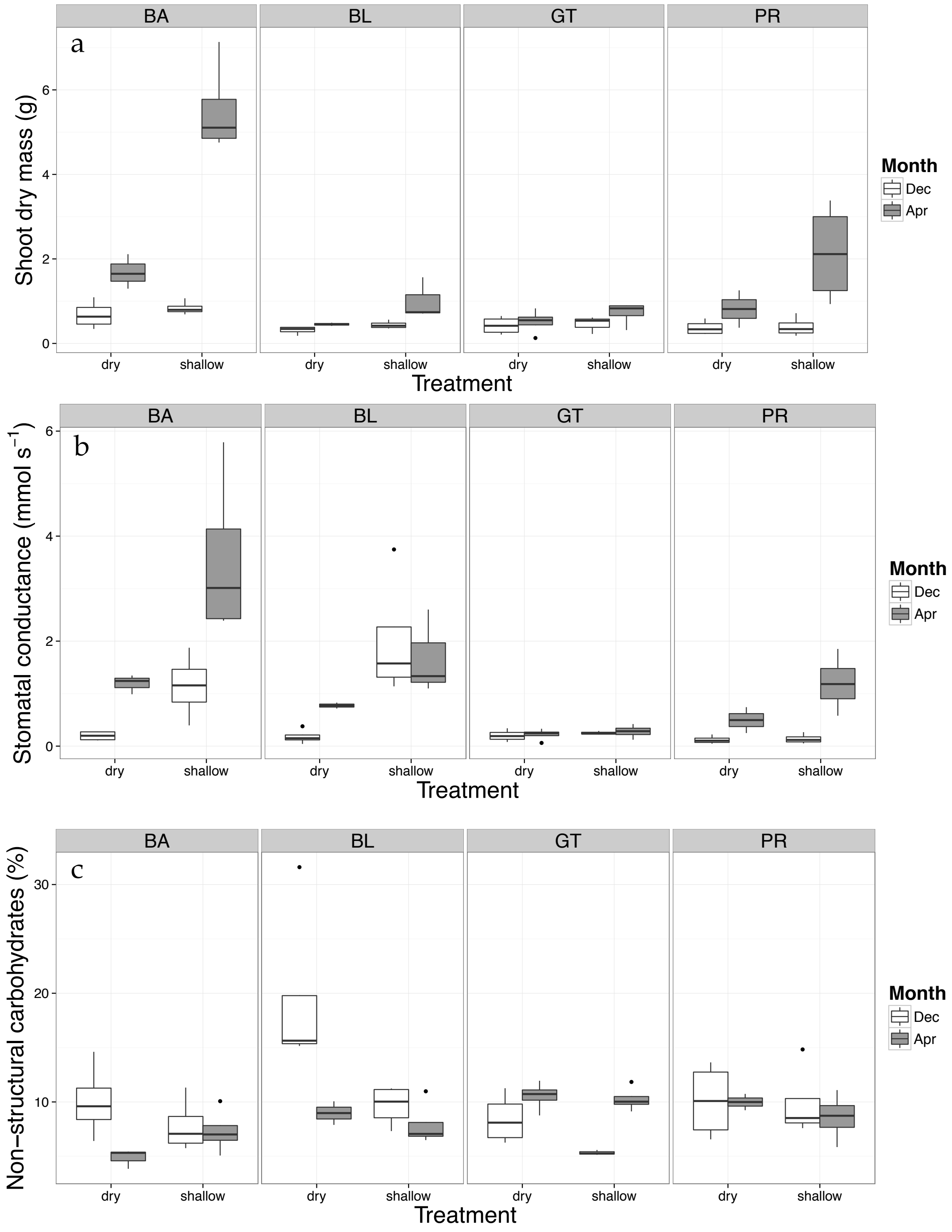

Figure 5.6 Data collected from "experiment 2" for each of the four species (*Banksia attenuata "BA"*, *Banksia littoralis "BL"*, *Gompholobium tomentosum* "GT" and *Pultenaea reticulata "PR"*) from each of the two treatments, no water table and no water addition (dry) and a shallow water table provided but no water addition (shallow). **(a)** Shoot dry mass, **(b)** average of daily shoot stomatal conductance adjusted by leaf area, and **(c)** non-structural carbohydrates (starch and sugars in % of dry mass). Data was collected from three to four individuals from each species from each treatment; with exception for BL and PR that the "dry" treatment had only two replicates. For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

5.5 DISCUSSION

Growth of deep roots

Seedlings of *B. attenuata* grew roots to approximately 112.5 cm deep and were thus able to access water from deeper layers and probably from the capillary fringe which is a zone that contains groundwater held by capillary action, sitting just above the water table (Eamus et al., 2006; Freeze and Cherry, 1979). This was reflected in higher stomatal conductance rates of seedlings that were kept moist as well as the ones that had access to deep water compared to those in the "dry" treatment. At the same time, under drier conditions, *B. attenuata* seedlings could reduce stomatal conductance to under 50 mmol m^{-2} s^{-1}, thereby avoiding severe desiccation as shown in their leaf water potentials that were relatively high. Such contrasting water use responses of plants able to access deep water have been observed for *B. attenuata* adults and juveniles in field and under glasshouse conditions (Canham et al., 2012; Enright and Lamont, 1992b; Groom, 2004). Seedlings of *B. littoralis* were also able to grow relatively deep roots and to reduce stomatal conductance in drier conditions, although stomatal conductance rates were still higher than the other species in similar conditions. They had, however, lower mid-day water potentials under drier conditions, suggesting that they may have a greater desiccation tolerance response than *B. attenuata* seedlings. Indeed, *B. littoralis* has previous been described in the literature for having relatively high stomatal conductance rates and lower water potentials under drier conditions (Groom, 2004, 2002).

Seedling roots of *G. tomentosum* did not reach the water table or capillary zone, which was approximately at 100 to 120 cm, only growing to 69.2 cm in length and the species showed low stomatal conductance rates during the driest summer months, being under 100 mmol m^{-2} s^{-1} for the "dry" treatment, as well as for the "shallow" treatment in which seedlings had no access to deep water due to their inability to reach the water table. At the same time, it appears that seedlings were slightly more drought stressed in the "dry" treatment, as pre-dawn water potential values were increasingly lower for "shallow" and "dry" treatments. This response suggests that these seedlings were losing more water than seedlings from the other species, and they displayed higher tensions in their xylem vessels. Seedlings of *P. reticulata* presented similar responses to *G. tomentosum*. However, they had even lower stomatal conductance rates over the driest summer month (< 50 mmol m^{-2} s^{-1}) and less negative water potentials. This suggests that they were better hydrated than *G. tomentosum* seedlings. Although *P. reticulata* occurs in areas where water tables are at medium to shallow depths, they appeared to avoid water loss under dry conditions. The responses of *P. reticulata* to drought in the field are unknown, however it is shown that surface water or water accumulated in deeper layers (0.3–1.2 m) is sufficient to maintain some medium-rooted species under summer drought (Groom, 2004; Zencich, 2004).

Shoot and root growth was highest in seedlings from “wet” followed by “shallow” treatments for *Banksia* seedlings and higher in the “wet” treatment for the other two species. Thus, it is possible that a winter with sufficient rainfall to recharge groundwater and/or the presence of water at depth facilitates the establishment of seedlings. This is because it allows for more extensive root growth, which is able to progressively access more water from deeper layers as the near-surface soil water content decreases with increasing summer drought. Indeed, there has been evidence for increased establishment of seedlings early in the season as germination and early growth take place during a longer period of water availability (Padilla et al., 2007; Quintana et al., 2004) and perhaps that enables roots to reach deeper layers.

Soil water content in shallow layers (approximately to 60 cm below the surface) was different between treatments, with “dry” and “shallow” holding less water than the “wet” treatment. Although there were differences between species, in deeper layers, soil water content was similar between treatments, being approximately 2.11% to 3.76% to 180 cm depth and then reaching soil water capacity 8-11%). This result suggests that drought period from December to February was not sufficient to complete dry out the 200 cm long pipes subjected to the “dry” treatment. In a study conducted on the Swan Coastal Plain, soil water content was closer to 1% at 2 m depth (Zencich et al., 2002), whereas in this experiment, soil water content was approximately 4% from 120 cm to 180 cm and below this was fully hydrated. Therefore, seedlings could have had access to some deep water in all treatments while in the shallow layers water availability was substantially different between the “wet” and other two treatments.

Accumulation and use of non-structural carbohydrates

For *B. littoralis* seedlings, the accumulation and use of NSC likely played an important role in the maintenance of metabolism under dry conditions. This is due to the fact that seedlings consumed the reserves but did not grow as much as seedlings from “shallow” treatment, which had accumulated lower reserves, despite reasonably high stomatal conductance in April. The response of *B. littoralis* seedlings is in agreement with other studies (Adams et al., 2013; Galiano et al., 2011; Mitchell et al., 2013), which also found that, under dry conditions, accumulation and consumption of NSC was associated with metabolism maintenance. Under drier conditions, seedlings of *B. attenuata* stored NSC for later use in growth, as they accumulated more reserves and grew less; whereas under moist conditions, they did not accumulate NSC but grew more. The survival and growth during summer drought of *G. tomentosum* and *P. reticulata* seedlings are not related to storage and use of NSC. This is due to the fact that *G. tomentosum* accumulated more non-structural carbon reserves in April, independent of the treatment; and growth and stomatal conductance were not affected by either month or treatment (the null model was the best in both cases). Additionally, stomatal conductance and growth of *P. reticulata* were affected by month, independent of the treatment; and accumulation of NSC was not influenced by month nor by treatment.

Combined responses to summer drought

Across a gradient of water availability, *B. attenuata* is known to be able to survive in xeric environments and is considered a facultative phreatophytic species (Zencich et al., 2002). It uses water from deep sources during dry summer and early autumn months, and from the upper zone of the soil profile as winter rainfall recharges shallower zones (Zencich et al., 2002). In this study, *B. attenuata* seedlings used a combination of strategies to survive summer drought: they were able to grow deep roots in their first 6 months accessing relatively deep water, and they were able to reduce stomatal conductance rates when conditions were dry. Although results of the accumulation and use of NSC were less evident, they suggest that these reserves may also play a role in drought resistance since accumulation and use of carbohydrates were needed to at least support growth of *B. attenuata* seedlings under drier conditions. *B. littoralis* is almost exclusively dependent on shallower groundwater all year around (Zencich et al., 2002; Zencich, 2004) and it thus may lack the ability to quickly reduce water loss under prolonged dry conditions, being at risk of hydraulic failure if soil water content falls under 1% (Dodd et al., 1984; Groom, 2002). This species was able to survive summer drought by also growing roots to relatively deep soil layers and by reducing stomatal conductance rates, although less efficiently than *B. attenuata* seedlings. Surprisingly, accumulation and use of carbohydrates seems to be important for drought resistance of *B.littoralis* seedlings, as seedlings from the "dry" treatment accumulated and consumed more reserves, while stomatal conductance and growth were lower than seedlings from the "shallow" treatment.

Gompholobium tomentosum has been described to form medium roots (1– 2m; Groom et al., 2000) and therefore it is more dependent on rainfall than deep-rooted species. Under drier conditions, *G. tomentosum* seedlings were able to grow roots to medium depths, but did not reach the water table, which was 120 cm deep. They could, however, tolerate relatively higher water deficits, despite low stomatal conductance rates. Accumulation and use of NSC, on the other hand, was not important for drought resistance of *G. tomentosum* seedlings. Therefore, they are more likely to tolerate drought and eventually face hydraulic failure under prolonged drought, as also predicted by McDowell et al., (2008). Although seedlings of *P. reticulata* presented relatively similar responses to *G. tomentosum*, the mechanisms by which these seedlings survived summer drought were not as clear. NSC seems not to be important since no variation were detected across summer months for drought resistance of *P. reticulata* seedlings, at the same time, stomatal conductance rates and water potentials were relatively low.

The different strategies of these four Mediterranean species to survive summer drought as seedlings are complex and a combination of responses were involved. There was evidence of at least two strategies that these seedlings employed to survive drought. One strategy was to access water from deep soil layers combined with stomatal regulation and possibly use of NSC (*Banksia* seedlings). The second strategy appears to involve tolerance to water deficits, at least for *G. tomentosum*, despite reasonably low stomatal conductance rates. This last strategy suggests that these plants might tolerate drought by photosynthesizing the minimum necessary to maintain metabolism, and possibly slowing their metabolism down during summer drought (George, 2002). Seedlings of *G. tomentosum* and *P. reticulata* presented little changes in NSC dynamics and reduced stomatal conductance rates. Indeed, leaves of adults of *G. tomentosum* have been observed to turn yellow during summer and re-green when water availability increases in field conditions (George, 2002), which suggests that this species lowers its metabolism during drought. Responses of *P. reticulata*, however, were less clear with no previous observations found in the literature. In order to investigate desiccation tolerance characteristics, however, it is necessary to measure or document cell desiccation, which is outside of the scope of this study.

5.6 CONCLUSION

This study found evidence of two strategies to survive summer drought employed by seedlings of Southwestern Australian species, including: (1) reduction of stomatal conductance rates, accumulation and use of non-structural carbohydrates, and growth of deep roots; and (2) reduction of stomatal conductance rates despite no accumulation and use of non-structural carbohydrates. This information can indicate seedlings' requirements to survive drought, which is important when selecting species for ecological intervention as well as when predicting future scenarios. I acknowledge that my observations on the accumulation and use of non-structural carbohydrates by seedlings during summer drought remain preliminary and were limited as only shoots were analysed. Besides, my study was not designed to investigate characteristics related to dormancy and resurrection. It is also possible that there is trait plasticity, i.e. individuals from a drier area perform differently to those from wetter environments. Thus, further research is needed to examine if my results are generalizable to other similar species or situations within *MTEs*, which is required for more appropriate management and intervention of such areas.

CHAPTER SIX: CAN HYDRAULICALLY REDISTRIBUTED WATER ASSIST SURVIVAL AND GROWTH OF SURROUNDING SEEDLINGS DURING SUMMER DROUGHT?

6.1 ABSTRACT

Studies of plant interactions allow a better understanding of the role of key species in ecosystems, which can assist in the conservation, management and intervention of natural resources. Among the interactions, facilitation and competition are known to strongly affect ecosystem structure and function. In this study, I investigated whether there is facilitation between a deep-rooted species and surrounding seedlings through hydraulic redistribution during their first summer water deficit in a Mediterranean-type ecosystem. I conducted a field experiment, in which seedlings from two different species were isolated or not from donor plants, known to be able to hydraulically redistribute water. In addition, I conducted a glasshouse experiment, in which seedlings were growing with donor plants that were either accessing or not accessing the water table, or growing without a donor tree. Donor trees were either adult Banksia trees (in the field) or two years old Banksia seedlings (in the glasshouse). Survival, growth, stomatal conductance rates and soil water content were recorded during summer months; approximately, 5-8 months after seeds were sown. I also applied δ^2H enriched water to roots of donor plants to track water movement within and between plants. In the field, seedlings of *B. attenuata* that were isolated from the donor tree had the highest survival, growth and stomatal conductance rates. Seedlings of *G. tomentosum* survived and grew better with a donor tree with no access to the water table. In the glasshouse experiment, stomatal conductance rates were higher when growing with a donor tree (regardless of water table access) than when growing without a donor tree over the first summer months, but this relationship reversed towards the end of summer. Field seedlings from both treatments had a similar increase in leaf δ^2H, indicating that were able to absorb hydraulically lifted water. Moreover, access to the water table by donor plants caused a slight increase in leaf δ^{2}H of glasshouse seedlings. Donor plants were able to perform hydraulic redistribution and seedlings were able to absorb the hydraulically redistributed water. In the field, it is possible that root interactions occurred in deeper layers, but this needs further testing. Soil water content was higher on the side where the seedlings were interacting with a donor tree (field), and in seedlings interacting with donor plants that had access to the water table (glasshouse). However, seedlings performed better in the other treatments, suggesting that donor plants and seedlings could have been competing for a resource other than water. These results support recent findings from other studies where competition was more prominent than facilitation in extremely stressful conditions, such as the severe summer water deficits of Mediterranean-type climates. In this context, using *Banksia attenuata* trees as potential facilitators of water availability for neighbouring seedlings in rehabilitation projects might not be recommended during the driest periods of the year.

Keywords: facilitation, competition, hydraulic redistribution, water availability, ecological intervention, and Mediterranean- ecosystems

6.2 INTRODUCTION

Facilitation plays an important role in community assembly in many plant communities (Callaway and Walker, 1997b), as it reduces the negative influences of stressful environments by providing shade, increasing nutrient and water availability, and/or by ameliorating extreme temperatures. A typical example is nurse/donor plants with established canopies, beneath which germination and survival are much more likely. Facilitation can also result in altered species distribution patterns, greater species coexistence and enhanced diversity and productivity, all of which greatly influence ecosystem structure and function (Callaway, 1995). It was initially suggested that facilitation would play an important role in highly stressed environments, whereas competition would dominate in less-stressed ecosystems (Bertness and Callaway, 1993; Callaway, 2007). However, although facilitation may extend the range of conditions where an organism can occur, under very stressful conditions it might be insufficient to support the growth of all individuals involved. Therefore, facilitation is now generally assumed to enhance plant performance under moderate conditions only (Holmgren and Scheffer, 2010).

