

Mining for water: Plant-soil interactions on restored mine substrates

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SUMMARY

Waste rock is a major by-product of mining worldwide. It is most often piled into waste rock dumps, creating new landforms that pose substantial environmental and public health risks to local and downstream receptors. Engineered covers utilising water balance principles (ET covers) have proven effective at mitigating risks associated with hazardous waste and are becoming increasingly prevalent in semi-arid to arid environments. ET covers limit water percolation through waste by retaining water during rainfall, then releasing it back to the atmosphere during dry periods through evaporation and plant transpiration thus minimising the risk of contaminant release. Covers are generally constructed using locally available materials from the mining process, including stripped and stockpiled top soil, and non-reactive subsoils and waste rock. Soils used for cover construction often have altered hydrological properties making plant establishment and growth challenging. Therefore, the growth soil plays a deterministic role in the establishment and survival of vegetation on ET covers in water-limited systems, especially through moisture retention. The objective of this thesis was to increase the understanding of functional relationships between soil characteristics, depth, and water availability to plant water use strategies, physiological and morphological adaptations, and root distributions on mining soils and ET covers in water limited systems.

Vegetation function on covers, in particular growth, survival and patterns of water use, depends on key ecophysiological traits of the dominant species, such as investment in roots and leaf area, stomatal regulation, and tissue dehydration tolerance. A comparative glasshouse study was conducted to investigate the physiological and morphological traits associated with drought tolerance of nine semi-arid region *Acacia* species in a typical mine site restoration soil, and to determine if these traits correlated with their respective soil preferences. I found that *Acacia* species from this region differed significantly in a range of traits associated with drought tolerance, where species from alluvial, rocky, and one sandy soil species were more anisohydric than isohydric. These species had higher stomatal conductance at more negative leaf water potentials. Alluvial soil species had greater total biomass allocation to lateral roots, whereas two coarser-textured soil species had high allocation to tap roots. Differences in drought tolerance traits were generally poorly related with *a priori* preferred soil type classifications.

Two field studies followed, where an ET cover, comprised of two parts banded iron formation (BIF) and one part top soil was constructed atop a waste rock dump slope. The cover was spread into three soil depth treatments, instrumented with moisture sensors and meteorological instrumentation, then planted with six local woody native species seedlings. Plants of one of the species died (*Acacia alata* R.Br.), the other five species grew well. The first field study investigated the effects of cover thickness, soil moisture, and climatic drivers on growth, morphology, area based net photosynthesis (A), stomatal conductance (g_s) and water status (leaf water potentials; Ψ_l) of the five surviving experimental species (*Acacia acuminata* Benth., *Eucalyptus leptopoda* Benth., *E. loxophleba* Benth., *Melaleuca cordata* Turcz., *M. nematophylla* Craven) over two years. Shallow soils and confined roots reduced plant-available water leading to lower g_s and lower water status; mean root length (RL) in the 0.7 m cover was 39% and 70% higher than in the 0.5 m and 0.3 m covers, respectively, while mean g_s on the 0.3 m thick cover was 19 % and 24 % lower than the 0.5 m and 0.7 m thick covers, respectively. Water use was partly mediated by seasonal changes in VPD, and plants on thicker covers were least sensitive to increasing evaporative demand due to more plant-available water and larger root systems. All species displayed anisohydric behaviour, and while water potentials varied seasonally, apparent hydraulic gradients were approximately constant throughout the year due to effective stomatal control. However, given the observed differences in plant physiology, future changes in growth and survival between cover thicknesses are expected, especially as transpiring leaf area and root systems expand. In a severe drought year plants on thicker covers are expected to perform better as they have more access to soil water to meet increasing evaporative demands. In this study, species with low leaf area to root length ratio (LA:RL_{Total}) and soils that supported high RL led to higher water status. Selecting species with these traits, and the use of thicker covers will achieve robust ET cover systems capable of withstanding severe drought and warmer future climates.

The second field study quantified diurnal water use of saplings of two *Eucalyptus* species during hot summer days. Primary objectives were (1) investigate the influence of soil depth on plant water availability and on diurnal shifts in g_s , E , A , intercellular CO₂ concentrations (C_i), and leaf water potentials at pre-dawn (Ψ_{PD}) and mid-day (Ψ_{MD}); and (2) understand the impact of water availability on key physiological variables (g_s , E , A , C_i , Ψ_{PD} , and Ψ_{MD}) to determine whether they differed between species and soil depth in response to diurnal

fluctuations in VPD, temperature, irradiance, and soil moisture. I concluded that diurnal water use and A were mainly influenced by plant water availability as a function of soil depth, in conjunction with VPD, temperature, and irradiance. Although physiological responses to daily weather did not differ between the two species, responses were different for plants growing in shallow versus deep soil. Daily average g_s and E were significantly higher on thicker covers; g_s on the 0.3 m depth was 1.6 and 2 times lower, while E was 1.4 and 1.8 times lower compared to the 0.5 and 0.7 m soil depths, respectively. Overall, plants on deeper soils were less affected by high VPD, temperature, and irradiance due to increased plant available water, partly provided by unconfined roots resulting in higher overall g_s , E , A , and Ψ_{MD} . Besides g_s , non-stomatal factors also strongly limited afternoon A , with high temperature and intense light causing repairable damage to the photochemical reaction centres and associated enzymes.

I have obtained new evidence to support that revegetation on disturbed mining lands can be guided by ecophysiological and morphological trait-based selection criteria but must be underpinned by the geotechnical characteristics of the restoration substrate. I advise that deeper soils on ET covers enhance the probabilities of successful re-vegetation in situations where soil textures are conducive to water storage, as the impacts of diurnal and seasonal environmental fluctuations in VPD, temperature and irradiance were buffered by greater plant available water and unconfined roots in deeper soils. Moisture storage provided by deeper soils may also help reduce plant sensitivity to environmental factors over long-term shifts in climate. This study emphasises the importance of understanding the ecophysiology of plants on mined lands, such that insights gained here may improve the restoration success of revegetated disturbed sites in arid to seasonally dry systems.

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STATEMENT OF ORIGINAL CONTRIBUTION

The research presented in this thesis is an original contribution to the field of plant ecophysiology and restoration ecology. The hypotheses and experiments presented and discussed in this thesis are my own original ideas and writings with help of supervisors and co-authors. This thesis has been developed and completed during the course of enrolment in a PhD degree at the University of Western Australia, and has not been used previously for a degree or a diploma at any other institution.

Dr. Erik Veneklaas and Dr. Pieter Poot were the supervisors of this project and are cited as co-authors at the beginning of Chapters 2 – 4. They guided me through the process of formulating hypotheses, designing experiments, statistical analyses, and scientific writing.

Sebastian C. J. Lamoureux

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THESIS STRUCTURE

This thesis is in agreement with the Postgraduate and Research Scholarship Regulation 1.3.1.33 (1) of the University of Western Australia and is presented as a series of three scientific papers. There are five chapters in this thesis; a general introduction, three experimental chapters and a general discussion. The general introduction covers the broad background for the work presented in the thesis in order to justify the research objectives. A more focussed review of literature is presented in the introduction of each experimental chapter. The three experimental chapters are presented in the format of scientific papers that can be read individually or as a part of the whole thesis. Chapter 1 has been published in the Journal of Arid Environments, while the other two are in preparation for submission to peer-reviewed scientific journals. Each experimental chapter includes the following sections: abstract, introduction, materials and methods, results, discussion, acknowledgements, and references. When appropriate, appendices with supporting information were also added. This “thesis-as-a-series-of-papers” format results in some unavoidable repetition, especially in the materials and methods sections of chapters 2 to 4; I have tried to keep such repetition to a minimum.

THESIS DECLARATION



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The examination of the thesis is an examination of the work of the student. The work must have been substantially conducted by the student during enrolment in the degree. Where the thesis includes work to which others have contributed, the thesis must include a statement that makes the student's contribution clear to the examiners. This may be in the form of a description of the precise contribution of the student to the work presented for examination and/or a statement of the percentage of the work that was done by the student. In addition, in the case of co-authored publications included in the thesis, each author must give their signed permission for the work to be included. If signatures from all the authors cannot be obtained, the statement detailing the student's contribution to the work must be signed by the coordinating supervisor.

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Student Signature.....

Coordinating Supervisor Signature.....

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PUBLICATIONS ARISING FROM THIS THESIS

Primary authored publications

1. Lamoureux, S.C., Veneklaas, E.J., Poot, P. 2016. Informing arid region mine-site restoration through comparative ecophysiology of *Acacia* species under drought. *Journal of Arid Environments* **133**: 73-84.

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1. Lamoureux, S.C., Veneklaas, E.J., Poot, P., O’Kane, M.A. 2016. The effect of cover system depth on native plant water relations in semi-arid Western Australia. *Proceedings of the Eleventh International Conference on Mine Closure*, March 15-17, 2016, Perth, Australia.

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1. Lamoureux, S.C., Veneklaas, E.J., Poot, P. 2014. Comparative root ecophysiology of Australian dryland *Acacias* in a mine restoration soil under drought conditions. *Proceedings on the Sixth International Symposium on Physiological Processes in Roots of Woody Plants*, September 8-13, 2014, Nagoya, Japan.

CHAPTER 1

1. GENERAL INTRODUCTION

The thesis explores key aspects of plant soil interactions and their atmospheric drivers during short and long-term periods in a mine site restoration soil and on a newly constructed evapotranspirative (ET) cover system. It also focuses on species and community level differences in drought tolerance traits and water use “strategies”. Chapter 1 presents background information pertaining to the context and motivation for this thesis. Firstly, the major environmental risks associated with hazardous mine waste are discussed. The relevance of mine waste as a major issue concerning the mining industry today and in the future are further outlined. Secondly, engineered covers utilising the ‘store-and-release’ concept, the favoured method for sequestering hazardous mine waste from the environment are described. Design principles concerning the functioning of engineered covers are described for water limited systems, and reviewed through field and modelling studies. The key roles and interactions that vegetation have on engineered covers are also discussed. Knowledge gaps in the literature and the current state of practice regarding plant ecophysiology are identified and subsequently used to formulate the key research objectives of this study. Lastly, the structure of the thesis is outlined.

1.1. Context and Engineered Cover Systems Background

Degraded lands are estimated to cover 40 – 66 % of the world’s land (Kellner et al. 2011, Gibbs and Salmon 2015), making restoring 150 million hectares of disturbed and degraded land globally by 2020 a key target at the 2012 United Nations Rio+20 Conference on Sustainable Development (Menz et al. 2013). Some estimates of land degradation in dryland environments are between 10 – 20% (Reynolds et al. 2007), while others are as high as 70% (Dregne and Chou 1992). Arid environments account for approximately 41 – 47 % of the earth’s landmass, and constitute 75 % of Australia’s total area, the highest proportion of arid land of all continents (Gratzfeld 2003, Reynolds et al. 2007). Globally these areas contain many extractive resources (Gratzfeld 2003). For instance, live and pending mining tenements cover 91.8% of Western Australia’s Pilbara region (EPA 2014) with a revenue of \$73.7 billion in 2013-2014 from iron ore alone (DMP 2012).

Waste rock is a major by-product of mining worldwide and in Australia. It is most often piled into very large waste rock dumps (WRD's), creating new landforms that pose substantial environmental and public health risks to local and downstream receptors. Common risks include the release of low pH leachate and heavy metals from the base of WRD's, known as acid mine drainage (AMD), due to the oxidation of sulphide minerals in waste rock and subsequent release by percolating water. Engineered covers have proven effective at mitigating risks associated with hazardous waste (Albright et al. 2004, Albright et al. 2006). Covers often have multiples purposes reflected by specific design considerations. However, their general function is to limit water percolating through waste material minimising deep drainage, and to support plant communities that resemble those of the pre-mined landscape (Benson et al. 2001, Ayers et al. 2004, Abdel Rahman 2011).

Covers are generally constructed using locally available materials from the mining process, including stripped and stockpiled top soil, non-reactive subsoils and waste rock (Gwenzi et al. 2014). Cover materials are generally well-graded or multi-layered waste rock, top soil, or fine textured material designed for soil moisture storage (Khire et al. 2000, Ayers et al. 2004, Eamus et al. 2013). Covers utilising water balance principles are becoming increasingly prevalent in semi-arid to arid environments where annual potential evapotranspiration greatly exceed annual precipitation (Gee et al. 2006, Bohnhoff et al. 2009, Gwenzi et al. 2014). In the literature these covers are often mentioned as store-and-release covers, water balance covers, phytocaps, or evapotranspirative covers, hereafter referred to as ET Covers (Scanlon et al. 2005b, Bohnhoff et al. 2009, Gwenzi et al. 2014). These covers are designed to retain water during rainfall, then release a significant portion of pore-water back to the atmosphere during drier periods through evaporation of surface layers, and plant transpiration (Eamus et al. 2013, Arnold et al. 2015). Although ET covers rely on evaporation to remove a large portion of soil held water in arid systems, vegetation is key to controlling the water balance of ET covers by precluding deep drainage below the root zone through root water uptake (Scanlon et al. 2005b, Seyfried et al. 2005).

Drylands have been identified as ideal locations for landscape scale restoration opportunities because they are major resource hubs in need of restoration, and contain extractive companies capable of financing and implementing restoration research (Reynolds et al. 2007, Menz et al. 2013). Performance of ET covers has been mixed, and insights have mostly been gained by field studies of trial covers (e.g. Breshears et al. 2005, Fayer and Gee 2006) and by using water balance models (e.g. Berger 2000, Eamus et al. 2013) to assess hydrogeological cover objectives. The

effectiveness of ET covers in a semi-arid system with grass and shrub species was demonstrated by Fayer and Gee (2006) over a 17 year monitoring period by limiting deep drainage to the design objective of $< 0.5 \text{ mm yr}^{-1}$. However, Albright et al. (2004) observed higher than expected percolation rates due to preferential flow, inadequate soil water storage capacity, and low transpiration capability. He stressed the importance of examining site specific attributes such as cover vegetation and the soil water storage capacity of cover soils (Albright et al. 2004).

1.2. Cover System Models and Validation

Cover system models are central to cover system design due to the lack of long-term empirical data, and are used to understand long-term cover performance, including the effects of vegetation. Therefore, engineers and mine planners often use plant ecophysiological and water balance models to guide cover system construction. Cover soil and plant physiological characteristics are required for input into these models. However, the majority of models oversimplify plant contributions to ET cover performance. In a recent review, O'Kane Consultants (2014) highlighted deficiencies in current cover system models by identifying ecophysiological and physical characteristics of vegetation relevant to ET covers. The review generally concluded that models should account for physiological mechanisms governing transpiration. Other recommendations included the expansion of models to at least two-dimensions, better integration of root growth that responds to water and nutrients, and greater experimental validation of vegetation models including cover thickness, required soil properties, and root growth to support a given community type in a mining context (O'Kane Consultants 2014).

Scientific studies have also questioned the extent to which cover models accurately predict plant transpiration on ET Covers. For instance, Gwenzi (2011) determined that VADOSE/W (Krahn 2004) and the Soil-Plant-Atmosphere (SPA) model (Williams 2005) likely overestimated transpiration on a water limited engineered cover, as they are mostly driven by atmospheric water demand (VPD) rather than soil moisture, and make assumptions about root distribution for water capture (Gwenzi 2011). Deficiencies in vegetation modelling were also identified by Scanlon et al. (2005b) in a semi-arid to arid system where large decreases in water storage from spring through summer corresponded to substantial increases in plant cover from 1% to between 30 and 60% due to rapid spring/early summer growth. However, when a sensitivity analysis was performed on individual plant parameters (root depth, root-length, density, vegetation type, and LAI) using the

UNSAT-H model, variation in individual parameters had little to no effect on the model; rather, the model was most sensitive to either the presence or absence of vegetation (Scanlon et al. 2005b). Their results indicate the inability of current models to capture the opportunistic behaviour of vegetation as it responds to water storage changes especially for individual plant parameters. The use of two-way feedbacks between soil water storage and vegetation has been recommended to simulate vegetation more realistically on engineered covers (Scanlon et al. 2005b, Gwenzi 2011). Bohnhoff et al. (2009) identified the importance of validating models using field-measured water balance data from a 1.36 m layered cover in a semi-arid system. Poor estimates of ET and soil-water storage by all tested models (UNSAT-H, VADOSE/W, HYDRUS, and LEACHM) at the end of the study were attributed to changes in plant species composition. Variation in plant water use by different species and their interactions with hydrogeological cover system properties should be further explored for input into models, especially because plants and their associated traits are likely to vary in abundance and composition over time. Therefore, field studies on engineered cover systems are essential to improve cover system models, and the overall knowledge base regarding plant ecophysiological interactions on such systems.

1.3. Cover System Hydrogeology

The importance of hydrogeological soil properties has been examined in numerous field and modelling investigations (e.g. Nyhan et al. 1990, Benson et al. 2001, Albright et al. 2004, Benson et al. 2004, Albright et al. 2006, Eamus et al. 2013). In a semi-arid climate in the USA, Breshears et al. (2005) evaluated the effectiveness of a gravelly-sandy loam and found it able to store most of the rainfall such that 94 – 98% of precipitation could be evapotranspired. The presence of woody vegetation (*Pinus edulis* and *Juniperus monosperma*) and herbaceous plants was a key factor in removing excess soil water during winter conditions (Breshears et al. 2005). However, it was identified that the proportion of these plants could be optimised through varying soil textures and thicknesses due to its effect on vertical profile water (Breshears et al. 2005), and its influence on the relative proportions of woody and herbaceous plants (Breshears et al. 1997, Breshears and Barnes 1999). The opposite effect was observed by Gwenzi et al. (2011a) in a semi-arid bauxite residue disposal area where residue sand (>150 µm in diameter) ranging from three to six meters in depth exhibited low moisture retention and had a high saturated hydraulic conductivity (K_s). In this soil, plant water use was limited to 22% of annual total rainfall (693 mm) (Gwenzi 2011). Conclusions were that bauxite residue sand was not ideal for the construction of ET covers in

dryland systems due to high K_s and low soil moisture retention, which is not conducive for ET covers intended to restore near original ecosystem functionality (Gwenzi et al. 2011a). Modelling results based on three arid to semi-arid sites concluded that effective cover material should have a water storage capacity capable of storing the rainfall volume that can be expected in above-average wet months and years, plus account for intra and interannual variation in rainfall and climate variables that drive ET (Eamus et al. 2013). During simulations where rainfall ranged from 500 to 2000 mm annually, cover profiles consisting of pure clay and sandy clay prevented drainage for all levels of precipitation (Eamus et al. 2013). However, covers constructed with sandy loam and pure sand were only able to prevent drainage at an annual precipitation of up to 1500 mm and ≤ 500 mm, respectively (Eamus et al. 2013). Evaluating the efficiency of individual material properties only constitutes a portion of the interactions occurring on ET covers. Substantial consideration must be given to interactions between material properties and vegetation as plants are often included during the cover design process. The inclusion of vegetation on covers is of great benefit as they help achieve the store-and-release function by avoiding oversaturation and can improve the hydraulic properties of the cover.

1.4. Cover System Ecophysiology

The benefits of vegetation on ET covers are well documented and rely on root water uptake to increase ET through plant transpiration, thus limiting deep drainage to underlying mine waste (Benson et al. 2001, Ayers et al. 2004). Fayer and Gee (2006) demonstrated that increased vegetation cover significantly reduced drainage in a 1.5 m sandy loam cover compared to an un-vegetated plot. Moreover, Scanlon et al. (2005a) found that increased plant productivity resulted in reduced soil water storage thus precluding deep drainage below the root zone compared to an un-vegetated plot. Recently, however, (Arnold et al. 2015) determined that vegetation may only remove significant amounts of water through ET if plant coverage is high. In this regard, the importance of initial plant establishment and drought tolerance is key to the effectiveness of vegetation on ET covers in semi-arid to arid environments.

Feedbacks between soil characteristics and vegetation are central to plant community structure and composition (Li et al. 2012). They therefore play a major role in the establishment and survival of vegetation on ET covers in water-limited systems, especially through moisture retention. Plant available water is largely a function of texture, soil pore size and saturated hydraulic

conductivity (Bhaskar and Ackerly 2006, McDowell et al. 2008). Therefore, varying soil textures present different obstacles for plant water extraction (Bristow et al. 1984). As such, plant water uptake on different soil types is supported by a variety of morphological traits and contrasting water use strategies influencing vegetation community structure (Westoby and Wright 2006, McDowell et al. 2008). Plant available water in the growth medium is arguably the most important abiotic factor influencing vegetation and its services on ET covers.

Vegetation has a profound influence on many aspects of ET covers, which include cover hydrology, water redistribution, rainfall interception and soil hydraulics. This is especially true in arid lands where preferential infiltration in vegetated areas and soil moisture redistribution are two major processes influencing establishment and survival of vegetation (Ursino 2009, Li et al. 2012). However, the key plant parameter influencing ET cover performance is transpiration. Plant transpiration relies primarily on plant water availability which is largely a function of root distribution within the cover profile in combination with soil texture. With decreasing soil water potential, the associated decrease in soil hydraulic conductivity results in declining water transport towards plant roots (Seneviratne et al. 2010). This relationship was observed by (Arnold et al. 2015) on a semi-arid ET cover, where soil drying resulted in low root conductance and subsequent stomatal closure. Similarly, results by Gwenz et al. (2011b) highlighted the negative influence of chemical impedance of subsoil layers on vertical root growth of *Melaleuca nesophila* and *Acacia rostellifera*, restricting 90% of fine roots to the top 40 cm. Volumetric soil moisture fluctuations at this site accounted for 75% of the total variability in transpiration with winter rates approximately three times higher than summer rates due to soil moisture levels of 12% and 2%, respectively (Gwenz et al. 2012). The collective effects of high material saturated hydraulic conductivity and low moisture retention, combined with rainfall seasonality and shallow root systems translated into low transpiration rates due to low plant water availability in the root zone (Gwenz et al. 2011b, Gwenz et al. 2012). The aforementioned work (Gwenz et al. 2012) and other studies (e.g. Grigg et al. 2008, Poot and Veneklaas 2013) have demonstrated that lower summer transpiration rates were mediated by tight stomatal control in response to dry season moisture stress. However, stomatal regulation is only one of several physiological and morphological traits utilised by plants in dry-land environments to optimise plant function under moisture deficit.

Water use patterns vary markedly between species in dry-land environments, and are usually a function of combined physiological regulation and morphological adaptations. High stomatal

sensitivity to moisture stress is typical of isohydric stomatal regulation which prevents leaf water potentials (Ψ_1) from falling to extremes; other species operate under anisohydric regulation leaving stomates open but allowing leaf water potentials to become more negative to extract water at more negative soil water potentials (McDowell et al. 2008). Plants under drought stress may also limit water loss through the accumulation of leaf solutes allowing leaves to maintain turgor at lower soil water contents, and enabling them to generate more negative leaf water Ψ_1 (Warren et al. 2007, Bartlett et al. 2012). Some species maintain high solute concentrations throughout the year (i.e. low osmotic potential), while others ‘adjust’ concentrations prior to the dry season in response to drying soil (Turner and Jones 1980, Kramer and Boyer 1995). Physiological traits tend to be more plastic and respond quickly to moisture stress, whereas most morphological traits respond slowly, are less plastic and designed to avoid desiccation (Pohlman et al. 2005, Maseda and Fernandez 2016, Valladares and Sánchez-Gómez 2006). Morphological adaptations to moisture deficit include greater root depth and biomass allocation for increased water acquisition (Hernández et al. 2010), small diameter xylem vessels resistant to cavitation (Poot and Veneklaas 2013), and reduced leaf area to decrease water demand (Valladares and Sánchez-Gómez 2006). Physiological and morphological traits typically combine to form strategies that vary across habitats (Westoby and Wright 2006, McDowell et al. 2008, Mitchell et al. 2008), designed to avoid xylem embolism, one of the main causes of plant mortality (McDowell et al. 2008). Allocation to roots relative to shoots, root depth and density, and leaf physiology all operate together to regulate plant water extraction potential across varying soil textures (Sperry et al. 1998, Westoby and Wright 2006, Bartlett et al. 2012). Although some studies have attempted to link soil characteristics, soil water availability, plant water use strategies, physiological and morphological adaptations, more work must be undertaken to understand how these factors interact on ET covers in water limited systems.

2. RESEARCH SIGNIFICANCE, OBJECTIVES, AND OUTLINE

Linking plant ecophysiology with the hydrogeology of ET covers requires a clear understanding of restoration goals. Hobbs and Harris (2001) describe the importance of concise goal setting, which accounts for the system characteristics under restoration, the causes of degradation, and actions needed to restore various aspects of the system. Restoration of plant structural composition, functional success, and persistence are some of the primary restoration goals identified for degraded systems (Hobbs and Norton 1996, Higgs 1997). Restoration of a degraded site can be aided through

the use of past and nearby reference systems, however, restoration goals and decisions should focus on the characteristics of the system post disturbance and in the future, rather than its attributes in the past (Pfadenhauer and Grootjans 1999, Hobbs and Harris 2001). As previously discussed the key restoration goals of ET cover systems atop waste rock dumps are to limit deep drainage and support a stable plant community to restore near natural ecosystem functionality (Benson et al. 2001, Ayers et al. 2004). It is my opinion that for an ET cover, characteristics of the pre-disturbed site, natural sites in the area, and most importantly, the ET cover properties should be used in combination to guide appropriate and attainable restoration actions to satisfy ET cover goals. Specific native plant species from the area should be designated for ET cover re-vegetation based on their ecophysiological suitability given ET cover material properties, rather than selecting those that persisted pre-disturbance under a different set of hydrogeological material characteristics. Therefore, species most suitable for revegetation may be those native to the area, but new to the site in question and novel to the ET cover system itself.

To attain pre-defined ET cover objectives, however, emphasis must be placed on improved comprehension of the soil-plant-atmosphere continuum (Arora 2002, O'Kane Consultants 2014, Arnold et al. 2015). An improved understanding of interactions in the continuum is vital for the effective establishment and development of vegetation on ET covers. This continuum is seldom studied on vegetated engineered covers, especially in water limited systems. Potential for utilising ET covers as a platform for functional ecosystems exists but is dependent on our understanding of constraints and opportunities imposed by the soil, the climate and the plants. Better quantification of site specific factors on ET covers was corroborated by Gee et al. (2006), and recently by (Arnold et al. 2015) where soil properties, local plant community traits, and corresponding soil water storage were evaluated. Several studies have recommended specific research tasks intended to capture the dynamic interplay between soil texture and water, plant community types, and climate in order to design long term robust ET covers (e.g. Arora 2002, Gee et al. 2006, Gwenzi et al. 2012, Li et al. 2012, Arnold et al. 2013, O'Kane Consultants 2014, Arnold et al. 2015). Recommended research tasks can be summarised as:

- Improved *in situ* measurements of species and community level water use patterns and plant physiological and morphological adaptation to moisture stress on ET covers;

- Characterization of soils and their moisture dynamics in association with soil-plant-atmosphere feedbacks on ET covers;
- The effect of soil characteristics such as cover thickness, saturated hydraulic conductivity, and compaction in relation to plant establishment, persistence, water use, and vegetation community characteristics for ecosystem rehabilitation on ET covers;
- Improved selection of vegetation at the species and community level, matched according to hydrogeological and ecophysiological characteristics of the cover soil, vegetation, climate, and ecosystem services required of ET covers for a given location; and
- Cover system models which allow improved integration of plant physiological and morphological traits, cover thickness, and texture for the most appropriate ET cover design and vegetation community type selection for a given area.

Motivated by the aforementioned gaps in the state of knowledge and practice, this thesis is comprised of one glasshouse experiment, and two field investigations. These were designed to better understand key aspects of the soil-plant-atmosphere continuum during short and long-term periods, and at the species and community level in a mine restoration soil and on a newly constructed ET cover system.

Chapter 2 presents a comparative glasshouse study that investigated the physiological and morphological traits associated with drought tolerance of nine Pilbara *Acacia* species in a typical mine site restoration soil, and if these traits correlated with their respective soil preferences. Water use patterns and key physiological shoot, and morphological root and shoot traits associated with drought tolerance were explored at the species and community level. We hypothesised that, when faced with soil moisture deficit, *Acacia* species with a tendency to keep stomata open for longer, allowing further decreases of plant water potentials, would be associated with lower osmotic potentials at full hydration (π_{sat}) and greater biomass investment in lateral roots. We also expected such species to typically occur in more fine-textured alluvial soils than more coarse-textured sandy and rocky soils. This chapter has been published as follows: “Lamoureux, S.C., Veneklaas, E.J., Poot, P. 2016.

Informing arid region mine-site restoration through comparative ecophysiology of *Acacia* species under drought. *Journal of Arid Environments* 133: 73-84.”

Chapter 3 explores plant ecophysiology, plant water use, and plant response to soil moisture storage as a result of cover thickness, for six semi-arid native plant species on an ET cover over 2 years in semi-arid Western Australia. The primary objectives were to (1) determine if shallow covers compromise plant function in a semi-arid climate by assessing the influence of soil depth on moisture storage and its effect on physiological processes as they respond to seasonal environmental fluctuations (2) relate patterns of root distributions, functional traits, and growth for each species and cover thicknesses to observed physiological processes. We hypothesised that plant physiology would mostly be driven by seasonal shifts in soil volumetric water content (VWC), and that plants growing on thicker covers would have more access to soil moisture due to greater moisture storage and unconfined roots, resulting in overall larger g_s and decreased physiological sensitivity to dry summer atmospheric conditions. We also anticipated that species with higher g_s would have lower investment in leaf area relative to the size of their root system.

Chapter 4 quantifies diurnal plant water use of 2-year old *Eucalyptus* species from rocky soils (*Eucalyptus loxophleba*) and sandy soil (*Eucalyptus leptopoda*) on an engineered cover system at three soil depths in a semi-arid climate during summer. The primary objectives were (1) to investigate the influence of soil depth on plant water availability and its effect on diurnal shifts in stomatal conductance (g_s), E , area based net photosynthesis (A), intercellular CO_2 concentrations (C_i), pre-dawn (Ψ_{PD}), and mid-day (Ψ_{MD}) leaf water potentials; and (2) to better understand the impact of water availability on key physiological processes (g_s , E , A , C_i , Ψ_{PD} , and Ψ_{MD}) to determine whether they differed between species and soil depth in response to diurnal fluctuations in VPD, temperature, irradiance, and soil moisture. We tested the hypothesis that plants growing on deeper soils would have more access to soil moisture resulting in decreased sensitivity to increasing daily VPD, temperature, and irradiance. We also anticipated that plants on deeper soils would have higher g_s , E , A , Ψ_{PD} , and Ψ_{MD} .

Chapter 5 summarises the main results of the work presented in this thesis and provides a synthesis of its key contributions to the field of restoration ecology and ecohydrology on engineered cover systems in semi-arid to arid climates. It also discusses future research directions to further improve restoration technologies and knowledge on engineered covers.

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CHAPTER 2 Informing arid region mine-site restoration through comparative ecophysiology of *Acacia* species under drought

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PREFACE

This chapter was published as follows: “Lamoureux, S.C., Veneklaas, E.J., Poot, P. 2016. Informing arid region mine-site restoration through comparative ecophysiology of *Acacia* species under drought. *Journal of Arid Environments* 133: 73-84.” The rationale for this chapter was to begin the thesis with a glasshouse experiment examining physiological and morphological shoot traits, morphological root traits, and water use strategies associated with drought tolerance in a typical restoration soil. Establishing vegetation on disturbed soils in arid environments is notoriously difficult, therefore, I felt it was essential to examine traits associated with drought tolerance in these soils, and to investigate whether these traits were correlated with species’ preferred soil types based on *a priori* classification from undisturbed systems. This was done to focus the study prior to expanding the overall work into a field experiment in Chapters 3 and 4.



ABSTRACT

Establishing vegetation on disturbed sites in arid environments is difficult due to decreased water availability caused by altered soil properties: depth, compaction, and hydraulic characteristics. Plants cope with moisture stress through a combination of traits, including physiological strategies such as anisohydry and isohydry. We used a typical mine restoration substrate in a glasshouse pot experiment to investigate drought tolerance of nine Pilbara region *Acacia* species classified according to habitat preferences defined by preferred soil type: alluvial (fine textured), sandy, rocky, and generalists without a clear soil preference. Seedlings were examined to (1) determine physiological shoot, and morphological shoot and root traits associated with drought tolerance, and (2) identify if these traits were correlated with species' soil preferences. Species from alluvial, rocky, and one sandy soil species were more anisohydric. These species had higher stomatal conductance at more negative leaf water potentials. Alluvial soil species had greater total biomass allocation to lateral roots, whereas two coarser textured soil species had high allocation to tap roots. Soil preference was a poor predictor of plant water relations, presumably due to complex soil profiles in nature associated with widely differing hydraulic characteristics, and interactions among plant functional traits influencing water uptake, transport and loss.

1. INTRODUCTION

Plant establishment for vegetation restoration on mine-impacted sites is often challenging, especially in arid environments where moisture deficits pose a considerable hindrance. In such conditions, water availability may be further compromised by alteration of soil hydraulic characteristics by machinery (e.g. compaction), and lack of topsoil to construct profiles with the required depth and moisture holding capacity (Sheoran et al. 2010). Better understanding of physiological and morphological adaptations to drought may aid species selection and substrate design resulting in enhanced establishment of plant species in restored areas, such as former mine sites (Grierson et al. 2011). Although studies have presented useful information concerning species response to drought, some knowledge gaps exist regarding the traits and strategies used for assessment of drought tolerance (e.g. Valladares and Sánchez-Gómez 2006, Galmés et al. 2007, Bartlett et al. 2012). Remaining physiologically active under moisture stress hinges on the ability to tolerate high water

tensions in the xylem which enables continued moisture uptake from a drying soil (Bhaskar and Ackerly 2006). Although physiological adaptations are essential to drought tolerance, soils and plant-soil interactions strongly affect those adaptations, thus influencing plant available water across climates (Hacke et al. 2000, Sperry and Hacke 2002, Jury and Horton 2004).

Plant adaptations to drought in seasonally dry environments are essential for their survival. To maximise long-term carbon gain, and nutrient use efficiency (Gray 1983), many species in these environments are evergreen and have long-lived leaves with low nutrient concentrations, low leaf area to mass ratios, low photosynthetic rates, and high root:shoot ratios (Mooney and Dunn 1970, Kummerow et al. 1977, Ackerly 2004). Another advantage for survival in drought prone environments is high tolerance to low water potentials in xylem tissues to avoid cavitation. High wood density and its associated resistance to cavitation has been shown to be an effective drought adaptation (Hacke et al. 2001). Plants may also cope with moisture stress through physiological controls such as anisohydric and isohydric strategies. Under declining soil water potentials (Ψ_s), anisohydric plants typically have more negative leaf water potentials (Ψ_l), maintain higher stomatal conductance (g_s), and often have wider Ψ_l ranges (Barnes 1986, McDowell et al. 2008). Conversely, isohydric plants reduce g_s in response to decreasing Ψ_s thus maintaining more constant Ψ_l to avoid water transport failure (Sperry et al. 2002, McDowell et al. 2008). However, even within semi-arid to arid systems, plant adaptations to drought vary as different combinations of traits provide drought tolerance across varying soil types and associated hydraulic properties.

Plants grow on a range of soil types that have different hydraulic characteristics with textures ranging from heavy clays to coarse sands (Hacke et al. 2000). Plant water availability is largely a function of soil texture through the influence of pore size, saturated hydraulic conductivity, and moisture retention capacity (Bhaskar and Ackerly 2006, McDowell et al. 2008). Therefore, varying soil textures present different challenges for plant water extraction (Bristow et al. 1984) and species associated with different soil types often have different morphological traits and water use strategies to facilitate plant water uptake (Westoby and Wright 2006, McDowell et al. 2008), suggesting a close coordination between plant traits or strategies and soil profiles/textures (Westoby and Wright 2006). For instance, populations of shrub species growing on fine-textured soils generally have more negative Ψ_l than

populations of the same species growing on coarse textured soils (Sperry and Hacke 2002). These more negative Ψ_1 reflect the smaller and more abundant pore spaces in fine textured soils that hold water at more negative Ψ_s (Sperry and Hacke 2002). Additionally, plant rooting depth may be influenced by moisture retention across soil textures (Jackson et al. 2000). Plants on sandy soils are required to grow roots into deeper moist soil layers due to low soil moisture retention capacity of sands (Sperry and Hacke 2002). Deep growth in coarser textured soils is also supported by greater oxygen availability at depth, and easier root penetration, especially in sandy soils (Sperry and Hacke 2002). In finer textured soils deep root growth is reduced due to broader Ψ_s ranges as a result of smaller pore spaces, shallower wetting, and impedance to root penetration (Sperry and Hacke 2002). This stresses the importance of quantifying drought adaptation across soil types within the same water limited environment.

The Pilbara region in NW Australia is semi-arid to arid, and is prone to periods of severe seasonal drought (Van Vreeswyk 2004). Despite severe moisture deficits it hosts a large plant diversity with 1,094 native vascular species, including 150 conservation significant species, on 21 soil groups forming 44 plant-soil associations (Van Vreeswyk 2004, EPA 2014). The Pilbara is mostly dominated by *Triodia-Acacia-Eucalyptus* alliances, however, species within these genera vary across sub-regions and soil types (Beard 1975, Van Vreeswyk 2004), and thus are expected to exhibit functional differences reflecting adaptations to varying soil types. The region is rich in mineral resources producing more than 90% of Australia's iron ore (DMP 2012). Active and pending mining tenements cover 91.8% of the Pilbara, and thus large areas will require restoration of native flora due to mining disturbance (EPA 2014). Because mining restoration substrates differ from naturally occurring soils in depth, compaction, and hydraulic characteristics, physiological and morphological plant responses to drought stress in these substrates are largely unknown. Therefore, it is of value (1) to examine how the ecophysiological and morphological mechanisms of drought tolerance differ among common Pilbara species when grown on a common mine site restoration substrate, and (2) to determine whether potential differences are related to species' preferred soil types. The latter would suggest that specific adaptations to local soil types or profiles occur and are expressed even when species are grown on a common substrate.

In this study, nine Pilbara *Acacia* species were grown in a typical mine site restoration substrate in a glasshouse experiment with the following aims: (1) determine the physiological shoot, and morphological shoot and root traits associated with drought tolerance, and (2) determine if these traits differed between their preferred soil types. We hypothesised that, when faced with soil moisture deficit, *Acacia* species with a tendency to keep stomata open for longer, allowing further decreases of plant water potentials, would be associated with lower osmotic potentials at full hydration (π_{sat}) and greater biomass investment in lateral roots. We also expected such species to typically occur in more fine-textured alluvial soils than more coarse-textured sandy and rocky soils.

