The influence of depth-to-groundwater on the ecology of woodland vegetation

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This research is supported by an Australian Government Research Training Program Scholarship.

Signature of student: .....

Date: .....

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Abstract

Groundwater-dependent ecosystems (GDEs) must have access to groundwater to maintain their ecological integrity. Groundwater extraction for human needs, however, is threatening GDEs globally. Consequently, an understanding of relationships between naturally occurring spatial gradients in depth-to-groundwater (DGW) and the ecological properties of vegetation assemblages is urgently needed. Currently, little is known about relationships between DGW and the ecology of mesic woodlands within GDEs. I used field work, desktop analyses and a novel experimental system to further our understanding of ecological relationships between DGW and woodland vegetation assemblages.

Plant species composition varied significantly with DGW across mesic woodland vegetation within the Kangaloon study region of south-eastern Australia, with spatial shifts in abundance of nine understorey species driving most of this variation. The compositional differences among assemblages were not underpinned by interspecific variation in several important plant traits (e.g. LMA, plant height, seed mass) in desktop analyses of literature-based trait data and in separate analyses using fresh field collections from the study region.

In a glasshouse experiment, I grew seedlings from seeds of *Hakea dactyloides* collected from both the shallow and deep ends of the DGW gradient at Kangaloon. Both shallow and deep seedlings were exposed to two treatments that simulated differences in soil-water infiltration rates between shallow (slow-draining) and deep (fast-draining) ends of the gradient. Seedlings demonstrated varying degrees of phenotypic plasticity in a range of traits to track changes in water availability of the local environment. For instance, seedlings derived from both populations reduced stomatal conductance and transpiration rates in the fast-draining treatment to increase water use efficiency. There

was little evidence for local adaptation to differentiate the seedlings of populations from the two ends of the DGW gradient.

In a complementary study of arid-zone woodlands of the Ti Tree Basin in central Australia, I found that woodland assemblages with high total plant abundance were correlated with shallow DGW. In addition, the proportion of perennial species increased and the proportion of annual species decreased as DGW increased, and the number of shrub species increased with increasing DGW. These findings, so different from mesic woodlands, indicate that relationships between DGW and the ecology of woodland plant assemblages are not broadly generalizable between ecogeographic regions.

My research provides compelling evidence that DGW influences the ecological properties of vegetation assemblages in idiosyncratic ways between different regions. This research contributes important baseline information vital for the sustainable management of woodland vegetation of GDEs.

Chapter 1: General introduction

1.1 Groundwater as a global resource

Groundwater is the largest freshwater resource in the world, comprising over 97% of all freshwater available for life on Earth (Griebler *et al.* 2001). Located in the saturated sub-surface zone of the soil profile and stored in underground rock crevices in aquifers, groundwater plays an important role in the world's hydrological cycle via transpiration, surface expression of groundwater feeding rivers, lakes, streams and wetlands, and subsequent evaporation (Eamus *et al.* 2006a). In many regions across the world, groundwater stored within aquifers provides the only reliable source of drinking water (Watson *et al.* 1997). As human populations have increased in size over the last 100 years, the use of groundwater for human needs has also substantially risen (Konikow and Kendy 2005; Wada *et al.* 2010; Dalin *et al.* 2017).

Although groundwater has been readily accessed by humans for millennia via naturally occurring springs, oases and as baseflow discharge into rivers, it has only been during the past 100 years that large-scale exploitation of groundwater resources has become a global issue (Gleick and Palaniappan 2010). Indeed, there is growing concern that increased reliance on groundwater for human consumption and industry will have detrimental and potentially irreversible impacts on the flora, fauna and ecosystems that must have access to groundwater (Murray *et al.* 2003; Chen *et al.* 2006; Elmore *et al.* 2006; Kløve *et al.* 2014). Such ecosystems are known as groundwater-dependent ecosystems, or GDEs (Hatton and Evans 1998), and they are found in a diverse range of biomes across the world, including springs, riparian zones, wetlands and in terrestrial vegetation communities. Some GDEs, including 65 wetlands in Australia, have been recognised as important habitats for migratory birds, rare plants and invertebrates, and

as such have been protected by the international Ramsar convention on wetlands since 1971. However, we are only just beginning to understand the critical role that groundwater plays in regulating the ecological structure and function of GDEs (Hatton and Evans 1998; Elmore *et al.* 2006; O'Grady *et al.* 2006; Froend and Sommer 2010; Zhu *et al.* 2013; Zolfaghar *et al.* 2014).

1.2 Groundwater-dependent ecosystems in Australia

Groundwater-dependent ecosystems are a geographically small, yet diverse, valuable and important feature of the Australian landscape (Murray *et al.* 2003; Eamus 2009). In Australia, GDEs fall into three broad categories: surface terrestrial, surface aquatic, and subterranean ecosystems (Nevill *et al.* 2010). Subterranean GDEs consist of submerged caves, cave streams, wet passages and aquifers in karst, pseudo karst, calcrete and fractured rock, and in water-filled interstitial spaces between sediments (Hancock *et al.* 2005; Tomlinson and Boulton 2008). The biological communities within subterranean GDEs are largely invertebrate fauna and micro-organisms known as stygofauna (Humphreys 2008). Examples of subterranean GDEs in Australia occur within sedimentary aquifers in the Gnangara Groundwater Mound located in the Swan Coastal Plain of south-western Australia, and in the Great Artesian Basin, which is one of the largest groundwater reservoirs in the world, lying under approximately 22% of the Australian continent.

Surface aquatic GDEs include permanent springs, streams, rivers, swamps and wetlands that are reliant on the surface expression of groundwater and whose functioning also depends on connectivity to local aquifers (Nevill *et al.* 2010). As these surface aquatic GDEs typically occur at the lowest points in the landscape, they regularly intercept water from unconfined aquifers. In fact, many Australian rivers and

streams rely on groundwater base flow over the majority of the year (Boulton *et al.* 2008), and this is especially important during dry seasons in seasonally dry climates or perennially in arid and semi-arid regions. Examples of surface aquatic GDEs in Australia include Stirling Swamp in the arid zone of the Northern Territory (NT GOV 2009), Porters Creek Wetland on the New South Wales Central Coast (Payne *et al.* 2012), and temperate montane peat swamps in the upper Blue Mountains in New South Wales (Benson and Baird 2012).

Surface terrestrial GDEs are comprised of land-based vegetation communities which occur over much of inland and coastal Australia (Fig. 1.1). In Australia, these GDEs are represented by a broad range of vegetation communities such as forests, woodlands, shrublands, sedgelands and swamplands. The vegetation of sedgelands and swamplands are dominated by low-growing sedges and graminoid species (Benson and Baird 2012; Keith and Myerscough 1993). Shrublands contain a diverse range of plant species in the midstorey and understorey canopy layers, with forests and woodlands also containing a diverse range of species in the overstorey. These upper canopy species tend to have roots that can extend, in some instances, to ~70 m below the ground surface into the saturated zone to access groundwater (Canadell *et al.* 1996).

Surface terrestrial GDEs exhibit varying degrees of dependency on groundwater from total dependence (obligate dependency) to partial or seasonal dependence (facultative dependency) (Hatton *et al.* 1998). Artesian mound springs are examples of obligate dependency, where the absence of groundwater from these ecosystems would result in the entire collapse and loss of the ecosystem (Eamus *et al.* 2006a). Paperbark (*Melaleuca* spp.) swamps and River Red Gum (*Eucalyptus camaldulensis*) stands show facultative dependency (Eamus *et al.* 2006a). In facultative GDEs, groundwater is used when available, but these ecosystems are also able to persist for months or even years

when groundwater is unavailable (Eamus 2009). Plants which access groundwater either directly from the groundwater table or from the capillary fringe directly above the groundwater table are often referred to as groundwater-dependent vegetation (Eamus *et al.* 2006b; Nevill *et al.* 2010). The capillary fringe, is the zone where water has moved upwards through capillary forces (attractive forces) between water and soil particles. Whilst the capillary fringe is less than one metre thick, capillary forces are dependent on soil particle size; sandy soils have a thinner capillary fringe than silt because average pore size is larger in sands and so the capillary forces are weaker (Eamus *et al.* 2006a).

Across surface terrestrial GDEs, depth-to-groundwater (DGW) from the soil surface ranges from shallow, where groundwater is within the rooting zone of vegetation, to deep, where groundwater is mostly below the reach of plant roots. Topographic variation across GDEs is responsible for the spatial variation in DGW observed in these landscapes. In soil, sub-soil, or permeable material immediately above the water table, i.e. the capillary fringe, moisture moves upwards through capillary action where it is utilised by plants (Nevill *et al.* 2010). While deep-rooted plant species are capable of accessing groundwater deep below the soil surface, species with shallower root systems that are unable to access groundwater directly can in some instances benefit from increased water availability via hydraulic lift from the capillary fringe to shallower depths during periods of water stress (Naumburg *et al.* 2005; Froend *et al.* 2006).

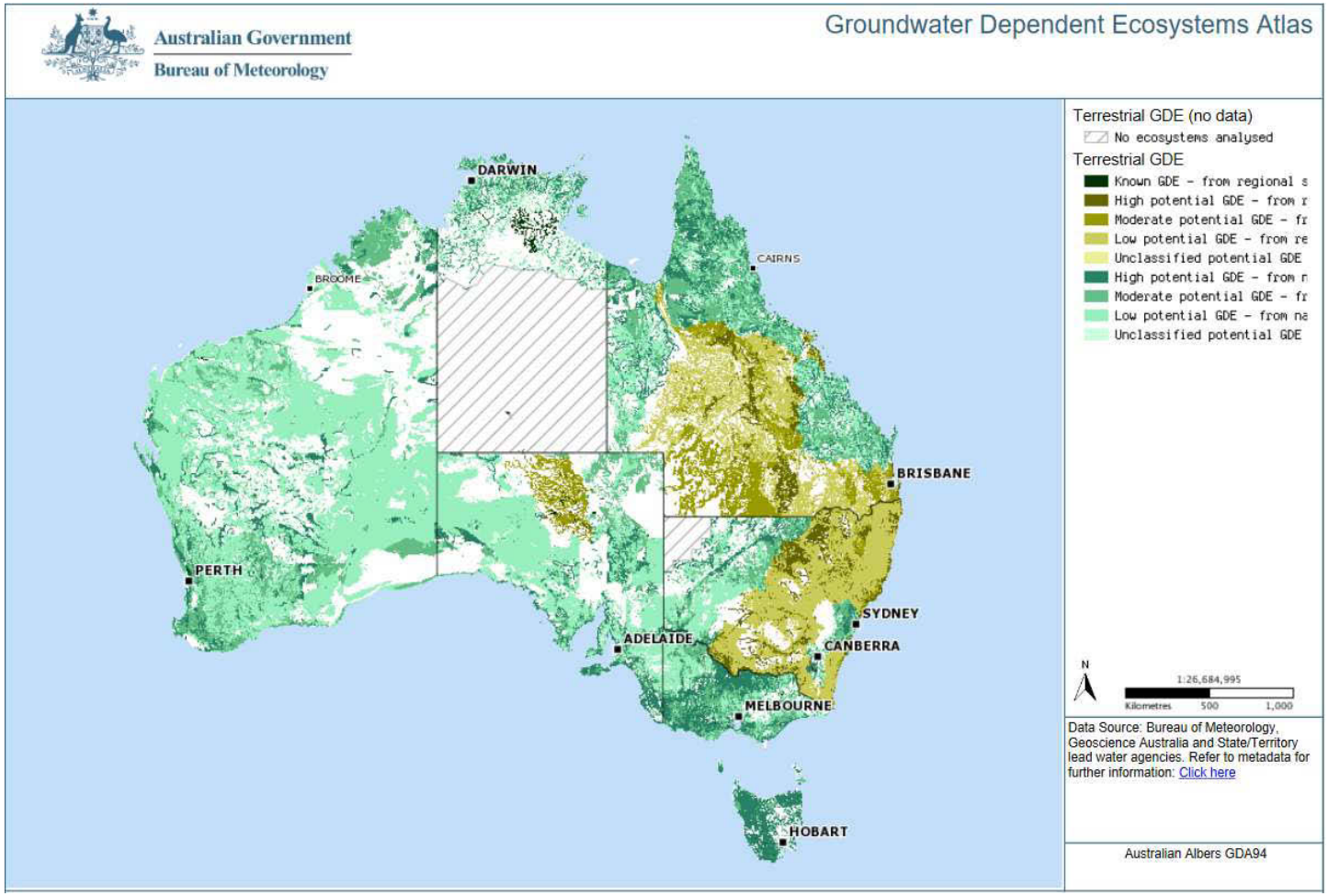


Figure 1.1. Current state of knowledge about the widespread distribution of terrestrial GDEs in Australia (Source GDE Atlas of Australia, BOM 2017).

1.3 Threats to terrestrial groundwater-dependent ecosystems in Australia from groundwater extraction

Arguably, the biggest threat to GDEs is over-extraction of groundwater for human use. The critical issue for surface terrestrial GDEs is the depth of the water table below the ground surface (i.e. depth-to-groundwater) and the preclusion of access to groundwater by roots should groundwater extraction lower the water table beyond the depth at which roots can obtain water. In Australia, the utilization of groundwater for human consumption has been increasing at an exponential rate (Nevill *et al.* 2010). In some regions of Australia groundwater extraction has increased by 90% (1985–2000) (Khan 2008). As the demand for groundwater resources increases, the need to mitigate the environmental impacts of groundwater development is also increasing. Historically in Australia, the majority of groundwater was extracted for use in irrigation (48%), 33% was extracted for urban and industrial use and 19% was extracted for stock watering and rural use (SOE 2001). In many regions of Australia, extraction now exceeds recharge, with the consequence that groundwater levels have declined (NLWRA 2001). Such declines in groundwater regimes threaten native flora and fauna as well as ecosystem services (Chen *et al.* 2006; Murray *et al.* 2006; Sommer and Froend 2011; Zhu *et al.* 2013) and ultimately the structure and function of GDEs (Groom *et al.* 2000; 2001). Demands for groundwater can alter the regimes of GDEs that have developed over thousands or even millions of years (Murray *et al.* 2006). In recognition of the demands for groundwater and the threats posed to terrestrial GDEs via groundwater extraction, there has been growing appreciation of the value of groundwater for the conservation and management of native biodiversity and for the provision of ecosystem services.

Should groundwater extraction lower water tables beyond depths from which roots can obtain water, plant species and their populations with full dependence on

groundwater will either die or decline in abundance while vegetation assemblages will shift in composition (Froend and Sommer 2010; Nevill *et al.* 2010; Sommer and Froend 2014). The additional effects of drought are likely to exacerbate the impacts of groundwater extraction. Thus, there is increasing concern amongst water resource managers about how GDEs will respond over the long and short term to changes in altered groundwater regimes (Shafroth *et al.* 2002; O'Grady *et al.* 2006; Froend and Sommer 2010).

1.4 The Influence of depth-to-groundwater on groundwater-dependent vegetation

The consequences of groundwater extraction, such as tree mortality and a reduction in tree abundances, have been observed in the south-western region of Western Australia (e.g. Hatton and Evans 1998; Groom *et al.* 2000). Additionally, groundwater extraction has been shown to have a direct impact on vegetation structure and function resulting in declines in species diversity, a significant loss in over-storey species with a decline in the populations of species, and a shift in composition from mesic-adapted vegetation to drought-adapted vegetation (e.g.Stromberg *et al.* 1996; Groom *et al.* 2000; 2001; Zencich *et al.* 2002; Cooper *et al.* 2003; Chen *et al.* 2004; 2006; Elmore *et al.* 2006; Froend and Drake 2006; Froend and Sommer 2010; Sommer and Froend 2011; Zhu *et al.* 2013; Sommer and Froend 2014).

In GDEs in arid and semi-arid regions, DGW from the soil surface has a strong influence on plant ecophysiology and the ecological structure and function of vegetation assemblages in Australia (Zencich *et al.* 2002; Canham *et al.* 2009; Froend and Sommer 2010; O'Grady *et al.* 2010; Sommer and Froend 2011; Sommer and Froend 2014) as well as in other parts of the world (e.g. Stromberg *et al.* 1996; Scott *et al.* 1999; Cooper

et al. 2003; Chen *et al.* 2006; Miller *et al.* 2010; Brooksbank *et al.* 2011; Zhu *et al.* 2013). For example, vegetation growing in shallow groundwater areas are more sensitive to drought-related stress compared to vegetation growing in areas with deep groundwater (Scott *et al.* 1999; Cooper *et al.* 2003; Groom 2004; Froend and Drake 2006; Eamus 2009; Zolfaghar *et al.* 2015a) as plant root systems are more likely to access and maintain a functional connection with shallow groundwater (Canham *et al.* 2009; Miller *et al.* 2010; Brooksbank *et al.* 2011), thus providing additional water for growth and transpiration (Scott *et al.* 1999; Naumburg *et al.* 2005). With respect to ecological patterns, studies from the arid region of western China have shown that vegetation growing in areas with shallow groundwater display increased species diversity compared to vegetation growing in areas with deep groundwater (Chen *et al.* 2004, 2006; Zhu *et al.* 2013), while in the western United States, it has been shown that increased vegetation cover was correlated with areas overlying shallow groundwater Elmore *et al.* 2006). In the south-western region of Australia studies have shown that vegetation growing in areas with shallow groundwater are characterized by mesic-adapted species, increased structural complexity, and higher floristic diversity and abundance compared with vegetation growing in areas with deep groundwater (Froend and Sommer 2010; Sommer and Froend 2011; Sommer and Froend 2014). In mesic regions of Australia, a small body of work is beginning to show that spatial variation in DGW has an important influence on plant physiology and productivity (Zolfaghar *et al.* 2014; Zolfaghar *et al.* 2015a; 2015b). For example, vegetation growing in shallow groundwater areas has larger biomass and higher net primary productivity compared with vegetation in areas with deep groundwater (Zolfaghar *et al.* 2014).

While it has been well documented that DGW exerts considerable influence on the ecological properties of vegetation assemblages in arid and semi-arid regions, there

is a major gap in our understanding of the relationships between the ecological properties of vegetation assemblages and natural spatial variation in DGW across mesic environments.

1.5 Research novelty and significance

In this thesis, I primarily sought to understand and provide insight into ecological relationships between spatial variation in DGW and the structure and function of vegetation assemblages in a mesic environment. In addition, while previous studies have focussed extensively on shifts in the structure and function of a variety of vegetation assemblages in response to changes in DGW as a result of groundwater extraction (e.g.Stromberg *et al.* 1996; Groom *et al.* 2000; 2001; Zencich *et al.* 2002; Cooper *et al.* 2003; Chen *et al.* 2004; 2006; Elmore *et al.* 2006; Froend and Drake 2006; Froend and Sommer 2010; Sommer and Froend 2011; Zhu *et al.* 2013; Sommer and Froend 2014), I have focused on understanding shifts in the ecological properties of vegetation including species composition, abundance and diversity, as well as shifts in plant traits including leaf mass per area, plant height and seed mass, along naturally-occurring gradients in DGW. That is, variation in DGW across the landscape that is not a result of groundwater extraction. My rationale for this approach is that analyses of these relationships will provide fundamental information about how terrestrial GDEs are structured and how they function in many natural mesic systems around the world that are free of extraction activities. Such baseline information will be valuable for natural resource management, where natural resource management decisions, ecological restoration targets and monitoring programs can be facilitated with the baseline data arising from my research.

1.6 Research approach and study regions

The main approach that I have used in this thesis is to examine variation in the ecological properties of vegetation assemblages as a function of natural DGW gradients. Importantly, while my research focussed mainly on a mesic GDE, I included an examination towards the end of the thesis of an arid-zone GDE which has also not been exposed to significant groundwater extraction. This allowed a comparison of the role of DGW in driving ecological patterns in two contrasting Australian environments. In my work, I used a combination of approaches that included extensive field-based vegetation surveys and plant collections, data mining with sophisticated statistical analyses, and a novel, manipulative experimental system to further our understanding of relationships between naturally-occurring gradients in DGW and landscape variation in vegetation properties.

The two study regions utilised for this research were situated in a mesic woodland and an arid-zone woodland. The mesic woodland was located in the pristine *Eucalyptus* woodlands and forests of Kangaloon, to the north of the township of Robertson and east of the township of Bowral, NSW. The Kangaloon region is situated in the Upper Nepean catchment on the Woronora Plateau in the Southern Highlands of south-eastern Australia. Kangaloon lies within the mesic/temperate climatic zone and experiences warm summers and cool winters with a relatively high mean annual rainfall of approximately 1067 mm (2000–2012, Bureau of Meteorology station no. 68243). Recent studies from the Kangaloon region have demonstrated that spatial variation in DGW has a strong influence over a number of plant physiological and structural properties (Zolfaghar *et al.* 2014; Zolfaghar *et al.* 2015a; 2015b; Zolfaghar *et al.* 2017), suggesting that overstorey species at the shallow end of the DGW show facultative dependency on groundwater. In contrast, the arid-zone woodland was located in the Ti

Tree basin 200 km north of Alice Springs. The Ti Tree basin encompasses Mulga woodlands, *Corymbia* savanna, with rivers fringed by River Red Gum woodlands and lies within the tropical arid zone of central Australia. The Ti Tree basin experiences hot summers and warm winters and a relatively low mean annual rainfall of 319.9 mm (BOM 2016).

In the manipulative experiment, I constructed specific equipment for use in a glasshouse environment to control whether seedlings experienced fast or slow soil-water infiltration rates that represented low vs high water availability. This system was designed to simulate contrasting field conditions along the DGW gradient between the shallow and the deep ends of the gradient in the mesic woodland study region. A pilot study in the field showed that water moved more quickly through the soil at the deep end compared to the shallow end of the gradient. I conducted a full experiment to specifically ask whether local adaptation and/or phenotypic plasticity in a range of seedling traits of the species *Hakea dactyloides*, a common species of the region, were key elements of variation in plant growth along the gradient in response to differences in soil-water infiltration rates. Local adaptation can be defined as the pattern of divergent selection pressures that cause populations to evolve in response to specific environmental conditions (Joshi *et al.* 2001; Kawecki and Ebert 2004). Phenotypic plasticity is the ability to modify expression of a genotype or rendering different phenotypes under different environmental conditions, so that plants are better suited to meet local conditions (Schlichting 1986; Valladares *et al.* 2007). Understanding the roles of local adaptation and phenotypic plasticity in this model species of the region can offer important insight into the factors regulating the abundance and distribution of species along naturally-occurring DGW gradients.

1.7 Thesis aims

I addressed four specific aims in this thesis:

1. To identify vegetation properties, such as species composition, richness and abundance, that vary in relation to a naturally-occurring gradient in DGW in mesic *Eucalyptus* woodlands (Chapter 2).
2. To identify plant traits that vary in relation to a naturally-occurring gradient in DGW in mesic *Eucalyptus* woodlands (Chapters 3 and 4).
3. To determine whether local adaptation differentiates seedling growth patterns of *Hakea dactyloides* between populations from shallow and deep ends of a naturally-occurring gradient in DGW (Chapter 5).
4. To identify vegetation properties that vary in relation to a naturally-occurring gradient in DGW in arid-zone woodlands (Chapter 6).

1.8 Chapter structure and descriptions

I address aims 1 to 4 of the thesis in the following chapters:

Chapter 2 explores relationships between DGW and variation in plant species composition, species richness and total plant abundance along a naturally occurring DGW gradient in mesic *Eucalyptus* woodlands. This chapter is based on extensive field work and vegetation surveys that I conducted in the Kangaloon area, and is work that is currently in press at the journal *Community Ecology*.

Chapter 3 describes a desktop study that seeks to identify whether seven important plant traits, collected from the literature in a data mining exercise for the plant species of the vegetation assemblages identified in Chapter 2, vary significantly along the natural DGW gradient.

In Chapter 4, I go beyond the data mining exercise and focus explicitly on three strategically important plant traits – leaf mass per area (LMA), plant height and seed mass – using fresh data on these traits that I collected from the study sites of Chapter 2. This chapter sought to overcome the potential problems of using single values of traits obtained from the previous data mining exercise for species that occur at multiple sites along the DGW gradient.

In Chapter 5, I present the results of a glasshouse experiment designed to investigate patterns of local adaptation and phenotypic plasticity in response to changes in soil-water availability between *Hakea dactyloides* seedlings derived from the deep and shallow ends of the DGW gradient. In particular, I compare seedling growth traits and ecophysiological traits between deep and shallow populations and between conditions of high vs low water availability.

In Chapter 6, I examine relationships between plant assemblage structure and composition along a naturally occurring DGW gradient in arid-zone woodlands of central Australia. This chapter is based on field work and vegetation surveys that I conducted in the Ti Tree basin.

Chapter 7, the final chapter of the thesis, provides a discussion of the key results and the implication of the findings for the management of GDEs. This chapter concludes with a discussion of potential future research directions arising from the results of this thesis.

Chapter 2: Does depth-to-groundwater influence the ecological properties of mesic woodlands?

This chapter has been published as: Hingee MC, Eamus D, Krix DW, Zolfaghar S, Murray BR (2017) Patterns of plant species composition in mesic woodlands are related to a naturally occurring depth-to-groundwater gradient. *Community Ecology* 18: 21–30.

Chapter 3: Relating interspecific variation in plant traits to depth-to-groundwater: A desktop study

3.1 Introduction

Determining how vegetation properties such as species composition, richness and abundance vary along environmental gradients is a critical first step in understanding how plant assemblages respond to shifting environmental conditions. In Chapter 2 of this thesis, I showed that plant species composition varied significantly among my study sites in relation to increasing depth-to-groundwater (DGW). I not only found that shifts in the abundance of several key species in the understorey contributed substantially to vegetation changes along the gradient, I also identified three groups of plant species, each of which only occurred at either shallow, intermediate or deep sites. The aim of this chapter is to better understand how plant assemblages vary along a DGW gradient by complementing the previous chapter's analysis of vegetation properties with an analysis of variation in seven plant traits along the gradient.

Analysis of trait variation along environmental gradients can offer insight into mechanisms that underpin shifts in plant species composition (Pate *et al.* 1990; Murray *et al.* 2002; Maharjan *et al.* 2011; Yan *et al.* 2013), because such trait variation reflects successful strategies of species for tolerating contrasting environments (Díaz *et al.* 1998; Fonseca *et al.* 2000; Thuiller *et al.* 2004; Pekin *et al.* 2011). The environmental conditions of any site can be thought of as a filter that sorts species on the basis of traits that lead to successful establishment, survival and reproduction (Díaz *et al.* 1999; Cingolani *et al.* 2007; Venn *et al.* 2011). As a result, prevailing environmental conditions contribute to selection for suites of traits and thus the mix of species found in local plant assemblages (Díaz *et al.* 1998, 1999; Lavorel and Garnier 2002; Yan *et al.*

2013). Thus, complementary analyses of how both species composition and plant traits vary along a DGW gradient have the potential to offer crucial insight into the structure and function of plant assemblages in a groundwater-dependent ecosystem (GDE).

Here, I seek to identify whether seven important plant traits vary significantly along the natural DGW gradient described in Chapter 2. In section 3.2 below, I establish a series of testable predictions which relate variation in each trait to DGW. My approach in this chapter is to use a desktop analysis employing a range of botanical sources to compile information on the seven plant traits for the 120 plant species identified in the previous chapter. These traits include three continuous traits – leaf size, fruit size and plant height – as well as four categorical traits – growth form, plant longevity, dispersal mode and leaf type. I examine variation in these plant traits across sites and explore relationships between traits and DGW. I also include in the analyses an additional 14 environmental attributes covering a range of important physical, chemical and soil attributes. I address the following questions: (1) Is there coordinated variation in plant traits along the DGW gradient? (2) Can groups of plant species that only occur in either shallow, intermediate or deep sites be distinguished from each other on the basis of their traits?

3.2 Predictions

I test the following seven predictions relating variation in plant traits to the DGW gradient. These predictions have been established for the study region where the same rainfall regime is experienced across the study sites (see Chapter 2). Across the sites, drier soil conditions with faster soil-water infiltration rates are found at deep sites compared with shallow sites (see Chapter 5). This provides a gradient of declining soil water availability along the increasing DGW gradient under the similar rainfall

conditions and a selection pressure for coping with comparatively drier conditions at deep sites.

(1) Leaf size

There is unequivocal empirical evidence in the literature showing that leaf size declines with decreasing mean annual rainfall (e.g. Beard 1946; Cowling and Campbell 1980; Givnish 1984; Stone and Bacon 1995; McDonald *et al.* 2003; McLean *et al.* 2014). In general, smaller leaves are able to conserve water more efficiently due to lower transpiration rates as a result of reduced leaf surface area compared to larger leaves (Givnish 1984; Taiz and Zeiger 2010). For the plant assemblages occurring in my study area, I predict that species occurring at the deeper, drier sites are more likely to have smaller leaves than species at shallow sites.

(2) Leaf type

Compound leaves are less common in moist environments, where simple leaves predominate (Givnish 2010). Compound leaves have shorter airflow paths across their surfaces relative to simple, entire leaves of the same total surface area (McDonald *et al.* 2003). Consequently, compound leaves are able to transpire less than simple entire leaves of the same surface area (Givnish 1978; Schuepp 1993; Givnish 2010). Compound leaves may therefore act as an alternative to branching by reducing leaf sizes in low rainfall environments. I predict that species at deep sites are more likely to possess compound leaves compared with species at shallow sites.

(3) Plant height

There is considerable evidence in the literature showing that plant height increases with increasing mean annual rainfall (e.g. Cowling and Lamont 1985; Williams *et al.* 1996; Fonseca *et al.* 2000; Falster and Westoby 2003; Moles *et al.* 2009). Sites with higher water availability favour increased rates of photosynthesis, which allow for greater allocation to productive leaf tissue, increased biomass allocation and higher growth rates, which result in an increase in maximum plant height (Binkley *et al.* 2010; Givnish *et al.* 2014). I predict that species at deep sites are more likely to be shorter than species at shallow sites.

(4) Growth form

Plant growth form is a trait that is frequently used to classify species into groups that can be associated with eco-physiological functional adaptation (Pérez-Harguindeguy *et al.*, 2013). The development of vascular tissue (present in forbs, graminoids, trees, shrubs and climbers) is one such adaptation that has allowed efficient transport of water from roots to the leaves in these plants allowing them to persist in dry environments (Atwell *et al.* 1999; Rowe and Speck 2005; Taiz and Zeiger 2010). In contrast, ferns require free water for reproduction (Watkins *et al.* 2007) and are therefore generally found in moist environments (Benson and McDougall 1993). I predict that tree, shrub, forb, graminoid, and climber growth forms are more likely to be found at deep sites, while ferns are more likely to be found at shallow sites.

(5) Fruit size

Previous work in Australia has shown that larger-fruited species occur in areas experiencing lower average annual rainfall (Murray and Gill 2001). Furthermore, a tight

relationship exists between fruit size and seed size in eucalypt and tropical rainforest species, with larger fruits producing larger seeds (Gill *et al.* 1992; Carpenter *et al.* 2003). Larger seeds with greater mass have larger seed reserves which helps improve seedling survival (Leishman *et al.* 2000) in the face of environmental hazards such as drought. I predict that species at deep sites are more likely to produce larger fruits than species at shallow sites.

(6) Plant longevity

Plant longevity is defined as the time period from establishment until no live part remains of the respective individual plant (Pérez-Harguindeguy *et al.* 2013). Plant longevity has been proposed as a key trait for understanding how plants deal with environmental stress such as drought (Diaz *et al.* 2007). For example, in arid environments many plant species have developed strategies to escape drought which is attained through short life cycles (e.g. annuals and biennials) that allow plants to grow, reproduce and avoid transpirational water loss before the environment becomes too dry (Borchert 1994; McKay *et al.* 2003; Markesteijn and Poorter 2009). In addition, studies from South Africa have linked the dominance of perennial shrubs to high rainfall environments (Cowling *et al.* 1999). I predict that annuals and biennials are more likely to be found at deep sites compared with shallow sites.

(7) *Seed dispersal*

In low rainfall environments, wind dispersal of seeds is thought to be advantageous (Gentry 1982, 1988) because lower growth rates and more open canopies allow the penetration of higher wind velocities into forest and woodland canopies, which makes wind dispersal more effective (Givnish 1998). Conversely, in high rainfall

environments, higher growth rates with larger and more closed canopies and calmer wind speeds work against wind dispersal, which favour vertebrate seed dispersal (Bullock *et al.* 1995; Givnish 1998). Furthermore, recent studies from the Kangaloon region have shown that canopy species growing in areas with shallow DGW have larger aboveground biomass and net primary productivity compared to canopy species growing in areas with deep DGW (Zolfaghar et al. 2014). I predict that seeds of species at deep sites are more likely to be wind dispersed, while seeds of species at shallow sites are more likely to be vertebrate dispersed.

3.3 Methods

3.3.1 Study sites and species

Descriptions of data collection for plant species composition and environmental attributes at the 16 study sites are described in detail in Chapter 2.

3.3.2 Trait data collection

I compiled a literature-based dataset of seven traits for all plant species identified during the vegetation surveys conducted for Chapter 2. Trait data were obtained from the following published sources: *Flora of New South Wales* (Harden 1990-1993), *Native Plants of the Sydney District* (Fairley and Moore 1995), *Field Guide to the Native Plants of Sydney* (Robinson 2003) and several issues of *Cunninghamia* focussed on the ecology of Sydney plant species (e.g. Benson and McDougall 2005). All species and trait data are presented in Appendix D.

Data for three continuous variables were collected including maximum plant height (m), fruit length (mm) and leaf size (cm^2). Leaf size was estimated by entering maximum leaf length and width measurements into the formula leaf size = width x

length x 0.7 (Kraft *et al.* 2008). Data for four categorical variables were collected including growth form, leaf shape, plant longevity and dispersal mode. Growth form categories included: (1) tree, (2) shrub, (3) graminoid, (4) forb, (5) fern and (6) climber. Leaf shapes were grouped into categories of: (1) simple and (2) compound. Longevity categories were: (1) annuals and biennials, (2) perennials and (3) plants living for an indefinitely defined timeframe. Dispersal mode categories included: (1) no investment in structural modifications for dispersal, (2) dispersal by ants, (3) dispersal by vertebrate animals, (4) wind dispersal and (5) dispersal by force (i.e. seeds contained within a specialised pods which open explosively).

3.3.3 Analytical approach

I used two complementary multivariate matrix approaches to quantify and test relationships between plant traits and both DGW and the set of environmental attributes. First, RLQ analysis was used to provide a broad qualitative overview of the associations between plant traits and environmental attributes (Dolédec *et al.* 1996). Pairs of ordinations were constructed via a generalised singular value decomposition, which related traits to environmental attributes while taking into account species abundances (Brown *et al.* 2014). Second, relationships between plant traits and environmental attributes were quantified using fourth-corner analysis (Legendre *et al.* 1997). Fourth-corner analysis is a permutational method that tests the significance of associations between plant traits and environmental attributes while taking into account species abundances (Pekin *et al.* 2011). Complementary use of RLQ and fourth-corner analyses represents an integrated approach to analyse trait-environment relationships (Kleyer *et al.* 2012).

I then employed univariate statistical analyses to individually examine how each plant trait varied along the DGW gradient. Simple linear regressions were used to examine relationships between each quantitative plant trait and DGW, while either a logistic or a multinominal regression was used to quantify relationships between categorical plant traits and DGW. The goal here was to determine whether a univariate approach that did not take into account species abundances or the potentially confounding influence of other plant traits produced different, potentially misleading results compared to the combined RLQ and fourth-corner analyses.

