Trees and groundwater on the water-limited Eyre Peninsula: an ecohydrological perspective

submitted by

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Declaration of originality

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any other university; and that to the best of my knowledge and belief does not contain any material previously published or written by another person, except where due reference is made in the text.

The contribution for the authorship of the three published journal papers which are presented in this thesis as Chapters 2, 3 and 4 are declared to be as follows:

- **Chapter 2**: Water use strategies of two co-occurring tree species in a semi-arid karst environment (Swaffer 70 %, Holland 5 %, Doody 5 %, Li 15 %, Hutson 5 %).
- **Chapter 3**: Rainfall partitioning across two co-occurring, morphologically distinct tree species in a semi-arid environment (Swaffer 85 %, Holland 5 %, Doody 5 %, Hutson 5 %).
- **Chapter 4**: Comparing ecophysiological traits and evapotranspiration of an invasive exotic, *Pinus halepensis* in native woodland over a karst aquifer (Swaffer 90 %, Holland 10 %).

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Brooke Aily Swaffer

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Abstract

This thesis investigated the ecohydrological linkages between native and exotic tree species and karstic groundwater systems on Eyre Peninsula, South Australia. It focuses on two issues of global importance: the declining status of fresh water resources in water-limited environments and the rate of evapotranspiration from native and non-native trees encroaching across groundwater recharge areas. The work focussed on karstic groundwater systems, a geological setting where the water flux dynamics from the surface through to groundwater is a complex process involving storage in the unsaturated zone and diffuse and preferential recharge pathways. This geological complexity and the processes associated with this behaviour are not well understood, despite karst aquifers often being the source of drinking water supplies. As a result, uncertainty regarding the ecohydrological processes in this environmental setting remains large.

In addition to the complex nature of the karst substrate, the presence of vegetation growing above the groundwater lens will further influence recharge and discharge dynamics. Water use by trees, as well as the partitioning of precipitation into interception, stemflow and throughfall, was considered to be an important ecohydrological process affecting this groundwater system.

This study examined whether groundwater level decline could be attributed to changes in land cover, reflecting differences in evapotranspiration rates and pathways of water flux across morphologically distinct, locally common tree species. Pre-European settlement the region was characterised by a grassy woodland dominated by Allocasuarina *verticillata* (Lam.) L. Johnson (drooping sheoak). Since the establishment and subsequent cessation of grazing across

groundwater recharge areas, *Eucalyptus diversifolia* ssp. *diversifolia* Bonpl. (coastal white mallee) has expanded in range. Furthermore, the non-native *Pinus halepensis* Mill. (Aleppo pine), originally planted as a wind-break, has since naturalised and invaded significant areas of the rocky, shallow, calcrete soils often characteristic of karstic systems. Invasion by an aggressive exotic plant species into this water-limited environments was commonly believed to further exacerbate water scarcity issues.

Fundamentally, this thesis seeks to address concerns regarding the effect native trees, or encroachment by exotic trees, exerts over groundwater flux in a water-limited environment. The belief that the vegetation will detrimentally affect the groundwater charge rates inevitably attracts debate regarding the ability of active management of vegetation to improve water yield.

I applied both plot-scale and remotely-sensed methodologies to examine total evapotranspiration (*ET*) flux, and used these to construct a water balance for the three tree species in question, as well as for a grassland site. Water use strategies and ecophysiological characteristics were examined using leaf water potential and soil matric potential, and twig water sources were traced using the stable isotopes of water. The funnelling of water from canopies via tree surfaces to enhance infiltrate around the base of tree boles was explored as a mechanism though which soil water content could sustain transpiration during dry periods. Global literature suggested that variability in methodological approach significantly affected the reported results, which I demonstrated using two years of rainfall partitioning data.

The results of this study indicated that *ET* losses from native vegetation associations were equivalent to long term precipitation. Despite a shallow groundwater depth of < 5 m, use of groundwater to sustain transpiration requirements was not clearly demonstrated by the studied tree species, suggesting that while recharge will be reduced by the presence of these trees, *ET* was most likely supported by soil water stores rather than groundwater extraction. The reliance on soil moisture, rather than groundwater, was further supported by actual *ET* remaining well below (one third of) potential *ET*, therefore a significant constraint was evident on the system. However, the encroachment of the invasive *Pinus halepensis* was considered likely to have contributed to declining groundwater levels, based on higher rates of sap flux per unit sapwood area compared to the native species. Comparison of *ET* rates before and after removal of *P. halepensis* suggested an annual water saving of ~ 50 mm; however it was recommended more than two years of post-removal data be used to assess the likelihood of realising long term water savings.

Irrespective of stand and morphological differences, the water use characteristics of the native *E. diversifolia* and *A. verticillata* were remarkably similar , demonstrating the evolutionary capacity of these species to maximise the use of the available precipitation. Furthermore, rainfall channelled as stemflow is believed to play an important ecohydrological role in this environment. Infiltration directly adjacent tree root systems provides a water store which can be used during times of precipitation deficit. In addition, I suggest that the water holding capacity of porous geological substrate has played an important role in buffering inter- and intra-annual rainfall variability and needs to be considered when characterising karstic groundwater systems.

The findings described in this thesis add to our knowledge of evapotranspiration rates of vegetation in semi-arid systems. I have demonstrated the value of using both plot scale field investigation and remotely sensed data to address important knowledge gaps and improve the management of regionally significant groundwater supplies. The results of this research are expected to inform water resource policy as competition for fresh water increases, expected to intensify following predicted climate change scenarios.

1. Introduction

1.1. General introduction

This research aims to improve our understanding of the behaviour of, and limitations on, water use by native and non-native trees in the water-limited Eyre Peninsula region of southern Australia. Most notably, I sought to characterise the hydrological characteristics of dominant tree species to assess their potential impact on the water balance of a regionally significant groundwater resource.

This chapter introduces the issues of importance relevant to this thesis; water scarcity, global groundwater decline, the ecohydrological links to native and exotic tree species, and the detrimental impact of woody plant invasions. I provide some contextual background regarding the history of water resources on Eyre Peninsula, highlighting the critical role of these groundwater systems in supporting the social and economic requirements of the region. Finally, the drivers for the selection of each tree species included in the research are discussed.

1.1.1. Global freshwater scarcity

Fresh water resources are essential to sustain societal, economic and environmental requirements. However, pressure on these resources continues to increase owing to population growth and economic expansion, and is exacerbated by hydrological changes induced from climate change (Hanasaki *et al*., 2013). Groundwater – earth's most plentiful freshwater resource (Aeschbach-Hertig and Gleeson, 2012) – plays a critical role in meeting global freshwaterdemands, yet its depletion is widespread across both local and global scales (Gleeson *et al*., 2012).

Groundwater stress, defined as a level of consumption likely to affect the long term availability of groundwater, the extent of groundwater-dependent surface water and/or the viability of groundwater-dependent ecosystems has been estimated to affect 1.7 billion people globally (Gleeson *et al.*, 2012). Consequently, current strategies for managing groundwater resources could be argued to have had limited success. This is due at least in part to the concealed nature of the resource, which rendersdirect observations difficult, and the typical time lags between resource development and an observed response (e.g. lowering of groundwater levels) from over-exploitation. Thus, there is an urgent need for future management strategies to pursue alternative science-based methods for societies to value, manage and characterise groundwater systems in a sustainable manner.

Australia is well positioned to lead global research into water security issues. Long referred to as the driest inhabited continent (Davidson, 1969), recent severe drought (Van Dijk *et al.*, 2013), reliance on irrigated agriculture and climate change predictions led to the Australian Government initiating widespread water reform – the National Water Initiative. Through these reforms, Australian governments agreed on actions to deliver a cohesive national approach to the manner in which the country manages, measures, plans, prices and trades water. Solutions to such issues have necessitated innovative approaches and collaboration of scientists and policy makers alike to implement sound science-based natural resource management policies.

The work presented in this thesis is directly linked to the National Water Initiative through funding awarded to address key knowledge gaps in order to develop and improve hydrological models used to manage Eyre Peninsula groundwater resources. This thesis is coupled, specifically, to Theme 1: Evapotranspiration (*ET*) and Recharge – determining *ET*, interception and their effect on rainfall-recharge processes under vegetation.

1.1.2. Ecohydrology – linking vegetation and water

Attempting to address the global competition for fresh water has led to the traditionally separate disciplines of climate, soil, vegetation and water being united – commonly referred to as ecohydrology (*sensu* Rodriguez–Iturbe, 2000) which now occupies a central niche within the field of hydrological research. The recognition that current and future environmental problems are so complex that interdisciplinary collaboration is required to address them (Wilcox and Newman, 2005) has significantly advanced the recognition and appreciation of the intrinsically related components of natural systems (Wagener *et al.*, 2010).