2. MATERIALS AND METHODS

2.1. Study Species

Nine Pilbara *Acacia* species from four contrasting preferred soil types were chosen for this study. Species were categorised according to their soil preferences based on evidence gathered from literature, online flora descriptions (Flora Base, <http://florabase.dpaw.wa.gov.au/>) and expert consultations. Soil preference categories are as follows:

- alluvial (fine textured) soil species: *Acacia aneura* Benth., *A. citrinoviridis* Tindale & Maslin, and *A. cowleana* Tate;
- sandy soil species: *A. coriacea* subsp. *pendens* R.S.Cowan & Maslin and *A. stellaticeps* Kodela, Tindale & D.A.Keith;
- rocky soil species: *A. pruinocarpa* Tindale and *A. maitlandii* F.Muell.; and
- generalist species: *A. bivenosa* DC. and *A. ancistrocarpa* Maiden & Blakely.

Although study species were categorised according to these soil preferences, we recognise that within-species variation in distribution patterns exists, e.g. related to ecotypes or interactions with climate. Soil preferences indicated here reflect the preferred soil types in which these species are most commonly found.

2.2. Experimental Design

The experiment was conducted in a glasshouse mimicking the climate in the species' native habitats. Air temperature was approximately 32°C during the day and 25°C at night (Appendix A). During the growth stage (14 August 2013 – 14 February 2014), automated shade cloths on the glasshouse structure were raised when outside temperature reached 30 °C or more which reduced sunlight by 60%. During the experimental period (15-25 February 2014) plants received natural light which peaked on average at approximately 1500 $\mu\text{mol m}^2 \text{s}^{-1}$ during mid-day; shade cloths were not deployed, however, the glasshouse structure reduced light by 25%. Plants were grown in 2.8 L free-draining pots (diameter 16.5 cm, height 16.5 cm). Pots were lined with a fine synthetic mesh and a thin layer of gravel (0.40 kg) to retain soil, and inhibit root growth out of the pots. Each pot was filled with soil (2.60 kg; see details below), and then topped with a thin layer of gravel (0.30 kg) to limit soil water evaporation. Soil was kept moist and allowed to settle for one week prior to sowing.

Acacia species seeds were collected from the Pilbara and obtained from the Botanical Gardens and Parks Authority (Kings Park, Perth, Western Australia). Seeds were scarified in near-boiling water prior to sowing. Ninety pots were sown with five seeds from one of nine *Acacia* species. Ten additional pots were left without plants to quantify soil evaporation rates. A standard block design was used across five benches in the glasshouse, each *Acacia* species having two pots (one well-watered, one water-stressed) per bench. Pots were randomised within each block. Pots were watered to field capacity during the growing stage, by hand for the first month, and by micro-irrigation three times daily for one minute thereafter. After six months, one healthy seedling (of representative size for its species) was kept per pot, and the extras were cut at the stem-soil interface.

Some mortality occurred during the well-watered phase of the experiment reducing the number of replicates for some species. Each species had five replicates for drought and control treatments, with the exception of *A. maitlandii*, *A. cowleana* and *A. stellaticeps* with four plants for both treatments. All pots were hand-watered and weighed to a constant soil water content of 0.1395 g g^{-1} on the afternoon before the start of the experimental treatments (February 15, 2014). Watering then ceased for all drought plants, but continued for control plants which were watered daily to the same initial water content. All plants had phyllodes rather than true leaves during experimental conditions; all physiological measurements were

conducted on the youngest fully grown phyllodes. Phyllodes will be referred to as leaves hereafter.

2.3. *Soil Analysis*

Soil for the experiment was collected from a restored waste rock dump at Mount Goldsworthy (20°21'04.6 S, 119°31'42.4 E, 92 m a.s.l.) in the Pilbara. The top 50 cm were collected using a backhoe, then transported in barrels to the University of Western Australia Plant Growth Facilities (PGF), Perth, Western Australia (31°59'03.2 S, 115°53'10.3 E, 5.2 m a.s.l.). Soil was air dried in a drying room for 7 days and then sieved to discard particles >5 mm. The soil was analysed in the Wesfarmers CSBP Ltd. Soil and Plant Analysis Laboratory (Bibra Lake, Western Australia) to determine physical and chemical properties, including soil texture (size classes: 0.02 > sand < 2.0, 0.002 > silt < 0.02, clay < 0.002 mm; %; Modified Pipette Procedure), electrical conductivity (1:5 soil: water extract), pH (1:5 soil: water extract and 1:5 soil: CaCl₂ 0.01M solution), organic carbon (Walkley-Black method), ammonium nitrogen (extracted in 1M KCl), nitrate nitrogen (extracted in 1M KCl), plant available phosphorus (Colwell P, bicarbonate extraction), potassium (Colwell K, bicarbonate extraction), sulphur (extracted in KCl at 40°C), exchangeable cations (aluminium, calcium, magnesium, potassium, and sodium; Mehlich No. 3 test), extractable copper, iron, manganese and zinc (DTPA extraction), and boron (hot CaCl₂ method) (Appendix B). Plants were fertilised twice during the growth period with an application of 0.1 L of liquid fertiliser onto the soil at a concentration of 0.005 kg L⁻¹ containing 95 mg of nitrogen, 42 mg of phosphorus, and 79 mg of potassium.

A soil water retention curve was produced for the experimental soil. Water was added to dry soil at concentrations ranging from 0.0041 to 0.104 ml g⁻¹, mixed in air-tight plastic bags, allowed to equilibrate for 24 hours, and measured using a WP4C dewpoint water potential meter (Decagon Devices Inc., Pullman, WA, USA). Gravimetric soil moisture content (%) of analysed soil was then verified using the oven-drying method (Appendix C).

2.4. *Physiological Functional Traits*

Evapotranspiration (ET; water loss from pots with plants), evaporation (E; water loss from pots without plants), and pot water content were determined daily by weighing the pots at the

same hour every day during the experimental period. Whole-plant daily transpiration was estimated as $ET - E$ for every plant, and leaf area-based transpiration was calculated by dividing whole-plant transpiration by leaf area as determined at the final harvest. Leaf area (LA) was determined with a scanner and using WinRHIZO software (Regent Instruments, Quebec, Canada). Standardised daily leaf-area-based transpiration rates for each drought plant across all species were calculated as:

$$\text{Standardised Transpiration} = \frac{\text{Daily Transpiration/LA}}{\text{Mean (Control Plant Daily Transpiration/LA)}} \quad \text{Equation 1}$$

During the experimental period, starting 15 February 2014, stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{ s}^{-1}$) was monitored daily during mid-morning (08:00 – 10:00) on the same position on one fully grown leaf per plant leaf location, using a Leaf Porometer (Decagon Devices Inc., Washington, USA) calibrated before every use. Pot weighing for quantification of ET , E , and pot water content was conducted every afternoon (14:00 – 16:00). Daily measurements continued until individual drought pots reached a soil water content of 0.035 g g^{-1} (equivalent to -2.18 MPa according to the soil water retention curve), when plants were harvested. The harvest cut-off soil moisture content of 0.035 g g^{-1} was chosen to represent moderate stress, based on a pilot experiment, avoiding the risk of mortality in the least tolerant species. Leaves (one per measurement) for measuring leaf water potentials (Ψ_l ; MPa) and osmotic potential (π ; MPa) were harvested (15:00 -17:00) from plants that reached the pre-defined drought water content after pot weighing; these plants were harvested the following morning after the final measurement of g_s . The average soil water content at harvest was 0.0325 g g^{-1} (-2.64 MPa), with some variability among individual plants, as some plants were slightly over, or under the harvest threshold, and were likely to surpass the threshold by the following day. The number of days for each plant to reach pre-defined harvest conditions was recorded.

Leaf water potential was measured using a Pressure Chamber (PMS Instruments, Oregon, USA). Leaves were cut using a scalpel, wrapped in aluminium foil, placed in an airtight plastic bag, and kept cool for immediate transport and analysis in the nearby laboratory. A grass-compressing gland sealing system was used to hold leaves in place as they were not petiolate. Leaves for measurement of π were collected in the same manner. Each leaf sample was then cut in half lengthwise; one half for estimating leaf relative water content (RWC) and the other for osmotic potential. Samples for osmotic potential were frozen in a -80°C

freezer immediately after cutting, and thawed in their vials for one hour before sap expression (Turner 1981). Leaf osmotic potential was measured using a vapour pressure osmometer and sample chambers (PSYPRO CR-7 and C-52, Wescor Inc, Utah, USA). Filter paper discs were wetted in leaf sap then placed into sample chambers at an ambient temperature of 21°C. Osmotic potential at full turgor (π_{sat} ; i.e. at 100% RWC) was calculated using Equation 2-2 (Ludlow et al. 1983).

$$\pi_{sat} = \pi \times RWC \quad \text{Equation 2}$$

Osmotic adjustment was calculated as the difference in π_{sat} between droughted and control plants. Leaf RWC was measured using the method outlined by Ryser et al. (2008). Leaf fresh mass (FM_{Leaf}) was measured immediately after cutting. Leaf saturated mass (SM_{Leaf}) was measured after leaves were placed between moist paper towels for 24 h and covered with aluminium foil at room temperature. Leaf dry mass (DM_{Leaf}) was determined after samples were dried for 48 h in a 70°C oven. Leaf relative water content was computed using Equation 2-3.

$$RWC = \frac{FM_{Leaf} - DM_{Leaf}}{SM_{Leaf} - DM_{Leaf}} \quad \text{Equation 3}$$

2.5. Morphological Functional Traits

During harvest aboveground components were separated into leaves and stems. Fresh weights were measured for all aboveground components, and leaves were scanned to determine LA using WinRHIZO software. Roots were carefully washed atop a sieve to avoid loss of fine roots within 3 days of harvest, placed in plastic bags, and kept refrigerated at 5°C for up to 2 days prior to analysis. The majority of roots had not reached the bottom of the pots; in only a few pots did fine roots make contact with the synthetic fabric. Belowground components were separated into tap and lateral roots and weighed. Root scanning was conducted on sub-samples of each root system, due to their size. Sub-samples constituted of the tap root and lateral roots from one side of the tap root. Root length was determined for tap and lateral roots in each sub-sample using WinRHIZO software. All plant components (leaves, stems, tap roots, and lateral roots) were oven dried at 70°C for 48 h to determine the dry weight of each component. Specific leaf area (SLA ; $\text{cm}^2 \text{g}^{-1}$) was computed by dividing LA by leaf dry mass. The mass fraction of leaves (LMF ; g g^{-1}), stems (SMF ; g g^{-1}), tap roots

(RMF_{TR} ; g g^{-1}), and lateral roots (RMF_{LR} ; g g^{-1}) were calculated by dividing their respective dry weights by total plant dry weight. The tap root to lateral root mass ratio (TR/LR Ratio; g g^{-1}) of each plant was determined for whole root systems by dividing the dry mass of tap roots by the dry mass of lateral roots. The specific root length of lateral roots (SRL_{LR} ; cm g^{-1}) for each plant was determined by dividing lateral root length by the dry mass of lateral roots. The root length to leaf area ratio (RL/LA Ratio; cm g^{-1}) was computed by dividing total root length by LA for each plant.

Stem specific density (SSD; g mm^{-3}) was determined for each plant using the dimensional method (Pérez-Harguindeguy et al. 2013) where the oven-dry mass (at 70°C for 72 h) of a main stem section including bark (DM_{Stem}) was divided by its fresh volume. Stem volume (V_{Stem}) and SSD were computed as:

$$V_{\text{Stem}} = (0.5D)^2 \times \pi \times L \quad \text{Equation 4}$$

$$\text{SSD} = \frac{\text{DM}_{\text{Stem}}}{V_{\text{Stem}}} \quad \text{Equation 5}$$

where D and L are stem diameter and length, respectively, measured with a digital micrometer (Mitutoyo, Honshu, Japan).

2.6. Data Analysis

Data were analysed with RStudio statistical software (R v0.98.1028, R Foundation for Statistical Computing, R, 2013). Analysis of covariance (ANCOVA) was conducted to test differences in slopes and intercepts among regression lines for log-transformed standardised transpiration (response variable) with log-transformed Ψ_s (explanatory variable), and either soil type with nested species, or species as the categorical variables. Natural logarithmic transformations were applied to standardised transpiration and Ψ_s to achieve linearity thus satisfying assumptions of the ANCOVA. The same analysis was conducted for g_s (response variable). Tukey's multiple pairwise comparison test was used to analyse differences among species or soil groups if ANCOVA model outputs were significant.

Differences in Ψ_l , π_{sat} , LMF, SLA, SSD, RMFs, SRLs, TR/LR Ratio, and RL/LA Ratio for treatment, soil groups with nested species, and species were tested using mixed effects models with weighted variance structures to ensure homogeneity of variance as outlined in Zuur et al. (2009). When testing between soil type preferences, soil type was a fixed factor and species within soil types a random factor. When testing between species the fixed factor was species. Nested models were tested using five variance structures (VarFixed, VarIndent, VarPower, VarExp, and VarConstPower) and Analysis of Variance (ANOVA). Models were evaluated and selected using Akaike's information criterion (AIC; a measure of relative model performance for a given set of data), and plots of standardised residuals. Models with the lowest AIC value, and homogeneity of variance were chosen for further analysis. Selected models were subsequently tested using ANOVA at $\alpha = 0.05$. Tukey's multiple pairwise comparison test was used to analyse differences among species or soil groups if ANOVA model outputs were significant. Mean differences in π_{sat} for osmotic adjustment within species, between control and drought plants were calculated using paired one-tailed t-tests with a 95% confidence.

3. RESULTS

The experimental soil used had no major geochemical restrictions to plant growth (Appendix B). The soil was classified as a sandy loam with a soil moisture retention curve that displayed a gradual decline of Ψ_s with decreasing soil moisture content (Appendix C). Experimental plants appeared healthy throughout the growth period and showed no signs of nutrient deficiency or toxicity.

3.1. Physiological Functional Traits

Differences in log-transformed standardised transpiration (leaf-area-based transpiration of droughted plants, relative to that of well-watered plants), and the rate of decline (slopes in Figure 3-1) were apparent but not significantly different between species ($F_{8, 216} = 1.78$, $P > 0.05$) or preferred soil type with nested species ($F_{2, 4} = 6.85$, $P > 0.05$). Log-transformed standardised transpiration of *A. bivenosa* (generalist group) was most sensitive to soil water status, while *A. ancistrocarpa* (generalist group) was least sensitive (Figure 3-1). At the Ψ_s corresponding to 25% of the Ψ_s at field capacity (-2.2 MPa), these two species had reduced transpiration by 31% and 26%, reaching predefined drought conditions after 6 and 7 days,

respectively. Overall, species from the rocky soil group tended to be least sensitive to soil water status, with transpiration rates at 25% soil field capacity reduced by 28%. These species took 8 days to reach predefined drought conditions. Plant sizes varied among species and preferred soil types, therefore, the effect of plant size on standardised transpiration is thought to be negligible.

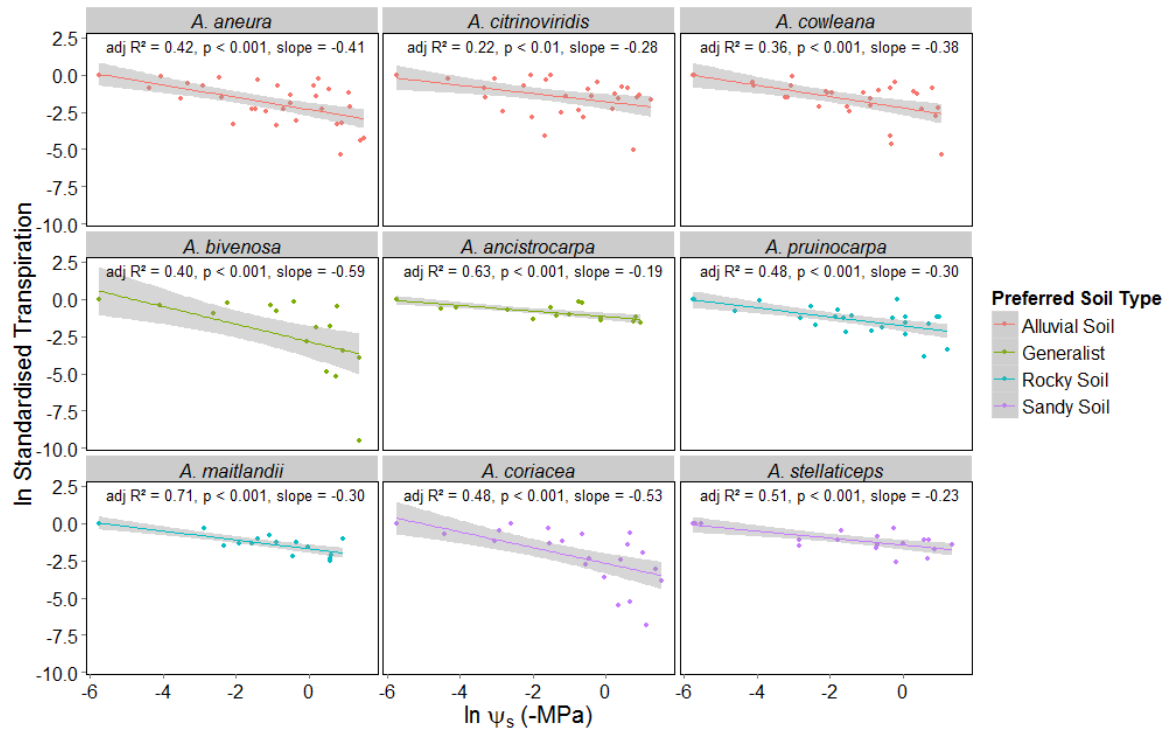


Figure 3-1. The natural logarithm of daily standardised transpiration during pot drying (transpiration of droughted relative to that of well-watered plants) as a function of the natural logarithm of soil water potential (Ψ_s ; -MPa) for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Grey shaded areas denote 95% confidence bands around the regression line. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

Patterns in the reductions of g_s as a function of decreasing Ψ_s ($R^2 = 0.12$, $P < 0.01$) largely coincided with those for standardised transpiration as a function of decreasing Ψ_s . Unlike transpiration, the rate (slope) of g_s decline as function of drying soil differed significantly among species ($F_{8, 219} = 10.41$, $P < 0.0001$) and was associated with preferred soil type with nested species ($F_{2, 4} = 7.75$, $P < 0.05$). Decline in g_s was highest in the generalist soil group but was only significantly ($P < 0.05$) different from the sandy soil group, and lowest in the rocky soil group with significantly ($P < 0.05$) lower rates compared to the alluvial and sandy soils groups (Figure 3-2). *A. pruinocarpa* of the rocky soil group was least sensitive to

decreasing Ψ_s compared to six other experimental species, and continued to have high rates of g_s ($P > 0.05$) at more negative Ψ_s (Figure 3-2). The absence of a response of early morning g_s to drying in *A. pruinocarpa*, despite a significant decrease in transpiration, may point to stomatal closure later during the day in this species.

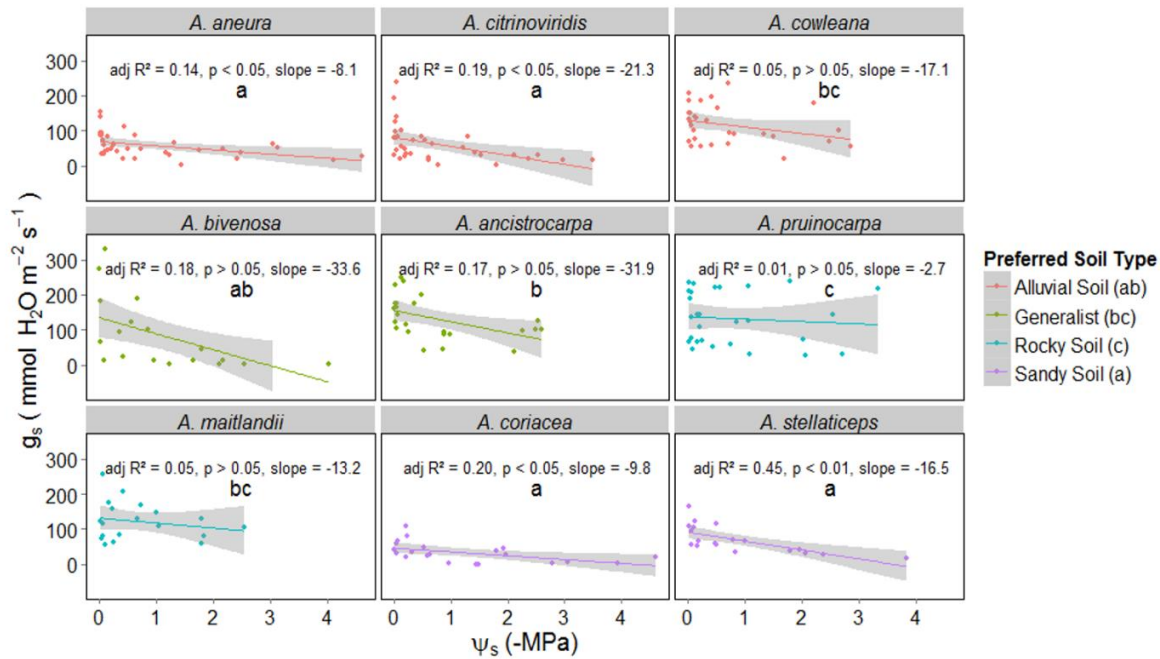


Figure 3-2. Daily morning (08:00 – 10:00) stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{s}^{-1}$) as a function of soil water potential (Ψ_s ; -MPa) for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Grey shaded areas denote 95% confidence bands around the regression line. Letters indicate significant differences ($P < 0.05$) between species, or preferred soil types. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

To explore if species' responses to drying soils tended towards isohydric or anisohydric behaviour, Figure 3-3 shows their sensitivity of g_s plotted against their change in Ψ_1 . The generalist Group (*A. bivenosa*, *A. ancistrocarpa*) had the highest rates of g_s decline, and along with *A. stellaticeps* the smallest differences between control and drought Ψ_1 . The tendency to keep Ψ_1 less negative through stomatal closure points to isohydric behaviour. The alluvial soil group (*A. aneura*, *A. cowleana*, and *A. cowleana*), rocky soil group (*A. maitlandii*, *A. pruinocarpa*), and *A. coriacea* had slow to moderate rates of decline, and the largest Ψ_1 differences between control and drought plants. These species kept their stomata open, reducing Ψ_1 , making them better adapted to extraction at more negative Ψ_s , or soil with higher clay content.

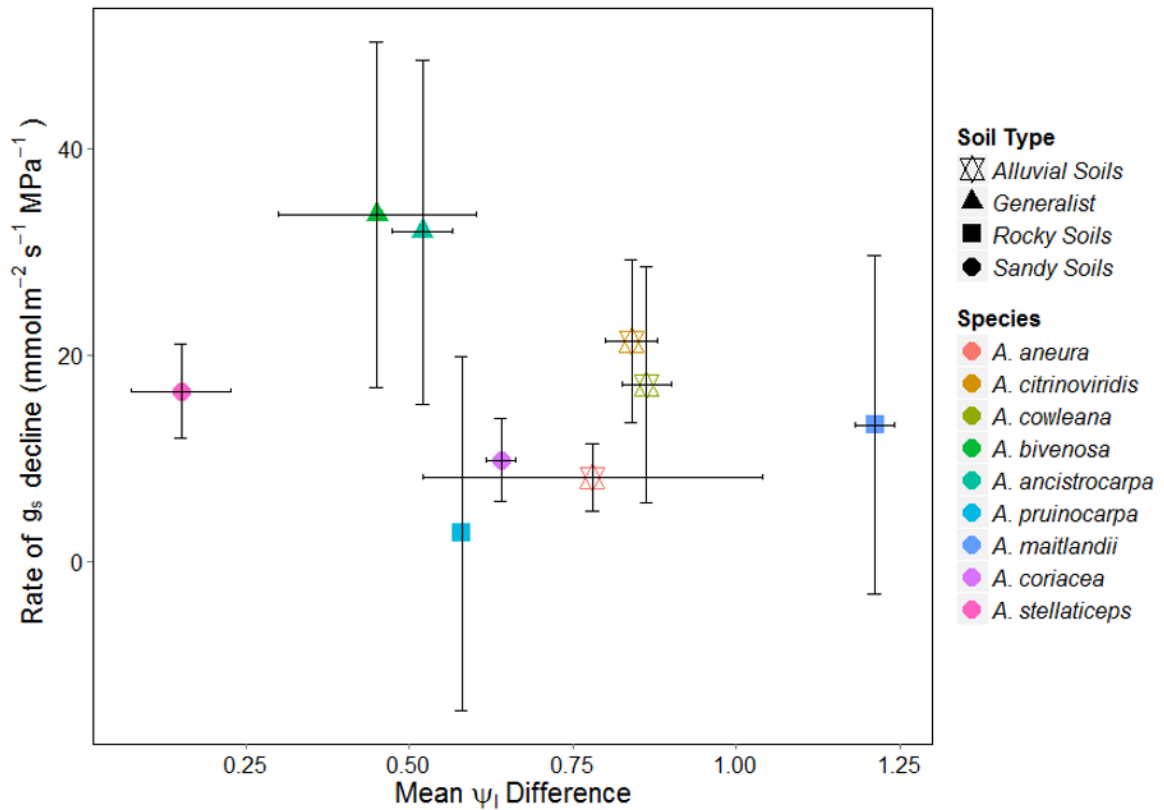


Figure 3-3. Rate of stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{s}^{-1}$) decline as a function of drying soil (slope) (\pm SE) plotted against the mean (\pm SE) Ψ_1 difference between control and drought plants for nine *Acacia* species and their preferred soil types from the West Australian Pilbara. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

There were significant differences in Ψ_1 between species ($P < 0.05$), with *A. aneura*, and *A. stellaticeps* having the most negative and least negative Ψ_1 for drought plants, respectively (Figure 3-4). However, there was no clear association between soil groups and Ψ_1 .

Difference between control and drought plant Ψ_1 for species in the alluvial soils group were similar, with changes in Ψ_1 ranging from -0.78 to -0.86 MPa (Figure 3-3, Figure 3-4), and were coupled with intermediate rates of g_s decline (Figure 3-3) and transpiration (Figure 3-1). However, the greatest Ψ_1 difference between control and drought plants occurred in *A. maitlandii* from the rocky soils group at -1.21 MPa (Figure 3-3), but was not significantly more negative compared to other species (Figure 3-4).

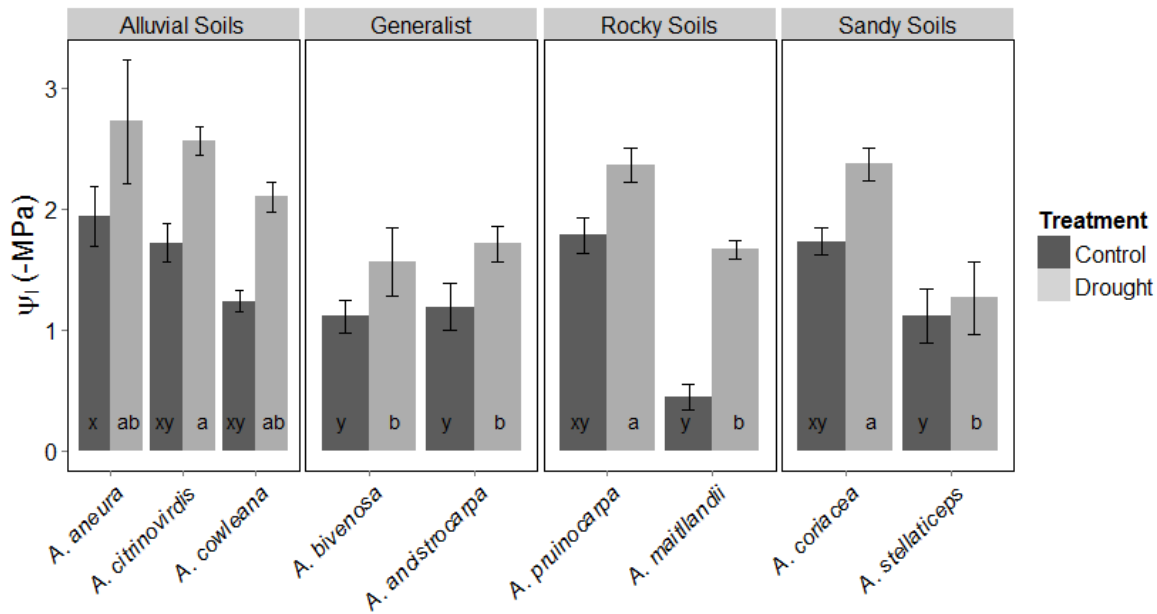


Figure 3-4. Mean (\pm SE) leaf water potential (Ψ_1 ; -MPa) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) between species within treatments. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

Overall Ψ_1 and leaf osmotic potential at full turgor (π_{sat}) of drought plants were negatively correlated ($R^2 = 0.21$, $P < 0.01$). There were significant differences in π_{sat} between species for drought plants ($P < 0.05$), with *A. ancistrocarpa* and *A. coriacea* having the least and most negative potentials, respectively (Figure 3-5). However, there was no clear association between soil group and leaf osmotic potential. Most species tended to have more negative osmotic potentials at full turgor in the drought treatment when compared to the control treatment, indicative of osmotic adjustment, although this was only significant for *A. aneura*, whereas several species including *A. cowleana* and *A. ancistrocarpa* showed no signs of osmotic adjustment.

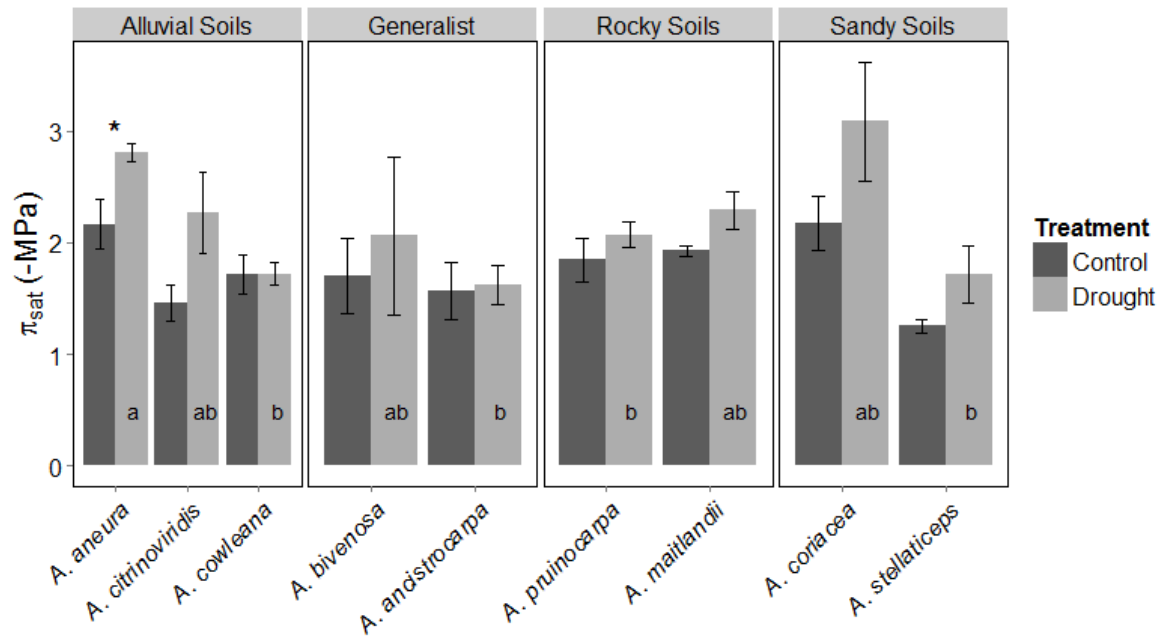


Figure 3-5. Mean (\pm SE) leaf osmotic potential at full turgor (π_{sat} ; -MPa) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Asterisks indicate significant ($P < 0.05$) osmotic adjustment between control and drought plants within species. Letters denote significant differences ($P < 0.01$) between species for drought plants; there were no significant differences between species for control plants. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

3.2. Morphological Functional Traits

There were significant biomass allocation differences among species and preferred soil type groups. For drought plants, the lateral root mass fractions (RMF_{LR}) of *A. aneura* and *A. ancistrocarpa* were the largest and smallest, respectively ($P < 0.05$) (Figure 3-6). The alluvial soil group RMF_{LR} was significantly ($F_{3, 5} = 6.08$, $P < 0.05$) higher compared to all other soil groups for drought plants only. No other significant ($P < 0.05$) associations between soil group and any other morphological functional trait were found for drought and control plants. For drought plants, *A. cowleana* and *A. aneura*, had the highest and lowest ($P < 0.05$) leaf mass allocations respectively (Figure 3-6). The same pattern of leaf mass allocations in *A. cowleana* and *A. aneura* held true for control plants ($P < 0.05$). Stem mass fraction (SMF) and tap root mass fraction (RMF_{TR}) differed between species for drought and control plants ($P < 0.05$). *A. coriacea* and *A. cowleana* SMF and RMF_{TR} were the highest and lowest for both treatments, respectively (Figure 3-6).

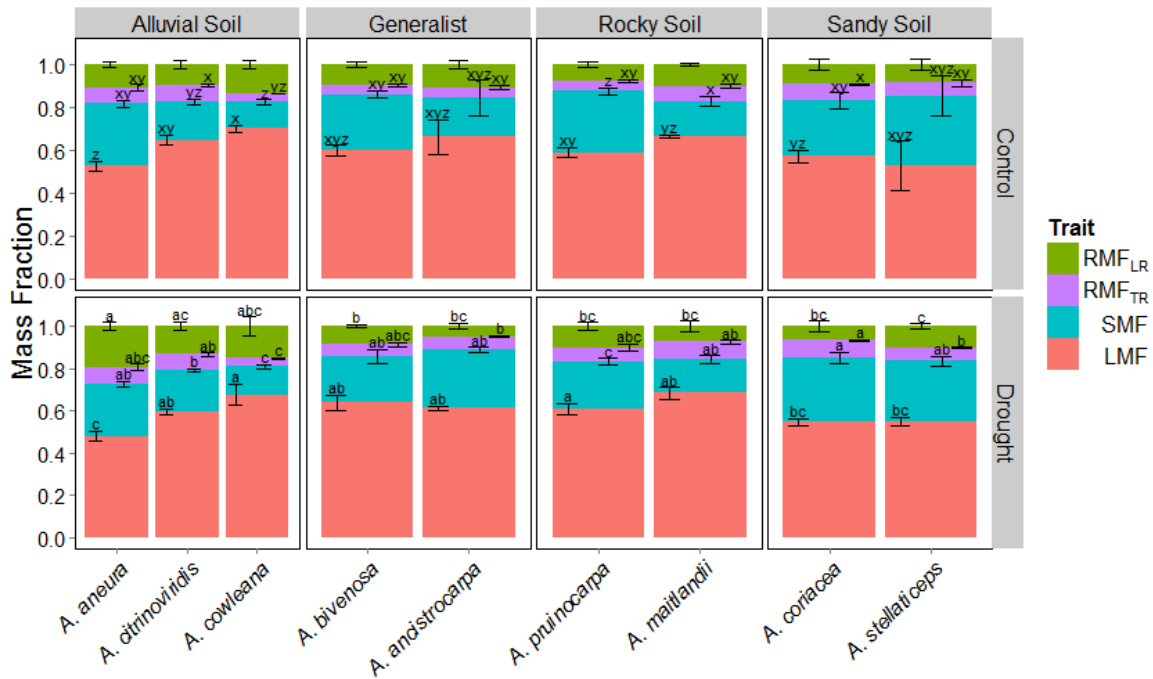


Figure 3-6. Mean (\pm SE) lateral root, tap root, stem and leaf mass fractions (g g^{-1}) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) between species within treatments and traits. The alluvial soil group RMF_{LR} was significantly ($P < 0.05$) higher compared to all other soil groups for drought plants only, differences in RMF_{LR} for control plants were non-significant. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

Significant ($P < 0.005$) differences in tap root to lateral root mass allocation of drought and control plants were apparent among species. For control and drought plants, *A. coriacea* and *A. cowleana* allocated the largest and least proportion of belowground biomass to their tap root, respectively. However, there were no clear differences between soil groups in tap root to lateral root mass allocation. A significant interaction between species and treatment was observed ($P < 0.05$, Appendix E), the ratio decrease in response to the drought treatment in two species from the alluvial soil group, whereas in other species there was either no response or an increase (Figure 3-7).