There is evidence that water can be facilitated by deep-rooted plants to neighbouring plants through “hydraulic lift” (Caldwell et al., 1998; Pang et al., 2013; Yu and D’Odorico, 2015) and hydraulic redistribution (Burgess et al., 1998; Caldwell et al., 1998). Hydraulic lift is the movement of water upward from deep wet layers to shallow drier layers within plant root systems (Richards and Caldwell, 1987), and hydraulic redistribution refers to the movement of water through plant root systems from moist to dry soil layers, at any depth, driven by a water potential gradient (Burgess et al., 1998; Caldwell et al., 1998). Hydraulic redistribution is a main mechanism of facilitation, and it has been shown to account for up to 80% of the water lost through stomatal conductance (Emerman and Dawson, 1996; Neumann and Cardon, 2012; Scholz et al., 2010). Moreover, this mechanism supports the maintenance of fine root function (Bauerle et al., 2008; Caldwell et al., 1998), prolongs the lifespan of mycorrhizal fungal hyphae in dry soils (Querejeta et al., 2007), and delays the onset of water stress (Caldwell et al., 1998; Meinzer et al., 2004). Hydraulic redistribution has been reported for a large number of taxa (Caldwell et al., 1998), across many ecosystems and environments (Emerman and Dawson, 1996; Prieto et al., 2009; Scholz et al., 2008), and it is thought to be important in the structure and functioning of many plant communities (Katul and Siqueira, 2010). Of particular interest is the role that this facilitation mechanism plays in community assembly in systems where the surface of the soil is very dry but there is adequate deep water, which would allow significant lift and redistribution of water.

In order to predict the future effects of current human activities on ecosystems, and to improve the conservation and management of natural resources, we must understand and acknowledge the potential effects of facilitation or competition on community assembly. Studying such interactions helps establishing the role of key species, which is needed to restore the functional characteristics of ecosystems (Bruno et al., 2003). Unfortunately however, to the best of my knowledge, intervention efforts have seldom accounted for the potentially important role of facilitation through hydraulic redistribution.

The *Banksia* woodlands of Southwest Australia have been heavily cleared and modified across their range, with many local attempts now in place to rehabilitate degraded areas and their ecological functions. *Banksia* trees are the dominant overstorey species in these formations, and many areas have remnant Banksia trees that can potentially facilitate the process of rehabilitation of such areas. This is due to the fact that they may positively influence the recruitment and survival of understorey of conspecific and other plants and seedlings by increasing shallow water availability. Hydraulic redistribution is known to occur in *Banksia* species (*B. prionotes*), and most species have dimorphic roots consisting of a deep tap-root that accesses groundwater and shallow lateral roots, that facilitate the uptake of nutrients and recent precipitation (Burgess et al., 2000; Dawson and Pate, 1996).

Due to the importance of *Banksia* in restoration of the fragile and highly impacted ecosystems of Southwest Australia, the aim of this study was to investigate the occurrence of facilitation through water redistribution between seedlings of two native plants and neighbouring adult *Banksia* trees. I predicted that seedlings of both deep-rooted (*B. attenuata*) and relatively shallow-rooted (*G. tomentosum*) species would absorb hydraulically redistributed water from a deep-rooted donor tree (*B. attenuata*), known to be capable of hydraulic redistribution, which would result in improved survival and growth, at least for shallow-rooted species. To address this hypothesis, I conducted two experiments, in which seedlings were grown in the field and the glasshouse with or without the presence of a putative donor tree. In the glasshouse experiment, the donor plants were manipulated so that some had access to deep water, whereas the others were solely reliant on shallow soil water. In all experiments, I measured the survival, growth and stomatal conductance rates of seedlings at the beginning, middle and end of the summer stress period. At the end of the experiment, I used δ^2H labelled water to track the water movement within and between plants to determine whether seedlings could absorb shallow water that was previously hydraulically lifted by the donor tree.

6.3 MATERIALS AND METHODS

Field experiment

The study site was situated approximately 38 km north of Perth, Southwest Australia on the Gnangara groundwater mound, a shallow unconfined aquifer. This area has a Mediterranean-type climate with mild, wet winters and hot, dry summers; mean annual rainfall of 797.7 mm, from which approximately 56% falls during winter months, whereas only nearest 5% falls during summer months (Bureau of Meteorology; Aschmann, 1973; Gentilli, 1972). Sites were located on the Bassendean Dune System which consists of highly leached quartz sand with negligible silt and clay, and hence the soils have a very low water holding capacity (McArthur, 1991; McArthur and Bettenay, 1960). There is also a wide range (3 to 30 m) in depth to the groundwater water table across the dunal landscape (Thackway and Cresswell, 1995), which causes differences in the availability of water to the plants. As a result of this gradient and these differences in water availability, there are differences in species assembly and plant tolerance to water deficits (Beard, 1990; Froend and Sommer, 2010; Sommer and Froend, 2014). The field site (31º42’30’’S and 115º54’30’E) was located in the mid-slope of the gradient of water availability, and is under a restoration program (Maher et al., 2008).

In April 2013, eight well-established (150 to 300 cm height) adult individuals of *Banksia attenuata* were selected, and the sparse surrounding vegetation was removed to plant seedlings to simulate an ecological intervention program and to reduce possible effects of roots from neighbouring plants on these seedlings. Three 60 cm deep barriers, made of impermeable plastic (polyethylene dampcourse), were buried in the soil at the edges of the donor trees' canopies (Fig. 6.1). The lengths of the barriers were also based on the lengths of the donor trees' canopies; for example, a 5 m length barrier was used when the donor plant's canopy was of 5 m (Fig. 6.1). Thirty seeds of each species were planted external to the barrier and therefore were isolated from the donor-tree, whereas another set of thirty seeds was planted in the internal side of the barrier allowing for possible interaction with the donor tree. These groups are refereed as "without barrier" (-B) and "with barrier" (+B). The third barrier was placed to limit the area of the +B group of seedlings similarly to the -B group (Fig. 6.1). I acknowledge that this design does not stop interactions between these seedlings and roots of neighbouring plants, but if these interactions exist, effects should be similar for both groups (+B and -B). After the barriers were installed, I calibrated volumetric soil moisture sensors (5TM and EC-TM, Decagon) for use in sandy soils, as by manufacturer protocol, and installed them at depths of 20 and 50 cm on each side of four donor plants, adjacent to where seeds were to be planted. Soil water content was recorded hourly by these sensors for 11 months until March 2014.

In July 2013, which was the wettest period of the year, 30 seeds of *Banksia attenuata* (*R.Br*) and 30 seeds *Gompholobium tomentosum* (*Labill*) species were planted on both sides of donor plants and protected with mesh fencing. These species were selected since they are abundant in undisturbed vegetation stands in the area and are known to form deep roots (*B. attenuata*) and shallow roots (*G. tomentosum*). Therefore each of the eight blocks consisted of one donor tree, three underground barriers (Fig. 6.1), and the two groups (+B and –B) of up to 30 seedlings. The groups of seedlings were equally oriented east west, so that both groups received similar amount of sunlight. To avoid seedling death they were watered once every two weeks with 1l of water, until the end of October, when the experimental treatment was applied.

Seedling survival was recorded every two weeks in November, December, January and February. From December to February, I also measured the stem length of all seedlings and the stomatal conductance rates for two seedlings of each species growing on each side of all eight donor plants, every two hours from 0530 to 1930 h with an AP4 Leaf Porometer (Delta T devices, Cambridge, UK).

At the end of the experiment in March 2014, leaf water potential was determined on one healthy mature leaf from each seedling previously used for stomatal conductance measurements and on two leaves of each donor tree. Predawn water potential measurements were made between 0400 and 0600 h and midday measurements between 1100 and 1300 h using a Scholander-type pressure chamber (PMS Instruments, Oregon, USA, Model 3005). Few *G. tomentosum* seedlings survived the summer drought so water potentials, isotope composition and dry mass of this species were not determined.

Leaf isotope composition was investigated in the donor trees to check whether the methodology worked and water got injected in the vessels of the plant, being transported to the leaves as expected, and in *B. attenuata* seedlings. There were not enough seedlings of *G. tomentosum* for this last part of the experiment. Leaf material of *B. attenuata* seedlings and donor plants was collected and rapidly sealed in two plastic bags and frozen at -20ºC for later analysis of δ^2H isotope signatures. On the following day, the top 40 cm of soil at the base of each donor tree was excavated and taproots exposed were injected with 20 ml of a solution of 25% deuterium and reburied. Leaf material of all seedlings and donor plants was collected again at 12, 24 and 48 hours after the injections and was prepared and stored as described above. Aboveground shoots of all surviving seedlings were harvested and returned to the laboratory for drying and weighing. Water extractions were performed at Edith Cowan University using cryogenic vacuum distillation following methods outlined in West et al., (2006) and then analysed for δ^2H at the University of Western Australia with a Picarro Liquid Water Isotope Analyser. Samples were corrected for possible organic contaminations based on ChemCorrect algorithm. Normalization was done based on three laboratory standards, each repeated twice, calibrated against international standards provided by IAEA: VSMOW2, SLAP2 and GISP (Coplen and B., 1996). The external error for non-enriched water samples (one standard deviation) is of 0.10 ‰ for δ18O and of 1.0 ‰ for δ2H. Technical details of the instrument and used procedure can be found in Skrzypek and Ford (2014).

Glasshouse experiment

In the glasshouse experiment, I set up three treatments: 1) seedlings growing by themselves (no donor plant), referred to as "NoD"; 2) seedlings growing with donor plants able to access a water table, "D+w"; and 3) seedlings growing with donor plants deprived of a water table, "D-w". To achieve this, I used 33 planter bags of 100 l, 23 of which were individually connected at the base to a 90 cm pipe that was placed inside a bucket of water (Fig. 6.1). Donor plants were established by placing plastic tubing inside the pipes going from the surface all the way to the bucket to make sure donor plants would grow deep roots through the pipe and reach the water in the bucket. All bags and plastic tubing were filled with white washed sand. I also placed volumetric soil moisture sensors (5TM and EC-TM, Decagon) at 20 cm depth in three bags from the "D-w", eight from "D+w" and five from "NoD" and recorded soil moisture hourly until the end of the experiment. In June 2013, a single one-year old *B. attenuata* seedling was planted in each plastic tube and, with approximately 8 g of Osmocote© fertilizer for native plants added. Sand was kept moist by automatic irrigation system set to 5 minutes three times a week. In March 2014, plastic tubes were removed to allow lateral roots to grow throughout the bag.

In June 2014, I started germinating the "receiver" seedlings by planting 10 seeds of *B. attenuata* and 10 of *G. tomentosum* in each bag, including the 10 bags without donor plants (NoD). I also added another 16 g of Osmocote© fertilizer for native plants in each set to minimize nutrient limitations and to isolate the effect of water. Plants were then allowed to germinate and grow under similar conditions, being watered three times a week for 5 minutes through the automatic irrigation system. In September 2014, to investigate whether all donor plants were accessing the water table after approximately 17 months of growth, I added a solution of LiCl (10 ml of 0.3 M) to the water in the buckets. Two small leaves were collected before and after the LiCl addition for the LiCl analyses that were performed at Edith Cowan University, through an initial sample digestion, with nitric and perchloric acids; followed by readings in an inductively coupled plasma optical emission spectroscopy (ICP–OES). Donor plants that had a clear increase in leaf LiCl were assigned to treatment "D+w", whereas donor plants with no increase in leaf LiCl were assigned to treatment "D-w". Buckets from donor plants in treatment "D-w" were emptied of water and kept this way for the remainder of the experiment. Also, excess seedlings were removed from all bags to make sure I had a similar number of plants in each bag (2 to 4 *B. attenuata* and 3 to 6 *G. tomentosum* seedlings per bag) and irrigation system was turned off.

The experiment was initiated in October 2014 when I progressively reduced watering. Towards the end of the experiment, in April 2015, I drilled a hole in the pipes of five bags from treatment "D+w" and five from "D-w", approximately 40 cm from the floor and injected 20 ml of a solution of 25% deuterium. I followed the same approach used in the field experiment to collect samples, but I only harvested plants from the bags that had δ^2H isotope added, plus five bags from the "NoD" treatment. The remaining bags were left intact but remained unwatered until the end of May so that survival of seedlings and donor plants could be monitored for a longer period. In November, January and April-May, I measured plant heights, as well as stomatal conductance rates every two hours from sunrise to sunset on all plants with a AP4 Leaf Porometer (Delta T devices, Cambridge, UK).

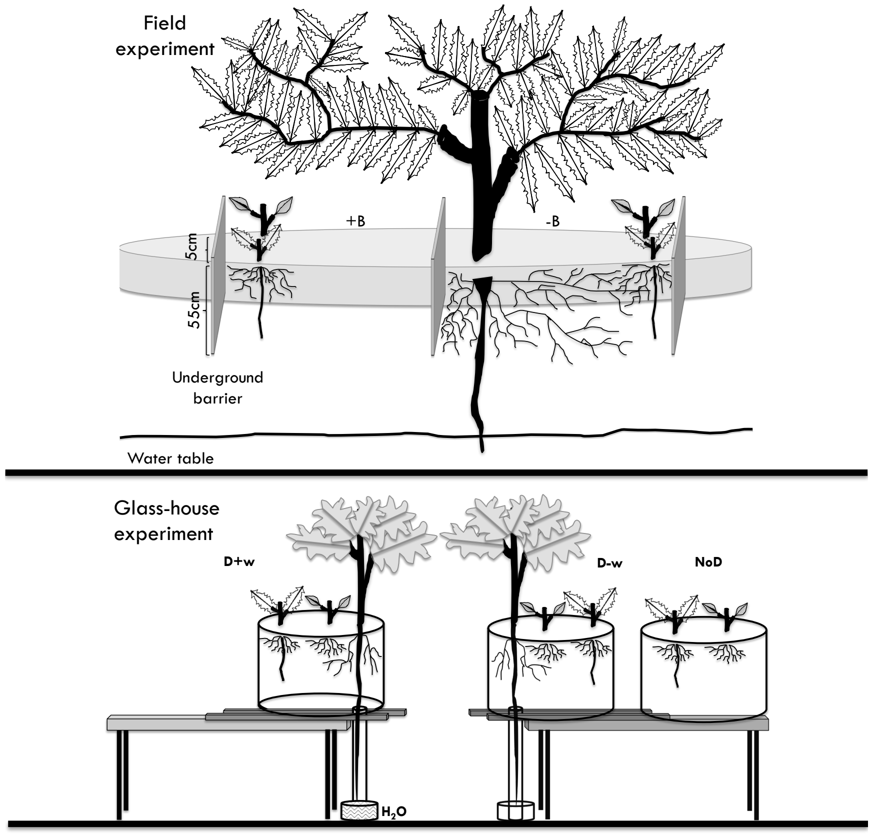

Figure 6. Experiment designs of the field and glasshouse studies. In the field experiment, seedlings were planted either without a barrier allowing for possible interactions with the donor plant or partially isolated because of a physical water-resistant plastic barrier positioned underground between the donor plant and receiver seedlings. In the glasshouse experiment, seedlings were growing without a donor plant, or were growing with a donor plant that was accessing a water table, or with a donor plant that was deprived of a water table.

Data analysis

Field and glasshouse experiments were analysed using R (version 3.0.2), as described below. For the field experiment, “treatment” refers to whether seedlings were isolated or not of a donor tree. In the glasshouse experiment, “treatment” referred to whether seedlings interacted (D) or not (NoD) with a donor tree, and additionally whether this donor tree had access (D+w) to the water table or not (D-w). “Month” refers to the three periods when the length and stomatal conductance measurements were made (November-December, January and February-May); although for soil moisture data, “month” represents each of the measured months (“Oct”, ‘Sep”, “Nov”, “Dec”, Jan”, Feb” and “Mar”). “Time” refers to the time of the day when stomatal conductance rates were measured (5:30-7:30, 7:30-9:30, 9:30-11:30, 11:30-13:30, 13:30-15:30, 15:30-17:30 and 17:30-19:30). “Period” refers to the time of the day when water potential measurements were made (pre-dawn and mid-day). “Hour” refers to the hourly measurement of soil moisture. “Collection” refers to the collection time before and 12, 24 and 48 hours after the isotope injections (initial, h12, h24 and h48). “Species” refers to the two species used, *B. attenuata* (BA) and *G. tomentosum* (GT). Also, the identities of the donor tree and of the individual seedlings measured were included as random effects. The random effect of donor was necessary because certain seedlings (up to 10) shared the donor tree they were interacting with. On the other hand, individual was included as a random effect when individual plants were measured more than once, which means that these observations were not independent. I also added neighbour as a covariate (fixed effect) in all models to account for the number of seedlings growing on each side of the donor tree (field experiment) or in each bag (glasshouse experiment).

I applied the function “*survdiff ()*” from the package ‘survival’ (Therneau, 2015; Therneau and Grambsch, 2000) to investigate the differences in survival of seedlings between the treatments for each species separately. I used the function “*survfit()”*, from the same ‘survival’ package to plot the curves. The number at risk is compared to the number of events at each unique death time and this information is used to generate the survival curves. Next, I used linear mixed modelling, function “*lmer()’* (lme4 package; Bates et al., 2015) to investigate differences between the treatments in growth (seedling length) and stomatal conductance rates over three periods of the year, for each species independently. In this analysis, I included “month”, “treatment” and “time” (“time” being only applied for stomatal conductance analysis) as fixed factors. Then, with same analysis approach, I investigated differences in shoot dry mass and leaf water potentials, in which "treatment" was used as a fixed factor for both analyses, whereas "period" was used as a second fixed factor for the water potential analysis only.

Glasshouse data of leaf water isotope composition was log transformed to better visualization, as donor trees presented very high concentrations of δ^2H (varying between 1000 and 15000 ‰) and seedlings presented lower concentrations (varying between 25 to 125 ‰); and outliers detected were excluded from the analysis. Both field and the corrected glasshouse isotope data were analysed with linear mixed effects models, in which "treatment" and "collection" were included as fixed factors. Lastly, soil moisture data was analysed with "treatment", "month" and "hour" included as fixed factors. I also repeated the soil moisture analysis changing the covariate "neighbour" for an estimate of total stomatal conductance per bag; since the results were similar, I kept the simplest version of the models with "neighbour" as a covariate. For all linear mixed effects models, the Akaike information criterion corrected for low sample sizes (AICc) was used to select the most parsimonious model (the model with the lowest AICc), where and differences >2 in AICc values are considered meaningful (Burnham and Anderson, 2004, 2002).

6.4 RESULTS

Field experiment

There was an increase in δ^2H in the leaves of the donor plants and *B. attenuata* seedlings from both treatments after the injections (Appendix 6; Fig. 6.2). Soil water content variation was best explained by the model that included "month", "treatment" and their interaction (Appendix 6). The sensors positioned at 20 cm depth recorded similar amount of soil moisture, and this amount was higher than the sensors positioned at 50 cm (Fig. 6.3). Overall, soil water content measured with all sensors decreased over summer months (Fig. 6.3) and increased in April and May.