Ecohydrology refers to the understanding of the hydrological mechanisms that underlie the distribution, structure and functioning of ecosystems, including the effects of biotic processes, such as tree water use, on the water cycle (Nuttle, 2002). This thesis has adopted the definition of ecohydrology provided by Eamus *et al.*, (2006):

"the study of how the movement and storage of water in the environment and the structure and function of vegetation are linked in a reciprocal exchange".

The availability of water to plant roots, from both soil moisture and accessible groundwater, is one of the key links between climate, hydrology and vegetation. Water availability is a major determinant of terrestrial vegetation structure and productivity on a global scale (Nemani *et al*., 2003), but the coupling between water and vegetation is particularly close in dryland environments (Jackson *et al*., 2009). Drylands are drought prone regions, defined by an annual potential evaporation rate that exceeds annual precipitation (Newman *et al*., 2006). Warm dryland regions, including those of the Eyre Peninsula in Australia, are often subject to high variability in rainfall patterns both within and among years (Schwinning and Sala, 2004); consequently, characterisation of dryland environments continues to challenge and stimulate scientists at a global scale.

Vegetation

Trees will influence the hydrology of their immediate environment in a number of ways, including; 1) interception and evaporation from plant surfaces during and after rainfall events (Crockford and Richardson, 1990c); 2) uneven soil moisture, due to both the spatial variability in which rainfall reaches ground surfaces (Llorens and Domingo, 2007) and canopy shading decreasing soil evaporation (Swaffer *et al*., 2014); 3) roots removing water from the subsurface profile for transpiration (Holland *et al*., 2006); and 4) improving water infiltration (Allison and Hughes, 1983) and redistributing water to different soil layers (Burgess *et al*., 1998).

With reference to the hydrology of Eyre Peninsula, and the focus of this thesis on trees and shallow groundwater systems, several of these processes will be examined through measurement of evapotranspiration (*ET*). Evapotranspiration refers to the aggregate of all forms of evaporation from wet surfaces, such as soils, wet leaf and bark surfaces, plus transpiration through plants (Eamus *et al*., 2006). The quantification of *ET* has received much attention in the literature for two reasons; 1) it is the major hydrological pathway through which water is returned to the atmosphere, and 2) it is the primary factor limiting the volume of water draining past the root zone and becoming available as recharge to water resources. Therefore, detailed estimates of evapotranspiration are essential for sound water resource management (Moore and Heilman 2011), particularly in water-limited areas where water is considered to be the key driver of ecosystem processes (i.e. vegetation productivity) in dryland environments (Schwinning *et al*., 2011).

Vegetation in dryland environments is typically comprised of ephemerals (which emerge in favourable conditions, namely after rainfall), succulents (which accumulate and store water) and non-succulent perennials (hardy grasses, shrubs and trees which can tolerate the harsh conditions). The ability of vegetation to withstand the water deficits characteristic of arid and semi-arid zones is due to a range of adaptive characteristics such as deep root systems, reduction in transpiring surfaces, tight stomatal control, and hardy, waxy tissues (sclerophyllous) which minimise water loss. Tree water use will increase along with the climatic drivers of *ET* in times of plentiful water, that is, actual *ET* will mirror potential *ET* if soil moisture conditions permit. However, when trees are under water stress, adaptations to prevent water loss are deployed to avoid loss of turgor and xylem cavitation.

Water

The ability of changes to woody plant cover to affect the ecosystem water budget has been explored in catchments (see reviews by Bosch and Hewlett 1982, Zhang *et al*., 2001, Brown *et al*., 2005), along river systems (Huxman *et al*., 2005, Nagler *et al*., 2008, Doody and Benyon 2010, Doody *et al*., 2011), and for groundwater resources (Holland and Benyon 2010, Moore *et al*., 2012).

A recent review of groundwater discharge and terrestrial systems within Australia by O’Grady *et al*., (2010) included studies of the Gnangara Mound in Western Australia (Groom *et al*., 2000, Zencich *et al*., 2002), shallow groundwater along the River Murray (SA) (Holland *et al*., 2006, Doody *et al*., 2009) and the Daly River (NT) (O’Grady *et al*., 2002), as well as an assessment of saline affected agricultural areas (White *et al*., 2002, Wildy *et al*., 2004a). Despite this broad scope, examination of terrestrial links to karstic groundwater systems has been limited to the forestry region of south-eastern Australia (Benyon and Doody, 2004, Benyon *et al*., 2006), and has ignored a number of other karst systems that are critical to the economic and social success of local communities. This thesis seeks to address the large knowledge gaps regarding the influence of native and exotic vegetation on groundwater recharge and discharge processes of the karstic groundwater systems on Eyre Peninsula, South Australia.

The term karst refers to a geological formation where soluble rock results in the presence of dissolution features (Schwinning 2008); these open conduits provide low resistance pathways for rainfall, soil moisture and groundwater, which often circumvent the granular or matrix component of the unsaturated zone (White 2002). The heterogeneous nature of karst systems means that water flow will occur not only through conduits, but also fractures, fissures, the soil matrix, and the connections between them (White 2002).

The karstic aquifers of Eyre Peninsula are characterised by surface soil properties with limited soil water holding capacities (Harrington *et al*., 2006). Such characteristics are known to result in high rates of infiltration and rapid transfer of water to aquifers via dissolution features (Huxman *et al*., 2005). Where vegetation cover alters these drainage patterns, the volume of groundwater recharge may also be adversely affected. These knowledge gaps for Eyre Peninsula groundwater resources, and the aquifer's role in sustaining the social, economic and environmental requirements of the region, motivated the examination of vegetation and groundwater ecohydrology addressed in this thesis.

1.1.3. Eyre Peninsula water supplies: a historical perspective

The quest to secure a reliable water supply has played a dominant role throughout the history of Eyre Peninsula that continues until today. Viewed by early surveyors as barren, stony and waterless, doubts about the region's settlement potential were demonstrated by the rejection of Port Lincoln as the state capital in 1836. Indeed, historian Sumerling (1987) writes:

"the theme of water ….. is intricately woven into every period and theme of the Eyre Peninsula so that its heritage has become what it is because of the lack of water".