To quantify relationships between plant traits of species that only occur in the shallow, intermediate or deep sites, I used multiple regression modelling within a logistic regression framework. All statistical analyses were conducted using R 3.1.3 (R Development Core Team 2015, R Foundation for Statistical Computing, Vienna, Austria).

3.3.4 Statistical analyses

3.3.4.1 Multivariate analyses across the DGW gradient

The RLQ analysis required multiple data tables taking the form of three input matrices (R, L and Q). For this purpose, I compiled a species trait matrix (Q), with species listed as rows and plant traits as columns, a species composition matrix (L) with species as rows, sites as columns and cells occupied by abundance data, and an environmental matrix (R) containing DGW and the environmental attributes (columns) and sites as rows (see Chapter 2 for information about DGW and environmental attributes). I used the 'rlq' function (library ade4) to implement RLQ analysis (Dolédec *et al.* 1996) to summarize the multivariate joint structure (M) between plant traits (Q), species abundances (L) and environmental attributes (R) (Fig. 3.1). First, I performed a separate

preliminary analysis for each data matrix. The species composition matrix (L) was analysed using correspondence analysis, which provided the optimal simultaneous ordination of species and site scores. The species trait matrix (Q) and the environmental data matrix (R) were analysed using principal component analysis, and weighted by the sites and species weights derived from the previous correspondence analysis of the species composition matrix (L). The species trait matrix (Q) contained both quantitative and categorical variables. In this case, I used the 'dudi.hillsmith' function as this allows for a mix of both quantitative and categorical variables. Next, I applied a single inertia analysis (RLQ analysis) on the cross-matrix of the R, L and Q data matrices. RLQ analysis is an extension of coinertia analysis (a two-table method, Dolédec and Chessel 1994), and simultaneously finds linear combinations of the environmental variables (matrix R) and linear combinations of the species traits (matrix Q) weighted by species abundances (matrix L) (Dray *et al.* 2003). RLQ analysis graphically summarises the main co-structure in the data matrices R, L and Q. To deal with multicollinearity among the environmental variables, six variables (OM, N, Clay, Mg, P and K) that were strongly correlated with each other and with Ca (correlations between < -0.65 and > 0.65; Table 2.1) were excluded from the analysis with Ca retained (Quinn and Keough 2002) to represent the suite of excluded correlated variables in the analysis (as described in Chapter 2).

I used fourth-corner analysis (Legendre *et al.* 1997) to quantify and test the significance of associations between plant traits and environmental attributes, described above using the 'fourthcorner' function (library ade4). I dealt with the issue of multicollinearity among the environmental variables using the procedure described above for the RLQ analysis. Fourth-corner analysis is a permutation method that tests the significance of associations (M) between the species composition matrix (L), the

species trait matrix (Q) and the environmental data matrix (R) (Fig. 3.1). To control for type I error, the significance of the link was determined via model 6 (Dray *et al.* 2014) using 9,999 permutations in all randomisation procedures and the false discovery rate method (Benjamini and Hochberg 1995) to adjust *P* values for multiple testing.

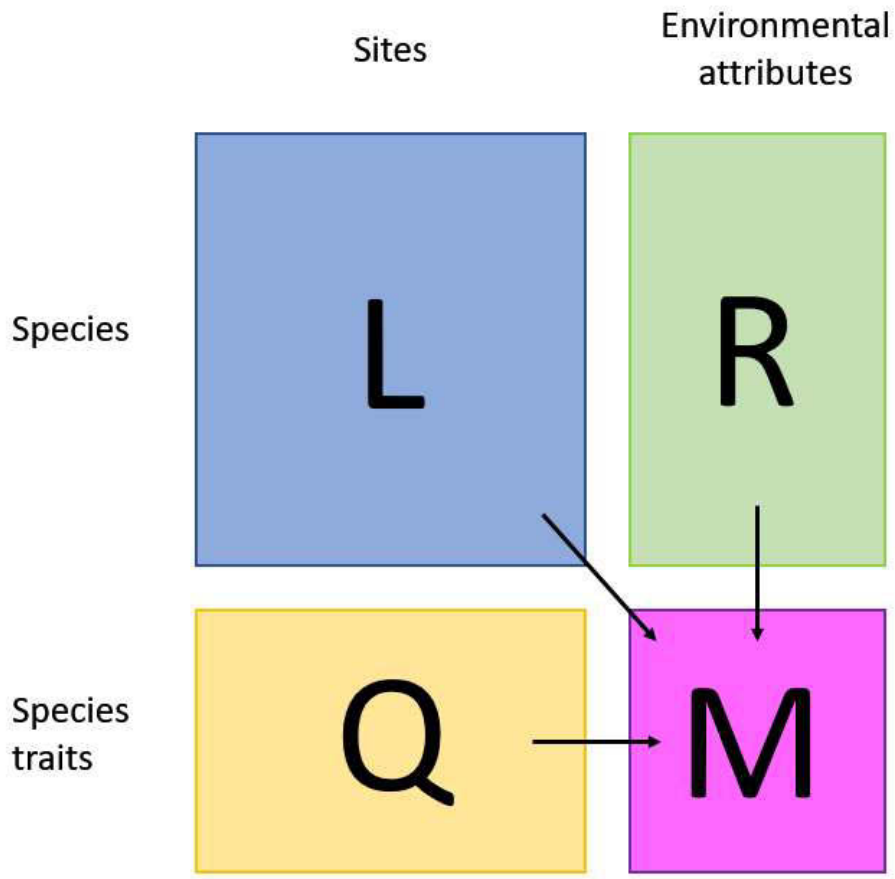


Figure 3.1. Conceptual representation of the multivariate matrix approach. A species composition matrix (L), species trait matrix (Q) and environmental data matrix are used to summarise the multivariate joint structure (M) (RLQ analysis) or significance of associations (M) (fourth-corner analysis).

3.3.4.2 Univariate analyses across the DGW gradient

For continuous variables in the univariate analyses, I used a series of simple linear regression models to examine relationships between each response variable (leaf size, maximum plant height and fruit length) and the explanatory variable DGW. In each model, sites were treated as replicates (n = 16 in each model). Log transformations prior to analyses were applied to leaf size, maximum plant height and fruit length to improve normality. I verified homogeneity of variance by inspecting standard model validation graphs of residuals versus fitted values of each model. Relationships between DGW and categorical variables were quantified using logistic and multinomial regression. I used a generalized linear model (McCullagh and Nelder 1989) to examine the relationship between leaf shape (response variable) and DGW. In this model, sites were treated as replicates (n = 16 in each model). Because this response variable had two categorical outcomes (i.e. simple and compound), a binomial error distribution and a logit link function was used in the model. Where response variables had more than two categorical outcomes, I used the function 'mlogit' (library mlogit) to implement multinominal regression to examine relationships between each response variable (i.e. growth form, longevity and dispersal mode) and DGW. In these models, sites were treated as replicates (n = 16 in each model).

3.3.4.3 Trait comparisons between shallow, intermediate and deep sites

To compare plant traits between groups of species that were identified as only occurring in either the shallow, intermediate or deep sites, I used multiple logistic regression modelling. The aim of this analysis was to build minimum adequate models to identify plant traits that were uniquely related to variation in DGW over and above other the other plant traits (Crawley 2007). In a series of models, paired sections of the

groundwater gradient were treated as the response variable and plant traits as the explanatory variables to allow the simultaneous consideration of multiple plant traits in a single analysis. Three models were built: the first compared shallow to intermediate sites, the second compared intermediate to deep sites and the third compared shallow to deep sites. A saturated model was fitted first, which included all plant traits. The process of model simplification followed, by applying the 'drop1' function (library stats), which dropped one explanatory variable in turn and each time applied an analysis of deviance test. I then omitted the most non-significant term from the next round of analysis and the process of model simplification continued. Initial model simplifications indicated problems with perfect separation, which can be a problem with analytical outputs (Gelman *et al.* 2008). I therefore, applied the Bayesian inference approach using the function 'bayesglm' (library arm) to obtain stable logistic regression coefficients (Gelman *et al.* 2008). To control for potential type I errors, the sequential Bonferroni correction procedure (Holm 1979; Rice 1989) was applied to adjust *P* values for multiple testing. After Bonferroni correction, the critical *P*-value for significance was 0.00833.

3.4 Results

3.4.1 Plant traits and environmental attributes: RLQ and fourth corner analyses

The co-structure between plant traits and environmental attributes was summarised primarily by the first two RLQ axes (48.6% and 24.9% of the cross-covariance for axis 1 and 2 respectively). Of note, several plant traits appeared to align with increasing DGW on the second axis, which also described decreasing soil pH, increasing soil EC and lower slope angles (Fig. 3.1a). These included shifts in growth from perennial forbs to grasses and shifts from wind dispersal of seeds to vertebrate seed dispersal (Fig.

3.1b). However, the complementary fourth-corner analysis showed that these patterns were not reflected by significant correlations between plant traits and DGW (Appendix E). Indeed, among the 70 possible associations between plant traits and environmental attributes or DGW, only five significant associations emerged prior to adjusting for multiple comparisons; after adjustment, all 70 correlations became non-significant (Appendix E).

3.4.2 Plant traits and groundwater depth: univariate analyses

Non-significant results from the fourth-corner analysis were supported by univariate analyses relating each plant trait on its own to DGW. There were no significant relationships between DGW and leaf size ($F_{1, 14} = 0.02$, $P = 0.90$), fruit length ($F_{1, 14} = 0.67$, $P = 0.53$) or maximum plant height ($F_{1, 14} = 0.90$, $P = 0.36$). Similarly, there were no significant relationships between DGW and leaf shape ($\chi^2 = 0.02$, d.f. = 1, $P = 0.88$), growth form ($\chi^2 = 3.03$, d.f. = 1, $P = 0.70$), longevity ($\chi^2 = 2.85$, d.f. = 1, $P = 0.42$) or dispersal mode ($\chi^2 = 4.34$, d.f. = 1, $P = 0.36$).

3.4.3 Logistic regression and minimum adequate models

The model simplification procedure, after application of Bonferroni corrections, showed that no plant traits differentiated significantly or uniquely between groups of species in the intermediate and deep, shallow and deep, or intermediate and shallow comparisons of the DGW gradient (Table 3.1).

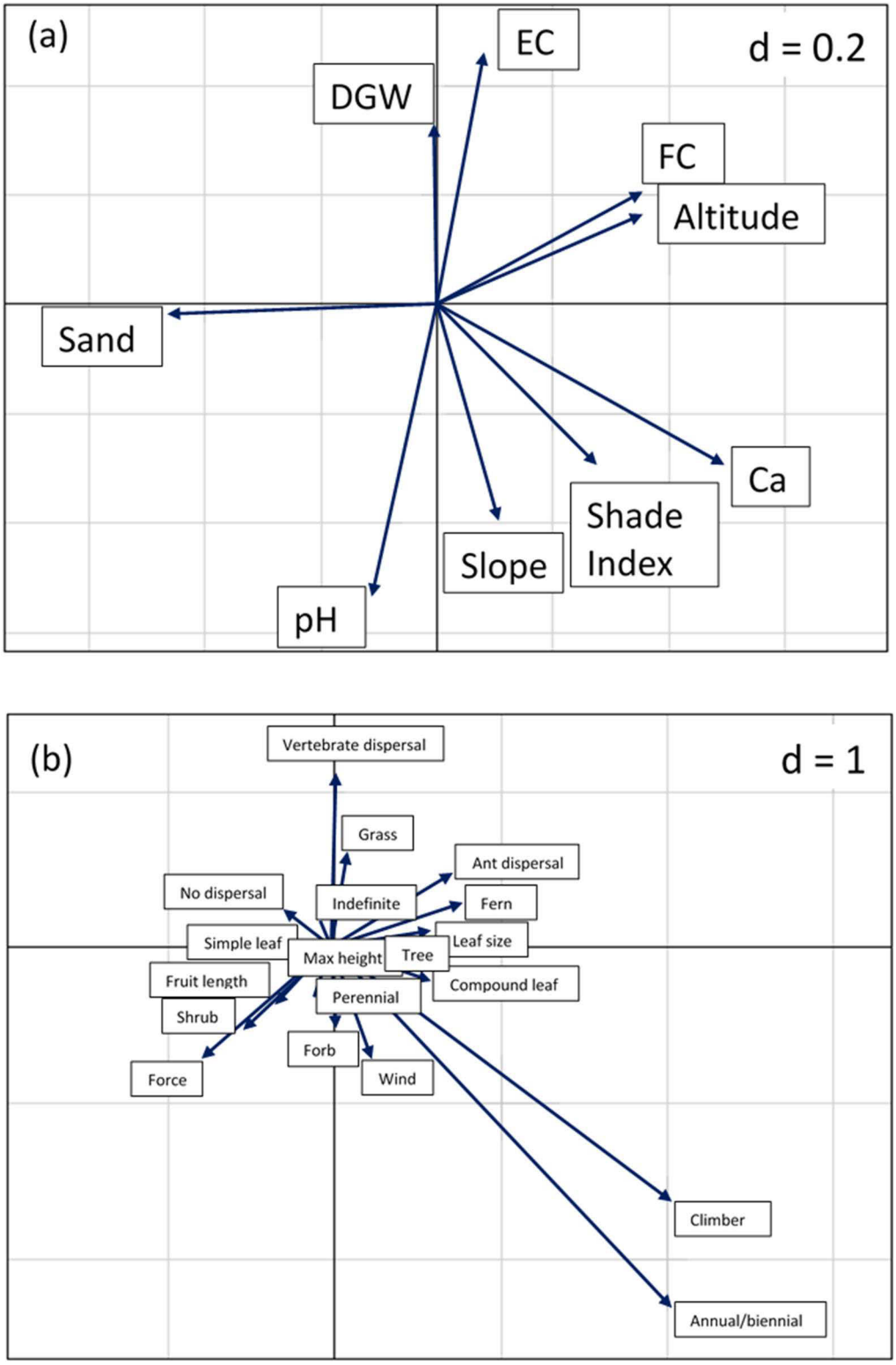


Figure 3.2. The first two axes of RLQ analysis showing (a) coefficients for environmental attributes and (b) coefficients for plant traits. The values of d represent the grid size, equivalent to x and y-axis values. Length and direction of the arrows represents the strength of the relationship along each axis.

Table 3.1. Minimum adequate model comparing plant traits between (a) shallow and intermediate, (b) shallow and deep and (c) intermediate and deep sections of the gradient to plant traits. Bonferroni correction critical *P* value = 0.00833.

Trait	d.f.	Change in deviance	*P*
(a) Shallow and intermediate			
Traits in MAM			
Growth form	4	11.60	0.04
Traits not in MAM			
Fruit length	1	0.10	0.75
Leaf shape	1	0.08	0.78
Longevity	2	2.13	0.35
Dispersal mode	4	5.87	0.21
Leaf size	1	1.97	0.16
Maximum height	1	2.15	0.14
(b) Shallow and deep			
Traits in MAM			
Longevity	2	7.58	0.02
Traits not in MAM			
Leaf shape	1	5.59	1.00
Dispersal mode	4	1.60	0.87
Leaf size	1	0.47	0.49
Fruit length	1	0.67	0.41
Growth form	4	7.12	0.21
Maximum height	1	3.75	0.05

Trait	d.f.	Change in deviance	*P*
(c) Intermediate and deep			
Traits in MAM			
Maximum height	1	2.71	0.09
Traits not in MAM			
Leaf shape	1	0.03	0.86
Growth form	4	1.90	0.75
Longevity	2	1.72	0.42
Leaf size	1	1.29	0.26
Fruit length	1	1.50	0.22
Dispersal mode	4	4.60	0.33

3.5 Discussion

This desktop analysis showed that variation in the seven plant functional traits were not significantly related to the DGW gradient, providing no support for any of the predictions. This was the case when considering variation in plant traits across the whole gradient as well as when comparing traits between groups of species that only occurred in the shallow, intermediate and deep sections of the gradient. I discuss two possible explanations, one methodological and one ecological, for the lack of any association between the plant traits and DGW.

With respect to the methodological explanation, the use of literature-based data for species values of the continuous traits (leaf size, height and fruit length) may not have captured a true representation of site-level trait values. That is, using one value to represent a trait for a species at each of the sites at which it occurs does not take into account intraspecific variation in the trait. In fact, intraspecific trait variation has

previously been shown to drive plant community assembly changes along environmental gradients (e.g. Hulshof *et al.* 2013; Carlucci *et al.* 2015). However, I initially decided to use the approach of collating literature data, as it is a common approach adopted in comparative studies on a wide range of topics given its advantage of low cost and it is less time consuming compared to collecting fresh data from the field. The disadvantage of this approach, however, is that obtaining a single trait value for each species irrespective of site, does not capture the true variation in trait values that may be influenced by environmental attributes at each site. Furthermore, there is often no way of knowing that trait values obtained from the literature or from online databases actually encompass the geographical locations that are the focus of a given study. As a consequence, using a single trait value for each species across all sites does not permit the analysis of trait variation within species occurring at each site along the DGW gradient. Therefore, it is important to acknowledge that the overall outcome of this methodological issue is that the inability of this study to detect significant variation in plant traits in relation to DGW may be an artefact of not using truly representative values of traits for each species at each site.

Nevertheless, it is also important to provide an ecological explanation for the lack of relationships between the plant traits and DGW. This explanation may apply to the continuous traits, despite the lack of information about their variation at the intraspecific level. More importantly, however, this explanation is more appropriate for the categorical traits (growth form, longevity, dispersal mode and leaf shape) as these are much less likely to vary intraspecifically across the gradient. I suggest that the similarity in traits across the gradient is because plant species of all growth forms, longevities, dispersal modes and leaf shapes have evolved to fill all available niche positions along the gradient. Niche differentiation (Ackerly *et al.* 2000) in other plant

traits would then play a role in permitting the many plant species in these woodland assemblages to coexist.

In the next chapter, I overcome the limitations of the methodological problem of using trait data mined from published sources. I perform extensive field based measurements of three strategic plant functional traits, within the leaf-height-seed (LHS) plant ecological strategy scheme of Westoby (1998). I investigate interspecific variation in leaf mass per area, plant height and seed mass in relation to DGW in the Kangaloon study region.

Chapter 4: Variation in functional traits among perennial woody plant species along a depth-to-groundwater gradient in *Eucalyptus* woodlands

4.1 Introduction

A key goal of functional and trait-based ecology is identifying how plant traits vary among species along environmental gradients (Tilman 1988; Grime *et al.* 1997; Reich *et al.* 2003; Bernhardt-Römermann *et al.* 2008; Lacourse 2009; Messier *et al.* 2010). Such trait variation describes successful plant strategies for tolerating shifting environmental conditions (Díaz *et al.* 1998; Fonseca *et al.* 2000; Thuiller *et al.* 2004; Pekin *et al.* 2011). Plant functional traits are the properties of plants that have an impact on plant growth, fitness, function and performance (Violle *et al.* 2007), all of which ultimately influence ecosystem functioning (Lavorel and Garnier 2002). Thus, quantifying interspecific variation in plant functional traits along spatial gradients in depth-to-groundwater (DGW) has the potential to offer crucial insight into the functioning and dynamics of plant assemblages in groundwater-dependent ecosystems (GDEs), while also identifying the mechanisms that help shape plant species distribution patterns (Dudley 1996; Violle *et al.* 2011; Fraaije *et al.* 2015; Lawson *et al.* 2015).

In Chapter 3 of this thesis, I examined variation in woodland plant species' traits in a mesic GDE and explored relationships between plant traits and DGW. Surprisingly, I found that none of the seven plant traits were influential in explaining the significant shifts in plant species composition along the DGW in the study system outlined in Chapter 2. This was the case when considering variation in plant traits across the whole gradient as well as when comparing traits between groups of species that only occurred in the shallow, intermediate and deep sections of the gradient. One possible explanation

for the lack of significant relationships between DGW and the plant traits is that the manner of data collection biased the analyses. That is, all trait data used in Chapter 3 were collected from literature sources. It could be argued that data for continuous traits such as leaf mass per area (LMA), plant height and seed mass are better collected within the relevant study region. This is because aggregating data from across a very broad region like the Australian continent to give one value of a trait for a species has the potential to ignore important regional and local trait variation.

Here, I seek to identify patterns in plant functional traits along a natural DGW gradient in a mesic woodland GDE using fresh, field-collected data. My approach in this chapter is to overcome limitations of the methodological problem of using trait data obtained from published biological sources by performing extensive field-based measurements of three strategic plant functional traits, within the leaf-height-seed (LHS) plant ecological strategy scheme devised by Westoby (1998). This is a theoretically and empirically well-established strategy scheme that with only three plant traits, captures a considerable magnitude of variation in plant life history and ecology (e.g. Lavergne *et al.* 2003; Laughlin *et al.* 2010; De Frenne *et al.* 2011). Whilst there have been few studies that have examined LHS relationships along latitudinal and successional gradients (e.g. Garnier *et al.* 2004; De Frenne *et al.* 2011), I examine variation in leaf mass per area (LMA), plant height and seed mass across sites and explore interspecific relationships in these three strategic plant functional traits in relation to DGW. Specifically, I ask the question, do LMA, plant height and seed mass vary in a systematic way in relation to DGW?

4.2 Methods

4.2.1 Study sites

This study was performed at the 16 field sites described in detail in Chapter 2 (section 2.2.1).

4.2.2 Study species and their functional traits

A total of 26 perennial plant species that occurred at multiple sites along the DGW gradient (Chapter 2) were the focus of this study (Table 4.1). I collected trait data for each of the 26 species at all sites along the DGW gradient at which they were recorded (Tables 4.1, 4.2). Where there were insufficient individuals at a site to meet the minimum sampling requirements for a species (described below), I sampled individuals of the species that occurred outside, but not more than 10 m away from, each site.

Data for the three functional traits were collected in the field using standardised protocols (e.g. Cornelissen *et al.* 2003) between January 2013 and December 2013. I used not only well-developed approaches for trait measurements, but widely-used approaches so that the data and findings in this study would be readily comparable with studies from across the world (Díaz *et al.* 1998; Vaughton and Ramsey 1998; Lamont *et al.* 2002; Meir *et al.* 2002; Poorter *et al.* 2008; Qi *et al.* 2015). For each of the 26 species, plant heights (m) were measured *in situ* from ten mature individuals at each site. Plant height was measured as the distance between the upper boundary of the foliage (i.e. excluding inflorescences) and ground level. I used a standard tape measure to measure the height of species < 1.5 m tall, and a digital hand held clinometer (Haglöf Electric Clinometer, HEC; Sweden) for species taller than 1.5 m. When using the digital hand held clinometer, a sufficient distance from each individual plant was adopted where the height of the upper most part of the canopy could be observed and thus easily

measured. Plant heights were taken from 10 replicate measurements in the field. For the measurement of LMA, five fully-expanded, hardened, healthy, outer canopy leaves were collected from five individuals of each species at each site (Cornelissen *et al.* 2003). Harvested samples (twigs with leaves attached) were immediately wrapped in wet paper towel, sealed in plastic bags, transported from the field in a cool box and stored under refrigeration. The fresh area (cm^2) of each leaf was measured within 36 h of harvest using a Canon CanoScan CS5600F Scanner (Canon Australia Pty Ltd) and leaf area was calculated using Image J imaging software (Abràmoff *et al.* 2004). Here I refer to 'leaf' as any green structure that carries out the majority of the plant photosynthesis. For needle-like leaves (e.g. as found in *Allocasuarina* species), leaves were assumed to have a circular cross section and leaf area was adjusted by $\pi/2$ (Wright *et al.* 2002; Wright and Westoby 2002; 2003). All leaves were then placed in a drying oven at 60 °C for a minimum of 72 h until a constant mass was reached and the final dry mass recorded (Cornelissen *et al.* 2003). LMA was then calculated as the ratio of leaf dry mass to leaf area ($g\ m^{-2}$).

For seed mass measurements, seeds were harvested from three individuals of each species at each site (Cornelissen *et al.* 2003). The mass of mature seeds was measured as seed coat plus embryo and endosperm (Cornelissen *et al.* 2003) after the removal of all dispersal structures (wings, comas, pappus, elaiosomes and fruit flesh). Seeds were assessed as alive and viable by visual inspection and a squeeze test (Sawma and Mohler 2002). Forceps were lightly pressed on either side of the seed and light pressure was applied to the seed. Seeds were considered alive and viable if the seed did not crack or deform under light pressure. Visual inspection identified seeds that were not appropriate for mass measurements due to clear evidence of pre-dispersal seed predation (holes in the seeds), disease (the presence of fungal growth or seed

degeneration) or abortion (flattened, empty seeds). Seeds were then oven dried at 80 °C for a minimum of 48 h until constant mass was reached (Cornelissen *et al.* 2003). Individual mean seed mass was calculated from three replicate samples, measured as the total oven dry mass (in mg) of between five and 500 individual seeds divided by the number of seeds in the sample. The number of seeds in a batch depended on the species being assessed, with fewer seeds for large-seeded species (e.g. 5 seeds per plant for *Hakea* species) and more seeds for small-seeded species (e.g. 500 seeds per plant for *Melaleuca* species).

Table 4.1. Mean trait data for the 26 perennial plant species that occurred at multiple sites along the DGW gradient. Sites describes the number and identity of the study sites at which each species occurred (see Chapter 2); LMA represents leaf mass per area, CV represents coefficient of variation.

Species	Family	Sites	Mean LMA ($g\ m^{-2}$)	Mean height (m)	Mean seed mass (mg)	CV LMA (%)	CV height (%)	CV seed mass (%)
Acacia longifolia	Fabaceae (Mimosoideae)	1, 2, 3, 6, 7, 10, 11, 15	186.35	3.42	20.57	28.96	25.68	25.58
Acacia terminalis	Fabaceae (Mimosoideae)	6, 8, 10, 11, 15	203.37	2.44	11.44	13.12	18.43	15.46
Banksia paludosa	Proteaceae	4, 5, 6, 9, 10, 11, 15	238.39	0.91	10.43	10.74	33.01	19.84
Banksia spinulosa	Proteaceae	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16	202.52	1.54	12.70	15.94	28.53	21.40
Bossiaea obcordata	Fabaceae (Faboideae)	6, 7, 8, 9, 11, 13, 15	82.58	1.08	3.47	11.65	30.26	17.02

Species	Family	Sites	Mean LMA ($g\ m^{-2}$)	Mean height (m)	Mean seed mass (mg)	CV LMA (%)	CV height (%)	CV seed mass (%)
Dampiera stricta	Goodeniaceae	2, 4, 5, 7, 8, 9, 10, 11, 15, 16	121.12	0.28	no data	26.24	24.57	no data
Eucalyptus globoidea	Myrtaceae	1, 2, 3, 6, 7, 11, 12, 13, 14, 15	218.65	23.07	0.54	13.39	22.64	23.02
Eucalyptus piperita	Myrtaceae	1, 2, 3, 6, 7, 8, 10, 11, 12, 13, 14, 15	190.21	24.38	0.74	12.15	20.68	28.66
Eucalyptus radiata	Myrtaceae	1, 2, 3, 5, 7, 8, 11, 12, 13, 14	140.86	19.03	0.51	18.62	18.99	35.49
Eucalyptus sclerophylla	Myrtaceae	2, 4, 5, 6, 8, 9, 11, 13, 14, 15, 16	246.06	23.78	0.63	18.13	9.23	133.36
Eucalyptus sieberi	Myrtaceae	2, 10, 11, 15	262.75	19.38	1.61	13.47	9.35	14.53

Species	Family	Sites	Mean LMA ($g\ m^{-2}$)	Mean height (m)	Mean seed mass (mg)	CV LMA (%)	CV height (%)	CV seed mass (%)
Hakea dactyloides	Proteaceae	1, 4, 5, 6, 8, 9, 10, 11, 13, 15, 16	220.31	2.44	15.26	23.11	48.40	22.54
Hibbertia sericea	Dilleniaceae	1, 2, 3, 6, 7, 8, 10, 11, 14, 15	108.57	0.27	no data	23.11	33.35	no data
Isopogon anemonifolius	Proteaceae	4, 5, 6, 9, 11, 15, 16	250.35	0.86	2.79	9.97	34.75	17.32
Leptospermum juniperinum	Myrtaceae	5, 7, 10, 11, 16	138.60	1.98	0.11	12.34	32.27	13.85
Leptospermum trinervium	Myrtaceae	2, 3, 4, 5, 6, 11, 16	135.03	2.36	0.13	12.21	34.95	15.13
Leucopogon lanceolatus	Myrtaceae	1, 2, 7, 8, 12, 13, 14	135.77	1.59	5.28	13.88	40.16	12.59
Lomatia silaifolia	Myrtaceae	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, 15, 16	170.22	0.32	no data	17.08	59.47	no data
Melaleuca linariifolia	Myrtaceae	2, 3, 10, 12	120.20	5.87	0.02	21.59	54.39	6.93

Species	Family	Sites	Mean LMA ($g\ m^{-2}$)	Mean height (m)	Mean seed mass (mg)	CV LMA (%)	CV height (%)	CV seed mass (%)
Persoonia laurina	Proteaceae	5, 9, 16	198.70	0.55	15.96	7.69	27.60	15.80
Persoonia levis	Proteaceae	6, 7, 8, 10, 11, 15	185.92	2.95	0.95	9.98	26.18	26.33
Persoonia linearis	Proteaceae	1, 7, 8, 14, 16	157.66	2.83	0.66	16.78	37.23	24.88
Petrophile sessilis	Proteaceae	6, 8, 11, 15	255.39	1.95	3.14	12.79	34.77	26.70
Pultenaea elliptica	Fabaceae (Faboideae)	4, 5, 8, 9, 15, 16	104.37	0.50	no data	16.67	38.13	no data
Telopea speciosissima	Proteaceae	1, 6, 7, 11, 15	162.71	2.28	47.18	12.13	27.25	15.49
Tetratheca thymifolia	Elaeocarpaceae	1, 6, 7, 11, 15	100.85	0.26	no data	21.21	38.08	no data

Table 4.2. Assemblage-level trait data for the 16 study sites across the DGW gradient. DGW = depth-to-groundwater, ALM = assemblage level mean, CWM = community weighted mean (see section 4.2.3).

Site	DGW (m)	ALM LMA ($g\ m^{-2}$)	ALM height (m)	ALM seed mass (mg)	CWM LMA ($g\ m^{-2}$)	CWM height (m)	CWM seed mass (mg)
1	2.35	159.80	9.48	11.83	151.75	9.79	5.28
2	4.24	173.69	10.00	3.09	165.71	7.39	5.82
3	4.60	118.92	11.45	7.09	113.40	8.29	9.97
4	6.78	205.95	2.98	5.91	217.98	0.98	11.41
5	8.00	189.79	3.59	5.39	164.51	0.84	10.18
6	9.89	205.57	5.70	6.04	192.77	3.80	4.88
7	12.36	156.69	7.52	6.87	113.00	2.59	5.83
8	16.29	154.34	4.88	3.72	124.93	1.66	4.54
9	16.77	181.71	2.60	9.42	158.18	2.31	12.19
10	17.76	172.03	4.95	8.43	163.35	3.92	13.09
11	21.96	189.44	6.70	8.28	134.98	1.87	5.28

Site	DGW (m)	ALM LMA ($g\ m^{-2}$)	ALM height (m)	ALM seed mass (mg)	CWM LMA ($g\ m^{-2}$)	CWM height (m)	CWM seed mass (mg)
12	27.43	144.48	13.13	2.96	167.72	14.73	1.58
13	29.12	148.58	10.18	3.62	155.63	10.58	2.80
14	32.12	171.78	8.65	3.30	141.35	0.80	4.19
15	37.54	194.99	5.58	9.69	186.39	3.20	9.63
16	43.65	187.21	2.77	6.90	182.80	2.41	10.78

4.2.3 Statistical analyses

First, I calculated the mean value of each trait for each individual species at each of the 16 sites (Table 4.1). I then used these species' means to calculate an assemblage level mean (ALM) for each trait at each site (Table 4.1). The ALM values were calculated as the mean across all species at a site for each trait (Table 4.2). I also calculated the coefficient of variation (CV) and the community weighted mean (CWM) values of each trait (Table 4.2). The use of CV values examined the idea that interspecific variation in traits may be greater at particular groundwater depths, although no *a priori* hypotheses were established as this was an exploratory exercise. The ALM and CWM trait values were also calculated separately for overstorey species (woody, perennial tree species with foliage height > 5m) and understorey species (woody, perennial shrub species with foliage height < 5m) across the 16 sites. Community weighted mean trait values were calculated as:

$$CWM = \sum_{i=1}^{n} p_{ik} \times trait_{ik}$$

Where CWM is community weighted mean trait value, $p_i$is the relative abundance of species i in site k and $trait_i$ is the mean trait value of species i in site k (Violle *et al.* 2007). The logic of using CWM values is that plant traits of the most abundant species in a community are assumed to reflect the prevailing environmental conditions. For example, a number of studies have shown that plant trait-abundance relationships are driven by prevailing environmental conditions, with species possessing optimal trait values occurring in higher abundances for a given location (e.g. Cornwell and Ackerly 2010; Yan *et al.* 2013). This suggests that the most abundant species in a community possess the ideal trait values best suited to local environmental conditions (Kumordzi *et*

al. 2014). I therefore used CWM in my analyses as these measurements weight trait values in terms of the abundances of species within an assemblage.

For each trait, the ALM and CWM values and the CV (three separate response variables) were related to DGW and the 14 environmental attributes measured for the sites in Chapter 2 using minimum adequate regression models with sites treated as replicates (Crawley 2007). The response variables were log transformed where necessary prior to analyses to meet parametric assumptions. Minimum adequate models produced a model for each response variable that contained only those explanatory variables able to explain significant and unique variation in the response variable over and above the effects of all other explanatory variables (Crawley, 2007). In building minimum adequate models, DGW and the environmental attributes were first included in a full model. The process of model simplification followed, by applying the 'drop1' function (library stats) to the full model, which dropped one explanatory variable in turn and each time applied an analysis of deviance test. I then omitted the most non-significant term from the next round of analysis and the process of model simplification continued (Crawley, 2007). To manage multi-collinearity among the environmental variables, six variables (OM, N, Clay, Mg, P and K) that were strongly correlated with each other and with soil calcium (Ca) (correlations between < -0.65 and > 0.65; Table 2.1) were excluded from the analysis with Ca retained (Quinn and Keough 2002) to represent the suite of excluded correlated variables in the analysis (as described in Chapter 2).

All statistical and graphical analyses were performed using R 3.1.3 (R Core Team 2015), with packages stats (R Core Team 2015) ggplot2 (Wickham 2009) and gridExtra (Auguie 2015).

4.3 Results

My results indicate substantial variation in LMA, height and seed mass among species across each of the 16 study sites (Fig. 4.1). In the following sections I describe trait variation for ALM, CWM and CV in relation to DGW and the 14 environmental attributes.