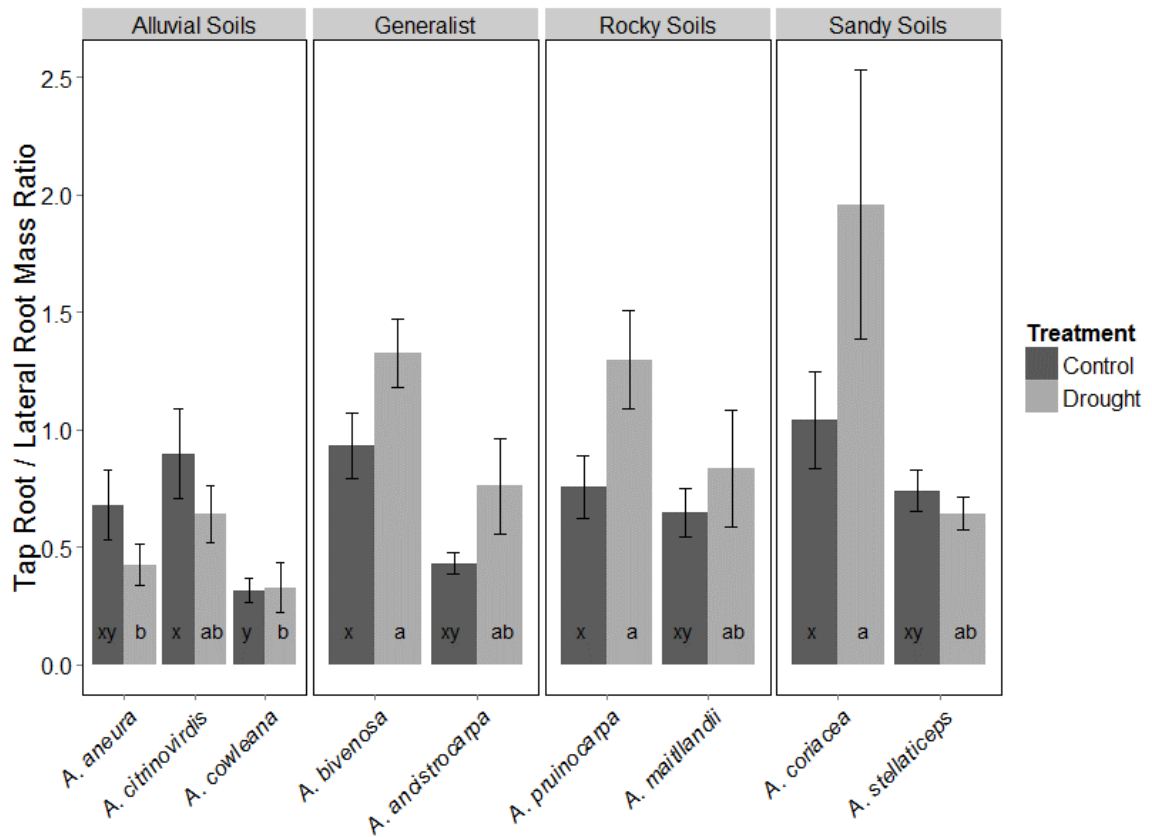


Figure 3-7 Mean (\pm SE) tap root to lateral root mass ratio (g g^{-1}) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) between species within treatments. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

Significant ($P < 0.05$) differences in specific lateral root length (SRL_{LR}) were detected among species for drought and control plants, but not between soil groups. *A. maitlandii* and *A. stellaticeps* had the highest SRL_{LR} for drought and control plants, respectively (Appendix D). Conversely, *A. coriacea* had the lowest SRL_{LR} in both treatments. Root length to leaf area ratio (RL/LA) was significantly ($P < 0.05$) different among species, but not preferred soil type. *A. aneura* had the highest ratio for both treatments indicating it invested more in below ground water acquisition per unit transpiring leaf area (Appendix D). The lowest ratios were measured in *A. bivenosa* and *A. coriacea* for control and drought plants, respectively. Specific leaf area (SLA) differed significantly ($P < 0.05$) amongst species, but was not related to soil group. *A. maitlandii* had the highest SLA for drought and control plants, while *A. pruinocarpa* and *A. coriacea* had the lowest SLA for control

and drought plants, respectively (Appendix D). Stem specific densities (SSD) differed significantly ($P < 0.05$) between species for drought plants only, but were unrelated to preferred soil type in either treatment. The highest and lowest SSDs were attributed to *A. coriacea* and *A. cowleana*, respectively (Appendix D).

4. DISCUSSION

Acacia species in this experiment varied markedly in their physiological and morphological responses to drought. Under drought stress species vary in their degree of anisohydry (Tardieu and Davies 1992, Tardieu and Simonneau 1998). Anisohydric plants typically maintain higher g_s at more negative Ψ_1 under declining soil water content, and often have a wider range of Ψ_1 (McDowell et al. 2008, Klein 2014). These plants usually occupy habitats predisposed to drought (McDowell et al. 2008), however, some plants in these regions tend towards isohydric behaviour. Results from this study are discussed in terms of a continuum from less to more anisohydric.

Species with a higher degree of anisohydry in this experiment tended to be from the alluvial soil group (*A. aneura*, *A. citrinoviridis*, *A. cowleana*), rocky soil group (*A. pruinocarpa* and *A. maitlandii*), and *A. coriacea* from the sandy soil group. In this experiment these species had the highest capacity to extract tightly bound water from the tested mine substrate and thus had the largest “water use envelope” (Sperry et al. 2002) of all experimental species. Their Ψ_1 tended to be more negative for control and drought plants, while g_s remained relatively high at more negative Ψ_1 under drought conditions. Similarly, on a coarse textured natural site Gwenzi et al. (2014) observed that *A. pruinocarpa* Ψ_1 was most negative (-3.5 MPa) with the greatest difference between wet and dry season conditions, and had the highest rates of gas exchange over both seasons compared to co-occurring *A. bivenosa*, *A. inaequilatera*, and *A. pyrifolia*. Also observations on *Eucalyptus wandoo* and *E. accedens* in the south-west of Western Australia showed that these species had lower Ψ_1 and accessed water held at lower Ψ_s in clay-rich layers, as compared to two other *Eucalyptus* species occurring on coarser textured soil (Poot and Veneklaas 2013). Low minimum Ψ_1 confer a competitive advantage to these species during seasonal drought by allowing them to access tightly bound water (Poot and Veneklaas 2013). Unlike *A. coriacea*, however, *A. stellaticeps* in the same sandy soil group exhibited less anisohydric

behaviour. This would suggest that the latter species is less able to extract water from more fine-textured soils, and would prefer deep sandy profiles, whereas *A. coriacea* may also occur on shallower sandy profiles underlain by more fine textured soils. Topographic variation within sandy landscapes (such as dunes, interdunes and sandplains), as well as soil hydraulic characteristics, root system distributions, and the interactions between all these factors may influence local water availability and allow a range of strategies to be successful (e.g. Grigg et al. 2008a, Grigg et al. 2008b, Hoy 2014). For example, Rosenthal et al. (2005) showed that plants on dune ridges had improved water status due to deep roots and increased water availability with depth (Rosenthal et al. 2005), while others found low water stress and high growth of plants with shallower roots in depressed parts of sandy landscapes due to proximity to the ground water table (Gries et al. 2003).

Water uptake by the more anisohydric species *A. aneura*, *A. citrinoviridis*, and *A. coriacea* may have been supported by more negative osmotic potentials and the accumulation of solutes (osmotic adjustment). More negative osmotic potentials are associated with a lower turgor loss point and allow greater tissue dehydration (Bartlett et al. 2012). Species with more negative osmotic potentials also tend to have cell walls that are less elastic under moisture deficit protecting tissue integrity especially during re-hydration (Mitchell et al. 2008, Bartlett et al. 2012). All three aforementioned experimental species had the most negative osmotic potentials, had comparatively lower SLAs, and tended to show osmotic adjustment, although *A. aneura* was the only species with significant adjustment. Similar to other studies, these three species also had the three most negative Ψ_1 , which is largely determined by osmotic potential (Mitchell et al. 2008, Bartlett et al. 2012, Hoy 2014). However, the positive relationship between π_{sat} and Ψ_1 considering all nine species ($R^2 = 0.23$, $P < 0.001$ data not shown) was considerably weaker than the relationships reported between π_{sat} and Ψ_1 at turgor loss point reported by other authors. For instance, Hoy (2014) found a strong relationship between these two factors for eight species in the Great Sandy Desert of Western Australia, as did Mitchell et al. (2008) in southwestern Western Australia, and Bartlett et al. (2012) across species in a global dataset. It is likely that leaves sampled at the end of our drought treatment had not reached turgor loss points, causing a poor correlation with osmotic potential. Alternatively, the role of tissue properties such as elasticity may have a bigger role in differentiating Ψ_1 among these closely-related plants.

Nonetheless, the low π_{sat} of *A. citrinoviridis*, *A. coriacea*, and especially *A. aneura* partly explains their low Ψ_1 and improved water extraction ability.

Leaf physiological responses to drought observed in the alluvial soil group tended towards anisohdry, which was likely associated with high investment in lateral roots. Species in the group had significantly higher RMF_{LR} compared to other soil groups (Figure 3-6) and tended to have the lowest TR/LR Ratios (Figure 3-7). Greater total root mass and root biomass allocation to lateral roots reduces the risk of hydraulic failure at negative Ψ_s , especially in superficial fine textured soils. Increasing the number of lateral roots expands the root area capable of water uptake reducing the demand for water uptake per unit area of root (West et al. 2008, Comas et al. 2013). Therefore, more anisohydric physiological behaviour exemplified by the alluvial soil group was likely supported by lateral root characteristics that helped to facilitate continued water extraction under drying soil conditions.

Species displaying more isohydric water relations were from the generalist soil group (*A. bivenosa* and *A. ancistrocarpa*), and *A. stellaticeps* (sandy soil group). In agreement with other studies, these species were found to be conservative water users through stomatal closure during water stress resulting in moderately negative Ψ_1 (Grigg et al. 2008a, Hoy 2014). Conservative water users can survive periods of mild drought providing they have positive carbon status, but cannot access tightly bound water at very negative Ψ_s , leaving them more susceptible to cavitation as soil continues to dry (Szota et al. 2011).

Drought avoidance as soils continue to dry can be facilitated by plant access to water at greater depth through formation of deep tap roots (White et al. 2000, McDowell et al. 2008), or via hydraulic redistribution from deeper tap roots to lateral roots through dimorphic rooting patterns (e.g. *A. ancistrocarpa*; Grigg et al. 2008a). In this study, TR/LR was largest in two coarse textured soil species that were more anisohydric, it was also statistically significant for one generalist species, *A. bivenosa* that was less anisohydric (Figure 3-7). Gwenzi et al. (2014) previously reported high tap root biomass investment for *A. bivenosa* with comparatively shallow fine roots in the top 15 cm, and a main tap root that reached approximately 0.5 m-depth. It displayed a dimorphic rooting system, which may be an inherent drought response evolved as an adaptation that may explain *A. bivenosa*'s occurrence in a variety of habitats. Conversely, the high tap root biomass investment of

more anisohydric *A. coriacea* and *A. pruinocarpa* from coarse textured soils is likely an adaptation to access water deeper in the profile, which is characteristic of species growing on coarse textured soils (Hacke et al. 2000, Sperry and Hacke 2002). For instance, on a coarse textured material *A. pruinocarpa* had a lack of fine roots at the surface, but a deep root system with access to deep moisture (Gwenzi et al. 2014). Therefore, high tap root biomass investments of species in this study are likely associated with typical rooting strategies; one supporting a more dimorphic root system (*A. bivenosa*), and the other placing much more emphasis on deep tap roots (*A. coriacea* and *A. pruinocarpa*), characteristic of species from coarse textured soils. However, each above-mentioned species had a counterpart within the same soil group that did not have significantly high tap root allocation. Thus observed seedling rooting patterns were not specific to a particular preferred soil type. This may indicate that (1) there may be high spatial heterogeneity even within habitats classified under a specific soil type, (2) that different trait combinations can lead to successful adaptation to these harsh habitats, (3) that our classification of species into their preferred soil types is not accurate, or (4) that drought responses of seedlings grown in pots are different from those of adult plants in the field. All above explanations would necessitate further research on the reliability of species soil/habitat classification and the local heterogeneity in soil profiles and landscapes.

5. CONCLUSION

Our data suggest that (1) *Acacia* species from the semi-arid Pilbara of Western Australia differ significantly in a range of traits associated with drought tolerance, showing a continuum from less to more anisohydric behaviour, (2) differences in drought tolerance traits were in most cases poorly related with preferred soil type, and (3) *a priori* classification of species into preferred soil types may help with inferring drought tolerance but is not a good predictor.

This study demonstrates the range of drought tolerance traits within and between preferred soil types, in the genus *Acacia* growing in a mine site restoration substrate under drought conditions. Species groupings into preferred soil types should be completed from site specific empirical data rather than *a priori* classification. In light of results presented here, drought tolerance traits of young *Acacia* seedlings are more likely species-specific than

related to soil type preferences when growing in a mine site restoration substrate. It should be noted, however, that the classification based on soil preference used here does not capture all traits that affect plant water availability. Given more accurate empirical evidence, the potential to implement ecophysiological and morphological trait-based selection criteria for revegetation of local native flora on disturbed sites, underpinned by the geotechnical characteristics of the restoration substrate still exists. Trait-based selection criteria could utilise physiological and morphological traits identified in this and other studies to match the most appropriate local native flora to the geotechnical characteristics of a restoration substrate, or conversely, to define the geotechnical conditions required to provide niches for this flora. Land restoration practitioners would benefit from this information as it is key to successful restoration of mined lands in arid to semi-arid systems, improving the overall success of revegetation projects on disturbed lands.

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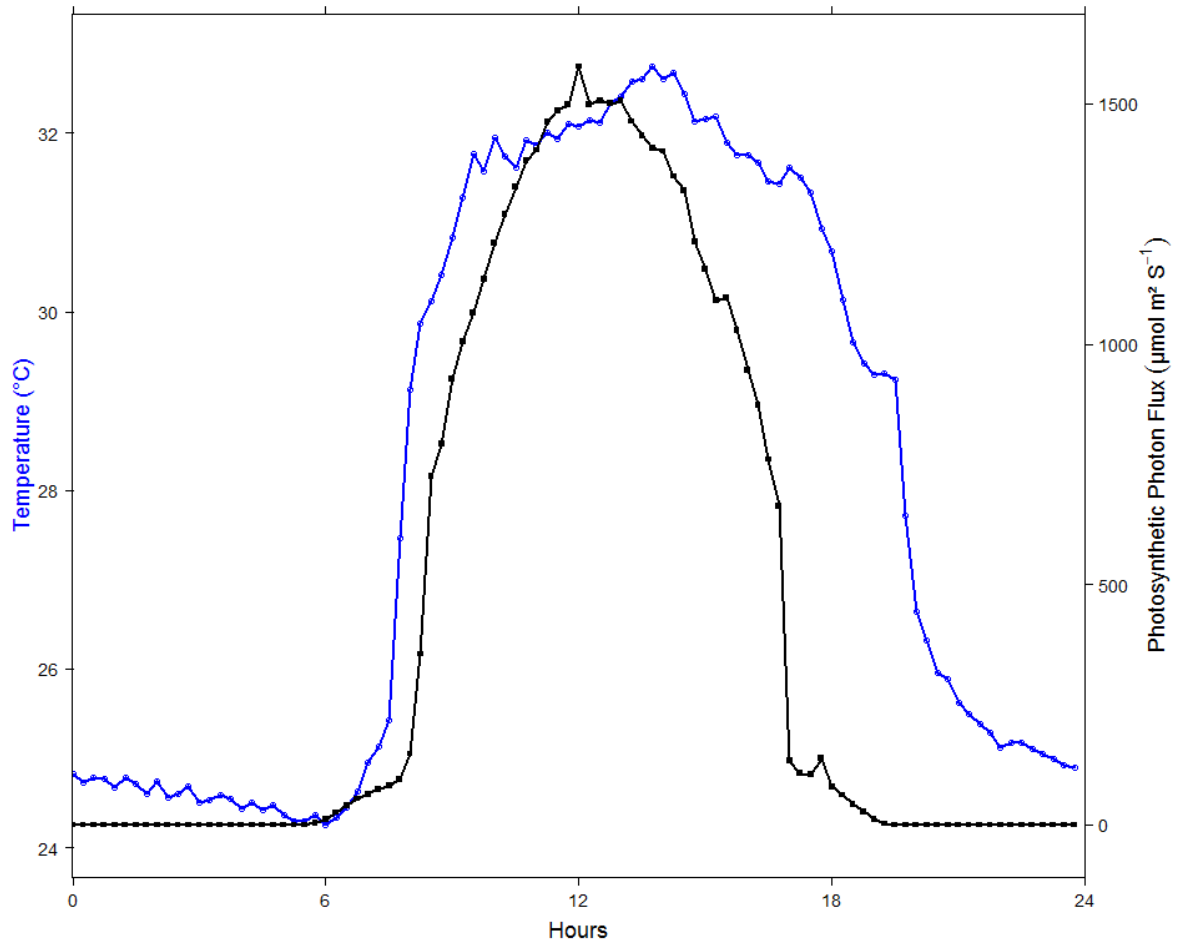
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8. APPENDICES

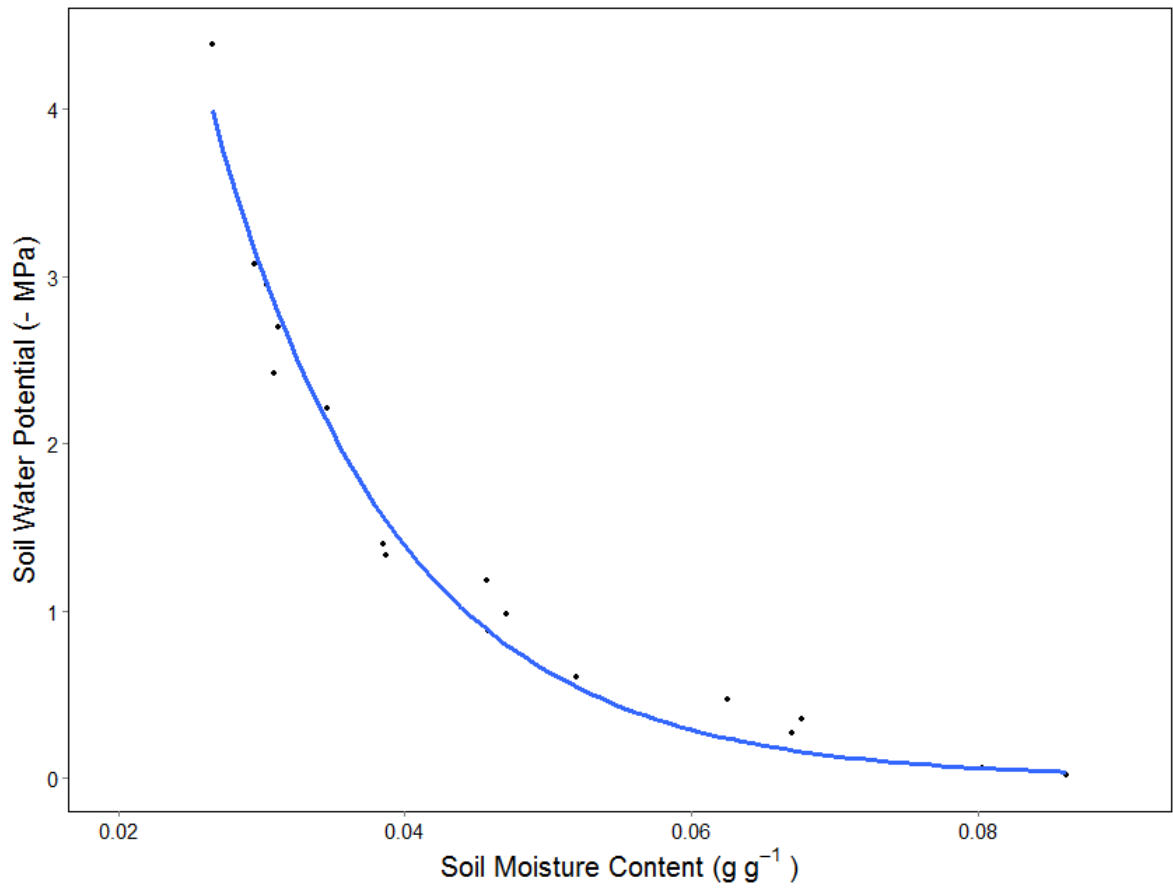


Appendix A Average daily glasshouse temperatures ($^{\circ}\text{C}$) and photosynthetic photon flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) outside the glasshouse, measured at 15 minute intervals through the sampling period (15 – 25 February 2014).

Appendix B Physical and chemical properties of restoration substrate sourced from Mt. Goldsworthy, Pilbara, Western Australia.

Analyte	Units	Value
Clay	%	13.4
Sand	%	69.7
Silt	%	16.9
pH Level (H ₂ O)	pH	8.6
pH Level (CaCl ₂)	pH	7.9
Conductivity	dS m ⁻¹	0.11
Ammonium Nitrogen	g kg ⁻¹	0.001
Nitrate Nitrogen	g kg ⁻¹	< 0.001
Phosphorus Colwell	g kg ⁻¹	0.003
Potassium	g kg ⁻¹	0.12
Sulphur	g kg ⁻¹	0.23
Organic Carbon	g kg ⁻¹	2.1
Copper	g kg ⁻¹	0.0007
Iron	g kg ⁻¹	0.006
Manganese	g kg ⁻¹	0.005
Zinc	g kg ⁻¹	0.005
Boron	g kg ⁻¹	0.005
Aluminum	meq/100g	0.06
Calcium	meq/100g	4.75
Magnesium	meq/100g	1.73
Potassium	meq/100g	0.21
Sodium	meq/100g	0.44

The soil was analysed by the CSBP Soil and Plant Analysis Laboratory (Bibra Lake, WA) to determine physical and chemical properties, including soil texture (classes: 0.02 > sand < 2.0 , 0.002 > silt < 0.02, clay < 0.002 mm; %; Modified Pipette Procedure), electrical conductivity (1:5 soil: water extract), pH (1:5 soil: water extract and 1:5 soil: CaCl₂ 0.01M solution), organic carbon (Walkley-Black method), ammonium nitrogen (extracted in 1M KCl), nitrate nitrogen (extracted in 1M KCl), plant available phosphorus (Colwell P, bicarbonate extraction), potassium (Colwell, bicarbonate extraction), sulphur (extracted in KCl at 40°C), exchangeable cations (aluminium, calcium, magnesium, potassium, and sodium; Mehlich No. 3 test), extractable copper, iron, manganese and zinc (DTPA extraction), and boron (hot CaCl₂ method).



Appendix C Soil water potential (Ψ_s ; -MPa) and gravimetric soil moisture content (g g^{-1}) of the soil used in the experiment ($R^2 = 0.95$, $y = 30.897e^{-75.73x}$).

Appendix D Summary of statistical results for mean differences between species and preferred soil type categories with species nested within preferred soil type categories, for standardised transpiration response to decreasing Ψ_s (MPa⁻¹), response of stomatal conductance (g_s) to decreasing Ψ_s (mmol m⁻² s⁻¹ MPa⁻¹), leaf water potential (Ψ_1 ; MPa), osmotic potential at full turgor (π_{sat} ; MPa), specific leaf area (SLA; cm² g⁻¹), stem specific density (SSD; g cm⁻³), root length to leaf area ratio (RL/LA Ratio; cm cm⁻²), leaf mass fraction (LMF; g g⁻¹), stem mass fraction (SMF; g g⁻¹), lateral root mass fraction (RMF_{LR}; g g⁻¹), tap root mass fraction (RMF_{TR}; g g⁻¹), total specific lateral root length (SRL_{LR}; cm g⁻¹), tap root to lateral root ratio (TR/LR Ratio; g g⁻¹), and total plant dry mass (g) of control (well-watered) and drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species from the West Australian Pilbara region. Letters indicate significant differences (P<0.05) among species or preferred soil type groups within treatments. For each species n = 5, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where n = 4. Degrees of freedom for species and preferred soil type groups were eight and three, respectively.

Factor	ln Transpiration (slope)	g_s (slope)	Ψ_1		π_{sat}		SLA		SSD		RL/LA Ratio	
Species	Drought	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
<i>A. aneura</i>	-0.41	-8.10 ^a	-1.94 ^x	-2.72 ^{ab}	-2.17	-2.81 ^a	47.2 ^z	47.3 ^c	0.46	0.46 ^{bc}	9.70 ^x	15.1 ^a
<i>A. citrinoviridis</i>	-0.48	-21.30 ^a	-1.72 ^{xy}	-2.56 ^a	-1.46	-2.27 ^{ab}	52.8 ^z	53.6 ^c	0.52	0.62 ^{ab}	3.97 ^{xy}	6.63 ^{ab}
<i>A. cowleana</i>	-0.38	-17.10 ^{bc}	-1.24 ^{xy}	-2.10 ^{ab}	-1.72	-1.72 ^b	96.2 ^x	72.8 ^b	0.38	0.42 ^c	7.77 ^{xy}	7.73 ^{ab}
<i>A. bivenosa</i>	-0.59	-33.60 ^{ab}	-1.11 ^y	-1.56 ^b	-1.7	-2.06 ^{ab}	71.1 ^y	62.6 ^{abc}	0.46	0.49 ^{abc}	2.16 ^y	3.20 ^{abc}
<i>A. ancistrocarpa</i>	-0.19	-31.90 ^b	-1.19 ^y	-1.71 ^b	-1.57	-1.62 ^b	103.0 ^{xyz}	67.8 ^{abc}	0.5	0.44 ^c	5.69 ^{xyz}	4.93 ^{abc}
<i>A. pruinocarpa</i>	-0.3	-2.70 ^c	-1.78 ^{xy}	-2.36 ^a	-1.85	-2.07 ^b	43.0 ^z	48.5 ^c	0.51	0.62 ^{ab}	6.95 ^z	5.19 ^c
<i>A. maitlandii</i>	-0.3	-13.20 ^{bc}	-0.45 ^y	-1.66 ^b	-1.93	-2.29 ^{ab}	119.4 ^x	97.2 ^a	0.63	0.46 ^{bc}	3.97 ^x	5.57 ^a
<i>A. coriacea</i>	-0.53	-9.80 ^a	-1.73 ^{xy}	-2.37 ^a	-2.18	-3.09 ^{ab}	42.7 ^z	43.5 ^c	0.6	0.67 ^a	4.27 ^z	2.91 ^c
<i>A. stellaticeps</i>	-0.23	-16.50 ^a	-1.11 ^y	-1.26 ^b	-1.25	-1.72 ^b	74.7 ^{xyz}	73.3 ^b	0.49	0.54 ^{abc}	4.46 ^{xyz}	4.96 ^b
Soil Categories	Drought	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Alluvial Soil	-0.42	-15.5 ^{ab}	-1.63	-2.46	-1.78	-2.27	63.2	56.8	0.46	0.51	7.1	9.98
Generalists	-0.39	-32.75 ^{bc}	-1.15	-1.64	-1.63	-1.84	87.4	65.2	0.48	0.47	3.92	4.06
Rocky Soil	-0.3	-7.95 ^c	-1.12	-2.01	-1.89	-2.18	71.7	70.1	0.56	0.55	5.83	5.36
Sandy Soil	-0.38	-13.15 ^a	-1.42	-1.82	-1.72	-2.4	56.9	56.7	0.58	0.61	4.35	3.82

Factor	LMF		SMF		RMFLR		RMFTR		SRL _{LR}		TR/LR Ratio		Total Plant Dry Mass	
Species	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
<i>A. aneura</i>	0.53 ^z	0.48 ^c	0.29 ^{xy}	0.25 ^{ab}	0.11	0.19 ^a	0.07 ^{xy}	0.08 ^{abc}	3986 ^{xy}	3609 ^{ab}	0.68 ^{xy}	0.42 ^b	1.33 ^{yz}	1.35 ^b
<i>A. citrinoviridis</i>	0.65 ^{xy}	0.60 ^{ab}	0.18 ^{yz}	0.20 ^b	0.1	0.13 ^{ac}	0.08 ^x	0.08 ^{ab}	3157 ^y	3002 ^b	0.90 ^x	0.64 ^{ab}	2.62 ^y	2.99 ^b
<i>A. cowleana</i>	0.70 ^x	0.68 ^a	0.12 ^z	0.13 ^c	0.14	0.15 ^{abc}	0.04 ^{yz}	0.04 ^c	7005 ^x	5421 ^a	0.32 ^y	0.33 ^b	0.45 ^z	1.14 ^b
<i>A. bivenosa</i>	0.62 ^{xyz}	0.61 ^{ab}	0.27 ^{xy}	0.27 ^{ab}	0.06	0.05 ^b	0.05 ^{xy}	0.06 ^{abc}	3289 ^y	5988 ^{ab}	0.93 ^x	1.33 ^a	8.46 ^x	8.95 ^a
<i>A. ancistrocarpa</i>	0.60 ^{xyz}	0.64 ^{ab}	0.26 ^{xyz}	0.22 ^{ab}	0.1	0.09 ^{bc}	0.04 ^{xy}	0.05 ^b	6883 ^x	5019 ^a	0.43 ^{xy}	0.76 ^{ab}	1.15 ^{yz}	1.86 ^b
<i>A. pruinocarpa</i>	0.67 ^{xy}	0.68 ^a	0.16 ^z	0.16 ^c	0.1	0.07 ^{bc}	0.07 ^{xy}	0.08 ^{abc}	3684 ^{xy}	4268 ^a	0.76 ^x	1.30 ^a	2.18 ^y	2.41 ^b
<i>A. maitlandii</i>	0.59 ^{yz}	0.61 ^{ab}	0.28 ^x	0.23 ^{ab}	0.08	0.10 ^{bc}	0.05 ^{xy}	0.07 ^{ab}	6147 ^x	6133 ^a	0.65 ^{xy}	0.84 ^{ab}	0.82 ^{yz}	1.13 ^b
<i>A. coriacea</i>	0.57 ^{yz}	0.55 ^{bc}	0.26 ^{xy}	0.30 ^a	0.09	0.07 ^c	0.08 ^x	0.08 ^a	2189 ^y	2508 ^b	1.04 ^x	1.96 ^a	8.07 ^x	9.54 ^a
<i>A. stellaticeps</i>	0.59 ^{xyz}	0.61 ^{bc}	0.28 ^{xyz}	0.23 ^{ab}	0.08	0.1	0.05 ^{xy}	0.07 ^b	7267 ^x	4399 ^a	0.65 ^{xy}	0.84 ^{ab}	2.35 ^{yz}	3.45 ^{ab}
Soil Categories	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Alluvial Soil	0.62	0.58	0.2	0.19	0.11	0.16 ^a	0.06	0.06	4552	3910	0.63	0.46	1.54	1.87
Generalists	0.61	0.62	0.26	0.25	0.08	0.07 ^b	0.05	0.06	5086	5503	0.68	1.04	4.81	5.41
Rocky Soil	0.63	0.65	0.22	0.19	0.09	0.09 ^b	0.06	0.08	4607	5097	0.7	1.07	1.67	1.84
Sandy Soil	0.58	0.58	0.27	0.26	0.08	0.08 ^b	0.06	0.07	4446	3349	0.84	1.4	5.52	6.83

Appendix E Summary of statistical results between treatment and species for leaf water potential (Ψ_1 ; MPa), osmotic potential at full turgor (π_{sat} ; MPa), specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$), stem specific density (SSD; g cm^{-3}), root length to leaf area ratio (RL/LA Ratio; cm cm^{-2}), total plant dry mass (g), leaf mass fraction (LMF; g g^{-1}), stem mass fraction (SMF; g g^{-1}), lateral root mass fraction (RMF_{LR} ; g g^{-1}), tap root mass fraction (RMF_{TR} ; g g^{-1}), total specific lateral root length (SRL_{LR} ; cm g^{-1}), and tap root to lateral root ratio (TR/LR Ratio; g g^{-1}) of control (well-watered) and drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species from the West Australian Pilbara region. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$. Significance is indicated by ‘*’ $P < 0.05$, ‘**’ $P < 0.01$, ‘***’ $P < 0.001$, and ‘****’ $P < 0.0001$.

Factor	df	F-Values					
		Ψ_1	π_{sat}	SLA	SSD	RL/LA Ratio	Total Plant Dry Mass
Treatment	1	62.58****	11.44**	1.78	0.55	0.62	3.05
Species	8	13.27****	5.81****	11.27****	4.19***	5.76****	11.54****
Treatment x Species	8	1.36	1.18	1.19	1.09	0.93	0.25
Error	83						
Factor	df	F-Value					
		LMF	SMF	RMF_{LR}	RMF_{TR}	SRL_{LR}	TR/LR Ratio
Treatment	1	2.34	0.93	0.08	2.15	3.21	0.09
Species	8	8.40****	19.79****	4.29***	19.25****	14.72****	6.35****
Treatment x Species	8	0.69	1.36	1.52	0.53	2.43	2.1*
Error	83						

CHAPTER 3 The positive effects of thicker engineered covers: A 2-year ecophysiological study

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PREFACE

After identifying some of the traits and water use strategies conducive to drought tolerance for nine Pilbara *Acacia* species in Chapter 2, it was logical to expand from the glasshouse and examine these traits and strategies under field conditions on an engineered cover (Chapters 3 and 4). In seasonally dry environments soil depth is a key determinant of rainfall storage capacity. Therefore, an engineered cover utilising the “store and release” concept (ET Cover) was built atop a waste rock dump slope at three cover thicknesses. The ET cover was instrumented with soil moisture and meteorological sensors to quantify environmental factors, and five local native species seedling were planted across the cover system. Plant ecophysiology was quantified over 2 years in an attempt to better understand the effects of cover thickness on long-term soil moisture storage, plant physiology, drought tolerance traits, root distributions, and growth, and their interactions with seasonal climatic fluctuations on an ET cover. This chapter is in preparation for submission to a peer reviewed scientific journal.



ABSTRACT

The ecophysiology of five semi-arid woody species (*Acacia acuminata* R.Br., *Eucalyptus loxophleba* Benth., *E. leptopoda* Benth., *Melaleuca cordata* Turcz., and *M. nematophylla* Craven) saplings was investigated over 2 years on an engineered cover system with thicknesses of 0.3, 0.5 and 0.7 m in a semi-arid climate. Objectives were to (1) determine if plant function was negatively affected by shallow soil depth through its effect on moisture storage (2) relate physiological processes to observed patterns of root distributions, functional traits, and growth for all species and cover thicknesses. Shallow soils and confined roots reduced plant-available water leading to lower stomatal conductance (g_s) and lower water status. Water use was partly mediated by seasonal changes in VPD, and plants on thicker covers were least sensitive to increasing evaporative demand due to more plant-available water and larger root systems. All species displayed anisohydric behaviour, and while water potentials varied seasonally, apparent hydraulic gradients were approximately constant throughout the year due to effective stomatal control. However, given the observed differences in plant physiology future changes in growth and survival between cover thicknesses are expected, especially as transpiring leaf area and root systems expand. In a severe drought year plants on thicker covers are expected to perform better as they have more access to soil water thus meeting increasing evaporative demands. In this study, species with low leaf area to root length ratio (LA:RL_{Total}) and soils that supported high RL led to higher water status. Selecting species with these traits among others not examined here, and the use of thicker covers will achieve robust ET cover systems capable of withstanding severe drought and warmer future climates.

1. INTRODUCTION

The relationship between plant functioning and engineered cover design is pivotal to effective cover system performance. Covers are generally constructed atop hazardous mine waste using available materials from the mining process, including non-reactive subsoils and waste rock, and stripped and stockpiled top soil, or mixtures of both (Gwenzi et al. 2014). Cover materials are generally well-graded or multi-layered waste rock, top soil, or fine textured material suited for soil moisture storage (Khire et al. 2000, Ayers et al. 2004). Cover systems utilising the ‘store and release’ concept (ET covers) are grounded on water balance principles, whereby infiltrating

rain water is stored for subsequent release by evapotranspiration (Albright et al. 2004, Gee et al. 2006, O’Kane and Ayres 2012, Eamus et al. 2013). Several ecohydrological studies evaluating the importance of vegetation on engineered covers have demonstrated its importance in water uptake and limiting deep drainage (Breshears et al. 2005, Scanlon et al. 2005, Yunusa et al. 2010). For instance, Nyhan et al. (1990) demonstrated that a cover designed to increase water holding capacity at the root zone resulted in twice the amount of plant biomass, which increased evapotranspiration by 8.4% and reduced water percolation by a factor of four compared to a conventional cover in a semi-arid region. Performance of the engineered cover was enhanced through superior vegetation establishment by increasing the top soil layer thickness, and utilising a capillary barrier to slow the vertical movement of water making it more available to plant roots for a longer period (Nyhan et al. 1990). The performance of vegetated engineered covers is therefore contingent on the successful establishment and persistence of vegetation, which itself requires reliable plant available water.

Due to diversity in plant structure and function, species differ in their water requirements and ability to extract water from different soils. Although field and modelling studies have documented the applied hydrological benefits of vegetation on engineered covers (e.g. Scanlon et al. 2005, Fayer and Gee 2006, Eamus et al. 2013), few have disentangled the water use patterns and ecophysiology of individual species from different community types. Typically these studies characterise evapotranspiration not plant water use (e.g. Breshears et al. 2005, Fayer and Gee 2006), which yields little information pertaining to actual transpiration of individual species or community types. However, a few recent studies have endeavoured to investigate the finer scale ecophysiology of vegetation on engineered covers (e.g. Grigg et al. 2010, Gwenzi et al. 2012). Because species and community types vary in their ecophysiological traits, an understanding of such variation is important for revegetation of engineered covers. For instance, on a waste management site in temperate Eastern Australia Yunusa et al. (2010) concluded that a grass cover restricted evapotranspiration to 75% of rainfall due to shallow rooting and seasonal growth compared to a woodland at 85% of rainfall. At the species scale, Veneklaas and Poot (2003) found that species differences in rooting depth (i.e. deep versus shallow rooting) were related to seasonal water use patterns in a *Banksia* woodland on deep sands in south-western Australia. Water use differences at

the community and species level are apparent but little empirical information exists for vegetation on engineered covers.

Species and community level water uptake, however, is dependent on soil characteristics. Plant behaviour and soil water interact dynamically allowing vegetation to remain physiologically active. Soil texture greatly influences plant available water due to variation in pore size and its effect on water potentials and saturated hydraulic conductivity (Hacke et al. 2000, Sperry and Hacke 2002, Jury and Horton 2004). Soil texture thus results in different challenges for plant water extraction (Bristow et al. 1984). Under moisture stress plants from different soil textures use variable morphological traits and contrasting water use strategies to facilitate plant water extraction. In semi-arid to arid systems, remaining physiologically active under moisture stress hinges on the ability to maintain turgor despite water loss (Bartlett et al. 2012) and to tolerate high tension in the xylem, especially in root xylem tissue, and to supply water from the soil to the leaves to balance transpirational losses (Seyfried et al. 2005, Bhaskar and Ackerly 2006).

Maintaining adequate contact at the root-soil interface is central to water extraction as it influences the ability to remain physiologically active in water limited systems as coarse soil textures are not conducive to good contact (Sperry et al. 1998) and have poor soil moisture retention characteristics. However, in many situations water extraction is also dependent on the available soil volume for root exploration. To preclude deep drainage in semi-arid to arid environments it has been suggested that species must extend roots to depths of 2 – 3 m and generate soil water potentials < -1 MPa for long periods with sufficient root density for effective water extraction (Walvoord et al. 2002, Seyfried et al. 2005). Interestingly, maximum rooting depths of shrubs in natural water-limited systems was approximately 1.8 m in the comprehensive Schenk and Jackson (2002) study, suggesting that investment in deeper roots is not adaptive as drainage to deeper soil layers may be too infrequent due to low rainfall and effective plant water uptake. By comparison, studied engineered covers in water-limited systems have had thicknesses ranging from 0.2 – 5.2 m, and were often comprised of material combinations such as non-acid-forming waste rock, sand, cobbles, clay and top soil resulting in unnatural site hydrogeology (e.g. Nyhan et al. 1990, Benson et al. 2004, Breshears et al. 2005, Scanlon et al. 2005, Fayer and Gee 2006, Gwenzi et al. 2014). Blanket recommendations regarding ET cover thickness have been made in the literature,

where it was suggested that in semi-arid to arid systems a 1 m thick cover underlain by a capillary barrier was adequate to minimise drainage to $\leq 1 \text{ mm yr}^{-1}$ (Scanlon et al. 2005). This however was met with opposition where it was argued that covers should be designed according to site specific conditions where local plant community traits, soil types and corresponding soil water storage are evaluated (Gee et al. 2006).