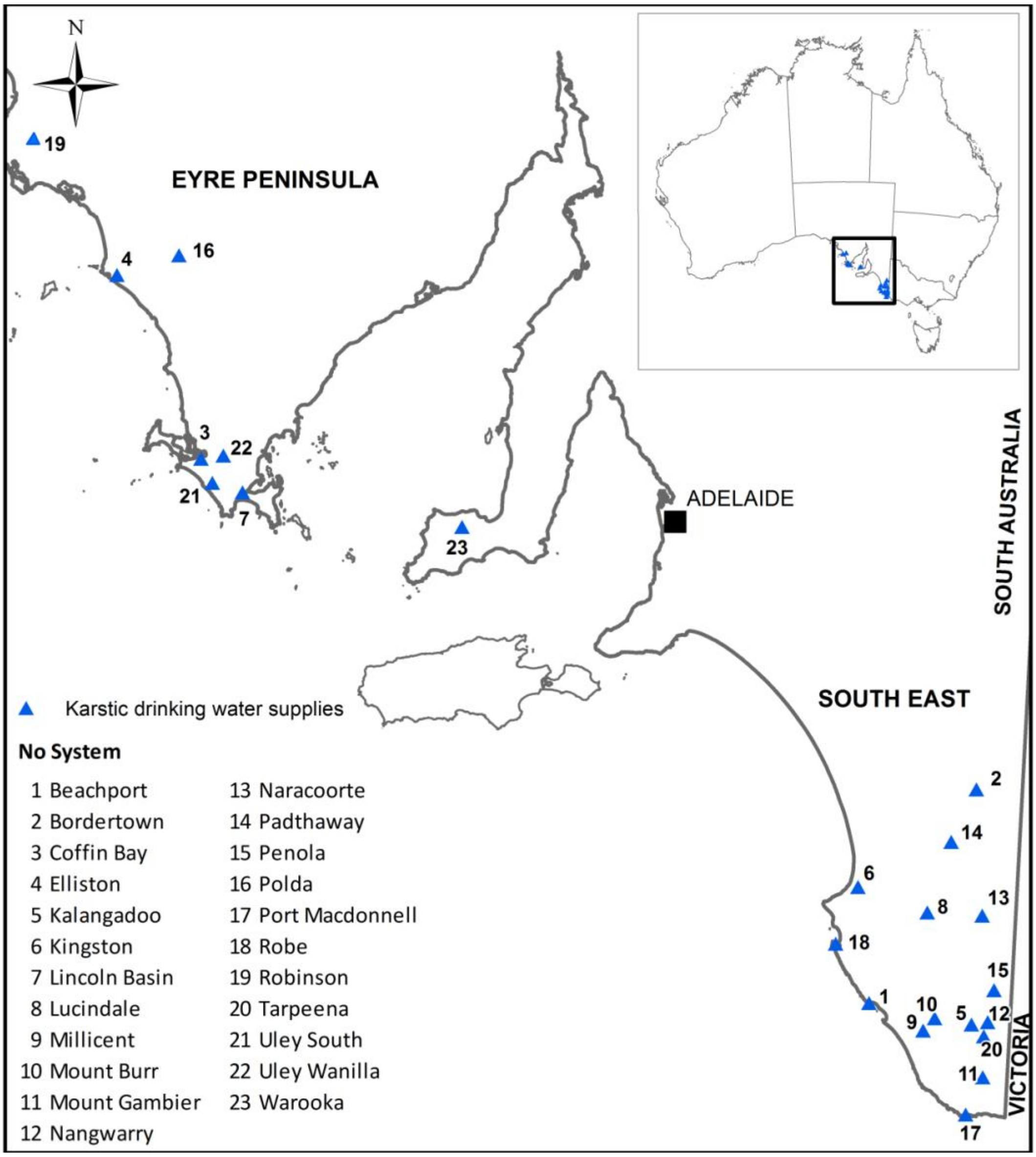

Figure 1.1.3.1 Map showing the extent of regional drinking water supplies supported by karst aquifers on a) the Eyre Peninsula and b) the South-East of South Australia. Groundwater supplies, while only supplying around 6 % of the state's water needs, represents an important water source for the regional communities of South Australia (SA Water, 2014).

Prior to the 1880's, European settlers on Eyre Peninsula were responsible for ensuring their own water supplies. Between 1880 and 1922 the Government established over 500 tanks, wells and small reservoirs along important stock routes to assist the fledgling population meet their freshwater requirements. A Royal Commission (1916) was appointed to investigate water supply options for Eyre Peninsula resulting, principally, in the recommendation to build the Tod River Reservoir. The reservoir remained the primary source of water for the region for decades, despite the presence of fresh groundwater supplies being known since 1802. Historic accounts report a lack of enthusiasm for using groundwater resources for public water supplies because of skepticism regarding their water yield and sustainability (Sumerling, 1987).

Eventually, the expansion of the region necessitated supplementary water supplies, and the first of the underground water resources was developed in 1950 − Uley Wanilla (EWS 1991). When water from the aquifer proved plentiful, local communities preferred Uley Wanilla water over the reservoir. Consequently, Lincoln Basin groundwater supplies were developed in 1960, followed by Uley South, which commenced extraction in 1974 and remains the most heavily relied upon resource; today Uley South provides approximately 70 % of the region’s water needs.

Unfortunately, these groundwater systems have seen substantial decline in water levels over recent decades (Figure 1.1.4.1). The remote nature of the region also limits alternative supply options should the groundwater resources fail. Thus, there is concern that current management strategies may inadvertently result in the resources failing, and drives the need to address long standing knowledge gaps for the system. Resource managers recognise that the interactions between vegetation and the karst aquifers of Eyre Peninsula are poorly understood, possibly leading to an inaccurate conceptual understanding. For example, trees have been reported to rely on the water held in the upper limestone matrix above a karst aquifer on the Yucatan Peninsula and despite the prolonged dry season, did not tap into the fresh groundwater resources as expected (Querejeta *et al*., 2007).

1.1.4. Common vegetation associations

Much of the land covering the fresh groundwater basins on Eyre Peninsula is, as noted by the early surveyors, stony and barren. Where trees were present, the dominant associations were *Eucalyptus diversifolia*, subsp. *diversifolia* Bonpl. (coastal white mallee), which forms a scrub, and *Allocasuarina verticillata* (Lam.) L. Johnson (drooping sheoak), which develops as an open, grassy woodland (Crocker 1946). Both associations were common to areas where sheet limestone and thin, poor soils often associated with karst regions were present.

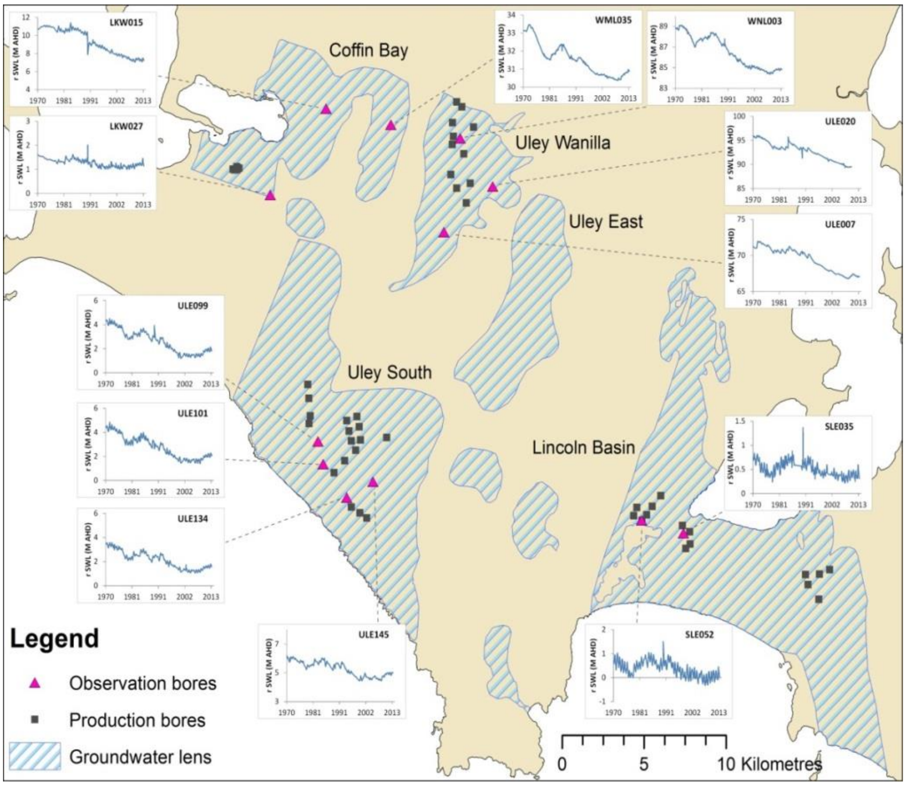

Figure 1.1.4.1 Groundwater level trends for the quaternary groundwater lenses of the Southern Basins Prescribed Wells Area, Eyre Peninsula

When the region was settled, much was used for pastoral grazing and broadacre clearing was avoided where possible (Bishop and Venning, 1986). Unlike *E. diversifolia* stands, the *A. verticillata* woodland was usually grazed in its natural state because of the presence of edible grasses such as *Stipa* spp. and *Danthonia* spp. However, the grassy woodland declined rapidly after settlement. Major rabbit plagues dominated the region during 1885 – 1892, and combined with heavy grazing pressure that resulted in damage to fragile topsoil , culminated in the loss of many *A. verticillata* trees (Bishop and Venning, 1986).

As the expanse and density of *A. verticillata* woodland declined, strong winds combined with shallow root systems and thin soils resulted in many trees falling over. As a result, since European settlement nearly all of the once expansive *Allocasuarina verticillata* woodland has died over western Eyre Peninsula (Bishop and Venning, 1986).

The recharge area of the Uley South groundwater lens is one such area where *A. verticillata* trees were common, but are now virtually absent from the landscape. The remains of trees are evident as dead trunks and strewn across the low lying area of the lens. Of particular interest are the remnants of *A. verticillata* trees within the isolated stands of *Eucalyptus diversifolia*. When these *E. diversifolia* stands became established is unclear; however the presence of *A. verticillata* remains would suggest that the emergence of the *E. diversifolia* has occurred after the *A. verticillata* decline. In addition, juvenile *E. diversifolia* trees are present around the outer edge of each stand, suggesting range expansion is occurring.

The hydrological implications of the change from an *A. verticillata* dominated landscape, to one with *E. diversifolia* encroachment are unclear, and is the major focus of this thesis.