4.3.1 ALM values of traits in relation to DGW and the environmental attributes

High LMA was significantly and positively related to high sand content (Fig 4.2a) while increased height was significantly and positively related to high shade and high soil Ca (Fig. 4.2b, Fig. 4.2c). There were no significant relationships between seed mass and DGW or any of the other environmental attributes for all species (Table 4.3). Significant patterns were detected in LMA and height in the overstorey (Table 4.4). In the overstorey, high LMA was significantly and negatively related to low field capacity (FC) and significantly and positively related to high sand content (Fig. 4.3a, Fig. 4.3b), increased height was significantly and negatively related to low altitude and significantly and positively related to high Ca (Fig. 4.3c, Fig. 4.3d). There were no significant relationships between seed mass and DGW or any of the other environmental attributes in the overstorey (Table 4.4). In the understorey, high LMA was significantly and negatively related to low shade and significantly and positively related to high sand content (Fig. 4.4a, Fig. 4.4b). In the understorey, I also found that increased height was significantly and positively related to high shade (Fig. 4.4 c). There were no significant relationships between seed mass and DGW or any of the other environmental attributes in the understorey (Table 4.5).

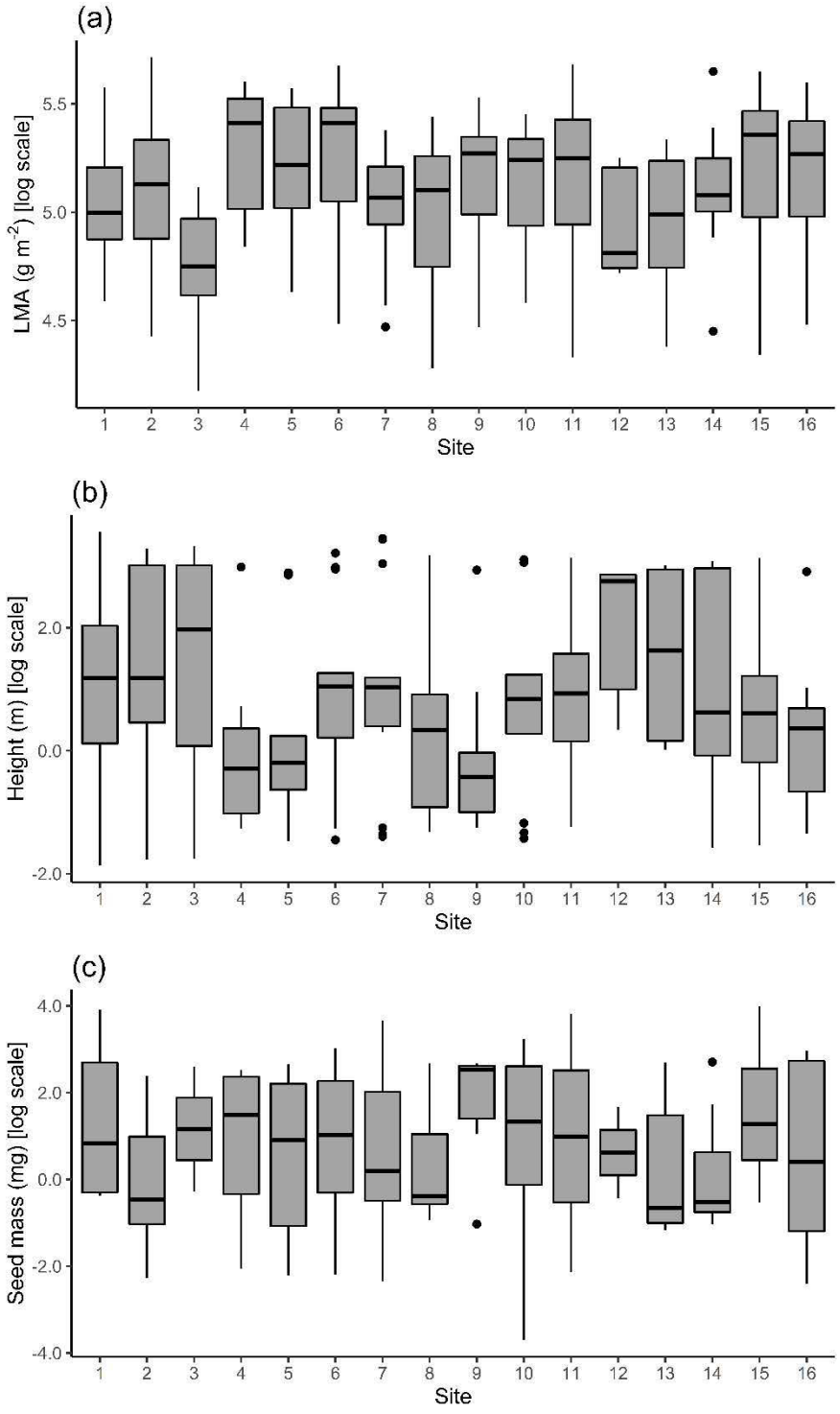


Figure 4.1. Boxplots describing variation in (a) leaf mass area (LMA), (b) height and (c) seed mass trait values among species at each of the 16 study sites. Thick horizontal line represents the median, upper and lower horizontal line represents upper and lower quartile respectively, the length of upper and lower vertical lines represents the maximum and minimum values, and points represent outliers.

Table 4.3. Minimum adequate models relating ALM values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for all species. Significant *P*-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	Sand	1	8574.30	**0.00008**
Traits not in MAM				
	Altitude	1	1768.20	0.49
	EC	1	1845.60	0.38
	Ca	1	2064.90	0.19
	Shade index	1	2286..50	0.20
	Slope	1	2426.90	0.33
	DGW	1	2637.10	0.25
	pH	1	2832.90	0.28
	FC	1	3222.40	0.15
(b) Height				
Traits in MAM				
	Shade index	1	73.88	**0.009**
	Ca	1	88.14	**0.002**
Traits not in MAM				
	DGW	1	30.29	0.96
	FC	1	30.46	0.77
	Sand	1	31.29	0.51
	EC	1	33.93	0.26
	Slope	1	39.75	0.11
	pH	1	42.90	0.27
	Altitude	1	48.46	0.16
(c) Seed mass				
Traits in MAM				
	Slope	1	106.71	0.06
Traits not in MAM				
	DGW	1	57.40	0.92
	FC	1	57.59	0.82
	Shade index	1	58.42	0.63
	Altitude	1	59.69	0.56
	pH	1	61.01	0.55
	EC	1	64.11	0.37
	Ca	1	78.97	0.07
	Sand	1	85.67	0.25

Table 4.4. Minimum adequate models relating ALM values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for overstorey species. Significant P-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	FC	1	8872.30	**0.02**
	Sand	1	16940.10	**0.00007**
Traits not in MAM				
	EC	1	3646.00	0.61
	Shade index	1	3794.20	0.42
	Altitude	1	3892.70	0.52
	Ca	1	4391.10	0.17
	pH	1	5115.20	0.12
	Slope	1	5789.10	0.16
	DGW	1	6343.00	0.23
(b) Height				
Traits in MAM				
	Altitude	1	0.28	**0.006**
	Ca	1	0.31	**0.002**
Traits not in MAM				
	Slope	1	0.09	0.96
	EC	1	0.09	0.67
	FC	1	0.10	0.41
	Sand	1	0.10	0.57
	pH	1	0.12	0.11
	Shade index	1	0.14	0.13
	DGW	1	0.17	0.05
(c) Seed mass				
Traits in MAM				
	FC	1	0.08	0.35
Traits not in MAM				
	Ca	1	0.51	0.59
	pH	1	0.53	0.40
	DGW	1	0.57	0.29
	Altitude	1	0.61	0.30
	Shade index	1	0.69	0.16
	Slope	1	0.73	0.33
	EC	1	0.80	0.21
	Sand	1	1.02	0.05

Table 4.5. Minimum adequate models relating ALM values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for understorey species. Significant *P*-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	Shade index	1	3913.00	**0.009**
	Sand	1	6350.1	**0.0001**
Traits not in MAM				
	Altitude	1	1200.00	0.61
	Slope	1	1215.50	0.65
	Ca	1	1270.40	0.40
	DGW	1	1406.10	0.20
	FC	1	1639.30	0.12
	EC	1	2138.60	0.05
	pH	1	2544.60	0.10
(b) Height				
Traits in MAM				
	Shade index	1	7.66	**0.03**
Traits not in MAM				
	Altitude	1	3.05	0.94
	FC	1	3.11	0.57
	EC	1	3.20	0.49
	Sand	1	3.46	0.27
	DGW	1	3.78	0.23
	Ca	1	4.39	0.12
	pH	1	5.05	0.14
	Slope	1	5.65	0.18
(c) Seed mass				
Traits in MAM				
	Ca	1	184.77	0.20
Traits not in MAM				
	FC	1	112.82	0.91
	DGW	1	113.05	0.86
	Shade index	1	114.08	0.70
	pH	1	116.86	0.53
	EC	1	119.89	0.52
	Altitude	1	123.23	0.51
	Sand	1	145.69	0.10
	Slope	1	166.55	0.14

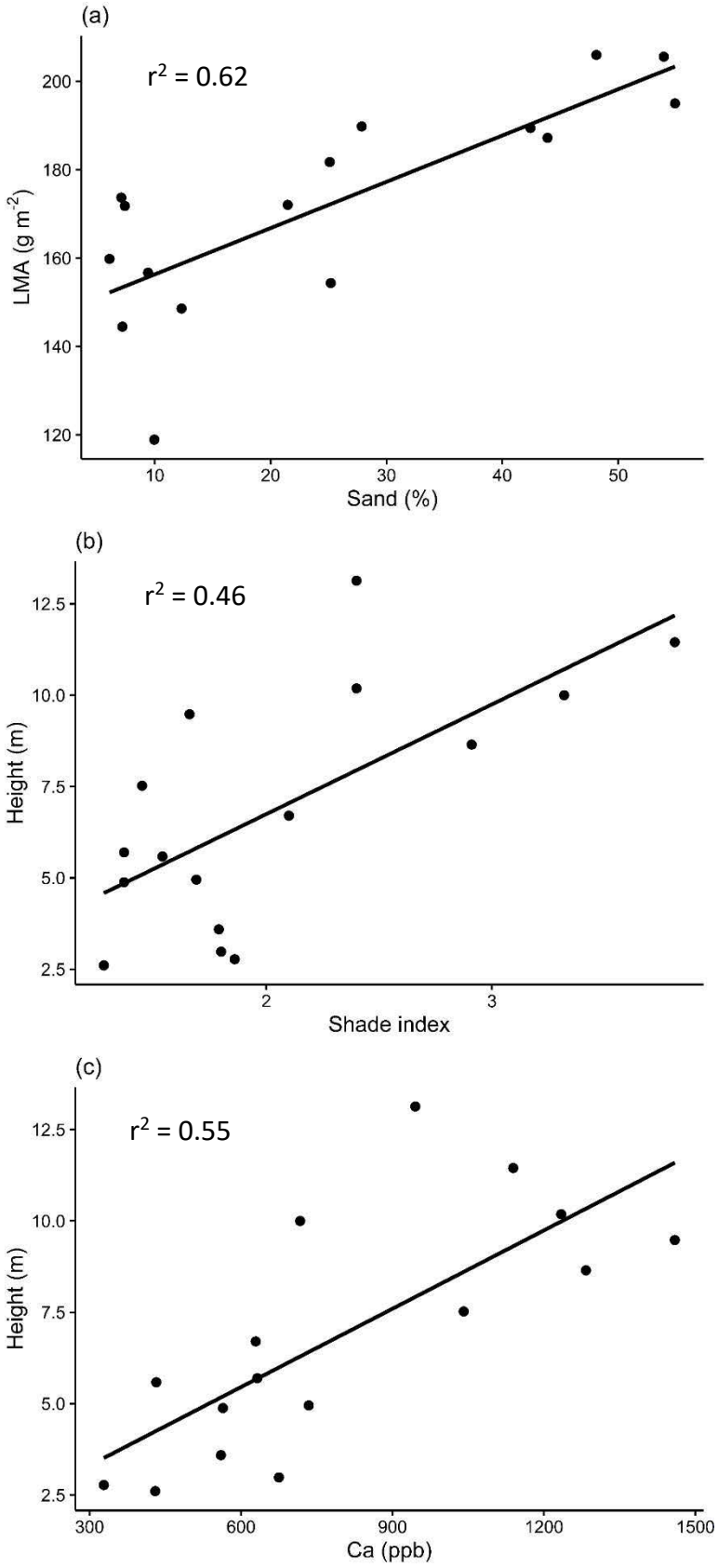


Figure 4.2. Significant relationships between ALM values of (a) LMA and sand, (b) height and shade index, and (c) height and Ca for whole plant assemblages.

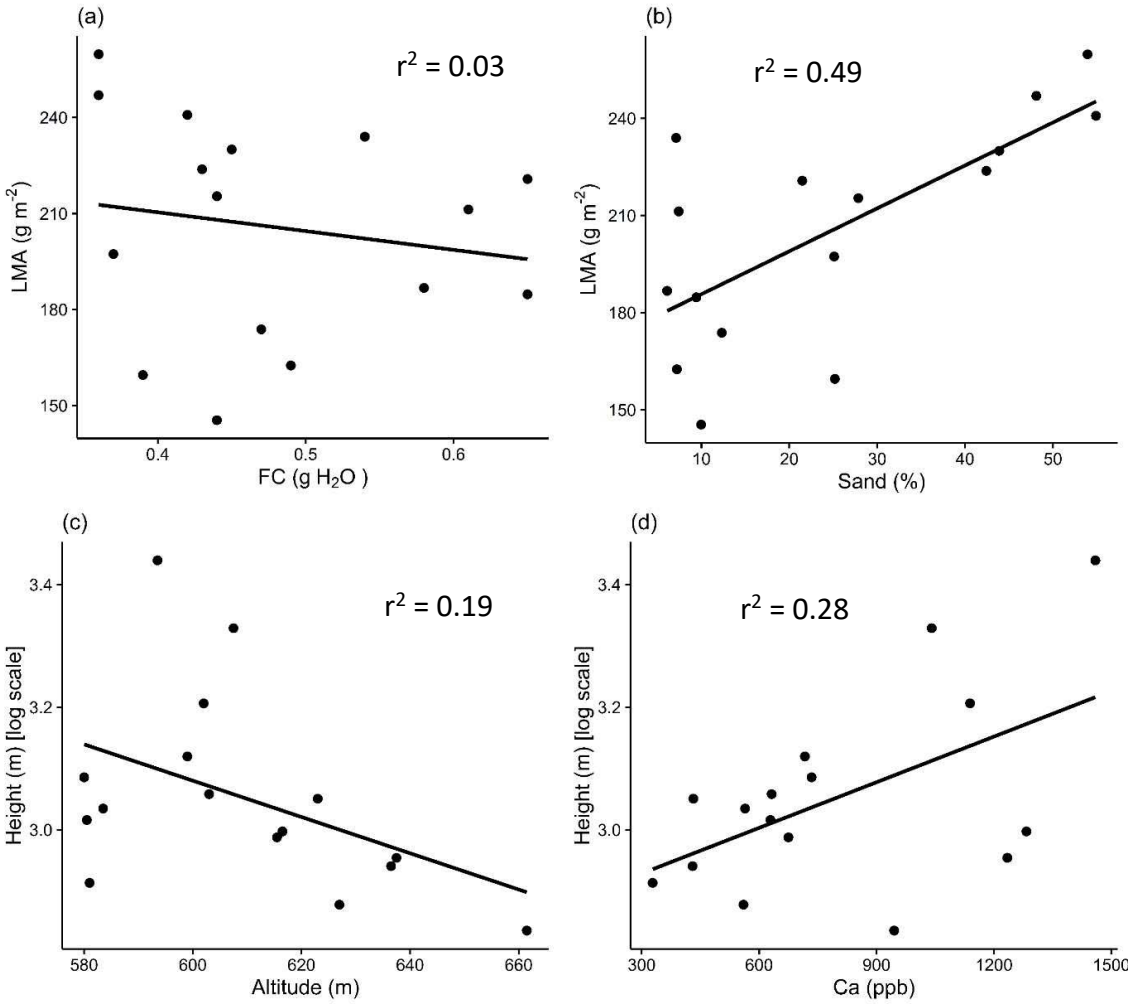


Figure 4.3. Significant relationships between ALM values of (a) LMA and FC, (b) LMA and shade index, (c) height values and altitude, and (d) height values and Ca for overstorey species. FC represents field capacity.

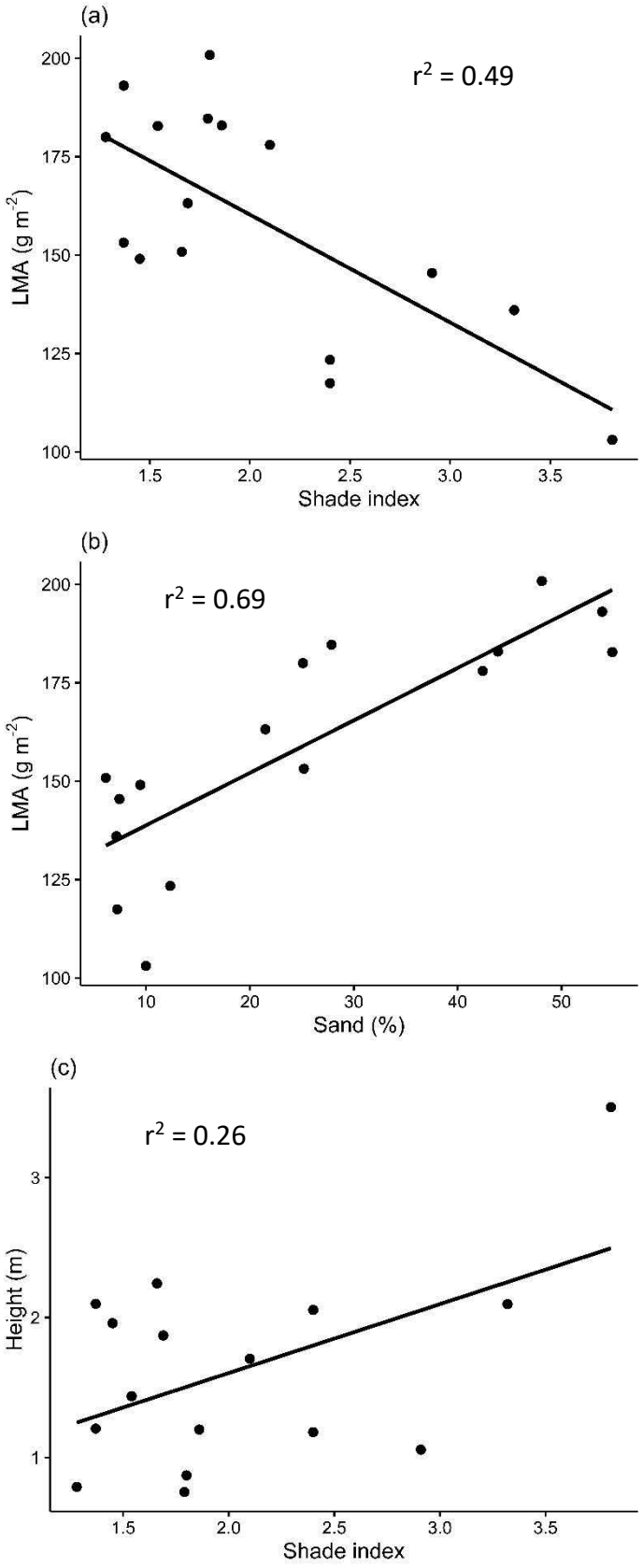


Figure 4.4. Significant relationships between mean (a) LMA and shade index, (b) LMA and sand, and (c) height and shade index for understorey species.

4.3.2 CWM trait values in relation to environmental attributes

I found a significant and positive relationship between high CWM LMA and high sand content (Table 4.6; Fig. 4.5a). There was a significant and negative relationship between increased CWM height and shallow DGW and a significant and positive relationship between high altitude, pH and EC values (Table 4.6; Fig. 4.5b, Fig. 4.5c, Fig. 4.5d, Fig. 4.5e). There was a significant and negative relationship between increased CWM seed mass and low Ca (Table 4.6; Fig. 4.5f). In the overstorey, there was a significant and positive relationship between high CWM LMA and high sand content (Fig. 4.6a), while there was a significant and negative relationship between increased CWM height and low altitude (Fig. 4.6b) and a significant and positive relationship between high Ca (Fig. 4.6c). There were no significant relationships between CWM seed mass and DGW or any of the other environmental attributes in the overstorey (Table 4.7). In the understorey, there was a significant and positive relationship between high CWM LMA and high sand content (Table 4.8; Fig. 4.7). There were no significant relationships between either CWM height or CWM seed mass for understorey species and DGW or any of the other environmental attributes (Table 4.8).

Table 4.6. Minimum adequate models relating CWM values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for all species. Significant *P*-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	Sand	1	12409.90	**0.006**
Traits not in MAM				
	pH	1	2877.00	0.52
	Shade index	1	2961.10	0.50
	Ca	1	3501.30	0.10
	FC	1	4076.50	0.12
	DGW	1	4994.30	0.07
	EC	1	5819.50	0.12
	Slope	1	6295.10	0.26
	Altitude	1	7681.50	0.07
(b) Height				
Traits in MAM				
	DGW	1	7.57	**0.02**
	Altitude	1	8.04	**0.01**
	pH	1	7.66	**0.02**
	EC	1	11.88	**0.0004**
Traits not in MAM				
	FC	1	4.17	0.82
	Sand	1	4.18	0.87
	Slope	1	4.27	0.55
	Ca	1	4.40	0.50
	Shade index	1	5.46	0.06
(c) Seed mass				
Traits in MAM				
	Ca	1	195.98	**0.03**
Traits not in MAM				
	Altitude	1	112.99	0.99
	Slope	1	112.99	0.98
	EC	1	113.15	0.88
	Shade index	1	116.33	0.51
	Sand	1	123.46	0.33
	FC	1	127.45	0.48
	pH	1	136.03	0.31
	DGW	1	144.87	0.32

Table 4.7. Minimum adequate models relating CWM values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for overstorey species. Significant *P*-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	Sand	1	19717.00	**0.001**
Traits not in MAM				
	pH	1	5070.40	0.62
	Slope	1	5152.50	0.61
	EC	1	5329.90	0.46
	Ca	1	6223.90	0.12
	Shade index	1	6956.50	0.18
	DGW	1	8271.30	0.10
	Altitude	1	9063.20	0.23
	FC	1	10194.1	0.17
(b) Height				
Traits in MAM				
	Altitude	1	0.270	**0.003**
	Ca	1	0.312	**0.001**
Traits not in MAM				
	Slope	1	0.101	0.91
	Shade index	1	0.101	0.76
	EC	1	0.103	0.63
	Sand	1	0.107	0.43
	FC	1	0.110	0.49
	pH	1	0.124	0.16
	DGW	1	0.158	0.05
(c) Seed mass				
Traits in MAM				
	FC	1	1.08	0.35
Traits not in MAM				
	Ca	1	0.51	0.59
	pH	1	0.53	0.40
	DGW	1	0.57	0.29
	Altitude	1	0.61	0.30
	Shade index	1	0.69	0.16
	Slope	1	0.73	0.33
	EC	1	0.80	0.21
	Sand	1	1.02	0.05

Table 4.8. Minimum adequate models relating CWM values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for understorey species. Significant *P*-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	Sand	1	15130.60	**0.0002**
Traits not in MAM				
	Altitude	1	2273.30	0.65
	EC	1	2430.20	0.30
	Ca	1	2709.30	0.19
	Shade index	1	3153.20	0.12
	FC	1	3735.20	0.10
	DGW	1	4392.70	0.11
	Slope	1	5354.40	0.08
	pH	1	6344.90	0.10
(b) Height				
Traits in MAM				
	Slope	1	6.22	0.13
Traits not in MAM				
	Altitude	1	3.21	0.98
	FC	1	3.27	0.60
	Sand	1	3.40	4.21
	Ca	1	3.51	0.48
	EC	1	3.77	0.28
	DGW	1	4.02	0.31
	pH	1	4.60	0.14
	Shade index	1	5.39	0.11
(c) Seed mass				
Traits in MAM				
	Ca	1	181.86	0.20
Traits not in MAM				
	Altitude	1	130.12	0.84
	EC	1	130.61	0.81
	Slope	1	130.93	0.84
	Sand	1	137.11	0.39
	DGW	1	141.76	0.47
	FC	1	145.21	0.54
	Shade index	1	154.00	0.33
	pH	1	164.14	0.31

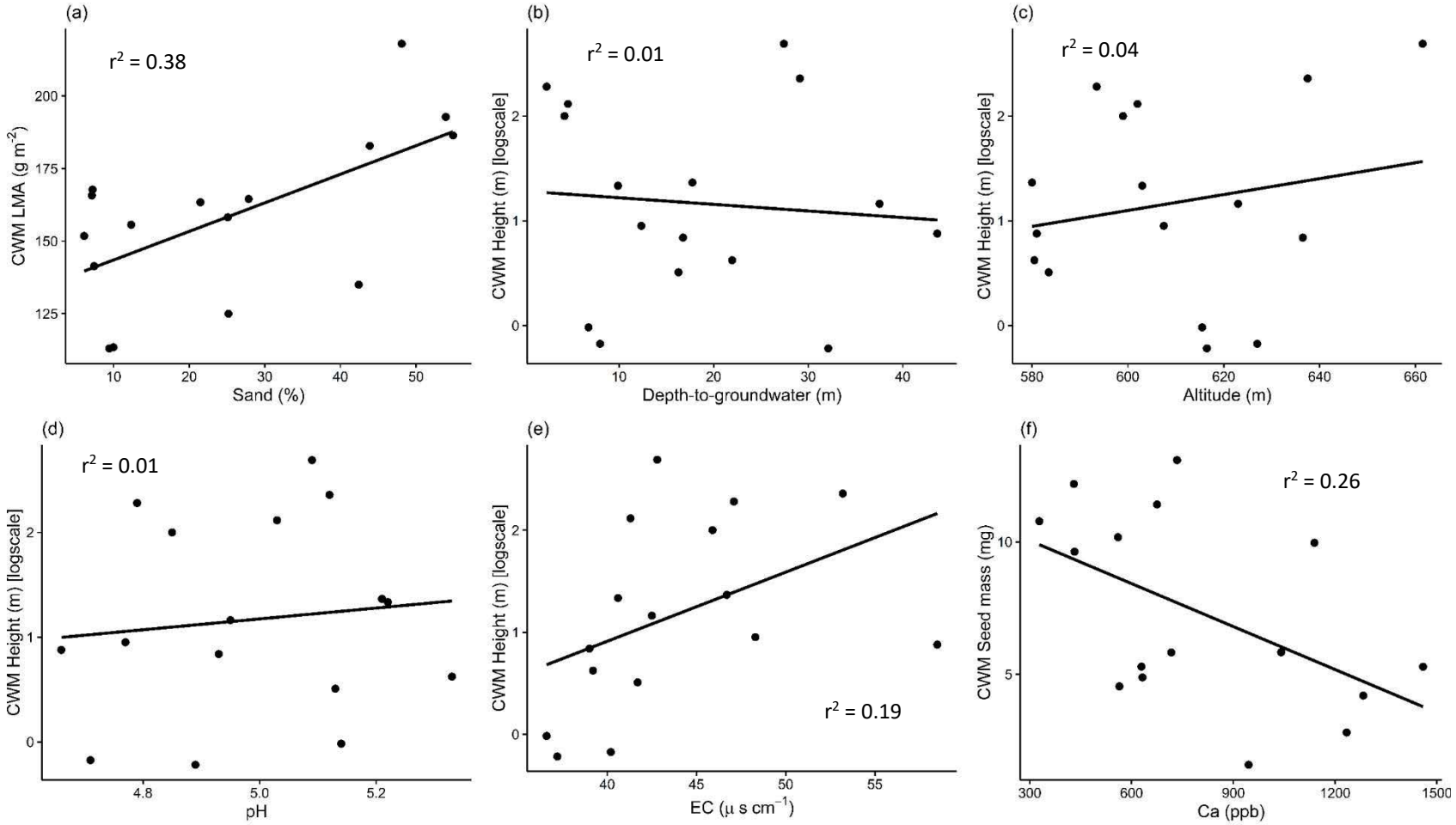


Figure 4.5. Significant relationships between CWM (a) LMA and sand, (b) height and DGW, (c) height and altitude, (d) height and pH, (e) height and EC, and (f) seed mass and Ca for whole assemblages.

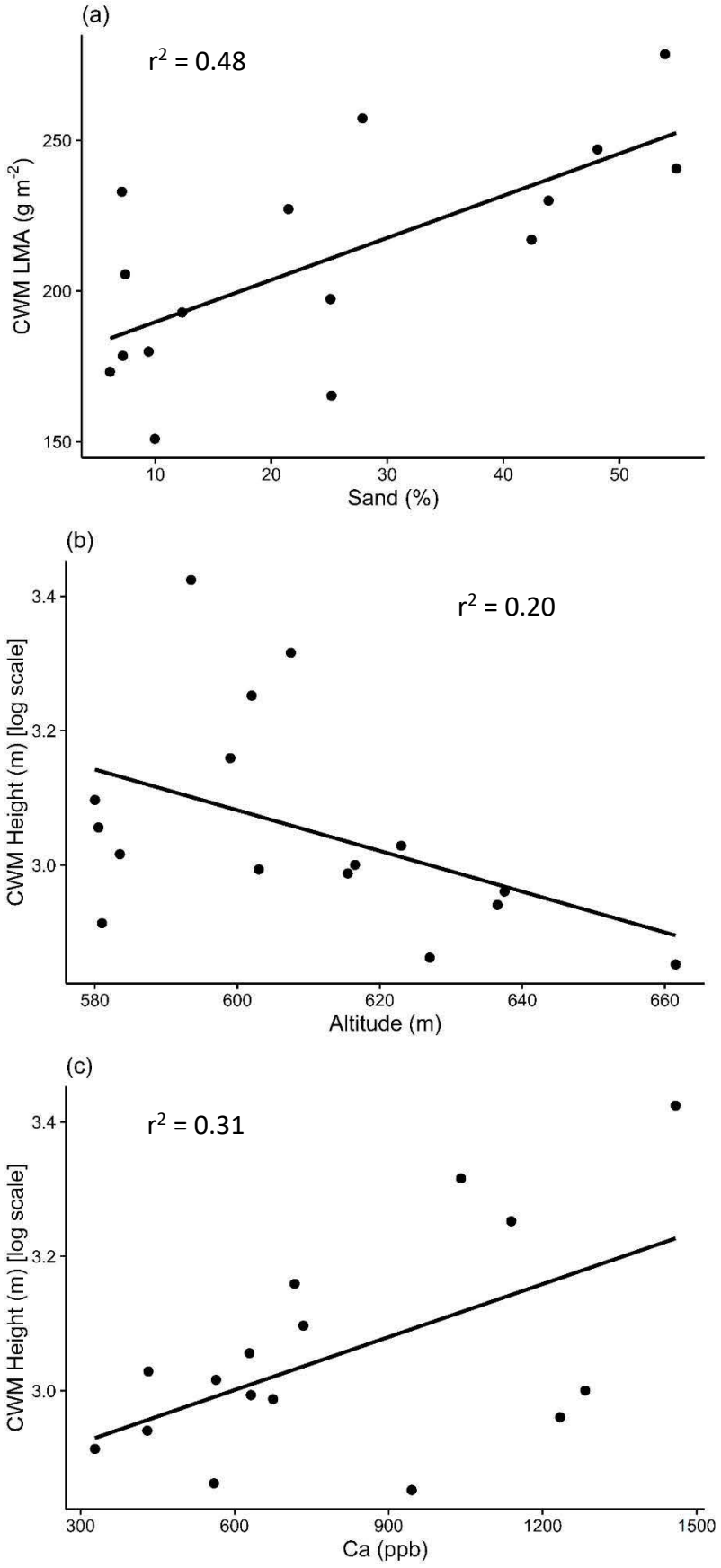


Figure 4.6. Significant relationships between CWM (a) LMA and sand, (b) height and altitude, and (c) height and Ca for overstorey species.

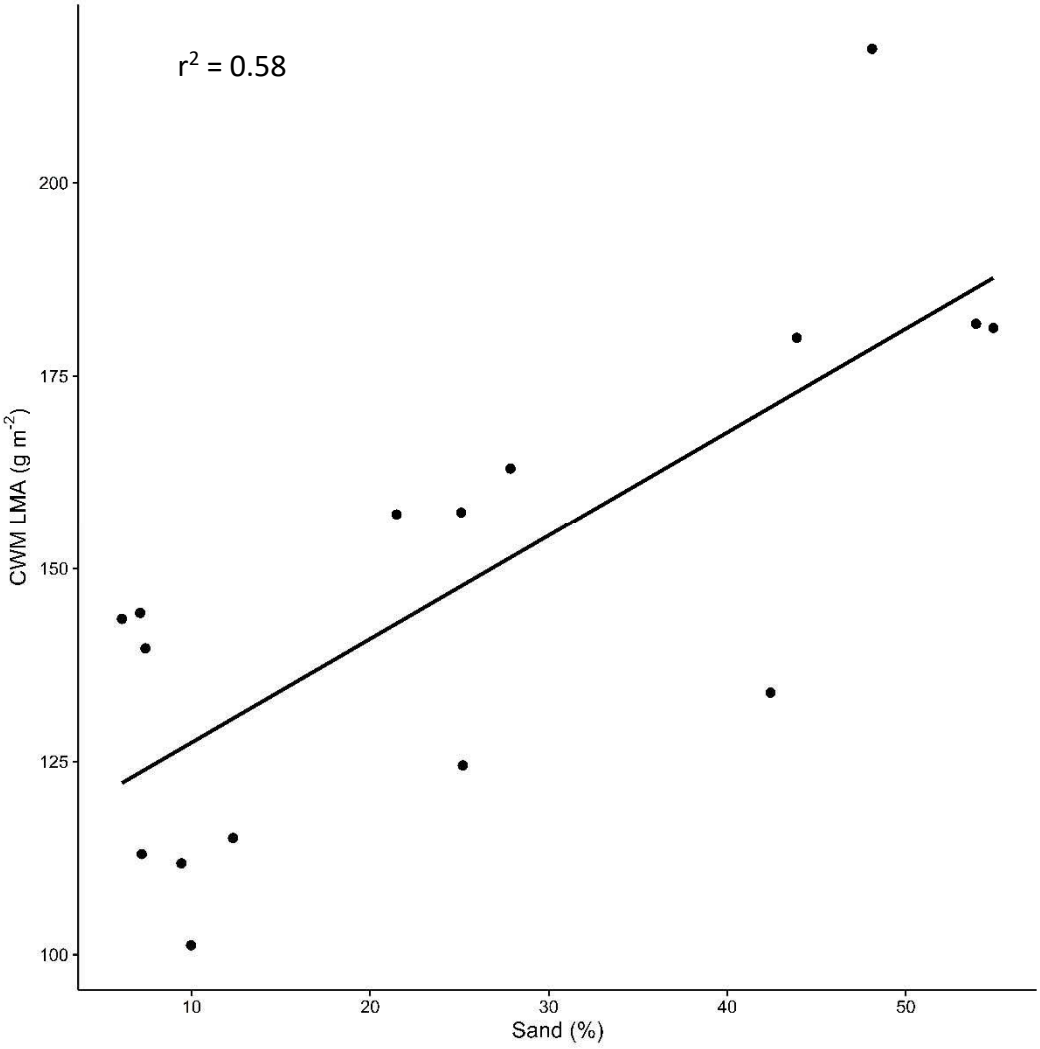


Figure 4.7. Significant bivariate relationship between CWM LMA and sand for understorey species.