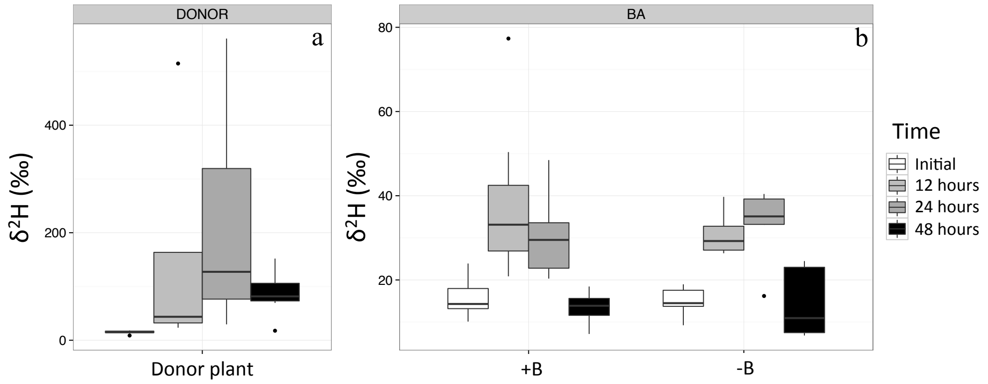

Figure 6.2: Leaf water isotope composition (‰VSMOW) measured at the end of the field experiment of initial and 12, 24 and 48 hours after injections of a deuterium enriched solution into taproots of donor plants. Measurements were made on: **a)** donor plants and **b)** *B. attenuata* seedlings from both treatments, with a barrier to isolate seedlings (+B) and without the barrier (-B). For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

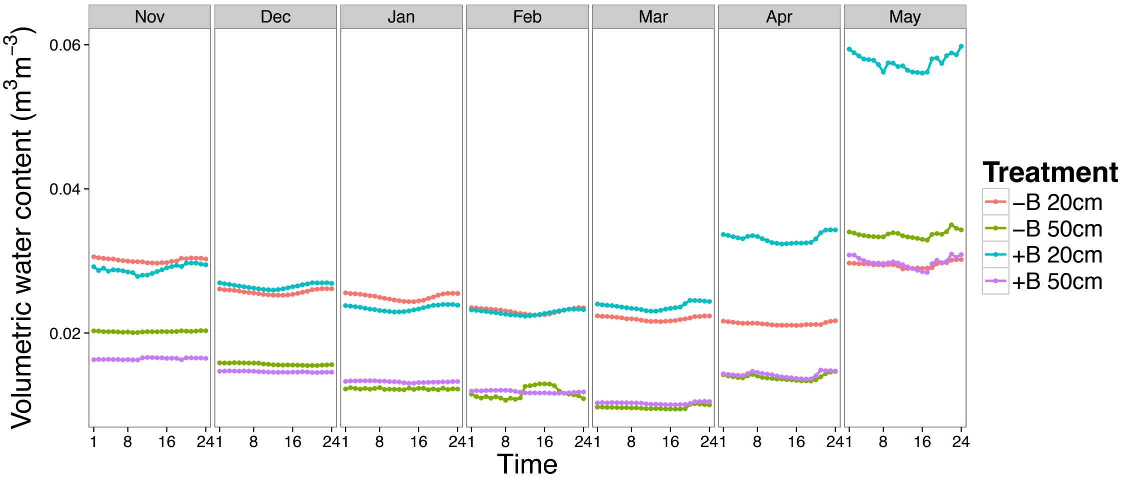

Figure 6.3: Daily average of soil volumetric water content that was measured hourly from November 2013 to May 2014 with dielectric sensors placed in 3-4 blocks in both sides of the donor tree (at the depth of 20 cm and 50 cm) and in both treatments (with a barrier to isolate seedlings, +B, and without the barrier, -B).

B. attenuata seedlings survival was similar for both treatments, despite of seedlings from -B appeared to have marginally higher mortality than seedlings from +B (chisq = 3.4, df = 1, p = 0.065, Fig. 6.4). There were no differences in the survival curves of *G. tomentosum* seedlings between groups (+B and -B; chisq =0 .2, df = 1, p = 0.695, Fig. 6.4) with both groups showing very high mortality (Fig. 6.4).

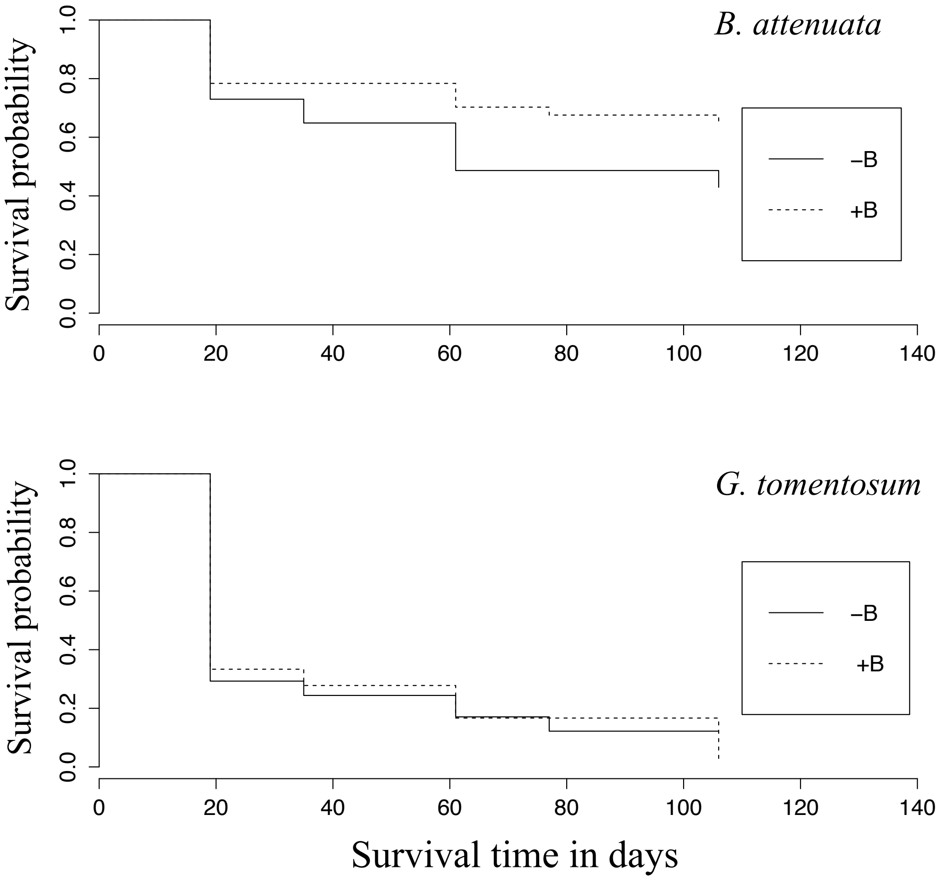

Figure 6.4: Survival data collected from the field experiment and analysed with the function "*survdiff()*" (R verison 3.0.2). Survival probability is shown for *B. attenuata* and *G. tomentosum* seedlings from both groups –isolated from the donor plant (+B) and potentially interacting with it (-B).

Stomatal conductance rates of *B. attenuata* were affected by the month, the time of the day and the interactions between these two factors (Appendix 6 and Fig. 6.6). *B. attenuata* seedlings had the highest stomatal conductance rates early in the day and in December, which progressively decreased over the next two months (Fig. 6.6). Seedlings from +B had slightly higher stomatal conductance rates than seedlings from -B, average of 134.39, 73.82 and 45.36 mmol m^{-2} s^{-1} for December, January and

February, respectively (Appendix 6 and Fig. 6.6). Conversely, stomatal conductance rates of *G. tomentosum* were affected by time, month and treatment and the interaction between treatment and month and between treatment and time (Appendix 6 and Fig. 6.6). In December and January, individuals of *G. tomentosum* from +B had higher stomatal conductances rates, averages of 108.57 and 61,57 mmol m^{-2} s^{-1} respectively, than individuals from –B, averages of 65.51 and 38.59 mmol m^{-2} s^{-1} respectively, and this trend appeared to reverse in February. However, for this last period, there were not enough replicates to test it (Fig. 6.6). *Banksia attenuata* seedlings had greater growth in shoot and in shoot dry mass in the +B treatment (Appendix 6; Figs. 6.6 and 6.5). *G. tomentosum* seedlings from the +B also had higher growth rates than the ones from -B (Appendix 6; Fig. 6.6). Seedlings of *B. attenuata* from -B had similar leaf water potential values to the seedlings from +B, although values were different for pre-dawn and mid-day measurements (Appendix 6; Fig. 6.5).

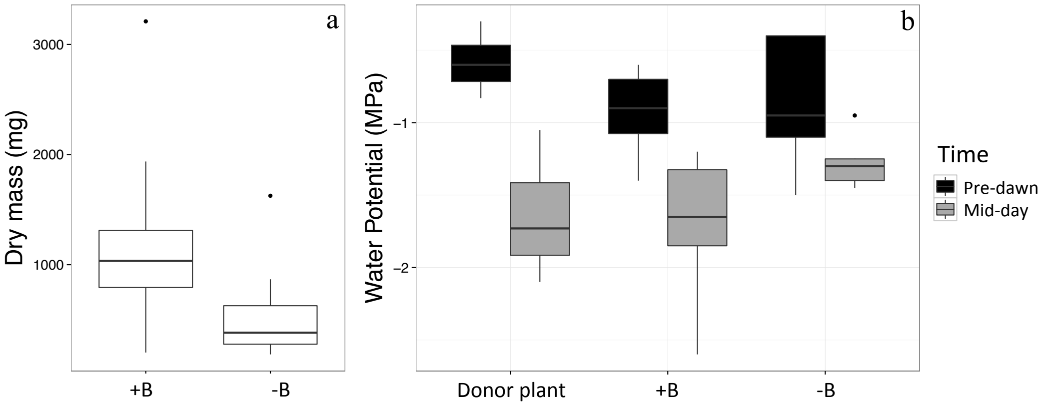

Figure 6.5: Measurements performed at the end of the field experiment of: **a)** Dry mass of *Banksia attenuata* seedlings from both treatments; **b)** Pre-dawn and mid-day leaf water potential measured in the donor plants and in *B. attenuata* seedlings from both treatments, with a barrier to isolate seedlings (+B) and without the barrier (-B). For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

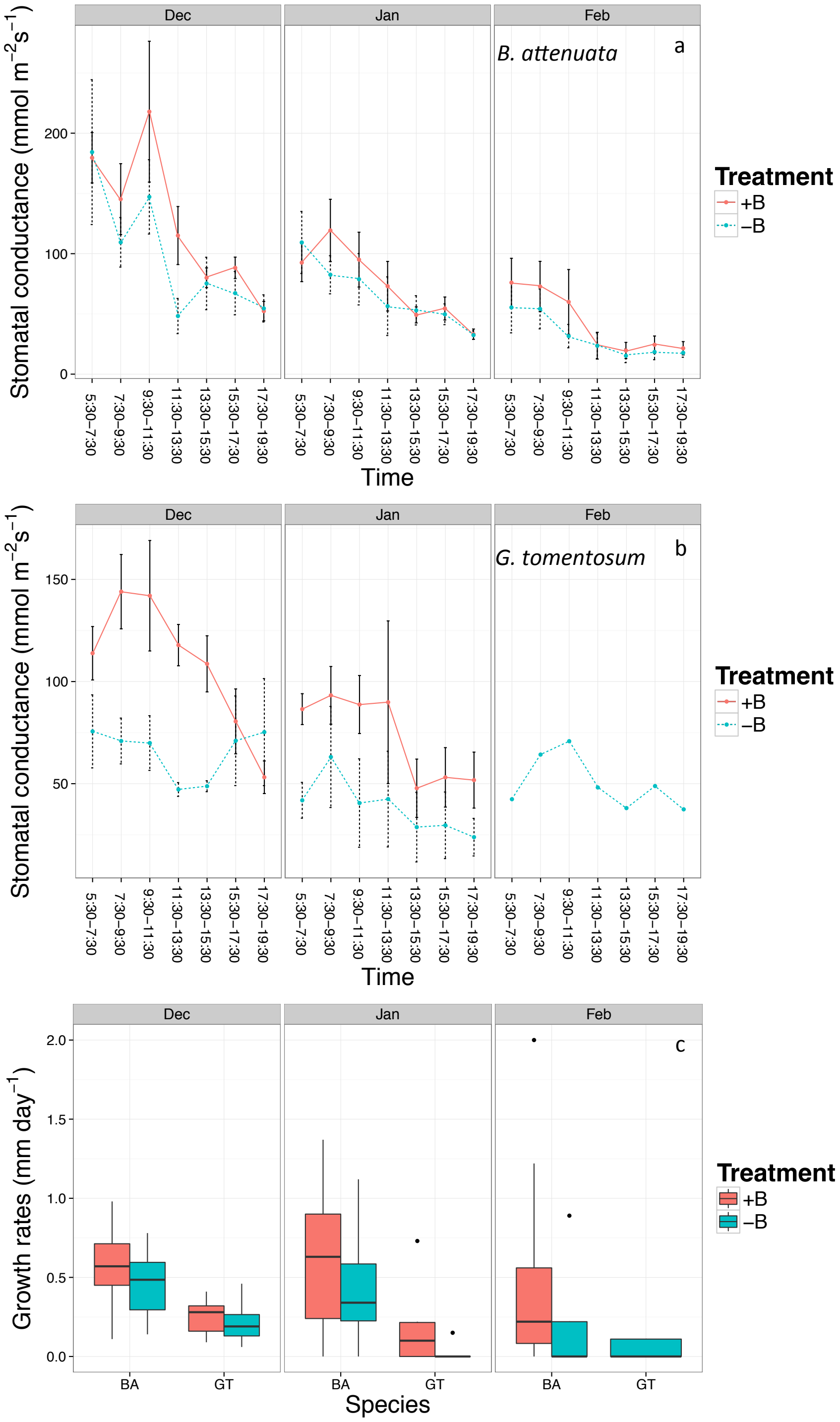

Figure 6.6: Field measurements performed in December, January and February of stomatal conductance measured in: **a)** *Banksia attenuata* (BA); and **b)** *Gompholobium tomentosum* (GT). **c)** Growth rates measured in the same three periods of BA and GT. Means and standard errors are shown for all line graphs, with the exception for GT measured in February, due to the small number of replicates (n= 5-30, depending of the month). Measurements were made in both treatments: with a barrier (+B) and without the barrier (-B). For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

Banksia attenuata and *G. tomentosum* seedlings grown with donor plants all showed an increase in leaf δ^2H in both treatments. This increase was higher for seedlings from the D+w treatment than for the D-w treatment (appendix 7 and Fig. 6.7). Soil moisture was affected by month, treatment and their interaction (appendix 7), with the treatment D+w showing the highest soil water content (Fig. 6.8).

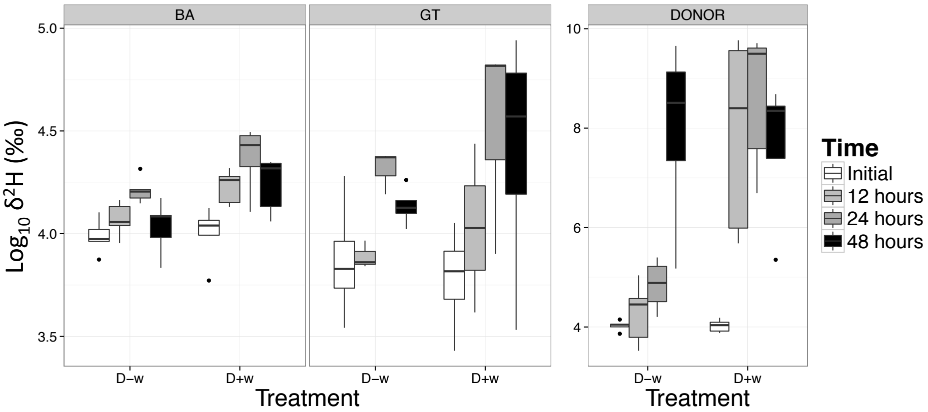

Figure 6.7: Glasshouse measurements of leaf water isotope composition (‰VSMOW) of donor plants and seedlings of *Banksia attenuata* and of *Gompholobium tomentosum* for both treatments: with donor trees accessing water table (D+w) and with donor trees deprived of a water table (D-w). Collections were made before (initial) and 12, 24 and 48 hours after injections of a deuterium enriched solution adjacent to the deep roots of donor plants. Data was log transformed to better visualization, as scales were very different for donor trees and seedlings. For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

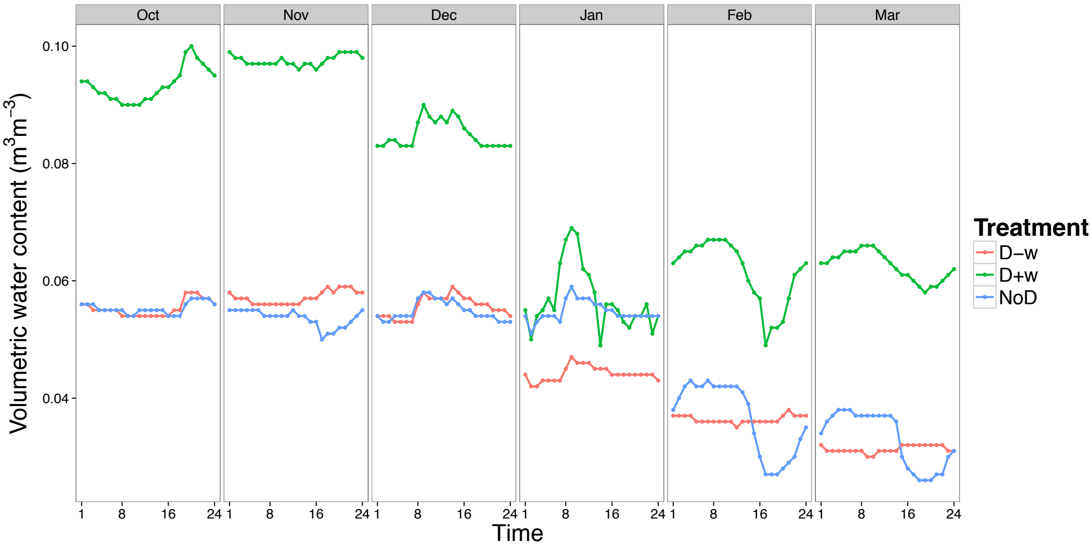

Figure 6.8: Daily average of soil volumetric water content measured in the glasshouse experiment hourly from October 2014 to March 2015. Dielectric sensors were placed 10 cm deep in 8 bags from the treatment with donor trees accessing water table "D+w", 5 from the treatment without a donor tree "NoD" and 3 from the treatment with donor trees deprived of a water table "D-w" treatments.