1.1.5. Hydrological implications of invasive species

Globally, investigating the hydrological impact of exotic plant invasions has received significant attention (Sala *et al*., 1996, Owens and More, 2007, Nagler *et al*., 2008, Doody and Benyon, 2011), as the presence of non-native species are believed to alter hydrological processes and detrimentally impact ecosystems and biodiversity (Doody *et al*., 2014). Invasion by exotic tree species into previously unoccupied niche habitats has been reported to significantly increase evapotranspiration losses from a given area (Doody and Benyon, 2011), and leads to demands to actively manage vegetation to improve water yield (Wilcox 2002, Shafroth *et al*., 2005).

The Eyre Peninsula's largely hostile environment, with poor soils and irregular rainfall, has suited a particular assembly of exotic plants. The most notable of these is *Pinus halepensis* Mill. (Aleppo Pine), native to the Mediterranean region, which is well adapted to the thin, calcareous soils of the Eyre Peninsula. *Pinus halepensis* was introduced to the Eyre Peninsula by the forestry industry in the early 1800's (Way, 2006). While unsuccessful as a forestry species, *P. halepensis* served as an excellent windbreak and was subsequently widely planted as stock shelter belts and around farmhouses. *Pinus halepensis* populations have since naturalised, and are capable of invading intact native vegetation and forming severe infestations (Quarmby, 2004). One such area where *P. halepensis* has had an acute impact is on the Uley Wanilla groundwater reserve. Uley Wanilla has historically been grazed since European settlement. However, since the dedication of the land for watershed protection in 1948, a *Eucalyptus diversifolia* – *Leucopogon parviflorous* – *Melaleuca lanceolata* association has regenerated across much of the landscape. Subsequent to the native shrubland regeneration, further landscape modification by recent *P. halepensis* infestations is believed to have exacerbated the recent decline in groundwater recharge rates.

While quantitative estimates of *P. halepensis* density across Uley Wanilla are lacking, qualitative assessments estimate that some areas have *P. halepensis* canopy cover of 100% (Quarmby, 2004). As *Pinus* canopies have been found to intercept more water than *Eucalyptus* trees (Crockford and Richardson, 1990c) and transpire at rates of up to 2.1 mm day^{-1} (Schiller and Cohen 1998), we might expect that *P. halepensis* water use and the rate of evapotranspiration from infested areas will increase, thereby reducing groundwater recharge rates in comparison to shrubland comprised only of native trees and shrubs. This concept has, therefore, been included as a focus of this thesis.

1.2. Focus of thesis

The aim of the thesis is to increase our understanding of the patterns of and constraints in water use by both native and non-native trees (*E. diversifolia, A. verticillata* and *P. halepensis*) in the semi-arid Eyre Peninsula region of southern Australia. In particular, I sought to characterise the water use physiology of these regionally significant species to assess the potential impact of their presence on the water balance of the Uley South and Uley Wanilla groundwater lens. The thesis thus examines the ecohydrological functioning of these water limited ecosystems (using a range of methodologies) in order to aid the management of these groundwater resources, so important for the drinking water requirements of the region.

The Eyre Peninsula, as described in section 1.1.3, has a long history of water scarcity issues. This has, at least in part, contributed to the current community pressure on government agencies to sustainably manage the Southern Basins. This scrutiny has intensified with ongoing groundwater level decline and uncertainty concerning future annual rainfall patterns, which have a history of being variable (for example, Station #18017, which has an annual rainfall range of 365 – 925 mm, Bureau of Meteorology, 2010) as well as a Notice of Prohibition preventing extraction from the Polda lens in an attempt to promote stabilisation and recovery of groundwater level and quality after a period of significant degradation.

Actively managing vegetation to improve water yield is a commonly suggested approach for addressing water scarcity issues resulting from reduced groundwater recharge. However, an understanding of actual *ET* from the various vegetation associations, required to inform the feasibility of any management intervention, has been lacking in this area. While it is well understood woodlands and forests have higher *ET* rates than do grasslands (Zhang *et al.*, 2001), it is surprising how seldom tree removal has successfully increased water yield (see review by Doody *et al.*, 2011). This uncertainty motivated this research, which focusses on furthering our understanding of hydrological impacts of landuse changes brought about by vegetation removal, encroachment and invasion.

This thesis outlines the processes that determine patterns of the temporal and spatial water availability in dryland regions, focussing on karst systems, and the manner through which the hydrology may be affected by the differences in catchment vegetation. It provides an overview of the water use physiology of trees in this dryland area, giving particular reference to the impact of trees on groundwater recharge rates. This provides context for the experimental rationale that is presented in Chapters 2 to 4 of this thesis, including the potential applications of this research to improve water management strategies amidst rapid development of semi-arid and arid regions of Australia. Finally, Chapter 5 summarises the contributions to knowledge generated through this work, and highlights areas where future work could build upon the findings of this research.

1.3. Objectives

This thesis explored the key ecohydrological characteristics of the karst aquifers on Eyre Peninsula, which remain poorly understood despite their regional significance in supporting the local economy. The research focussed on common woody trees, and their interaction with the hydrological processes occurring across the landscape. Ultimately, this outcome of this work will inform groundwater management strategies and policies, and ensure the capacity of these resources to continue to support the water use requirements of the Eyre Peninsula region.

Further to these goals, the objectives of this thesis were to:

1. Assess the evapotranspiration losses for two co-occurring, but morphologically distinct native tree species and a non-vegetated area, and consider whether *ET* was being supplemented by groundwater (Chapter 2),

2. Investigate the rainfall partitioning pathways of two common tree species with inference to the ecohydrological significance of stemflow, and place the results in the context of global rainfall partitioning studies (Chapter 3),
3. Investigate the hydrological implications of the invasive *Pinus halepensis* in comparison to native woodland species, using detailed plot scale assessments of tree water use and remotely-sensed evapotranspiration (Chapter 4).

1.4. Journal publications

Chapter 2, 3 and 4 are three international peer-reviewed journal papers that were delivered through undertaking this research. The first has been published in *Hydrological Processes,* and both the second and third have been published in *Ecohydrology*. The references are as follows;

> Swaffer BA, Holland KL, Doody TM, Li C, Hutson J. 2014. Water use strategies of two co-occurring tree species in a semi-arid karst environment. *Hydrological Processes* **28**: 2003-2017
>
> Swaffer BA, Holland KL, Doody TM, Hutson J. 2014. Rainfall partitioning, tree form and measurement scale: a comparison of two co-occurring, morphologically distinct tree species in a semi-arid environment *Ecohydrology* DOI: 10.1002/eco.1461
>
> Swaffer BA, Holland KL. 2014. Comparing ecophysiological traits and evapotranspiration of an invasive exotic, *Pinus halepensis* in native woodland overlying a karst aquifer. *Ecohydrology* DOI: 10.1002/eco.1502

It should be noted that the introduction of each journal paper, and henceforth thesis chapter, contain the necessary literature review pertaining to each of the chapter objectives. Further to this, each discussion section provides, at length, the necessary references to place the findings of this work into the context of other national and international studies.

2. Water use strategies of two co-occurring tree species in a semi-arid karst environment[1]

2.1. Introduction

Karst aquifers form an important component of freshwater resources worldwide; 25% of drinking water supplies are sourced from a geological setting covering around 10% of the earth's land surface (Schwinning, 2008; Estrada-Medina *et al.*, 2010). The delivery of high quality water into karst aquifers is facilitated through features developed through dissolution of the soluble rock (Schwinning, 2008; Ward *et al.*, 2009). Water-enlarged cracks, fissures and channels allow rapid recharge of surface water to groundwater and can result in significant stores of water within the porous unsaturated zone (Perrin *et al.*, 2003). The degree of complexity associated with this preferential water movement can be challenging to characterise, particularly in semi-arid areas where recharge-producing rainfall may be highly irregular or episodic. Reliance on rainfall-derived recharge may also result in karst aquifers being particularly vulnerable to climatic change (Loáiciga *et al.*, 2000), most notably where rainfall is variable, and groundwater abstraction supports community, agricultural or industrial needs.

The extent of groundwater recharge will be further influenced by the coverage of vegetation. The presence of canopies will reduce the volume of precipitation infiltrating the soil (Domingo *et al.*, 1998, Huber and Iroume 2001) and reduce the volume of water in the unsaturated zone available for recharge. In addition, where tree roots are able to access persistent recharge pathways, i.e. cracks and

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[1] Swaffer BA, Holland KL, Doody TM, Li C, Hutson J. 2014. Water use strategies of two co-occurring tree species in a semi-arid karst environment. *Hydrological Processes* **28**: 2003-2017

fissures, the occurrence of the vegetation is likely to substantially reduce groundwater replenishment through reduced infiltration and increased tree transpiration.