ET covers are in part designed to enhance soil moisture storage (Yunusa et al. 2010), but the hydraulic properties of the cover material utilising common mine waste are often not conducive to water storage (Gwenzi et al. 2014). One of the simplest methods of increasing the water storage capacity of engineered covers is to increase the thickness of the plant growth medium. Enright and Lamont (1992) determined that *Banksia* survival on rehabilitated mine sites in southwestern Australia was limited by a combination of impedance to root penetration into denser subsoils restricting roots to surface layers, inadequate soil water in the topsoil layer, and an inability of roots to keep pace with the drying front. They suggested that an increase in total cover depth, with an unconsolidated sandy layer below the topsoil, would allow seedlings to establish root systems to depths where water was less limiting during the hot and dry summer (Enright and Lamont 1992). Similarly, and also in southwestern Australia, Szota et al. (2007) observed *Eucalyptus marginata* stand densities that were 2.4 times greater on a ripped restored site consisting of top soil, sandy gravel and kaolinitic clay subsoil with a total thickness of at least 1.4 m, versus a ripped restored site with a total thickness of 0.6 m with the absence of the kaolinitic clay subsoil. Greater tree density at the thicker site was attributed to better material properties resulting in deeper tap and sinker roots into a soil with higher moisture storage capacity, and greater overall soil volume to exploit (Szota et al. 2007). In agreement with Enright and Lamont (1992), it was shown that trees on the site where roots were restricted to the top 0.5 – 0.6 m of the soil experienced drought stress much earlier during summer (Szota et al. 2007). Gwenzi et al. (2014) further corroborated the importance of soil texture for the purpose of increasing soil water storage. He determined that enhanced soil moisture storage resulting from increased cover thickness was not achievable at a bauxite residue sand ($> 150 \mu\text{m}$) area, due to high saturated hydraulic conductivity and poor water retention (Gwenzi et al. 2011a, Gwenzi et al. 2014). It is important to recognise that engineered cover thickness is site and material specific (Albright et al. 2004, Gee et al. 2006), and should be based on the interactions of

several factors including texture, climate, and plant community requirements (Arora 2002, Arnold et al. 2015).

Compared to natural systems, our understanding of plant ecophysiology, in particular water relations, and plant response to soil moisture storage as a result of cover thickness on engineered covers is lacking. To address these knowledge gaps, the current study examined the ecophysiological behaviour of five semi-arid native plant species over 2 years on an experimental ET cover at three different cover thicknesses in semi-arid Western Australia. The primary objectives were to (1) assess the influence of soil depth on moisture storage to determine if shallow covers compromise plant function in a semi-arid climate (2) relate physiological processes to observed patterns of root distributions, functional traits, and growth for each species and cover thickness. We hypothesised that plant physiology would mostly be driven by seasonal shifts in soil volumetric water content (VWC), and that plants growing on thicker covers would have more access to soil moisture due to greater moisture storage and unconfined roots, resulting in overall larger g_s and decreased physiological sensitivity to dry summer atmospheric conditions. We also anticipated that species with higher g_s would have lower investment in leaf area relative to the size of their root system.

2. MATERIALS AND METHODS

2.1. Study Site

The study was conducted at Mt. Gibson Mining's Extension Hill Operation (29°34'14.7 S, 117°10'15.6 E, 344 m a.s.l), located approximately 293 km North East of Perth, in the Yalgoo region of Western Australia. Climate in the Yalgoo region is characterised as semi-arid, with low mean annual rainfall of approximately 338 mm and high annual potential evaporation exceeding 2200 mm (Bureau of Meteorology, <http://www.bom.gov.au>). The region experiences strongly seasonal weather conditions that are hot and dry from December to March and relatively cool from June to September, during which the majority of rainfall occurs. The region is dominated by *Acacia* and *Eucalyptus* open woodland and shrubland on sandy and alluvial plains, in addition to rocky outcrops and banded ironstone communities (McKenzie et al. 2003). The area is diverse containing 706 native plants in 50 major habitats (Payne 1997). Dominant plant species, geology, soils, landforms, and climate of the Yalgoo region are described in Payne (1997).

2.2. Experimental Design

The cover system trial plots were constructed atop a waste rock dump slope (18°) with a NE aspect. Waste rock in the dump is dominated by Banded Iron Formation (BIF), hematite, and goethite comprising 94.8 % of the total waste volume (Landloch Pty. Ltd. 2012). Overall the waste rock was classified as non-acid forming, with the exception of Felsic Volcanics that contained pyrite, which represented 0.1 % of the total waste rock volume (Landloch Pty. Ltd. 2012). The ET cover material used was comprised of a 2:1 BIF/topsoil mix. Topsoil was sourced from pre-mining stockpiles of no more than 2 m high. The cover material was mixed by spreading from the top of the dump to its bottom using a bulldozer with GPS capabilities. The cover was spread into three thicknesses; 0.3 m, 0.5 m, and 0.7 m at a precision of ± 0.1 m, with three replicates totalling nine cover plots (Appendix A). Monitoring locations were established at two slope positions (A and B) on every cover plot. The centres of slope positions A and B were situated 6.25 m and 13.75 m from the waste rock dump top, respectively, for a total of 18 monitoring plots.

Six locally occurring native species from two contrasting community types were chosen for this study (Table 2-1). All seeds used for propagation were from the local region. Species in this area were classified as typically occurring in a sandy or rocky community type, based on evidence gathered from pre-mining vegetation surveys (Bennet Environmental Consulting 2000, Griffin and Associates 2005, ATA Environmental 2006), literature (Payne 1997, McKenzie et al. 2003, Meissner and Caruso 2008), expert consultations, on-site knowledge, and the online database Flora Base (<http://florabase.dpaw.wa.gov.au/>).

Table 2-1 Species planted on cover system trial plots and the soil types in which they occur in the Mt. Gibson mining area.

Rocky Soil	Sandy Soil
<i>Eucalyptus leptopoda</i> (Benth.)	<i>Eucalyptus loxophleba</i> (Benth.)
<i>Acacia alata</i> (R.Br.)	<i>Acacia acuminata</i> (Benth.)
<i>Melaleuca nematophylla</i> (Craven)	<i>Melaleuca cordata</i> (Turcz.)

E. leptopoda was grown from seed collected by Mt. Gibson Iron in the outdoor shade house at the University of Western Australia (UWA) Plant Growth Facilities (PGF), Perth, WA (31°59'S, 115°53'E), while *E. loxophleba* and *A. acuminata* were purchased from Australian Native Nursery, Oakford, WA (32°13'S, 115°54'E), *M. nematophylla* and *M. cordata* from Wongan Trees, Wongan Hills, WA (30°53'S, 116°44'E), and *A. alata* from Apace Nursery,

North Fremantle, WA (32°02'S, 115°45'E). All seedlings were approximately 6 months old and of similar height within species.

In early July 2014, seedlings were planted at the centre of every cover system plot in a 6 x 6 configuration 0.5 m apart. The outermost plants were 1 m from the edge of every plot. Within each vegetated plot, six seedlings per species were planted at random using a random number generator. A total of 648 seedlings were planted, 108 per species for every cover thickness (Appendix B). A pump-fed drip (1.6 L hr⁻¹) irrigation system was constructed and assembled onsite shortly after planting. Seedlings were irrigated twice weekly with potable water for 15 minutes representing an approximate input of 7.2 mm week⁻¹ during the first monitoring year to ensure establishment and survival over hot summer months. Drip lines were extended between every seedling row on all plots to ensure equal watering. Irrigation ceased May 1, 2015 for the remainder of the monitoring period. The entire monitoring area was enclosed with rabbit proof fencing to deter herbivory by small mammals.

2.3. Soil Analyses

Representative bulk soil samples were collected from across all plots for physical and chemical analysis. Soil samples were analysed in the Wesfarmers CSBP Ltd. Soil and Plant Analysis Laboratory (Bibra Lake, WA) to determine electrical conductivity (1:5 soil:water extract), pH (1:5 soil:water extract and 1:5 soil:CaCl₂ 0.01M solution), organic carbon (Walkley-Black method), ammonium nitrogen (extracted in 1M KCl), nitrate nitrogen (extracted in 1M KCl), plant available phosphorus (Colwell P, bicarbonate extraction), potassium (Colwell), sulphur (extracted in KCl at 40 °C), exchangeable cations (aluminium, calcium, magnesium, potassium, and sodium; Mehlich No. 3 test), extractable copper, iron, manganese and zinc (DTPA extraction), and boron (hot CaCl₂ method). Samples were analysed by Soil Water Group (East Perth, WA) for physical characteristics, including soil texture (classes: 0.02 > sand < 2.0, 0.002 > silt < 0.02, clay < 0.002 mm; %; Modified Pipette Procedure), particle size analysis > 0.075 mm (sieve analysis; Australian Standard 1289.3.6.1), and laboratory saturated hydraulic conductivity (K_{sLab}) (constant head method; Australian Standard 1289.6.7.1).

Field saturated hydraulic conductivity (K_{sField}) was measured in triplicate at every soil sample location using a Philip-Dunne permeameter (Muñoz-Carpena et al. 2002). The design, field

protocol, and calculations of K_{sField} followed detailed procedures outlined by (Muñoz-Carpena et al. 1998) and Muñoz-Carpena et al. (2002). At each sampling location a 100 mm deep hole was created using a 100 mm auger. The permeameter was inserted into the hole and filled with water to 200 mm mark. Time was recorded when the water level dropped to 100 mm and 200 mm marks. The procedure for calculating K_{sField} from Philip-Dunne measurements is outlined in Muñoz-Carpena et al. (2002) based on Philip (1993). The summary equation used to calculate K_{sField} was:

$$K_{sField} = \left(\frac{\pi^2 r_o \tau_{max}(\alpha)}{8 t_{max}} \right) \quad \text{Equation 6}$$

where r_o is half the internal diameter of the permeameter, τ_{max} is a dimensionless time variable calculated from t_{max} , α is a factor accounting for permeameter and soil characteristics, and t_{max} is the time taken for the permeameter to empty.

Initial and final soil moisture was measured for each augered hole using a sampling ring on the auger to a depth of 30 mm prior to the addition of water, and below the bottom of the hole after a sample run (Muñoz-Carpena et al. 2002). Samples were then oven-dried at 105°C for 24 h and soil moisture content calculated. Dry soil bulk density (ρ_b ; kg m⁻³) was computed using the soil volume for the wet and oven-dried soil samples (Blake and Hartge 1986). Total porosity (Φ ; m³ m⁻³) of soil samples was calculated as:

$$\Phi = 1 - \frac{\rho_b}{\rho_s} \quad \text{Equation 7}$$

where ρ_s is particle density assumed to be 2650 kg m⁻³ (McKenzie et al. 2002).

At each soil sample location initial and final soil surface compaction (C ; kg m⁻²) was assessed using a Humboldt H-4200 soil penetrometer (Humboldt, Illinois, USA) for unconfined compressive strength at a mean volumetric soil moisture content of 0.051 cm³ cm⁻³ for initial values and 0.11 cm³ cm⁻³ for final values. A soil water characteristic curve (SWCC) was produced for the cover soil (Appendix C). Known water volumes were added to dry soil, mixed in air-tight plastic bags, allowed to equilibrate for 24 hours, and measured using a WP4C dewpoint water potential meter (Decagon Devices Inc., Pullman, WA, USA). Gravimetric soil moisture content (%) of analysed soil was then verified using the oven-drying method.

2.4. Quarterly Field Monitoring

Continuous soil volumetric water content (VWC) was measured using CS616 moisture content reflectometers split between one CR 800 and two CR 10X dataloggers routed via AM 16/32B multiplexers (Campbell Scientific Inc., Logan, Utah, USA). Data acquisition was split between three monitoring stations (three dataloggers) as a precaution in case of datalogger failure. Dataloggers were programmed to record volumetric water content (VWC) readings every four hours. Moisture sensors were installed on every cover plot prior to planting seedlings. Sensors were installed at 0.2 m depth intervals starting at a soil depth of 0.1 m, down to the maximum depth of every plot. Total profile water (mm) for each cover thickness was computed assuming that sensors measured VWC of a 0.2 m depth interval (0.1 m above and 0.1 m below the sensor) except for deepest sensor which measured VWC of the 0.1 m above the waste rock only.

Meteorological variables on the plots were measured by an automated weather station (central monitoring station; CR 800). The station measured air temperature and relative humidity (CS215 Campbell Scientific Inc., Logan, Utah, USA), wind speed and direction (Wind Sentry 03002, RM Young Pty. Ltd., Traverse City, Michigan, USA), solar radiation (SP-110 pyranometer, Apogee Instruments Inc., Logan, Utah, USA), and rainfall using a tipping bucket rain gauge (CS 703, Campbell Scientific Inc., Logan, Utah, USA). Meteorological variables were measured at 15 minute intervals. Vapour pressure deficit (VPD; kPa) was calculated by first computing saturated vapour pressure (SVP; Pa) from the measured air temperature (T ; °C) using Equation 8, then by using relative humidity (RH; %) in Equation 9:

$$SVP = 610.7 \times 10^{7.5T/(237.3+T)} \quad \text{Equation 8}$$

$$VPD = \frac{\left(\frac{100-RH}{100}\right) \times SVP}{1000} \quad \text{Equation 9}$$

Plant physiological monitoring was conducted quarterly on one randomly chosen representative sapling per species for every plot. Pre-dawn (04:00 – 05:30; Ψ_{PD}) and mid-day (13:00 – 15:00; Ψ_{MD}) leaf water potentials (MPa) were measured using a Pressure Chamber (PMS Instruments, Oregon, USA). Leaves were cut using a scalpel, placed in an airtight plastic bag, and placed in the dark on ice for immediate analysis at the field site. A

compressing gland sealing system designed for grass leaves was used to hold leaves in place as most were not petiolate. The difference between Ψ_{MD} and Ψ_{PD} (Ψ_{MD-PD}) was also determined, and is a relative measure of plant water status used as an indicator of the hydraulic gradient between soils and leaves. During morning (06:30 – 10:30) sapling transpiration (E ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), area based net photosynthesis (A ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and intercellular CO_2 concentrations (C_i ; $\mu\text{mol CO}_2 \text{ mol}^{-1}$) were measured using an LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). Once gas exchange values had stabilised five readings per plant were taken. *Eucalyptus* species and *A. acuminata* seedlings were sampled by clamping the leaf chamber onto a leaf, *Eucalyptus* species filled the leaf chamber, therefore, leaf area for these species was equal to the area of the chamber; *A. acuminata* had phyllodes of constant width, therefore, leaf area in the chamber was determined by measuring the length and width of the leaf. The *Melaleuca* species were sampled by placing 2 – 3 leaves on a short length of stem in the sample chamber due to their small size. Leaves were cut after measurements, sealed in plastic bags, cooled and taken to the laboratory where leaf areas were determined using a scanner and WinRHIZO software (Regent Instruments, Quebec, Canada). In the chamber red and blue LEDs provided a photosynthetic photon flux density of $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Reference gas concentration of CO_2 was set at $405 \mu\text{mol mol}^{-1}$ and leaf chamber CO_2 concentrations were $395\text{-}400 \mu\text{mol mol}^{-1}$. The leaf chamber thermocouple was adjusted to be below the leaf, and the energy balance equation was used to calculate leaf temperature (LI-COR Inc. 2011). Plant physiological variables were computed through equations derived by von Caemmerer and Farquhar (1981).

Non-destructive morphological traits were monitored quarterly on all seedlings. Plant height (H_{max}) was taken using a meter stick from the base of the plant to the highest point of the foliage by extending all parts of the plant upwards (Pérez-Harguindeguy et al. 2013). Stem diameter was measured using digital callipers (Mitutoyo, Honshu, Japan) by averaging two perpendicular measurements at the base of the seedling. Crown width was measured using a metre stick above the crown of seedlings by averaging two perpendicular measurements starting with the largest width. Seedling survival was determined from the start of irrigation in July 2014 and again once irrigation ceased in May 2015.

2.5. *Plant Harvest and Root Sampling*

Plants were harvested in February 3, 2016 for destructive morphological trait and root analyses. One representative seedling for each species was harvested from every plot for a total of 90 plants. A 200 mm length of branch measured from the terminal end of the branch towards the plant stem was cut, placed in a plastic bag and immediately kept cool. Branch sub-samples were rehydrated in the lab by placing the cut end of the stem in water for 24 hours. Leaves were stripped from the branch sub-sample, weighed and scanned to determine branch leaf area (LA_b ; mm^2) using WinRHIZO software (Regent Instruments, Quebec, Canada). Leaves were then dried at 70°C for 48 h and weighed. Sapwood area (SA; mm^2) was calculated by excluding the bark and phloem, and measured with a digimatic micrometer (Mitutoyo, Honshu, Japan). The pith and heartwood of stems were not excluded from the measurement due to their very small size. The leaf area:sapwood ratio (LA:SA; $\text{mm}^2 \text{mm}^{-2}$; i.e. inverse of the Huber value) was measured by dividing LA_b by SA (Pérez-Harguindeguy et al. 2013). Leaf mass per area (LMA; kg m^{-2}) was computed by dividing leaf dry mass by LA_b . Leaf dry matter content (LDMC; g g^{-1}) was calculated by dividing the oven dry mass of leaves by their fresh weight. Specific leaf area (SLA; $\text{m}^2 \text{kg}^{-1}$) was calculated by dividing LA_b by leaf dry mass. Carbon-13 isotope fractionation ($\delta^{13}\text{C}$) was determined for dry leaf samples from each stem sub-sample at the West Australian Biogeochemistry Centre (UWA). The rest of the plant was separated into leaves and stems and weighed. Leaves and stems were oven dried at 70°C for 48 h to determine the dry weight of each component. The mass fraction of leaves (LMF; g g^{-1}), stems (SMF; g g^{-1}) was calculated by dividing their dry weight with total plant dry weight. Plant leaf area (LA_p ; m^2) was computed by multiplying the branch sub-sample SLA with the total dry weight of leaves.

Trenches were dug using a backhoe and smooth bucket at the base of each harvested plant. Pits were dug approximately 0.1 m from the plant stem, and dug to the maximum depth of each respective cover thickness (i.e. 0.3 m, 0.5 m, 0.7 m) and 0.25 m on either side of the stem (Appendix D). Pit walls were smoothed using shovels and small water sprayers to remove excess soil and expose roots. Sampling frames divided into 0.05×0.05 m grid cells (0.0025 m^2) were used to count the number of roots in each cell (Appendix D). Root length density (RLD; m m^{-3}) was estimated for each plant and cover thickness from the sum of point

intersections with the observation plane, then dividing by the area of the observation plane for each respective cover thickness (N ; m^2) assuming an isotropic root distribution:

$$RLD = 2N \quad \text{Equation 10}$$

as described by Melhuish and Lang (1968), and in further detail by van Noordwijk et al. (2000). Root length (RL; m) was calculated by multiplying the area of the sampling frame (m^2) with RLD for each plant and cover thickness. As a measure of relative investment, plant leaf area to total root length ratios ($LA:RL_{Total}$; $m^2 m^{-1}$) were computed by dividing LA_p and RL.

2.6. Data Analysis

Data were analysed with RStudio statistical software (R v0.98.1028, R Foundation for Statistical Computing, R, 2013). To determine if cover material hydraulic and physical characteristics were homogeneous across all cover thicknesses and slope positions, differences in K_{sField} , K_{sLab} , C , ρ_s , and Φ were tested using generalised least squares (glS) models using package nlme (Pinheiro et al. 2015) with weighted variance structures to ensure homogeneity of variance as outlined in Zuur et al. (2009). When testing between cover depth and slope position, fixed factors were cover thickness and slope position. Models were tested using five variance structures (VarFixed, VarIndent, VarPower, VarExp, and VarConstPower) and Analysis of Variance (ANOVA). Models were evaluated and selected using Akaike's information criterion (AIC); a measure of relative model quality for a given set of data, and plots of standardised residuals. Models with the lowest AIC value, and homogeneity of variance were chosen for further analysis. Selected models were subsequently tested using ANOVA at $\alpha = 0.05$. In further plant morphological and physiological tests there was no indication that slope position was a significant factor in any of the analyses, therefore it was excluded. The above-mentioned analyses were also conducted to test for differences in total profile water between cover thicknesses where cover thickness was the fixed factor; and to test for differences in height growth, stem growth, crown growth, LMA, LDMC, log LA:SA, SMF, LMF, $\delta^{13}C$, Ψ_{MD} , log RLD, log RL, and log LA:RL_{Total} where species and cover thickness were fixed factors. Where appropriate transformations were applied to satisfy assumptions of normality for statistical tests. Differences in C_i were tested with species, cover thickness, and season as fixed factors.

Where ANOVA model outputs were significant, Tukey's multiple pairwise comparison test was used to analyse differences within factors.

The package nlme and gls models were also used to test for differences in log-transformed g_s , square root-transformed $\Psi_{\text{MD-PD}}$, and log-transformed Ψ_{PD} (response variables) between species and cover thickness (categorical variables) using three separate models (Model- g_s , Model- $\Psi_{\text{MD-PD}}$, and Model- Ψ_{PD}) where explanatory variables were weighted VWC; and the VPD closest to the g_s measurements for Model- g_s and Model- $\Psi_{\text{MD-PD}}$, and the average night time VPD (00:00 – 05:00) for Model- Ψ_{PD} . Transformations were applied to satisfy assumptions of normality. Models were tested using the same variance structures and model discrimination procedures for gls models previously described. Selected models were subsequently tested using ANOVA at $\alpha = 0.05$. Tukey's multiple pairwise comparison test was used to analyse differences among species or cover thicknesses if ANOVA model outputs were significant. To gain an understanding of the variability explained by each model, predicted values were computed using the function predict.lme in package nlme, then plotted against observed values for each response variable. A linear regression between model predictions and observed values was then performed for each response variable; Model- g_s ($P < 0.001$, adj. $R^2 = 0.37$, Appendix E), Model- $\Psi_{\text{MD-PD}}$ ($P < 0.001$, adj. $R^2 = 0.30$, Appendix F), and Model- Ψ_{PD} ($P < 0.001$, adj. $R^2 = 0.21$, Appendix G). Analysis of covariance (ANCOVA) was conducted to test differences in slopes and intercepts among regression lines for A (response variable) with log-transformed g_s with species as the categorical variable. A log transformation was applied to g_s to achieve linearity thus satisfying assumptions of the ANCOVA. Tukey's multiple pairwise comparison test was used to analyse differences among species if ANCOVA model outputs were significant.

Horizontal spread of roots was assessed by combining data of quadrants (0.0025 m^2) of both sides (left and right) of the soil profile grid; root count proportions were then summarised for 0.1 m depth intervals by joining two vertical quadrants. These data were then square root-transformed to satisfy normality, and differences among species, soil depth intervals, and lateral position were tested using gls models and ANOVA at $\alpha = 0.05$. Fixed factors were species, root depth interval and lateral root spread. Models were tested using the same variance structures and model discrimination procedures for gls models previously described.

Tukey's multiple pairwise comparison test was used to analyse differences among species, soil depth intervals, and lateral position if ANOVA model outputs were significant.

Engineered covers also aim to achieve ecosystem and hydrological functions that resemble natural ecosystems (Yunusa et al. 2010), therefore comparing observed root distributions to typical woody ecosystems is of interest. Observed cumulative root proportions for each cover thickness (species combined) were compared to the asymptotic single-parameter global model (Gale and Grigal 1987, Jackson et al. 1996):

$$Y = 1 - \beta^z \quad \text{Equation 11}$$

where Y is the cumulative root fraction at any soil depth, Z is soil depth measured from the surface (cm) and β is a dimensionless root extinction parameter. The Jackson et al. (1996) is the most popular model used to describe root depth distributions of terrestrial ecosystems based on 250 empirical root studies. Jackson et al. (1996) derived β parameter values for many ecosystems including tropical grassland / savannah ($\beta = 0.972$), desert ($\beta = 0.975$), and sclerophyllous shrubland ($\beta = 0.964$), among others. High β values (≈ 0.97) correspond to greater root proportions at deeper soil depths, while low values β (≈ 0.92) imply greater root proportion at the soil surface (Jackson et al. 1996). The β parameter was computed for each cover thickness with combined species roots using nonlinear least squares regression (nls) in package stats (R Core Team 2016) to determine if β were similar to a sclerophyllous shrubland ($\beta = 0.964$), as this is the dominant ecosystem in the study area.

3. RESULTS

3.1. Soil analyses

No major geochemical restrictions to plant growth were apparent (Appendix H), and seedlings did not show signs of nutrient deficiency or toxicity. The cover soil was characterised as a coarse sandy loam with a large gravel fraction (Appendix I). At the start of the experiment, mean K_s (field and lab) was 0.20 m day^{-1} with no difference between slope position or cover thickness, and within the range of those reported for deep alluvial soils ($0.05 - 14.5 \text{ m day}^{-1}$) (Botros et al. 2009), and other engineered covers ($8.6 \times 10^{-7} - 0.86 \text{ m day}^{-1}$) (Ogorzalek et al. 2008, Bohnhoff et al. 2009). Dry bulk density (ρ_s) and porosity Φ differed ($P < 0.01$) between slope positions where slope position A had higher ρ_s and Φ

compared to slope position B. Mean ρ_s , Φ , and compaction (C) for the cover soil were $1424 \pm 59 \text{ kg m}^{-3}$, $0.46 \pm 0.023 \text{ m}^3 \text{ m}^{-3}$, and $23509 \pm 1133 \text{ kg m}^{-2}$, respectively. After 2 years, field K_{sField} increased to 0.38 m day^{-1} with no differences between slope position or cover thickness. An increase in K_s over time is common in Australian mining soils due to self-filtration and mobilisation of fine particles from top soil layers with deposition at depth leading to higher K_s at the soil surface caused by the remaining coarse fraction (Dikinya et al. 2008, Gwenzi et al. 2011a). At experiment end, cover material ρ_s , Φ , and C were $1617 \pm 140 \text{ kg m}^{-3}$, $0.47 \pm 0.019 \text{ m}^3 \text{ m}^{-3}$, $21504 \pm 1306 \text{ kg m}^{-2}$, respectively, and homogeneous across cover thicknesses and slope positions.

3.2. *Climate*

Seasonal patterns were evident for all climate variables (Figure 3-1); large rainfall events were spread throughout the year, with the majority of rainfall occurring in early autumn with some isolated showers in late summer. A large rainfall event occurred on 1 April 2015 when 71.6 mm fell in one day. Temperature, VPD, irradiance, and wind speed were all considerably higher during summer months. The predominant wind direction during the entire study period was SE-SSE.

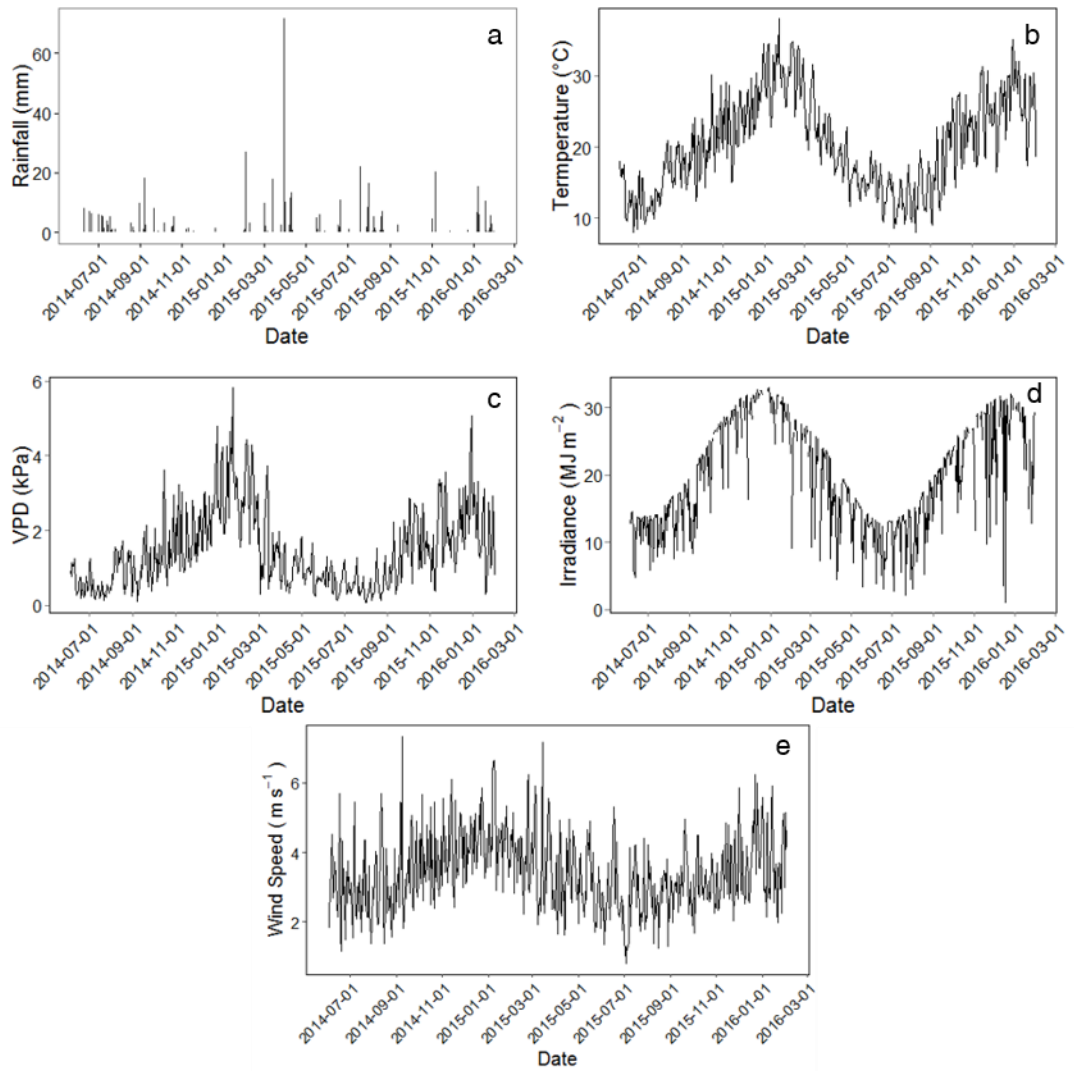


Figure 3-1(a) Total daily rainfall, (b) mean daily air temperature, (c) vapour pressure deficit (VPD), (d) solar irradiance, and (e) wind speed over the entire study period on an engineered cover system with an 18° slope with a NE aspect.

3.3. Soil moisture, plant growth, and roots

Changes in soil VWC corresponded well to rainfall events, especially at shallower sensor depths (Figure 3-2). VWCs were on average lower in the 0.3 m cover thickness treatment, presumably due to faster drying of a smaller volume of stored water through evaporation and transpiration (Figure 3-2). VWCs were higher at mid-depth in the 0.5 m thick cover than in the 0.7 m thick cover, likely because of the entry of a similar amount of water into a smaller soil volume (Figure 3-2). Total profile water was significantly ($P < 0.001$) higher in thicker covers $0.7\text{ m} > 0.5\text{ m} > 0.3\text{ m}$ due to increased moisture storage provided by extra soil volume (Figure 3-2c). Increases of total profile water clearly correspond with rainfall events. Soil

moisture content after cessation of irrigation remained similar to the irrigated period, and did not reach lower minimum values, due to the occurrence of several rainfall events. The large rainfall event (71.6 mm) on 1 April 2015 recharged soil moisture across all covers, and subsequent smaller events ensured that soil moisture was not depleted further (Figure 3-2). Total rainfall for the non-irrigated period was 242 mm, which is just below the long-term (1982-2016) average of 278 mm (Bureau of Meteorology, <http://www.bom.gov.au>).

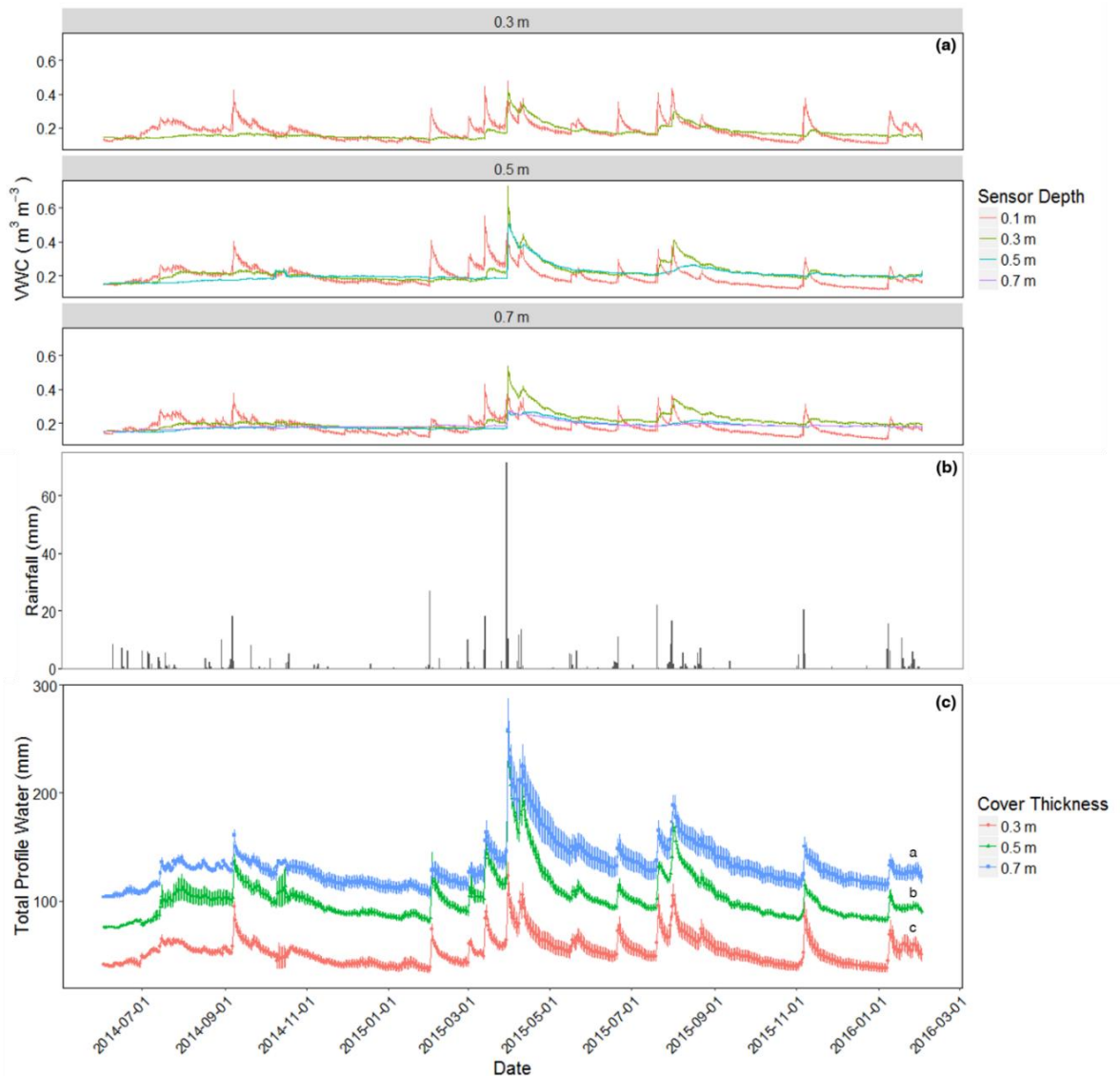


Figure 3-2 (a) Volumetric water content (VWC) at four sensor depths (0.1 m, 0.3 m, 0.5 m, 0.7 m), (b) total daily rainfall, and (c) total profile water (\pm SE) across three cover thickness treatments (0.3 m, 0.5 m, 0.7 m; six replicates per cover thickness) over the entire study period on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Total profile water contents were significantly ($P < 0.001$) different between cover thickness treatments.

Seedling survival remained above 90% for all species, with the exception of *Acacia alata*. All *A. alata* seedlings died within 3 months after planting. Plant height, stem diameter, and crown diameter growth were only significantly ($P < 0.001$) different between species of different genera and not cover thickness (Appendix J – Appendix L). Seedlings of the

surviving *Acacia* species reached the greatest height, followed by the *Eucalyptus* species, and then the *Melaleuca* species (Appendix J). In contrast, stem growth was larger for *Eucalyptus* species than for *Acacia* species, with *Melaleuca* species having the smallest stem growth (Appendix K). The crown diameter of *A. acuminata* could not be accurately determined due to its sparse leaves and columnar shape. Both *Eucalyptus* species had larger crowns than the *Melaleuca* species (Appendix L). Larger crown diameters in *Eucalyptus* species were physically supported by larger stem diameters (Appendix K and Appendix L). Amongst the *Eucalyptus* species, *E. leptopoda* had a significantly larger crown than *E. loxophleba* (Appendix L). Crown diameter growth stagnated at the end of the experiment (Appendix L; early summer to mid-summer), however, the considerable increase in height during this period (Appendix J) suggests that crowns were still growing but shifted from horizontal expansion to height growth in a likely response to increased foliage density. There was little evidence to suggest seasonality in growth.

LMA, LDMC, LA:SA, SMF, and LMF differed between species ($P < 0.001$) but not between cover thicknesses. LMA was significantly ($P < 0.001$) lower in the two *Eucalyptus* species and highest ($P < 0.001$) in *M. nematophylla* (Appendix M). LDMC was highest in *A. acuminata* ($P < 0.001$), and lowest in the *Eucalyptus* species and *M. nematophylla* (Appendix M). LA:SA was highest in both *Eucalyptus* species and lowest in *M. nematophylla* ($P < 0.001$; Figure 3-3a) suggesting differences in relative area-based rates of transpiration and sap flow. *A. acuminata*'s SMF and LMF were highest ($P < 0.001$) and lowest ($P < 0.001$), respectively Figure 3-3b).