Banksia attenuata seedlings survived best in the D+w treatment, approximately 58%, and in the NoD treatment, approximately 53%, than when growing in the D-w treatment, in which only approximately 29% survived, (chisq = 10.5, df = 2, p = 0.005, Fig. 6.9). Conversely, *G. tomentosum* seedlings survived more in the "D-w" treatment, approximately 90%, compared to the treatments D+w, which approximately 53% of the seedlings survived, and to "NoD", which approximately 61% of the seedlings survived (chisq = 17.8, df = 2, p = 0.0001, Fig. 6.9).

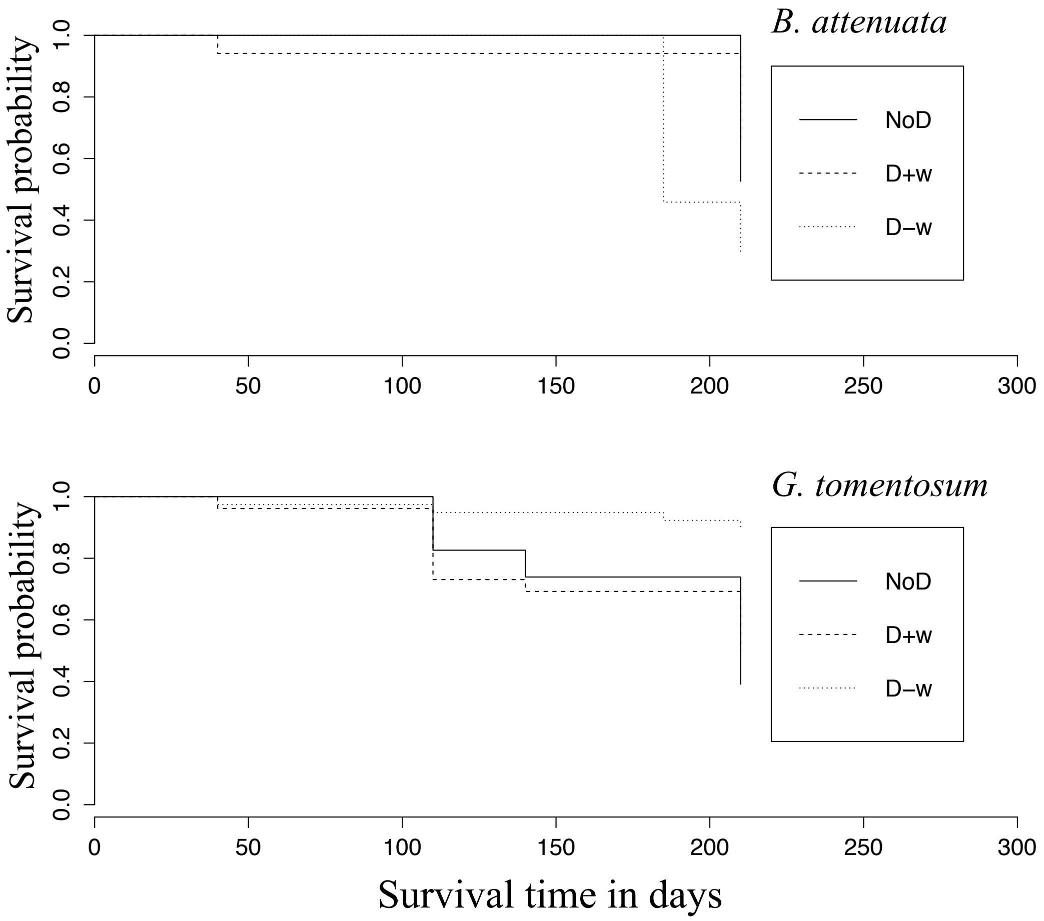

Figure 6.9: Survival data collected from the glasshouse experiment and analysed with the function "*survdiff()*" (R verison 3.0.2). Survival probability is shown for *Banksia attenuata* and *Gompholobium tomentosum* seedlings from all groups –growing without a donor plat (NoD), growing with a donor plat accessing water table (D+w), and growing with a donor plant deprived from a water table (D-w).

Stomatal conductance rates of both *B. attenuata* and *G. tomentosum* were affected by month, time, treatment and the interactions between month and treatment and month and time (Appendix 7 and Fig. 6.10). The highest stomatal conductance rates recorded for the *B. attenuata* seedlings were in November and they progressively decreased over the following months (Fig. 6.10). *B. attenuata* seedlings growing in the NoD treatment had the highest stomatal conductance rates, followed by seedlings in the D+w treatment, and the seedlings from D-w had the lowest rates. By May, there was no discernible difference in stomatal conductance from *B. attenuata* seedlings between treatments (Fig. 6.10). Seedlings of *G. tomentosum* had higher stomatal conductance rates over the first two periods (November to January) than later in the experiment (April-May; (Appendix 7 and Fig. 6.10). Individuals growing in both D+w and D-w treatments had higher stomatal conductance rates in the first period, but this relationship changed over the next two periods (Appendix 7 and Fig. 6.10). In the second period, seedlings growing in the NoD treatment and in the D+w had similarly high stomatal conductance rates, with the exception of mid-day measurements, in which seedlings from NoD showed a higher peak (Appendix 7 and Fig. 6.10). In the last period, stomatal conductance rates were higher in the seedlings from the NoD treatment, followed by the seedlings from the D-w and then those from the D+w treatment (Appendix 7 and Fig. 6.10).

The growth of *B. attenuata* and *G. tomentosum* seedlings were influenced by month, treatment and their interaction (appendix 7). Seedlings of *B. attenuata* from the NoD treatment recorded similar growth to the other treatments in the first two periods of measurement but had the highest growth during the third (driest) period (Fig. 6.10). In the first period of measurement, *G. tomentosum* seedlings from the D-w treatment had higher growth than the other seedlings. During the second period, seedlings from D-w grew more than seedlings from NoD. In the third period, seedlings from all treatments had similar growth (Fig. 6.10). Seedlings of *B. attenuata* from the NoD treatment had the highest dry mass, whereas seedlings from the other two treatments had similar dry mass values (Appendix 7 and Fig. 6.11). *G. tomentosum* seedlings, had similar dry mass values in all treatments (Appendix 7 and Fig. 6.11).

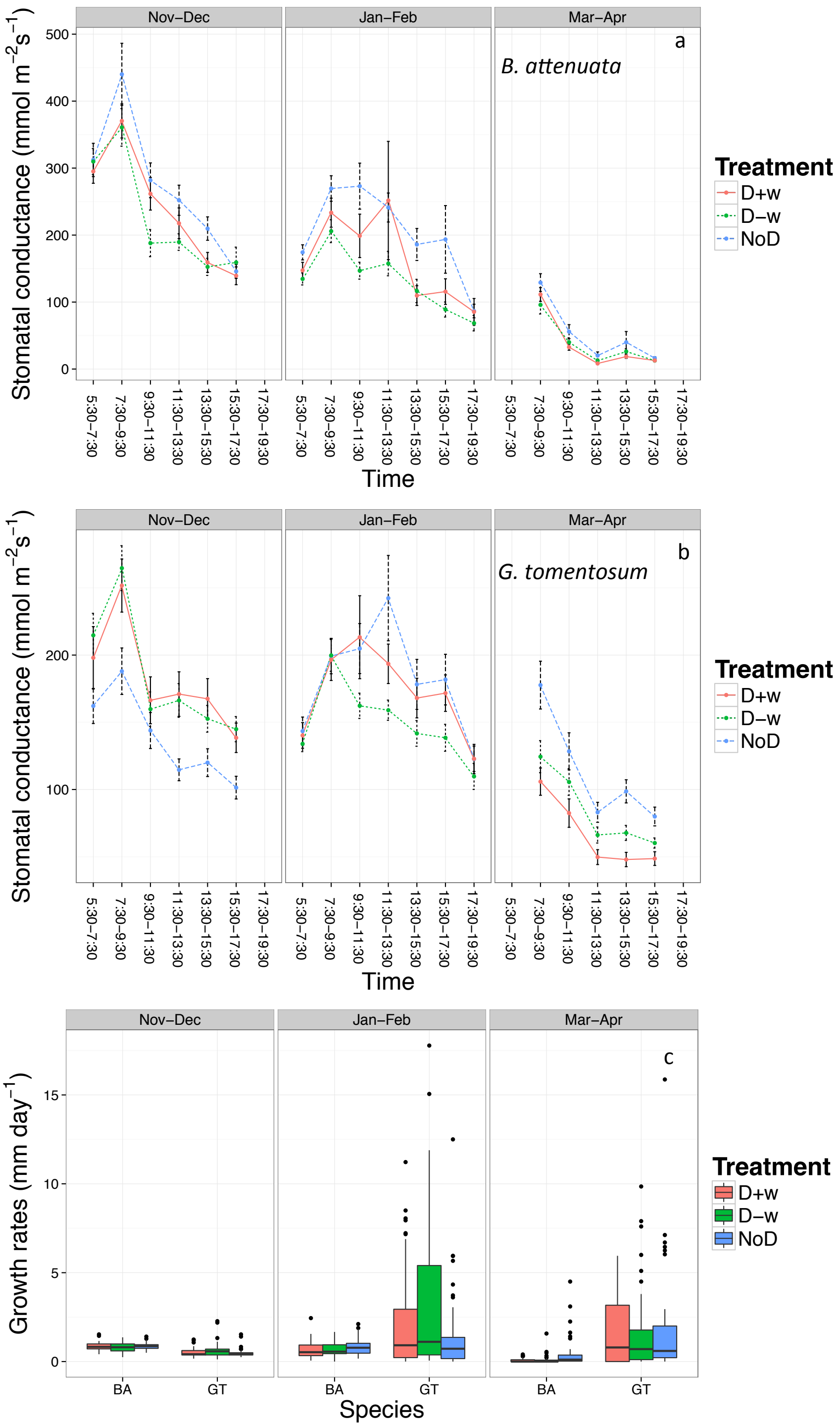

Figure 6.10: Glasshouse measurements performed in November-December, January-February and March-April of stomatal conductance measured in **a)** *Banksia attenuata* and in **b)** *Gompholobium tomentosum*. **c)** Growth rates measured in the same three periods of *B. attenuata* (BA) and *G. tomentosum* (GT). Measurements were made in all treatments: with donor trees accessing water table (D+w), with donor trees deprived of a water table (D-w) and without a donor tree "NoD". For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

For donor plants and *B. attenuata* seedlings, leaf water potential values showed the expected pattern of pre-dawn values close to zero and mid-day values ranging from -1 to -2 (MPa, Appendix 7 and Fig. 6.11). However, there were no differences between treatments (Appendix 7 and Fig. 6.11). For *G. tomentosum*, leaf water potential measurements of pre-dawn and mid-day were more negative for “NoD” and “D-w” treatments, whereas for “D+w”, pre-dawn values were closer to zero (Appendix 7 and Fig. 6.11).

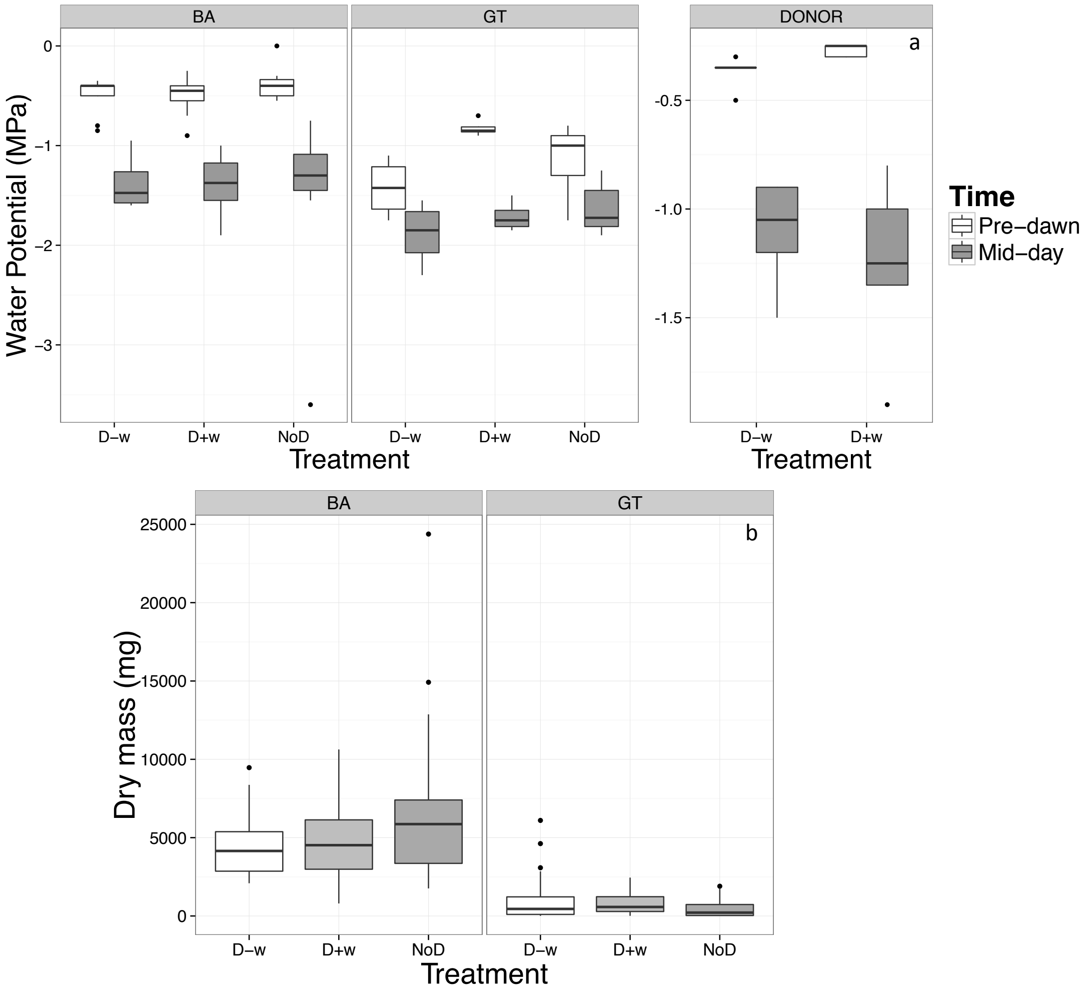

Figure 6.11: Glasshouse measurements performed at the end of the experiment of: **a)** Pre-dawn and mid-day leaf water potential measured in the donor tree, in *Banksia attenuata* and *Gompholobium tomentosum* seedlings from all treatments, with donor trees accessing water table (D+w), with donor trees deprived of a water table (D-w) and without a donor tree “NoD”. **b)** Dry mass of *B. attenuata* and *G. tomentosum* seedlings from all treatments. For the boxplot graphs, boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, whiskers represent approximately 2 SDs of the distributions, and open circles represent outliers.

6.5 DISCUSSION

Hydraulic redistribution and water uptake by seedlings

I found strong evidence that donor plants are able to redistribute water and that recipient *B. attenuata* seedlings are able to absorb the hydraulically redistributed water in my field and glasshouse experiments. The experiment setup in the glasshouse was constructed in a way to prevent, or at least reduce, recipient seedlings of *B. attenuata* reaching the water table. Thus, the increased leaf δ^2H of recipient seedlings of *B. attenuata* in both experiments is evidence of the use of hydraulically redistributed water by these recipient seedlings. For *G. tomentosum* seedlings, the increase of δ^2H in the leaves was entirely attributed to hydraulic redistribution from an adjacent donor tree in the glasshouse experiment since, in addition to the experiment setup, all roots in the pipes were inspected at harvest time and no *G. tomentosum* roots were found in the pipes (*B. attenuata* and *G. tomentosum* form roots that are distinguished by colour). These results support recent findings on the absorption of hydraulically lifted water by neighbouring plants (Egerton-Warburton et al., 2007; Liste and White, 2008; Ludwig et al., 2004; Pang et al., 2013).

Seedlings of *B. attenuata* are able to grow roots at least to 1-1.5 m deep in the first six months of growth (Canham, 2011; Groom, 2004; Chapter 5 of this thesis). So, they are probably investing in early growth of deep roots and thus may access water from deep layers within their first summer drought. Therefore, seedlings of deep-root species such as *B. attenuata* are then less likely to rely in hydraulically redistributed water from a donor tree to survive summer drought, despite being able to absorb it. Seedlings of *G. tomentosum*, on the other hand, have shallow-medium roots, being able to grow roots to approximately 70cm within first six months (Groom et al., 2000; Chapter 5 of this thesis; Pate and Bell, 1999). Soil water availability at such depth can be lower than 1% (Zencich et al., 2002) during summer months in some areas of Southwest Australia and, therefore, the absorption of hydraulically redistributed water by shallow-medium species might be crucial for survival over first summer drought.