Ecosystem evapotranspiration (*ET*) is a complex process driven by multiple climate-soil-vegetation interactions (Verstraeten *et al*., 2008). Water is transferred from soils in the unsaturated zone, then through vegetation and into the atmosphere. *ET* includes water flux from plant transpiration, soil evaporation and intercepted rainfall and, assuming run-off is small, often represents the dominant pathway for water loss in vegetated landscapes (Zhang *et al*., 2001, Brown *et al*., 2005). In semi-arid or arid regions, *ET* from forested systems is often nearly equal to gross precipitation (P_g) (Zhang *et al*., 2001) as vegetation communities evaporate or transpire almost all of the incoming rainfall (Ellis *et al*., 2005). In these environments, the growth of perennial plants will be predominantly water limited and are reported to be in stable equilibrium with their environment, having evolved the capacity to mediate water stress (Eagleson, 1982, Ellis and Hatton 2008). Where previous studies of *ET* rates in semi-arid regions have been confined to deeper soils, this research presents the first account of *ET* over a fresh, shallow karst groundwater system in a semi-arid environment. It is unclear how annual *ET* rates from dominant tree species compare to annual P_g in a karst environment, characterised by a skeletal soil profile and shallow (<5 m), fresh groundwater.

Shallow soil profiles experiencing prolonged summer drought are likely to lack sufficient water holding capacity to support the transpiration requirements of trees and shrubs (Zencich *et al*., 2002). In Mediterranean climates, such as that across southern Australia, winter dominated rainfall recharges soil and groundwater stores, replenishing the profile which progressively dries out as summer progresses (Ayalon *et al*., 1998, Perrin *et al*., 2003). Faster water depletion in the surface soils would be expected in the presence of root systems and high evaporative demand from the atmosphere. Therefore, if transpiration is to continue through the drier months, water would need to be sourced from progressively deeper, wetter locations within the profile. The ability of trees to extract water held at lower water potentials are traits often associated with drought tolerant species (El-Lakany 1983, O'Grady *et al*., 1999; Carter and White 2009); therefore, consideration of the physiological characteristics of tree species will be important in order to understand their hydrological influence on the unsaturated zone.

This study examined the influence of woody vegetation occurring across a semi-arid region where a fresh water aquifer exists close to the soil surface. Quantifying the ecohydrological characteristics of naturally occurring vegetation has yet to be examined in this area, and this work focuses on two locally prevalent, co-occurring, but morphologically distinct tree species – *Eucalyptus diversifolia* subsp. *diversifolia* Bonpl. (coastal white mallee) and *Allocasuarina verticillata* (Lam.) L. Johnson (drooping she-oak).

Few studies appear in the literature for *Casuarina* or *Allocasuarina* spp., and those reported are often restricted to the actinorhizal characteristics of the genus (e.g. Franche *et al*., 1997, Obertello *et al*., 2005) or salinity effect on growth (e.g. Sun and Dickinson 1995; Walsh *et al*., 1995, Cramer *et al*., 1999). However, Mitchell *et al*., (2008a) used physiological traits to define *Allocasuarina* *campestris* and *Casuarina humilis* as drought-suppressed, narrow leaved shrubs. Mitchell *et al*., (2008b) reported the leaf water potentials for *C. humilis* and *A. campestris* were lower than – 4.5 and – 6 MPa (pre-dawn), during summer water deficit. *Allocasuarina verticillata* extends across the south-eastern section of Australia on skeletal soils derived from sandstone or limestone (Florabank, 2011). To my knowledge, this is the first study to investigate the water use strategies of *A. verticillata*.

Similarly, while mallee-form eucalypts are widespread across southern Australia, studies are generally restricted to deeper soil regions in Western Australia (e.g. Nulsen *et al*., 1986, Wildy *et al*., 2004a, b, Mitchell *et al*., 2008a, Carter and White 2009, Mitchell *et al*., 2009). *Eucalyptus diversifolia* is found on coastal and sub-coastal regions across southern Australia (Wright and Ladiges 1997). In the eastern part of its range, including Eyre Peninsula, it is found mostly on consolidated calcareous sands (Wright and Ladiges 1997). The extensive range of mallee-form eucalypts across southern Australia highlights the necessity to consider their function in a wide variety of geological settings.

Both tree species occur across the topographically low lying area where the water table is close to the surface and are morphologically distinct. *Eucalyptus diversifolia* possess multiple, smooth stems, simple leaves, with extensive lignotuber and deep root systems characteristic of drought avoidance (Wright and Ladiges 1997, Wildy *et al*., 2004a). *Allocasuarina verticillata* is characterised by a single trunk with thick, rough bark, long thin vertical phyllodes and a shallow root system that is characteristic of the genus (Sun and Dickinson 1995). Presenting a water balance for these tree species will help to clarify their influence on recharge and discharge processes of the karst aquifer. Water balance techniques have been successfully applied over a wide range of habitats and tree species throughout Australia (e.g. Cook *et al.*, 1998, Benyon and Doody, 2006, Benyon *et al.*, 2006, Mitchell *et al.*, 2009, Doody *et al.*, 2009, Feikema *et al.*, 2010, Holland and Benyon, 2010, Doody and Benyon, 2011). Investigating the source of tree water in shallow groundwater regions using environmental tracers can support water balance assessments. The use of the stable isotopes of water are based on the assumption that isotopic fractionation does not occur during plant uptake (Thorburn *et al.*, 1993a, b, Cramer *et al.*, 1999). This has become a standard tool for trying to quantify the source of tree water (Ehleringer and Dawson, 1992; Thorburn *et al.*, 1993a,b, Brunel *et al.*, 1995) and has been applied successfully for plants with access to stream water and soil water (Ehleringer and Dawson, 1992), soil water and groundwater (Zencich *et al.*, 2002, Holland *et al.*, 2006, Queretjeta *et al.*, 2006), rainfall and soil water (Brunel *et al.*, 1997) and riverwater and groundwater (O'Grady *et al.*, 2006 a, b).

The objectives of the paper were to (1) quantify the *ET* losses of these two vegetation communities, (2) consider if the trees were accessing groundwater to sustain transpiration requirements and (3) infer the effect of these vegetation communities on the regional water balance. Tree water use strategies were determined based upon the relative components of *ET* (transpiration, soil evaporation and interception), and by using soil and leaf water potentials in association with twig water isotope ratios measured over the dry season. Tree water sources were determined by comparison of the isotopic signature of twig water to the range of potential water sources in the landscape.

2.2. Methods

Regional climate

The study site was located on Uley South Water Reserve, southern Eyre Peninsula, South Australia (34.77ºS, 135.54ºE), which has a Mediterranean-type climate with hot dry summers and cool, moderately wet winters. The region is classed as semi-arid, based on the ratio of rainfall to potential *ET* being < 0.5 to > 0.2 (Anon 1999). A weather station (Davis Wireless VP Plus ISS, California USA) recording 15 min readings of temperature, wind speed, wind direction, relative humidity and rainfall was installed from the 23 September 2009 until 30 January 2010. The relationship between on-site data and daily rainfall and potential *ET* (ET_p) data near Port Lincoln (Big Swamp #18017) was used to estimate the long-term average rainfall of 502 mm year^{-1} ($r^2 = 0.99$) and ET_p of 1524 mm year^{-1} ($r^2 = 0.61$) (Bureau of Meteorology 2010).

Study area

Uley South is a topographically closed, internally draining fresh groundwater lens. The lens is bound by topographic rises, mobile sand dunes and coastal cliffs to 140 m AHD (Harrington *et al.*, 2006). Soils in the Uley South reserve are typically skeletal calcareous aeolianite (Crocker 1946, Harrington *et al.*, 2006), less than 30 cm deep. A sheet limestone sub-stratum is often present, visible on local rises. The soils have low water holding capacity and are nutrient deficient (Wen 2005). The groundwater is fresh; recorded salinity across 42 observation bores averaged 1071 EC (SD = 368, n = 171) (DEWNR, 2011) and is an important drinking water source for the region.

Three 20 x 20 m plots were used to sample a stand of *E. diversifolia*, *A. verticillata* and a bare site between 19 July 2008 and 18 July 2010. These sites were selected based on their close proximity to the water table, to increase the likelihood that groundwater could be used by the trees. Logistical constraints prevented the replication of *ET* measurements at other locations across the topographical profile; however, leaf area index (*LAI*) was measured at several other locations for comparative purposes. These sites were chosen to cover varying elevations (topographical lows and highs) and also the range of Normalised Difference Vegetation Index reflectance based on remote sensing images. The results of the *LAI* comparisons are presented in Figure 2.2.1.