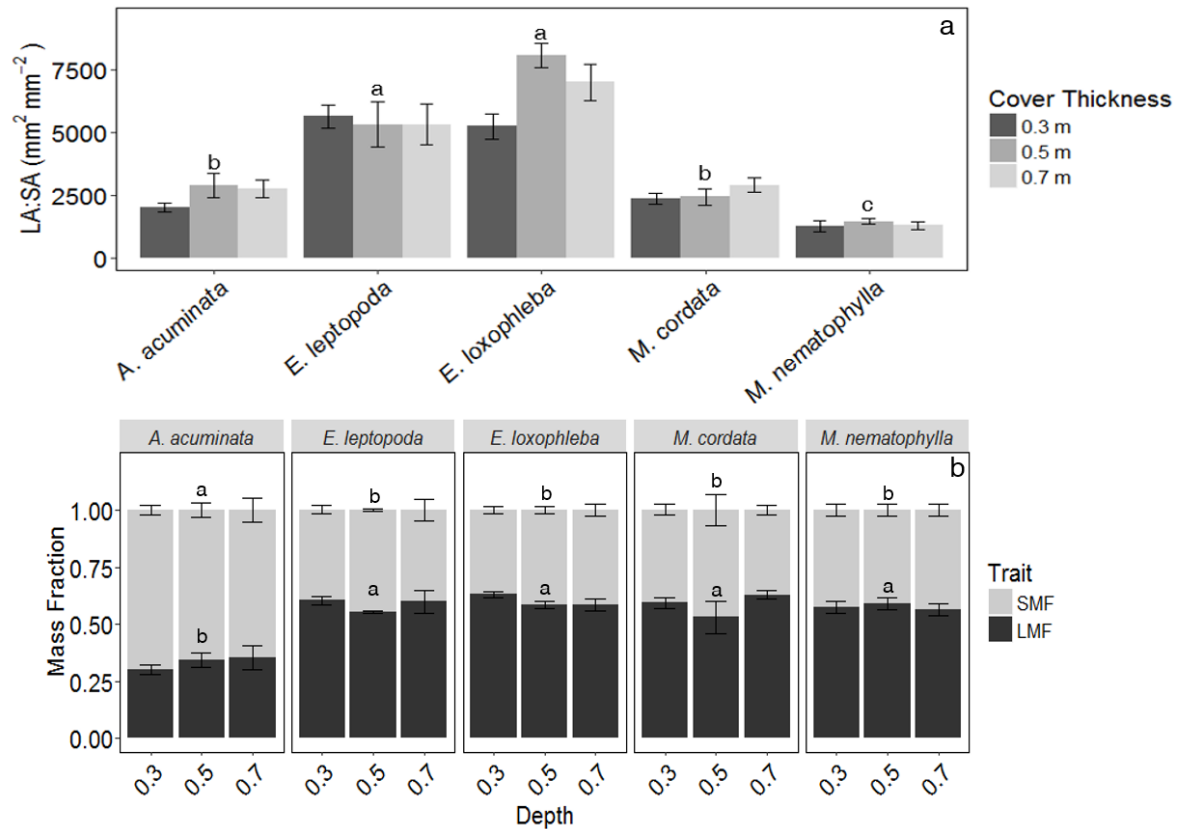


Figure 3-3 (a) Mean (\pm SE) leaf area to sapwood area ratio (LA:SA; mm² mm⁻²) from 200 mm long branches (b) mean (\pm SE) stem and leaf mass fractions (SMF and LMF; g g⁻¹) based on aboveground biomass. All samples were taken from five local native saplings on three cover system thicknesses grown over 572 growth days on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. For (a) letters denote significant ($P < 0.001$) differences between species, and for (b) letters denote significant ($P < 0.001$) differences among species within traits. There were no differences between cover thicknesses for LA:SA, SMF, and LMF. For each species and cover thickness $n = 6$.

Mean RL differed significantly ($P < 0.001$) between cover thicknesses where RL in the 0.7 m cover was 39% and 70% higher than in the 0.5 m and 0.3 m covers, respectively (Figure 3-4a). Mean LA:RL_{Total} and RL were lowest and highest, respectively, for each species on the 0.7 m thick cover, indicating a shift in root/shoot balance disfavouring roots on thinner covers (Figure 3-4). Mean LA:RL_{Total} differed significantly ($P < 0.001$) between cover thickness and species (Figure 3-4b). Plants on the 0.7 m thick cover had LA:RL_{Total} that were on average 55% lower compared to the 0.3 m cover. *M. nematophylla* had the lowest ($P < 0.05$) LA:RL_{Total} with a value that was 90% and 91% lower than that of the two highest species *E. loxophleba* and *E. leptopoda*, respectively ($P < 0.001$; Figure 3-4b).

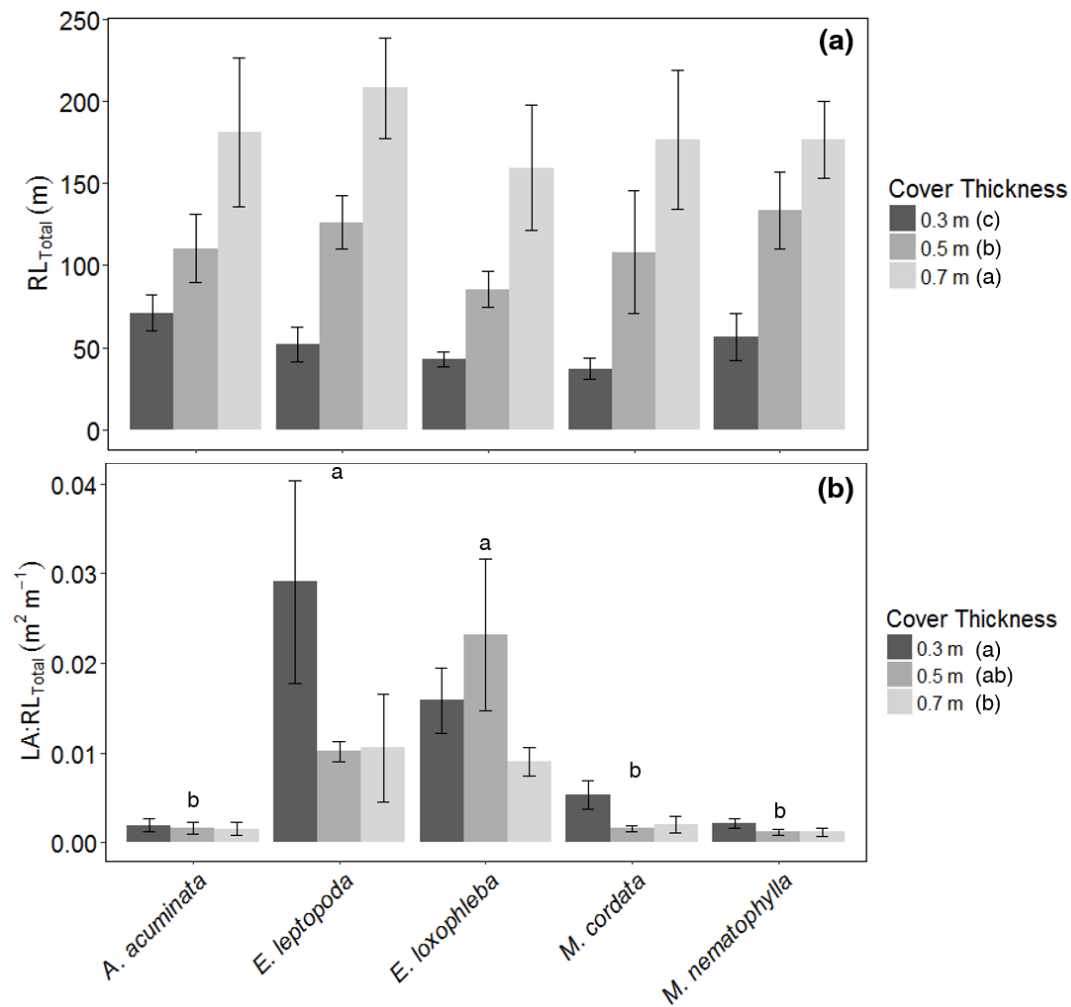


Figure 3-4 (a) Mean (\pm SE) total root length (RL_{Total}; m) (b) mean (\pm SE) leaf area to total root length ratio (LA:RL_{Total}; m² m⁻¹) of saplings from five local native species on three cover system thickness treatments after 572 growth days. Values represent the mean (n = 6) values of plant leaf area and of roots on a vertical pit face below the plant for each species and cover thickness. Plants were on an engineered cover system with an 18° slope and NE aspect in semi-arid Western Australia. For (a) letters denote significant differences among cover thickness (P < 0.001), and for (b) letters denote significant differences among species (P < 0.05) and cover thicknesses (P < 0.001), respectively.

In the 0.3 m thick cover the highest root proportions for *A. acuminata*, *E. leptopoda*, and *E. loxophleba* were found in the top 0.2 m of soil, and *M. cordata* and *M. nematophylla* had the highest root proportion in the 0.1 – 0.2 m interval (Figure 3-5). In the 0.5 m thick cover relative root distributions were quite similar between species with the exception of *M. cordata*, which had its roots more homogeneously distributed with a significant amount in the deepest layer. Root profiles of most species in the 0.7 m thick cover were similar, with the majority of roots in the top 0.5 m of the profile and very few roots in the uppermost

surface layer. However, *A. acuminata* differed from all others in having the highest root densities in the top 0.3 m of the profile with much lower densities further down the profile. In general, the greatest root densities were found at shallow depths in thin covers, and at greater depths in thicker covers (Figure 3-5 and Figure 3-6). Root proportions were found to decrease significantly ($P < 0.01$) with distance from the stem on the 0.5 m thick cover only (Figure 3-5). Mean RLD for the 0.3 m, 0.5 m, and 0.7 m covers were $4724 \pm 434 \text{ m m}^{-3}$, $3523 \pm 324 \text{ m m}^{-3}$, and $2928 \pm 256 \text{ m m}^{-3}$, respectively, and differed significantly ($P < 0.01$) between cover thickness but not species.

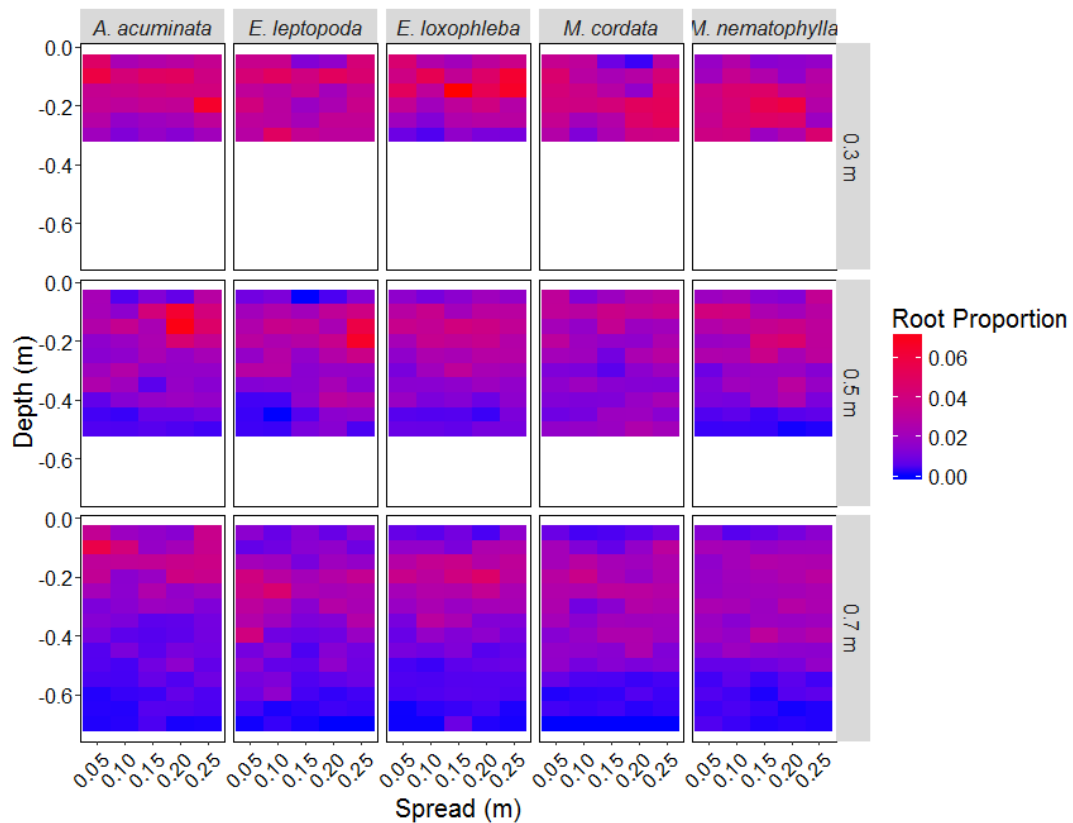


Figure 3-5 Heat maps of mean root proportions per sampling quadrant of saplings from five local native species on three cover system thickness treatments after 572 growth days. Values represent the mean ($n = 6$) proportion of roots collapsed by horizontal spread per 0.0025 m^2 on a vertical pit face below the plant. Plants were on an engineered cover system with an 18° slope and NE aspect in semi-arid Western Australia.

Root depth distributions differed between species at each cover thickness as demonstrated by a significant species/root depth interaction ($P < 0.001$). This was mainly caused by the behaviour of *M. cordata* in thinner covers, and *A. acuminata* in the thickest cover (Figure 3-5 and Figure 3-6). As expected, the deeper soil profiles allowed root exploration of a

much larger soil volume and as a consequence allocated proportionally less roots to the top 0.2 m of the soil: the 0.3 m thick cover had 67 – 82 % of all species roots in the top 0.2 m of soil, and the 0.5 m and 0.7 m thick covers only had 40 – 59 % and 33 -52 % of their roots in this top layer, respectively (Figure 3-6). For comparison Figure 3-6 also shows the average root distribution of sclerophyllous shrublands ($\beta = 0.964$; Jackson et al. 1996) expected for this region. The calculated root extinction coefficients ($\beta \pm SE$) were 0.938 ± 0.107 for the 0.3 m cover, 0.957 ± 0.093 for the 0.5 m cover, and 0.965 ± 0.092 for the 0.7 m m. The thickest cover resembled the root distribution of sclerophyllous shrublands most closely, but with lower than predicted root densities at the surface. The relatively even density of roots at all depths on the 0.3 m thick cover contrasts with the attenuation seen in deeper soils (Figure 3-5 and Figure 3-6).

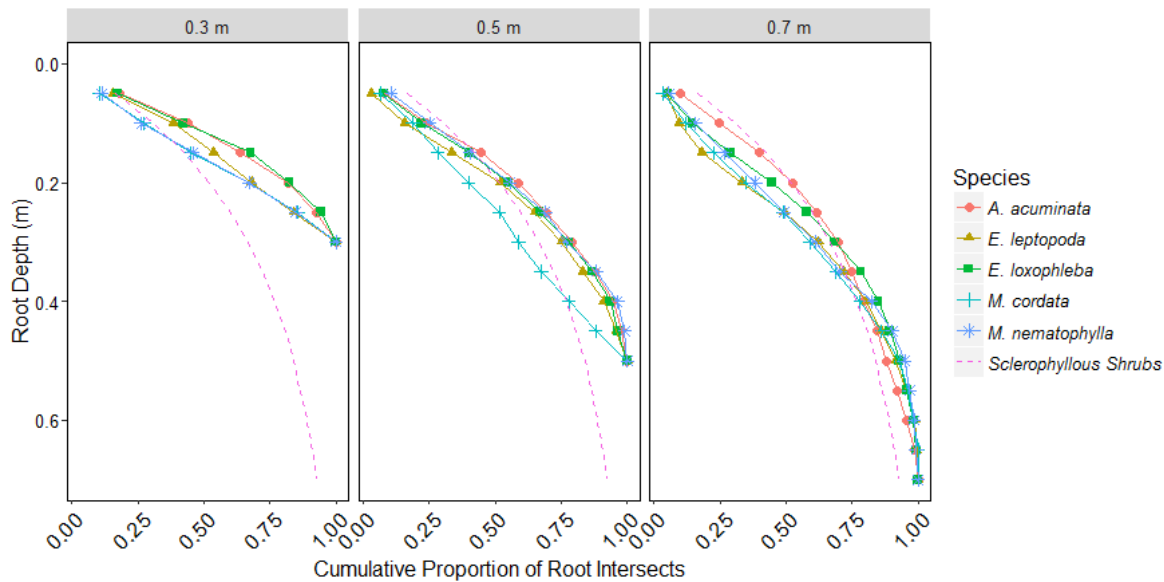


Figure 3-6 Cumulative proportion of root intersects for saplings of five local native species with depth, after 572 growth days on three cover system treatments of different depths. The model for sclerophyllous shrubland (dashed pink line) reported by Jackson et al. (1996) is included in each graph. The engineered cover system was located on an 18° slope with NE aspect in semi-arid Western Australia. For each species and cover thickness $n = 6$.

3.4. Plant Physiology

Seasonal trends in g_s were apparent, increasing in autumn and decreasing in summer (Figure 3-7). The large spike in g_s (Figure 3-7) was 43 days after the largest single rainfall event of 71.6 mm (Figure 3-1a). This rainfall event was the only one during the

experimental period which was large enough to substantially increase soil water content below 0.5 m depth (Figure 3-2a), even during the May 2015 sampling event, and highlights the effects of large rain pulses on plant water use. In some species the response to the large rainfall event was much larger than in others, however, no clear differences amongst cover depth treatments were apparent, although the majority of species tended to have slightly higher g_s on the 0.7 m thick cover.

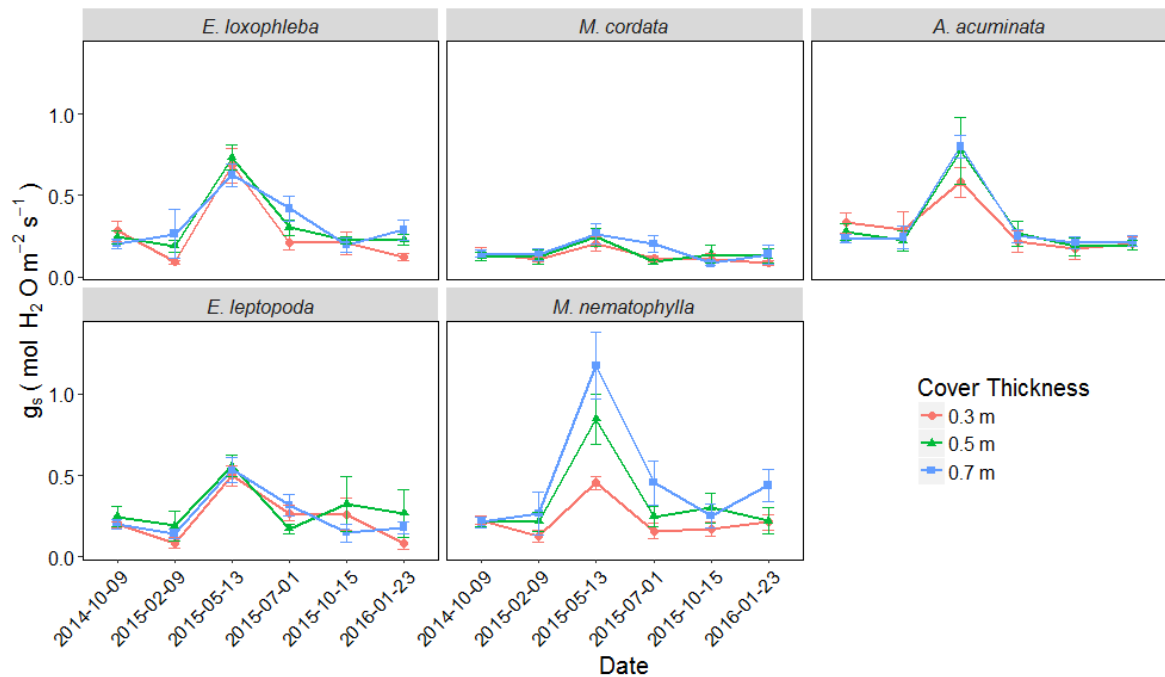


Figure 3-7 Mean (\pm SE) morning stomatal conductance (g_s ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) during each sampling event for saplings of five local native species across three cover thicknesses on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. For each species and cover thickness $n = 6$.

Although there were no clear effects of cover thickness treatments on g_s (Figure 3-8), it declined more strongly with increasing VPD in the shallow cover thickness treatments (Figure 3-8; VPD/cover thickness interaction $P < 0.05$). g_s was also significantly ($P < 0.001$) related to weighted VWC. As evident in Figure 3-8, it was the two eucalypt species that were more sensitive to high VPD when growing in shallow soils. Differences between species were also significant ($P < 0.001$) where *M. cordata* had significantly ($P < 0.001$) lower mean g_s compared to all other species (Figure 3-8).

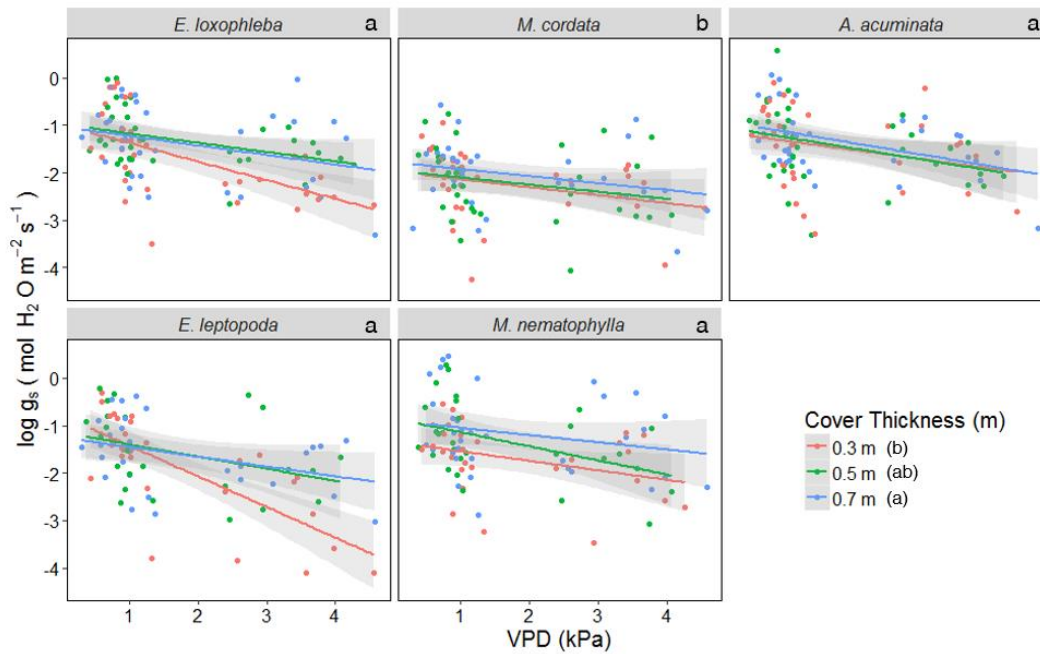


Figure 3-8 Morning log-transformed stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) as a function of vapour pressure deficit (VPD; kPa) for saplings of five local native species across three cover thicknesses on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Letters denote significant differences between species ($P < 0.001$) and cover thickness ($P < 0.01$), respectively.

Both Ψ_{PD} and Ψ_{MD} declined strongly in summer (Figure 3-9). Ψ_{PD} was significantly ($P < 0.001$) related to weighted VWC and night time VPD, with no significant difference between species or cover thicknesses (Figure 3-9 and Figure 3-10). The addition of VPD to the model explained 9 % more variance, and Ψ_{PD} decreased with increasing VPD.

Significant differences in Ψ_{MD} occurred between cover thicknesses ($P < 0.01$), where Ψ_{MD} on the 0.3 m thick covers was most negative ($P < 0.001$), and Ψ_{MD} on the 0.7 m cover thicknesses was least negative ($P < 0.01$) (Figure 3-9). Differences among species were also apparent ($P < 0.001$), where *A. acuminata* ($P < 0.05$) and *M. cordata* ($P < 0.05$) had the least negative Ψ_{MD} , and *E. leptopoda* ($P < 0.05$) and *E. loxophleba* ($P < 0.05$) were most negative (Figure 3-9).

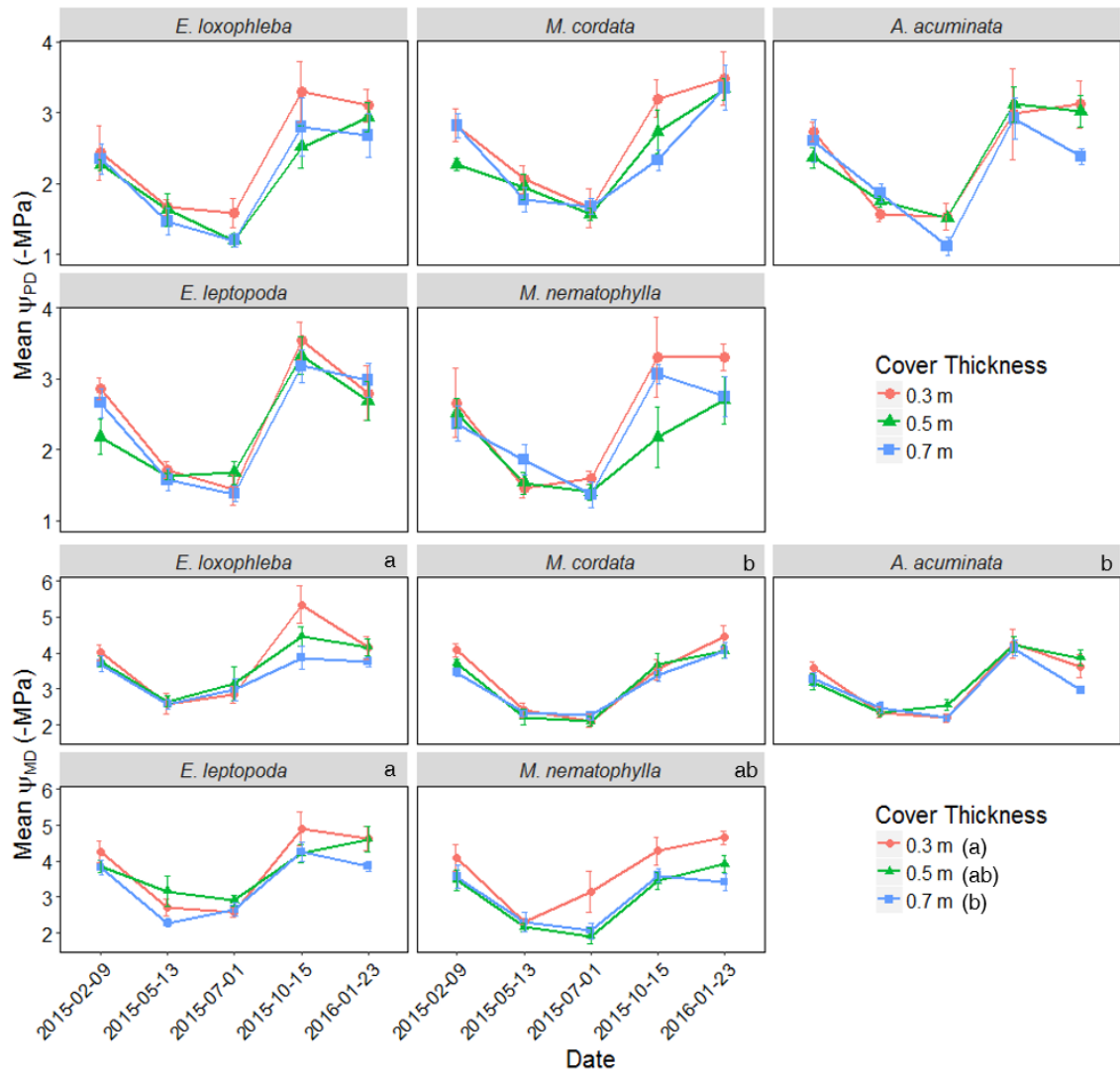


Figure 3-9 Mean (\pm SE) pre-dawn leaf water potential (Ψ_{PD} ; -MPa) and mean (\pm SE) mid-day water potential (Ψ_{MD} ; -MPa) for all sampling events for saplings of five local native species across three cover thicknesses on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. For Ψ_{PD} letters denote significant ($P < 0.05$) differences between cover thicknesses. For Ψ_{MD} letters denote significant ($P < 0.05$) differences between species and between cover thicknesses.

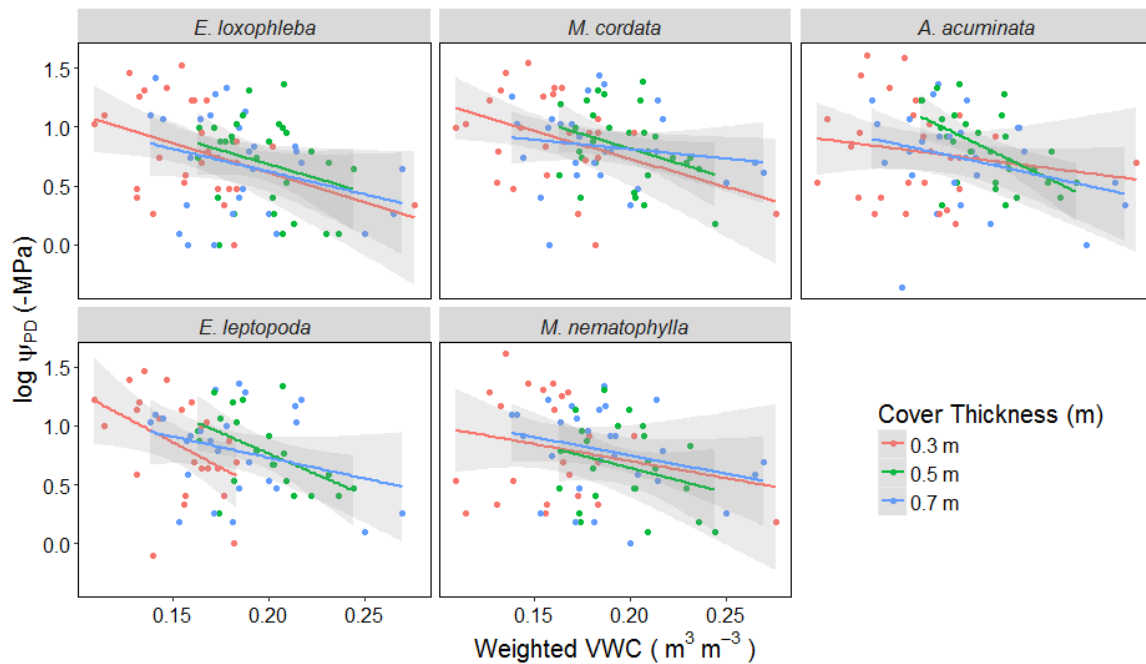


Figure 3-10 Log-transformed Ψ_{PD} (-MPa) as a function of weighted VWC ($\text{m}^3 \text{m}^{-3}$) for saplings of five local native species across three cover thicknesses on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Log Ψ_{PD} was significantly ($P < 0.0001$) related to weighted VWC and VPD.

Mean Ψ_{MD-PD} was significantly ($P < 0.05$) lower on the 0.7 m thick cover than on the 0.5 m thick cover suggesting that plants on the thicker cover had greater access to soil moisture or had the lowest hydraulic conductances (Figure 3-11). Ψ_{MD-PD} responded differently to increasing VPD in some species (Figure 3-11; VPD/Species interaction $P < 0.001$).

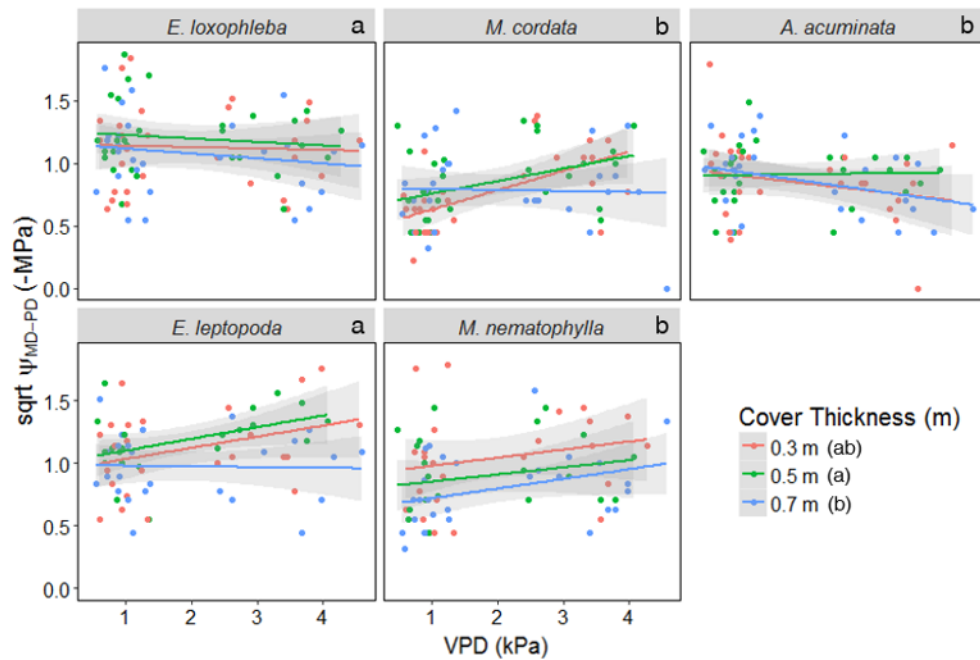


Figure 3-11 Square root-transformed Ψ_{MD-PD} (-MPa) as a function of VPD (kPa) for saplings of five local native species across three cover thicknesses cover system with an 18° slope with a NE aspect in semi-arid Western Australia. There was a significant effect of cover thickness on Sqrt Ψ_{PD} ($P < 0.05$), and a significant interaction between species and VPD ($P < 0.001$).

Ψ_{MD} was strongly related with Ψ_{PD} ; slopes of regression lines were significant ($P < 0.001$) for all species and almost parallel with the 1:1 line indicating anisohydric behaviour and scaling of g_s with hydraulic capacities to constrain Ψ_{MD-PD} (Figure 3-12). Intercepts for *E. loxophleba* and *E. leptopoda* were larger relative to other experimental species indicating a larger hydraulic gradient, suggesting greater transpiration for a given hydraulic conductance ($P < 0.001$; Figure 3-12). The *Eucalyptus* species were also found to have greater transpiring leaf area relative to sapwood cross-sectional area, LA:SA (Figure 3-3).

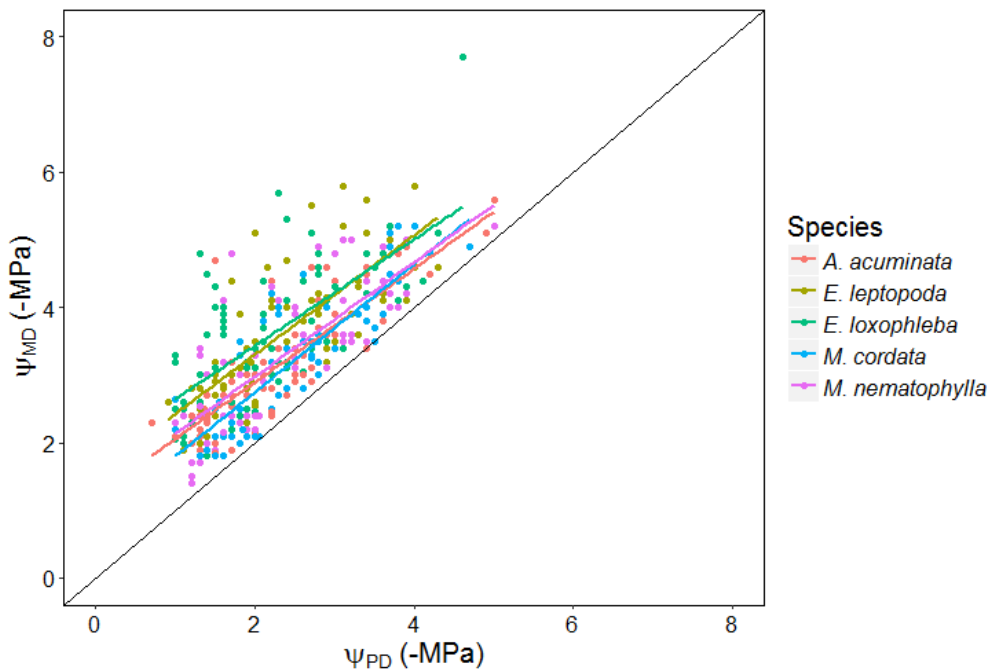


Figure 3-12 Predawn (Ψ_{PD}) and midday leaf water potentials (Ψ_{MD}) for all sampling events of saplings from five local native species across three cover thicknesses on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. The diagonal line is the 1:1 line.

Over 2 years of growth the rate of A increase as a function of increasing $\log g_s$ differed significantly ($P < 0.001$) among species (Figure 3-13). Increase in A was significantly higher for *A. acuminata* ($P < 0.001$) and *M. nematophylla* ($P < 0.001$) when compared to species with the lowest response, *E. loxophleba* and *M. cordata* (Figure 3-13). This indicates a high capacity to assimilate carbon per unit water lost. Despite their large height, stem diameter, and crown diameter (Appendix J – Appendix L), *E. leptopoda* and *E. loxophleba* did not have greater A at a given g_s than *A. acuminata* (Figure 3-13). Carbon-13 isotope fractionation ($\delta^{13}C$) was significantly different between species ($P < 0.001$), but not cover thickness (Figure 3-14). *E. leptopoda* ($P < 0.001$) and *E. loxophleba* ($P < 0.01$) had less negative values compared to all other experimental species (Figure 3-14). Gas-exchange-based estimates of C_i showed a significant species/season interaction ($P < 0.001$), due to significantly lower C_i in summer than in winter ($P < 0.01$) in the two *Eucalyptus* species.