4.3.3 Relating CV values to environmental attributes

There were no significant relationships between CV values for LMA and DGW or any of the environmental attributes (Table 4.9). However, I found that high CV values for height were significantly and negatively related to low altitude (Table 4.9; Fig. 4.8a) and that high CV values for seed mass were significantly and positively related to increased DGW (Table 4.9; Fig. 4.6b), significantly and negatively related to low altitude (Table 4.9; Fig. 4.6c) and significantly and positively related to high Ca (Table 4.9; Fig. 4.6d).

Table 4.9. Minimum adequate models relating CV values of (a) LMA, (b) height and (c) seed mass to DGW and environmental attributes for whole assemblages. Significant *P*-values shown in bold.

Trait		d.f.	Change in deviance	*P*
(a) LMA				
Traits in MAM				
	Slope	1	0.012	0.29
Traits not in MAM				
	DGW	1	0.006	0.74
	Shade index	1	0.006	0.72
	pH	1	0.007	0.53
	FC	1	0.007	0.34
	EC	1	0.007	0.27
	Altitude	1	0.009	0.09
	Sand	1	0.010	0.17
	Ca	1	0.011	0.26
(b) Height				
Traits in MAM				
	Altitude	1	3.22	**0.03**
Traits not in MAM				
	Ca	1	1.29	0.73
	Shade index	1	1.33	0.48
	EC	1	1.39	0.41
	Slope	1	1.50	0.27
	DGW	1	1.61	0.27
	FC	1	1.72	0.32
	pH	1	1.96	0.15
	Sand	1	2.43	0.06
(c) Seed mass				
Traits in MAM				
	DGW	1	0.69	**0.04**
	Altitude	1	0.76	**0.01**
	Ca	1	0.99	**0.002**
Traits not in MAM				
	Slope	1	0.50	0.82
	EC	1	0.50	0.70
	pH	1	0.51	0.65
	Sand	1	0.52	0.61
	Shade index	1	0.53	0.63
	FC	1	0.54	0.57

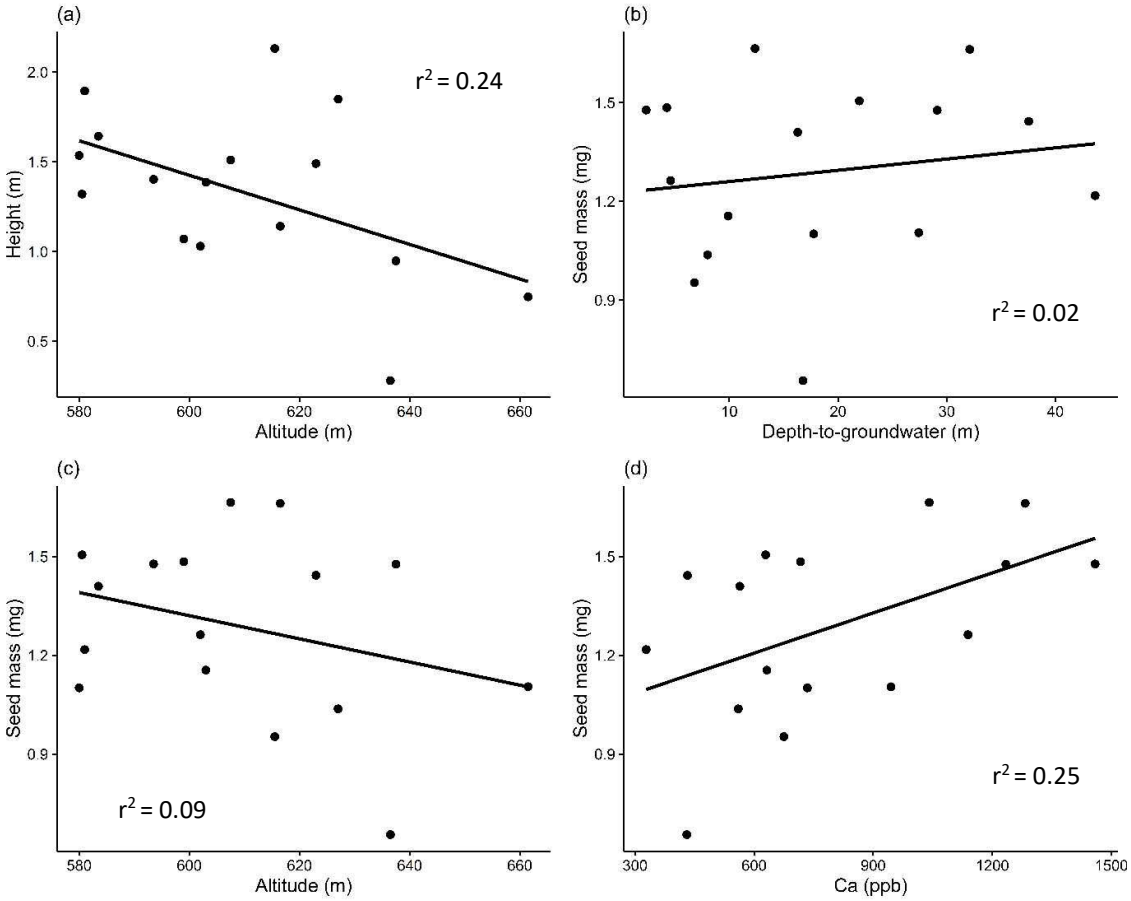


Figure 4.8. Significant relationships between CV values for (a) height and altitude, (b) seed mass and DGW, (c) seed mass and altitude, and (d) seed mass and Ca.

4.4 Discussion

In this study I set out to answer the question, do LMA, plant height and seed mass vary in a systematic way in relation to DGW when trait data are based on fresh field data from the study region? I found that when whole assemblages were considered, and after partialling out the influence of the environmental attributes by using minimum adequate models, DGW was only significantly related to CWM height and CV values of seed mass. Below I discuss relationships between DGW and plant height and seed mass and

also provide possible explanations for the observed significant relationships between environmental attributes and LMA, plant height and seed mass.

Measured plant trait values for the species considered in this study are generally in the mid to high range when compared to the global database of plant traits (Kattge *et al.* 2011). Plant trait values reported here represent only a small proportion of the range of trait values for the species found across mesic woodland study region. When the whole assemblage at each site was considered, I found that increased CWM height was significantly and negatively related to shallow DGW in conjunction high altitude, pH and EC values (Table 4.6; Fig. 4.5). My finding is consistent with previous studies which have found that plant height increases with increasing water availability (e.g. Cowling and Lamont 1985; Williams *et al.* 1996; Fonseca *et al.* 2000; Falster and Westoby 2003; Moles *et al.* 2009). Sites with higher water availability have lower hydraulic limitation (Zolfaghar *et al.* 2015a) and favour increased rates of photosynthesis, which allow for greater allocation of resources to productive leaf tissue, biomass allocation to aboveground tissues and higher growth rates, resulting in an increase in plant height (Binkley *et al.* 2010; Givnish *et al.* 2014).

When the overstorey at each site was considered, I found that neither ALM height nor CWM height were significantly related to DGW (Table 4.4, Table 4.7). This finding contradicts previous work in the Kangaloon region, where tree height was found to increase as DGW decreased (Zolfaghar *et al.* 2014). I suggest that the discrepancy between my findings and the results of the Zolfaghar *et al.* (2014) study, is that in my study overstorey species were considered to be plants > 5m which included larger shrubs, that may not be able to permanently access groundwater at the intermediate or deep sites, whereas the Zolfaghar *et al.* (2014) study considered upper canopy species only that are more easily able to access groundwater.

I found that high CV in seed mass was significantly and positively related to deep DGW, high Ca (an element which represented a several other soil attributes in the model) and low altitude (Table 4.9; Fig. 4.8b). This means that there is a greater interspecific variation in seed mass at the deeper end of the gradient. I suggest that this not only means that a broader range of seed-size strategies can be maintained at deep sites, but that larger values of seed mass tend to occur at the deeper end of the DGW gradient. Previous studies from Australia and other regions of the world, where seed mass has been found to increase across sites from high water availability to low water availability (e.g. Baker 1972; Schimpf 1977; Sorensen and Miles 1978; Stromberg and Patten 1990; Wright and Westoby 1999; Butler *et al.* 2007; Peco *et al.* 2009). Seeds with large mass have increased seed reserves and produce larger, faster growing seedlings that are capable of developing larger root systems (Jurado and Westoby 1992; Mack 1998; Milberg *et al.* 1998; Bond *et al.* 1999) that are capable of exploiting soil moisture at deeper soil depths. Seeds with larger mass also have a higher percentage germination (Cideciyan and Malloch 1982; Weis 1982), more rapid emergence rates from deeper depths within the soil (Wulff 1986; Weller 1989) and less stringent requirements for germination (Winn 1985), thereby increasing the chance for germination and establishment in low water availability sites. In contrast, seeds with low mass have decreased seed reserves, may germinate earlier (Black and Wilkinson 1963) but are capable of being dispersed further than seeds with large mass (Jackson 1981; Howe and Richter 1982), increasing the chance of reaching more favourable germination sites and decreasing competition for resources among parental plants and offspring.

I found that high ALM and CWM LMA were significantly and positively related to high sand content for the whole assemblage as well as for overstorey and understorey

species, demonstrating that sand content was the most influential environmental attribute responsible for driving variation in ALM and CWM LMA. Although this study did not specifically set out to test predictions about soil particle size relationships with LMA, it should be noted that soils with high sand content have lower water holding capacity than sites with a large clay content (Handreck and Black 2002; Dexter 2004), which signifies decreased water availability for plants. Leaves with high LMA are advantageous in environments with low water availability and previous studies have found that LMA increases across sites following a gradient from high water availability to low water availability (e.g. Mooney *et al.* 1978; Specht and Specht 1989; Schulze *et al.* 1998; Cunningham *et al.* 1999; Niinemets 2001; Swenson *et al.* 2012). Species growing in low water availability environments possess leaves with high LMA, which are thicker, denser and harder than leaves of species in high-rainfall sites (Poorter *et al.* 2009). This suite of traits that are correlated with high LMA contribute to reductions in leaf water loss (via transpiration) and increase the ability of leaves to withstand desiccation without wilting. These features also enable leaves to avoid tissue and airspace compression arising from leaf embolism which can impede photosynthesis or even lead to leaf death (Small 1973; Orians and Solbrig 1977; Grubb 1986; Turner 1994; Cunningham *et al.* 1999; Niinemets 2001).

I found significant positive linear relationships between ALM height and soil Ca content (an element which represented several other soil attributes in the model) for whole assemblages and overstorey species, indicating that soil Ca content, and thus several other soil attributes, was the most important environmental attribute influencing variation in height for all species and overstorey species. I also found that a significant positive relationship emerged between CWM height and altitude for whole assemblages and a significant negative relationship emerged between CWM height and altitude for

overstorey species, indicating that altitude was the most important environmental attribute influencing variation in CWM height for whole assemblages and overstorey species. Although this study did not specifically set out to test predictions about soil fertility or other environmental attribute relationships with plant height, it is worth noting that previous studies have also found that plant height varies in response to soil fertility and altitude (e.g. Fonseca *et al.* 2000; Moles *et al.* 2009; Pellissier *et al.* 2010). Given the established relationships between soil fertility, altitude and plant height, it is clearly important to account for their potential confounding influence on plant functional traits when examining the role of DGW.

The results of this study have shown that ALM values of LMA, plant height and seed mass did not vary in a systematic way in relation to DGW. I did, however, find that significant relationships emerged between DGW and both CWM height and CV seed mass for whole assemblages. However, I believe it important to note that both of these relationships with DGW were not highly significant (CWM height $P = 0.02$, CV seed mass $P = 0.04$), and if the large number of statistical analyses performed with this dataset are taken into account, the statistical significance of the findings means that they should be interpreted cautiously. In fact, I would suggest that the results of this study, when considered together with those of the previous chapter, demonstrate that interspecific variations in the functional traits examined in this thesis, when investigated at the whole assemblage level, are probably not largely driven by spatial changes DGW. Thus, these functional traits do not explain the observed variation in plant species composition along the DGW gradient (Chapter 2). Investigations into other ecological and ecophysiological factors must be sought, to explain the important compositional patterns presented in Chapter 2. Recent insights from the ecological literature, have outlined the importance of using within species trait variation for unravelling species

diversity and compositional patterns along water availability gradients (Engelbrecht *et al.* 2007; Jung *et al.* 2010; Violle *et al.* 2012; Shipley *et al.* 2012). Thus, in the next chapter, I begin this search by examining within-species strategies in seedling traits as a function of the DGW gradient.

Chapter 5: Patterns of local adaptation and phenotypic plasticity in seedling traits of *Hakea dactyloides* along a depth-to-groundwater gradient

5.1 Introduction

Widely-distributed plant species experience a broad range of conditions along environmental gradients (Gimeno *et al.* 2009; Ramírez-Valiente *et al.* 2010). Some widespread species demonstrate phenotypic plasticity in their traits in response to such gradients (Valladares *et al.* 2007; Nicotra *et al.* 2010). Phenotypic plasticity is the ability to modify phenotypic expressions of genotypes in response to the environment, so that plants are better able to meet local conditions (Bradshaw 1965; Schlichting 1986). For example, seedlings of some species growing in dry soil conditions are able to improve water exploitation in the deeper soil layers by producing longer and deeper root systems, compared with seedlings of the same species growing in high soil moisture conditions where shorter and more branched root systems are produced to increase water uptake in the upper soil layers (Guevara *et al.* 2010; Ramírez-Valiente *et al.* 2010). Alternatively, generations of natural selection operating on sessile plants, which typically have spatially limited genetic dispersal through seeds and pollen, can lead to adaptive genetic differentiation in traits, resulting in local adaptation in plant populations (Sork *et al.* 1993; Galloway and Fenster 2000; Kawecki and Ebert 2004; Savolainen *et al.* 2007). For instance, along a combined altitudinal and soil moisture gradient, seedlings replanted in their own 'home' habitats produce more seeds and have higher survival rates compared to seedlings of the same species transplanted in 'away' habitats (Joshi *et al.* 2001; Giménez-Benavides *et al.* 2007).

Reciprocal transplant experiments are commonly employed to compare plant performance between home and away habitats to detect local adaptation (e.g. Joshi *et al.* 2001; Macel *et al.* 2007). Local adaptation is observed when plants perform better under environmental conditions at home sites compared to their performance when transplanted to away sites, where environmental conditions can be different in various ways (e.g. Kindell *et al.* 1996; Joshi *et al.* 2001; Giménez-Benavides *et al.* 2007; Leimu and Fischer 2008). In these transplant experiments, the effects of multiple abiotic and biotic factors on transplanted plants are often hard to disentangle, making it difficult to unequivocally detect the effects of specific environmental factors posited to provide selection pressures on plant performance (Macel *et al.* 2007). One way to overcome this problem is to use a common garden approach (Kawecki and Ebert 2004), where a specific environmental factor can be manipulated in the glasshouse (or laboratory) while controlling for other environmental factors (Kawecki and Ebert 2004; Ramírez-Valiente *et al.* 2010). Local adaptation in common garden experiments is detected when plants perform better when grown under glasshouse conditions where the specific environmental factor matches the home habitat, compared with contrasting conditions for that factor found at away habitats. Identifying local adaptation using such techniques is not only important for understanding spatial dynamics in plant assemblages along environmental gradients (Galloway and Fenster 2000; Doebeli and Dieckmann 2003; Urbieta *et al.* 2008), it can also guide the effective conservation and management of species (e.g. Hällfors *et al.* 2016).

In mesic landscapes such as the Kangaloon study region of this thesis, pronounced spatial variation in DGW not only provides a gradient of access to groundwater, which has been shown to influence species composition (see thesis Chapter 2), it also provides a spatial gradient of water infiltration rates into the soil

(Sophocleous 2002). Water infiltration is defined as the downward entry of water that penetrates the soil surface; the velocity at which water passes through soil is termed water infiltration rate (Mao *et al.* 2016). Water infiltration rate is an indicator of a soil's ability to allow water movement into and through the soil profile where it is temporarily stored, making it available for root uptake (Doran and Jones 1996). Soils with high water infiltration rates have lower water-holding capacity, leading to reduced water availability for plants, in contrast to soils with low water infiltration rates which have high water-holding capacity and increased water availability for plants (Franzluebbers 2002; Handreck and Black 2002).

To confirm the link between DGW and water infiltration rate for the Kangaloon study region, I performed a pilot study (Fig. 5.1) that showed that where groundwater is close to the ground surface (i.e. shallow sites), water infiltration rates were significantly slower than where groundwater is further from the ground surface (i.e. deep sites). Thus, under the same broad rainfall conditions across the Kangaloon study region, plants growing at deep groundwater sites with faster rates of water infiltration through the soil are exposed to selection pressures linked to combatting conditions of comparatively lower water availability. Effectively, reduced water availability at the deep sites is a critical limiting resource for seedling establishment (Williams and Hobbs 1989), which I predict will influence seedling morphological and physiological traits. In particular, I predict that seedlings of species with populations at both shallow and deep groundwater sites will show population differentiation as a function of variation in water infiltration rates. Seedlings at deep sites (i.e. high infiltration rates) are predicted to possess leaves with higher leaf mass per area (LMA) than seedlings at shallow sites, with higher LMA enabling leaves to have higher water use efficiency (WUE) (Wright and Westoby 2002; McDonald *et al.* 2003; Donovan *et al.* 2007; Poorter *et al.* 2009).

Water use efficiency is the ratio of net photosynthetic capacity to stomatal conductance (Field *et al.* 1983; Pérez-Harguindeguy *et al.* 2013). Seedlings at deep sites are also predicted to possess longer root systems, larger root biomass and larger root:shoot ratios than seedlings at shallow sites. The allocation of increased resources to belowground traits provides an advantage in exploiting moisture at greater depths in the soil profile (Lloret *et al.* 1999; Schenk and Jackson 2002) where soil moisture is more reliable and less likely to be affected by evapotranspiration (Wetzel and Chang 1987; Lloret *et al.* 1999).

I examined seedling traits of *Hakea dactyloides*, a species that I observed growing across the DGW gradient from shallow to deep sites at Kangaloon. I used a glasshouse manipulation experiment to compare seedling traits between plants sourced from shallow and deep sites. Specifically, seeds were collected from shallow and deep populations of *H. dactyloides*, germinated and subsequently grown under controlled conditions to examine the effects of contrasting water infiltration rates on seedling traits. I manipulated water infiltration rates so that they were either high to represent low water availability, thus matching conditions at deep sites, or low to represent high water availability, matching conditions at shallow sites. Plants derived from both shallow and deep sites were exposed to both soil water conditions in the glasshouse. I measured a total of 14 seedling traits to examine plant responses to the manipulated differences in water availability. Specifically, I compared seedling growth traits and ecophysiological traits between deep and shallow populations and between conditions of high vs low water availability to determine if local adaptation differentiates seedling growth patterns of *H. dactyloides* between populations from the shallow and deep ends of the DGW gradient.

Seedling performance is a critical component of seedling establishment and can be influential in the maintenance of plant populations (Castro *et al.* 2004; Forbis and Doak 2004; Benard and Toft 2007). Therefore, detecting local adaptation in traits associated with seedling performance in the populations of a plant species may provide information on the processes that effect the spatial distributions of a plant species (Galloway and Fenster 2000; Doebeli and Dieckmann 2003) in landscapes that occur along a depth-to-groundwater gradient.

5.2 Study predictions

In my experimental system, *H. dactyloides* seedlings may vary their WUE, leaf mass per area (LMA) and belowground biomass in response to treatments that differ in water availability. If seedlings from the shallow and the deep ends of the gradient respond plastically to low vs high differences in water availability, then I predict that seedlings from both populations will possess high WUE, high LMA and increased belowground biomass under dry (deep) conditions and low WUE, low LMA and reduced belowground biomass under wet (shallow) conditions (Fig. 5.2a). However, one or both of the populations may demonstrate local adaptation in WUE, LMA and belowground biomass. If the deep populations are locally adapted to dry soils at the deep end of the gradient (Fig. 5.2b), then their seedlings will have high WUE, high LMA and increased belowground biomass when grown under fast infiltration rates (i.e. home conditions) and also maintain high WUE, high LMA and increased belowground biomass when grown under slow infiltration rates that represent wet soils at the shallow end of the gradient (i.e. away conditions). If shallow populations are locally adapted to wet soils at the shallow end of the gradient (Fig. 5.2c), then their seedlings will have low WUE, low LMA and decreased belowground biomass when grown under slow infiltration rates

(i.e. home conditions) and also maintain low WUE, low LMA and decreased belowground biomass when grown under fast infiltration rates that represent dry soils at the deep end of the gradient (i.e. away conditions). I measured stomatal conductance and rate of photosynthesis to calculate WUE. I also measured transpiration to explore how this physiological trait varied in relation to WUE. To explore how other leaf traits varied in relation to any changes in LMA I measured leaf length, leaf width and leaf number, whilst I measured root length to ascertain whether this trait varied in relation to any changes in belowground biomass.

With respect to aboveground growth, *H. dactyloides* seedlings may vary their aboveground biomass in response to treatments that differ in water availability. If seedlings from the shallow and the deep ends of the gradient respond plastically to low vs high differences in water availability, then seedlings from both populations will demonstrate a decrease in aboveground biomass under dry (deep) conditions and an increase in aboveground biomass under wet (shallow) conditions (Fig. 5.2d). However, one or both of the populations may demonstrate local adaptation in seedling biomass. If deep populations are locally adapted to dry soils at the deep end of the gradient (Fig. 5.2e), then their seedlings will show a decrease in aboveground biomass when grown under fast infiltration rates (i.e. home conditions) and also demonstrate a decrease in aboveground biomass when grown under slow infiltration rates that represent wet soils at the shallow end of the gradient (i.e. away conditions). If shallow populations are locally adapted to wet soils at the shallow end of the gradient (Fig. 5.2f), then their seedlings will show an increase in aboveground biomass when grown under slow infiltration rates (i.e. home conditions) and also maintain an increase in aboveground biomass when grown under fast infiltration rates that represent dry soils at the deep end of the gradient (i.e. away conditions). I also measured leaf biomass, final height and

root:shoot ratio to explore how other aboveground growth traits varied in relation to any changes in aboveground biomass.

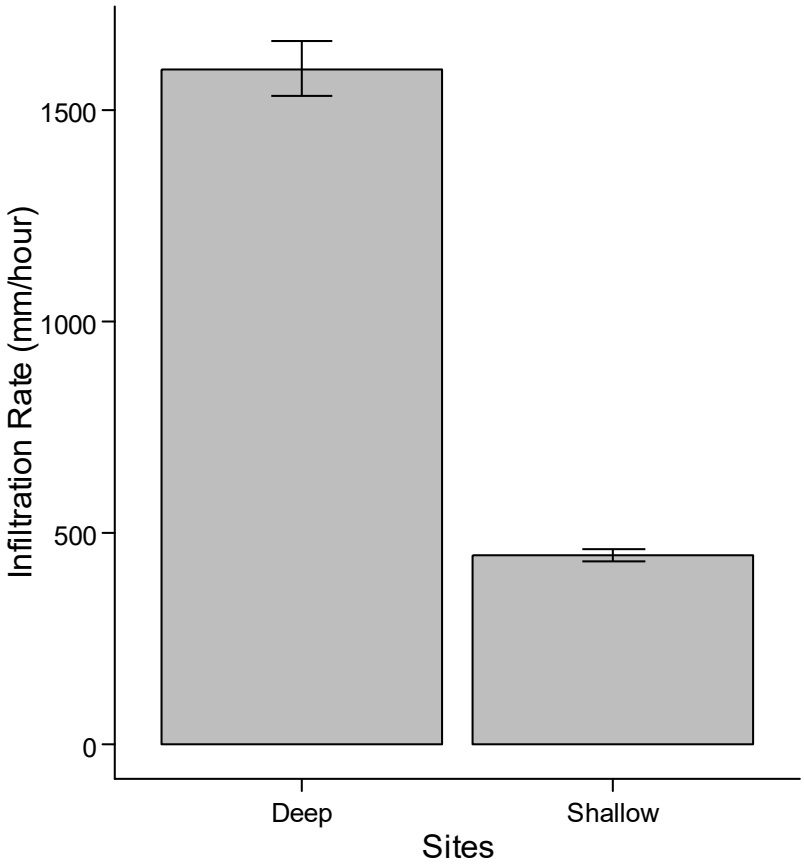


Figure 5.1. Mean (± SE) soil infiltration rates for deep (water table 43.65 m below the ground surface) and shallow (water table 2.32 m below the ground surface) sites measured using an infiltrometer (Handreck and Black 2002) and four replicate measurements placed randomly at each site. The deep and shallow sites differed significantly in their water infiltration rates (ANOVA, $F_{1,6} = 292.5$, $P < 0.001$), with 3.5x faster infiltration rates at the deep (1597.5 ± 65.4 mm/hour) compared with shallow sites (445.6 ± 16.3 mm/hour).

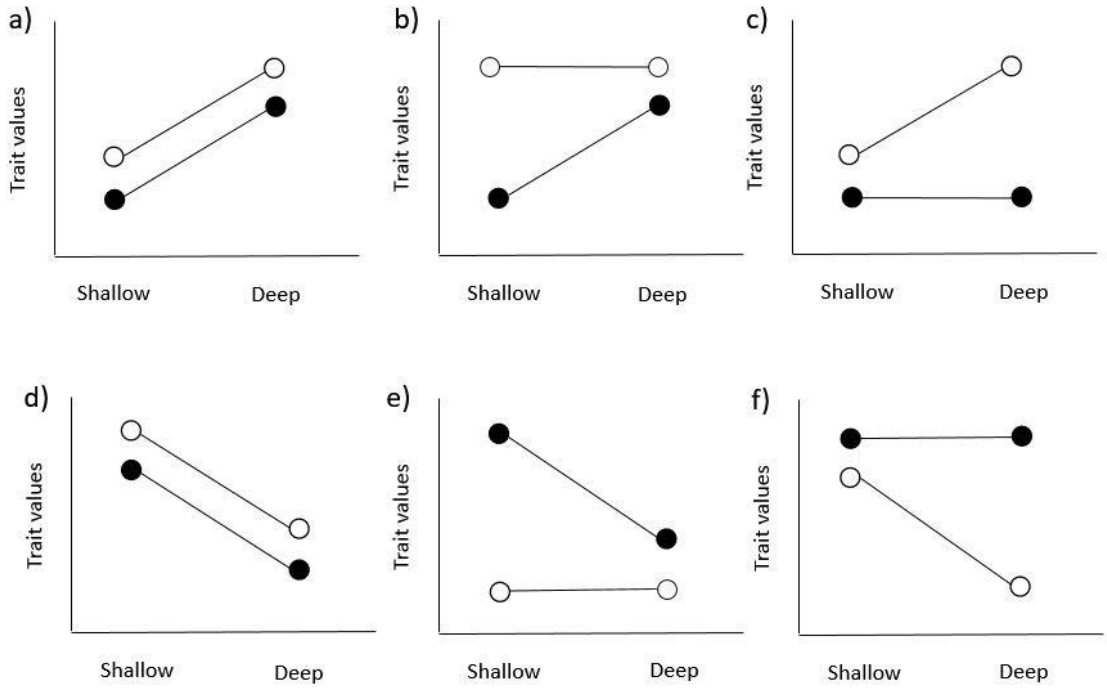


Figure 5.2. Prediction plots for seedling trait responses between the fast and slow infiltration treatments separated into shallow (closed circles) and deep (open circles) populations: a) phenotypic plasticity in both populations for WUE, LMA and belowground biomass; b) local adaptation in the deep population and phenotypic plasticity in the shallow population for WUE, LMA and belowground biomass; c) local adaptation in the shallow population and phenotypic plasticity in the deep population for WUE, LMA and belowground biomass; d) phenotypic plasticity in both populations for aboveground biomass; e) local adaptation in the deep population and phenotypic plasticity in the shallow population for aboveground biomass; f) local adaptation in the shallow population and phenotypic plasticity in the deep population for aboveground biomass.

5.3 Methods

5.3.1 Study region

Seeds of *H. dactyloides* were sourced from the Kangaloon study region as described in detail in Chapter 2 (Section 2.2.2).

5.3.2 Study species selection

Hakea dactyloides (Proteaceae) is an Australian native perennial shrub (Fig. 5.3) that is widespread along the coast and tablelands of south-eastern Australia, where it grows mostly on sandy soils in heath, dry sclerophyll forests and woodlands (Benson and McDougall 2000). Leaves of the species are either linear, lanceolate, oblanceolate or obovate in shape, up to 100 mm long and 20 mm wide with three to five longitudinal veins (Harden 1990-1993). The shrub produces small flowers that occur in clusters within the leaf axis during spring and produce moderate amounts of nectar to attract vertebrate and invertebrate pollinators (Benson and McDougall, 2000). Flowering is followed by the production of woody seed pods about 25 mm long by 20 mm wide, containing two winged seeds. Seeds are released from seedpods after death of the branch or plant (Benson and McDougall, 2000).

Hakea dactyloides is found across the study region, with field work performed for Chapter 2 of this thesis reporting that the species increases in abundance from moderately abundant at deep groundwater sites to highly abundant at shallow sites (Fig. 5.4). Seeds of *H. dactyloides* were separately collected from populations at the shallow and deep sites in the Spring of 2013. Seeds were collected from a minimum of 10 random reproductive individuals at two collection sites, the deepest site and the shallowest site, then transported back to the laboratory in paper bags.

Figure 5.3. Hakea dactyloides bearing woody seed pods. Leaves of the species as in this photo reach up to 10 cm in length.

5.3.3 Experimental design

All seeds were sown in a mixture of four parts coarse river sand to one-part peat moss and placed in glasshouse conditions where they were irrigated twice daily. Once seedling emergence was observed, irrigation was reduced to maintain 'moist' soil conditions without oversaturation. After germination and when the first true leaves appeared, seedlings were transplanted into 150 mm pots, irrigated daily and grown on until they were large enough to be transplanted into the main experiment. At the five-leaf stage, seedlings of similar size from each population were individually transplanted into single 300 mm diameter one-metre long PVC pipes and placed in the glasshouse. Thus, there was no evidence of genetic variation between plants from each population

before the start of the experiment. The glasshouse temperature was kept between 19°C and 36°C during the day and 10°C and 18°C at night.

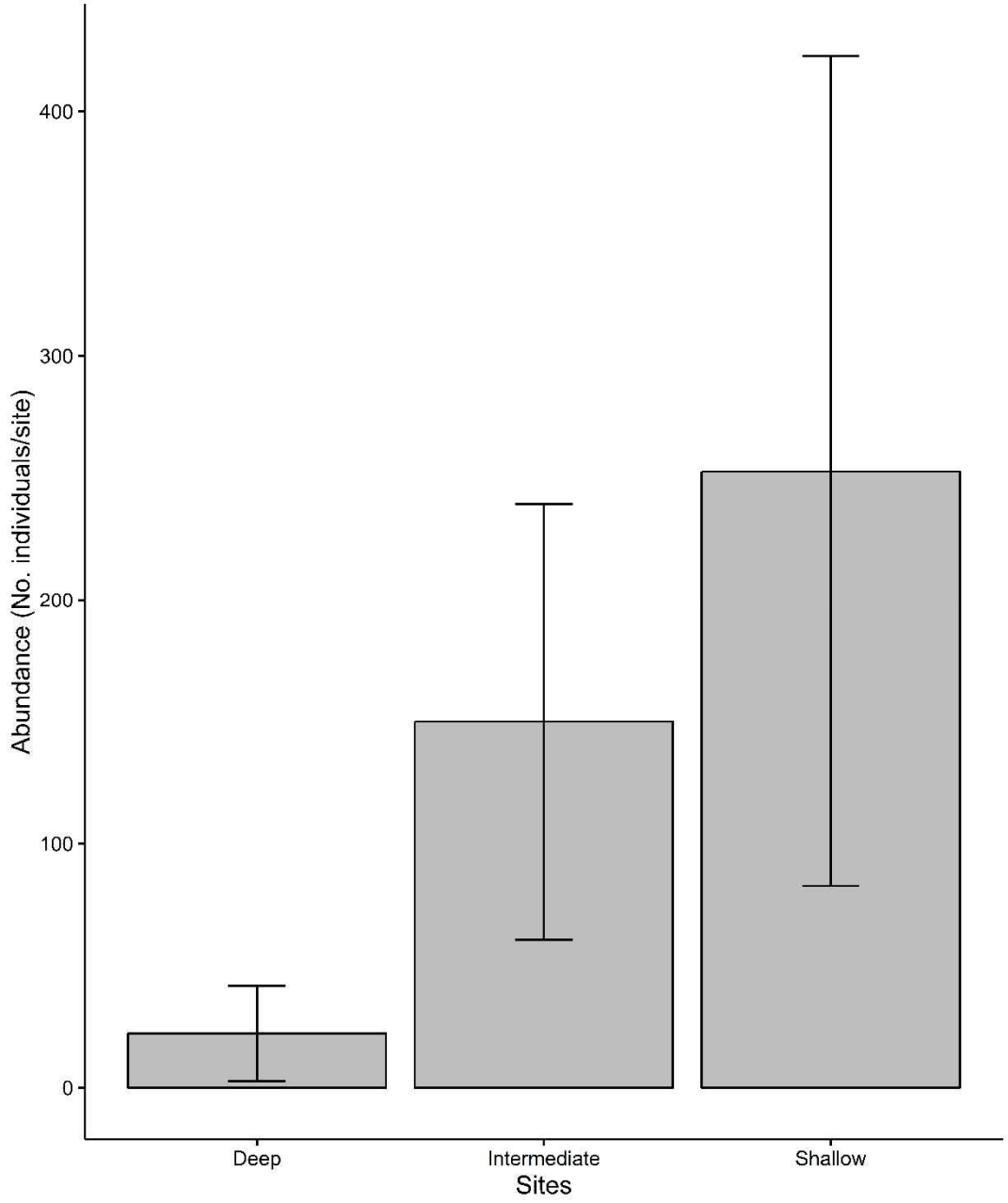


Figure 5.4. The mean (± SE) abundance of *Hakea dactyloides* increases from moderately abundant at deep sites to highly abundant at shallow sites.

The PVC pipes were filled with a mix of four parts coarse river sand, two parts Australian Native Potting Mix® and one-part perlite. Low phosphorous slow release Osmocote® Native Gardens fertiliser (17.9N:0.8P:7.3K) was mixed into the soil at the recommended rate. The final mix had mildly acidic pH (~5.1) similar to the soils at both the shallow and deep sites. Pipes were supported in the glasshouse in a steel framed table with rows spaced approximately 10 to 30 cm apart (Fig. 5.5).

The experimental period ran over 12 weeks in which two water treatments were imposed, including fast-draining and slow-draining treatments. At the beginning of the experiment, each tube in both the fast-draining and slow-draining treatments was watered to the top of the tube. Thus, all tubes in both treatments started with the same height of the saturated zone. Each tube had a total of 11 holes predrilled into it that were subsequently plugged to maintain a water tight seal. Slow-draining tubes had holes spaced 23.8 mm vertically apart down the length of the tube, while fast-draining tubes had holes spaced 83.3 mm down the tube. This meant that fast-draining tubes had holes drilled the full length of the tube while the slow draining tube only had holes drilled down to nearly a third of the way down the tube. These two distances simulated the relative differences in drainage rates between shallow (slow-draining) and deep (fast-draining) habitats, with fast-draining tubes draining at a rate 3.5 times faster than the shallow-draining tubes (see Fig 5.1).