Although impermeable barriers were placed in the field experiment (55 cm deep) to control interaction between donor plants and seedlings (Fig. 6.1), it appears that the barriers were not effective in preventing donor-seedling root interaction, as suggested by the increase in concentration of deuterium in the recipient seedlings in both the +B and -B. It is possible that donor plants used in the experiment may have had deeper lateral roots than the barriers, which interacted with deep roots of the recipient seedlings that were likely reaching 1-1.5 m depth at that stage (Canham, 2011; Groom, 2004; Chapter 5 of this thesis). Surprisingly, soil water content measured in the field through the sensors was higher at 20 cm than at 50 cm. This result suggests that hydraulic lift could have been affecting soil moisture at shallower layers of both treatments. Such differences in soil moisture could also be resulted from differences in the hydrophobicity of soils at the two depths, being higher (more hydrophobic) at shallower layers and hence little water could drain to deeper layers.

In the glasshouse experiment, the increased of δ^2H in the leaves of seedlings from the D-w treatment, suggests that donor plants of the D-w treatment could have been accessing residual deep water near the bottom of pipes, including the δ^2H spiked water, despite the water tables from D-w having been removed at the beginning of the experiment (October 2014). It also suggests that under drier conditions there maybe still hydraulic redistribution. On the other hand, the D+w treatment had the highest soil water content, which is consistent with the slighter higher values for leaf δ^2H from seedlings growing in this treatment. Donor plants from the D-w treatment showed a delayed response in taking up the deuterium-enriched water in relation to the ones growing in the D+w treatment and as a result the seedlings had slightly lower values of leaf δ^2H. Therefore, it is possible that the magnitude of hydraulic lift in the D+w treatment was higher than in the D-w. Clearly, soil water content of (D+w) was higher than of the other two treatments. This result can be an evidence of hydraulic lift as water could have been transported from deeper layers to shallower lawyers through the roots of donor plants. It could also mean that donor plants were using water from deeper layers and therefore shallower layers continued hydrated for a longer period than D-w and NoD did.

Interaction between donor plants and receiver seedlings

Contrary to my hypothesis, the results suggest that although seedlings from *G. tomentosum* and *B. attenuata* were able to absorb hydraulically redistributed water, the interaction with donor plants was not beneficial for the survival and growth of the seedlings. These findings are consistent with an updated version of the stress-gradient hypothesis, which currently states that competition is more prominent than facilitation when conditions are extremely stressful, with facilitation more evident in moderate conditions (Holmgren and Scheffer, 2010). There has also been evidence that supports this revised hypothesis, in which donor plants are shown to not affect or negatively affect water availability under very dry conditions (Aguiar and Sala, 1994; Kitzberger et al., 2000; Tielbörger and Kadmon, 2000). Similarly, a study conducted in the savannah of northern Tanzania found that the facilitative effects of hydraulic lift for neighbouring species was overwhelmed by competition for water (Ludwig et al., 2004). Indeed, summer water deficits in Southwest Australia represent a major recruitment bottleneck for seedling in Mediterranean-type ecosystems (Castri, 1973) and conditions are too dry to support facilitation between deep-rooted plants and deep-rooted and shallow-rooted seedlings. In fact, the impact of competition was greater than the benefits conferred from hydraulic redistribution, at least during the driest months studied. Therefore, competition could be affecting more intensively this plant community than previously considered.

It is also possible that both facilitation and competition are affecting the community in different times of the year. One possible evidence for this is that seedlings of *G. tomentosum* growing with donor plants that were accessing water presented higher stomatal conductance rates over first summer months when conditions were less dry, and this relationship reversed towards the end of summer, when conditions became drier (Fig. 6.10). Wright et al., (2014) has recently found similar outcome when testing facilitation and competition between pines and neighbours, as the effects of these interactions changed during the year.

Another possible hypothesis is that seedlings and donor plants were competing for resources and maybe water was not the most limiting of them, despite of the initial addition of nutrients. It is known that soils from the Bassendean Dune System, where the field experiment was conducted, are extremely poor in nutrients with phosphorus, nitrogen and manganese being the most scarce (Laliberté et al., 2012; Shane and Lambers, 2005b; Twidale and Campbell, 1988). Therefore, plants that interacted less with the donor tree in the field could have had access to more nutrients than seedlings that grew in the side where interaction with the donor tree was stronger. There is some evidence that in arid environments and soils with course texture, maximum lateral roots spread is greater and in nutrient patches the overlap zone of root growth increases

(Casper et al., 2003). Consequently, lateral roots of donor plants could have limited the growth of seedlings' lateral roots besides the stronger competition in the available nutrient patches. Possibly similar competition for nutrients happened in the glasshouse experiment, where soils were probably low in nutrients (only small amounts of slow-release fertiliser were added to pure sand). It has also been shown that some plants maximize root length in order to pre-empt nutrient supplies when coming into contact with neighbours (Craine and Dybzinski, 2013). Yet, the competition for nutrients between donor plants and seedlings hypothesis needs further testing since it was beyond the scope of this study.

6.6 CONCLUSION

This study showed that interaction between deep-rooted plants and surrounding seedlings is complex. Despite sharing redistributed water accessed by deep-rooted donor plants, seedlings of *B. attenuata* (deep-rooted species) and *G. tomentosum* (shallow-rooted species) did not benefit in terms of improved survival, growth and water relations, at least during their first summer months. The mechanisms behind these interactions remain unclear. It is likely that donor plants and seedlings competed for water or possibly another resource, most likely nutrients. Therefore, this study does not support the idea of using donor plants for improving establishment of seedlings in such ecosystems during their first summer drought, at least with these specific species. It is possible, however, that different species behave differently, but this needs further testing. I suggest that future studies should aim at: (1) unveiling the mechanisms by which hydraulically redistributed water is transferred from one plant to the other; (2) testing the facilitation hypothesis with better control of other components, such as nutrients; and possibly with different combinations of soil water and nutrient levels, and (3) establishing whether facilitation is affecting the community in a different period of the year and how it could improve survival and growth of seedlings.

CHAPTER SEVEN: GENERAL DISCUSSION

Over the last few decades the world has changed extensively at faster rate and over larger scales than in previous centuries (Steffen et al., 2004). In *MTEs,* where drought is predicted to increase in frequency and intensity (IPCC, 2014; Klausmeyer and Shaw, 2009), assembling native ecosystems that can withstand drier conditions should be the goal for effective management and intervention of such areas. The studies reported in this thesis together provided insights into a trait-based approach that restoration practitioners might use to achieve effective ecological intervention of *MTEs*. These studies have added to our understanding of plant resistance to water deficits, particularly on the different functional strategies that native plants use to survive summer drought in Southwest Australia. At a broader scale, the studies provided information on the functioning and assembly of a Mediterranean ecosystem, which is relevant when planning ecological intervention.

Functional traits and ecological intervention

The use of functional traits has recently been described to be a powerful tool for ecological intervention (Gondard et al., 2003; Laughlin, 2014; Lavorel, 2013), providing restoration practitioners with qualitative information and a framework whereby suitable species might be selected for intervention efforts. The use of functional traits associated with water relations has not been sufficiently explored and applied in ecology. Yet, it can be used as an important tool for ecological intervention of *MTEs*, by assisting with the selection of species more suited to drier conditions. The identification of "most suitable species" will depend on the specific area to be rehabilitated and on its existent functional types. For example, it might be beneficial to use species less dependent on deep water for an area where the water table is declining. Therefore, knowing the hydraulic functional types present in an ecosystem should be the first step for ecological intervention of *MTEs*. Based on this idea, I used a functional approach to assess the different water-use strategies of adult plant species growing on the Swan Coastal Plain (SCP) in Southwest Australia. However, before attempting to develop the traits based framework a literature review (Chapter 2) was needed to identify key functional traits since there are a vast number of plant traits that have been employed in ecological intervention (Ostertag et al., 2015; Pywell et al., 2003; Weiher et al., 1999). The seven traits selected for investigation are all associated with carbon assimilation and water loss, which together comprise an important trade-off that shapes most plant responses to water stress (Farquhar et al., 1980). The key functional traits selected included leaf carbon isotope composition, leaf nitrogen and phosphorus contents, leaf mass per area, leaf water potential at turgor loss point (π_{tlp}), and anatomy of xylem vessels.

These traits provided theoretical insights into water-use strategies of Mediterranean species when analysed in combination. For example, a plant that presents high values of leaf *[N]*, *[P]*, A_{max}, low values of $\delta^{13}C$ and of π_{tlp}, and a few large xylem vessels is expected to sustain high stomatal conductance rates and high hydraulic efficiency during dry conditions. Besides, it was possible to theorise on the different hydraulic functional types. Phreatophytes could be included in the group that presents high stomatal conductance rates and high hydraulic efficiency during dry conditions because these plants can access deep water and therefore sustain such responses. Drought tolerant species, on the other hand, are expected to present high stomatal conductance rates identified through high values of leaf *[N]*, *[P]*, A_{max}, high values of π_{tlp}, low values of $\delta^{13}C$, and high hydraulic safety, identified by many small xylem vessels (Chapter 2, Fig. 2.2). Such knowledge allows for identification of the habitat where species can potentially survive. Although there is a continuum in trait values, it will be interesting to investigate the range of trait values that characterise each functional group as well as possible overlaps in functional responses. For instance, what is the range of trait values that characterise phreatophytic species? This is certainly a promising avenue to advance the use of functional traits for identification of functional types.

The framework developed from the literature review (Chapter 2) enabled the application of the trait-based approach (Chapter 4), since it was possible to identify a few traits that matched pre-determined criteria (suitable methodologies for Mediterranean species, efficacy of measurement, association with resistance to water deficits and with effect traits). Also, the conceptual analysis of these traits provided the basis for determining a plant's response to water deficits that can be used for restoration practitioners. An issue that emerged from the literature review was the need to refine the osmometry methods used to extract sap for measuring π_{tlp}. The standard methodology (pressure-volume curves) is very time-consuming and therefore impractical for restoration practitioners that need to assess a vast number of plant species in Mediterranean ecosystems.

Through an experiment specifically designed to test the accuracy of *SE osmometry* to measure π_{tlp} (Chapter 3), I was able to demonstrate that this alternative technique can be used to measure π_{tlp} of small leaved sclerophylls species typical of *MTEs*. This technique proved to be efficient since 30-40 rather than 6-8 samples can be analysed in a day and it is suitable for species with small and large leaves. Results showed that there were no differences between the rehydration and no rehydration treatments, so I support the idea of using *SE osmometry* technique without prior rehydration, as previously suggested in the literature (Kubiske and Abrams, 1991a, 1991b, 1990; Meinzer et al., 2014). More accurate measures, on the other hand, might be obtained during periods of extreme differences in water availability, in which natural rehydration (Mediterranean winter) and dehydration (Mediterranean summer) of leaf tissue take place. I suggest further experimentation to test this idea, more specifically measuring the same individuals during winter and summer for a range of species from different taxonomic groups and with a diversity of leaf shapes and sizes.

In Chapter 4, I measured the seven functional traits selected in the literature review and three other traits (water potential at pre-dawn and at mid-day and leaf nitrogen isotope composition) in species from the SCP. The six leaf traits — D_{max}, D_s, *LMA*, *WD*, $\delta^{13}C$, and *TLP* — appeared to be key traits underlying plant strategies in relation to water use since they explain trait variation and are easy to measure. With simple analyses it was possible to form distinct group of species that present similar strategies to cope with water stress, which is valuable information for ecological intervention of such ecosystems. Also, it revealed the traits that contributed the most for each group and for each site as well as the similarities within and between groups and within and between sites, which is a key information when restoring through reassembly (Funk et al., 2008). In particular, the similarity found between functional groups and "root-type" groups is strong evidence that the analyses of functional traits provided accurate information of the functioning of this community. This is due to the fact that the SCP is strongly influenced by groundwater and, as a consequence, root depth is an important attribute (Zencich et al., 2002); which might not be the case of communities less affected by groundwater. This study provided practical and relevant information for management and intervention of the SCP, offering a robust approach for species selection. For the SCP example, species can be selected based on their requirements, such as connection to a shallow or deep water table. In a broad sense, this knowledge adds to our understanding of the dynamics between plant traits and ecological filters, which has become extremely important for biodiversity conservation in the face of climate change (Chapter 2, Fig.2.1). This approach can be applied to similar communities within *MTEs*, thus enhancing effective ecological intervention. The application of the trait-based approach (Chapter 4) supported the use of functional traits for efficiently screening species functional types.

I also discussed some limitations and concerns, which trait-based ecology approaches encounter and these need to be taken into account. One concern was the amount of plasticity that species may present, i.e. functional traits values can be different depending on the plants' specific habitat. In this study, *Banksia attenuata* was included in two different functional groups because individuals were collected from two distinct sites. This is evidence that plasticity should always be considered in functional trait approaches. The application of a theory-based approach often presents limitations and thus further testing towards refining this method is required, especially in determining when plasticity can be disregarded (Shipley et al., 2015). However, there has been recent evidence that a functional ecology approach, when used for ecological intervention projects, can succeed in catering for different demands, such as increasing survival or seedling recruitment of native species (Ostertag et al., 2015), predicting the success of particular restoration practices (Sandel et al., 2011), predicting the likely performance of species in restored vegetation (Pywell et al., 2003), and providing an indication of the potential success of a completed restoration project (Engst et al., 2016).

Functional traits, plant development and ecosystem properties

For *MTEs*, the first summer is critical for seedlings survival (Hallett et al., 2014; Lloret et al., 2005; Rokich, 2016) and thus seedling responses to summer drought might play an important role in community assembly. To provide a more complete assessment of community dynamics and assembly, I investigated the strategies that juveniles employ to survive summer drought. However, I acknowledge that the application of the functional approach for seedlings is challenging due to the fact that it is difficult to identify seedlings under similar stage of development in the field. Moreover, many species are very small in their first year of development, which makes it difficult to collect sufficient material for measurements of functional traits. Therefore, it is necessary greater control within experiments and a greater number of replicates to assess ecological strategies of juveniles or to identify the critical thresholds at each stage of development which would make predictions of species functional types more accurate.

Under water limitation, plants can reduce stomatal conductance and rely on their stored non-structural carbohydrates to survive periods of water deficits. In contrast they might tolerate drought by maintaining high hydraulic and stomatal conductance, photosynthesis and growth and by having a hydraulic system robust enough to withstand high tensions in the xylem vessels. Other plants have less reliance on stomatal control since they grow deep roots to access water stored in deeper layers thus avoiding serious water deficits (Eamus et al., 2006; Le Maitre et al., 2000; McDowell et al., 2008). These are three quite different ways that species from the SCP might use to survive drought. To evaluate the significance of each of these mechanisms, two experiments were conducted to investigate water relations, stomatal regulation and accumulation and use of non-structural carcohydrates (NSC) in seedlings from Southwestern Australian species during their first summer drought. Although the experiments (Chapter 5) were limited due to high mortality, evidence for at least two different ecological strategies were observed: 1) use of water from deep soil layers, stomatal regulation and possibly use of NSC (*Banksia* seedlings); and 2) tolerance to water deficits, despite reasonably low stomatal conductance rates (at least for *G. tomentosum)*. Whether the two strategies identified in this study can be generalised to other species and situations remains unclear. I suggest further investigation on the strategies of juveniles to survive summer drought in the SCP, including the development of a better and more efficient protocol to assess such drought responding strategies for juveniles in *MTEs*. In particular, it will be interesting to determine which functional traits will be more appropriate for measuring small samples since seedlings are very small in their first year of development.

Since functional trait approaches do not take into consideration important interactions within an ecosystem, I investigated the facilitation and competition effects of a particular phenomenon –hydraulic redistribution– on seedling survival during their first summer drought (Chapter 6). I found that “recipient” seedlings grew and transpired more when interacting less with donor plants (field experiment, Chapter 6) or without a donor plant (glasshouse experiment, Chapter 6), at least during summer months. These results suggested that seedlings and donor plants (known to hydraulically redistribute water) were competing. Such findings agree with recent studies testing the "stress-gradient hypothesis" (Holmgren and Scheffer, 2010). To understand the complexity of facilitation and competitive interactions further research is needed for more species from *MTEs*, known to be limited by nutrients and not just by water availability. I predict that facilitation could be more or less important in SCP communities in different periods of the year, possibly affecting more the community when drought conditions are moderate (Holmgren and Scheffer, 2010). Evidence for this emerges from the *G. tomentosum* seedlings growing with donor plants that were accessing water, which showed higher stomatal conductance rates during the moist early summer months and the relationship reversed at the end of summer, when conditions became drier. Therefore, it will be interesting to design experiments able to identify such interactions as well as possible shifts from facilitation to competition throughout the year. Knowledge of when species compete or benefit from facilitation is critical for the establishment of seedlings and hence for ecological intervention of such areas. Also, it would be beneficial to have experiments designed to exclude the possibility of nutrients limitation (glasshouse) or by understanding seedling and soil nutrient status (field).

Ecological intervention and trait-based ecology

A new framework has recently been proposed to assist in the selection of the type of intervention required for the restoration or rehabilitation of ecosystems (Miller and Bestelmeyer, 2016). From this conceptual diagram it is evident that management of "novel ecosystems" is an option when restoration does not intend to recover the historical state but the altered ecosystem is functional and self-sustaining (Fig.7.1; Miller and Bestelmeyer, 2016). On the other hand, "Partial restoration" is more appropriate when restoration aims to restore an ecosystem's historical state, but complete recovery is not feasible (Fig.7.1; Miller and Bestelmeyer, 2016). The trait-based approach proposed in this thesis can specifically assist with species selection when "management of novel ecosystems" and "partial restoration of ecosystems" are required for *MTEs* (Fig.7.1). In both scenarios, using species with traits that match future environmental and climate conditions can be crucial for success (Prober et al., 2015), rather than using fixed assemblages and restoration targets that are frozen in time. Another perspective is to achieve an initial target (based on current conditions) and then allow the functional traits to self-sort the system after that point, reaching a successful restoration state. For these three contexts "management of novel ecosystems", "partial restoration of ecosystems" and "restoration", I suggest the use of the six functional traits– D_{max}, D_s, *LMA*, *WD*, $\delta^{13}C$, and *TLP*– to efficiently identify the ecological strategies employed by Mediterranean species to resist water deficits. This can be achieved when measurements of these traits are examined with multivariate analyses and with SIMPER or other types of "cluster analyses". As a result, it is possible to determine functional types, to establish the similarities and dissimilarities between and within groups, and to identify the traits that are most associated with each functional group.