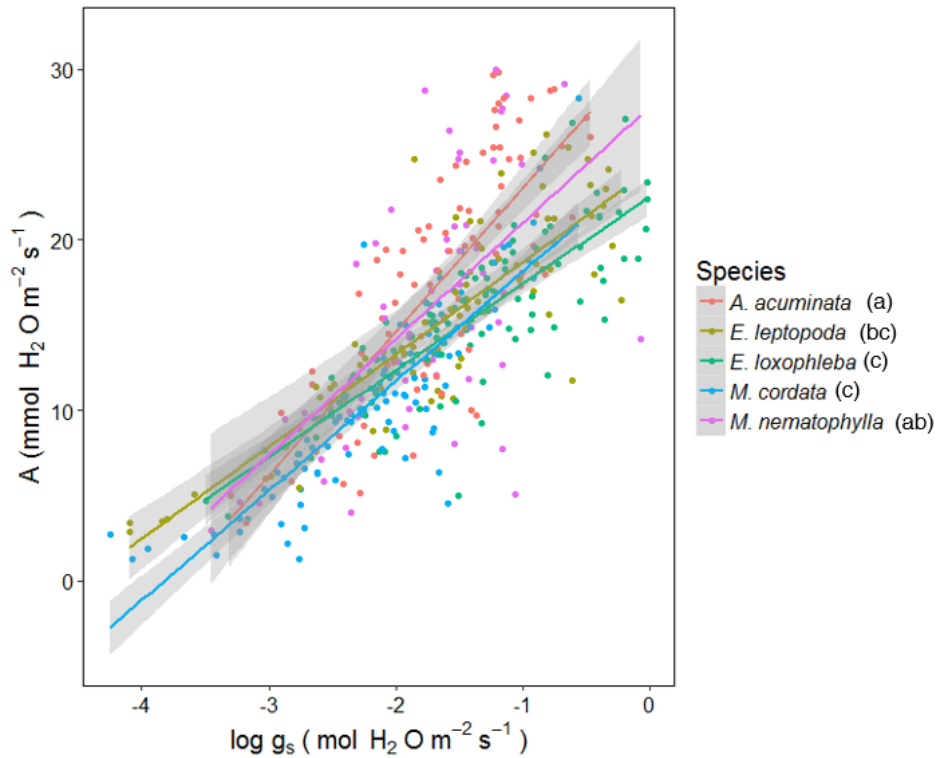


Figure 3-13 The relationship between morning photosynthesis (A ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) and (\log) stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) for saplings of five local native species growing on three cover thicknesses on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Grey shaded areas denote 95% confidence bands around the regression line. Letters indicate significant differences in slope ($P < 0.001$) between species, for each species $n = 6$. *A. acuminata* (adj. $R^2 = 0.59$, slope = 31.5, $P < 0.001$), *E. leptopoda* (adj. $R^2 = 0.73$, slope = 24.1, $P < 0.001$), *E. loxophleba* (adj. $R^2 = 0.68$, slope = 22.5, $P < 0.001$), *M. cordata* (adj. $R^2 = 0.75$, slope = 24.6, $P < 0.001$), *M. nematophylla* (adj. $R^2 = 0.35$, slope = 27.8, $P < 0.001$).

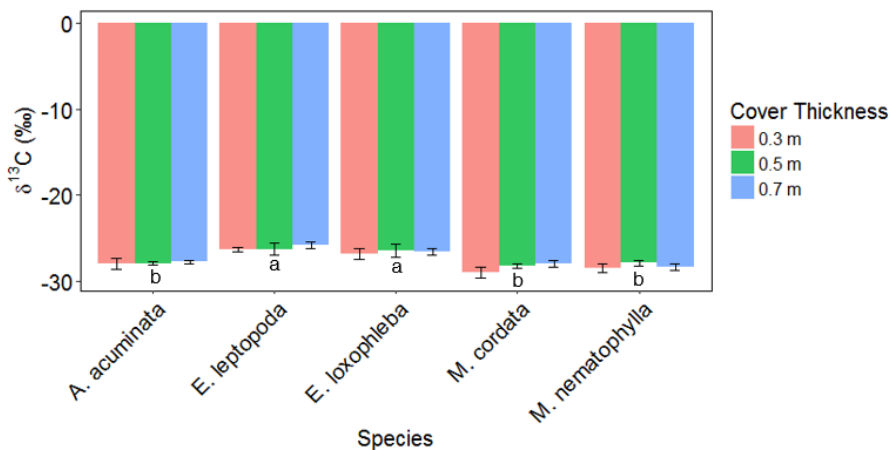


Figure 3-14 Carbon isotope fractionation ($\delta^{13}\text{C}$; \pm SE) in leaves for saplings of five local native species after 572 growth days on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Letters denote significant ($P < 0.01$) differences between species. For each species and cover thickness $n = 6$.

4. DISCUSSION

This study of five native woody species growing in ET covers of different depths confirms that shallow soils reduce plant-available water and root growth, leading to decreased water use, and enhanced stress. Although aboveground plant growth differences between cover thicknesses were not apparent over the 2-year study period, presumably due to the presence of irrigation during the first growth year leading to significant soil water storage, plants produced much fewer roots in shallow soil. Negative impacts of thinner covers on growth and survival are expected in the long-term given the observed reduced root growth, higher root densities and drier soils, with the corresponding differences in physiology.

As hypothesised, total profile water increased with cover thickness; this was attributed to the extra soil volume resulting in increased moisture storage. Higher moisture availability was also confirmed by the correlation between Ψ_{PD} and weighted VWC (Figure 3-10), and the lack of differences in Ψ_{PD} between species (Figure 3-9). This supports the widely accepted idea that Ψ_{PD} reflects soil water potential, based on the theory that Ψ_{PD} should equilibrate overnight with the water potential of the soil accessed by roots (Schulze and Hall 1982, Richter 1997). However, in this study Ψ_{PD} were not fully equilibrated to soil water potentials as they were generally more negative than expected (Figure 3-2a, Figure 3-9 and Appendix C). The main factors explaining incomplete equilibrium documented in

the literature are nocturnal transpiration (Bucci et al. 2005, Zeppel et al. 2010) and heterogeneous soil moisture causing hydraulic redistribution between roots (Burgess et al. 1998, Burgess and Bleby 2006, Brooksbank et al. 2011). The correlation between Ψ_{PD} and night-time VPD provides further evidence for nocturnal transpiration which has been found to contribute 15 – 25 % of total daily water loss in other woody species (Bucci et al. 2005). During hydraulic redistribution water is transported from roots in moist soil to roots in dry soil (Burgess et al. 1998). Water flowing to dry roots and soils is unlikely to flow back to stems and foliage, therefore, foliage rehydration is slowed down and equilibrium may not be achieved. For instance, Brooksbank et al. (2011) found that the net water volume redistributed by lateral roots in *E. kochii* subsp. *borealis* was equivalent of up to 27 % of transpiration in semi-arid Western Australia.

Despite the incomplete equilibration between plants and soil, it was clear that drier soils caused greater challenges for plant water uptake: both Ψ_{PD} and Ψ_{MD} were correlated with VWC, with an additional effect of cover thickness, as Ψ_{MD} on the 0.3 m covers were more negative than on 0.7 m covers (Figure 3-9). While Ψ_{MD} shifted seasonally with Ψ_{PD} (Figure 3-9 and Figure 3-11) there were interesting differences in Ψ_{MD-PD} between covers with different thicknesses: Ψ_{MD-PD} were smallest on the 0.7 m cover, and largest on the 0.5 m cover (Figure 3-11). It is common to interpret differences in Ψ_{MD-PD} as differences in the hydraulic gradient between soils and leaves, and to explore its interdependence with differences in transpiration rates and differences in hydraulic conductances. Given the evidence of incomplete equilibration between plant and soil at predawn, and lack of transpiration estimates at midday (when Ψ_{MD} was measured), we cannot quantitatively explain the lower Ψ_{MD-PD} of the 0.7 m and 0.3 m covers. However, on the 0.7 m covers which had the highest water availability (Figure 3-2c) and high total root length (Figure 3-4b), it is likely Ψ_{MD-PD} were small due high hydraulic conductances, whereas the plants with relatively low Ψ_{MD-PD} on the 0.3 m cover would have been due to early stomatal closure. Predawn water potentials on the 0.3 m cover were already very negative (Figure 3-9), requiring conservative water use to avoid extremely low Ψ_{MD} , leading to smaller Ψ_{MD-PD} (Figure 3-11). On thinner covers, the greater root density in a smaller soil volume (Figure 3-6) led to faster water depletion around roots, causing lower plant-available water and lower hydraulic conductance, ultimately leading to g_s that was not sustained during times of greater evaporative demand (Figure 3-8 and Chapter 4). In contrast, plants on

thicker covers had more roots (Figure 3-4b) in larger soil volumes that held more moisture, especially at greater thicknesses (Figure 3-2c). Grigg et al. (2008) observed similar results in an arid-zone dune system, where greater soil depth allowed greater soil water availability and unrestricted root growth which resulted in higher g_s . Although water availability was much improved for plants on deep covers, differences in physiology were relatively moderate, possibly due to conservative water use, partly driven by stomatal closure in response to extreme atmosphere evaporative demand (Chapter 4).

The relatively even lateral root distribution across covers may partially be due to complete root closure (analogous to canopy closure) characteristic of mature stands as suggested by Gwenz et al. (2011b). The idea of a mature state on the 0.7 m thick cover is partly corroborated by the comparison with the Jackson et al. (1996) global model of cumulative root proportions with soil depth (Figure 3-6). The comparison demonstrated that root depth distribution on the 0.7 m thick cover was most similar to that of sclerophyllous shrublands, the vegetation type common in this region. Whether or not current root depth distributions remain the same will depend on how species composition and plant density develop in future climates. Interestingly, total root system size differed between covers and was mostly determined by cover thickness (Figure 3-4b), while root densities were similar across covers but tended to increase with available soil volume. This suggests that plants try to avoid placing roots too close to one another, indicating the presence of mechanisms whereby roots sense the presence of 'self roots' (Falik et al. 2003, Schenk 2006), or resource depletion zones around existing roots (Casper and Jackson 1997) leading to growth away from other roots. There was also a clear reduction in root density with soil depth, and a clear tendency to place fewer roots near the surface and more at depth when soil volume was non-limiting on thicker covers (Figure 3-4b, Figure 3-5, and Figure 3-6). Schenk (2006) observed similar patterns where root growth responded to the availability of a larger soil depth rather than the presence of a competitor, and he proposed that a combination of increased volume available for root growth (Falik et al. 2005) and reduced root self-inhibition (Falik et al. 2003, Gruntman and Novoplansky 2004) were likely factors contributing to the observed pattern. In contrast to reports in other semi-arid systems (e.g. Montaña et al. 1995, Briones et al. 1996) and calculated root proportions from the Jackson et al. (1996) model, maximum root densities in this study were not in the top soil layers, but slightly deeper (Figure 3-5 and Figure 3-6). In terms of water use efficiency there may be

advantages to high densities of shallow fine roots, in particular in situations with frequent small rainfall pulses (Sala and Lauenroth 1982). However, this study shows that soil water recharge occurs mainly after large rain pulses and that shallow soil water is vulnerable to rapid evaporation in hot summer conditions (Figure 3-2a), as has been reported in other seasonally dry environments (Bleby and McGrath 2012). High temperatures, fast soil drying and infrequent large rainfall pulses present challenging conditions for plant roots in upper soil layers. Therefore, maintaining roots in unfavourable shallow soils is costly, requiring tighter stomatal control at the cost of carbon gain, more maintenance respiration, and cavitation resistant vessels (Eissenstat 1997, Schwinning and Ehleringer 2001), thus sustaining roots in upper soil layers is not always advantageous.

Cover thickness had a clear effect on soil moisture storage (Figure 3-2c) and on plant water use: mean g_s on the 0.3 m thick cover was 19 % and 24 % lower than the 0.5 m and 0.7 m thick covers, respectively (Figure 3-8). During summer when soil moisture became more limiting and evaporative demands increased, g_s , Ψ_{PD} , and Ψ_{MD} were reduced more on shallower covers (Figure 3-7, Figure 3-8, Figure 3-9, and Figure 3-10). Prior et al. (1997) reported similar patterns in *E. tetradonta* saplings, where g_s was more sensitive to increasing VPD at more negative Ψ_{PD} experienced during the dry season than when Ψ_{PD} were less negative during the wet season. Like in previous studies in southwestern Australia (e.g. Mappin et al. 2003, Veneklaas and Poot 2003, Mitchell et al. 2008), mean g_s was higher during winter compared to summer. The seasonal contrast in whole-day g_s is likely to be higher than suggested by the morning g_s data, because in summer g_s tends to decline fast after an early-morning maximum (Chapter 4) whereas in winter this is often not the case (Lambers et al. 2014). Similarly, this decline was faster in plants on shallower soils compared to deeper soils (Chapter 4). Plants on thicker covers (0.5 m and 0.7 m) were less sensitive to seasonal increases in VPD due to increased access to soil water, exemplified by higher g_s , Ψ_{MD} , and Ψ_{MD-PD} (Figure 3-8, Figure 3-9, and Figure 3-11). It is worth noting that these plants had better water status despite having higher g_s (i.e. higher transpiration rates). In contrast, on shallower covers the combination of lower g_s , and more negative Ψ_{MD} indicates limited water availability due to low VWC and potential loss of soil-to-root hydraulic conductivity due to the depletion of water around roots (Lombardini 2006) and/or cavitation-induced conductance loss in the plant (Sperry et al. 1993).

Not only was variation in g_s apparent between cover thicknesses, species differences in water use were also detected. Variation among species was linked to differences in physiological strategies, functional traits, and root densities. *M. cordata* had the lowest mean g_s compared to all other experimental species (Figure 3-8). Its intermediate LA:SA (Figure 3-3a) suggests that it may have an intermediate capacity to supply water to its leaves, since SA generally correlates well with stem hydraulic conductance (White et al. 1998). LA:SA ratio reflects the balance between demand and supply of water (Carter and White 2009, Gotsch et al. 2010), with arid environment species generally having lower ratios to cope with greater atmospheric evaporative demand and lower soil water availability (Mencuccini and Grace 1995, Gotsch et al. 2010, Gleason et al. 2012), thus avoiding damaging water potential gradients and minimising reductions in g_s . In arid to semi-arid environments, vessel tensions and within-plant hydraulic gradients are larger compared to mesic species allowing them to access tightly bound soil water. Tolerance to larger hydraulic gradients is associated with narrower high density xylem vessels (Hacke et al. 2001, Hacke et al. 2006), however, narrower vessels reduce stem conductivity (even at higher densities) and may reduce water transport capacity to leaves (Zanne et al. 2010, Gleason et al. 2012) reducing g_s . Here we see that *M. cordata* had slightly more negative Ψ_{PD} but one of the least negative Ψ_{MD} (Figure 3-9) leading to a smaller hydraulic gradient, presumably associated with its low g_s (Figure 3-8). The combination of a small hydraulic gradient and an intermediate LA:SA (Figure 3-3a) likely contributed to avoidance of damaging reductions in water potentials, and maintenance of relatively low but stable g_s for *M. cordata* (Figure 3-8) in response to increasing water stress (Figure 3-2) and evaporative demand (Figure 3-1c).

A. acuminata had a similarly intermediate LA:SA (Figure 3-3a), but higher g_s (Figure 3-8) and better overall water status (Figure 3-9). This was due to its high root densities (Figure 3-6), low LA:RL_{Total} (Figure 3-4b), which increased its capacity to supply water to leaves. For a given g_s , *A. acuminata* achieved an *A* that was 23% and 28 % higher compared to the lowest species *E. leptopoda* and *E. loxophleba*, respectively; and 12 % greater compared to the next highest species *M. nematophylla* (Figure 3-13). As up to three quarters of leaf nitrogen (N) is present in the photosynthetic machinery (Evans and Seemann 1989), there is a strong positive relationship between leaf N concentration and photosynthetic capacity (Evans 1989, Wright et al. 2004). This provides a direct link between leaf N concentration

and growth, partly constrained by the efficiency with which N is used during photosynthesis (Warren and Adams 2006). Therefore, water use, A , and N are linked due to the dependence of A on g_s where open stomata increase the photosynthetic use efficiency of N (Warren and Adams 2006). Therefore, in the potentially nitrogen fixing *A. acuminata* high A for a given g_s is most likely explained by high leaf N concentrations as it generally correlates with photosynthetic capacity. Despite not having a large crown, *A. acuminata* was also the tallest of the five species which is reflected by its high SMF (Figure 3-3b), and height (Appendix J).

Although *M. nematophylla* had the second largest A response to increasing g_s (Figure 3-13), it did not result in greater aboveground growth (Appendix J – Appendix L). *M. nematophylla* had the lowest mean LA:RL_{Total} indicating a low investment in photosynthesising leaf area and large allocation to roots (Figure 3-4b). Among species the LA:RL_{Total} for *M. nematophylla* was 91% and 92% lower compared to the two highest species *E. loxophleba* and *E. leptopoda*, respectively. *M. nematophylla* demonstrated a number of traits considered typical for drought-adapted species (Zimmer and Grose 1958, Mooney et al. 1978): low LA:RL_{Total} (Figure 3-4b), low growth (Appendix J – Appendix L), and high LMA (Appendix M) which is positively related to leaf thickness (Niinemets 1999, 2001). In a global study comprised of multiple leaf forms, including needle leaved shrubs (like *M. nematophylla*), A per unit LA was found to increase with LMA and leaf thickness (Niinemets 1999). The increase in photosynthetic capacity is due to accumulation of photosynthetic proteins per unit LA with increasing leaf thickness (Niinemets 2001). Therefore high rates of carbon assimilation in response to increasing g_s by *M. nematophylla* are partly explained by high LMA (Appendix M). Additionally, as SA generally correlates well with stem hydraulic conductance (White et al. 1998), its low LA:SA (Figure 3-3a) suggests that it had a high capacity to supply water to its leaves. As such, its large investment in roots and its low LA:SA increased its capacity to supply water to leaves, partly explaining its ability to maintain high g_s (Figure 3-8) and Ψ_{MD} (Figure 3-9) among experimental species.

Although the response of A to increasing g_s in *E. leptopoda* and *E. loxophleba* suggested that these species had a lower intrinsic water-use-efficiency (WUE) than the other species (Figure 3-13), this was not confirmed by $\delta^{13}C$ which is an indicator of long-term intrinsic

WUE (Figure 3-14). Carbon isotope fractionation ($\delta^{13}\text{C}$) suggested lower average C_i , but gas exchange measurements showed similar to higher C_i in these species. Low mesophyll conductance is a potential explanation for values of $\delta^{13}\text{C}$ that are higher (less negative) than expected (Barbour et al. 2010). The need in these eucalypt species to keep g_s relatively high to achieve a given A , may partly explain why these species also have the greatest average hydraulic gradients ($\Psi_{\text{MD-PD}}$) among the five species (Figure 3-11 and Figure 3-12).

All five species in this study displayed seasonal shifts in both Ψ_{PD} and Ψ_{MD} , resulting in relatively constant internal water potential gradients from roots to shoots (measured here as $\Psi_{\text{MD-PD}}$; Figure 3-11 and Figure 3-12). This behaviour is anisohydric (Martínez-Vilalta et al. 2014) or, using the term coined by Franks et al. (2007), isohydrodynamic, and requires whole-plant hydraulic conductance to scale with transpiration rate. It is interesting that all five species displayed this behaviour, and it would be worthwhile investigating how widespread this is in woody plants in these climatic conditions, and what the influence of soil hydraulic properties may be. Detailed diurnal patterns of stomatal regulation and water potentials during summer days are considered in greater detail for the two eucalypt species in Chapter 4.

5. CONCLUSION

The 2-year ecophysiological study presented here demonstrates that plants on thicker soil covers have greater access to soil water, not only due to a larger water storage capacity, but also due to better root growth. The deeper soils and root systems are presumably similar to analogue natural sclerophyllous shrubland. Species in the deeper soils had higher g_s , decreased sensitivity to seasonal shifts in VPD, and better water status. Although biomass allocation, leaf traits and growth rates varied between the five species, they all displayed anisohydric behaviour. While water potentials varied seasonally, apparent hydraulic gradients were approximately constant throughout the year as a result of effective stomatal control. Future changes in growth and survival between cover thicknesses are expected given the observed differences in physiology. In a severe drought year we expect plants on thinner covers to experience severe drought stress and possibly mortality due to less stored water and confined shallow root systems that would be unable to cope with evaporative demands, especially as plants continue to grow larger. The study also demonstrates the

close coordination between plant functional traits such as LA:RL_{Total}, RL and LA:SA, water use strategies, and growth, which should be considered prior to re-vegetation efforts. We expect improvements in the success of restoration efforts if thicker soil covers conducive to water storage are used, and an empirical understanding of traits highlighted in this study are considered when designing engineered covers.

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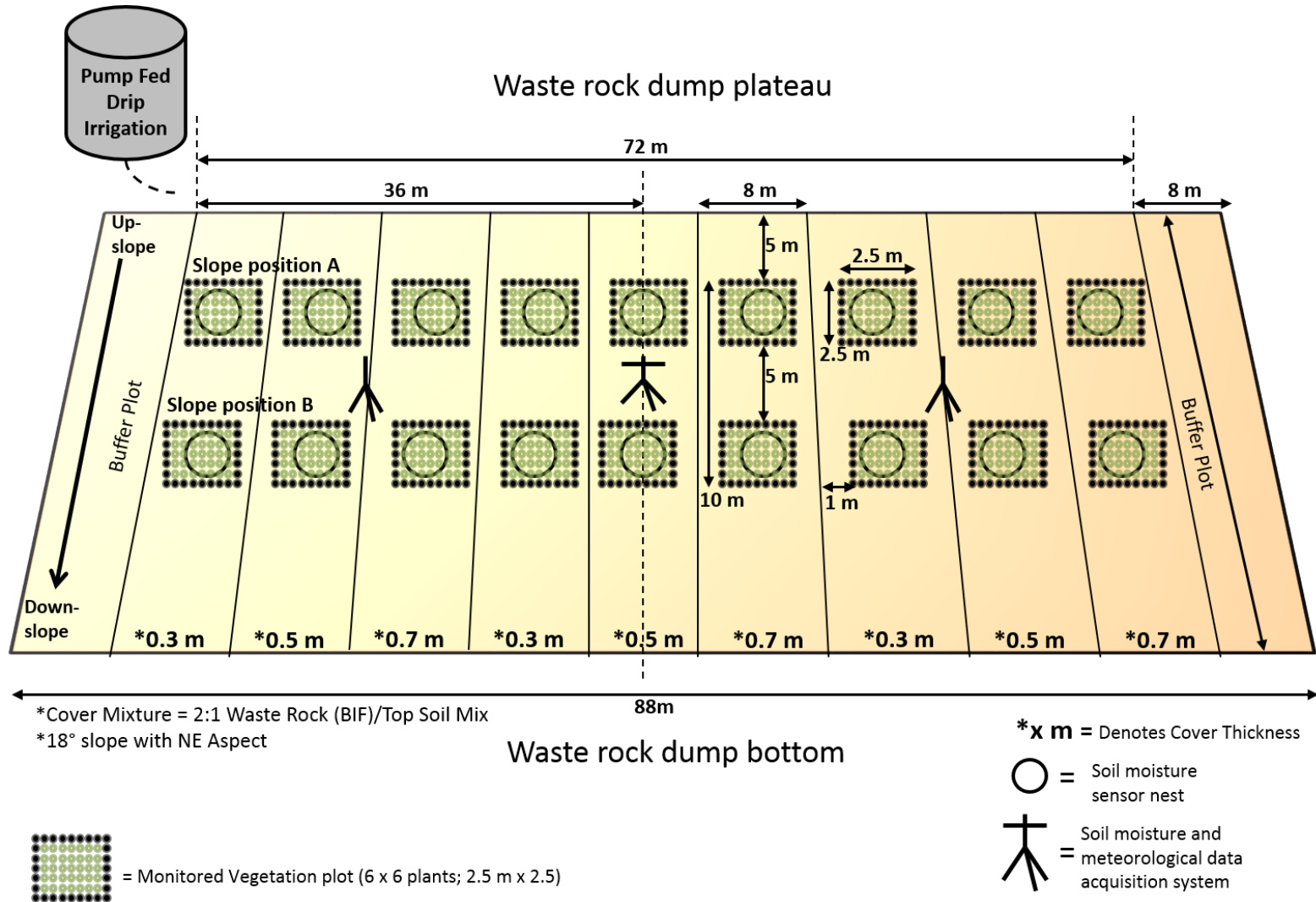
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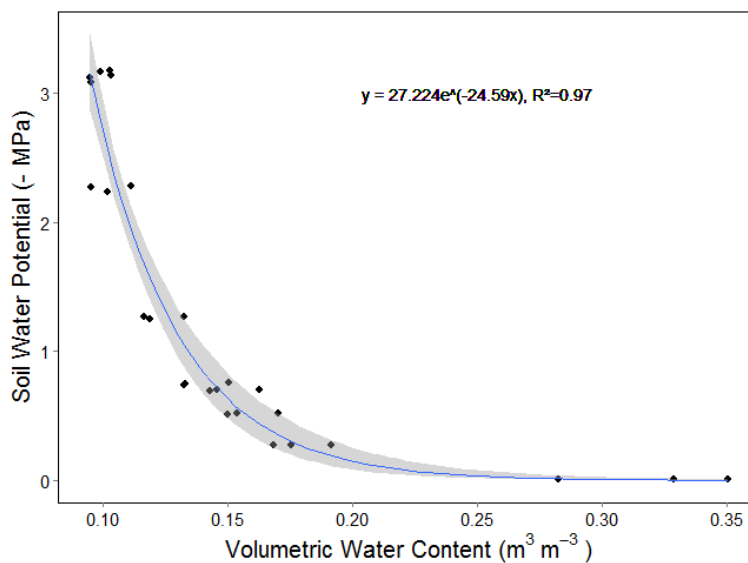
8. APPENDICES



Appendix A Sketch of the trial area from the perspective of facing the slope; meteorological monitoring was located at the central monitoring station.



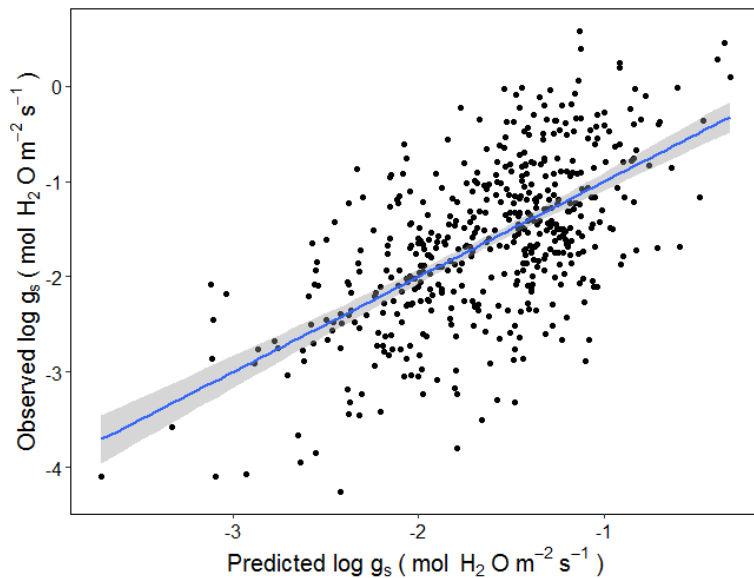
Appendix B Photo of the trial area looking north prior to harvest on February 3, 2016. Natural *Eucalyptus* woodlands are visible at the bottom of the slope, while the trial area contains five species (*Acacia acuminata*, *E. leptopoda*, *E. loxophleba*, *M. cordata*, and *M. nematophylla*) native to the area. All three soil moisture monitoring stations are visible, meteorological monitoring instrumentation was located on the centre station.



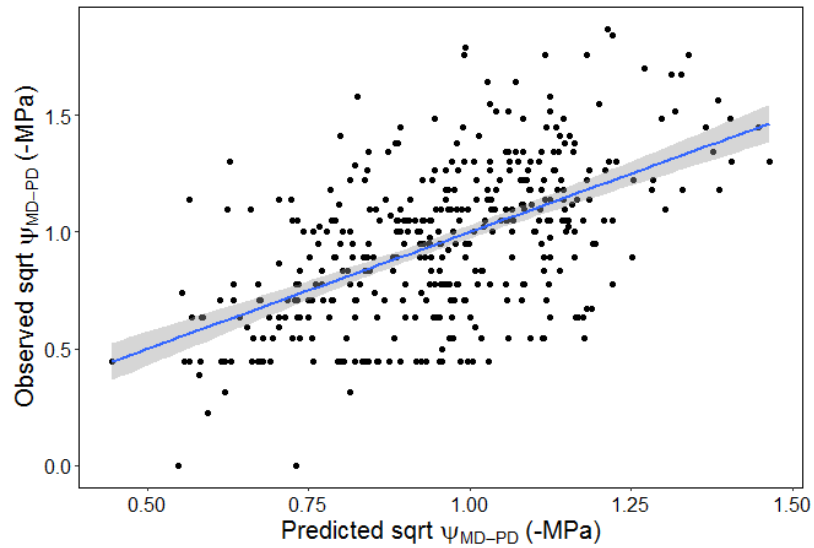
Appendix C Soil water potential (-MPa) and volumetric soil moisture content ($\text{m}^3 \text{m}^{-3}$) of the cover material determined using a WP4C dewpoint water potential meter and the oven drying method. Grey shaded areas denote 95% confidence bands around the regression line.



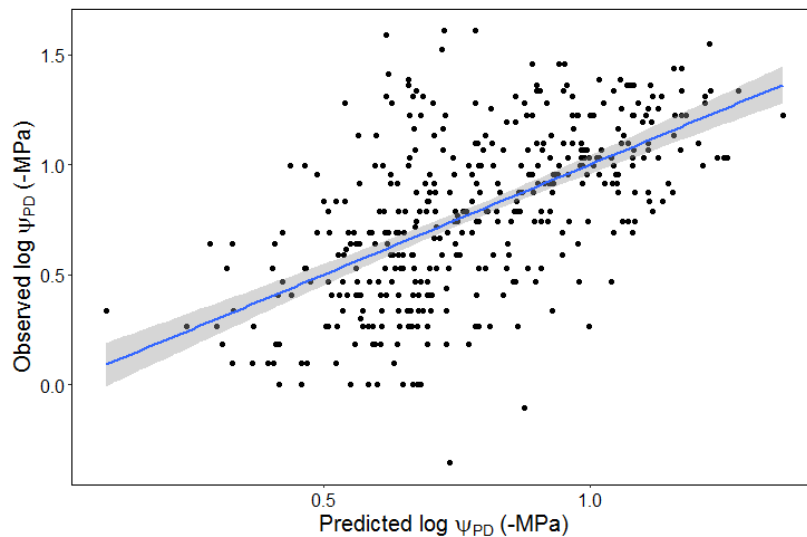
Appendix D a) Using a backhoe to create a soil pit 0.1 m from a plant stem b) sampling pit and frame with 0.0025 m² squares used to count the number of roots in each cell on a 0.3 m thick cover.



Appendix E Linear regression of observed and predicted log-transformed g_s values ($P < 0.001$, $R^2 = 0.37$) from Model- g_s where $\log g_s$ was the response variable, species and cover thickness were categorical variables, and weighted VWC and VPD where explanatory variables. Grey shaded areas denote 95% confidence bands around the regression line.



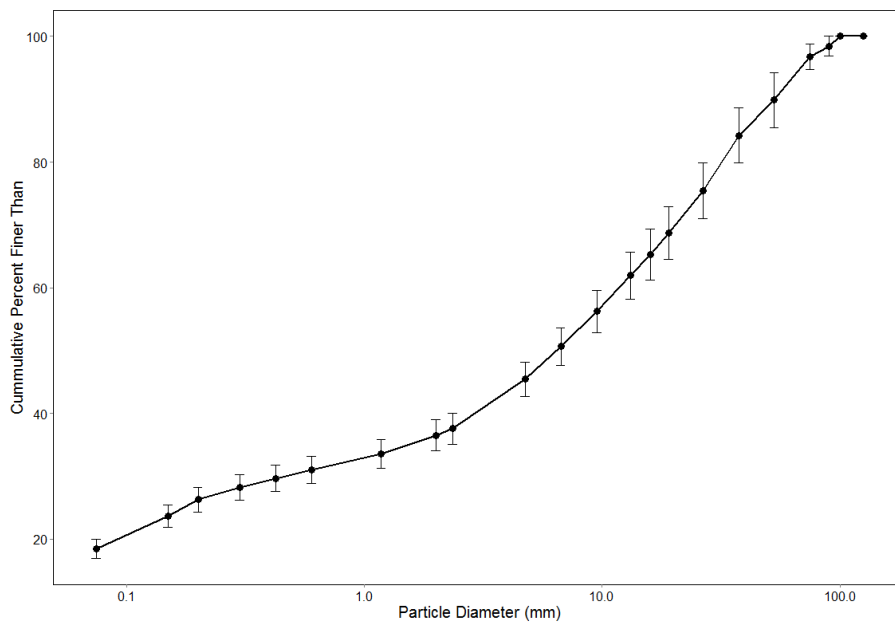
Appendix F Linear regression of observed and predicted square root-transformed Ψ_{MD-PD} values ($P < 0.001$, adj. $R^2 = 0.30$) from Model- Ψ_{MD-PD} where $\text{sqrt } \Psi_{MD-PD}$ was the response variable, species and cover thickness were categorical variables, and weighted VWC and VPD were explanatory variables. Grey shaded areas denote 95% confidence bands around the regression line.



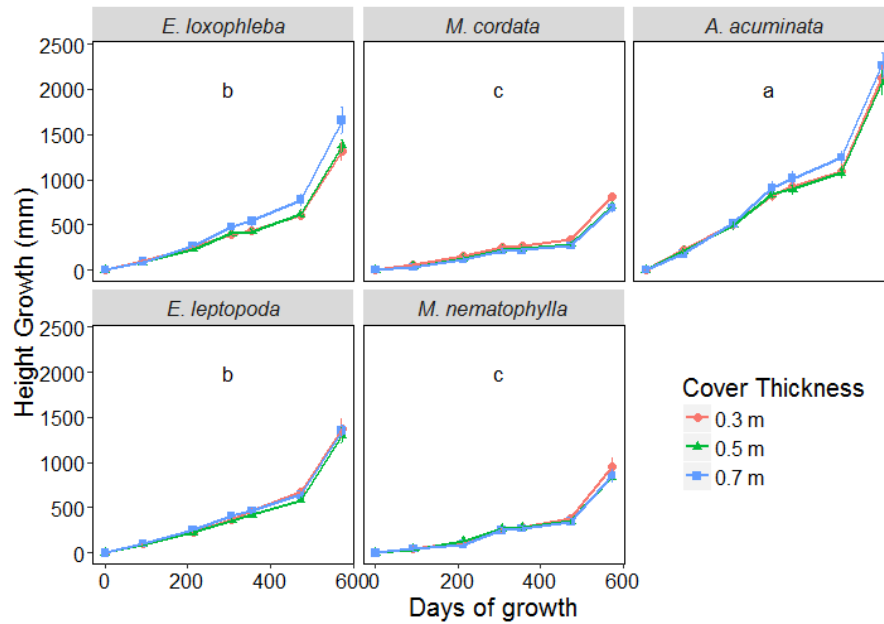
Appendix G Linear regression of observed and predicted log-transformed Ψ_{PD} values ($P < 0.001$, $R^2 = 0.21$) from Model- Ψ_{PD} where $\text{log } \Psi_{PD}$ was the response variable, species and cover thickness were categorical variables, and weighted VWC and night time (00:00 – 05:00) VPD were explanatory variables. Grey shaded areas denote 95% confidence bands around the regression line.

Appendix H Soil physical and chemical properties of the engineered cover material from the cover trial field plots. Refer to text for methods.

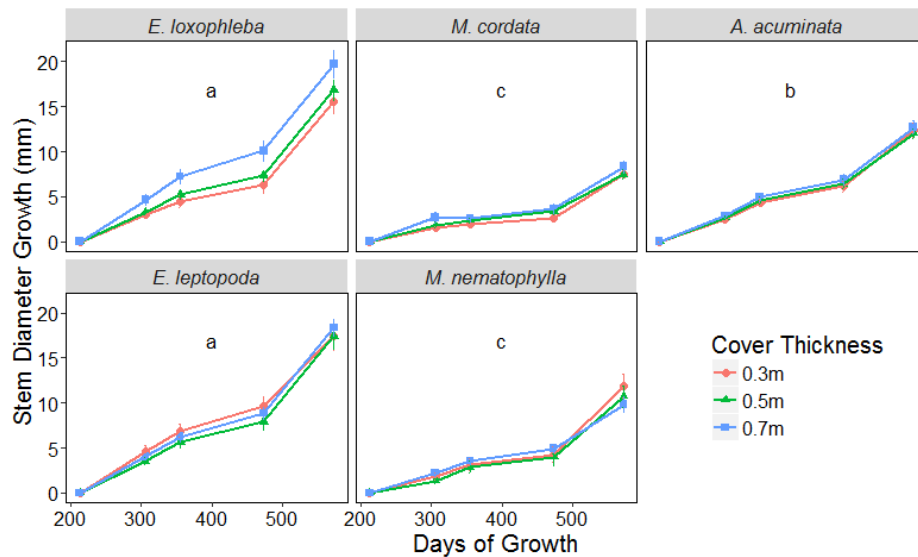
Analyte	Value	Analyte	Value
% Clay	16.4	Copper (g kg ⁻¹)	0.001
% Sand	72.9	Iron (g kg ⁻¹)	0.01
% Silt	10.7	Manganese (g kg ⁻¹)	0.004
pH Level (H ₂ O)	5.58	Zinc (g kg ⁻¹)	0.0002
Conductivity (dS m ⁻¹)	0.31	Aluminum (meq/100g)	0.12
Ammonium Nitrogen (g kg ⁻¹)	0.01	Calcium (meq/100g)	1.51
Nitrate Nitrogen (g kg ⁻¹)	0.01	Magnesium (meq/100g)	0.74
Phosphorus Colwell (g kg ⁻¹)	< 2	Potassium (meq/100g)	0.20
Potassium (g kg ⁻¹)	0.08	Sodium (meq/100g)	1.16
Sulphur (g kg ⁻¹)	0.09	Boron (g kg ⁻¹)	0.001
Organic Carbon (g kg ⁻¹)	55.83		



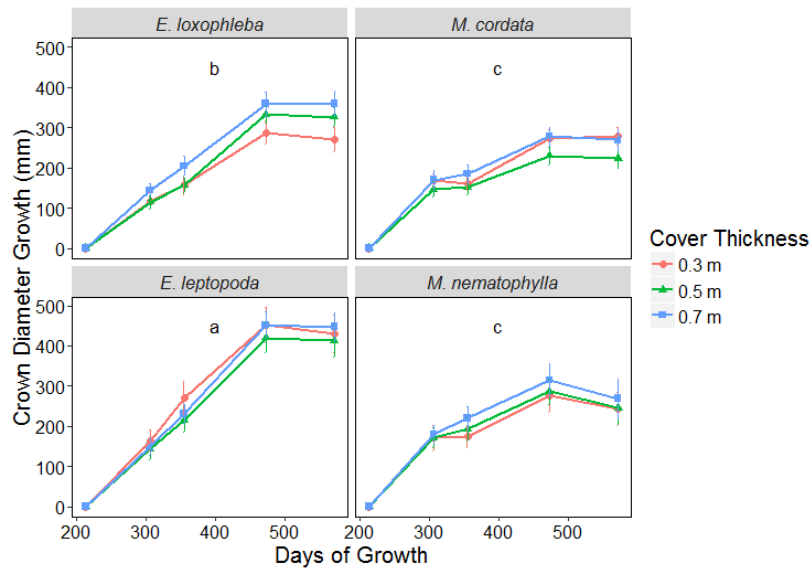
Appendix I Semi-logarithmic plot of mean (\pm SE) particle size distribution of the cover system material used in the experiment. The y-axis is linear while the x-axis is in logarithmic scale.