At the beginning of the first week of the experiment, the top-most plug of each tube was removed, and not replaced during the experiment, to allow water to drain from the tube. Each subsequent week, the next top-most plug was removed without replacement from each tube. During the third week of the experimental period, for example, the slow-draining tubes had been drained to a depth of 71.4 mm from the top of the tube while the fast-draining tubes had been drained to a depth of 249.9 mm from

the top of the tube. Thus, after each successive week of the experiment, as plugs were removed, the rate of infiltration differed between the two treatments. There were ten replicates of each combination of the two populations for each of the two treatments (i.e. 40 plants in total). Tubes were arranged in a randomised block design to control for variation in solar radiation and temperature in the glasshouse.

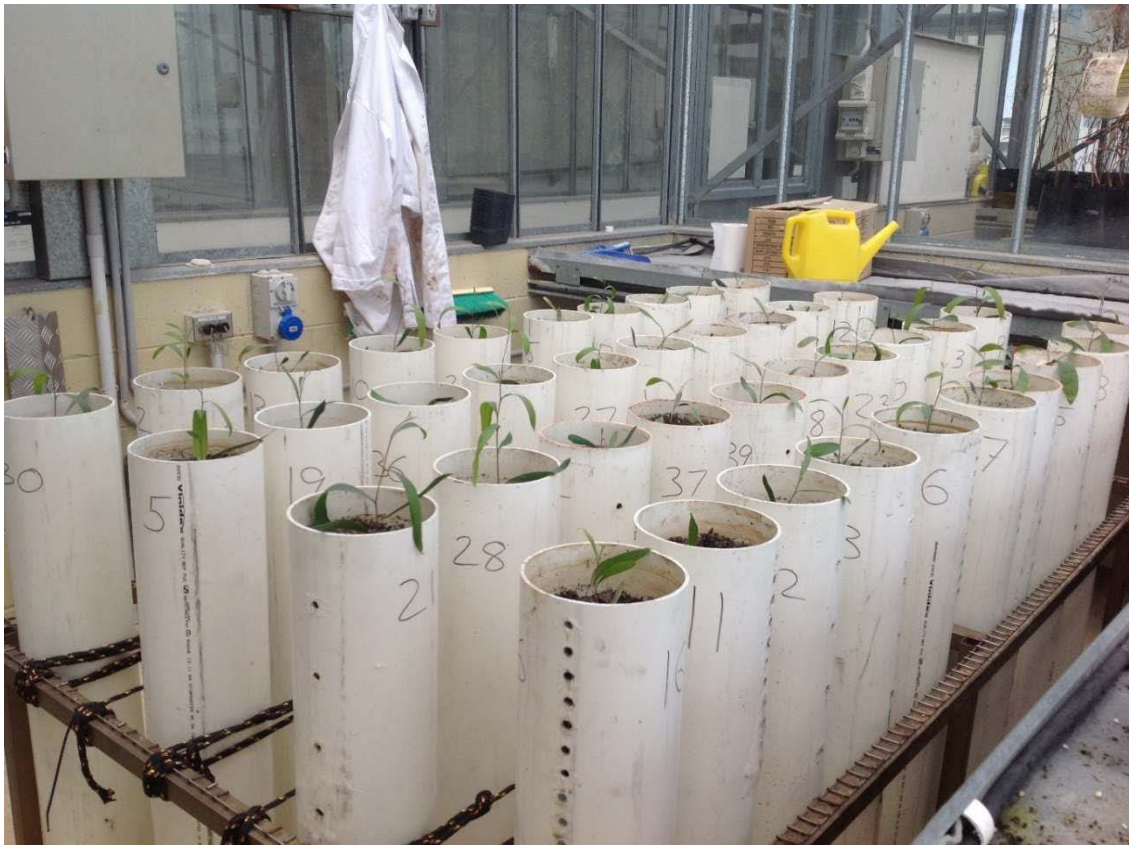


Figure 5.5. Water treatment set up in a randomised block design in the glasshouse, showing seedlings during week 7 of the experimental period.

5.3.4 Measurements

A total of 14 traits were measured. Traits were chosen based on literature assessments of key physiological, leaf, belowground growth and aboveground growth traits associated with plant trait responses to variation in water availability. Physiological trait

measurements were taken on five of the ten replicates (five replicates from each treatment and population were randomly selected) and included stomatal conductance (g_s; mol H_2O $m^{-2}s^{-1}$), rate of photosynthesis (A; µmol CO_2 $m^{-2}s^{-1}$), transpiration (E; mmol H_2O $m^{-2}s^{-1}$), and instantaneous water-use efficiency (WUE; µmol CO_2 mmol H_2O). A LI-COR® 6400 was used on a sunny day to measure E, g_s and A at ten weeks at ambient temperature (30 – 32 ºC) and light (~250 – 300 µmol m^{-2} s^{-1}) conditions. Sample CO_2 concentration was approximately 420 µmol mol^{-1} and leaf -to-air vapour pressure deficit was between 1.0 and 2.3 kPa. One fully expanded leaf from each replicate was clamped in the standard leaf cuvette (internal area 6 cm^2) without shading. For each measurement, g_s, A and E were monitored graphically (Li-COR 'view' mode) to assure that data were taken only after the parameters stabilized and before stomates began to close. Measurements were adjusted for leaf area, placed in the IRGA chamber using Image J software (Abràmoff *et al.* 2004). Instantaneous WUE was calculated as A over g_s. Harvesting for leaf, belowground growth and aboveground growth trait measurements took place at twelve weeks and were measured on all ten replicates. Leaf trait measurements included leaf mass per area (LMA), final leaf length, final leaf width and final leaf number. Belowground growth trait measurements included final root length and belowground biomass. For harvesting, plant tubes were cut using a circular saw set at a depth equal to the thickness of the PVC pipes. The PVC pipe containing the plant was then laid on its side and the soil was washed away from the plant roots. Intact plants were laid out and final root lengths were recorded immediately before desiccation could occur. For the calculation of LMA, a leaf from each replicate was scanned using a Canon CanoScan CS5600F Scanner (Canon Australia Pty Ltd) and leaf area was calculated using Image J imaging software (Abràmoff *et al.* 2004). Leaves were then placed in a drying oven at 60°C for a minimum of 72 h until constant mass was reach

and the final dry mass was recorded (Cornelissen *et al.* 2003). LMA was then calculated as the ratio of leaf dry mass to leaf area (g m^{-2}). Roots were washed to remove all soil prior to being oven dried. All biomass material was oven dried for 72 h until a constant mass was reached and weighed on an electronic balance. Aboveground growth trait measurements included aboveground biomass, final plant height, leaf biomass and root:shoot ratio.

5.3.5 Statistical analysis

All statistical analyses were conducted using R 3.1.3 (R Development Core Team 2015, R Foundation for Statistical Computing, Vienna, Austria). An initial exploratory analysis indicated that seeds from shallow sites were significantly heavier than seeds from deep sites (one-way ANOVA $F_{1, 4} = 13.78$, $P < 0.05$). I therefore included seed mass as a covariate in the models described below.

I implemented separate two-way ANCOVAs for each of the 14 traits, using the function 'aov '(library stats). Log transformations were applied where necessary prior to analyses to meet parametric assumptions. The full model included population (deep vs shallow), treatment (fast vs slow), the covariate seed mass and the population x treatment interaction. In this context, I specified a systematic order of tests that represented a pathway of effects of low water availability. The pathway first investigated the four key physiological traits (Gs, A, E and WUE) that represent the first set of plant physiological responses that underpin plant growth. In this context, a plant's first response to low water availability is to regulate g_s (Taiz and Zeiger 2010), thereby controlling the influx of atmospheric CO_2, essential for photosynthesis, in exchange for transpirational water loss, thereby influencing WUE (Vahisalu *et al.* 2008). These analyses were followed in sequence, by comparisons of leaf traits (LMA, leaf length,

leaf width and leaf number), belowground growth traits (belowground biomass and root length), and finally aboveground growth traits (aboveground biomass, leaf biomass, final height and root:shoot ratio).

5.4 Results

Overall, I found significant effects of treatment, population or treatment x population interactions in 11 out of the 14 seedling traits (Table 5.1, Table 5.2).

5.4.1 Physiological traits

Both shallow and deep populations demonstrated phenotypic plasticity in three physiological traits, with g_s, E and WUE differing significantly between fast vs slow water treatments; there was no significant effect of population and no significant interaction (Table 5.1, Fig. 5.6). Seedlings from both populations responded similarly to the different water treatments by decreasing g_s in response to reduced water availability in the fast-draining treatments (Fig. 5.6a). Both populations also responded similarly to the different treatments by decreasing transpiration rates in response to reduced water availability in the fast-draining treatments (Fig. 5.6b), which ultimately led to increased WUE by both populations in the fast-draining treatments (Fig. 5.6c).

Table 5.1. Two-way ANCOVA results for (a) physiological, (b) leaf, (c) belowground growth and (d) aboveground growth traits of *H. dactyloides* seedlings grown from seeds sourced from shallow and deep populations, subjected to fast and slow water infiltration treatments. Significant values shown in bold (d.f. = 1, 15).

Trait	Treatment		Population		Seed mass		Treatment x Population	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
(a) Physiological traits								
WUE	5.67	**0.03**	0.22	0.65	0.26	0.62	0.03	0.87
g_s	11.13	**0.005**	0.26	0.62	1.34	0.27	1.84	0.20
E	8.5	**0.01**	0.02	0.89	1.85	0.19	0.60	0.45
A	1.52	0.24	0.77	0.39	0.01	0.99	0.77	0.39
(b) Leaf traits								
LMA	0.50	0.49	4.51	0.05	0.02	0.89	3.12	0.10
Final leaf width	3.66	0.08	0.27	0.61	2.58	0.13	8.73	**0.01**

Trait	Treatment		Population		Seed mass		Treatment x Population	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Final leaf length	1.09	0.31	7.36	**0.02**	0.27	0.61	0.39	0.54
Final leaf number	0.03	0.87	7.64	**0.01**	0.16	0.69	1.91	0.19
(c) Belowground growth traits								
Belowground biomass	10.00	**0.006**	22.57	**0.0003**	1.63	0.22	7.41	**0.02**
Final root length	16.05	**0.001**	0.44	0.52	0.74	0.40	1.42	0.25
(d) Aboveground growth traits								
Aboveground biomass	0.41	0.53	16.37	**0.001**	0.09	0.77	4.24	0.06
Leaf biomass	0.95	0.36	25.20	**0.0002**	0.25	0.62	4.74	0.05
Final height	0.13	0.72	2.16	0.16	0.03	0.86	1.87	0.19
Root:shoot ratio	4.88	**0.04**	0.21	0.66	0.89	0.36	0.04	0.84

Table 5.2. Summary of plant trait responses demonstrating local adaptation and/or phenotypic plasticity among shallow and deep populations. Prediction represents responses for each trait according to the corresponding prediction plots in Fig. 5.2.

Trait	Local adaptation	Phenotypic plasticity	Prediction
WUE		Shallow and deep	a
g_s		Shallow and deep	d
E		Shallow and deep	d
Final leaf width	deep	shallow	e
Belowground biomass	shallow	deep	c
Final root length		Shallow and deep	a
Root:shoot ratio		shallow	a

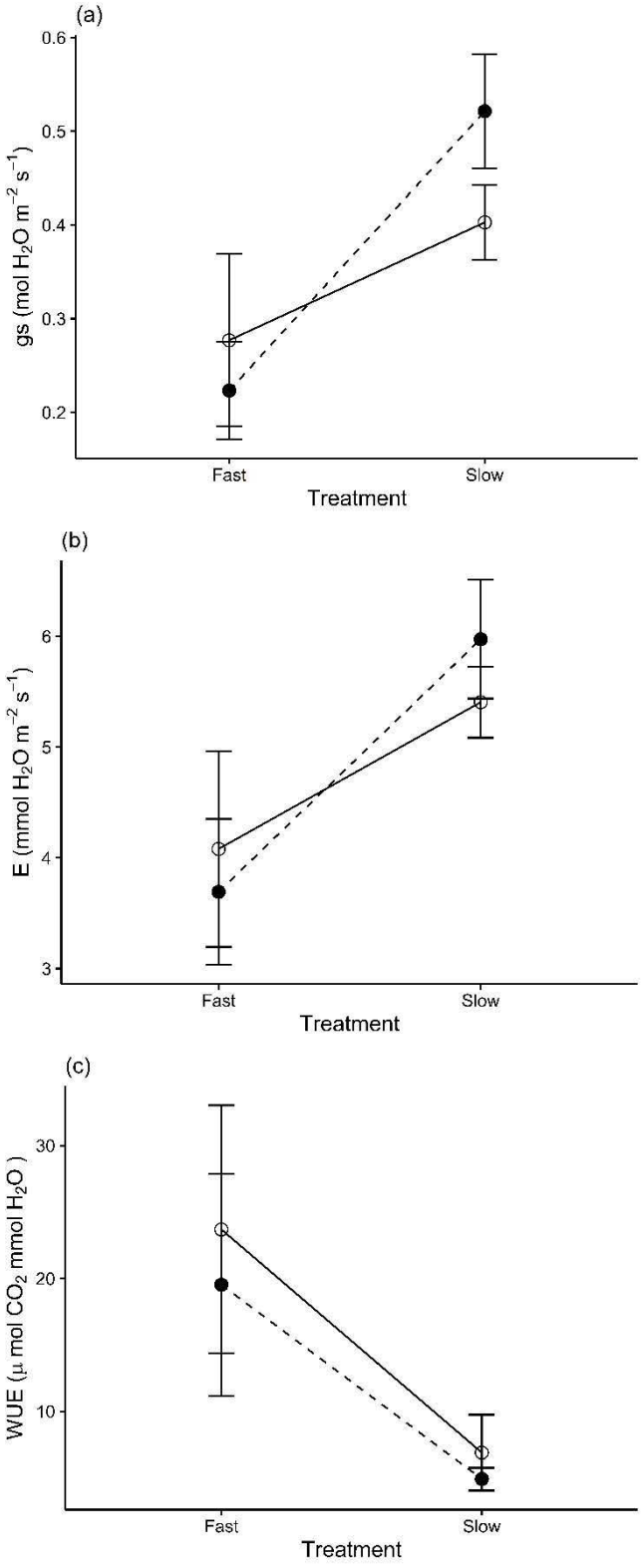


Figure 5.6. Comparison of mean (±SE) physiological trait responses: (a) g_s, (b) E, and (c) WUE between fast and slow-draining treatments separated into shallow (closed circles, dashed lines) and deep (open circles, solid lines) populations.

5.4.2 Leaf traits

Seedlings from the shallow population demonstrated phenotypic plasticity for final leaf width with narrower leaves in the fast treatment compared with wider leaves in the slow-draining treatment, in contrast to the deep population which demonstrated local adaptation with moderately narrow leaves maintained under both treatments (Fig. 5.7a). Both populations differed significantly in final leaf length and final leaf number regardless of treatment, with the shallow population always producing more and longer leaves than the deep population (Fig. 5.7b, 5.7c). Contrary to expectation, LMA did not differ significantly between populations or water treatments and there was no significant treatment x population interaction for LMA (Table 5.1).

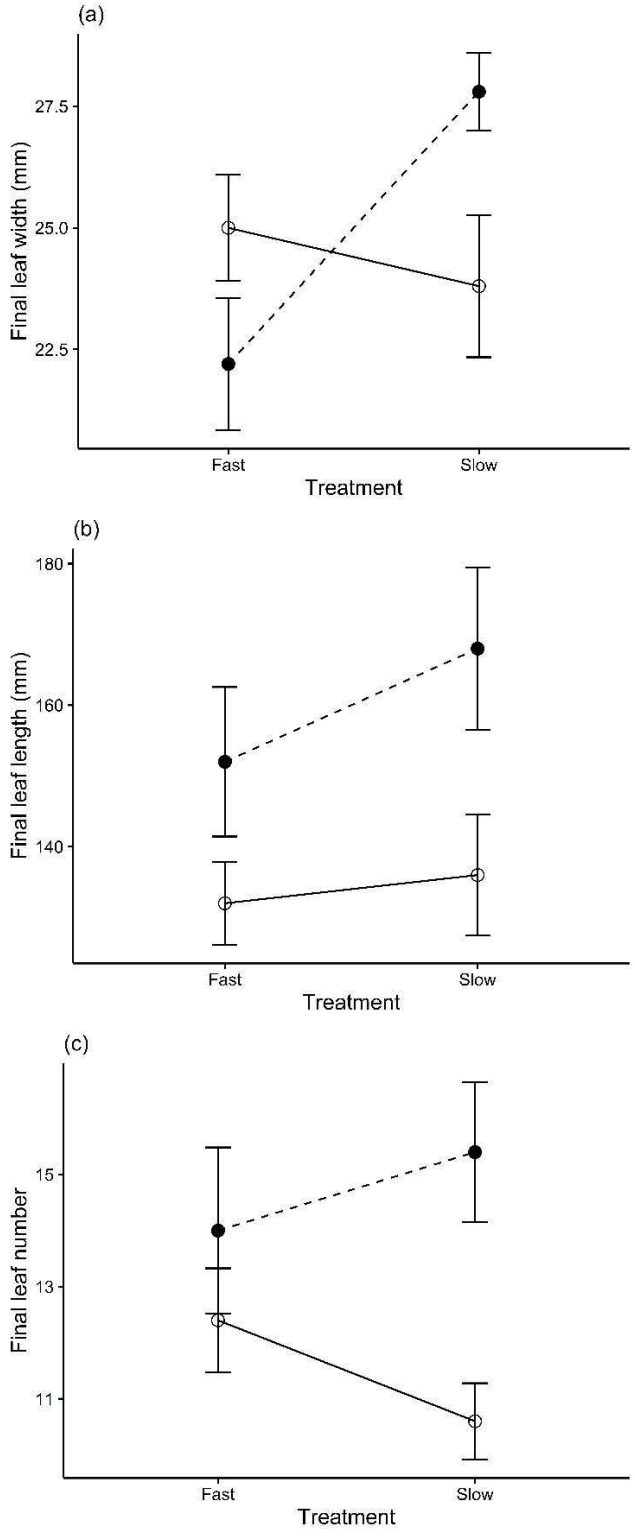


Figure 5.7. Comparison of mean (±SE) leaf trait responses for: (a) final leaf width, (b) final leaf length, and (c) final leaf number between fast and slow-draining treatments separated into shallow (closed circles, dashed lines) and deep (open circles, solid lines) populations.

5.4.3 Belowground growth traits

The deep population demonstrated phenotypic plasticity in belowground biomass, with significantly higher belowground biomass in the fast-draining treatment compared with much lower belowground biomass in the slow-draining treatment. This was in stark contrast to the shallow population which, surprisingly, invested in higher belowground biomass than the deep population in both the fast and the slow-draining treatments (Fig. 5.8a). In addition, both populations demonstrated phenotypic plasticity for final root length (Fig. 5.8b). Seedlings from both populations responded similarly to the different water treatments by significantly increasing root length in response to reduced water availability in the fast-draining treatments (Fig 5.7b).

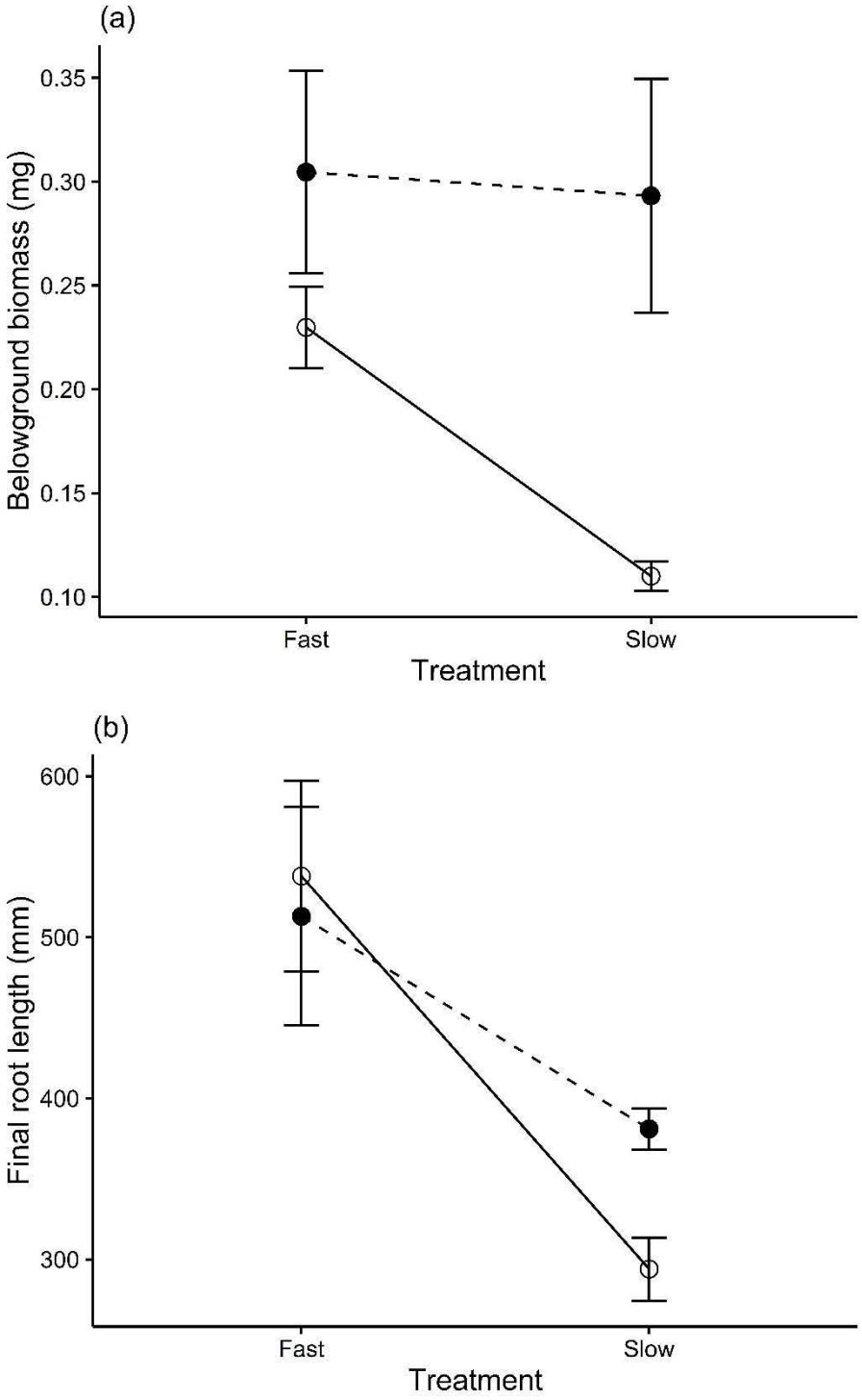


Figure 5.8. Comparison of mean (±SE) belowground trait responses for: (a) aboveground biomass, and (b) final root length between fast and slow draining treatments separated into shallow (closed circles, dashed lines) and deep (open circles, solid lines) populations.

5.4.4 Aboveground growth traits

Both populations differed significantly in aboveground biomass and leaf biomass regardless of treatment, with the shallow population always producing higher aboveground biomass and leaf biomass compared with the deep population (Fig. 5.9a, 5.9b). Seedlings from the shallow population responded plastically to the different water treatments by significantly increasing root:shoot ratio in response to reduced water availability in the fast-draining treatment (Fig. 5.9c).

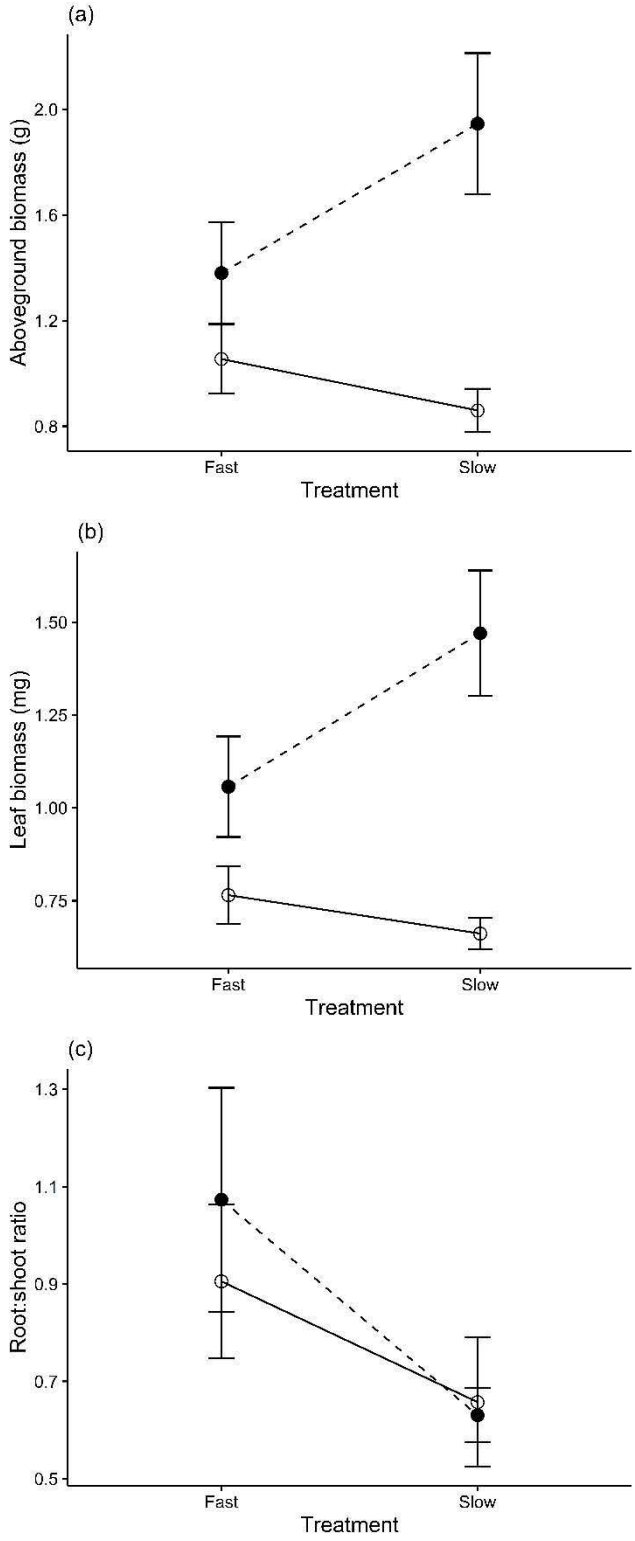


Figure 5.9. Comparison of mean (±SE) growth trait responses for: (a) aboveground biomass, (b) leaf biomass, and (c) root:shoot ratio between fast and slow draining treatments separated into shallow (closed circles, dashed lines) and deep (open circles, solid lines) populations.

5.5 Discussion

In this study, I set out to determine if local adaptation differentiates seedling growth patterns of *H. dactyloides* among populations from the shallow and deep ends of the DGW gradient. I tested for local adaptation in seedlings of *H. dactyloides* from the shallow and deep populations using a controlled glasshouse manipulation experiment. The responses of populations to contrasting water availabilities were compared using a systematic approach which characterised a sequential order of events, from physiological traits through to growth traits, which represents a plant's response to the effects of low water availability. I found contrasting results in seedling trait responses among populations and between treatments. My results indicated substantial variation in physiological plasticity. In addition, plastic and genetic morphological variation was evident in seedling traits among treatments and between populations, providing evidence for local adaptation in two morphological traits. Below, I describe differences in local adaptation of seedling traits between populations and among treatments. Next, I compare differences in phenotypic plasticity of seedlings traits from the shallow and deep populations. Finally, I consider how my results are likely to influence seedling establishment for seedlings of *H. dactyloides* in the shallow and deep habitats.

I found significant treatment effects in three out of four physiological traits (Table 5.1), demonstrating that the majority of physiological traits examined in this study displayed phenotypic plasticity. Seedlings from both populations appeared to display adaptive phenotypic plasticity, by demonstrating the ability to alter g_s, transpiration and WUE in response to variation in water availability. Seedlings from both populations were able to reduce g_s and lower transpiration rates in the fast draining treatments, which ultimately led to increased WUE by both populations in response to reduced water availability (Fig. 5.6a, 5.6b, 5.6c). Reduced water availability can lead to

water deficits in leaf tissues resulting in xylem cavitation (Vilagrosa *et al.* 2003). Xylem cavitation is the breakage of the water column under tension within a plant (Zimmermann 1983). Once cavitation occurs and the xylem becomes air filled (embolism), the main water transport pathway within a plant becomes blocked causing dehydration, death of leaves and organs (Taiz and Zeiger 2010) and ultimately reducing seedling survival and establishment. To avoid cavitation plants must regulate transpiration by reducing stomatal conductance (Cochard *et al.* 1996). Although this study did not directly test seedlings vulnerability to cavitation, the reduction in stomatal conductance and transpiration in the fast-draining treatments can be inferred as cavitation avoidance since reduced stomatal conductance works to actively prevent xylem cavitation (Sperry and Pockman 1993; Sparks and Black 1999).

Reduced stomatal conductance and transpiration have previously been linked to conservative water use (Vilagrosa *et al.* 2003). Conservative water use was evident in my results where WUE significantly increased in both populations in response to reduced water availability, indicating drought avoidance strategies were displayed by seedlings from both populations. Such drought avoidance strategies have been frequently reported in species from habitats with strong water limitations (Gullo and Salleo 1988; Salleo and Lo Gullo 1993). Phenotypic plasticity that was detected in physiological traits, is likely to enhance seedling establishment in *H. dactyloides* in both populations irrespective of habitat type.

I found significant population effects in two leaf traits, final leaf length and final leaf number and a significant interaction effect in one leaf trait, final leaf width (Table 5.1). These results indicate that the shallow population demonstrated phenotypic plasticity while the deep population demonstrated local adaptation for final leaf width (Fig. 5.7a). Small, short and narrow leaves with reduced leaf numbers are leaf traits that

that are correlated with reduced water availability (Stone and Bacon 1995; McDonald *et al.* 2003; McLean *et al.* 2014). Smaller, shorter and narrower leaves with reduced leaf numbers are advantageous in hot and dry environments due to reduced total leaf surface areas, higher heat exchange capacity and lower transpiration rates compared with more leaves that are longer, wider and larger with increased surface areas (Givnish 1984; Fonseca *et al.* 2000; Ackerly *et al.* 2002; McDonald *et al.* 2003). Leaves that are larger and wider are considered advantageous in moist environments as the larger leaf surface area allows for increased photosynthetic rates and higher growth rates (Taiz and Zeiger 2010). Therefore, increased leaf widths are likely to enhance seedling establishment in the shallow population as water availability increases, while the deep population displaying moderately narrow leaves across both treatments, is likely to increase seedling establishment in the deep population demonstrating local adaptation at home sites.

There were significant treatment, population and interaction effects for belowground biomass and a significant treatment effect for final root length (Table 5.1). Seedlings from the shallow population surprisingly, invested in higher belowground biomass than the deep population in both the fast and the slow-draining treatments (Fig. 5.8a) demonstrating that seedlings from the shallow population were locally adapted to conditions of increased water availability. In contrast seedlings from the deep population were able to increase belowground biomass in response to conditions of low water availability in the fast-draining treatments (Fig. 5.8a), while both populations were able to increase final root length in response to conditions of low water availability in the fast-draining treatments (Fig. 5.8b). These results demonstrate that phenotypic plasticity was evident in the deep population for belowground biomass and in both populations for final root length. As water availability decreases the allocation of a

plant's biomass shifts from aboveground biomass to belowground biomass (Poorter and Nagel 2000) in order to capture water as the most limiting resource. Indeed, empirical studies have shown that the allocation of increased biomass to root systems increases with reduced water availability (Yin *et al.* 2005; Zhang *et al.* 2005) and are common traits shared by many species growing in water-limited environments (Vonlanthen *et al.* 2010). Increased belowground biomass provides an advantage for plants growing in water-limited environments by producing more roots to increase soil moisture uptake at variable depths within the soil profile and allowing individual plants to successfully compete for water resources (Caldwell *et al.* 1986; Schenk and Jackson 2002). My results contradict the empirical evidence from the literature, where seedlings from the shallow population invested in higher belowground biomass compared to the deep population across both treatments (Fig. 5.8a). In contrast, the trend from empirical studies of increased belowground biomass accumulation as a results of reduced water availability were evident in my results where seedlings from the deep population developed increased belowground biomass in response to reduced water availability (Fig. 5.8a).

Previous studies from south-western Australia involving seven different *Hakea* species have found that during the early stages of seedling development, all of the *Hakea* species studied allocated more resources to developing belowground biomass compared to the later stages of growth (Poot and Lambers 2003), thus providing increased chances of seedling survival during early establishment. In addition, the development of longer and deeper root systems may enable more efficient use of soil water by assisting in a seedlings ability to exploit water at deeper soil levels, where soil water remains more readily available through periods of low rainfall (Dodd *et al.* 1984; Canadell and Zedler 1995; Hilbert and Canadell 1995), whilst increasing competitive

advantage during establishment. This trend for increased allocation to belowground biomass in *Hakea* seedlings (Poot and Lambers 2003), provides support for my results where I found that where seedlings from the shallow population invested in higher belowground biomass compared to the deep population across the different water treatments. Therefore, increased root biomass and longer root systems are likely to to further enhance seedling establishment by populations of *H. dactyloides* displaying phenotypic plasticity as water availability is reduced, while local adaptation in belowground biomass provides increased performance for shallow populations at home sites.

I found significant population effects in two aboveground growth traits, aboveground biomass and leaf biomass and a significant treatment effect for root:shoot ratio (Table 5.1). Seedlings from the shallow population responded to reduced water availability by increasing root:shoot ratio in the fast-draining treatment (Fig. 5.9c), demonstrating that the shallow population displayed phenotypic plasticity. Aboveground biomass accumulation is an important factor that indicates long-term viability of plant populations (Rice and Emery 2003). Indeed, increased aboveground biomass accumulation and the allocation of more biomass accumulation to leaves, allows for increased light interception, higher photosynthetic rates and higher relative growth rates, but comes at a cost of higher water requirements (Huante *et al.* 1992; Zerihun *et al.* 2006; Markesteijn and Poorter 2009). Thus, reduced aboveground biomass and leaf biomass are advantageous in habitats with low water availability where conservative water use strategies are prominent through higher investment in belowground biomass, slower growth rates and the ability to forage for water in deeper soil layers (Markesteijn and Poorter 2009). These features are likely to increase competitive performance and seedling establishment in populations growing in home

environments. Higher root:shoot ratios are commonly seen in plants that occur in habitats with low water availability and is considered to be an adaptation to low rainfall environments (Hilbert and Canadell 1995). Plants with higher root:shoot ratios may possess lower internal water deficits by maintaining a higher rate of water uptake by root systems relative to the rate of transpirational water loss (Passioura 1983). Therefore, higher root:shoot ratios are likely to to further enhance seedling establishment by both populations of *H. dactyloides* as water availability is reduced.