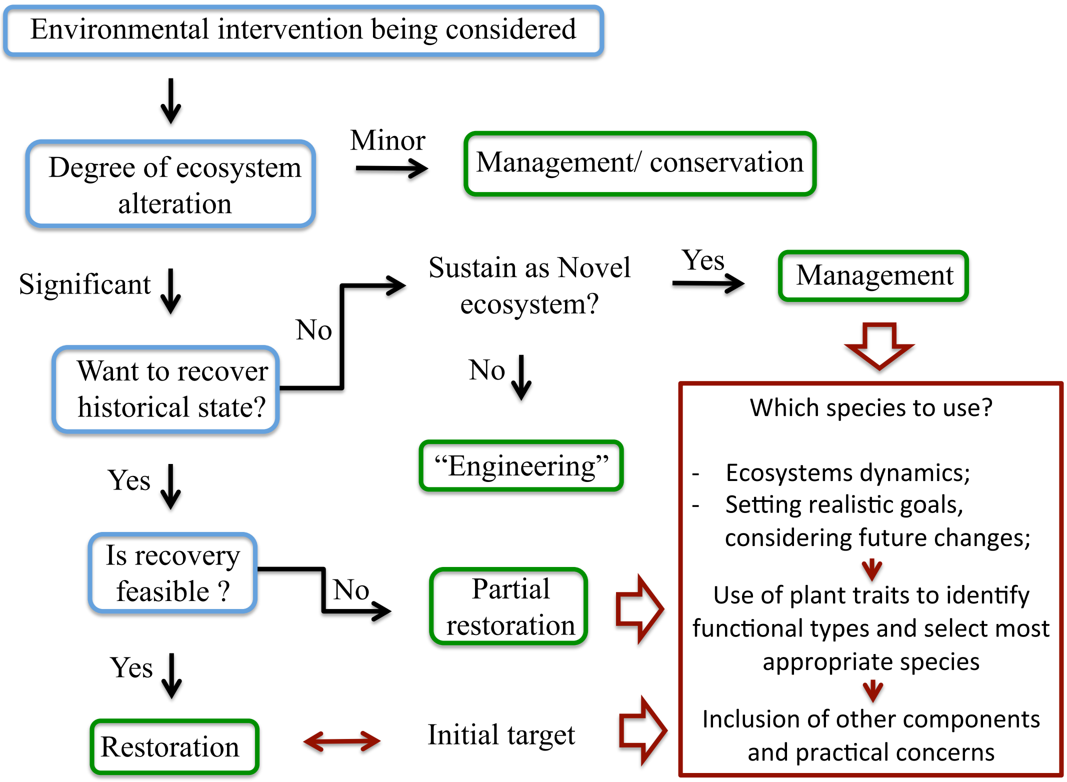

Figure 7.1: Diagram with possible alternatives for ecosystem intervention. The trait-based approach (in red) can provide a screening of functional types present in a community, which is essential for selecting appropriate species for "partial restoration" and "management" of ecosystems under the risk of environmental and climate changes. Also, it can be used for "restoration", first reaching an initial target, and then allowing for the functional traits to self-sort the system after that point. Figure modified from Miller and Bestelmeyer, (2016).

Finally, I conclude that the studies undertaken in this thesis have contributed to the identification of functional traits that can provide the basis for screening species' functional profiles in relation to water use, at least for adult plants of *MTEs*. The application of such a framework will accelerate our knowledge of community assembly and functioning, which will bring us closer to succeed in restoring ecosystems facing climate and other changes. The trait-based approach provides a rigorous first step in species selection and can be applied with relatively little effort by restoration practitioners. Inclusion of other components might also be required to refine and improve the accuracy of any trait-based framework. Components that need consideration include an understanding of juvenile vs. adult ecological strategies employed to resist summer drought; the role of important interactions, such as competition and facilitation; and an ability to determine when plasticity in response to the stressor is not important. As much as the trait-based approach to ecological intervention has value it should be recognised that there are many practical concerns that feed into final species choices including characteristics related to fire resistance, pollination type, fruit dispersion, availability of nutrients, culturally important species, and seedling cost and availability.

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APPENDIX

Appendix 1: Southwestern Australian species, taxonomic family, and mean ± standard error values per species (n=4) for the leaf parameters: Area (A), thickness (T), leaf dry mass content (LDMC), specific leaf area (SLA) and density (D) (Chapter 3).

Species	**Family**	**A (mm^2)**	**T (mm)**	**LDMC ($mg\ g^{-1}$)**	**SLA ($m^2\ kg^{-1}$)**	**D ($g\ cm^{-3}$)**
Adenanthos cygnorum Diels.	Proteaceae	76.15 ± 3.62	0.54 ± 0.03	406.33 ± 8.66	4.19 ± 0.44	0.45 ± 0.02
Astroloma macrocalix Sond.	Ericaceae	11.44 ± 2.12	0.20 ± 0.04	361.67 ± 81.74	6.18 ± 1.79	0.99 ± 0.07
Banksia attenuata R.Br.	Proteaceae	367.90 ± 45.28	0.58 ± 0.11	551.69 ± 26.47	3.06 ± 0.18	0.63 ± 0.11
Banksia ilicifolia R.Br.	Proteaceae	563.79 ± 34.56	0.54 ± 0.03	424.16 ± 13.14	3.58 ± 0.18	0.53 ± 0.03
Banksia menziesii R.Br.	Proteaceae	1716.30 ± 113.49	0.49 ± 0.04	574.84 ± 28.35	2.75 ± 0.12	0.76 ± 0.08
Eremaea pauciflora Endl.	Myrtaceae	3.35 ± 0.64	0.34 ± 0.03	302.65 ± 34.00	4.44 ± 0.70	0.72 ± 0.12
Gompholobium tomentosum Labill.	Fabaceae	51.48 ± 4.97	0.37 ± 0.02	407.92 ± 50.21	4.38 ± 0.38	0.65 ± 0.08
Kunzea glabrescens Toelken	Myrtaceae	6.11 ± 0.36	0.30 ± 0.04	248.74 ± 11.47	6.26 ± 0.15	0.56 ± 0.07
Melaleuca preissiana Schauer	Myrtaceae	9.81 ± 0.63	0.30 ± 0.02	260.93 ± 13.26	6.34 ± 0.52	0.54 ± 0.03
Pultenaea reticulata (Sm.) Benth.	Fabaceae	18.37 ± 0.86	0.25 ± 0.01	244.03 ± 76.11	4.90 ± 0.24	0.83 ± 0.03
Regelia ciliata Schauer	Myrtaceae	5.28 ± 0.56	0.21 ± 0.01	212.45 ± 20.69	5.97 ± 0.48	0.83 ± 0.12

Appendix 2: Southwestern Australian species and mean ± standard error values per species (n=4) for each of the following: osmotic potential at full turgor, osmotic potential at turgor loss point, cell elasticity, apoplastic fraction; and osmometer osmotic potential at full turgor (Chapter 3).

Species	*P-V* Osmotic Potential (Mpa)	Turgor loss point (Mpa)	Elasticity (MPa)	Symplastic fraction	Apoplastic fraction	Osm. osmotic potential (Mpa)
Non-rehydrated						
Adenanthos cygnorum Diels.	-0.95 ± 0.13	-1.37 ± 0.05	10.06 ± 2.83	0.64 ± 0.42	0.36 ± 0.08	-1.45 ± 0.02
Astroloma macrocalix Sond.	-1.04 ± 0.09	-1.30 ± 0.08	9.71 ± 0.80	0.51 ± 0.32	0.49 ± 0.18	-1.36 ± 0.14
Banksia attenuata R.Br.	-1.35 ± 0.21	-1.99 ± 0.16	21.16 ± 3.97	0.81 ± 0.37	0.19 ± 0.13	-1.94 ± 0.18
Banksia ilicifolia R.Br.	-1.30 ± 0.30	-1.81 ± 0.21	22.81 ± 4.74	0.72 ± 0.43	0.28 ± 0.07	-1.73 ± 0.07
Banksia menziesii R.Br.	-1.31 ± 0.26	-1.93 ± 0.30	18.16 ± 2.65	0.79 ± 0.48	0.21 ± 0.02	-1.68 ± 0.10
Eremaea pauciflora Endl.	-1.10 ± 0.13	-1.57 ± 0.13	15.78 ± 5.39	0.73 ± 0.38	0.24 ± 0.12	-1.53 ± 0.10
Gompholobium tomentosum Labill.	-1.05 ± 0.04	-1.25 ± 0.08	10.97 ± 1.55	0.34 ± 0.10	0.66 ± 0.40	-1.05 ± 0.04
Kunzea glabrescens Toelken	-1.23 ± 0.13	-1.79 ± 0.11	9.30 ± 2.91	0.40 ± 0.26	0.47 ± 0.24	-1.60 ± 0.05
Melaleuca preissiana Schauer	-	-	-	-	-	-2.12 ± 0.20
Pultenaea reticulata (Sm.) Benth.	-0.93 ± 0.11	-1.13 ± 0.07	13.96 ± 1.96	0.56 ± 0.29	0.31 ± 0.21	-1.07 ± 0.05
Regelia ciliata Schauer	-1.21 ± 0.18	-1.78 ± 0.25	9.18 ± 1.36	0.59 ± 0.26	0.41 ± 0.24	-1.80 ± 0.04
Rehydrated						
Adenanthos cygnorum Diels.	-0.96 ± 0.16	-1.34 ± 0.19	9.72 ± 2.46	0.65 ± 0.41	0.35 ± 0.09	-1.55 ± 0.07
Astroloma macrocalix Sond.	-0.92 ± 0.05	-1.24 ± 0.08	12.66 ± 2.92	0.68 ± 0.34	0.32 ± 0.16	-1.15 ± 0.11
Banksia attenuata R.Br.	-1.29 ± 0.07	-1.93 ± 0.08	20.83 ± 6.56	0.73 ± 0.35	0.27 ± 0.15	-2.01 ± 0.13
Banksia ilicifolia R.Br.	-1.64 ± 0.19	-2.11 ± 0.15	35.71 ± 5.38	0.75 ± 0.43	0.25 ± 0.07	-1.80 ± 0.05
Banksia menziesii R.Br.	-1.15 ± 0.28	-1.79 ± 0.32	13.15 ± 4.73	0.78 ± 0.43	0.22 ± 0.07	-1.57 ± 0.16
Eremaea pauciflora Endl.	-1.03 ± 0.09	-1.48 ± 0.11	12.28 ± 3.94	0.71 ± 0.33	0.29 ± 0.17	-1.48 ± 0.07
Gompholobium tomentosum Labill.	-0.95 ± 0.03	-1.19 ± 0.01	11.40 ± 3.96	0.52 ± 0.31	0.48 ± 0.19	-0.98 ± 0.03
Kunzea glabrescens Toelken	-1.06 ± 0.17	-1.70 ± 0.11	16.32 ± 6.82	0.57 ± 0.41	0.27 ± 0.09	-1.52 ± 0.05
Melaleuca preissiana Schauer	-1.72 ± 0.35	-2.27 ± 0.43	35.83 ± 2.62	0.81 ± 0.35	0.19 ± 0.15	-1.93 ± 0.10
Pultenaea reticulata (Sm.) Benth.	-0.73 ± 0.06	-1.01 ± 0.09	12.55 ± 3.58	0.49 ± 0.08	0.51 ± 0.58	-1.06 ± 0.09
Regelia ciliata Schauer	-0.98 ± 0.12	-1.56 ± 0.16	10.42 ± 0.98	0.77 ± 0.34	0.23 ± 0.16	-1.06 ± 0.25

Appendix 3: Site of occurrence of the fifteen plant species studied (Chapter 4), taxonomic family, type of roots, habit and mean ± stand errors (n=5) of the 12 functional traits measured: average and maximum diameter of xylem vessel (*D_{ave}*, *D_{max}*), number of xylem vessels per mm^2 (*D_s*), leaf mass per area (*LMA*), wood density (*WD*), leaf concentration of phosphorus (*[P]*) and of nitrogen (*[N]*), leaf isotope composition of nitrogen (δ^{15}N) and of carbon (δ^{13}C), water potential at turgor loss point (*TLP*), pre-dawn (*PD*) and mid-day (*MD*) water potentials.

Site	Species	Family	Root type	Habit	*D_{ave} (µm)*
ENHD	*Banksia attenuata R.Br.*	Proteaceae	Deep	Tree	22.58 ± 2.22
	Beaufortia elegans Shcauer	Myrtaceae	Medium	Shrub	22.95 ± 2.02
	Banksia hookeriana Meisn.	Proteaceae	Deep	Shrub	26.05 ± 1.46
	Melaleuca leuropoma Craven	Myrtaceae	Shallow	Shrub	23.26 ± 1.15
ENSW	*Banksia carlinoides Meisn.*	Proteaceae	Shallow	Shrub	20.10 ± 1.22
	Beaufortia elegans Schauer	Myrtaceae	Medium	Shrub	22.60 ± 1.54
	Melaleuca leuropoma Craven	Myrtaceae	Shallow	Shrub	22.06 ± 0.71
GNHD	*Adenanthos cygnorum Diels*	Proteaceae	Medium	Shrub	29.45 ± 1.11
	Banksia attenuata R.Br.	Proteaceae	Deep	Tree	21.72 ± 0.83
	Banksia menziesii R.Br.	Proteaceae	Deep	Tree	25.20 ± 0.72
	Sholtzia laxiflora Benth.	Myrtaceae	Medium	Shrub	26.11 ± 1.35
	Verticordia nitens Lindl.	Myrtaceae	Deep	Shrub	24.99 ± 2.36
GNMD	*Adenanthos cygnorum Diels*	Proteaceae	Medium	Shrub	28.45 ± 2.07
	Banksia attenuata R.Br.	Proteaceae	Deep	Tree	22.84 ± 1.10
	Banksia menziesii R.Br.	Proteaceae	Deep	Tree	23.77 ± 1.29
	Eremaea pauciflora Endl.	Myrtaceae	Medium	Shrub	25.74 ± 3.36
	Sholtzia laxiflora Benth	Myrtaceae	Medium	Shrub	24.92 ± 1.22
GNMS	*Banksia attenuata R.Br.*	Proteaceae	Deep	Tree	24.04 ± 1.28
	Banksia ilicifolia R.Br.	Proteaceae	Deep	Tree	21.49 ± 0.74
	Banksia menziesii R.Br.	Proteaceae	Deep	Tree	21.90 ± 1.71
	Hibbertia subvaginata Steud.	Dilleniaceae	Shallow	Shrub	17.52 ± 0.87
	Regelia inops Schauer	Myrtaceae	Shallow	Shrub	31.26 ± 3.21
	Scholtzia involucrata Endl.	Myrtaceae	Medium	Shrub	19.47 ± 0.52
GNSW	*Adenanthos cygnorum Diels.*	Proteaceae	Medium	Shrub	23.82 ± 0.44
	Banksia attenuata R.Br.	Proteaceae	Deep	Tree	24.50 ± 1.91
	Banksia ilicifolia R.Br.	Proteaceae	Deep	Tree	21.13 ± 0.67
	Banksia menziesii R.Br.	Proteaceae	Deep	Tree	22.82 ± 1.57
	Eremaea beaufortioides Benth.	Myrtaceae	Deep	Shrub	30.02 ± 1.01
	Scholtzia involucrata Endl.	Myrtaceae	Medium	Shrub	21.97 ± 0.62

Appendix 3 (continued)

Site	Tag	D_{ave} (µm)	D_{max} (µm)	D_s (Nº mm^{-2})	LMA (g m^{-2})
ENHD	Ba1	22.58 ± 2.22	35.11 ± 3.30	475.00 ± 53.82	469.40 ± 9.40
	Be1	22.95 ± 2.02	33.70 ± 3.37	491.20 ± 69.96	223.02 ± 11.10
	Bh1	26.05 ± 1.46	39.82 ± 2.21	439.20 ± 34.28	355.36 ± 21.57
	Ml1	23.26 ± 1.15	33.84 ± 3.15	499.20 ± 51.90	461.97 ± 12.95
ENSW	Bc2	20.10 ± 1.22	30.60 ± 2.29	419.20 ± 18.91	406.18 ± 14.08
	Be2	22.60 ± 1.54	33.79 ± 2.30	508.80 ± 39.59	267.72 ± 56.12
	Ml2	22.06 ± 0.71	32.52 ± 0.94	508.80 ± 60.27	469.40 ± 22.13
GNHD	Ac3	29.45 ± 1.11	48.23 ± 1.30	264.00 ± 14.91	297.59 ± 10.85
	Ba3	21.72 ± 0.83	31.53 ± 2.10	579.00 ± 85.53	359.08 ± 16.49
	Bm3	25.20 ± 0.72	42.94 ± 2.14	564.00 ± 42.61	477.09 ± 23.68
	Sl3	26.11 ± 1.35	39.99 ± 1.64	326.67 ± 9.66	226.01 ± 8.77
	Vn3	24.99 ± 2.36	41.01 ± 4.71	261.00 ± 13.05	461.03 ± 29.78
GNMD	Ac4	28.45 ± 2.07	44.29 ± 2.22	330.40 ± 31.86	267.17 ± 8.31
	Ba4	22.84 ± 1.10	35.54 ± 1.65	568.80 ± 43.70	394.99 ± 18.24
	Bm4	23.77 ± 1.29	37.74 ± 2.61	544.00 ± 58.47	475.79 ± 22.97
	Ep4	25.74 ± 3.36	38.39 ± 7.10	332.80 ± 57.15	241.89 ± 25.39
	Sl4	24.92 ± 1.22	37.45 ± 1.95	300.80 ± 28.46	223.84 ± 8.14
GNMS	Ba5	24.04 ± 1.28	38.80 ± 1.81	530.40 ± 58.47	368.93 ± 11.59
	Bi5	21.49 ± 0.74	30.76 ± 1.96	591.00 ± 111.53	374.86 ± 9.73
	Bm5	21.90 ± 1.71	32.85 ± 2.99	592.00 ± 96.73	424.54 ± 21.95
	Hs5	17.52 ± 0.87	24.09 ± 1.01	340.00 ± 21.09	183.24 ± 9.05
	Ri5	31.26 ± 3.21	45.97 ± 4.28	374.40 ± 91.22	136.48 ± 7.56
	Si5	19.47 ± 0.52	31.51 ± 1.60	463.00 ± 21.59	177.63 ± 7.67
GNSW	Ac6	23.82 ± 0.44	38.06 ± 0.14	308.00 ± 23.43	255.78 ± 65.87
	Ba6	24.50 ± 1.91	36.81 ± 3.14	482.40 ± 59.55	420.12 ± 8.68
	Bi6	21.13 ± 0.67	31.24 ± 1.47	630.40 ± 58.15	409.60 ± 20.90
	Bm6	22.82 ± 1.57	37.04 ± 1.88	507.20 ± 55.89	499.75 ± 36.42
	Eb6	30.02 ± 1.01	46.27 ± 1.88	283.00 ± 20.37	286.40 ± 31.48
	Si6	21.97 ± 0.62	35.37 ± 1.77	390.00 ± 24.08	274.57 ± 14.84