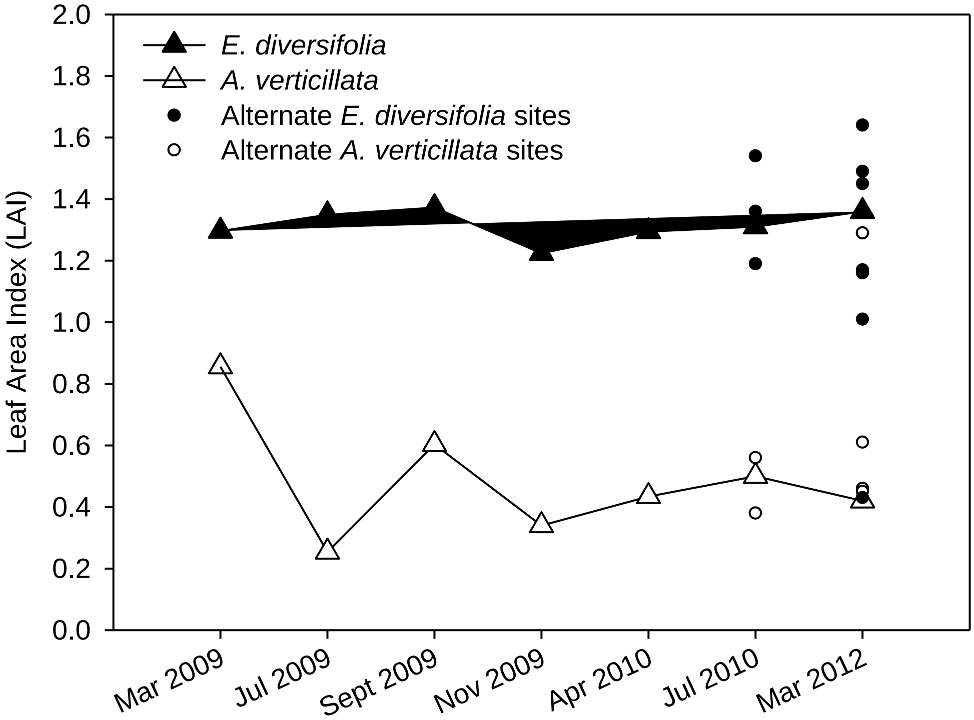

Figure 2.2.1 Comparative Leaf Area Index (LAI) assessments across multiple 20 x 20 m *Eucalyptus diversifolia* and *Allocasuarina verticillata* plots. Filled symbols represent the *E. diversifolia* LAI associated with observation bore ULE101 (triangles) and alternate sites (circles). Open symbols represent *A. verticillata* LAI associated with ULE099 (triangles) and alternate sites (circles). Elevations of the alternative sites varied between 5 and 90 m AHD.

A groundwater level monitoring bore (ULE099) was located near the *A. verticillata* site. A second observation bore (ULE101) was near *E. diversifolia* and the bare site (DEWNR 2011). To further account for potential site differences, a small number of *E. diversifolia* trees outside the *A. verticillata* plot were included in measurements of leaf water potentials (Ψ) and stable isotopes of water. The results of the *E. diversifolia* individuals occurring adjacent to the *A. verticillata* site are denoted by reference to the bore number (i.e. *E. diversifolia* 099). Standing water level (depth to groundwater below the ground surface) ranged between 3.6 and 4.4 m for both bores during the study period (Figure 2.2.2).

LAI was estimated using digital photographs from an Olympus FE-240 (7.1 megapixel) camera and transformed using Matlab R2009b and the Image Processing Toolbox (The Mathworks Inc., MA, USA). Fifty images per plot were taken using automatic exposure and upward looking images 1.5 m from the ground avoiding midday hours. The method was based on that proposed by Macfarlane *et al*., (2007) and Fuentes *et al*., (2008). Five images were taken at an interval of 10 m along two transects that were spaced 20 m apart. A Matlab script (Fuentes *et al*., 2008) was used to calculate the gap fraction for each image. The image was divided into either foliage or sky using the blue light band (450 – 495 nm) to select a suitable threshold value. The gap criterion was set to 0.75 and an extinction coefficient (k) of 0.5 was used following Macfarlane *et al*., (2007).

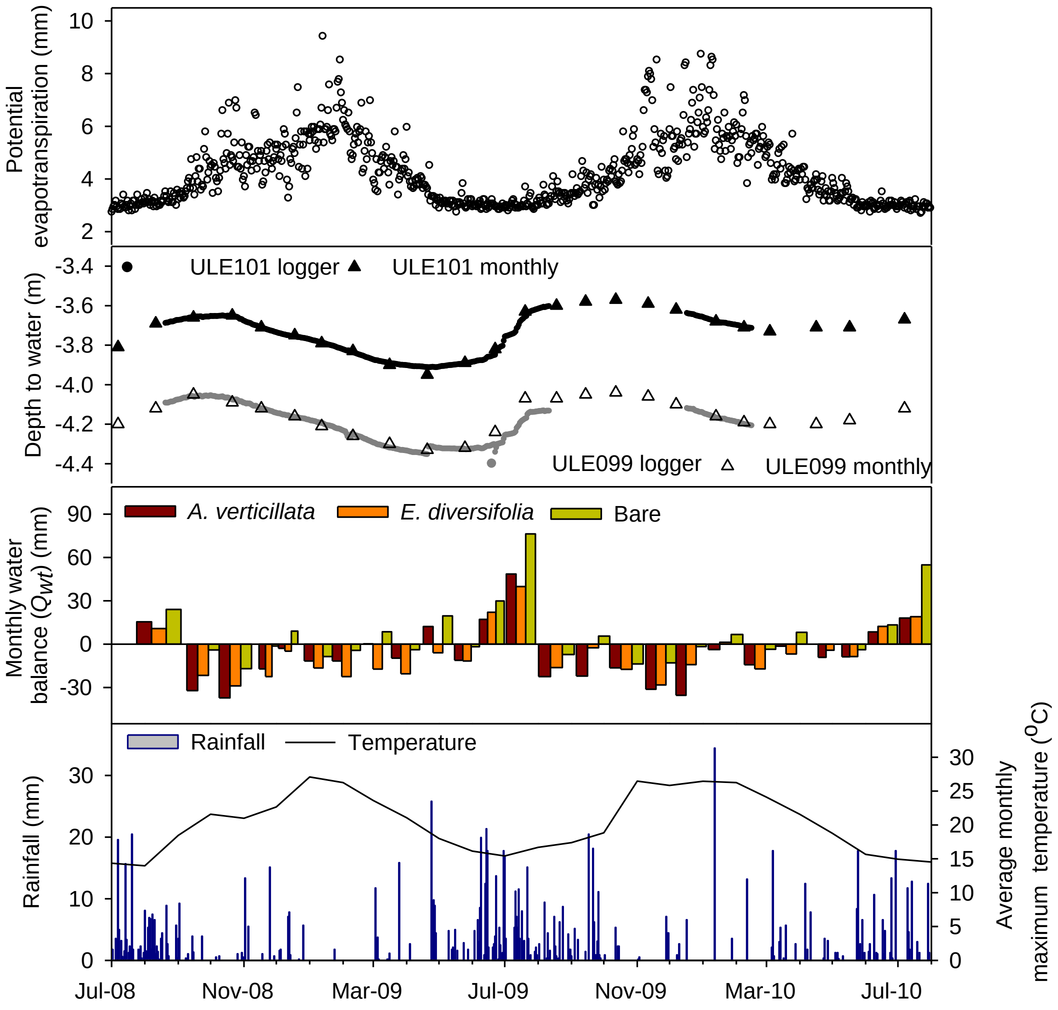

Figure 2.2.2 Project timeline and climate trends, including potential evapotranspiration rate (ET_p) (mm), depth to groundwater (DTW) (m), monthly water balance (Q_{wt}) (mm), daily rainfall (mm) and mean monthly maximum temperature (°C).

Water balance

Plot water balance was determined from

$$Q_{wt} = P_g - (T + I + E + \Delta S + R_o)$$

Where

Q_{wt} = net water balance with either deep drainage occurring (positive value) or water loss (a negative value) (mm)

P_g = gross precipitation, measured by standard rain gauges and a weather station in a nearby open location (mm)

T = plot transpiration, determined from the heat pulse velocity of instrumented stems (mm)

I = interception losses, estimated from measurements of stemflow and throughfall (mm)

E = surface soil evaporation, measured using weighing mini-lysimeters (mm)

ΔS = change in volumetric soil storage and was assumed to be zero over the two year measurement period, in part due to the inherent difficulty of obtaining replicate representative samples of the karst substrate. Zhang *et al.*, (2001) that over long periods of time, it is reasonable to assume net changes in soil water storage would be zero. Similar water balance studies have also assumed negligible changes in soil storage over similar timeframes to this investigation (i.e. Doody *et al.*, 2009, Feikema *et al.*, 2010). This assumption was, however, assessed by adjusting *ET* by a conservative estimate based on the range of values reported in the literature where soil storage change was measured (Benyon and Doody, 2006, Benyon *et al.*, 2006, Holland and Benyon, 2010). The results of the uncertainty analysis indicate the minor influence an annual soil water change of 10% of annual ET would have made to the overall water balance (Figure 2.2.3)

R_o = surface run-off was assumed to be zero, owing to a lack of relief at each plot, the relatively low volume of rainfall and high permeability of the karst substrate.