Despite the evidence for potential local adaptation among populations in one leaf trait, final leaf width, and one belowground growth trait, belowground biomass, not one group of traits demonstrated a consistent home site performance advantage, regardless of which traits were used to evaluate local adaptation. There may be several reasons why I found limited evidence for local adaptation in groups of seedling traits among the deep and shallow populations in this study. Local adaptation may be hindered by gene flow and/or a lack of genetic variation (Hirst *et al.* 2016) in traits associated with adapting to the shallow or deep habitats. High rates of gene flow may decrease genetic divergence among sites (Newman and Tallmon 2001) and reduce local adaptation (Santon and Galen 1997) especially if gene flow acts against conditions of contrasting water availability. The limited evidence for local adaptation in groups of seedling traits among the deep and shallow populations found in this study may also be explained by the different conditions under which seedlings were grown in failed to distinguish between the performance of different genotypes. Local adaptation may only be evident under certain environmental conditions such as soil properties of the field sites, which were not replicated in the present study, and may also take a longer time to be expressed (Dorman *et al.* 2009; Bennington *et al.* 2012) than the experimental period of this study. It may also be possible that local adaptation is expressed in different life-

history stages, such as seed production, which was not tested in this study. While water infiltration is also influenced by soil properties such as soil texture (Shukla 2013), soil organic matter (Franzluebbers 2002) and soil porosity (Lipiec *et al.* 2006), the influence of these soil properties on water infiltration rates were not fully tested here. Future studies examining patterns of local adaptation and phenotypic plasticity along a DGW gradients should also take into account the influence of such soil properties on water infiltration rates.

In conclusion, this study has demonstrated that although evidence for local adaptation was detected in one leaf trait, and one belowground growth trait seedling trait responses among the shallow and deep populations did not translate into a consistent home site advantage where local adaptation was prevalent in all trait groups. The majority of physiological traits showed more evidence for phenotypic plasticity than local adaptation among populations. My results show that seedlings of *H. dactyloides* from both populations, responded plastically to reduced water availability by reducing g_s and E, thereby increasing WUE, whilst increasing root lengths in the fast-draining treatments. The shallow populations responded plastically to reduced water availability by decreasing leaf widths and increasing and root:shoot ratios in the fast-draining treatments, while the deep population responded plastically to reduced water availability by increasing belowground biomass in the fast-draining treatment. My results also show that the deep population displayed local adaptation by maintaining moderately narrow leaf widths across both treatments while the shallow population invested in higher belowground biomass than the deep population across the different water treatments. The phenotypic plasticity responses of these traits to variation in water availability are likely to enhance seedling establishment in the shallow and deep habitats and may allow both populations to successfully establish in a wide range of soil moisture conditions,

whilst locally adapted populations are likely to demonstrate enhanced seedling performance among populations growing in home environments.

Chapter 6: Ecological properties of arid-zone woodlands are related to a depth-to-groundwater gradient in central Australia

6.1 Introduction

In arid and semi-arid regions of the world, water availability is the principal limiting factor influencing plant growth, productivity, recruitment and vegetation dynamics (Hadley and Szarek 1981; Smith *et al.* 1995; Reynolds *et al.* 1999; Li *et al.* 2001). Groundwater is an especially important resource for vegetation in deserts, where it plays a predominant role in groundwater-dependent ecosystems (GDEs) in supporting plant species richness and diversity (Chen *et al.* 2004; Zhu *et al.* 2013). A major threat to desert GDEs throughout the world is that groundwater is being extracted for human needs at an increasing rate (Konikow and Kendy 2005; Eamus *et al.* 2006b; Wada *et al.* 2010). Although groundwater has been readily accessed by humans for millennia via naturally occurring springs, oases and as base-flow discharge into rivers in deserts, it has only been during the past 100 years that exploitation of groundwater resources has become a global concern (Gleick and Palaniappan 2010). Whilst about 40% of global groundwater extraction occurs in arid and semi-arid regions, the scarcity of rain means that only 2% of groundwater recharge occurs in these regions (Wada *et al.* 2010). Considering that about two-fifths of the world's terrestrial surface area is arid or semi-arid and more than 38% of the world's population lives there, managing groundwater resources sustainably is a major social, economic and environmental priority for the world (Glazer and Likens 2012).

Recent studies have begun to demonstrate that declines in depth-to-groundwater (DGW) as a result of groundwater extraction can have serious impacts on the ecological properties of vegetation in arid and semi-arid GDEs, leading to declines in species

richness and diversity (Stromberg *et al.* 1996; Chen *et al.* 2006; Cooper *et al.* 2006; Hao *et al.* 2010; Zhu *et al.* 2013). Given the potential for deleterious consequences of groundwater extraction on vegetation and associated ecosystem services (Murray *et al.* 2006), there is a pressing need to provide a comprehensive understanding of relationships between DGW and the ecological properties of vegetation. Such an understanding must include fundamental knowledge of relationships between naturally occurring gradients in DGW across landscapes and the structure and composition of vegetation assemblages (Jakeman *et al.* 2016). At present, however, there is a paucity of information about relationships between naturally occurring gradients in DGW in areas that have not yet experienced groundwater extraction. Determining how landscape variation in DGW relates to ecological properties such as species composition, richness, diversity and abundance can provide insight into links between water resource availability and plant community assembly. Furthermore, we will be better placed to predict, manage and mitigate the broader ecological impacts of groundwater extraction.

In this chapter, I investigate relationships between a naturally occurring gradient in DGW and the ecological properties of vegetation in arid-zone woodlands of central Australia. Specifically, I examine variation in species composition, species richness, total plant abundance, species diversity, Pielou's evenness, plant growth form and plant longevity in relation to the DGW gradient in this arid ecosystem that has not been subject to groundwater extraction. I adopt analytical techniques that allow identification of any role played by DGW in shaping the ecological properties of vegetation, independently of a range of other physical and chemical features of the environment that could influence vegetation properties.

6.2 Methods

6.2.1 Study region

The study region was located 200 km north of Alice Springs and 180 km north of the Tropic of Capricorn (22.3°S, 133.2°E, 600 m a.s.l.) within the Ti Tree groundwater basin in arid Australia. The basin covers an area of approximately 5500 km^2 and comprises red kandosol soils with gently undulating sand plains of Aeolian origin, with alluvial deposits along ephemeral drainage lines (Cleverly *et al.* 2013). The Ti Tree basin contains three main vegetation types that include Mulga woodlands, *Corymbia* savanna (Harrington et al. 2002), and rivers fringed by River Red Gum woodlands (Chen et al. 2014). Mulga woodlands are characterised by a short (3-7 m) overstorey of *Acacia* species including *Acacia aneura* and *A. aptaneura* that form a discontinuous canopy, and an understorey of shrubs, forbs, and a mixture of C_3 and C_4 grasses (Cleverly et al. 2016). The vegetation of *Corymbia* savanna is characterised by a sparsely distributed overstorey of *Corymbia opaca* ranging from 10 to 15 m in height with an understorey spinifex (*Triodia schinzii*) and scattered forbs and tussock grasses (Cleverly et al. 2016). River Red gum woodlands are characterised by an overstorey dominated by *Eucalyptus camaldulensus* var. *obtusa* ranging from approximately 15 to 20 m in height, with a mixed understorey of shrubs, forbs and C_3 and C_4 grasses. Depth-to-groundwater becomes increasingly shallow, from approximately 50 m in the southwest and southeast corners of the basin to less than 2 m in the north where groundwater discharges to the land surface via evapotranspiration. Groundwater recharge occurs in the southern and central parts of the basin as slow percolation through the soil and also as flood recharge associated with ephemeral rivers (Cook *et al.* 2008).

The region is situated within a tropical arid zone with hot summers and warm winters. The mean annual temperature of the region is 30.8 °C, with a mean maximum daily temperature of 40.8 °C (January) and a mean minimum daily temperature of 18.9 °C (June). The region has a long-term mean annual rainfall of 319.9 mm and a long-term median rainfall of 299.0 mm, with a mean monthly minimum of 4.4 mm in August and a mean maximum of 64.1 mm in February and a median monthly minimum of 0.0 mm in July and August and a median maximum of 45.7 mm in January (BOM 2016). As this system is on the edge of the monsoonal influence in northern Australia, rainfall is uneven the during year, falling predominantly in December-February, with the smaller amounts in other months being insufficient to penetrate the soil profile (Chen *et al.* 2016). Rainfall also varies dramatically across years, varying from very wet to extended drought, depending on the prevailing climate models (Cleverly et al. 2016).

6.2.2 Study sites, vegetation surveys and ecological properties of plant assemblages

Selection of study sites (Fig. 6.1) was informed by availability of DGW data from groundwater monitoring bores across the region (Fig. 6.2, Northern Territory Government). I established 10 sites ranging in DGW from 2.55 m at the shallow end to 49.40 m at the deep end of the gradient (Fig. 6.1, Appendix F). At each site, a 100 m line transect was centred within 20 m of the monitoring bore, with 50 m of each transect placed on either side of the bore. Vegetation surveys using point transects were conducted after substantial rainfall occurred across the region, between mid-March to mid-April 2014. All aboveground plant species that intersected the transect at 1 m intervals were identified. The abundance of each species at each site was measured as the number of intersections per transect. In total, 38 plant species (36 native species and two exotic species) were recorded across the sites (Table 6.1).

Seven ecological properties of the plant assemblages at each site were quantified. Species richness was measured as the number of species per site. Total plant abundance was calculated by summing the abundances of all species at a site. Simpson's diversity index (1) and Pielou's evenness (2) were calculated as:

$$(1) \quad D = \sum_{i=1}^{S} P_i^2$$

$$(2) \quad JSW = (-\sum P_i \, LnP_i)/LnS$$

Here, S = total number of species in the i^{th} transect and P_i = is the relative importance of species i (total number of individuals of species i/total number of all individuals per site). Plant longevity (annual, perennial) and growth form (tree, shrub, graminoid, forb) of each species were defined using online sources AusGrass2 (ausgrass2.myspecies.info/), Plantnet (plantnet.rbgsyd.nsw.gov.au/) and FloraBase (florabase.dpaw.wa.gov.au/) (Table 6.1). Species composition was quantified using the identities of all species and their abundances at each site in multivariate analyses (see below).

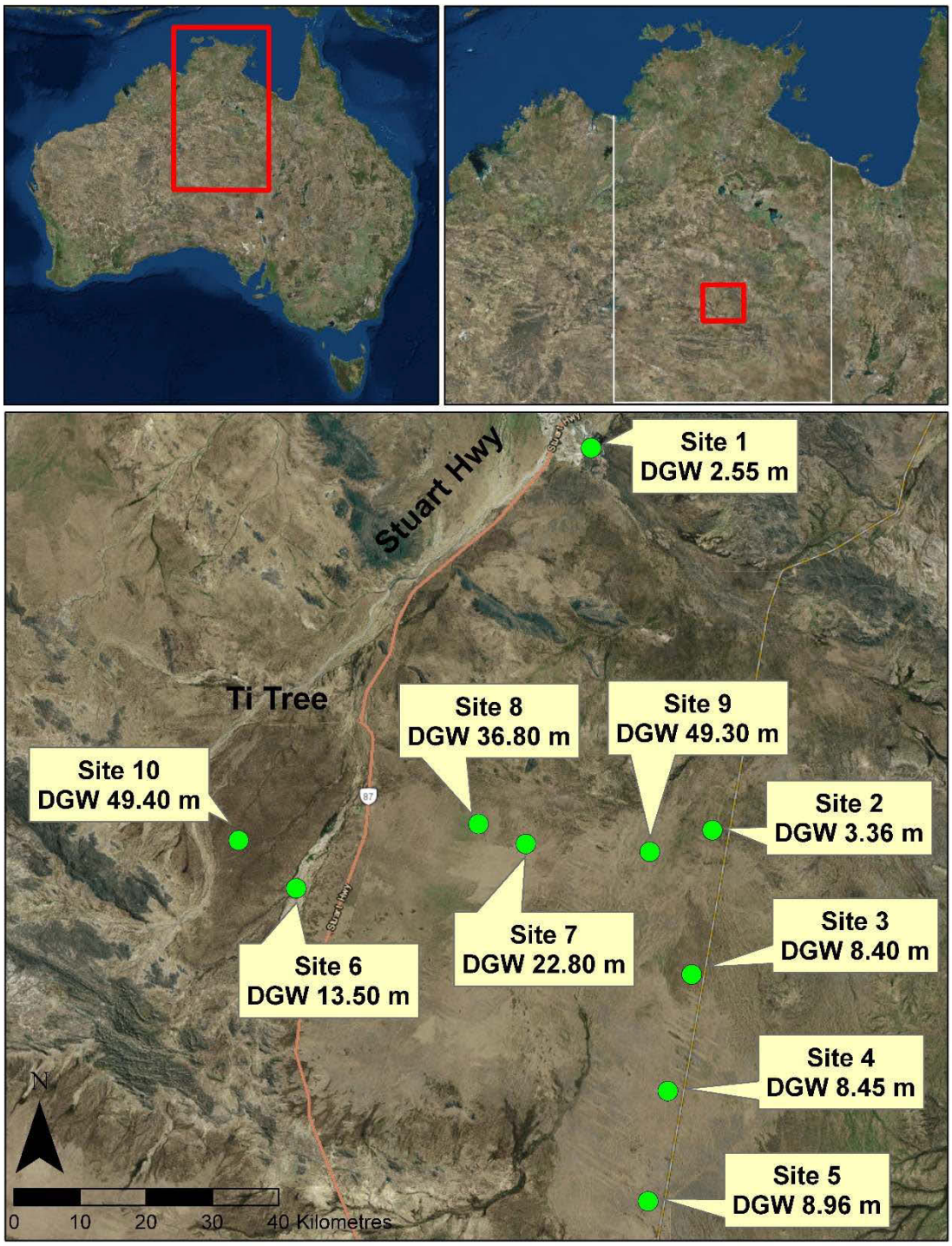


Figure 6.1. Map of the study region and sites (1 to 10), located in the Ti Tree basin 200 km north of Alice Springs. Yellow dashed line represents railway.

6.2.3 Environmental attributes

Data were collected for 12 environmental attributes at each site (Table 6.2). A shade index was determined as the ratio of the amount of photosynthetically active radiation (PAR; measured in μmol m^{-2} s^{-1}) above the canopy (or in a nearby open area) to the amount of PAR measured at 1.5 m above ground level beneath the canopy. Three measurements were taken along each transect at each site between 11:00 a.m. and 2:00 p.m. with a Li-Cor PAR sensor (model NO. 250A). Four to six soil samples were collected along each transect at each site, bulked and mixed thoroughly to obtain a representative sample. Samples were obtained via a soil corer (10 cm diameter) to a depth of 15–20 cm, after brushing aside any surface leaf litter. Prior to analyses, bulked soil samples were air dried at ambient laboratory conditions for approximately two weeks. Soil peds were crushed by hand using a mortar and pestle and passed through a mesh (2 mm) screen. Soil pH and electrical conductivity (EC) were determined using a WTW multi-probe (model NO. 3400) on soil extracts with a 1:5 ratio of soil to water after shaking for one hour. Organic matter content (OM) was estimated by loss on ignition by determining the difference in weight of soil samples dried at 105ºC overnight to remove residual water and at 400ºC for four hours to ash organic matter (Jones 2001). Field capacity (FC) determined via gravimetric methods (Hillel 1971) 24 hours after saturation of air dried soil samples with water. Estimates of soil particle size were determined through a particle size analyser (MALVERN Mastersizer 2000) after pre-treatment of 250 mg soil samples with 2 ml of 0.5% CALGON (sodium polyphosphate) solution for 48 h and in line ultra-sonication for five minutes. Two fractions were considered, fine fraction (clay) whose particles were < 0.39 μm and course fraction (sand) whose particles ranged from 62.5 μm to 2000 μm. Total nitrogen (N) was analysed using a carbon/nitrogen analyser (LECO Tru-Spec CN-628). Per cent

soil phosphorous (P), potassium (K), magnesium (Mg) and calcium (Ca) were analysed by inductively coupled plasma mass spectrometry (ICP-MS) after digestion with nitric acid (Krishnamurty *et al.* 1976).

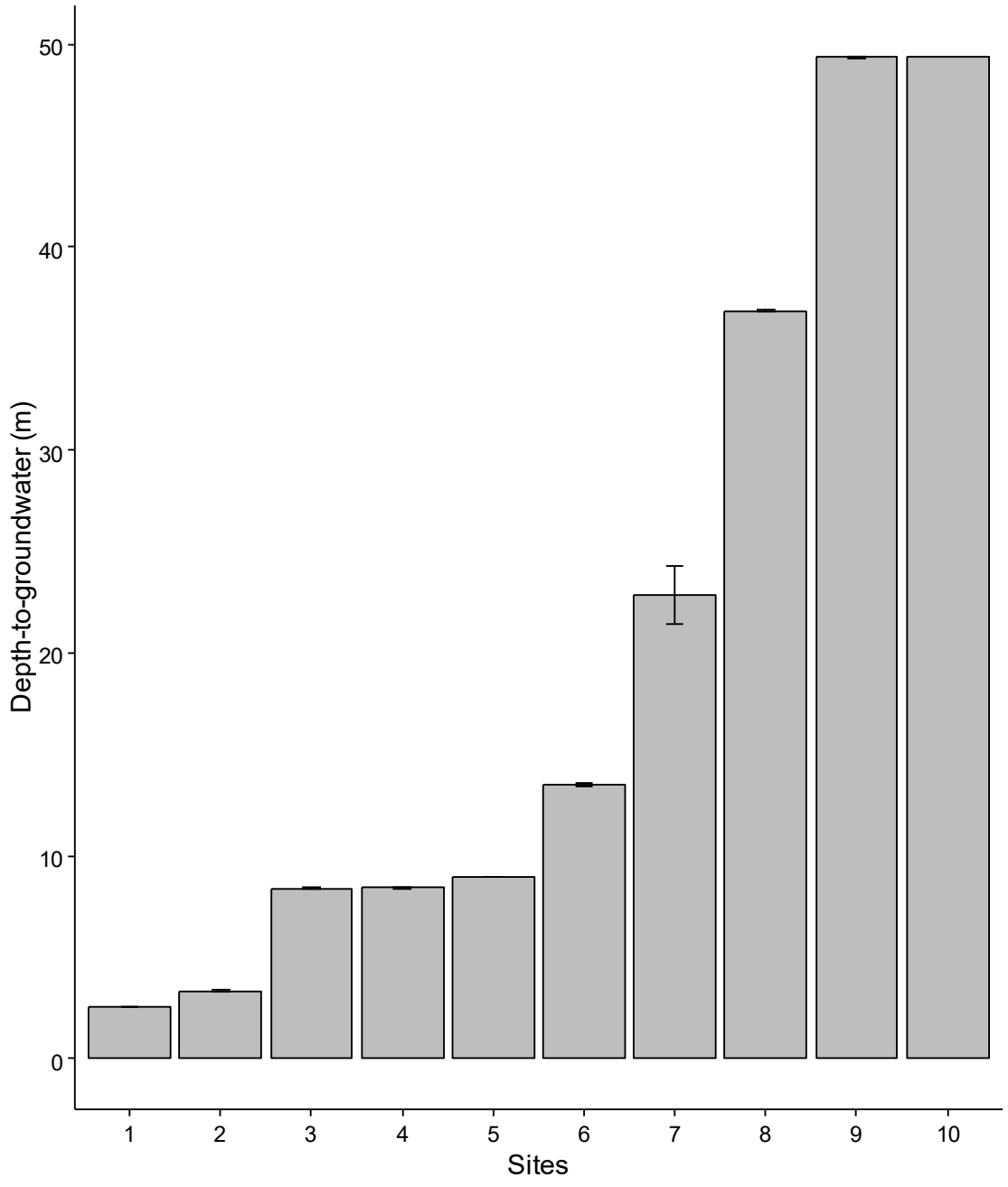


Figure 6.2. Mean (± SE) depth-to-groundwater at the study sites (1982–2012 data, Northern Territory Government).

Table 6.1. Plant species recorded across the 10 study sites in the Ti Tree basin with information on status (native, exotic), growth form (tree, shrub, graminoid, forb), longevity (annual, perennial), distribution (number of sites) and mean abundance across the groundwater gradient.

Species	Status	Growth form	Longevity	Distribution	Mean abundance
Abutilon otocarpum	Native	Shrub	Perennial	2	0.4
Acacia aneura	Native	Shrub	Perennial	3	3.9
Acacia apteneura	Native	Shrub	Perennial	2	2.4
Acacia kempeana	Native	Shrub	Perennial	1	0.6
Acacia melleodora	Native	Shrub	Perennial	1	0.1
Acacia sericophylla	Native	Shrub	Perennial	1	0.7
Acacia tetragonophylla	Native	Shrub	Perennial	1	0.1
Acacia victoriae	Native	Shrub	Perennial	1	0.7
Androcalva loxophylla	Native	Shrub	Perennial	2	3.0
Aristida holathera	Native	Graminoid	Annual	10	7.3
Aristida inaequiglumis	Native	Graminoid	Perennial	1	0.6

Species	Status	Growth form	Longevity	Distribution	Mean abundance
Boerhavia coccinea	Native	Forb	Perennial	1	0.3
Cenchrus ciliaris	Exotic	Graminoid	Perennial	1	4.5
Cenchrus setiger	Exotic	Graminoid	Perennial	2	4.7
Cleome viscosa	Native	Forb	Annual	2	2.4
Dactyloctenium radulans	Native	Graminoid	Annual	2	0.5
Enneapogon polyphyllus	Native	Graminoid	Annual	3	0.6
Eragrostis eriopoda	Native	Graminoid	Perennial	5	0.6
Eremophila latrobei ssp. *glabra*	Native	Shrub	Perennial	3	0.9
Eucalyptus camaldulensis var. *obtusa*	Native	Tree	Perennial	1	0.5
Euphorbia tannensis ssp. *eremophila*	Native	Shrub	Perennial	1	0.1
Evolvulus alsinoides var. *villosicalyx*	Native	Forb	Perennial	3	1.0
Hakea lorea ssp. *lorea*	Native	Shrub	Perennial	2	0.5
Indigofera colutea	Native	Forb	Annual	1	1.1

Species	Status	Growth form	Longevity	Distribution	Mean abundance
Hibiscus burtonii	Native	Shrub	Perennial	1	0.1
Indigophora linifolia	Native	Forb	Perennial	1	0.5
Maireana villosa	Native	Shrub	Perennial	1	0.1
Melaluca glomerata	Native	Shrub	Perennial	1	1.7
Paraneurachne muelleri	Native	Graminoid	Perennial	1	0.2
Portulaca filifolia	Native	Forb	Annual	2	1.4
Ptilotus obovatus	Native	Shrub	Perennial	1	0.1
Rhagodia eremaea	Native	Shrub	Perennial	1	0.4
Senna artemisioides ssp. *artemisioides*	Native	Shrub	Perennial	2	0.3
Tephrosia sp. *Willowra*	Native	Shrub	Perennial	1	0.4
Trachymene incisa	Native	Forb	Perennial	1	0.3
Tribulus astrocarpus	Native	Forb	Annual	2	0.3
Tribulus eichlerianus	Native	Forb	Perennial	2	0.7
Triodia schinzii	Native	Graminoid	Perennial	8	12.8

Table 6.2. Environmental attributes at each of the 10 study sites in the Ti Tree basin. DGW = depth-to-groundwater, FC = field capacity, EC = electrical conductivity, OM = organic matter content.

Site	DGW	Shade index	FC ($gH_2O.g\ soil^{-1}$)	pH	EC ($\mu s.cm^{-1}$)	N ($mg.g^{-1}$)	OM (%)	Clay (%)	Sand (%)	Mg (ppb)	P (ppb)	K (ppb)	Ca (ppb)
1	2.55	1.00	0.23	8.51	1394.25	0.40	1.71	9.39	62.38	134501.87	0.01	20345.06	0.72
2	3.36	1.00	0.23	7.41	65.50	0.43	1.75	9.08	49.61	30433.31	0.04	35795.27	0.34
3	8.40	1.20	0.19	6.53	22.50	0.08	4.68	10.15	69.25	6226.62	0.07	9956.59	0.75
4	8.45	1.28	0.15	6.61	21.50	0.19	1.29	10.27	65.89	3336.60	0.11	6225.65	0.58
5	8.96	1.00	0.19	5.25	18.30	0.23	4.13	9.87	68.50	4099.84	0.09	6477.22	0.76
6	13.50	1.00	0.16	5.49	11.40	0.05	1.92	3.45	83.55	2850.91	0.09	4679.87	0.49
7	22.84	1.05	0.17	6.91	29.60	0.17	1.17	10.97	64.77	5728.58	0.06	8294.84	0.61
8	36.84	1.00	0.15	6.26	10.40	0.07	1.21	6.41	77.07	3761.61	0.07	5808.48	0.44
9	49.34	1.00	0.17	6.12	16.30	0.14	1.34	10.32	67.23	4832.34	0.07	7238.33	0.40
10	49.40	1.29	0.19	6.10	18.30	0.26	2.35	12.61	61.82	4300.71	0.10	8047.59	0.53

6.2.4 Statistical analyses

Total plant abundance, species richness, Simpson's diversity index and Pielou's evenness (response variables) were related to DGW and the environmental attributes (explanatory variables) using multiple regression analysis (Lyons and Hutcheson 1986; Crawley 2007). General linear modelling with a normal error structure and an identity link was employed for continuous response variables (Simpson's diversity index, Pielou's evenness). Generalized linear modelling with a Poisson error structure and a log link was employed for response variables that were counts (total plant abundance, species richness). Where over-dispersion was detected in Poisson regression models, the standard errors were corrected using quasi-likelihood estimation (Zuur *et al.* 2009). For each response variable, the minimum adequate model (MAM) was determined, which is a model containing only those explanatory variables able to explain significant and unique variation in the response variable (Crawley, 2007). This modelling identified whether DGW or any environmental attributes were able to explain significant variation in the response variables over and above the effects of all other explanatory variables.

To account for multicollinearity among the environmental attributes in regression modelling, a principal component analysis (PCA) was used to convert the large number of correlated environmental attributes (Table 6.3) into a reduced set of linearly uncorrelated variables. DGW was kept separate from the calculation of principal components given my specific aim to identify its independent contribution to the ecological properties of plant assemblages along the DGW gradient. Component loadings were rotated (using varimax rotation) following extraction to maximize the sum of the variances of the squared loadings. The PCA produced three major independent principal components (PC1, PC2 and PC3) that together explained 83.5% of variation in environmental attributes (Table 6.4). PC1 represented comparatively high

soil FC, pH, EC, N, Mg, K and low soil P. PC2 represented more open conditions (low shade scores), low soil OM, low clay content, high soil pH and high sand content. PC3 represented low soil EC, OM, Ca and sand content.

In building MAMs, DGW and PC1, PC2, PC3 were first included in a full model. The process of model simplification followed, which dropped one explanatory variable in turn and each time applied an analysis of deviance test. The most non-significant term was omitted from the next round of analysis and the process of model simplification continued (Crawley, 2007).

Plant longevity was related to DGW, PC1, PC2 and PC3 using multiple logistic regression models (McCullagh and Nelder 1989). Generalized linear modelling with a binomial error distribution and a logit link was employed for longevity as this response variable had two categorical outcomes (i.e. annual and perennial). A MAM was built using the procedure described above to determine whether DGW or the environmental attributes were able to explain significant variation in plant longevity over and above the effects of all other explanatory variables.

Canonical correspondence analysis (CCA) was employed to relate growth form to DGW and PC1, PC2 and PC3 as the response variable growth form had four categorical outcomes and logistic regression was not appropriate. A forward-fitting model selection procedure was applied to determine if DGW was significantly and independently related to growth form while accounting for the effects of the PCs. Redundancy was verified by checking variance inflation factors (VIF) and relationships were tested using Monte Carlo permutation tests (N = 1000). This same CCA procedure was then employed to relate variation in species composition among sites to DGW.

All statistical analyses were conducted using R 3.1.3 (R Core Team 2015), with packages stats (R Core Team 2015), vegan (Oksanen *et al*. 2015), mlogit (Croissant 2013), ggplot2 (Wickham 2009) and gridExtra (Auguie 2015).

Table 6.3. Pairwise correlation matrix among environmental attributes and depth-to-groundwater (DGW). FC = field capacity, EC = electrical conductivity, OM = organic matter content. * P < 0.05, ** P < 0.01, *** P < 0.001.

Attributes	1	2	3	4	5	6	7	8	9	10	11	12	13
1. DGW	1.00												
2. Shade	0.14	1.00											
3. FC	-0.36	-0.22	1.00										
4. pH	-0.37	-0.07	0.65*	1.00									
5. EC	-0.36	-0.24	0.62	0.76*	1.00								
6. N	-0.34	-0.08	0.86**	0.66*	0.54	1.00							
7. OM	-0.30	0.19	0.18	-0.33	-0.13	-0.12	1.00						
8. Clay	0.22	0.53	0.30	0.23	0.03	0.40	0.17	1.00					
9. Sand	0.17	-0.15	-0.73*	-0.59	-0.21	-0.86**	0.06	-0.67*	1.00				
10. Mg	-0.41	-0.28	0.72*	0.82**	0.99***	0.64*	-0.14	0.03	-0.32	1.00			
11. P	0.36	0.55	-0.76*	-0.83**	-0.79**	-0.58	0.14	0.01	0.41	-0.85**	1.00		
12. K	-0.44	-0.26	0.83**	0.68*	0.36	0.82**	-0.09	0.09	-0.78**	0.51	-0.68*	1.00	
13. Ca	-0.44	0.21	0.11	0.05	0.35	-0.04	0.65	0.27	0.12	0.27	-0.08	-0.27	1.00

Table 6.4. Principal component analysis on the environmental attributes with loadings on the first three components (PC1, PC2 and PC3).

	PC1	PC2	PC3
Eigenvalue	5.89	2.25	1.89
Cumulative % variation explained	49.1	67.8	83.5
Eigenvectors			
Shade	-0.10	-0.51	0.04
FC	0.37	-0.11	-0.02
pH	0.36	0.40	-0.01
EC	0.32	0.14	-0.35
N	0.36	-0.15	0.17
OM	-0.06	-0.35	-0.41
Clay	0.11	-0.56	0.09
Sand	-0.30	0.33	-0.33
Mg	0.36	0.14	-0.27
P	-0.36	-0.22	0.13
K	0.34	0.01	0.27
Ca	0.03	-0.28	-0.63

6.3 Results

Total plant abundance, species richness, and species diversity, but not Pielou's evenness, were all related significantly and independently to one or more of DGW and environmental PCs (Table 6.5). High total plant abundance was related to shallow groundwater depth (Fig. 6.3a) and high values of PC1 (high FC, pH, EC, N, Mg, K and low P; Fig. 6.3b). High species richness was related to low values of PC2 (high pH, sand content, open conditions and low OM and clay content; Fig. 6.3c). High species diversity was related to low values of PC2 (Fig. 6.3d) and low values of PC3 (low EC, OM, Ca and sand content; Fig. 6.3e). Removing exotic plant species from analyses led to the disappearance of the significant relationship between DGW and total plant abundance (d.f. = 1, change in deviance = 7.87, $P = 0.44$).

Plant species composition varied among sites significantly and uniquely in relation to PC1 and PC3 ($P = 0.001$; Fig. 6.4). Low values of PC1 were associated with species composition at the two shallowest sites, while high values of PC1 were associated with species composition at all other sites. Low values of PC3 were associated with the second shallowest site (site 2) and the intermediate to deep sites (Site 10, 9, 8, 7, 6 and 4), while high values of PC3 were associated with shallowest site (site 1) and two intermediate depth sites (sites 3, 5). Highly variable suites of plant species characterized assemblages among the sites (Table 6.6). Of note, however, was that one third of the total 38 species were found only at either of the two shallowest sites (8 species, e.g. *Indigofera colutea*, *Dactyloctenium radulans*, *Cenchrus ciliaris* and *Cenchrus setiger*) or the two deepest sites (5 species, e.g. *Acacia melleodora*, *Hibiscus burtonii*, *Euphorbia tannensis* and *Trachymene incisa*).

Plant longevity was significantly and independently related to DGW (Table 6.7). The proportion of perennial species increased and the proportion of annual species

decreased as DGW increased (Fig. 6.5). Only one of the seven annual species was found at the deepest sites, and that species (*Aristida holathera*) was widespread and the only species to be found at all ten sites. Plant growth form was significantly and uniquely related to DGW and PC3 ($P = 0.01$; Fig. 6.6). Comparatively more shrub species were found at deep groundwater sites than shallow sites. Low PC3 values were weakly associated with an increase in forb species, while high PC3 values were weakly associated with an increase in tree species.

Table 6.5. Minimum adequate models (MAM) relating (a) total abundance, (b) species richness, (c) Simpsons diversity index and (d) Pielou's evenness to depth-to-groundwater (DGW), PC1, PC2 and PC3. Significant P-values shown in bold.

Attributes		d.f.	Change in deviance	*P*
(a) Total abundance				
Attributes in MAM				
	DGW	1	18.82	**0.01**
	PC1	1	25.17	**< 0.001**
Attributes not in MAM				
	PC2	1	10.59	0.21
	PC3	1	12.61	0.16
(b) Species richness				
Attributes in MAM				
	PC2	1	11.83	**0.02**
Attributes not in MAM				
	DGW	1	3.25	0.53
	PC1	1	3.90	0.42
	PC3	1	0.47	0.49
(c) Simpson's diversity index				
Attributes in MAM				
	PC2	1	0.08	**0.02**
	PC3	1	0.09	**0.01**
Attributes not in MAM				
	DGW	1	0.04	0.89
	PC1	1	0.04	0.70
(d) Pielou's evenness				
Attributes in MAM				
	PC3	1	0.07	0.12
Attributes not in MAM				
	DGW	1	0.04	0.30
	PC1	1	0.05	0.18
	PC2	1	0.06	0.15

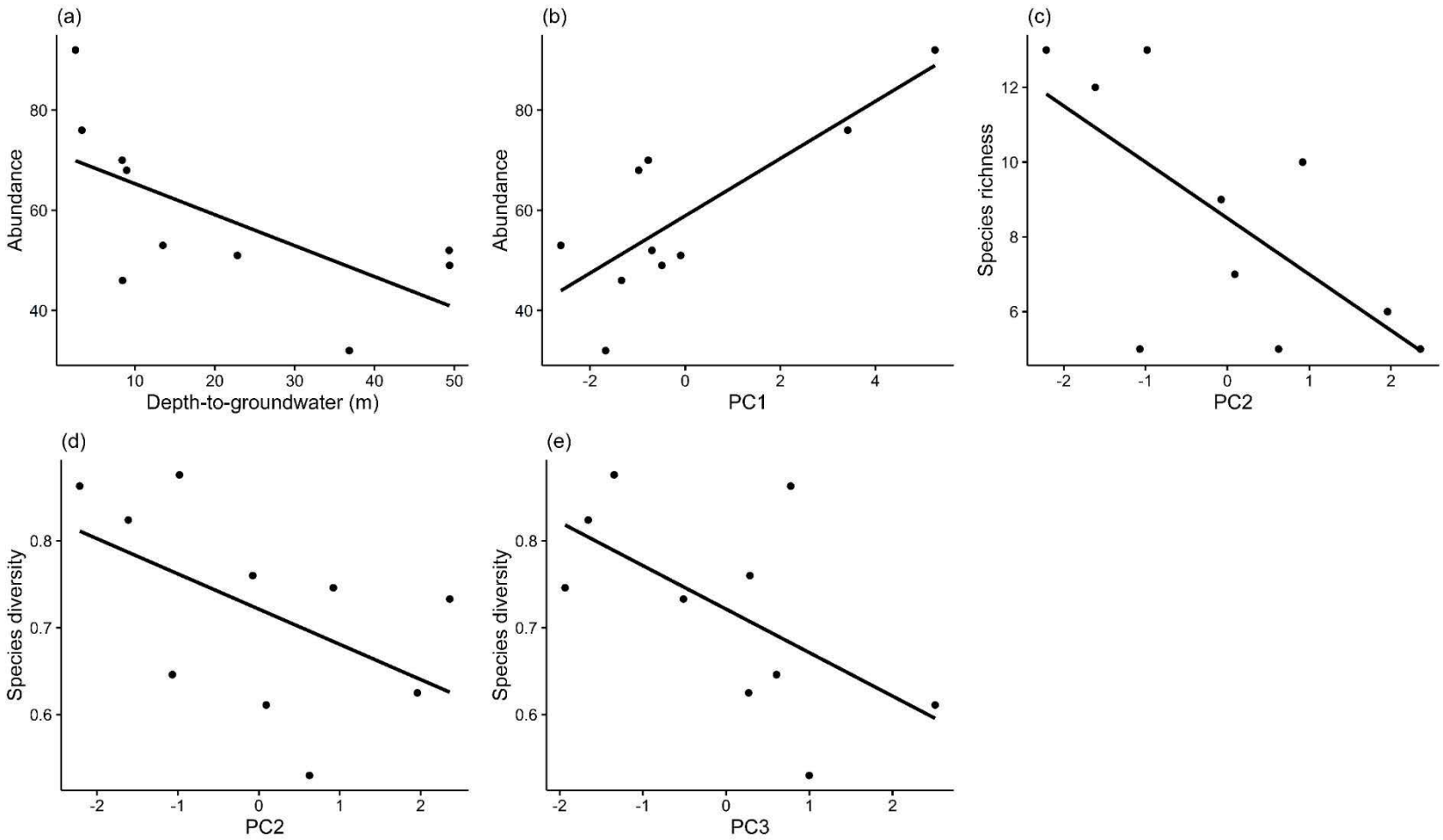


Figure 6.3. Bivariate relationships between (a) total plant abundance and DGW, (b) total plant abundance and PC1, (c) species richness and PC2, (d) species diversity and PC2 and (e) species diversity and PC3. Points represent sites.