Appendix 3 (continued)

Site	Tag	*WD (g cm^{-3})*	*[P] (mg g^{-1})*	*[N] (mg g^{-1})*	$\delta^{15}N$
ENHD	Ba1	0.65 ± 0.02	0.14 ± 0.01	6.8 ± 0.3	0.43 ± 0.27
	Be1	0.71 ± 0.05	0.15 ± 0.02	9.7 ± 0.4	0.91 ± 0.23
	Bh1	0.59 ± 0.03	0.18 ± 0.02	7.4 ± 0.3	0.44 ± 0.21
	Ml1	0.83 ± 0.05	0.20 ± 0.03	8.2 ± 0.5	-0.60 ± 0.44
ENSW	Bc2	0.71 ± 0.18	0.16 ± 0.02	5.7 ± 0.2	1.39 ± 0.18
	Be2	0.94 ± 0.13	0.21 ± 0.03	9.1 ± 0.5	1.21 ± 0.14
	Ml2	0.90 ± 0.04	0.18 ± 0.03	7.6 ± 0.3	0.08 ± 0.28
GNHD	Ac3	0.59 ± 0.01	0.16 ± 0.02	7.0 ± 0.4	1.32 ± 0.27
	Ba3	0.56 ± 0.01	0.15 ± 0.01	7.1 ± 0.3	-2.13 ± 0.33
	Bm3	0.51 ± 0.02	0.17 ± 0.02	6.9 ± 0.2	-2.86 ± 0.49
	Sl3	0.92 ± 0.04	0.19 ± 0.01	8.8 ± 0.5	0.21 ± 0.44
	Vn3	0.98 ± 0.02	0.16 ± 0.02	8.8 ± 0.2	-0.78 ± 0.22
GNMD	Ac4	0.55 ± 0.02	0.22 ± 0.03	6.0 ± 0.1	1.26 ± 0.31
	Ba4	0.54 ± 0.02	0.21 ± 0.02	7.0 ± 0.3	-2.52 ± 0.20
	Bm4	0.52 ± 0.01	0.18 ± 0.01	7.2 ± 0.3	-2.72 ± 0.17
	Ep4	0.57 ± 0.04	0.25 ± 0.02	8.4 ± 0.3	-0.72 ± 0.39
	Sl4	0.91 ± 0.06	0.27 ± 0.06	8.1 ± 0.2	-0.26 ± 0.26
GNMS	Ba5	0.58 ± 0.02	0.20 ± 0.00	8.1 ± 0.4	-0.26 ± 0.09
	Bi5	0.50 ± 0.02	0.18 ± 0.01	6.8 ± 0.3	-0.71 ± 0.38
	Bm5	0.52 ± 0.01	0.19 ± 0.01	8.1 ± 0.3	-0.50 ± 0.48
	Hs5	1.04 ± 0.29	0.34 ± 0.01	11.6 ± 0.7	1.85 ± 0.34
	Ri5	0.55 ± 0.02	0.37 ± 0.03	13.7 ± 0.4	2.19 ± 0.24
	Si5	0.67 ± 0.14	0.46 ± 0.09	12.9 ± 0.7	2.92 ± 0.24
GNSW	Ac6	0.68 ± 0.03	0.15 ± 0.01	7.1 ± 0.3	1.11 ± 0.56
	Ba6	0.57 ± 0.03	0.18 ± 0.01	7.7 ± 0.3	-1.16 ± 0.32
	Bi6	0.50 ± 0.02	0.13 ± 0.01	6.6 ± 0.2	-1.63 ± 0.21
	Bm6	0.50 ± 0.03	0.18 ± 0.01	7.5 ± 0.2	-1.90 ± 0.27
	Eb6	0.70 ± 0.04	0.26 ± 0.01	9.9 ± 0.8	0.43 ± 0.18
	Si6	1.03 ± 0.13	0.22 ± 0.03	10.5 ± 0.5	2.78 ± 0.34

Appendix 3 (continued)

Site	Tag	$\delta^{13}C$	*TLP (MPa)*	*PD (MPa)*	*MD (MPa)*
ENHD	Ba1	-25.55 ± 0.29	-2.05 ± 0.16	-1.28 ± 0.09	-2.18 ± 0.10
	Be1	-27.47 ± 0.26	-2.68 ± 0.17	-3.55 ± 0.39	-7.00 ± 0.00
	Bh1	-26.19 ± 0.25	-2.03 ± 0.18	-1.41 ± 0.15	-2.17 ± 0.16
	Ml1	-27.88 ± 0.36	-2.42 ± 0.15	-3.37 ± 0.26	-6.08 ± 0.39
ENSW	Bc2	-28.58 ± 0.10	-3.83 ± 0.20	-3.55 ± 0.20	-5.77 ± 0.26
	Be2	-28.00 ± 0.17	-2.92 ± 0.17	-4.70 ± 0.20	-7.00 ± 0.00
	Ml2	-28.25 ± 0.10	-2.43 ± 0.21	-4.50 ± 0.32	-6.92 ± 0.08
GNHD	Ac3	-29.23 ± 0.21	-1.73 ± 0.04	-0.38 ± 0.04	-1.26 ± 0.04
	Ba3	-29.09 ± 0.23	-2.05 ± 0.04	-0.65 ± 0.15	-2.03 ± 0.14
	Bm3	-28.58 ± 0.27	-1.89 ± 0.04	-0.45 ± 0.07	-1.63 ± 0.16
	Sl3	-28.88 ± 0.26	-1.67 ± 0.13	-0.96 ± 0.04	-1.62 ± 0.12
	Vn3	-27.80 ± 0.47	-1.79 ± 0.14	-0.84 ± 0.03	-1.93 ± 0.13
GNMD	Ac4	-28.88 ± 0.20	-1.64 ± 0.02	-0.34 ± 0.03	-1.27 ± 0.06
	Ba4	-28.46 ± 0.28	-2.30 ± 0.13	-0.29 ± 0.05	-1.75 ± 0.16
	Bm4	-29.32 ± 0.27	-2.15 ± 0.18	-0.28 ± 0.04	-1.40 ± 0.10
	Ep4	-28.81 ± 0.44	-1.90 ± 0.15	-0.53 ± 0.07	-2.37 ± 0.08
	Sl4	-28.87 ± 0.45	-1.91 ± 0.07	-0.84 ± 0.11	-1.81 ± 0.10
GNMS	Ba5	-28.18 ± 0.34	-1.95 ± 0.03	-0.13 ± 0.02	-1.19 ± 0.16
	Bi5	-28.59 ± 0.21	-1.90 ± 0.02	-0.09 ± 0.00	-0.89 ± 0.15
	Bm5	-28.43 ± 0.48	-1.80 ± 0.05	-0.26 ± 0.06	-1.17 ± 0.10
	Hs5	-28.95 ± 0.23	-1.60 ± 0.05	-1.04 ± 0.21	2.36 ± 0.16
	Ri5	-27.54 ± 0.56	-1.61 ± 0.08	-0.45 ± 0.13	-1.76 ± 0.06
	Si5	-29.89 ± 0.51	-1.65 ± 0.06	-0.70 ± 0.13	-1.82 ± 0.07
GNSW	Ac6	-30.74 ± 0.54	-1.66 ± 0.06	-0.13 ± 0.02	-1.27 ± 0.09
	Ba6	-29.08 ± 0.23	-2.13 ± 0.09	-0.28 ± 0.03	-2.11 ± 0.21
	Bi6	-30.49 ± 0.34	-2.09 ± 0.06	-0.10 ± 0.01	-2.19 ± 0.12
	Bm6	-29.98 ± 0.39	-1.95 ± 0.06	-0.24 ± 0.05	-1.89 ± 0.11
	Eb6	-28.75 ± 0.15	-1.64 ± 0.12	-0.55 ± 0.04	-1.90 ± 0.08
	Si6	-30.32 ± 0.31	-1.90 ± 0.07	-0.58 ± 0.12	-1.82 ± 0.11

Appendix 4: Summary of the models created through linear mixed effect modelling and selected using the Akaike information criterion (AICc) to investigate whether stomatal conductance, shoot length and dry mass, root depth and dry mass, soil water content, and water potentials varied between the treatments for each of the 4 species of “Experiment 1” (Chapter 5). The best four models plus the null of each analysis are represented in this table. Month is referred as “MN”, time as “T”, treatment as “TR”, species as “SP”, period as “PR” and depth as “DP”. Degree of freedom is referred as “*k*”, weight as “Wt”, cumulative weight as “Cum.Wt” and log-likelihood as “LL”.

Fixed effects	*k*	AIC_c	ΔAIC_c	Wt	Cum.Wt	LL
Stomatal conductance rates of *B. attenuata*						
MN + T + TR + (MN X T) + (MN X TR)	**30**	**3141.16**	**0.00**	**0.50**	**0.50**	**-1536.50**
MN + T + TR + (MN X TR)	**18**	**3142.12**	**0.95**	**0.31**	**0.81**	**-1551.63**
MN + T + TR + (MN X T) + (MN X TR) + (T X TR)	42	3143.98	2.82	0.12	0.93	-1521.63
MN + T + TR + (MN X TR) + (T X TR)	30	3145.07	3.91	0.07	1.00	-1538.46
Null	4	3261.43	120.26	0.00	1.00	-1626.64
Stomatal conductance rates of *B. littoralis*						
MN + T + TR + (MN X T) + (MN X TR)	**30**	**2201.54**	**0.00**	**0.99**	**0.99**	**-1064.57**
MN + T + TR + (MN X TR)	18	2211.30	9.76	0.01	1.00	-1085.54
MN + T + TR + (MN X T) + (MN X TR) + (T X TR)	42	2225.22	23.68	0.00	1.00	-1057.52
MN + T + TR + (MN X TR) + (T X TR)	30	2232.89	31.34	0.00	1.00	-1080.24
Null	4	2321.10	119.56	0.00	1.00	-1156.44
Stomatal conductance rates of *G. tomentosum*						
MN + T + TR + (MN X T) + (MN X TR)	**30**	**3397.88**	**0.00**	**1.00**	**1.00**	**-1665.89**
MN + T + TR + (MN X T) + (MN X TR) + (T X TR)	42	3422.78	24.90	0.00	1.00	-1663.23
MN + T + TR + (MN X TR)	18	3428.21	30.33	0.00	1.00	-1695.03
MN + T + TR + (MN X TR) + (T X TR)	30	3451.26	53.38	0.00	1.00	-1692.58
Null	4	3701.79	303.91	0.00	1.00	-1846.84
Stomatal conductance rates of *P. reticulata*						
MN + T + TR + (MN X T) + (MN X TR)	**30**	**2948.27**	**0.00**	**1.00**	**1.00**	**-1440.59**
MN + T + TR + (MN X T) + (MN X TR) + (T X TR)	42	2966.35	18.07	0.00	1.00	-1433.95
MN + T + TR + (MN X TR)	18	2993.43	45.16	0.00	1.00	-1477.47
MN + T + TR + (MN X T)	26	2997.10	48.83	0.00	1.00	-1469.91
Null	4	3213.34	265.06	0.00	1.00	-1602.60
Stomatal conductance rates with all species for "dry" treatment collected in February						
SP + T	**13**	**1084.98**	**0.00**	**0.81**	**0.81**	**-527.63**
T	10	1088.28	3.30	0.16	0.96	-533.05
SP + T + (SP x T)	31	1091.14	6.16	0.04	1.00	-502.17
SP	7	1117.58	32.60	0.00	1.00	-551.25
Null	4	1121.19	36.21	0.00	1.00	-556.41
Growth in length of *B. attenuata*						
MN	**5**	**163.35**	**0.00**	**0.86**	**0.86**	**-75.74**
MN + TR	7	167.72	4.37	0.10	0.96	-74.99
MN + TR + (MN X TR)	11	169.35	6.00	0.04	1.00	-68.60
Null	3	214.20	50.85	0.00	1.00	-103.75
TR	5	218.31	54.96	0.00	1.00	-103.22

Appendix 4 (continued)

Fixed effects	*k*	AIC_c	Δ AIC_c	Wt	Cum.Wt	LL
Growth in length of *B. littoralis*						
MN	**5**	**104.34**	**0.00**	**0.87**	**0.87**	**-45.67**
MN + TR	7	108.07	3.73	0.13	1.00	-43.93
MN + TR + (MN X TR)	11	121.66	17.31	0.00	1.00	-40.40
Null	3	133.38	29.04	0.00	1.00	-63.15
TR	5	135.67	31.33	0.00	1.00	-61.33
Growth in length of *G. tomentosum*						
MN + TR + (MN X TR)	**11**	**216.46**	**0.00**	**1.00**	**1.00**	**-93.66**
MN + TR	7	233.02	16.57	0.00	1.00	-108.15
MN	5	234.77	18.31	0.00	1.00	-111.69
Null	3	271.62	55.16	0.00	1.00	-132.54
TR	5	272.67	56.22	0.00	1.00	-130.64
Growth in length of *P. reticulata*						
MN + TR + (MN X TR)	**11**	**170.47**	**0.00**	**1.00**	**1.00**	**-69.84**
MN + TR	7	206.55	36.08	0.00	1.00	-94.63
MN	5	210.93	40.46	0.00	1.00	-99.63
TR	5	241.33	70.86	0.00	1.00	-114.83
Null	3	243.18	72.70	0.00	1.00	-118.27
Soil water content						
SP +DP + TR + (DP X TR)	**35**	**1652.29**	**0.00**	**0.88**	**0.88**	**-788.27**
DP + TR +(DP X TR)	31	1656.26	3.97	0.12	1.00	-794.89
SP +DP + TR + (DP X TR) + (SP X TR)	43	1667.67	15.38	0.00	1.00	-786.43
SP +DP + TR + (DP X TR) + (SP X DP)	71	1704.09	51.80	0.00	1.00	-768.33
Null	4	2267.63	615.34	0.00	1.00	-1129.77
Root depth						
SP	**6**	**464.27**	**0.00**	**0.72**	**0.72**	**-225.13**
SP +TR	**8**	**466.13**	**1.86**	**0.28**	**1.00**	**-223.26**
Null	3	481.20	16.93	0.00	1.00	-237.33
TR	5	483.71	19.45	0.00	1.00	-236.16
SP + TR + (SP X TR)	14	485.13	20.86	0.00	1.00	-222.39
Shoot dry mass						
SP +TR	**8**	**48.86**	**0.00**	**0.95**	**0.95**	**-14.63**
SP + TR + (SP X TR)	14	54.66	5.80	0.05	1.00	-7.15
SP	6	62.60	13.74	0.00	1.00	-24.30
TR	5	72.53	23.67	0.00	1.00	-30.57
Null	3	82.44	33.58	0.00	1.00	-37.95
Root dry mass						
SP +TR	**8**	**524.55**	**0.00**	**0.88**	**0.88**	**-253.10**
TR	5	529.76	5.20	0.07	0.94	-259.41
SP	6	530.31	5.76	0.05	0.99	-258.49
Null	3	534.38	9.82	0.01	1.00	-264.01
SP + TR + (SP X TR)	14	537.15	12.59	0.00	1.00	-250.75
Water potential						
SP + TR + PR	**10**	**137.71**	**0.00**	**0.53**	**0.53**	**-57.02**
SP + TR + PR + (SP x PR)	12	140.20	2.50	0.15	0.69	-55.41
SP + TR + PR + (TR x PR)	12	140.81	3.10	0.11	0.80	-55.72
SP + PR	8	141.69	3.98	0.07	0.87	-61.69
Null	4	150.80	13.09	0.00	1.00	-71.10

Appendix 5: Summary of the models created through linear mixed effect modelling and selected using the Akaike information criterion (AICc) to investigate whether non-structural carbohydrates, shoot dry mass, stomatal conductance, and water potentials varied between the treatments for each of the four species of "Experiment 2" (Chapter 5). All or the best four models plus the null of each analysis are represented in this table. Month is referred as "MN", time as "T", treatment as "TR", and period as "PR". Degree of freedom is referred as "*k*", weight is referred as "Wt", cumulative weight as "Cum.Wt" and log-likelihood as "LL".