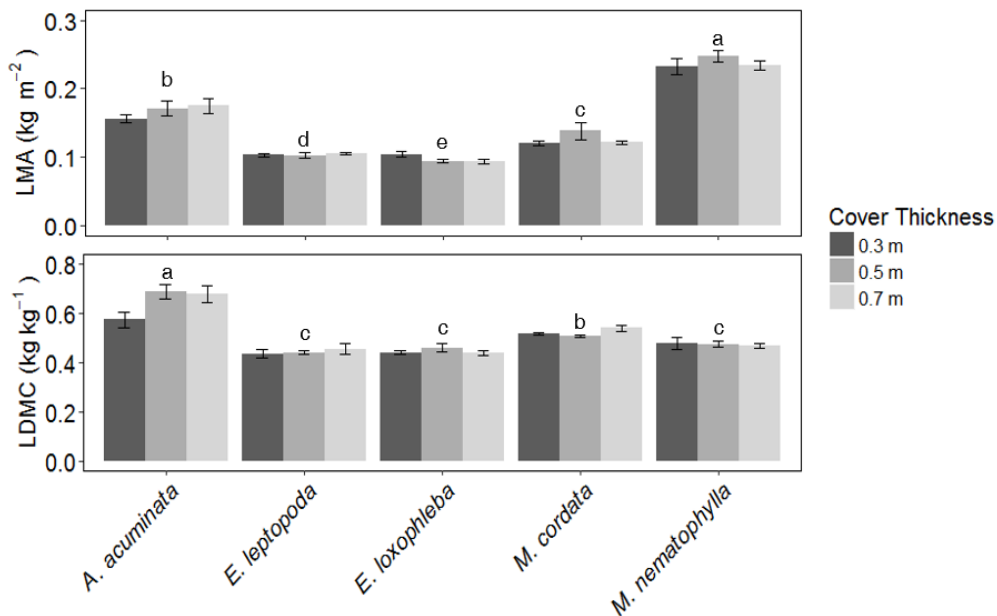
Appendix J Cumulative plant height growth (\pm SE) of saplings of five local native species over 572 growth days on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Letters denote significant ($P < 0.001$) differences between species. For each species and cover thickness $n = 6$.



Appendix K Cumulative stem growth (diameter) (\pm SE) of saplings of five local native species over 359 growth days on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Letters denote significant ($P < 0.001$) differences between species. For each species and cover thickness $n = 6$.



Appendix L Cumulative crown diameter growth (\pm SE) of saplings of five local native species over 359 growth days on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Letters denote significant ($P < 0.001$) differences between species. For each species and cover thickness $n = 6$.



Appendix M Leaf mass area (LMA; \pm SE) and leaf dry matter content (LDMC; \pm SE) of saplings of five local native species after 572 growth days on an engineered cover system with an 18° slope with a NE aspect in semi-arid Western Australia. Letters denote significant ($P < 0.001$) differences between species. For each species and cover thickness $n = 6$.

CHAPTER 4 Shallow soils negatively affect water relations and photosynthesis in two semi-arid *Eucalyptus* species

Sebastian C. Lamoureux, Pieter Poot, Erik J. Veneklaas

PREFACE

After quantifying long-term trends in plant ecophysiology on the ET cover (Chapters 3) it was of interest to examine diurnal plant water use and associated mechanisms in response to plant available water as a function of soil depth, and of the daily shift in vapour pressure deficit, temperature, and irradiance. Daily plant water use and associated physiological processes were studied for two *Eucalyptus* species on the ET cover during summer. Disentangling daily physiological processes to understand the finer scale mechanisms driving plant ecophysiology during hot summer days was important as it provides empirical evidence linking soil depth with increased moisture availability and improved plant water status when plants are most vulnerable during summer. This chapter is in preparation for submission to a peer reviewed scientific journal.



ABSTRACT

Two *Eucalyptus* species (*E. leptopoda* and *E. loxophleba* Benth.) saplings were investigated on a 2-year old engineered cover system at three soil depths in a semi-arid climate during summer. Objectives were (1) to assess the influence of soil depth on plant water availability in a semi-arid climate; and (2) to better understand the impact of water availability and diurnal environmental fluctuations on key physiological processes such as transpiration (E), stomatal conductance (g_s), net photosynthesis (A), intercellular CO₂ concentration (C_i), and mid-day leaf water potential (Ψ_{MD}) as they relate to regulation of plant water status. Physiological responses of plants to environmental factors differed between soil depth but not species. Plants on deeper soils were less affected by high VPD, temperature, and irradiance due to increased plant available water, partly provided by their less confined roots, resulting in higher overall g_s , E , A , and Ψ_{MD} . Stomatal closure reduced A but not C_i , indicating that temperature and radiation were also significantly contributing to the decline in A . Greater soil depth in natural (semi-)arid ecosystems and on vegetated engineered covers may minimise plant sensitivity to higher temperature, VPD and irradiance through plant available moisture, especially in areas most vulnerable to climate warming and drying.

1. INTRODUCTION

Plant establishment and survival in water-limited ecosystems are largely governed by soil water availability (Noy-Meir 1973, Grayson et al. 2006). In these systems water availability is mainly determined by unpredictable rainfall events, evapotranspiration, soil depth, and soil hydraulic properties. Soil depth is a key determinant of rainfall storage capacity, and in seasonally dry climates, it correlates strongly with vegetation type (Beard and Pate 1984, Borchert 1994, Li et al. 2012). Generally there is close coordination between plant water use strategies, soil textures and depths (Westoby and Wright 2006, Li et al. 2012) as varying textures present different challenges for plant water extraction (Bristow et al. 1984), and species from different soil types and depths often differ in water use strategies (Westoby and Wright 2006, McDowell et al. 2008).

Arid ecosystems characteristically have very low leaf area indices (LAI, m² of leaf area per m² of ground area) due to the lack of reliable access to soil water. Aridity-adapted species are generally considered to be conservative water users, i.e. they have low rates of water use per unit biomass due to low rates of transpiration (E). Nevertheless, maximum stomatal conductance (g_s) of arid species is not necessarily low (Körner 1995), indicating that effective stomatal regulation is vital to balancing water use to optimise growth and survival. In arid environments, plants with high stomatal conductance (g_s) can achieve high photosynthesis (A) and rapid growth, and can avoid high leaf temperatures by evaporative cooling of foliage in hot summer conditions, but risk depleting stored water. Low g_s is a safer strategy but reduces growth and is ineffective if neighbouring plants have higher water use and growth rates. The stomatal aperture can be reduced, in response to environmental cues such as low soil moisture availability and high vapour pressure deficit (VPD), directly as a result of the turgor loss of guard cells (hydropassive closure), and indirectly through signalling by production of abscisic acid (ABA) in roots and leaves (hydroactive closure) (Luan 2002, Lombardini 2006, Brodribb et al. 2014). While it is well-known and broadly described that optimal stomatal regulation must take into account available soil moisture, plant water status, and evaporative conditions, it is less clear which of the environmental or plant internal factors should take prevalence in determining stomatal opening for plants that differ in drought tolerance or drought exposure. In the context of conservative plant water use, a question of particular interest is how plants may optimise soil water uptake: being too conservative reduces a plant's competitive strength, being too profligate depletes soil water that may be needed for survival until the next (unpredictable) rainfall event. Plants that have evolved in soils with lower water storage capacity (shallow soils, sandy soils) and in areas with prolonged dry periods are therefore more likely to be conservative. As soil moisture varies with depth, which is dependent on soil physical properties as well as plant root distribution and water uptake, its influence on stomatal regulation is difficult to resolve, but likely to vary among species that differ in their soil and climatic preferences.

In addition to the complex effect of soil moisture on plant water status, g_s also varies diurnally in response to a decline in plant hydration from a pre-dawn maximum to a diurnal minimum, and due to fluctuations in atmospheric evaporative conditions. Mid-day depression and early morning peaks in g_s , A , and E have been described in numerous studies (e.g. Eamus and Cole 1997, Prior et al. 1997). Because A and E are both influenced by g_s , leaves need to balance

between maximising carbon gain, while minimising evaporative water loss (Lombardini 2006). While E is fully determined by g_s and environmental conditions, A is determined by g_s , environmental conditions, and the biochemical status of the photosynthetic apparatus, which varies with antecedent condition. For instance, A may be reduced by a combination of dehydration stress and high temperatures potentially causing reversible photoinhibition, by diverting excess light and heat energy away from Photosystem II (PS II), or even permanent damage of PS II, which might be exacerbated under warmer future climate conditions (Chaves 1991, Ball et al. 1995, O'Sullivan et al. 2017). The relationships between daily environmental shifts, g_s , E , and A are complex, especially when evaluated against soil water availability as a function of soil depth, and thus requires further quantification. Therefore, direct measurement of diurnal trends across a range of soil depths will improve the understanding of mechanisms that regulate daily plant water use and how this affects A .

In this study, diurnal water use and A of *Eucalyptus leptopoda* (Benth.) and *E. loxophleba* (Benth.) saplings was investigated on a 2-year old engineered cover system at three soil depths in a semi-arid climate during summer conditions. *E. loxophleba* and *E. leptopoda* were chosen as study species due to their prevalence in the area, where they occur in two contrasting community types. *E. leptopoda* generally occurs in drier inland areas and prefers rockier habitats, as opposed to *E. loxophleba* which occurs on deeper sandy soils (Figure 1-1). The primary aims were to investigate the influence of soil depth on plant-available water, plant water status, water use and photosynthesis, and to determine whether the two species differ in their response to soil depth. It was hypothesised that when growing on deeper soils, plants of both species would have more access to soil moisture resulting in (1) higher pre-dawn (Ψ_{PD}), and mid-day (Ψ_{MD}) leaf water potentials, g_s , E , A and intercellular CO_2 concentrations (C_i); and (2) decreased sensitivity to increasing daily VPD, temperature, and irradiance. We also anticipated that *E. leptopoda*, the species from rockier habitats, would have more conservative water use and tolerate more negative water potentials than *E. loxophleba*, the species occurring on deeper and sandier soils.

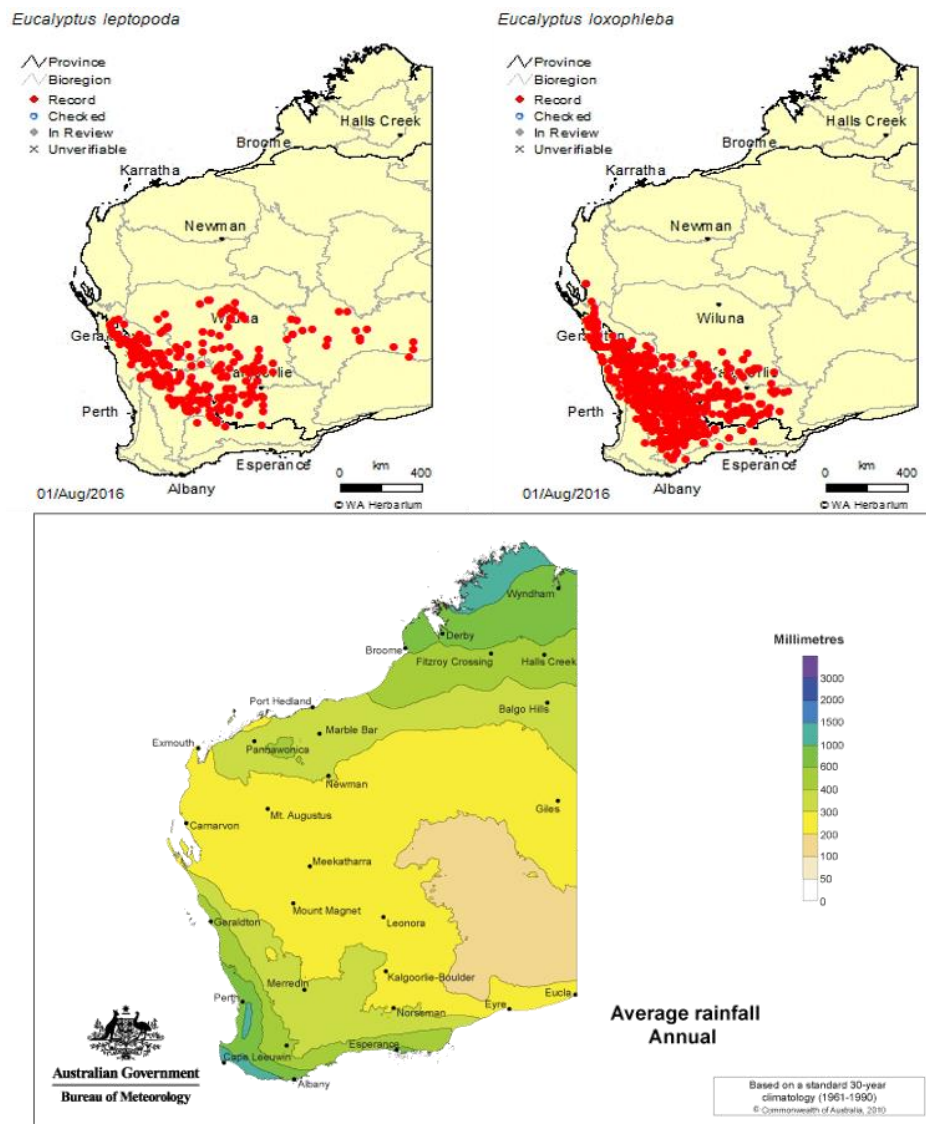


Figure 1-1 The distribution of *E. leptopoda* (Benth.) and *E. loxophleba* (Benth.) (FloraBase, <http://florabase.dpaw.wa.gov.au/>), and average annual rainfall (Bureau of Meteorology, <http://www.bom.gov.au/>) for Western Australia.

2. MATERIALS AND METHODS

2.1. Site Description and Weather

Monitoring was conducted on constructed soils atop a compacted waste rock dump slope (18°) with a North East aspect. The constructed soils were composed of two parts waste rock (banded iron formation, non-toxic), to one part topsoil sourced from stripped stockpiles of no more than 2 m high. The cover material was spread from the top of the dump to its bottom using a bulldozer with GPS capabilities. The cover soil mixture was a coarse sandy loam,

with low saturated hydraulic conductivity of $0.18 - 0.22 \text{ m day}^{-1}$ and no major geochemical restrictions to plant growth. The trial had three soil depth treatments; 0.3 m, 0.5 m, and 0.7 m at a precision of $\pm 0.1 \text{ m}$, a full description of the trial areas is available in Chapter 3. In this experiment there were three replicate plots for every depth, totalling nine plots.

The location of the trial was Mt. Gibson Mining's Extension Hill Operation ($29^{\circ}34'14.7 \text{ S}$, $117^{\circ}10'15.6 \text{ E}$, 344 m a.s.l.), at approximately 293 km North East of Perth, in the Yalgoo region of Western Australia. This semi-arid region is characterised by hot summers from October to March, and warm to cool winters from April to September during which the majority of rainfall occurs. Mean annual rainfall is approximately 338 mm with annual potential evaporation exceeding 2200 mm (Bureau of Meteorology, <http://www.bom.gov.au>). The region is dominated by *Acacia* and *Eucalyptus* open woodlands and scrub on sandy and alluvial plains, in addition to rocky outcrops and banded ironstone formations (McKenzie et al. 2003). The area is diverse and classified into 50 major habitats comprising 706 native plant species (Payne 1997). Dominant plant species, geology, soils, landforms, and climate of the Yalgoo region are fully described in Payne (1997).

The presence of *E. leptopoda* and *E. loxophleba*, and association with local community types in the area was based on evidence gathered from pre-mining vegetation surveys (Bennet Environmental Consulting 2000, Griffin and Associates 2005, ATA Environmental 2006), literature (Payne 1997, McKenzie et al. 2003, Meissner and Caruso 2008), expert consultations, on-site knowledge, and Flora Base (<http://florabase.dpaw.wa.gov.au/>). In Western Australia, distributions of *E. leptopoda* and *E. loxophleba* overlap, but the distribution range of *E. leptopoda* is further inland with an approximate annual rainfall of 100 – 400 mm, whereas *E. loxophleba* occurs in areas with annual rainfall of 200 – 600 mm (Figure 1-1). In the study area, *E. loxophleba* is predominantly found on sandy loam to loamy sand sites, whereas *E. leptopoda* generally occurs on granite outcrops and rocky loams. *E. loxophleba* seedlings were procured from the Australian Native Nursery, Oakford, WA ($32^{\circ}13'53.0 \text{ S}$, $115^{\circ}54'21.4 \text{ E}$, 24 m a.s.l.), while *E. leptopoda* was grown from seed collected by Mt. Gibson Mining in an outdoor shade house at the University of Western Australia Plant Growth Facilities, Perth, WA ($31^{\circ}59'03.2 \text{ S}$, $115^{\circ}53'10.3 \text{ E}$, 5.2 m a.s.l.). Study species (*E. leptopoda* and *E. loxophleba*) were grown in the same plots together with three other locally occurring species (*Acacia acuminata*, *Melaleuca nematophylla*, and *M. cordata*) which were

not studied in this experiment. Seedlings were planted in early July 2014, and were spaced 0.5 m apart, with none of the measured plants being less than 1 m from the edge of the plot. Each species had six seedlings per plot at random positions, at planting the mean heights of *E. leptopoda* and *E. loxophleba* seedlings were 101 mm and 149 mm, respectively.

2.2. *Environmental Parameter Measurements*

Meteorological variables on the plots were recorded at 15 minute intervals and compiled hourly using a CR 800 data logger (Campbell Scientific Inc., Logan, Utah, USA) and an automated weather station installed on site. The station measured air temperature and relative humidity (CS215, Campbell Scientific Inc., Logan, Utah, USA), wind speed and direction (Wind Sentry 03002, RM Young Pvt. Ltd., Traverse City, Michigan, USA), solar radiation (SP-110 pyranometer, Apogee Instruments Inc., Logan, Utah, USA), and rainfall using a tipping bucket rain gauge (CS 703, Campbell Scientific Inc., Logan, Utah, USA). Vapour pressure deficit (VPD) and absolute humidity were calculated from measured air temperature and relative humidity.

Field volumetric soil water content (VWC; $\text{m}^3 \text{m}^{-3}$) was measured hourly using CS616 moisture content reflectometers split between one CR800 and two CR10X data loggers routed via AM 16/32B multiplexers (Campbell Scientific Inc., Logan, Utah, USA). Sensors were installed in soil pits prior to planting seedlings. The first was installed at 0.1 m depth and from there at 0.2 m depth intervals to the maximum soil depth. Fine material was placed around each sensor to ensure adequate contact prior to backfilling. Data acquisition was split between three data loggers as a precaution in case of data logger failure. Total profile water (mm) for each soil depth was computed assuming that the sensors measured VWC of a 0.2 m depth interval (0.1 m above and 0.1 m below the sensor) except deepest sensor at the waste rock interface which measured VWC of the 0.1 m above the sensor only.

2.3. *Physiological and Morphological Measurements*

Sampling occurred over 3 sunny summer days, at one replicate plot for every soil depth treatment per day. Two representative plants per species were sampled consecutively on the same leaf in every plot each hour from 06:00-17:00. Once all plants had been measured, sampling resumed at the first plot the following hour. A total of nine plots and 18 plants per

species were sampled (three plots for every soil depth) over the course of 3 days. Gas exchange measurements were conducted in the field using a LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). Temperature and vapour pressure in the leaf chamber were kept at ambient conditions, and the LI-COR red and blue LED light source was used to achieve a constant photosynthetic photon flux density of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaf chamber CO_2 concentrations were 395-400 $\mu\text{mol mol}^{-1}$. Leaf temperature was measured by the leaf chamber thermocouple. Calculation of area based net photosynthesis (A ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO_2 concentrations (C_i ; $\mu\text{mol CO}_2 \text{ mol}^{-1}$) was according to the LI-COR manual.

Leaf water potentials (MPa) were measured at predawn (Ψ_{PD} ; 04:00 – 05:00) and ‘midday’ (Ψ_{MD} ; 14:00 – 15:00) for every sapling using a Pressure Chamber (PMS Instruments, Oregon, USA). Leaves were cut using a scalpel, placed in an airtight plastic bag in the dark over ice, and then measured within 10 minutes at the field site. Sapling height was measured from the base of the plant to the highest point of the foliage by extending all parts of the plant upwards (Pérez-Harguindeguy et al. 2013). Crown width was measured in two perpendicular directions. Stem diameter was measured at the base of the sapling using digital callipers (Mitutoyo, Honshu, Japan) in two perpendicular directions.

2.4. Data Analysis

Data were analysed with RStudio statistical software (R v0.98.1028, R Foundation for Statistical Computing, R, 2013). Differences in g_s , E , A , Ψ_{PD} , Ψ_{MD} , height, crown width, and stem diameter between species and soil depth, VWC within sensor depths among soil depths, and total profile water between soil depths were tested with generalised least square (gls) models using the package nlme (Pinheiro et al. 2015). In all models species and soil depth were fixed factors. Models were tested using five variance structures (VarFixed, VarIndent, VarPower, VarExp, and VarConstPower) to ensure homoscedasticity as outlined in Zuur et al. (2009). Models were evaluated and selected using Akaike’s information criterion (AIC; a measure of relative model performance for a given set of data), and plots of standardised residuals. Models with the lowest AIC value, and homogeneity of variance were chosen for further analysis. In selected models the ANOVA command was used to test significance (α

= 0.05) of the factors and covariates in the model. Tukey’s multiple pairwise comparison test was used to analyse differences among species or soil depths if ANOVA model outputs were significant.

3. RESULTS

At the time of the experiment (December 15 – 17, 2015) saplings were 2 years old, and sampled plants were significantly smaller in the 0.3 m soil depth compared to the 0.5 m and 0.7 m soil depths (Table 3-1). Averaged for the two species, plants in the 0.3 m soil depth plots were 27 % shorter and had 28 % and 29 – 35 % smaller crown width and stem diameter respectively, compared to the 0.5 and 0.7 m soil depth plots.

Table 3-1 Dimensions of 2-year old *E. leptopoda* and *E. loxophleba* saplings grown in plots with three different soil depths (0.3 m, 0.5 m, 0.7 m). Asterisks and letters denote significant ($P < 0.05$) differences between species and between soil depths, respectively.

Soil Depth	Plant Height (mm)		Crown Width (mm)		Stem Diameter (mm)	
	<i>E. leptopoda</i>	<i>E. loxophleba</i>	<i>E. leptopoda</i> *	<i>E. loxophleba</i> **	<i>E. leptopoda</i>	<i>E. loxophleba</i>
0.3 m	670.0 ^b	657.5 ^b	670.8 ^b	468.3 ^b	15.5 ^b	11.0 ^b
0.5 m	898.3 ^a	926.7 ^a	879.2 ^a	691.7 ^a	20.0 ^a	17.1 ^a
0.7 m	891.7 ^a	929.2 ^a	893.3 ^a	688.3 ^a	22.1 ^a	18.5 ^a

Water content as total profile water was significantly ($P < 0.001$) higher in deeper soils 0.7 m $>$ 0.5 m $>$ 0.3 m due to increased moisture storage provided by extra soil volume (Figure 3-1). A significant rainfall event occurred approximately 1.5 months prior to sampling, when 25.4 mm fell from November 7th – November 8th, 2015 (Figure 3-1). This input of rain can be observed as approximate 25 mm increase in total profile water on that day in all three depth treatments (Figure 3-1).

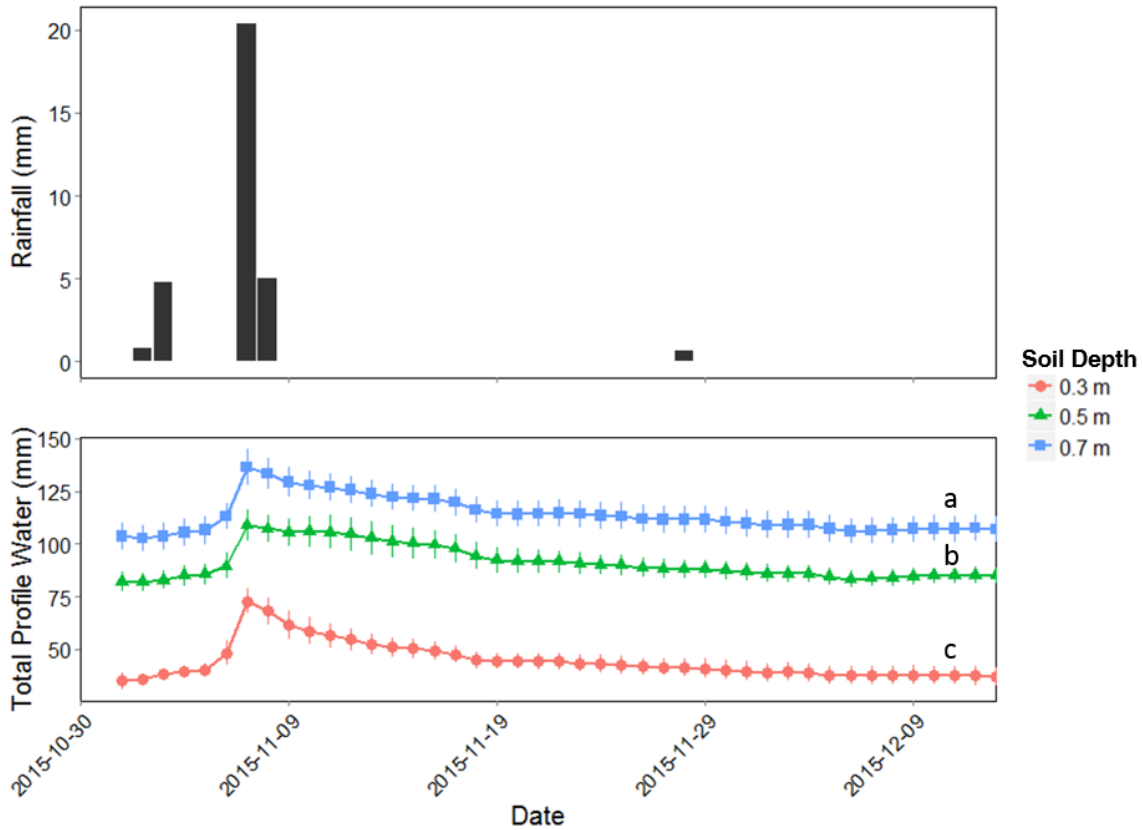


Figure 3-1 Rainfall and total profile water (\pm SE) in plots with three different soil depths (0.3 m, 0.5 m, 0.7 m) from 2015-11-01 to 2015-12-13, the 1.5 month period leading up to the days of gas exchange measurements. Letters indicate significant ($P < 0.001$) differences between soil depths.

During the days of the gas exchange measurements, VWC was lowest at shallow sensor depths (Figure 3-2). Although VWC at sensor depths of 0.3 m and 0.5 m were significantly ($P < 0.001$) higher on the 0.5 m deep soil compared to the 0.7 m deep soil (Figure 3-2), total profile water remained highest in the deepest soils (0.7 m > 0.5 m > 0.3 m; Figure 3-1).

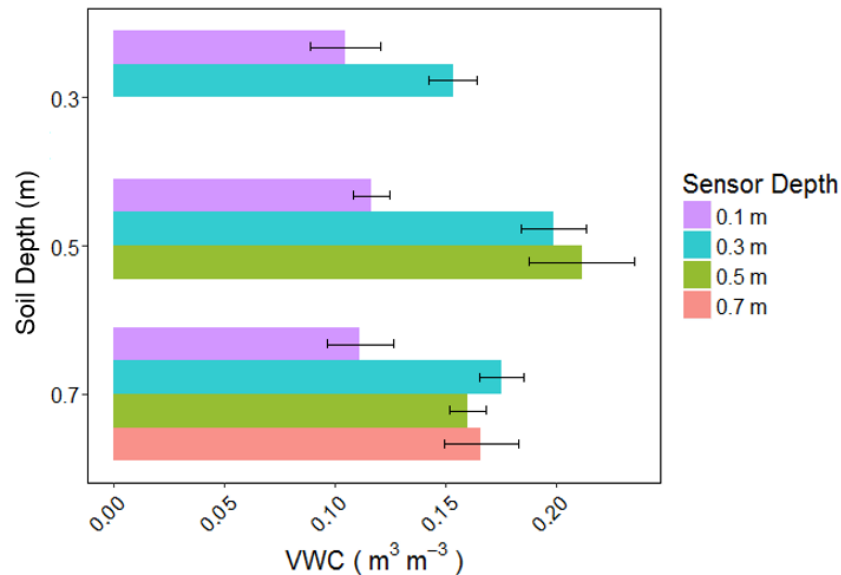


Figure 3-2 Volumetric water content (VWC; \pm SE) during the days of gas exchange measurements (2015-12-15 to 2015-12-17) across all sensor depths (0.1 m, 0.3 m, 0.5 m, 0.7 m) for each soil depth treatment (0.3 m, 0.5 m, 0.7 m) in a 2-year old trial.

On measurement days VPD increased quickly from 0.55 to 5.06 kPa (9-fold) from 05:00 and only started decreasing from 18:00 (Figure 3-3). The fast increase in VPD during the day was mostly due to a fast rise in temperature, although a 2-fold decrease in absolute humidity also contributed to the increase of VPD (Figure 3-3). Air temperature continued to rise until 18:00 when it reached a maximum of approximately 35°C, coinciding with the minimum absolute humidity (Figure 3-3), as well as a 2-fold increase in wind speed, and a change in wind direction from south to southwest at 19:00. Irradiance peaked at 14:00; there was no cloud cover except for a very short interval at 12:00 on one of the sampling days (Figure 3-3). During the sampling period sunrise and sunset were at 05:05 and 19:08, respectively (Geoscience Australia, <http://www.ga.gov.au>).

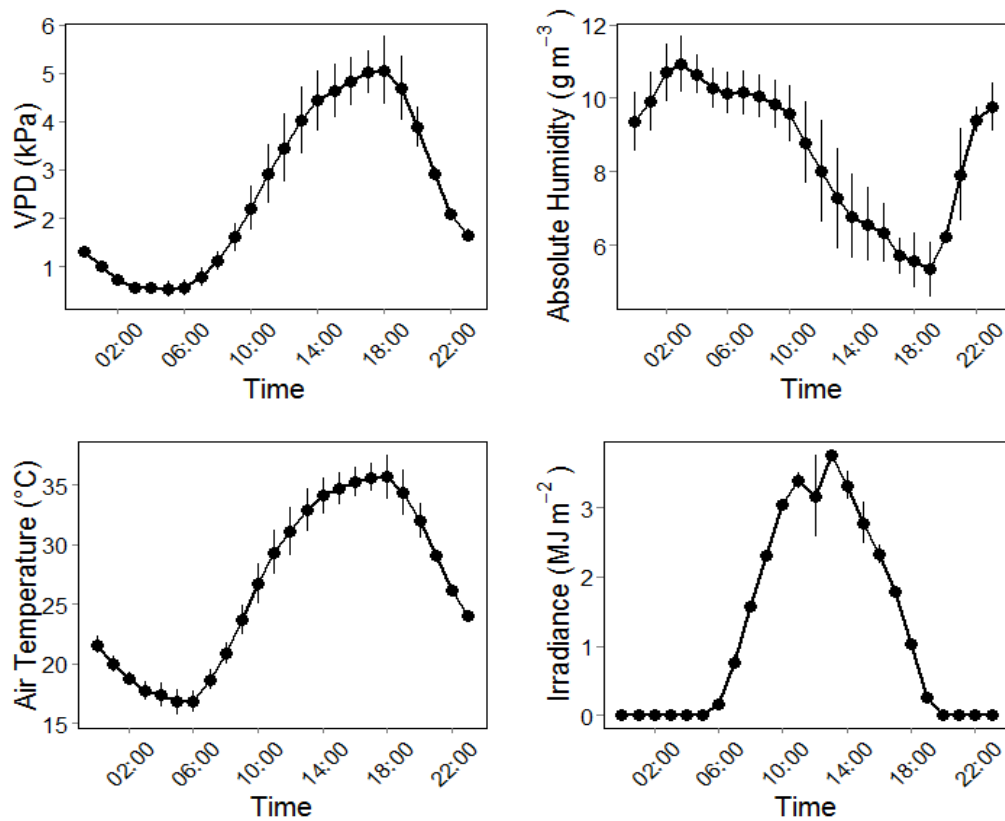


Figure 3-3 Diurnal variation in mean hourly (\pm SE) vapour pressure deficit (VPD), absolute humidity, air temperature, and solar irradiance during the sampling period (2015-12-15 to 2015-12-17).

Stomatal conductance (g_s) of both species was higher on deeper soils with greater total profile water (Figure 3-4). Conductance was highest at 07:00 and strongly declined thereafter until 14:00 when it stabilised or increased slightly. In contrast, the change in transpiration (E) was smaller than that of g_s , with a slight decline from 09:00 in *E. loxophleba* and 10:00 in *E. leptopoda*, with a slight increase on the 0.7 m cover at 14:00 for both species (Figure 3-4). Differences in E between cover depth treatments were associated with total profile water with plants on deeper soils having higher transpiration rates (Figure 3-1 and Figure 3-4). Daily average g_s and E were significantly ($P < 0.001$) different between soil depths, decreasing with soil depth (0.7 m > 0.5 > 0.3). On the 0.3 m depth g_s was 1.6 and 2 times lower, while E was 1.4 and 1.8 times lower compared to the 0.5 and 0.7 m soil depths, respectively. In general, g_s and E of plants on deeper soils responded less strongly to environmental factors such as high afternoon VPD, temperature, and irradiance (Figure 3-3 and Figure 3-4). Photosynthesis (A) decreased sharply during the morning for both species, like g_s (Figure 3-4). g_s , E , and A did not differ significantly between species.

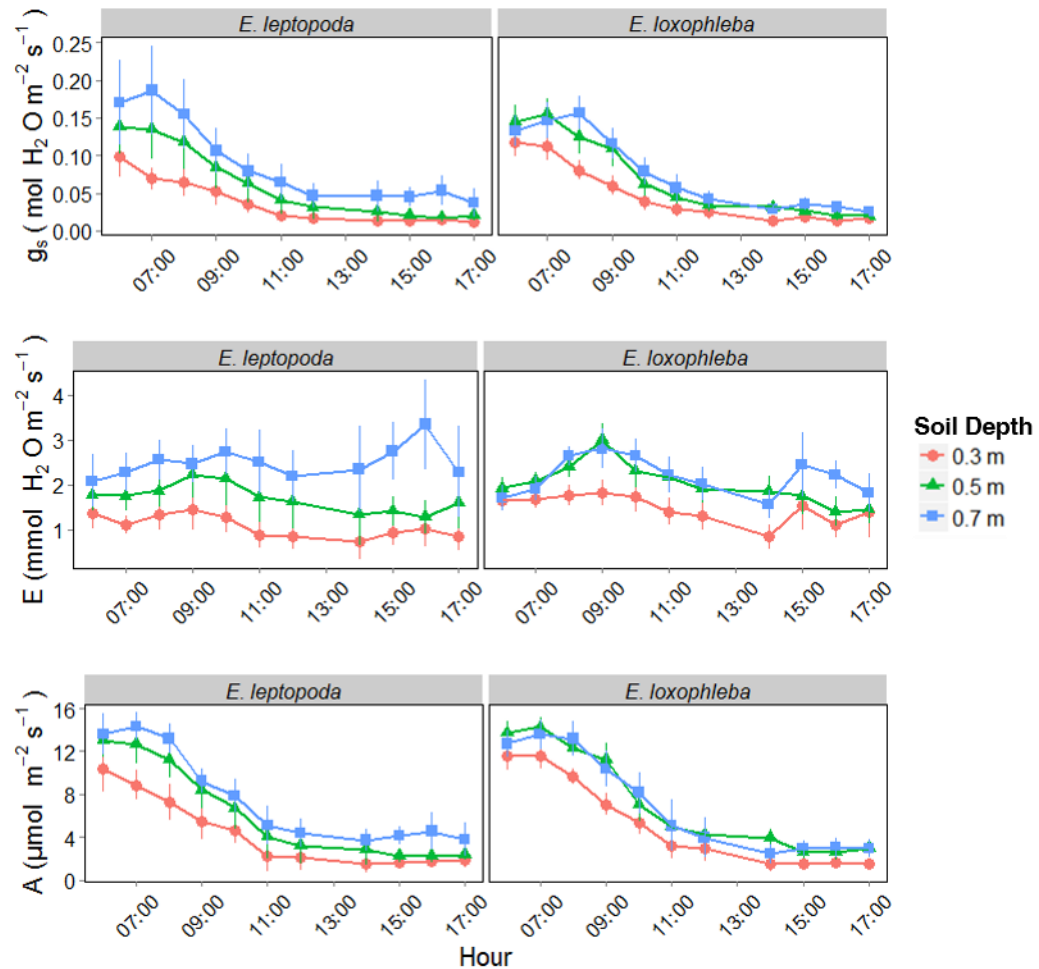


Figure 3-4 Mean hourly diurnal patterns of stomatal conductance (g_s ; \pm SE), transpiration (E ; \pm SE), and net photosynthesis (A ; \pm SE) for 2-year old *E. leptopoda* and *E. loxophleba* during the sampling period (2015-12-15 to 2015-12-17) across three soil depths (0.3 m, 0.5 m, 0.7 m).

If the reductions in A from morning to afternoon had been due to stomatal closure only, intercellular CO₂ concentrations (C_i) would be much lower in the afternoon, but this was not the case (Figure 3-5). During morning A decreased quickly without changes in C_i , suggesting that the biochemical capacity to fix CO₂ had decreased from the morning to the afternoon, likely due to excess radiation and temperature during the afternoon (Figure 3-3). However, most afternoon measurements show lower C_i (Figure 3-3) due to a slight increase in A during late afternoon (Figure 3-4 and Figure 3-5), suggesting a response to declining radiation (Figure 3-3).