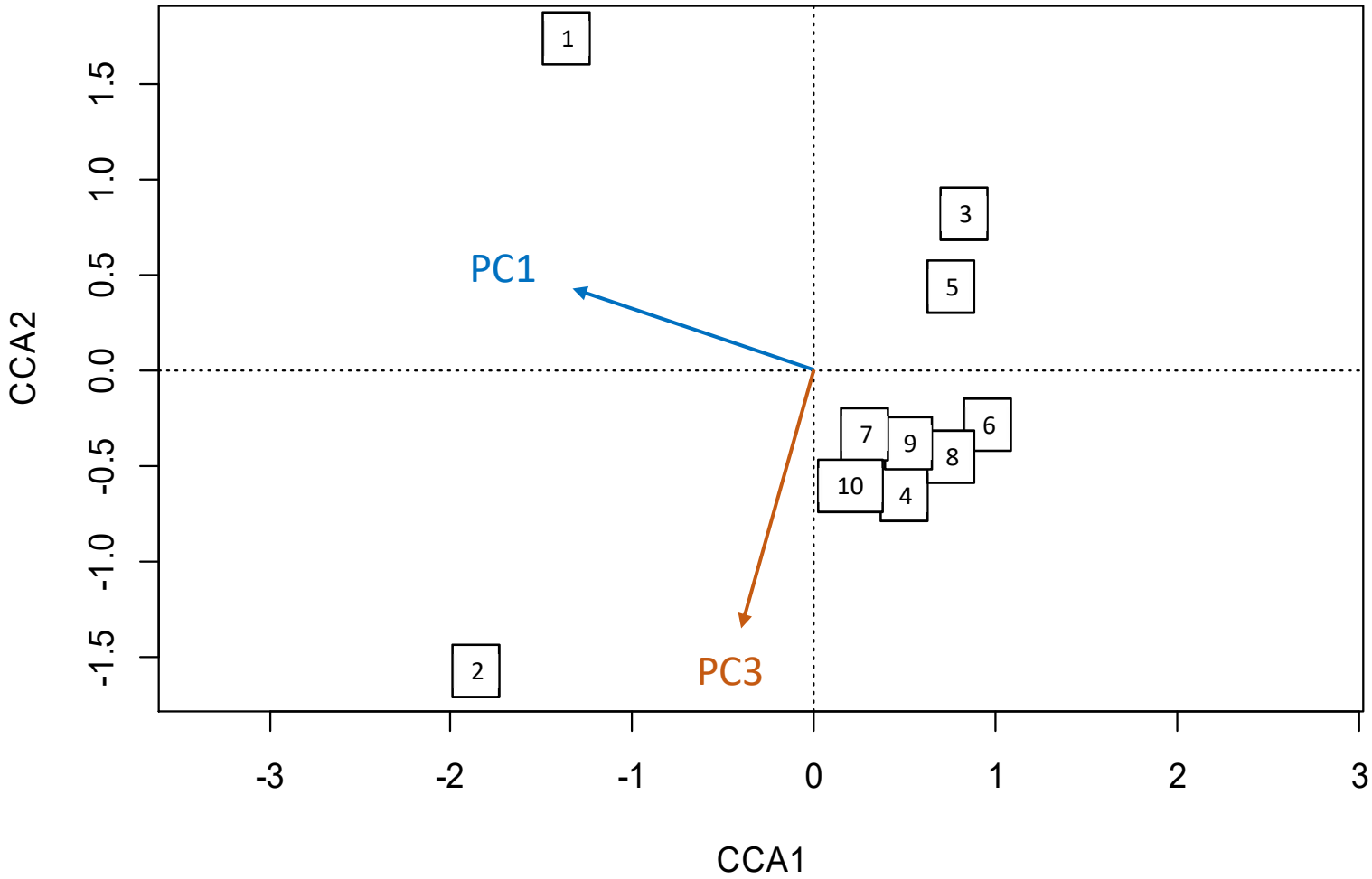
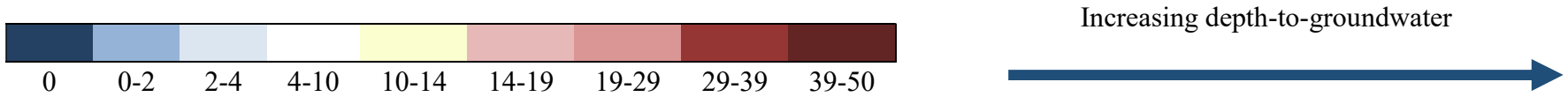
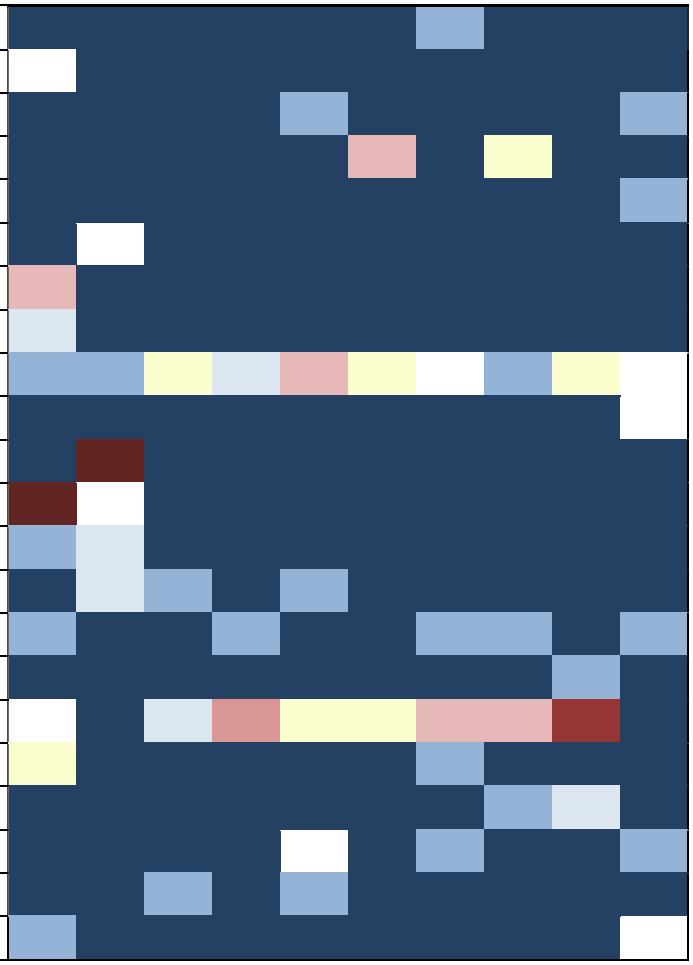


Figure 6.4. CCA ordination biplot showing relationships between significant environmental attributes (arrows) and the species composition of sites numbered 1 (shallow groundwater) to 10 (deep groundwater).

Table 6.6. Heat map showing abundances of all plant species as a function of depth-to-groundwater. The scale refers to number of individuals (abundance) of each species at each site.



			Sites									
Family	**Species**	**Status**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Amaranthaceae	*Ptilotus obovatus*	Native										
Amaranthaceae	*Rhagodia eremaea*	Native										
Apiaceae	*Trachymene incisa*	Native										
Capparaceae	*Cleome viscosa*	Native										
Chenopodiacae	*Maireana villosa*	Native										
Convolvulaceae	*Evolvulus alsinoides* var. *villosicalyx*	Native										
Euphorbiaceae	*Euphorbia tannensis* subsp. *eremophila*	Native										
Fabaceae/Caesalpinioideae	*Senna artemisioides* subsp. *artemisioides*	Native										
Fabaceae/Faboideae	*Indigofera colutea*	Native										
Fabaceae/Faboideae	*Indigophora linifolia*	Native										
Fabaceae/Faboideae	*Tephrosia* sp. *Willowra*	Native										
Fabaceae/Mimosoideae	*Acacia aneura*	Native										
Fabaceae/Mimosoideae	*Acacia aptaneura*	Native										
Fabaceae/Mimosoideae	*Acacia kempeana*	Native										
Fabaceae/Mimosoideae	*Acacia melleodora*	Native										
Fabaceae/Mimosoideae	*Acacia sericophylla*	Native										

Fabaceae/Mimosoideae	*Acacia tetragonophylla*	Native	
Fabaceae/Mimosoideae	*Acacia victoriae*	Native	
Malvaceae	*Abutilon otocarpum*	Native	
Malvaceae	*Androcalva loxophylla*	Native	
Malvaceae	*Hibiscus burtonii*	Native	
Myrtaceae	*Eucalyptus camaldulensis* var. *obtusa*	Native	
Myrtaceae	*Melaluca glomerata*	Native	
Nyctaginaceae	*Boerhavia coccinea*	Native	
Poaceae	*Aristida holathera*	Native	
Poaceae	*Aristida inaequiglumis*	Native	
Poaceae	*Cenchrus ciliaris*	Exotic	
Poaceae	*Cenchrus setiger*	Exotic	
Poaceae	*Dactyloctenium radulans*	Native	
Poaceae	*Enneapogon polyphyllus*	Native	
Poaceae	*Eragrostis eriopoda*	Native	
Poaceae	*Paraneurachne muelleri*	Native	
Poaceae	*Triodia schinzii*	Native	
Portulacaceae	*Portulaca filifolia*	Native	
Proteaceae	*Hakea lorea* subsp. *lorea*	Native	
Scrophulariaceae	*Eremophila latrobei* subsp. *glabra*	Native	
Zygophyllaceae	*Tribulus astrocarpus*	Native	
Zygophyllaceae	*Tribulus eichlerianus*	Native	

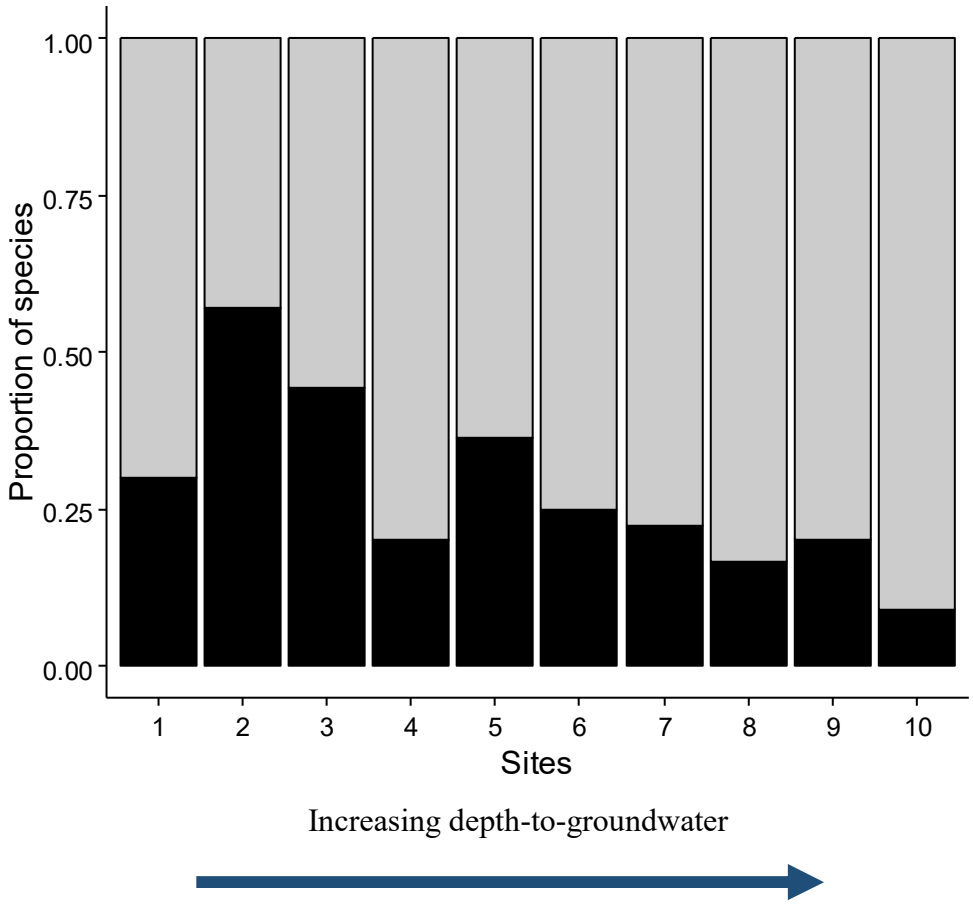


Figure 6.5. Spine plot showing the relative distributions of annual (black) and perennial (grey) species among sites.

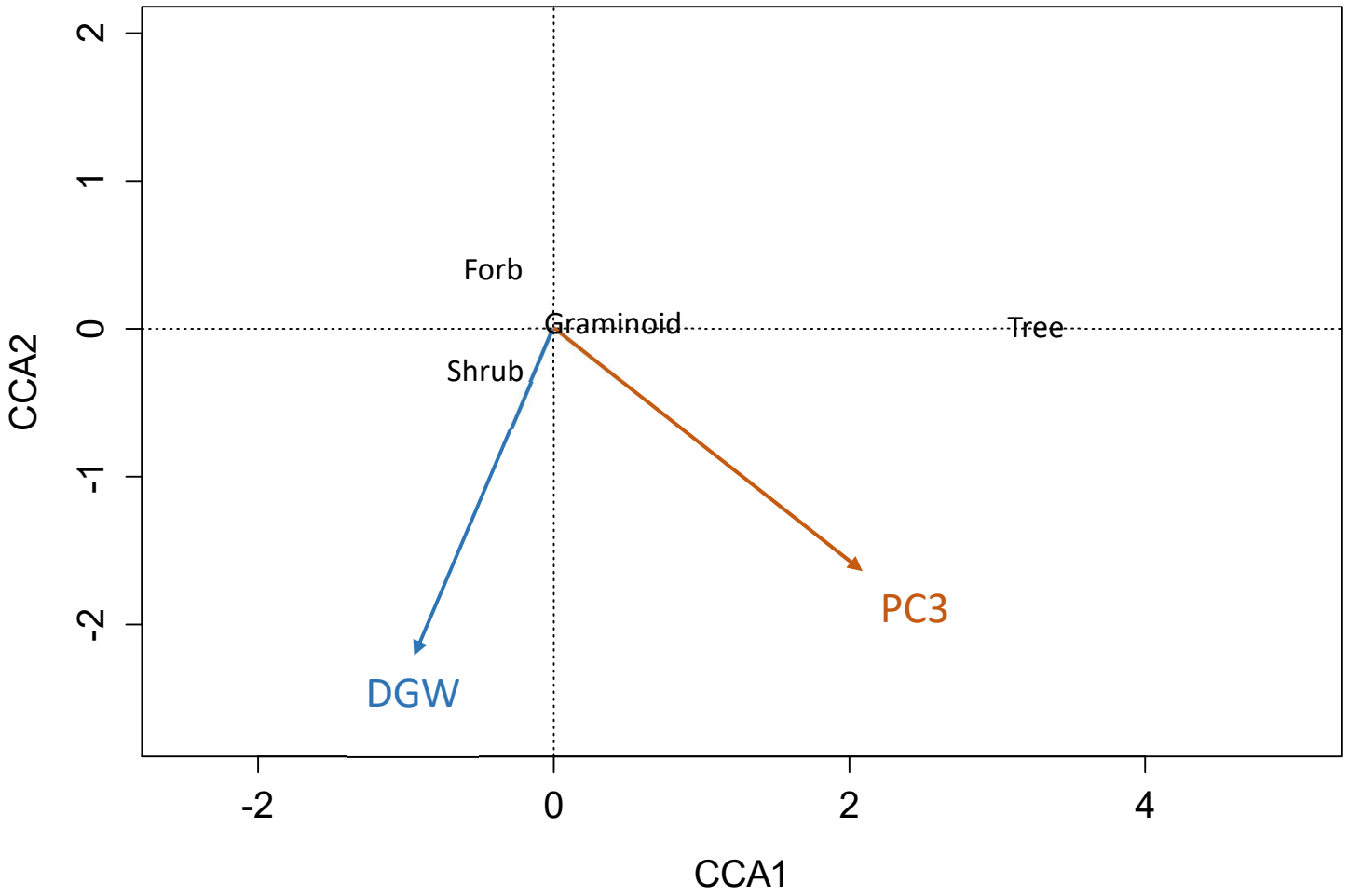


Figure 6.6. CCA ordination biplot showing relationships between significant environmental attributes (arrows) and plant growth form.

Table 6.7. Minimum adequate models relating plant longevity to depth-to-groundwater (DGW), PC1, PC2 and PC3. Significant P-values shown in bold.

Attributes		d.f.	Change in deviance	*P*
Attributes in MAM				
	DGW	1	92.13	**0.03**
Attributes not in MAM				
	PC1	1	91.14	0.96
	PC2	1	87.00	0.77
	PC3	1	87.41	0.52

6.4 Discussion

In this study I set out to answer the question: is a naturally occurring gradient in DGW related to the ecological properties of vegetation in arid-zone woodlands in central Australia? I found that DGW had a significant influence over three different ecological properties of the plant assemblages along the gradient. Total plant abundance was significantly related to DGW, with higher abundance found at the shallowest sites. Both plant longevity and plant growth form were significantly related to DGW, such that the proportion of perennial species increased and the proportion of annual species decreased as DGW increased and more shrub species were found at deep groundwater sites than shallow sites. I found that species richness, species diversity, Pielou's evenness nor species composition were significantly related to DGW. Below, I discuss relationships between DGW and the ecological properties of plant assemblages in the arid-zone woodland.

High total plant abundance was significantly related to shallow DGW (Fig. 6.3a) and high values of PC1 (high FC, pH, EC, N, Mg, K and low P; Fig. 6.3b). This finding is in contrast to the patterns found in the mesic woodland, where DGW was found to have no differential limiting effect on how many plants occurred in plant assemblages

across the gradient. However, this finding parallels results from previous work in the Mediterranean-type climate of south-western Australia where high total plant abundance was also related to shallow groundwater depth (Froend and Sommer 2010; Sommer and Froend 2011). Although the sites used in the Froend and Sommer (2010) and Sommer and Froend (2011) studies were also established around bores of known DGW, these two studies differ from the present study in that only two sites were used for monitoring before and after groundwater abstraction, whilst the relationship between total plant abundance and DGW was driven by the combined effects of drought and groundwater extraction. The high abundance relationship with shallow groundwater identified in the present study appears to be driven by the presence of two exotic grass species, *Cenchrus ciliaris* and *Cenchrus setiger,* that were found in high abundance at the two shallowest sites (Table 6.6). The increased field capacity of the soils at the two shallowest sites, suggest that these soils would enable higher water retention after rainfall events. I suggest that the combination of increased soil nutrient content and high field capacity are highly conductive for the growth of these exotic grass species, enabling them to become the dominant species in the landscape. Indeed, previous work has indicated that increased soil nutrients and higher field capacity are critical soil prerequisites for exotic plant invasion (Lake and Leishman 2004), particularly in low fertility soils such as the soils in my study area.

Variation in DGW was also related to both plant longevity and growth form. I found that the proportion of perennial species increased and the proportion of annual species decreased as DGW increased (Fig. 6.5) and that more shrub species were found at deep groundwater sites than shallow sites.

In the present study, the proportion of perennial species increased and the proportion of annual species decreased as DGW increased. This result contradicts

previous studies in arid environments which have shown that for arid sites receiving relatively lower rainfall, perennials are actively selected against due to the low probability of surviving extended drought periods (Ehleringer 1985), while studies from the arid-zone in South Africa have linked the dominance of perennials to higher rainfall sites (Cowling *et al.* 1999). In addition, short life cycles (e.g. annuals and ephemerals) are important strategies utilised by many plant species as a means of dealing with drought (Diaz *et al.* 2007). For example, in arid and semi-arid environments following short rainfall events, annual plant species plants are capable of utilising short pulses of soil moisture by germinating, growing and reproducing rapidly to avoid transpirational water loss before the environment becomes too dry (Borchert 1994; McKay *et al.* 2003; Markesteijn and Poorter 2009). As a result, annual plant species often form the main component of vegetation cover in arid and extreme arid zones following sufficient rainfall events (Kutiel and Lavee 1999).

I also found that significant relationships emerged between plant growth form and DGW and PC3 (low EC, OM, Ca and sand content). The finding that more shrub species were found at the deep groundwater sites compared to the shallow sites may be due to a number of traits that the shrub species possess which enable drought resistance, allowing more shrub species to persist at the deep groundwater sites. For example, *Acacia aneura*, the most abundant shrub across the deep groundwater sites, possess a suite of traits that facilitates a high degree of drought resistance (Cleverly *et al.* 2016). These traits include larger wood density, higher leaf mass per area (LMA, ratio of leaf dry mass to leaf area) and larger Huber values (ratio of sapwood cross-sectional area to leaf area) (O'Grady *et al.* 2009). Larger wood densities are correlated with enhanced resistance to xylem embolism, reducing soil-to-water hydraulic conductance and reduced transpiration rates (Wright *et al.* 2006; Zhang *et al.* 2009), higher LMA is

correlated with reduced transpiration rates (Poorter *et al.* 2009), while larger Huber values result in an increased capacity of sapwood to transport water to leaves (Magnani *et al.* 2002). Furthermore, recent work in Mulga Woodlands and *Corymbia* savanna in the Ti Tree Basin has shown that a storage reservoir of soil moisture accumulates within the rooting zone of *A. aneura* due to restrictions in water infiltration by a siliceous hardpan located below *A. aneura* (Cleverly *et al.* 2016). This suggests that other shrub species may also be utilising the soil moisture reservoir enabling more shrub species to persist with increasing DGW. While the siliceous hardpan may aid in soil water accumulation after rainfall events, it may also hinder access to the water table (Cleverly *et al.* 2016). Further studies examining the influence of the siliceous hardpan on vegetation properties may provide additional information and strengthen our understanding of ecological properties in these arid-zone woodlands.

I found that high species richness was significantly related to low values of PC2 (high pH, sand content, open conditions and low OM and clay content), and high species diversity was significantly related to low values of PC2 and low values of PC3 (low EC, OM, Ca and sand content), whilst species composition was significantly related to PC1 (high FC, pH, EC, N, Mg and low P) and PC3. In addition, I found a significant relationship between plant growth form and PC3. Although this study did not specifically set out to test predictions about soil relationships with species richness, species diversity, species composition and plant growth form, it should be noted that previous studies have found that species composition, richness, diversity and growth form varies in relation to a wide range of soil attributes, including EC, pH, OM, clay, Mg, N, P and K (e.g. Schulze 1982; McLendon and Redente 1991; Evans and Belnap 1999; Snyman 2002; Li *et al.* 2004; Zhu *et al.* 2013). Given the established relationship between soil fertility measures and species composition, richness, diversity and plant

growth form, it is important to account for their potential confounding influence on the ecological properties of vegetation when examining the role of DGW.

The results of this study have shown that there were significant relationships between DGW and total plant abundance, plant longevity and growth form in the arid-zone woodland. Whilst the majority of previous studies have showed a direct link between variation in vegetation properties in response to changes in DGW as a result of groundwater extraction in arid and semi-arid regions of Australia (e.g. Groom *et al.* 2000; 2001; Zencich *et al.* 2002; Froend and Drake 2006; Froend and Sommer 2010; Sommer and Froend 2011; Sommer and Froend 2014), the results in the present study have highlighted responses of vegetation properties to natural gradients in DGW, that is not a result of groundwater extraction. Thus, the results from this study have indicated that reductions in the level and volume of groundwater through reduced groundwater recharge or unsustainable groundwater extraction will alter the ecological properties of arid-zone woodland plant assemblages through reductions in total plant abundance, shifts in plant longevity and changes in plant growth form as DGW declines. Future studies examining how native vegetation assemblages respond when the influence of the invasive grass species, *C. ciliaris* and *C. setiger*, are removed may provide important baseline information that could be used for ecological restoration management.

Chapter 7: General discussion

7.1 Were the aims of the thesis met?

The overall aim of this thesis was to provide fundamental baseline information about how terrestrial GDEs are structured and how they function along naturally occurring gradients in DGW. The research presented in this thesis investigated ecological relationships between spatial variation in DGW and the structure and function of vegetation assemblages in mesic woodlands which have not been exposed to groundwater extraction. This thesis has identified several attributes of the ecological properties of vegetation assemblages that were linked to natural spatial variation in DGW across a mesic and an arid-zone GDE. Therefore, the broad aim of this thesis was fulfilled.

In order to identify details of the ecological properties that were linked to natural spatial variation in DGW, four specific aims were addressed in this thesis:

1. *To identify vegetation properties, such as species composition, richness and abundance, that vary in relation to a naturally-occurring gradient in DGW in mesic Eucalyptus woodlands.*

In Chapter 2, I investigated relationships between the vegetation properties of mesic woodland vegetation and a naturally occurring DGW gradient in south-eastern Australia. My findings in this chapter revealed contrasting results for different vegetation properties. I found that plant species composition varied significantly as a function of DGW, independently of 14 other environmental attributes. Patterns of differentiation of species composition along the DGW gradient were driven by nine species from the understorey that shifted in abundance across the shallow, intermediate

and deep sections of the gradient. In contrast, I found that neither plant species richness nor total plant abundance varied in relation to DGW or any of other environmental attributes.

2. *To identify plant functional traits that vary in relation to a naturally-occurring gradient in DGW in mesic Eucalyptus woodlands.*

I met this aim by examining patterns in plant functional trait variation along a natural DGW in mesic woodlands (Chapters 3 & 4). In Chapter 3, I investigated variation in seven plant functional traits in relation to DGW for the species identified from Chapter 2 using data obtained from published sources. I was unable to find a significant link between the seven plant functional traits and variation in DGW. One possible explanation that emerged for the lack of significant relationships between DGW and the plant traits was that the manner of data collection biased the analyses. I argued that data for continuous traits such as leaf mass per area (LMA), plant height and seed mass are better collected within the relevant study region. This is because compiling data from across a broad region like the Australian continent to give one value of a trait for a species has the potential to ignore important regional and local trait variation. In Chapter 4, I sought to overcome the shortfalls of using trait data obtained from published biological sources by performing extensive field based measurements focusing on three strategic plant functional traits – LMA, plant height and seed mass – using fresh data on these traits that I collected from the study sites of Chapter 2. For assemblage level mean trait values, I found that LMA, plant height and seed mass did not vary in a systematic way in relation to DGW. Whilst I found that significant relationships emerged between community weighted mean plant height and the coefficient of variation seed mass for whole assemblages, relationships were not highly

significant and if the large number of statistical analyses performed with this dataset are taken into account, the statistical significance of the findings means that they should be interpreted cautiously. Therefore, I consider that the results of Chapters 3 and 4 demonstrate that interspecific variations in the functional traits examined in this thesis, when investigated at the whole assemblage level, are not largely driven by spatial changes in DGW in a way that has substantial explanatory power for ecosystem functioning in this mesic GDE. Such functional trait variation may, however, be important in relation to DGW when considered in specific subsets of plant assemblages such as trees (Zolfaghar *et al.* 2014; Zolfaghar *et al.* 2015a).

3. *To determine whether local adaptation differentiates seedling growth patterns of Hakea dactyloides between populations from shallow and deep ends of a naturally-occurring gradient in DGW.*

In chapter 5, I compared seedling growth traits and ecophysiological traits between deep and shallow populations and between conditions of high vs low water availability. I tested predictions to determine if local adaptation differentiates seedling growth patterns of *Hakea dactyloides* between populations from the shallow and deep ends of the DGW gradient. I found evidence for local adaptation in one leaf trait and one belowground growth trait, with the deep population maintaining moderately narrow leaf widths across both treatments while the shallow population invested in higher belowground biomass across both water treatments. I also found evidence for phenotypic plasticity in three physiological traits, one leaf trait, two belowground growth traits and one aboveground growth trait among populations from the shallow and deep ends of the gradient. Seedlings from both populations demonstrated increased root lengths, reduced stomatal conductance and lower transpiration rates which led to increased water use efficiency in

the fast-draining treatments. Shallow populations decreased leaf widths and increased root:shoot ratios in the fast-draining treatments, while the deep population increased belowground biomass in the fast-draining treatment. The phenotypic plasticity responses of these traits to variation in water availability are likely to enhance seedling establishment in the shallow and deep habitats and may allow both populations to successfully establish in a wide range of soil moisture conditions, whilst locally adapted populations are likely to demonstrate enhanced seedling performance among population growing in home environments.

4. *To identify vegetation properties that vary in relation to a naturally-occurring gradient in DGW in arid-zone woodlands.*

The final research aim of this thesis was achieved in Chapter 6, where I investigated relationships between a naturally occurring gradient in DGW and the ecological properties of vegetation in arid-zone woodlands of central Australia. In contrast to the mesic woodlands, I found that that DGW had a significant influence over three different vegetation properties of the plant assemblages along the DGW gradient in the arid-zone woodlands. I found that high total plant abundance was significantly related to shallow groundwater depth, with the presence of two exotic grass species responsible for driving the significant abundance patterns detected along the gradient. I also found that plant longevity and plant growth form were both significantly related to DGW, while the proportion of perennial species increased and the proportion of annual species decreased as groundwater depth increased and that more shrub species were found at deep groundwater sites compared to the shallow sites. However, I found that species richness, species diversity, Pielou's evenness nor species composition were significantly related to DGW.

7.2 Mesic and arid-zone woodland responses to depth-to-groundwater

The research presented in this thesis, has shown that significant relationships emerged between DGW and total plant abundance, plant longevity and growth form in the arid-zone woodland. Whereas in the mesic woodland, plant species composition varied significantly among shallow, intermediate and deep sections of the groundwater gradient, with DGW correlated with variation in species composition independently of the other environmental attributes. My aim in comparing the responses of arid-zone and mesic woodlands was to identify if the relationships that emerged for mesic woodlands, described in Chapter 2 of this thesis, were consistent in the context of the ecological properties of arid-zone woodlands described in Chapter 6. The identification of a consistent mechanism operating irrespective of ecosystem type has enormous implications for the management of GDEs, as a single management program could then be implemented for GDEs across these two vastly different ecosystems. The different responses between these two vastly different ecosystems confirms that a single management program is not applicable and therefore cannot be successfully implemented across mesic and arid-zone GDEs. Future management programs should be individually evaluated and based on each specific ecosystem type.

The inherent different ecological responses of the mesic and arid-zone woodlands to DGW, may be attributed to the substantially different rainfall regimes displayed between these two vastly different ecosystems. In the arid-zone woodland, the comparative low rainfall regime means that water availability is the principal limiting factor influencing plant growth, productivity and recruitment (Hadley and Szarek 1981; Smith *et al.* 1995; Li *et al.* 2001). Thus, my research has shown that groundwater has an especially important influence on the ecological properties in this arid-zone woodland. The research presented in this thesis has also shown that even in the mesic woodland, an

ecosystem with comparatively high rainfall and where water is not a limiting factor for plant growth, DGW still exerts an important influence over species composition.

7.3 Research significance and management implications

The results from this thesis provide important baseline information on the ecological properties of woodland vegetation assemblages prior to groundwater extraction and also provide an understanding of the way vegetation assemblages in these systems may respond to future groundwater extraction. Importantly, my research has demonstrated that natural spatial variation in DGW has a considerable influence over the ecological properties of vegetation assemblages in mesic woodlands, whilst also having an important influence over vegetation properties of arid-zone woodlands. Specifically, my work demonstrates that in mesic woodlands, DGW has a profound influence over plant species composition, that is driven by detectable changes in the abundances of nine key understorey species. I also found evidence that partially supports my predictions that local adaptation differentiates seedling growth patterns of *H. dactyloides* between populations from the shallow and deep ends of the DGW gradient. Finally, my research provides compelling evidence that DGW exerts a strong influence over total plant abundance, plant longevity and plant growth from in arid-zone woodlands.

These findings have important implications for natural resource management and the conservation of GDEs, where the baseline information arising from this thesis can be used to set sustainable thresholds for groundwater extraction across the region, aid in the establishment of ecological restoration targets and also to help in establishing monitoring programs during any planned groundwater extraction. Future groundwater extraction across the Kangaloon region should be implemented in a sustainable way that takes into account the water requirements of the woodland vegetation assemblages

occurring across the region. Decisions for acceptable and sustainable thresholds for groundwater extraction may not necessarily be straight forward and are likely to require the implementation of an adaptive management strategy. Resource allocation during future monitoring efforts may be best directed towards two particular species, one of these species, *Entolasia stricta*, I found to be highly abundant at the shallow sites and declined in abundance across the intermediate to deep sites, the other species *Scutellaria humilis*, I found to be highly abundant at the shallow sites and absent from the intermediate and deep sites. During groundwater extraction, monitoring for declines in the abundances of these two species at the shallow sites may provide preliminary indications of impacts from declines in groundwater levels and help to identify acceptable predefined thresholds in the abundances of these species.

7.4 Future research directions

The research presented in this thesis provides detailed information on relationships between natural spatial variation in DGW and the ecological properties of vegetation assemblages in mesic and arid-zone woodlands. Whilst this research focused on relationships between natural spatial variation in DGW and woodland vegetation assemblages, future studies involving relationships between natural spatial variation in DGW and the ecological relationships of other vegetation associations such as shrublands, sedgelands and swamplands is warranted to determine if ecological properties in these vegetation associations match those found in this thesis.