ET represents the loss of water through transpiration, interception and soil evaporation, and is calculated by

$$ET = T + I + E$$

Interception losses (I) from plant canopy and stems were calculated from

$$I = P_g - (TF + SF)$$

TF = throughfall measurements, measured using eight randomly located collection troughs (mm)

SF = stemflow measurements, determined using randomly selected branches of varying size classes (mm).

TF was measured by eight randomly located galvanised steel troughs, each with a horizontal projection area of 0.1716 m^2 (1.2 m x 0.143 m). The throughfall in each container was averaged to determine mean plot TF. Litres of TF were converted into mm of precipitation by dividing the volume (L) collected by the trough catchment area (m^2). For larger trees (diameter at breast height (DBH) > 50 mm), SF was measured by winding split plastic tubing (16 mm diameter) twice around the trunk. Both were attached to the tree using staples and silicone following Crockford and Richardson (1990b). A total of nine sample trees (~ 10%

trees in plot) were randomly instrumented in the *E. diversifolia* site and six sample trees (50 % of trees in plot) in the *A. verticillata* site. Plot *SF* was calculated by scaling *SF* volume collected by instrumented trees to the volume expected at the plot scale using the sum of tree basal area over bark ($BAOB_t$) (m^2) following Mitchell *et al.*, (2009).

Soil evaporation was measured in all three sites using four randomly located mini-lysimeters containing 0.00194 m^3 of soil collected in situ. A standard rainfall gauge was positioned beside each lysimeter to record precipitation input into the soil column.

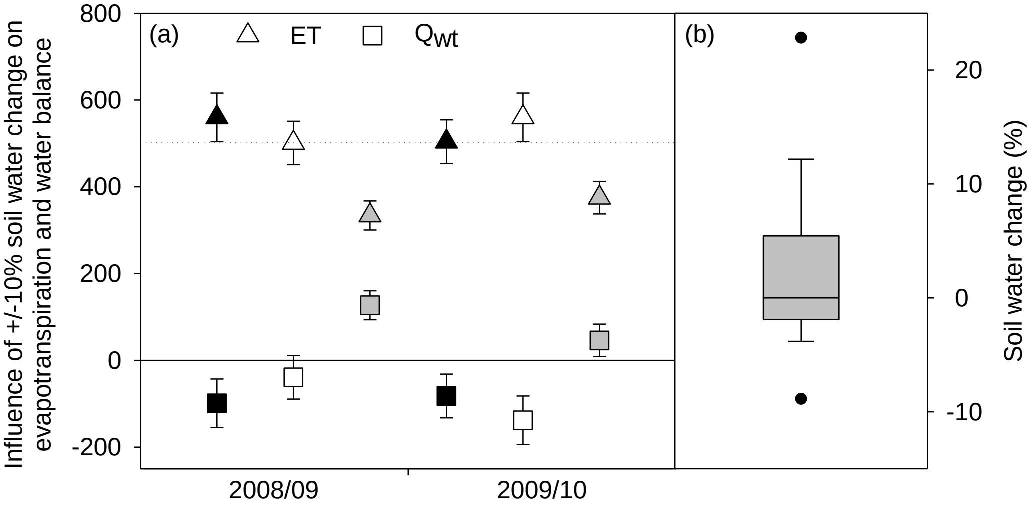

Figure 2.2.3 (a) Influence of a ± 10 % soil water change on annual actual evapotranspiration (*ET*) and subsequent water balance (Q_{wt}) for *Eucalyptus diversifolia* (black symbols), *Allocasuarina verticillata* (white symbols) and the bare site (grey symbols). Dotted lines represent the estimated long-term average rainfall for the area. (b) Published annual soil moisture changes where soil moisture was measured as part of a water balance assessment (Benyon *et al.*, 2006, Benyon and Doody 2009, Holland and Benyon 2010, n = 29). Box plots show the 25th and 75th percentile, with median values within. Whiskers show 10th and 90th percentiles, and symbols represent the 5th and 95th percentile.

Drainage collectors were installed below the column. The soil column was weighed each measurement period, and daily soil evaporation was estimated based on rainfall input and change in weight through time (Benyon *et al.*, 2006). The evaluation of effective precipitation (water which is available for uptake by plants) is derived from gross precipitation but takes into account various water losses prior to transpiration, i.e. soil evaporation, and interception losses. The National Water Commission (2011) defines effective precipitation as the portion of rainfall that is available for uptake by plants. Comparing effective precipitation with transpiration requirements is useful when estimating groundwater use by trees.

Effective precipitation (P_e) was calculated by

$$P_e = TF + SF - E$$

Tree transpiration rates were estimated from heat pulse measurements of sap velocity on six stems for the *E. diversifolia* site and five stems in the *A. verticillata* site every 30 min between 22 May 2008 and 19 July 2010 (except during equipment failure) and follow the sampling design of Hatton *et al.*, (1995). Stems were selected by first dividing into three size classes and randomly chosen within each size class. The heat pulse method is based on the compensation technique of Marshall (1958) and refined by Swanson and Whitfield (1981) and measured using sap-flow loggers (SF 300 probes, Greenspan Technology, Warwick, Queensland).

Radial profiles of heat pulse velocities were assessed by roving a set of four probes each with three pairs of thermocouple sensors spaced at 5, 12 and 21 mm

(Tranzflo NZ Pty, Palmerston North, New Zealand). The heat pulse method can be used for accurate measurements of sap flow in plant stems provided appropriate parameters are used to relate measured heat pulse velocity to estimated sap-flow velocity, including wood water fraction, measurement of probe separation and wound width (Thorburn *et al*., 1993b; Vertessy *et al*., 1997, Cramer *et al*., 1999, Bleby *et al*., 2004). Calibration techniques described by Steppe *et al*., (2010) also provide confidence in results.

Wood and water volumetric fractions of the sapwood were determined by weighing core samples, then oven drying samples at 105 °C and reweighing. Wood and water fractions ranged between 0.189 – 0.320 (wood) and 0.245 – 0.490 (water) for *E. diversifolia* and 0.235 – 0.446 (wood) and 0.281 – 0.537 (water) for *A. verticillata*. Species-specific wound widths of 0.34 mm for *E. diversifolia* and 0.31 mm for *A. verticillata* were determined following Swanson and Whitfield (1981). Probe separation values were measured by inserting blank probes at a distance equal to the depth of the sensor and recording variation from 10 mm above and 5 mm below the heater probe. Variance larger than 1 mm was rejected and holes redrilled.

A defined sap-flow velocity threshold was required and refers to the value of sap-flow velocity below which low sap flow cannot be distinguished from zero sap flow (Becker and Edwards 1999). The threshold was determined based on the mean value across four nights between midnight and 5 am.

The 99% confidence interval based on the standard deviation of values was then used as the no-flow threshold value (Benyon 1999).I note this threshold does not necessarily equate to zero transpiration, only that I have no means of verifying the value actually exceeds zero transpiration (Steppe *et al*., 2010). Daily measurements were scaled to estimate plot transpiration based on the relationship between sapwood area and tree size (using *DBH*) (Thorburn *et al*., 1993b, Vertessey *et al*., 1995, Benyon *et al*., 2006). Firstly, plot basal area over bark ($BAOB_p$) (m^2) was measured by recording tree *DBH* for all live trees in the plot and converted to an area, which included bark depth. Then, the depth of sapwood for instrumented trees was determined with sap-flow sensors after Hatton *et al*., (1995), and then used to calculate tree sapwood area using stem diameter, bark thickness and sapwood thickness. For trees where sapwood area and sapwood velocity were not measured, a linear regression estimated sapwood area using tree basal area over bark ($r^2 = 0.98$ for *A. verticillata*; $r^2 = 0.99$ for *E. diversifolia* (data not shown)). Plot sapwood area was calculated by summing the estimated sapwood area for all trees in the plot. Plot transpiration was calculated using the product of mean sap-flow velocity of measured stems and estimated plot sapwood area.