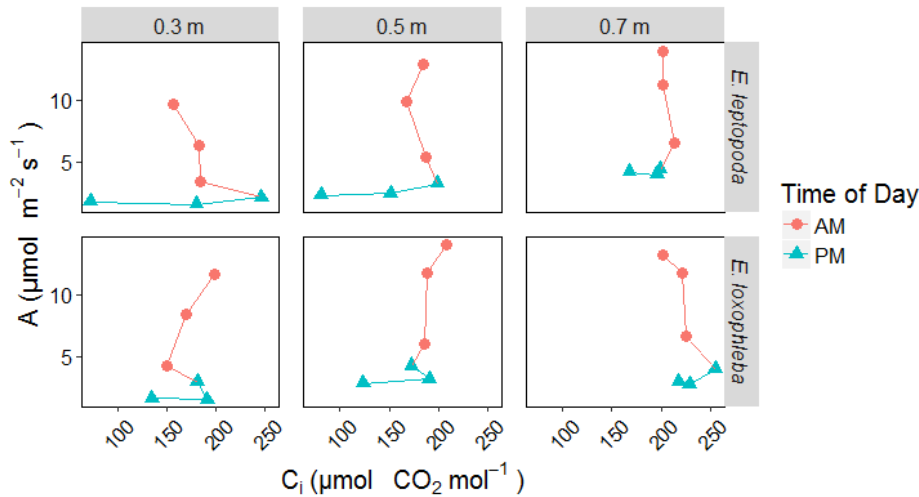


Figure 3-5 The relationship between mean intercellular CO₂ (C_i) and photosynthesis (A) during the sampling period (2015-12-15 to 2015-12-17) in the morning and afternoon averaged every 2 hours connected in time of day order across three soil depths (0.3 m, 0.5 m, 0.7 m) for 2-year old *E. leptopoda* and *E. loxophleba*.

Ψ_{PD} did not differ between soil depths (Figure 3-6). However, Ψ_{MD} were significantly ($P < 0.001$) less negative on the 0.7 m and 0.5 m soil depths compared to the 0.3 m depth (Figure 3-6 and Figure 3-7), despite having higher mean daily *E* rates (Figure 3-7). Ψ_{PD} and Ψ_{MD} did not differ significantly between species.

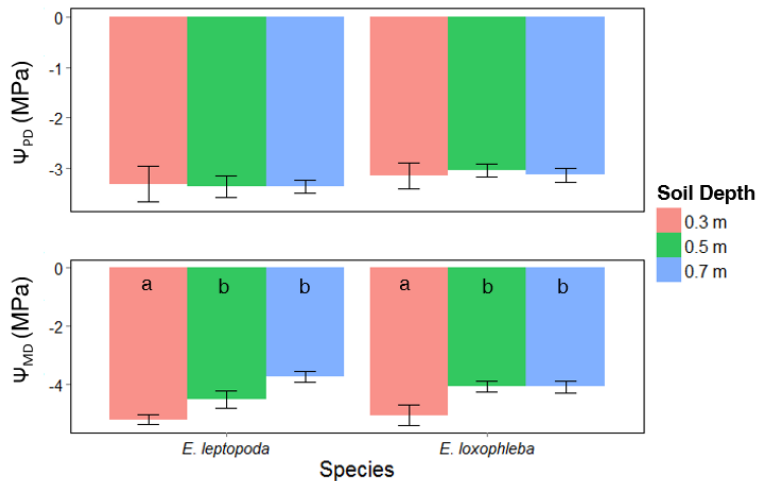


Figure 3-6 Mean daily (\pm SE) Ψ_{PD} and Ψ_{MD} for 2-year old *E. leptopoda* and *E. loxophleba* during the sampling period (2015-12-15 to 2015-12-17) across three soil depths (0.3 m, 0.5 m, 0.7m). Letters indicate significant ($P < 0.001$) differences between soil depths within species.

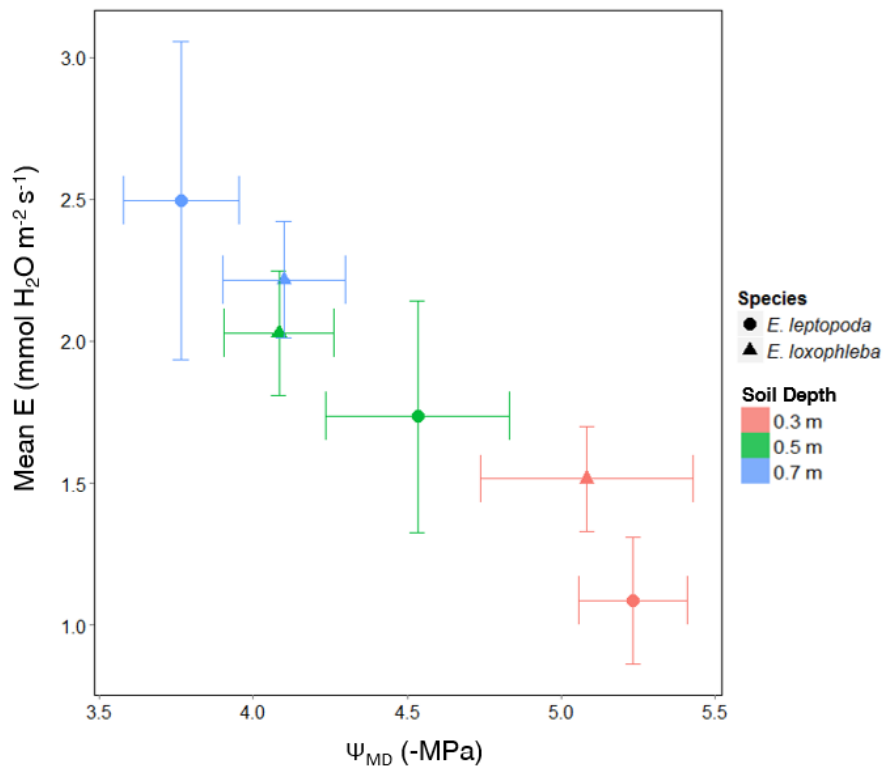


Figure 3-7 The relationship between Ψ_{MD} (\pm SE; MPa) and transpiration (\pm SE; E) during the sampling period (2015-12-15 to 2015-12-17) across three soil depths (0.3 m, 0.5 m, 0.7 m) for 2-year old *E. leptopoda* and *E. loxophleba*.

4. DISCUSSION

We examined the diurnal ecophysiology of two *Eucalyptus* species growing on soils that differed in depth only. Objectives were to (1) to assess the influence of soil depth on plant water availability in a semi-arid climate; and (2) to better understand the impact of water availability and diurnal environmental fluctuations on key physiological processes such as A and regulation of plant water status. Overall, shallower soil depths reduced E and sapling water status. Stomatal closure reduced A , but temperature and radiation were also significant co-contributors, especially on shallow soils.

As expected, total profile water was higher in the 0.7 m soil cover depth treatment, but interestingly the highest VWC was found in the 0.5 m soil depth treatment. Water distribution in soil is a function of physical processes including evaporation, infiltration, gravitational and capillarity-driven movement. There was little evidence to suggest water accumulation at the soil-waste rock interface due to lack of drainage, with sensors at a depth of 0.5 m on the

0.5 m deep cover treatment being very near field capacity. Air voids in the lower soil layers near the waste rock interface likely reduced the downward migration of water in the 0.5 m deep cover treatment compared to that in the 0.7 m deep treatment, resulting in a higher VWC in the 0.5 m soil depth (Faybishenko 1995, McLeod et al. 2015). Entrapped air can block soil pore spaces reducing their contribution to the downward movement of water flux and saturated hydraulic conductivity creating zones of “stagnant flow” (Faybishenko 1995, Ryan et al. 2000, McLeod et al. 2015). Nonetheless, total profile water remained significantly higher in the deepest soils (0.7 m > 0.5 m > 0.1 m) throughout the 1.5 month rain-free period preceding the experiment.

Experimental species *E. leptopoda* and *E. loxophleba*, typically associated with sandy and rocky soils, respectively, did not differ significantly in the measured traits or in their response to daily environmental fluctuations. It appears that the soil/rock mix of the study site was suitable for both species, and that either the progressive drought achieved in the experiment was not severe enough to cause different physiological responses in the species, or that the species are physiologically quite similar and the different habitat preferences are not associated to water relations. Saplings of both species were more stressed on shallower soils due to limited water storage and restriction of roots to shallow soil profiles, which has also been observed in other studies (e.g. Enright and Lamont 1992, Szota et al. 2007). In this experiment the percentage of roots in the top 0.2 m of soil decreased with increasing soil depth (Chapter 3). The percentage of *E. leptopoda* roots in the top 0.2 m of soil on the 0.3 m, 0.5 m, and 0.7 m soil depth treatments was 68%, 52%, and 33%, while the percentage of *E. loxophleba* roots was 82%, 55%, and 44%, respectively (Chapter 3). These observations suggest that the species preferring more rocky habitats allocated a larger percentage of its roots deeper in the soil. This behaviour has been described before in other species associated with rocky, shallow soil environments and would increase the chance of finding cracks in the underlying rock that may provide access to alternative water sources (e.g. Poot and Lambers 2008, Poot et al. 2012, Renton and Poot 2014). Plant roots on shallower soils were clearly constrained by limited soil volume decreasing their access to soil moisture, partly explaining their lower g_s , E , and Ψ_{MD} .

Soil depth had clear effects on the plants’ diurnal physiological behaviour, and these effects were the same for both species. Plants on deeper soils were less sensitive to high afternoon

VPD, temperature, and irradiance due to increased access to soil water exemplified by higher g_s , E , and Ψ_{MD} . As expected g_s declined during the day as VPD increased (Johnson and Ferrell 1983, Sharkey 1984). Overall g_s was higher on deeper soils that had greater total soil moisture, even in the comparison between 0.5 m and 0.7 deep cover treatments where the 0.5 m deep treatment had greater volume-weighted VWC. Although the g_s response to soil moisture deficit has commonly been observed (e.g. David et al. 1997, Eamus and Cole 1997, Prior et al. 1997), to our knowledge there have been no studies associating the response to soil depth under field conditions. Unlike g_s , E did not show significant diurnal reductions, however, the overall pattern of increasing E with soil depth was observed. Similar patterns have been described in natural systems, for instance, in two *Eucalyptus* species of Australian savannah woodland, when soil water availability was not limiting, O'Grady et al. (1999) found that E was higher in the dry season, as the negative effect of VPD on g_s was smaller than the positive effect of VPD on E . In our current study, where all plants were subjected to the same temperatures, irradiance and VPDs, we show experimentally that greater moisture availability due to deeper soils reduces plants' sensitivity to adverse atmospheric conditions and increases overall g_s , A , and E .

Diurnal declines in g_s also caused large reductions in A . As declines in A especially during the morning hours occurred with C_i remaining similar, non-stomatal factors must have been partly responsible (Lauer and Boyer 1992, Eamus and Cole 1997, Prior et al. 1997). Higher temperatures in the late morning and afternoon likely increased photorespiration rates relative to photosynthetic rates reducing overall photosynthetic efficiency (Lambers et al. 2008). High temperatures in combination with high light intensity may have also caused non-permanent damage to the photosynthetic apparatus (Chaves 1991), affecting the viscosity of lipids and proteins forming thylakoid membranes in PS II resulting in modification of structure and functionality with consequences to biochemistry (Lombardini 2006). Daily maximum temperatures for the study site in the 4 weeks prior to the gas exchange measurements ranged from 25 – 38 °C. Moreover, temperatures of leaves that hardly transpire are generally several degrees above ambient in these weather conditions, due to the small role of evaporative cooling. Therefore, high temperatures in the weeks prior to sampling may have caused damage to PS II for those plants on thinner covers resulting in decreased photosynthetic capacity. In addition to the effects of high temperature, tissue dehydration and the development of low leaf water potentials (Ψ_{MD} in this study) also have a direct negative

effect on the photosynthetic capacity of the mesophyll (Lawlor 2002). Plant photosynthetic capacity may have also been reduced by the direct damaging effects of high radiation loads on the photosynthetic metabolism of leaves that have been inhibited by high temperatures and water stress (Lombardini 2006). Ψ_{MD} and E (Figure 3-6 and Figure 3-7) increased with total profile water (Figure 3-1), which was a function of soil depth ($0.3 \text{ m} < 0.5 \text{ m} < 0.7 \text{ m}$), indicating that saplings on deeper soils had better water status due to the availability of water, despite having higher mean daily E rates (Figure 3-7). On shallower soils the combination of low E and Ψ_{MD} (and larger hydraulic gradient; $\Psi_{PD} - \Psi_{MD}$) suggests a lower leaf specific hydraulic conductivity, which may be due to cavitation-induced conductance loss in the plant (Sperry et al. 1993), and/or loss of soil-to-root hydraulic conductivity due to the depletion of water around roots (Lombardini 2006). Both processes would have contributed to a progressively more conservative use of water by these plants in these conditions.

5. CONCLUSION

The present study shows that the diurnal water use and photosynthesis of *E. leptopoda* and *E. loxophleba* was influenced by plant water availability as a function of soil depth, in conjunction with VPD, temperature, and irradiance. Although responses to environmental factors did not differ between species, physiological responses of the plants growing on shallow versus deeper soils were different. Overall, plants on deeper soils were less affected by high VPD, temperature, and irradiance due to increased plant available water, partly provided by unconfined roots, resulting in higher overall g_s , E , A , and Ψ_{MD} . Our results also highlight the potential negative effects of excess temperature and radiation causing repairable damage to the photochemical reaction centres and associated enzymes, in plants on shallow as well as deeper soils. As our study was done with relatively small plants that were reasonably widely spaced, it is to be expected that the level of stress imposed by shallow soils will increase as plants grow larger. With temperatures predicted to rise and rainfall predicted to decrease in the experimental area, moisture storage provided by deeper soils may help reduce plant sensitivity to the future climate. In natural (semi-)arid ecosystems, plants growing on shallow soils are likely to be most vulnerable to climate warming and drying. In newly engineered systems such as soil-vegetation covers over mine waste rock, implementation of greater soil depth, with soil textures conducive to water storage, will minimise plant sensitivity to higher temperature, VPD and irradiance.

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CHAPTER 5 GENERAL DISCUSSION

1. INTRODUCTION

The overall objective of the research presented in this thesis was to improve the quantitative understanding of how mine restoration soils and key aspects of engineered cover systems in arid climates affect plant water availability and exposure to drought stress, particularly cover thickness and its effect on moisture storage. Chapter 1 set the scene by introducing some of the environmental and public health risks associated with hazardous mine waste, highlighting the utility of engineered evapotranspiration (ET) covers for encapsulation in water limited systems, and emphasising the importance of soil-plant-atmosphere feedbacks in disturbed systems. The bulk of existing literature focuses on cover system performance from an engineering or hydrological standpoint (e.g. Nyhan et al. 1990, Benson et al. 2001, Albright et al. 2006), but vegetation affects water fluxes, and these effects vary with growth and water use patterns, so an ecohydrological perspective is important. Linking plant ecophysiology with the hydrogeology on ET covers requires empirical assessment of soil-plant-atmosphere interaction from time of establishment. Therefore this thesis aimed to link soil characteristics, depth, and water availability to plant water use strategies, physiological and morphological adaptations, and root distributions on a mining soil and ET cover in a semi-arid climate.

These objectives were addressed in the three core experimental chapters of the thesis (Chapters 2 – 4). I approached the research questions by starting with a glasshouse experiment, then scaling up to a real-world field study assessing soil-plant-atmosphere interactions over 2 years, then focusing on finer scale daily plant water use patterns during hot summer conditions. It was recognised that mining soil hydrological properties, plant physiological and morphological adaptations, ET cover thickness and construction, and their interactions in space and time all affect the ecohydrology of restored ecosystems. Accordingly, the thesis investigated physiological shoot, and morphological root and shoot traits associated with drought tolerance at the species level, and in terms of soil preference (Chapter 2); the effects of cover thickness, soil moisture, and climatic drivers on long-term trends in water use, plant water status, root distribution, and growth over two years (Chapter

3); and the influence of soil depth on plant water availability and its effect on diurnal shifts in water use between species and soil depth in response to daily fluctuations in weather (Chapter 4). The ecophysiological research presented in this thesis focused on a quantitative understanding of soil-plant-atmosphere interactions on engineered covers, adding new insights to the existing literature which has focused on cover system performance with vegetation as a contributing factor, or closing a site water balance (e.g. Breshears et al. 2005, Albright et al. 2006, Gee et al. 2006).

The purpose of this chapter is to discuss findings from experimental chapters preceding it, and highlight future research directions. The chapter first briefly restates identified knowledge gaps and research questions. Key findings of each chapter and their contributions towards solving the knowledge gaps are then highlighted, and discussed in a broader context in terms of this thesis and previous research. Finally, future research directions are discussed on the basis of findings from this work and past studies.

2. FINDINGS AND IMPLICATIONS

The glasshouse study and field experiments presented here offer important insights into the ecophysiology of mining soils and newly constructed ET covers. While findings from this research will not be representative of all mining soils and ET covers due to inherent differences in material characteristics, construction procedures, and the diversity of mining operations in semi-arid to arid regions, the principles and ideas presented here contribute to the overall knowledge base pertaining to soil-plant-atmosphere interactions on disturbed soils and ET covers.

2.1. Plant soil type preferences and drought resistant traits

One of the central themes explored throughout the thesis was the investigation of physiological and morphological traits associated with drought tolerance, and whether these differed among (semi-) arid species, and if these traits correlated with species' respective soil preferences (Chapters 2 – 4). Unsurprisingly traits associated with drought tolerance differed between species; *Acacia* species from a water-limited region differed significantly in a range of traits associated with drought tolerance (Chapter 2), and five semi-arid species from three genera differed markedly in their water use, functional traits, and water acquisition strategies

(Chapter 3). Species tended to differ in their degree of (an)isohdry, water use, biomass allocation, and traits responsible for acquisition, supply, and release of water. Unexpectedly, traits of species from these water-limited systems were mostly poorly related to their preferred soil types (Chapters 2 – 4), demonstrating a range of drought tolerance traits within and between these soil types. The poor associations may partly be explained by the inherent difficulties in classifying species into preferred soil types in (semi-) arid systems. Difficulties in classification may be due to (1) natural soils in semi-arid areas are often sand above fine-textured soil or rock (Litchfield and Mabbutt 1962), making it difficult to identify true soil preferences as these plants may display drought tolerance traits typical of fine or coarse-textured materials rather than those present at the soil surface, (2) the soils in the glasshouse (Chapter 2) and field experiments (Chapters 3 and 4) were characterised as sandy loams that have more even particle size distributions compared to most field soils, so traits conferring adaptations to more extreme soils may not have been fully expressed. It is also possible that soil texture is not a particularly good predictor of species distributions and their traits. In fact, the effect of soil texture on water availability is different in arid compared to mesic climates. Noy-Meir (1973) described the “inverse texture effect” as the effective capture of water on coarse soils in (semi-) arid systems after episodic rainfall followed by hot, dry conditions; which explains why in semi-arid regions the same species may occur at lower rainfall on coarse soils compared to higher rainfall on fine soils (Smith 1949, Noy-Meir et al. 1970). Larger pore spaces and coarse particle sizes of sandy and rocky soils result in higher saturated hydraulic conductivity and infiltration to deeper layers more quickly (Sperry and Hacke 2002). Weak capillary forces associated with coarse soils limit the upward migration of soil moisture consequently reducing evaporation (Alizai and Hulbert 1970). This is in contrast to fine textured soils where moisture is stored in shallow soil layers, and proportionally more water is lost to evaporation due to greater capillary rise (Seely and Louw 1980). The equilibrium between advantages (i.e. less evaporation) and disadvantages (more drainage) of the “inverse texture effect” occurs in an annual rainfall range of 300 – 500 mm (Noy-Meir 1973), in which this study area falls. Therefore, it is plausible that experimental species studied here may occur on both fine and coarse textured soils in the experimental area, resulting in poor association and lack of differences between identified preferred soil types in this study.

It should also be noted that the classification based on soil preference used here does not capture all traits that affect plant water availability. Therefore, in light of results presented here, I recommend further research into the understanding of water availability as a function of climate, soil properties, and the vegetation itself before more confident ecophysiological classification of species is possible. Such studies would be similar to the study presented in this thesis, but would be carried out in natural environments using the species present in their natural habitat. Once clear relationships between soils and species traits (or trait combinations) are demonstrated, species traits can be used to match the most appropriate local native flora to the geotechnical characteristics of a restoration substrate, or conversely, define the geotechnical conditions required to provide niches for this flora. Implementation of this information into a working model will help guide land restoration practitioners to the successful restoration of mined lands in (semi-) arid systems, improving the overall success of re-vegetation projects on disturbed lands.

Though it was difficult to ascribe physiological strategies and functional traits associated with drought tolerance to specific soil type preferences, certain strategies and traits conducive to survival under moisture stress were common among species. However, it was apparent that different trait combinations were capable of facilitating drought tolerance. Species tended to display a continuum from less to more anisohydric behaviour, and those with higher degrees of drought tolerance tended to be more anisohydric and/or had a combinations of morphological shoot and root traits that allowed them to utilise water efficiently and extract soil water effectively (Chapter 2 – 4). These species tended to have higher stomatal conductance (g_s) at more negative leaf and soil water potentials as evaporative demand increased, under more negative mid-day leaf water potentials (Ψ_{MD}) for a given pre-dawn leaf water potential (Ψ_{PD}), and a wider range between Ψ_{PD} and Ψ_{MD} (Chapter 2 – 4). These results are consistent with the literature which suggests that anisohydric species typically originate from drought-prone habitats (McDowell et al. 2008). In some instances this strategy was also supported by more negative osmotic potentials (*A. aneura*, *A. cowleana*, and *A. coriacea*; Chapter 2), high water use efficiency (*A. acuminata* and *M. nematophylla*; Chapter 3), and low leaf area to sapwood area (*A. acuminata*, *M. nematophylla*; Chapter 3). Root traits common to drought tolerance were high allocation to either lateral roots (*Acacia aneura*, *A. cowleana*, *A. cowleana*; Chapter 2) or to tap roots (*A. pruinocarpa* and *A. coriacea*; Chapter 2), high root length relative to leaf area (*A. acuminata*, *M. nematophylla*; Chapter 3), and

larger unconfined root systems as a result of deeper soils (Chapters 3 and 4). Not all drought tolerance traits and strategies were common among species, therefore different trait combinations were capable of supporting more anisohydric behaviour. Although the anisohydric behaviour conferred drought tolerance in this study, it is also important to note that this strategy is not immune to failure especially under a changing climate. Anisohydric plants risk hydraulic failure as they continue to transpire under low soil water potentials leading to greater risk of cavitation if drought is severe and prolonged, which may lead to increased tree mortality in response to climate change (McDowell et al. 2008). The majority of climate models agree that drought intensity, frequency, and surface temperatures will increase in Australia (Hughes 2003, IPCC 2013). Hotter air temperatures will increase atmospheric vapour pressure deficits (VPD) leading to greater demand on plant transpiration, and intensifying the effects of decreased rainfall (McDowell et al. 2008). Thus, the vulnerability of anisohydric species to hydraulic failure will increase if g_s does not decrease strongly under increasing VPD. Understanding plant physiological and morphological adaptations to drought stress and the associated soil-plant-atmosphere interactions under our current climate is vital so that we may begin to plan for our future climate where possible, such as on re-vegetated disturbed sites.

2.2. Cover depth effect on soil-plant-atmosphere interactions

The success of re-vegetation projects on disturbed lands and ET covers is largely dependent on the soil-plant-atmosphere interactions present in the system. Soil moisture, the spatial distribution of roots, atmospheric conditions, and canopy conditions all influence root water uptake, g_s , and transpiration. In (semi-) arid environments soil depth and texture reflect the most important edaphic properties affecting the water balance (Noy-Meir 1973, Pavlik 1980). Therefore, plant available water is closely related to material hydraulic properties and the spatial distribution of roots. Moreover, plant root distributions define water extraction zones, and transpiration represents a significant pathway to water loss in these systems. In addition to the complex effect of soil texture and moisture, transpiration is a function of g_s and environmental conditions; and area based net photosynthesis (A) is determined by g_s , environmental conditions, and the biochemical status of the photosynthetic apparatus, which varies with antecedent condition. g_s , however, varies diurnally in response to declines in plant water status from pre-dawn maximums to diurnal minimums, and due to fluctuations in

atmospheric evaporative conditions. As A and transpiration are both influenced by g_s , leaves are continuously finding the balance between minimising transpiration water loss, while maximising A (Lombardini 2006). Although mid-day depressions and early morning peaks in g_s , A , and transpiration have been described in numerous studies (e.g. Eamus and Cole 1997, Prior et al. 1997, O'Grady et al. 1999), few if any have done so on an engineered cover in response to plant available water as a function of soil depth. Due to the numerous interactions among material properties, plant water relations, transpiration, and atmospheric conditions it was of interest to investigate the influence of soil depth on plant water availability and its effect on diurnal shifts in g_s , transpiration, A , intercellular CO_2 concentrations (C_i), pre-dawn (Ψ_{PD}), and mid-day (Ψ_{MD}) leaf water potentials; and how this responded to diurnal fluctuations in vapour pressure deficit (VPD), temperature and irradiance (Chapter 4). Similar objectives were also investigated for long-term seasonal trends where the effects of cover thickness, soil moisture, and climatic drivers on g_s , transpiration, A , Carbon-13 fractionation ($\delta^{13}\text{C}$), C_i , Ψ_{PD} , and Ψ_{MD} were investigated over 2 years (Chapter 3).

Results from the long-term and diurnal assessments revealed that physiological responses were different for plants growing in shallow versus deep soil irrespective of species. Water use and A were mainly influenced by plant water availability as a function of soil depth and root system size, in conjunction with VPD, temperature, and irradiance (Chapters 3 and 4). Plants on deeper soils had higher overall g_s , transpiration, A , and Ψ_{MD} and were less affected by high VPD, temperature, and irradiance due to increased plant-available water that could be accessed by larger unconfined root systems (Chapters 3 and 4). Results also highlight the negative effects of excess temperature and radiation that caused repairable damage to the photochemical reaction centres and associated enzymes in plants limiting afternoon A in summer for plants on both shallow and deeper soils (Chapter 4). However, it is important to recognise that this work was only conducted over 2 years and that inter-annual climate variability may alter trends observed here. Semi-arid to arid regions are occasionally subjected to large infrequent rainfall events capable of recharging soil moisture, or alternatively high temperatures with the potential to induce severe drying. Therefore, longer ecophysiological time series data spanning several years would be beneficial to capture irregular events (Breshears et al. 2009). Nonetheless, the time series presented here is a good

initial step toward a better understanding of ecophysiology and the effect of soil depth on ET covers.

A major find in this thesis was the effect of cover thickness on root system depth and size. Roots decreased with soil depth on the thickest covers (0.5 m and 0.7 m), however, this pattern was less evident on the thinnest cover (0.3 m) indicating that roots were confined (Chapter 3). As expected, the thicker cover allowed root exploration of a much larger soil volume, and as a consequence allocated proportionally less roots to the top 0.2 m of the soil: the 0.3 m thick cover had 67 – 82 % of all species roots in the top 0.2 m of soil, and the 0.5 m and 0.7 m thick covers only had 40 – 59 % and 33 – 52 % of their roots in this top layer, respectively (Chapter 3). When compared to a global model of cumulative root proportions with depth (Jackson et al. 1996), root depth distributions on the thickest cover were most similar to that of a sclerophyllous shrubland, expected for this region, while the thinner covers did not (Chapter 3). The distribution of plant roots on the thickest cover may be an indication of positive plant health and successful restoration for this area. Additionally, the size of root systems increased with cover depth, where root lengths (RL) in the 0.7 m cover were 39% and 70% higher than in the 0.5 m and 0.3 m covers, respectively (Chapter 3). This is clear evidence that thicker covers promote deeper and larger root systems. On engineered cover in water limited systems where surface evaporation is high and soil hydraulic properties have been altered, shallow roots are not conducive to survival, especially during an extreme drought year or under a drying climate. Therefore, implementation of thicker soil covers of appropriate texture to store water will promote the growth of deeper and larger root systems from hostile surface layers to deeper layers where larger soil volumes can be explored, improving the re-vegetation success of engineered covers. Interestingly, aboveground growth was hardly affected by soil depth over the study period, resulting in lower relative investment in transpiring leaf area compared to roots, and suggesting that the risk of excessive growth causing potential vegetation collapse may not be imminent. It will be of great interest, however, to establish longer-term trials to document the evolution of restored plant communities, and to attempt to predict which species are able to persist, due to which traits.

2.3. Mine site restoration implications

Daily and long-term water use patterns as influenced by plant available water, soil depth, atmospheric conditions, and root distributions are key parameters in numerical modelling. Plant ecophysiological and water balance models are used by engineers and mine planners to guide cover system construction. However, due to lack of empirical work and *in situ* measurements, studies have questioned the extent to which models accurately predict plant transpiration and the opportunistic nature of plants (e.g. Scanlon et al. 2005, Gwenzi 2011), often making unrealistic assumptions regarding root distributions for water capture (Gwenzi 2011). Therefore, the diurnal and seasonal data gained in this research may be used to validate models using field measured data, and improve/develop vegetation modules through the implementation of ecophysiological relationships described here. Information gained pertaining to increased moisture storage and decreased plant sensitivity to increasing temperature, VPD and irradiance may also be used to design more robust ET covers for a changing climate. With temperatures predicted to rise and rainfall predicted to decrease in many (semi-) arid systems, moisture storage provided by deeper soils may help reduce plant sensitivity to the future climate. In natural (semi-) arid ecosystems, plants growing on shallow soils are likely to be most vulnerable to climate warming and drying (Padilla and Pugnaire 2007). The use of greater soil depth, with soil textures conducive to water storage, on ET covers will minimise plant sensitivity to higher temperature, VPD and irradiance, and improve plant survival and overall ET ecosystem function.

In (semi-) arid climates water availability is a major factor controlling seedling emergence and survival (Chapters 3 and 4). In this experiment seedlings were planted directly, and drip irrigated for the first year to subsidise rainfall by inputting 7.2 mm of water into the system every week, this clearly aided early establishment leading to seedling survival of 90 %. The benefits of drip irrigation systems in water limited systems is corroborated by earlier studies which found that this was an effective technique for re-vegetation of slopes due to leaching of harmful salts and phytotoxins from the root zone; conservative water use; adaptability to remote areas; promotion of deep root growth leading to better plant development; and decreased potential of runoff and erosion compared to other irrigation techniques (Bengson 1977, Tordoff et al. 2000). Drip irrigation is therefore a viable option for successful establishment of vegetation on slopes in water limited systems; however, long-term

maintenance of drip irrigation systems is neither feasible nor desirable, as engineered cover systems are designed to last in perpetuity without human intervention. Therefore, it is recommended that vegetation be closely monitored during an initial period of 0.5 – 2 years after planting or seeding depending on the weather and soil substrate, to determine if an appropriate amount of root proliferation and establishment has occurred, at which point irrigation can be reduced and eventually ceased. The re-vegetated system can then be left to undergo undisturbed successional processes, or alternatively; depending on the desired restoration goals, an adaptive management approach can be taken where human intervention can occur and irrigation can resume if required. While irrigation in the initial stages of restoration may seem ‘unnatural’ for arid ecosystems, rainfall in arid regions is usually highly variable, and large recruitment events are likely dependent on exceptional high-rainfall years (Holmgren et al. 2006). The goal of irrigation would be to mimic such rare favourable conditions.

This study also provides further evidence for the integration of non-acid forming or non-toxic mine waste (banded iron formation in this experiment) into cover material mixtures (Johnson et al. 1977, Bradshaw and Johnson 1992). In Australian (semi-) arid mining operations where top soil for spreading atop mine waste is often scarce, the use of mine waste in a cover material will support the efficient use of top soil stockpiles. Using waste as cover material alone is often not appropriate due to its very coarse (20 – 200 mm) or very fine (< 2 mm) size, low organic matter, and lack of soil microflora making plant establishment extremely difficult due to restrictive physical and biological characteristics (Bradshaw and Chadwick 1980, Tordoff et al. 2000). Coarse waste has low water holding capacity, leading to water deficit in surface layers, while fine waste (and tailings) is prone to greater erosion and surface compaction; characteristics that limit germination, establishment, and root penetration (Bradshaw and Chadwick 1980, Tordoff et al. 2000). However, waste rock in combination with a material that has better water holding capacity, is less erosive, and less susceptible to compressive forces may improve vegetation establishment on degraded sites that do not have enough high quality material alone. This may also limit the need for “borrow materials” during engineered cover construction, i.e. the harvesting of topsoil and other favourable substrates from non-mined locations, thus avoiding the additional environmental impact and associated permit procedures, and reducing the cost of rehabilitation by limiting trucking costs from borrow material sites to waste rock dumps. As highlighted by Arnold et al. (2013),

understanding a region's hydrogeology and ecohydrology is essential to guiding the reconstruction of landscapes and ecosystems. Therefore a quantitative understanding of cover material physical and hydraulic properties is essentially for vegetation establishment on engineered covers.

Although much of the work in this thesis centres on improving vegetation establishment, clear restoration outcomes must be established prior to re-vegetation. Water limited systems are hostile environments which cause inherent stress and plant mortality in natural systems, therefore, is maximal growth always the best restoration outcome? For instance, post-fire recruitment of *Eucalyptus incrassata* in semi-arid SE Australia caused stem densities of 7000 ha⁻¹ compared to 100 ha⁻¹ in an unburnt stand; however, natural mortality in the burnt stand was 75 % in 2 years, density dependent, and highest on upper dune positions presumably where soil moisture was lacking (Wellington and Noble 1985). Like in natural systems, poor and high quality sites exist in post-mine landscapes, which have a direct impact on survival and growth as observed by Szota et al. (2007) where stem density was 59 % higher on high quality sites. Similarly, a certain level of mortality is expected in recently restored systems, but if this mortality leads to a relatively sparse but species-rich plant community, as in the surrounding natural systems (Lambers et al. 2014), this should be considered as a positive restoration outcome. In (semi-) arid sites requiring re-vegetation, less may be more, considering that high densities may incite mortality. Prior to seeding or planting a restored site, natural analogue sites could be surveyed to better understand what tree densities can be supported by systems with different capacities to capture and store water.

3. IMPLICATIONS FOR FUTURE RESEARCH

This research provides valuable information pertaining to the ecophysiology of plants on mine site restoration soils and engineered cover systems, quantifying interactions within the soil-plant-atmosphere continuum. Considering that ecophysiological studies on engineered systems are limited, many research opportunities on the subject still exist. Results and ideas explored in this thesis may be expanded into future research, likely involving further field investigation and improvement to current ecohydrological and engineered cover system models.

Results presented in this thesis were based on two mine site restoration soils with similar textural characteristics (sandy loam and coarse sandy loam). Furthermore, the field study was based on one study site. Therefore, research should continue on soils with different characteristics and on field sites with contrasting climatic patterns over longer periods with the intention of capturing the effects of material and climatic variability on plant water use patterns. A synthesis of generalised conclusions using all available field data and material characteristics could then be performed to help guide the design and ecohydrological modelling of engineered covers using the most representative data.

This work focused on the processes governing the ecophysiology of a newly constructed and re-vegetated engineered cover. Albeit a good starting point, physiological data presented here was conducted on relatively young plants (approximately 0.5 – 2.5 years old), therefore, it would be beneficial to conduct a similar study involving mature trees and shrubs on an engineered cover. Larger stem diameters would allow for easier use of continuous transpiration measurements using sap flow sensors and provide an indication of plant physiological responses to soil depth, plant available water, and atmospheric drivers at a mature stage. The use of continuous transpiration measurements, in combination with portable chambers for quantification of bare soil evaporation, or the installation of an eddy covariance system to determine site evapotranspiration, may allow researchers to close water balance fluxes at various timescales. These techniques will also allow evapotranspiration to be partitioned into its respective components (i.e. transpiration and evaporation) and be extremely informative for cover system modellers.

As (semi-) arid ecosystems are prone to large episodic rainfall events, *in situ* measurements of surface run-off and erosion on vegetated engineered covers should also be a priority. The net effect of vegetation on reducing run-off and erosion, and increasing slope stability is typically positive. Studies have demonstrated that vegetation is capable of trapping and storing significant amounts of run-off water as run-on (e.g. Bergkamp 1998, Reid et al. 1999, Wilcox et al. 2003). Quantifying the amount of water being stored as run-on will help close the system water balance as it represents an important hydraulic input into the system, especially during heavy rainfall. The contributions of vegetation to slope stability and soil erosion as vegetation develops over time are also important factors when modelling the effect of vegetation on slopes. Low, dense canopies and their litter are effective at reducing raindrop

impact on the soil surface, and keep top soils moist aiding infiltration (Quinton et al. 1997). The presence of roots generally increases soil shear strength by binding soil particles reducing the movement of fine particles from upper to lower of slope positions (Tobias and Rickson 1994). Although studies have investigated the effect of vegetation cover on erosion (e.g. Quinton et al. 1997, Loch 2000), data is only available for a few community types and sites. Benefits derived from vegetation are site and species specific, therefore, further investigations across sites and species are required so that a synthesis of information is available to inform re-vegetation on disturbed slopes, and cover system models.

Comments have been made regarding the ability of current water balance and cover system models to predict the effects of dynamic plant water use and phenology on actual cover hydrology (Gee et al. 2006, Gwenzi 2011). Validation of models that incorporate plant water use and root distributions is uncommon due to the lack of *in situ* empirical data. The dataset presented here, and in similar studies, present a good opportunity to evaluate existing model codes, and the potential to develop and integrate new vegetation modules which comprise the ecophysiological relationships described in this thesis. Results in this study offer information for woody vegetation only, therefore, further field and associated modelling studies should explore the functional diversity of vegetation on ET covers. Water limited systems are comprised of species representing many functional types (i.e. grasses, annual herbs, shrubs, trees) with varying root distributions (e.g. shallow and deep rooted) and water use strategies. Accounting for plant functional diversity will allow for more accurate assessment of ecohydrology on ET covers, and may produce important information for engineered cover design.

4. CONCLUSION

This thesis has provided some valuable insights into the ecophysiology of plants growing on mine site restoration soils and ET covers in water limited systems. Re-vegetation efforts on disturbed soil in (semi-) arid climates will undoubtedly improve if researchers continue working towards a detailed understanding of drought tolerance traits and plant water use strategies. This research resulted in modest advancements in the understanding of interactions between plant available water, soil depth, plant physiology, and atmospheric drivers on daily and seasonal timescales. Findings suggest that when soils are conducive to

moisture storage, the larger amount of plant-available water stored in deeper soils decreases plant sensitivity when evaporative demands are high (e.g. midday and summer). This relationship was supported by unconfined root distributions capable of exploiting larger soil volumes. The thickness of cover soils is a key design feature for the restoration of disturbed sites in current and future climates, which will have a significant impact on the structure and composition of the developing vegetation, through interactive effects with soil type and climate. The abundance of disturbed mine lands and scarcity of natural soils reinforces the importance of future research into plant-soil interactions and atmospheric drivers on ET covers that utilise common mine waste. Potential for re-vegetation on such substrates exists and could be essential to restoring sustainable ecosystems in mine affected areas.

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