Whilst I conducted detailed investigations into ecosystem functioning via examinations of interspecific variation in plant functional traits as a function of the DGW gradient, there remains considerable scope for further research into intraspecific variation in plant functional traits as a function of the DGW gradient. Examination of

intraspecific variation in plant functional traits may help to explain the important compositional patterns presented in Chapter 2.

Patterns of local adaptation and phenotypic plasticity in seedling growth traits of *H. dactyloides* detected between populations from the shallow and deep ends of the DGW gradient, raises speculation that local adaptation may be evident and thus responsible for differentiating growth patterns in populations of other species occurring along the gradient. Therefore, research into patterns of local adaptation in seedling growth traits between populations for other species, populations and functional groups of species occurring along the gradient may provide important information that may help to explain the compositional patterns presented in Chapter 2.

Further research that incorporates relationships between natural spatial gradients in DGW and phylogenetic diversity of vegetation assemblages occurring across the gradient will complement the research presented in this thesis on taxonomic and functional diversity and may provide insight into evolutionary relationships of the vegetation assemblages occurring across the Kangaloon study area. Given that evolutionary relationships may affect ecological processes and dynamics due to integrated phenotypic differences among taxa (Felsenstein 1985; Harvey and Pagel 1991; Faith 1992), further research into phylogenetic diversity of vegetation assemblages occurring across the gradient may provide further information among species and inform our understanding of the compositional patterns presented in Chapter 2.

7.5 Conclusion

In this thesis, I investigated ecological relationships between natural spatial variation in DGW and the ecological structure and function of vegetation assemblages in mesic

woodlands and explored relationships between vegetation properties and natural spatial variation in DGW across arid-zone woodlands. In doing so I have identified the ecological properties of vegetation assemblages that are linked to natural spatial variation in DGW in mesic and arid-zone woodlands and have thus provided fundamental baseline information vital for aiding in management-based decisions and identifying future research directions. Given the paucity of studies examining relationships between the ecological structure and function of vegetation assemblages and naturally-occurring gradients in DGW, I believe that this thesis has provided information crucial for our understanding of the ecology of GDEs and the management of these important ecosystems.

Thesis appendices

Appendix A Geographic coordinates and depth-to-groundwater for the 16 study sites.

Site	Mean groundwater depth ± SE (m)	Easting	Northing	Altitude (m)
1	2.4 ± 0.01	0282646	6175634	594
2	4.2 ± 0.17	0282253	6176460	599
3	4.6 ± 0.05	0280538	6177081	602
4	6.8 ± 0.02	0274872	6178421	616
5	8.0 ± 0.53	0274861	6178013	627
6	9.9 ± 0.02	0281728	6176691	603
7	12.4 ± 0.18	0281342	6176748	608
8	16.3 ± 0.08	0273385	6182220	584
9	16.8 ± 0.01	0274862	6178217	637
10	17.8 ± 0.71	0280596	6178485	580
11	22.0 ± 0.22	0280351	6178785	581
12	27.4 ± 0.22	0276086	6179608	662
13	29.1 ± 0.45	0272931	6178094	638
14	32.1 ± 0.18	0276717	6181181	617
15	37.5 ± 0.26	0276518	6181481	624
16	43.7 ± 0.58	0274193	6184744	581

Appendix B. Study species and their abundances (number of individuals, see Methods for details) at each of the 16 sites.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acacia echinula	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
Acacia fimbriata	0	0	0	0	0	0	1	0	0	0	0	20	0	0	0	0
Acacia gunnii	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0
Acacia longifolia	5	30	40	0	0	20	30	0	0	20	500	0	0	0	35	0
Acacia rubida	0	0	0	0	0	0	0	500	0	0	0	0	0	0	0	0
Acacia saliciformis	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
Acacia terminalis	0	0	0	2	0	8	0	30	0	50	8	0	0	0	50	0
Acacia ulicifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0
Adiantum aethiopicum	0	0	50	0	0	0	0	0	0	0	0	100	0	0	0	0
Allocasuarina littoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Allocasuarina nana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Allocasuarina torulosa	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Austrostipa pubescens	0	1500	0	0	1500	0	100	0	500	500	30	0	0	50	500	1500
Backhousia myrtifolia	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Banksia ericifolia	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0
Banksia paludosa	0	0	0	40	100	30	0	0	5	7	6	0	0	0	30	0

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Banksia spinulosa	10	50	0	50	100	30	30	15	100	100	40	0	3	20	50	100
Billardiera scandens	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0
Blechnum wattsii	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Bossiaea obcordata	0	0	0	0	0	30	500	500	40	0	1000	0	8	0	50	0
Bossiaea buxifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0
Bossiaea heterophylla	0	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0
Cassinia uncata	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
Caustis flexuosa	0	0	0	500	0	50	0	0	0	0	0	0	0	0	0	0
Choretrum candollei	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
Choretrum pauciflorum	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0
Coprosma quadrifida	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Corymbia gummifera	0	0	0	0	0	0	0	0	0	15	20	0	0	0	0	0
Cyathochaeta diandra	0	0	0	1500	0	0	0	1500	0	0	1500	0	0	0	0	1000
Cymbonotus lawsonianus	0	0	1000	0	0	0	0	0	0	0	0	5	40	0	0	0
Dampiera stricta	0	20	0	20	50	0	500	1500	1500	30	8	0	0	0	100	500
Daviesia latifolia	0	0	0	500	0	0	0	100	50	0	0	0	0	0	0	0
Daviesia ulicifolia	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0
Dianella caerulea	20	10	50	0	5	0	9	0	0	0	40	15	10	0	4	0

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dichondra repens	15	0	1500	0	0	0	0	0	0	0	0	500	0	0	0	0
Dillwynia sericea	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0
Entolasia marginata	100	10	30	0	0	0	0	0	0	0	0	40	40	1500	0	0
Entolasia stricta	500	50	100	500	1000	1500	500	500	0	500	50	30	0	100	0	0
Eucalyptus cypellocarpa	0	0	4	0	0	0	0	0	0	0	0	7	1	0	0	0
Eucalyptus globoidea	8	20	20	0	0	10	5	0	0	0	4	40	30	5	15	0
Eucalyptus piperita	20	20	15	0	1	5	9	2	0	10	10	8	5	20	10	0
Eucalyptus radiata	30	3	8	0	2	0	8	3	0	0	3	8	5	15	0	0
Eucalyptus sclerophylla	0	3	0	50	30	30	0	20	50	0	2	0	9	15	20	50
Eucalyptus sieberi	0	8	0	0	0	0	0	0	0	50	30	0	0	0	6	0
Eucalyptus elata	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Eustrephus latifolius	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Gahnia sieberiana	0	1	4	0	0	0	0	0	0	0	0	0	8	0	0	0
Geitonoplesium cymosum	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycine clandestina	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0
Gompholobium glabratum	0	0	0	0	0	0	0	15	6	0	0	0	0	0	0	15
Gompholobium latifolium	0	0	0	0	0	0	0	0	0	0	30	0	0	0	15	0
Gonocarpus tetragynus	0	0	0	0	15	0	0	0	20	0	0	0	0	0	0	1500

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Gonocarpus teucrioides	0	0	50	0	0	0	1500	0	0	1000	500	5	500	6	30	0
Goodenia bellidifolia	15	0	100	100	20	0	500	100	100	0	0	20	500	500	20	0
Goodenia ovata	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0
Grevillea sericea	0	0	0	8	5	0	0	0	0	0	0	0	0	0	0	20
Grevillea baueri subsp. *baueri*	0	0	0	20	0	0	0	30	0	0	0	0	0	0	0	0
Hakea dactyloides	10	0	0	1000	500	6	0	100	500	50	100	0	4	0	7	100
Hakea salicifolia	0	0	0	0	0	0	0	0	0	0	0	30	50	0	0	0
Helichrysum rutidolepis	0	0	0	0	0	0	0	0	0	0	0	0	500	0	0	0
Helichrysum scorpioides	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
Hibbertia scandens	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
Hibbertia sericea	15	20	10	0	0	100	15	15	0	50	40	0	0	5	20	0
Hibbertia serpyllifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
Hydrocotyle laxiflora	0	0	50	0	0	0	0	0	0	0	0	100	50	0	0	0
Isopogon anemonifolius	0	0	0	50	50	20	0	0	30	0	20	0	0	0	20	50
Lagenifera stipitata	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0
Lambertia formosa	0	30	0	0	0	50	0	0	0	30	50	0	0	0	0	0
Lepidosperma laterale	0	0	8	0	0	0	0	0	0	500	0	0	8	0	0	0

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Leptomeria acida	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1
Leptospermum grandifolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0
Leptospermum juniperinum	0	0	0	0	5	0	40	0	0	40	4	0	0	0	0	5
Leptospermum polygalifolium	0	0	0	50	0	0	0	0	15	30	0	0	0	0	0	0
Leptospermum trinervium	0	15	7	1	20	20	0	0	0	0	1	0	0	0	0	2
Lepyrodia scariosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1500
Leucopogon lanceolatus	20	20	0	0	0	0	30	4	0	0	0	10	30	30	0	0
Leucopogon setiger	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0
Lindsaea linearis	0	10	20	0	0	50	0	0	0	50	50	0	0	0	0	500
Lindsaea microphylla	15	0	0	0	0	0	500	0	0	0	0	0	0	9	0	0
Lomandra filiformis subsp. *filiformis*	0	0	0	0	0	0	0	0	0	0	0	0	100	8	0	0
Lomandra longifolia	0	15	100	0	0	10	1500	0	9	10	4	1500	50	1500	20	0
Lomandra multiflora	0	0	0	0	0	0	0	30	30	0	0	0	0	0	15	15
Lomandra obliqua	0	0	0	0	0	20	0	0	0	0	50	0	0	0	5	50
Lomatia ilicifolia	5	10	0	100	10	100	0	0	0	0	0	0	0	1	0	0
Lomatia silaifolia	15	8	10	1000	20	20	50	30	30	0	30	0	0	1500	8	20
Melaleuca linariifolia	0	6	50	0	0	0	0	0	0	15	0	1	0	0	0	0

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mirbelia platylobioides	0	0	0	40	5	0	0	100	0	0	0	0	0	0	0	0
Monotoca scoparia	0	0	0	0	0	0	4	0	0	0	0	0	0	40	0	0
Olearia microphylla	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0
Pandorea pandorana subsp. *pandorana*	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Patersonia sericea	0	0	0	30	5	0	0	0	0	0	0	0	0	10	0	0
Persoonia chamaepitys	0	0	0	0	0	0	2	0	20	0	0	0	2	0	0	0
Persoonia laurina	0	0	0	0	50	0	0	0	30	0	0	0	0	0	0	1
Persoonia levis	0	0	0	0	0	20	8	2	0	4	50	0	0	0	10	0
Persoonia linearis	50	0	0	0	0	0	5	10	0	0	0	1	0	15	0	2
Persoonia oxycoccoides	0	0	0	2	2	0	0	10	0	0	0	0	0	0	0	0
Petrophile pedunculata	0	0	0	30	8	0	0	8	20	0	50	0	0	0	0	0
Petrophile sessilis	0	0	0	0	0	40	0	50	0	0	30	0	0	0	100	0
Pimelea linifolia subsp. *linifolia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0
Plantago sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
Poa sieberiana	0	0	1500	1500	1500	0	1500	50	1500	0	0	1500	1500	15	0	1500

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pomaderris phylicifolia	0	0	0	0	0	0	0	100	0	40	0	0	0	0	0	0
Pratia purpurascens	5	0	100	0	0	0	0	0	0	0	0	100	0	0	0	0
Prostanthera rugosa	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Pteridium esculentum	500	100	100	0	0	50	1500	0	0	30	6	1500	0	1500	20	0
Pultenaea blakelyi	50	0	100	0	0	0	15	0	0	0	0	0	0	0	0	0
Pultenaea elliptica	0	0	0	100	1000	0	0	30	50	0	0	0	0	0	20	50
Pultenaea linophylla	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0
Pultenaea retusa	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0
Pultenaea scabra	0	0	0	0	0	0	0	0	0	7	50	0	0	0	0	0
Ranunculus sp.	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0
Restio gracilis	0	100	0	0	0	1500	0	0	0	0	0	0	0	0	0	0
Rubus ulmifolius	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Schoenus melanostachys	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scutellaria humilis	1500	1500	1000	0	0	1500	0	0	0	0	0	0	0	0	0	0
Selaginella uliginosa	0	0	0	0	0	1500	0	0	0	0	0	0	0	0	0	0
Senecio hispidulus	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0
Taraxacum officinale	0	0	0	0	0	0	0	0	0	0	0	2	3	2	0	0
Telopea speciosissima	5	0	0	0	0	7	30	0	0	0	2	0	0	0	20	0

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Tetratheca thymifolia	20	0	0	0	0	0	0	20	0	40	0	0	0	500	15	0
Tristaniopsis collina	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthorrhoea resinosa	0	0	0	7	12	5	0	5	10	0	0	0	0	0	20	0
Unidentified climber	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
Unidentified groundcover	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0
Unidentified shrub 1	0	0	0	0	0	0	4	0	0	2	0	0	0	30	0	0
Unidentified shrub 2	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0
Unidentified shrub 3	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0
Unidentified shrub 4	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0
Unidentified shrub 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0
Unidentified shrub 6	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified shrub 7	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0

Appendix C. Examples of mesic woodland study sites across the DGW gradient.

Appendix D. Summary of plant traits for species identified from the 16 study sites (see Chapter 3). 'An/bien' = species that are annuals or biennials.

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Acacia echinula	Simple	0.09	40	2.00	Shrub	Indefinite	Force
Acacia fimbriata	Simple	1.75	9.5	6.00	Shrub	Perennial	Force
Acacia gunnii	Simple	0.35	40	1.00	Shrub	Indefinite	Force
Acacia longifolia	Simple	4.20	150	8.00	Shrub	Perennial	Vertebrate
Acacia rubida	Simple	0.39	120	10.00	Shrub	Perennial	Force
Acacia saliciformis	Simple	12.60	160	6.00	Shrub	Perennial	Force
Acacia terminalis	Compound	0.73	100	6.00	Shrub	Perennial	Ant
Acacia ulicifolia	Simple	0.21	60	2.00	Shrub	Perennial	Ant
Adiantum aethiopicum	Compound	18.10	1	0.50	Fern	Indefinite	Wind
Allocasuarina torulosa	Simple	0.49	33	25.00	Tree	Perennial	Wind
Allocasuarina littoralis	Simple	1.40	30	15.00	Tree	Perennial	Wind
Allocasuarina nana	Simple	0.45	24	2.00	Shrub	Perennial	Wind
Austrostipa pubescens	Simple	0.04	10	1.50	Grass	Indefinite	Vertebrate
Backhousia myrtifolia	Simple	18.38	6	15.00	Tree	Perennial	Wind

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Banksia ericifolia	Simple	0.14	20	6.00	Shrub	Perennial	Wind
Banksia paludosa	Simple	27.30	18	1.50	Shrub	Perennial	Wind
Banksia spinulosa var. *spinulosa*	Simple	7.00	24	3.00	Shrub	Perennial	Wind
Billardiera scandens	Simple	1.75	40	0.50	Climber	Indefinite	Vertebrate
Blechnum wattsii	Compound	21.00	1	1.00	Fern	Indefinite	Wind
Bossiaea obcordata	Simple	0.17	20	2.00	Shrub	Indefinite	Force
Bossiaea buxifolia	Simple	0.18	20	1.30	Shrub	Indefinite	Ant
Bossiaea heterophylla	Simple	0.84	40	1.00	Shrub	Perennial	Ant
Cassinia uncata	Simple	2.10	1	3.00	Shrub	Indefinite	Wind
Caustis flexuosa	Simple	15.75	5	0.90	Herb	Indefinite	None
Choretrum candollei	Simple	0.04	6	5.00	Shrub	Indefinite	Vertebrate
Choretrum pauciflorum	Simple	0.01	6	2.00	Shrub	Indefinite	Vertebrate
Coprosma quadrifida	Simple	5.25	5	4.00	Shrub	Indefinite	Vertebrate
Corymbia gummifera	Simple	44.80	20	30.00	Tree	Perennial	Wind
Cyathochaeta diandra	Simple	10.50	8	0.90	Herb	Indefinite	None
Cymbonotus lawsonianus	Simple	157.50	2.5	0.30	Herb	An/bien	Wind
Dampiera stricta	Simple	6.00	5	0.60	Shrub	Indefinite	Ant

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Daviesia latifolia	Simple	49.00	9	3.00	Shrub	Indefinite	Force
Daviesia ulicifolia	Simple	0.84	8	2.00	Shrub	Perennial	Force
Dianella caerulea	Simple	131.25	12	0.50	Herb	Indefinite	Vertebrate
Dichondra repens	Simple	4.38	1.4	0.10	Herb	An/bien	None
Dillwynia sericea	Simple	0.14	4	1.00	Shrub	Perennial	Ant
Entolasia marginata	Simple	1.05	1	2.00	Grass	Indefinite	None
Entolasia stricta	Simple	0.29	1	1.50	Grass	Indefinite	None
Eucalyptus cypellocarpa	Simple	35.00	10	30.00	Tree	Perennial	Wind
Eucalyptus elata	Simple	15.75	6	30.00	Tree	Perennial	Wind
Eucalyptus globoidea	Simple	21.88	7	30.00	Tree	Perennial	Wind
Eucalyptus piperita	Simple	29.40	8	20.00	Tree	Perennial	Wind
Eucalyptus radiata	Simple	15.75	6	30.00	Tree	Perennial	Wind
Eucalyptus sclerophylla	Simple	44.80	6	20.00	Tree	Perennial	Wind
Eucalyptus sieberi	Simple	29.40	11	45.00	Tree	Perennial	Wind
Eustrephus latifolius	Simple	24.50	20	6.00	Climber	Indefinite	Vertebrate
Gahnia sieberiana	Simple	168.00	4	2.00	Sedge	Indefinite	Vertebrate
Geitonoplesium cymosum	Simple	17.50	20	8.00	Climber	Indefinite	Vertebrate

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Glycine clandestina	Compound	5.60	5.3	2.00	Climber	Indefinite	None
Gompholobium glabratum	Compound	0.16	10	0.40	Shrub	Indefinite	Ant
Gompholobium latifolium	Compound	2.10	18	3.00	Shrub	Perennial	Force
Gonocarpus tetragynus	Simple	0.53	1.5	0.30	Herb	Indefinite	None
Gonocarpus teucrioides	Simple	1.05	1.5	0.40	Herb	Perennial	None
Goodenia bellidifolia	Simple	13.30	4	0.60	Herb	Perennial	Ant
Goodenia ovata	Simple	22.40	12	2.00	Shrub	Indefinite	Ant
Grevillea baueri subsp. *baueri*	Simple	1.47	14	1.00	Shrub	Indefinite	Wind
Grevillea sericea subsp. *sericea*	Simple	5.67	16	2.00	Shrub	Perennial	Wind
Hakea dactyloides	Simple	14.00	30	3.00	Shrub	Perennial	Wind
Hakea salicifolia	Simple	28.35	23	5.00	Shrub	Perennial	Wind
Helichrysum rutidolepis	Simple	4.48	1	0.40	Herb	An/bien	Wind
Helichrysum scorpioides	Simple	5.04	1	0.30	Herb	An/bien	Wind
Hibbertia scandens	Simple	14.00	40	4.00	Climber	Perennial	Vertebrate
Hibbertia sericea	Simple	0.28	4	1.00	Shrub	Indefinite	Ant
Hibbertia serpyllifolia	Simple	0.07	2	0.30	Shrub	Perennial	Ant
Hydrocotyle laxiflora	Simple	50.27	2	0.15	Herb	Indefinite	None

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Isopogon anemonifolius	Compound	3.08	16	1.50	Shrub	Perennial	Wind
Lagenifera stipitata	Simple	21.00	4	0.40	Herb	An/bien	Wind
Lambertia formosa	Simple	28.00	25	2.00	Shrub	Perennial	Wind
Lepidosperma laterale	Simple	56.00	4	1.00	Sedge	Indefinite	Ant
Leptomeria acida	Simple	0.01	7	3.00	Shrub	Perennial	Vertebrate
Leptospermum grandifolium	Simple	1.47	10	6.00	Shrub	Perennial	Wind
Leptospermum juniperinum	Simple	0.21	7	3.00	Shrub	Perennial	Wind
Leptospermum polygalifolium	Simple	0.70	10	4.00	Shrub	Perennial	Wind
Leptospermum trinervium	Simple	0.84	6	4.00	Shrub	Perennial	Wind
Lepyrodia scariosa	Simple	1.13	2	0.90	Rush	Indefinite	None
Leucopogon lanceolatus	Simple	1.19	3.3	3.00	Shrub	Perennial	Vertebrate
Leucopogon setiger	Simple	0.15	4.2	1.50	Shrub	Perennial	Ant
Lindsaea linearis	Compound	39.20	1	0.30	Fern	Indefinite	Wind
Lindsaea microphylla	Compound	21.00	1	0.50	Fern	Indefinite	Wind
Lomandra filiformis subsp. *filiformis*	Simple	10.50	3	0.30	Rush	Indefinite	Ant
Lomandra longifolia	Simple	52.50	5	1.00	Rush	Indefinite	Ant
Lomandra multiflora	Simple	18.90	5	0.90	Rush	Indefinite	Ant

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Lomandra obliqua	Simple	0.56	3	0.50	Rush	Indefinite	Ant
Lomatia ilicifolia	Simple	70.00	30	3.00	Shrub	Indefinite	Wind
Lomatia silaifolia	Compound	49.00	50	2.00	Shrub	Perennial	Wind
Melaleuca linariifolia	Simple	0.95	4	10.00	Shrub	Perennial	Wind
Mirbelia platylobioides	Simple	4.20	20	0.40	Shrub	Indefinite	Force
Monotoca scoparia	Simple	2.73	3.1	1.20	Shrub	Perennial	Vertebrate
Olearia microphylla	Simple	0.15	1	2.00	Shrub	Indefinite	Wind
Pandorea pandorana subsp. *pandorana*	Compound	33.60	60	4.00	Climber	Perennial	Wind
Patersonia sericea	Simple	25.20	30	0.60	Herb	Indefinite	Ant
Persoonia chamaepitys	Simple	0.13	8	0.20	Shrub	Indefinite	Vertebrate
Persoonia laurina	Simple	46.20	10	1.00	Shrub	Perennial	Vertebrate
Persoonia levis	Simple	78.40	10	4.00	Shrub	Perennial	Vertebrate
Persoonia linearis	Simple	3.57	13	3.00	Shrub	Indefinite	Vertebrate
Persoonia oxycoccoides	Simple	0.46	8	0.90	Shrub	Indefinite	Vertebrate
Petrophile pedunculata	Compound	89.60	30	2.50	Shrub	Indefinite	Wind
Petrophile sessilis	Compound	49.00	30	3.00	Shrub	Indefinite	Wind

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Pimelea linifolia subsp. *linifolia*	Simple	1.47	5	1.50	Shrub	Perennial	Ant
Poa sieberiana	Simple	14.70	1	1.00	Grass	Indefinite	None
Pomaderris phylicifolia	Simple	0.42	3	2.00	Shrub	Perennial	Wind
Pratia purpurascens	Simple	1.40	10	0.30	Herb	Indefinite	None
Prostanthera rugosa	Simple	0.32	5	1.50	Shrub	Indefinite	None
Pteridium esculentum	Compound	262.50	1	1.50	Fern	Indefinite	Wind
Pultenaea blakelyi	Simple	0.35	10	3.00	Shrub	Perennial	Force
Pultenaea elliptica	Simple	0.53	5	1.00	Shrub	Perennial	Ant
Pultenaea linophylla	Simple	0.78	6	1.00	Shrub	Indefinite	Ant
Pultenaea retusa	Simple	0.53	7	1.00	Shrub	Perennial	Force
Pultenaea scabra	Simple	1.46	7	1.50	Shrub	Indefinite	Force
Restio gracilis	Simple	105.00	5	1.00	Rush	Indefinite	Wind
Rubus ulmifolius	Compound	30.80	12	3.00	Shrub	Indefinite	Vertebrate
Schoenus melanostachys	Simple	0.09	2	1.25	Rush	Indefinite	None
Scutellaria humilis	Simple	2.52	2	0.15	Herb	Indefinite	Wind
Selaginella uliginosa	Compound	0.03	1	0.38	Fern	Indefinite	Wind
Senecio hispidulus	Simple	16.80	2.7	1.00	Herb	An/bien	Wind

Species	Leaf shape	Leaf size (cm^2)	Fruit length (mm)	Maximum height (m)	Growth form	Longevity (years)	Dispersal mode
Taraxacum officinale	Simple	280.00	4.5	0.40	Herb	An/bien	Wind
Telopea speciosissima	Simple	127.40	150	3.00	Shrub	Perennial	Wind
Tetratheca thymifolia	Simple	1.12	6	1.00	Shrub	Indefinite	Wind
Tristaniopsis collina	Simple	16.63	8	30.00	Tree	Indefinite	Wind
Xanthorrhoea resinosa	Simple	0.07	5	0.60	Rush	Perennial	Wind

Appendix E. Fourth-corner summary statistics showing *P* values before and after correcting for multiple comparisons using the false discovery rate method (* = P < 0.05).

Test	Stat	Obs	Std. Obs	Alter	*P*	Adjusted *P*
GW/Leaf shape	F	269.09	-0.52	greater	0.59	0.99
Slope/Leaf shape	F	1538.51	0.41	greater	0.20	0.99
Altitude/Leaf shape	F	940.79	-0.16	greater	0.40	0.99
Shade Index/Leaf shape	F	3.57	-0.82	greater	0.96	0.99
pH/Leaf shape	F	0.06	-0.82	greater	0.99	0.99
EC/Leaf shape	F	622.74	-0.19	greater	0.40	0.99
Ca/Leaf shape	F	2359.96	0.30	greater	0.25	0.99
FC/Leaf shape	F	1720.51	0.17	greater	0.29	0.99
Silt/Leaf shape	F	95.31	-0.64	greater	0.77	0.99
Sand/Leaf shape	F	332.68	-0.53	greater	0.61	0.99
GW/Leaf size	r	0.05	0.55	two-sided	0.61	0.99

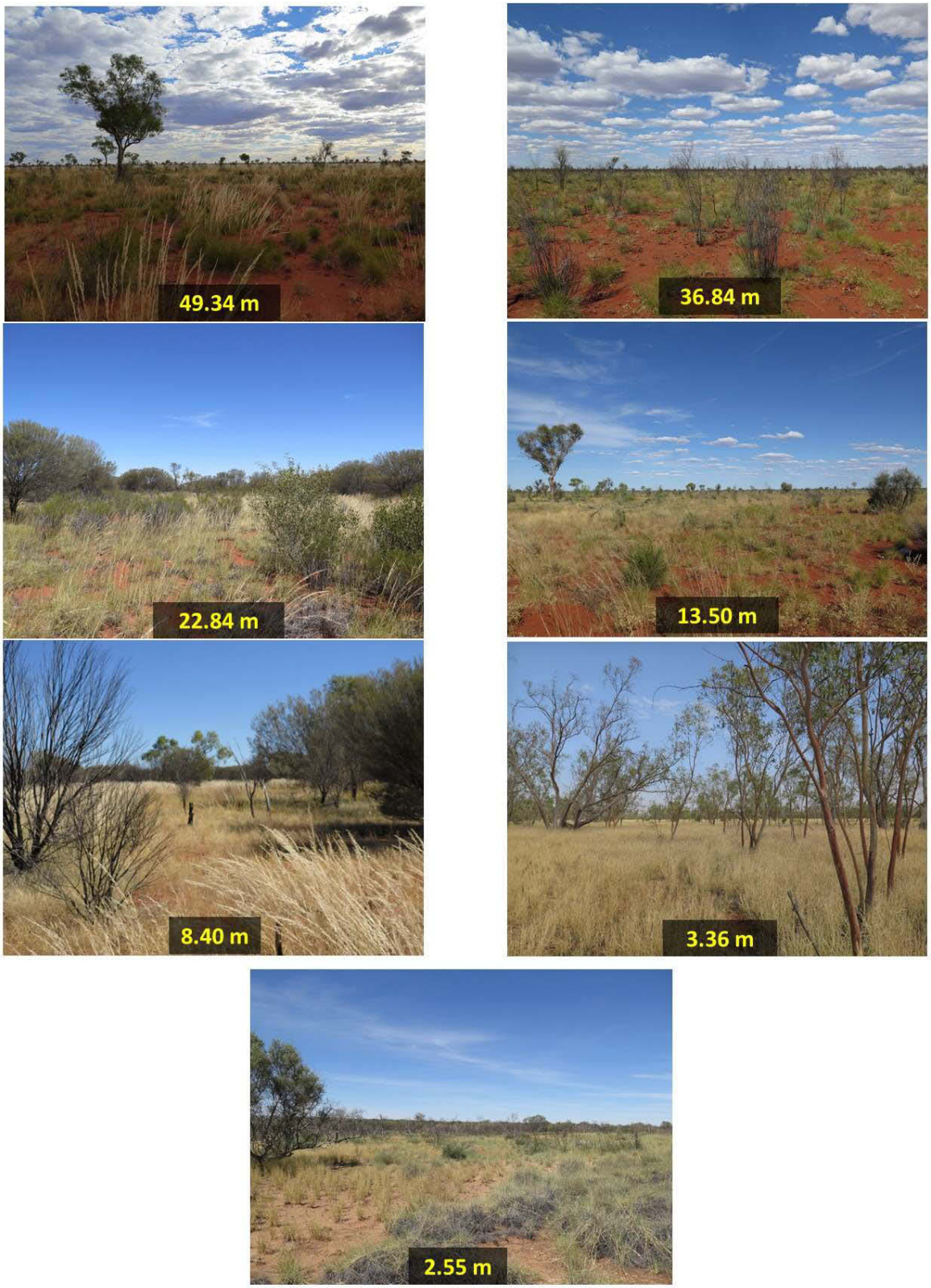
Test	Stat	Obs	Std. Obs	Alter	*P*	Adjusted *P*
Slope/Leaf size	r	0.06	0.64	two-sided	0.57	0.99
Altitude/Leaf size	r	0.17	1.60	two-sided	0.12	0.99
Shade Index/Leaf size	r	0.06	0.62	two-sided	0.59	0.99
pH/Leaf size	r	-0.07	-0.67	two-sided	0.54	0.99
EC/Leaf size	r	-0.02	-0.17	two-sided	0.89	0.99
Ca/Leaf size	r	0.24	2.36	two-sided	0.01*	0.28
FC/Leaf size	r	0.24	2.30	two-sided	0.01*	0.28
Silt/Leaf size	r	0.23	2.18	two-sided	0.02*	0.28
Sand/Leaf size	r	-0.24	-2.31	two-sided	0.01*	0.28
GW/Fruit length	r	0.02	0.16	two-sided	0.87	0.99
Slope/Fruit length	r	0.09	0.79	two-sided	0.42	0.99
Altitude/Fruit length	r	-0.11	-0.95	two-sided	0.36	0.99
Shade Index/Fruit length	r	-0.04	-0.42	two-sided	0.70	0.99

Test	Stat	Obs	Std. Obs	Alter	*P*	Adjusted *P*
pH/Fruit length	r	0.15	1.51	two-sided	0.13	0.99
EC/Fruit length	r	-0.17	-1.72	two-sided	0.08	0.88
Ca/Fruit length	r	-0.08	-0.66	two-sided	0.54	0.99
FC/Fruit length	r	-0.08	-0.70	two-sided	0.51	0.99
Silt/Fruit length	r	-0.12	-1.06	two-sided	0.31	0.99
Sand/Fruit length	r	0.11	0.97	two-sided	0.33	0.99
GW/Growth form	F	235.90	-1.19	greater	0.94	0.99
Slope/Growth form	F	264.52	-1.09	greater	0.92	0.99
Altitude/Growth form	F	918.56	-0.33	greater	0.58	0.99
Shade Index/Growth form	F	510.01	-0.75	greater	0.75	0.99
pH/Growth form	F	593.55	-0.51	greater	0.64	0.99
EC/Growth form	F	1272.44	0.71	greater	0.19	0.99
Ca/Growth form	F	641.72	-1.03	greater	0.89	0.99

Test	Stat	Obs	Std. Obs	Alter	*P*	Adjusted *P*
FC/Growth form	F	550.46	-0.97	greater	0.84	0.99
Silt/Growth form	F	189.53	-1.35	greater	0.96	0.99
Sand/Growth form	F	265.41	-1.32	greater	0.96	0.99
GW/Maximum height	r	0.01	0.05	two-sided	0.95	0.99
Slope/Maximum height	r	0.04	0.25	two-sided	0.81	0.99
Altitude/Maximum height	r	-0.02	-0.07	two-sided	0.95	0.99
Shade Index/Maximum height	r	-0.03	-0.31	two-sided	0.76	0.99
pH/Maximum height	r	0.06	0.54	two-sided	0.57	0.99
EC/Maximum height	r	-0.07	-0.65	two-sided	0.51	0.99
Ca/Maximum height	r	-0.02	-0.15	two-sided	0.89	0.99
FC/Maximum height	r	-0.01	-0.02	two-sided	0.99	0.99
Silt/Maximum height	r	-0.02	-0.07	two-sided	0.94	0.99
Sand/Maximum height	r	0.02	0.14	two-sided	0.90	0.99

Test	Stat	Obs	Std. Obs	Alter	*P*	Adjusted *P*
GW/Longevity	F	646.55	-0.53	greater	0.64	0.99
Slope/Longevity	F	547.39	-0.62	greater	0.67	0.99
Altitude/Longevity	F	215.90	-0.76	greater	0.82	0.99
Shade Index/Longevity	F	6989.67	3.38	greater	0.02*	0.28
pH/Longevity	F	556.64	-0.65	greater	0.70	0.99
EC/Longevity	F	381.03	-0.57	greater	0.74	0.99
Ca/Longevity	F	1922.68	0.25	greater	0.32	0.99
FC/Longevity	F	267.03	-0.87	greater	0.83	0.99
Silt/Longevity	F	1012.49	-0.26	greater	0.49	0.99
Sand/Longevity	F	1447.64	0.11	greater	0.37	0.99
GW/Dispersal mode	F	829.35	-0.35	greater	0.55	0.99
Slope/Dispersal mode	F	581.28	-0.54	greater	0.66	0.99
Altitude/Dispersal mode	F	519.59	-0.95	greater	0.83	0.99

Test	Stat	Obs	Std. Obs	Alter	*P*	Adjusted *P*
Shade Index/Dispersal mode	F	345.40	-0.95	greater	0.86	0.99
pH/Dispersal mode	F	1214.86	0.52	greater	0.26	0.99
EC/Dispersal mode	F	1081.99	0.35	greater	0.32	0.99
Ca/Dispersal mode	F	1107.36	-0.59	greater	0.67	0.99
FC/Dispersal mode	F	556.43	-0.91	greater	0.83	0.99
Silt/Dispersal mode	F	805.83	-0.52	greater	0.63	0.99
Sand/Dispersal mode	F	789.91	-0.66	greater	0.71	0.99



Appendix F. Examples of arid-zone woodland study sites across DGW gradient.

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