Fixed effects	*k*	AIC_c	ΔAIC_c	Wt	Cum.Wt	LL
Non-structural carbohydrates of *B. attenuata*						
Null	**3**	**81.14**	**0.00**	**0.44**	**0.44**	**-36.48**
MN	**4**	**81.16**	**0.03**	**0.43**	**0.88**	**-34.58**
TR	4	84.95	3.81	0.07	0.94	-36.47
MN + TR	5	85.83	4.69	0.04	0.98	-34.58
MN + TR + (MN x TR)	6	87.65	6.52	0.02	1.00	-32.58
Non-structural carbohydrates of *B. littoralis*						
MN + TR + (MN x TR)	**6**	**90.66**	**0.00**	**0.93**	**0.93**	**-33.33**
TR	4	97.99	7.33	0.02	0.96	-42.77
MN	4	98.65	7.99	0.02	0.97	-43.10
MN + TR	5	98.89	8.24	0.02	0.99	-40.70
Null	3	99.62	8.96	0.01	1.00	-45.61
Non-structural carbohydrates of *G. tomentosum*						
MN	**4**	**65.12**	**0.00**	**0.86**	**0.86**	**-26.56**
MN + TR	5	69.10	3.98	0.12	0.98	-26.22
MN + TR + (MN x TR)	6	72.84	7.71	0.02	1.00	-25.17
Null	3	76.31	11.19	0.00	1.00	-34.07
TR	4	78.86	13.73	0.00	1.00	-33.43
Non-structural carbohydrates of *P. reticulata*						
Null	**3**	**74.30**	**0.00**	**0.75**	**0.75**	**-32.95**
MN	4	77.89	3.59	0.12	0.87	-32.72
TR	4	77.98	3.68	0.12	0.99	-32.77
MN + TR	5	82.69	8.39	0.01	1.00	-32.60
MN + TR + (MN x TR)	6	89.01	14.71	0.00	1.00	-32.51
Shoot dry mass of *B. attenuata*						
MN + TR + (MN x TR)	**6**	**46.35**	**0.00**	**1.00**	**1.00**	**-11.92**
MN + TR	5	60.99	14.64	0.00	1.00	-22.16
MN	4	64.81	18.46	0.00	1.00	-26.41
TR	4	72.65	26.30	0.00	1.00	-30.33
Null	3	73.00	26.65	0.00	1.00	-32.41
Shoot dry mass of *B. littoralis*						
MN	**4**	**15.52**	**0.00**	**0.41**	**0.41**	**-1.26**
MN + TR	**5**	**16.81**	**1.29**	**0.22**	**0.63**	**0.88**
Null	**3**	**16.90**	**1.38**	**0.21**	**0.84**	**-4.12**
TR	4	17.67	2.15	0.14	0.98	-2.33
MN + TR + (MN x TR)	6	21.25	5.72	0.02	1.00	2.38
Shoot dry mass of *G. tomentosum*						
Null	**3**	**8.49**	**0.00**	**0.41**	**0.41**	**-0.16**
MN + TR	**5**	**9.66**	**1.16**	**0.23**	**0.65**	**3.51**
MN	**4**	**9.80**	**1.30**	**0.22**	**0.86**	**1.10**
TR	4	10.90	2.40	0.12	0.99	0.55
MN + TR + (MN x TR)	6	15.45	6.96	0.01	1.00	3.52

Appendix 5 (continued)

Fixed effects	*k*	AIC_c	Δ AIC_c	Wt	Cum.Wt	LL
Shoot dry mass of *P. reticulata*						
MN	**4**	**41.99**	**0.00**	**0.76**	**0.76**	**-14.77**
MN + TR	5	45.60	3.62	0.13	0.89	-14.05
Null	3	47.13	5.14	0.06	0.95	-19.36
MN + TR + (MN x TR)	6	48.42	6.44	0.03	0.98	-12.21
TR	4	48.95	6.97	0.02	1.00	-18.25
Stomatal conductance rates of *B. attenuata*						
MN + TR	**5**	**54.11**	**0.00**	**0.65**	**0.65**	**-18.72**
MN + TR + (MN x TR)	6	56.64	2.53	0.18	0.83	-17.07
MN	4	57.36	3.25	0.13	0.96	-22.68
TR	4	60.38	6.27	0.03	0.99	-24.19
Null	3	62.67	8.56	0.01	1.00	-27.25
Stomatal conductance rates of *B. littoralis*						
TR	**4**	**40.40**	**0.00**	**0.88**	**0.88**	**-13.70**
MN + TR	5	45.93	5.54	0.06	0.94	-13.68
Null	3	45.98	5.58	0.05	0.99	-18.66
MN	4	50.16	9.77	0.01	1.00	-18.58
MN + TR + (MN x TR)	6	51.96	11.56	0.00	1.00	-12.98
Stomatal conductance rates of *G. tomentosum*						
Null	**3**	**-19.42**	**0.00**	**0.67**	**0.67**	**13.80**
TR	4	-16.93	2.49	0.19	0.86	14.47
MN	4	-15.91	3.51	0.12	0.98	13.96
MN + TR	5	-12.64	6.79	0.02	1.00	14.65
MN + TR + (MN x TR)	6	-6.81	12.62	0.00	1.00	14.65
Stomatal conductance rates of *P. reticulata*						
MN	**4**	**22.35**	**0.00**	**0.73**	**0.73**	**-4.95**
MN + TR	5	25.05	2.70	0.19	0.92	-3.78
MN + TR + (MN x TR)	6	27.19	4.84	0.07	0.99	-1.59
Null	3	30.89	8.54	0.01	1.00	-11.24
TR	4	32.64	10.29	0.00	1.00	-10.10
Water potential of *B. attenuata*						
PR	**5**	**42.99**	**0.00**	**0.45**	**0.45**	**-15.46**
PR + TR	**6**	**44.59**	**1.59**	**0.20**	**0.66**	**-14.79**
PR + MN	6	45.76	2.77	0.11	0.77	-15.38
PR + TR + (PR X TR)	7	46.04	3.05	0.10	0.87	-13.95
Null	4	74.09	31.10	0.00	1.00	-32.38
Water potential of *B. littoralis*						
PR +MN + (PR X MN)	**7**	**-15.80**	**0.00**	**0.33**	**0.33**	**16.77**
PR +MN + TR + (PR X MN)	**8**	**-15.26**	**0.54**	**0.25**	**0.59**	**18.11**
PR + TR	6	-13.60	2.20	0.11	0.70	14.15
PR	5	-12.22	3.58	0.06	0.75	12.05
Null	4	85.53	101.33	0.00	1.00	-38.16
Water potential of *G. tomentosum*						
PR + MN + TR + (PR X MN) + (PR X TR) + (TR x MN) + (MN X PR X TR)	**11**	**-22.54**	**0.00**	**0.61**	**0.61**	**25.41**
PR + MN + TR + (PR X MN) + (MN X TR)	**9**	**-20.70**	**1.84**	**0.24**	**0.85**	**21.40**
PR +MN + TR + (PR X MN) + (PR X TR) + (TR + MN)	10	-18.11	4.43	0.07	0.92	21.61
PR + MN + (PR X MN)	7	-17.54	5.00	0.05	0.97	16.99
Null	4	76.80	99.34	0.00	1.00	-33.99

Appendix 5 (continued)

Fixed effects	*k*	AIC_c	Δ AIC_c	Wt	Cum.Wt	LL
Water potential of *P. reticulata*						
PR + MN + TR + (PR X MN) + (PR X TR)	**9**	**-36.54**	**0.00**	**0.37**	**0.37**	**30.37**
PR + MN + TR + (PR X MN)	**8**	**-35.37**	**1.17**	**0.21**	**0.57**	**28.09**
PR +MN + TR + (PR X MN) + (PR X TR) + (TR x MN)	10	-31.44	2.10	0.13	0.70	31.15
PR +MN + TR + (PR X MN) + (TR x MN)	9	-33.29	3.25	0.07	0.78	28.75
Null	4	41.18	77.72	0.00	1.00	-16.00

Appendix 6: Summary of the models created through linear mixed effect modelling and selected using the Akaike information criterion corrected for low sample size (AICc) to investigate whether growth, stomata conductance, water potentials, leaf δ2H and soil water content varied between the treatments of field experiment (Chapter 6). All or the best four models plus the null of each analysis are represented in this table. Month is represented as "MN", treatment as "TR", time as "TI", period as "PR", Hour as "H", and collection as "COL". In the first row, Degree of freedom is referred as "*k*", "Wt" is the weight, "Cum.Wt" is the cumulative weight, and "LL" is the log-likelihood.

Fixed effects	*k*	AIC_c	ΔAIC_c	Wt	Cum.Wt	LL
Growth in length of *B. attenuata*						
MN + TR	**8**	**88.44**	**0.00**	**0.75**	**0.75**	**-35.68**
MN + TR + (MN x TR)	10	91.69	3.25	0.15	0.89	-35.00
MN	7	92.38	3.94	0.10	1.00	-38.77
TR	6	99.80	11.36	0.00	1.00	-43.59
Null	5	102.47	14.03	0.00	1.00	-46.01
Growth in length of *G. tomentosum*						
MN + TR + (MN x TR)	**10**	**-27.44**	**0.00**	**0.71**	**0.71**	**27.39**
TR	6	-24.43	3.01	0.16	0.87	19.45
MN + TR	8	-23.15	4.30	0.08	0.95	21.82
Null	5	-20.90	6.54	0.03	0.98	16.31
MN	7	-20.57	6.88	0.02	1.00	18.98
Stomata conductance of *B. attenuata*						
TI + MN + TR + (MN x TI)	**9**	**2936.0**	**0.00**	**0.39**	**0.39**	**-1458.7**
TI + MN + (MN x TI)	**8**	**2937.4**	**1.41**	**0.19**	**0.58**	**-1460.4**
TI + MN + TR + (TR x TI) + (MN x TI)	**10**	**2937.6**	**1.56**	**0.18**	**0.76**	**-1458.4**
TI + MN + TR + MN x TI + MN x TR	10	2938.1	2.04	0.14	0.90	-1458.6
Null	5	3059.1	123.06	0.00	1.00	-1524.4
Stomata conductance of *G. tomentosum*						
TI + MN + TR + (MN x TR) + (TR x TI)	**10**	**1519.1**	**0.00**	**0.60**	**0.60**	**-748.8**
TI + MN + TR + (MN x TR) + (MN x TI) + (TR x TI)	**11**	**1520.8**	**1.75**	**1.75**	**0.85**	**-748.5**
TI + MN + TR + (MN x TR) + (MN x TI) + (TR x TI) + (MN x TI x TR)	12	1522.2	3.10	0.13	0.98	-748.0
TI + TR + MN + MN x TR	9	1526.3	7.26	0.02	0.99	-753.5
Null	5	1576.9	57.85	0.00	1.00	-783.3

Appendix 6 (continued)

Fixed effects	*k*	AIC_c	$\Delta\ AIC_c$	Wt	Cum.Wt	LL
Water potential of *B. attenuata*						
PR	**6**	**38.2**	**0.00**	**0.50**	**0.50**	**-10.64**
PR + TR	**7**	**38.7**	**0.50**	**0.39**	**0.88**	**-8.86**
PR + TR + (PR x TR)	8	41.2	2.99	0.11	0.99	-7.81
Null	5	47.1	8.89	0.01	1.00	-16.89
TR	6	49.3	11.05	0.00	1.00	-16.17
Dry mass *B. attenuata*						
TR	5	**562.1**	**0.00**	**0.97**	**0.97**	**-275.07**
Null	4	569.4	7.31	0.03	1.00	-280.08
Leaf δ2H of *B. attenuata*						
TI	**8**	**343.0**	**0.00**	**0.81**	**0.81**	**-161.51**
TI + TR	9	346.0	2.93	0.19	1.00	-161.40
TI + TR + (TI x TR)	12	354.7	11.68	0.00	1.00	-160.47
Null	5	371.2	28.20	0.00	1.00	-179.84
TR	6	373.4	30.35	0.00	1.00	-179.58
Leaf δ2H of Donor						
COL	**7**	**339.04**	**0.00**	**0.56**	**0.56**	**-159.41**
Null	**4**	**339.54**	**0.50**	**0.44**	**1.00**	**-164.82**
Soil moisture						
MN + TR + (MN x TR)	**17**	**-395551.9**	**0.00**	**1.00**	**1.00**	**197793.0**
MN + H +TR + MN:TR	40	-395527.5	24.38	0.00	1.00	197803.8
MN + H +TR + MN:TR + H:TR	63	-395482.3	69.59	0.00	1.00	197804.2
MN + H +TR + MN:TR + MN:H	178	-395263.4	288.48	0.00	1.00	197810.7
Null	4	-382965.6	12586.32	0.00	1.00	191486.8

Appendix 7 Summary of the models created through linear mixed effect modelling and selected using the Akaike information criterion (AICc) to investigate whether growth, stomata conductance, water potentials, leaf δ2H and soil water content varied between the treatments of glasshouse experiment (Chapter 6). All or the best four models plus the null of each analysis are represented in this table. Month is represented as "MN", treatment as "TR", time as "TI", period as "PR", and collection as "COL". In the first row, Degree of freedom is referred as "*k*", "Wt" is the weight, "Cum.Wt" is the cumulative weight, and "LL" is the log-likelihood.

Fixed effects	*k*	AIC_c	ΔAIC_c	Wt	Cum.Wt	LL
Growth in length of *B. attenuata*						
MN + TR + (MN x TR)	**13**	**444.9**	**0.00**	**0.87**	**0.87**	**-208.92**
MN + TR	9	448.7	3.78	0.13	1.00	-215.09
MN	7	455.8	10.91	0.00	1.00	-220.76
TR	7	545.8	100.50	0.00	1.00	-265.55
Null	5	551.8	106.86	0.00	1.00	-270.81
Growth in length of *G. tomentosum*						
MN + TR + (MN x TR)	**13**	**2032.4**	**0.00**	**0.90**	**0.90**	**-1002.8**
MN	7	2037.2	4.76	0.08	0.98	-1011.5
MN + TR	9	2040.0	7.54	0.02	1.00	-1010.8
Null	5	2096.9	64.52	0.00	1.00	-1043.4
TR	7	2099.5	67.05	0.00	1.00	-1042.6
Stomata conductance of *B. attenuata*						
TI + MN + TR + (MN x TR) + (MN x TI)	**28**	**25761.4**	**0.00**	**0.73**	**0.73**	**-12852.3**
TI + MN + TR + MN x TI	24	25763.6	2.21	0.24	0.97	-12857.5
TI + MN + TR + MN x TR + MN x TI + TI x TR	40	25768.7	7.32	0.02	0.99	-12843.5
TI + MN + TI x MN	22	25770.3	8.95	0.01	1.00	-12862.9
Null	5	26510.2	748.86	0.00	1.00	-13250.1
Stomata conductance of *G. tomentosum*						
TI + MN + TR + (MN x TR) + (MN x TI)	**28**	**32635.3**	**0.0**	**1.0**	**1.0**	**-16289.3**
TI + MN + TR + (MN x TR) + (MN x TI) + (TI x TR)	40	32647.6	12.3	0.0	1.0	-16283.2
TI + MN + TR + (MN x TR) + (MN x TI) + (TI x TR) + (TI x TR x MN)	58	32663.4	28.16	0.00	1.00	-16272.4
TI + MN + TI x MN	22	32710.2	74.95	0.00	1.00	-16332.9
Null	5	33311.8	676.51	0.00	1.00	-16650.9
Water potential of *B. attenuata*						
TI	**6**	**36.75**	**0.00**	**0.92**	**0.92**	**-11.71**
PR + TR	8	41.75	5.00	0.08	0.99	-11.69
PR + TR + (PR x TR)	10	46.56	9.81	0.01	1.00	-11.42
Null	5	131.36	94.61	0.00	1.00	-60.21
TR	7	135.94	99.19	0.00	1.00	-60.06
Water potential of Donor						
PR	**6**	**15.92**	**0.00**	**0.91**	**0.91**	**1.27**
PR + TR	7	20.69	4.78	0.08	0.99	1.32
PR + TR + (PR x TR)	8	24.87	8.95	0.01	1.00	2.11
Null	5	42.39	26.47	0.00	1.00	-14.05
TR	6	46.54	30.62	0.00	1.00	-14.04
Water potential of *G. tomentosum*						
PR + TR	**8**	**21.13**	**0.00**	**0.54**	**0.54**	**-0.32**
PR	**6**	**22.61**	**1.48**	**0.26**	**0.80**	**-4.07**
PR + TR + (PR x TR)	10	23.16	2.02	0.20	1.00	2.09
Null	5	53.38	32.25	0.00	1.00	-20.83
TR	2	54.63	33.50	0.00	1.00	-18.62

Appendix 7 (continued)

Fixed effects	*k*	*AIC*$_c$	Δ *AIC*$_c$	Wt	Cum.Wt	LL
Dry mass *B. attenuata*						
TR	**6**	**2177.1**	**0.00**	**0.90**	**0.90**	**-1082.2**
Null	4	2181.5	4.35	0.00	1.00	-1086.6
Dry mass *G. tomentosum*						
Null	**4**	**2234.4**	**0.00**	**0.70**	**0.70**	**-1113.1**
TR	6	2236.1	1.68	0.30	1.00	-1111.7
Leaf δ2H of *B. attenuata*						
COL + TR	**8**	**-43.78**	**0.00**	**0.94**	**0.94**	**32.21**
COL + TR + (COL x TR)	11	-37.86	5.92	0.05	0.98	34.64
COL	7	-35.68	8.10	0.02	1.00	26.59
TR	5	-26.15	17.63	0.00	1.00	18.96
Null	4	-21.67	22.11	0.00	1.00	15.41
Leaf δ2H of *G. tomentosum*						
COL	**7**	**24.75**	**0.00**	**0.87**	**0.87**	**-2.43**
COL + TR	8	28.74	3.99	0.12	0.99	-2.37
Null	4	33.73	8.98	0.01	1.00	-11.96
TR	5	36.18	11.43	0.00	1.00	-11.66
COL + TR + (COL x TR)	11	43.24	18.49	0.00	1.00	-1.82
Leaf δ2H of Donor						
COL + TR + (COL x TR)	**11**	**135.91**	**0.00**	**1.00**	**1.00**	**-51.88**
COL + TR	8	155.95	20.04	0.00	1.00	-67.49
COL	7	159.95	23.82	0.00	1.00	-71.00
TR	5	171.88	35.96	0.00	1.00	-80.00
Null	4	176.36	40.45	0.00	1.00	-83.58
Soil moisture						
MN + TR + (MN x TR)	**36**	**-19522.8**	**0.00**	**1.00**	**1.00**	**9797.7**
MN + TR + HR + (MN x TR)	59	-19490.4	32.39	0.00	1.00	9805.2
MN + TR + HR + (MN x TR) + (TR x HR)	105	-19397.1	125.65	0.00	1.00	9806.7
MN	14	-19239.8	282.93	0.00	1.00	9634.0
Null	4	-19259.7	1011.36	0.00	1.00	9259.7