Tree water sources

Samples for tree water sources were collected in March 2009 (late summer), November 2009 (late spring) and February 2010 (late summer). Comparing twig water isotopic signatures to the potential sources of rainfall, surface soil water and groundwater is possible using the natural abundance of hydrogen isotopes ($^2H/H$) and oxygen isotopes ($^{18}O/^{16}O$).

Australian natives do not alter the isotopic composition of water during uptake by the roots thereby allowing direct comparison (Thorburn *et al*., 1993a,b, Cramer *et al*., 1999). The range of isotopic signatures of rainfall samples (δ^2H and $\delta^{18}O$) was profiled using 25 cumulative monthly rainfall samples collected in the bare site between the 22 May 2008 and 19 July 2010.

Paraffin oil was added to the collection bottles to minimise evaporation. Twig samples from each tree were quickly immersed into kerosene in an airtight 200 ml glass jar to prevent any isotopic exchange. Twig water was extracted for isotopic analysis by azeotropic distillation with kerosene (Thorburn *et al*., 1993a). Surface soil samples were taken within 2 m from the base of four sample trees by hand. The shallow nature of the soil and hardness of the sheet calcrete prevented the use of a hand auger to sample deeper soils. Soil was placed into 500 ml glass jars and sealed for later analysis of isotopic ratio content and matric suction. Water for isotopic analysis was extracted from soil using azeotropic distillation as described by Revesz and Woods (1990).

Groundwater isotope samples were obtained after purging three well volumes to ensure the sample was taken from the aquifer (Barber and Davis 1987). Profiling water held within the karst unsaturated zone was undertaken during November 2009 through core drilling, using a steel NMLC Triefus Triple Tube core barrel (Pre-mat Drilling Pte Ltd, Singapore). All water samples were provided to the CSIRO Isotope Analysis Service, Adelaide and measured using a dual inlet stable isotope gas ratio mass spectrometer (PDZ Eurpoa Ltd, UK).

Groundwater samples were analysed for deuterium by equilibrating with hydrogen using a platinum catalyst for one hour using an automated Water Equilibration System (WES). Soil and twig water samples were analysed for deuterium by vaporising and circulating over uranium metal heated to 800 ºC (Dighton *et al*., 1997). All water samples (water, soil, twig) were analysed for oxygen isotope ratios by equilibrating with CO_2 at 50 ºC for 8 h using an automated WES following Epstein and Mayeda (1953). All isotopic concentrations are reported in standard delta notation in units per mil (%) relative to the Vienna-Standard Mean Oceanic Water.

A mixing model program (IsoSource) (Phillips and Gregg, 2003) was used to assess the feasible proportional contribution of each of the three possible tree water sources in November 2009 using $\delta^{18}O$; surface soil water, subsurface soil water and groundwater. The program provides the breadth of plausible source water solutions that could result in the end product signature (in this case the twig water). Only the November 2009 samples were used in the model, as this was the only time drilling was able to obtain subsurface soil water samples. End product isotope signatures were entered into the program, with a nominated source increment of 1 % and mass balance tolerance of ± 0.1 %.

Leaf and soil water potentials

Leaf water potential (Ψ) (MPa) (an indicator of plant water stress) was measured using a PMS 1000 pressure chamber (PMS Instrument Company, Albany, Oregon) on two or three replicate twigs for six sample trees taken 1 – 2 m above ground (Scholander *et al.*, 1965).

Leaves were bagged and placed in a humidified bomb chamber to minimise water loss during measurement (Turner 1988) before dawn (Ψ_p) (0400 – 0600 h) and at midday (Ψ_m) (1130 – 1330 h) (Australian Central Standard Time). Leaf water potentials were measured on the 2 – 4 March 2009 (late summer), 25 November 2009 (late spring) and 22 February 2010 (later summer). The March 2009 results may be influenced by the 20 mm rain event that fell between Ψ_m and Ψ_p.

Soil matric potentials were determined using the filter paper method (Greacen *et al*., 1989). This method is accurate for matric potentials between – 5 and – 1 MPa (Greacen *et al*., 1989). Three replicate Whitman No. 42 filter papers were placed in contact with soil, evenly distributed in a 500 ml glass jar. The jars were left to equilibrate in a temperature controlled environment for 7 days. The papers were weighed immediately after surplus soil was quickly removed with a paintbrush. The papers were then oven dried at 105 ºC for 24 h, and reweighed. Using known calibrations (provided in Greacen *et al*., 1989), the matric potential can be estimated from the water content of the filter papers. Reported values are the average of the three replicates for each sample. Osmotic potentials were not measured, but were assumed to be negligible due to the shallow, low salinity groundwater at the sites. Gravimetric water potentials were also not included, due their relative insignificance in comparison to measured matric potentials.

Statistical analyses

Statistical confidence intervals were based at the 95 % confidence level and estimated using the variation between measured and estimated water balance parameters. All statistical analysis was performed using SigmaPlot v11.0 (Systat Software Inc, USA).

For *I*, confidence intervals were based on the variation in mean *TF* between collection troughs and mean *SF* across the stem flow collectors. For *E*, confidence intervals were based on the variation in mean evaporation across the four lysimeters in each site, and confidence intervals for *T* were based on the variation in mean sap velocity across instrumented trees. The confidence intervals presented for *ET* are based on *T* as I have assumed the 95 % confidence interval for *ET* is equal to that for transpiration following Benyon *et al.*, (2006).

Significant differences in water balance components across plots were determined using parametric and nonparametric tests depending on normality and independence assumptions. Where assumptions were met, unpaired t-tests were applied to the data to test for variance between groups, for example *E. diversifolia ET* compared to *A. verticillata ET*. Where normality test failed, the Mann–Whitney Rank Sum Test was used to perform an unpaired t-test on the sum of the ranks. One-Way Repeated Measures Analysis of Variance (ANOVA) was used to test for significant differences in water balance parameters in a plot across the two water balance years, for example the volume of soil evaporation in the first year compared to the volume of soil evaporation in the second year for *A. diversifolia*. Seasonal differences in species' transpiration rates were assessed by assigning season as a factor to the daily sap flux data, and using an ANOVA to assess whether the difference in seasonal means was significant at the $p < 0.05$ level.

2.3. Results

Plot characteristics

Plot basal area over bark ($BAOB_p$) (m^2) was measured for all trees in May 2008, January 2009, March 2009, September 2009, February 2010 and July 2010. Despite the measurement period spanning two years, tree growth was relatively slow; *E. diversifolia* $BAOB_p$ increased 0.9 m^2 ha^{-1} (from 14.9 m^2 ha^{-1} to 15.8 m^2 ha^{-1}) and *LAI* averaged 1.32 (SD = 0.17, n = 7). *A. verticillata* $BAOB_p$ increased 0.7 m^2 ha^{-1} from 11.3 m^2 ha^{-1} to 12.0 m^2 ha^{-1} over the study period. *LAI* was lower for *A. verticillata* than for *E. diversifolia* and averaged 0.48 (SD = 0.15, n = 7).

LAI measurements (1.19 to 1.54 (*E. diversifolia*) and 0.36 to 0.50 (*A. verticillata*)) collected at seven different sites across the landscape were comparable to the vegetation associated with observation bores ULE101, and ULE099, suggesting these vegetation stands were typical of that growing across the groundwater reserve (Figure 2.2.1).

Water balance

During the 731 day monitoring period, P_g totalled 883 ± 80 mm. This equated to 461 ± 48 mm year^{-1} between 18 July 2008 and 16 July 2009, and 422 ± 33 mm year^{-1} between 17 July 2009 and 19 July 2010. P_g was 41 mm and 80 mm lower in each year than the estimated long-term average P_g of 502 mm year^{-1} (Table 2.3.1). *ET* at the bare site was the lowest of the three measurement sites and was estimated to be 709 ± 158 mm. This translated to 334 ± 68mm in the first year and 375 ± 90mm in the second year of measurement. The net water balance (Q_{wt}) for the bare plot was estimated to be 127 mm for the first year and 46 mm for the second year; however, this surplus of precipitation was not found to be statistically different from *ET* ($p = 0.80$ and $p = 0.67$) (

Figure 2.3.1).

ET losses for the *E. diversifolia* site totalled 1064 ± 269 mm. This equalled 560 ± 159 mm in the first year and 504 ± 110 mm in the second year. Q_{wt} was – 99mm and – 82mm over each of the water balance periods; however, the confidence intervals for *ET* were within the error margins calculated for P_g. Therefore, *ET* was not significantly different from P_g for either water balance year ($p = 0.17$ and $p = 0.46$).

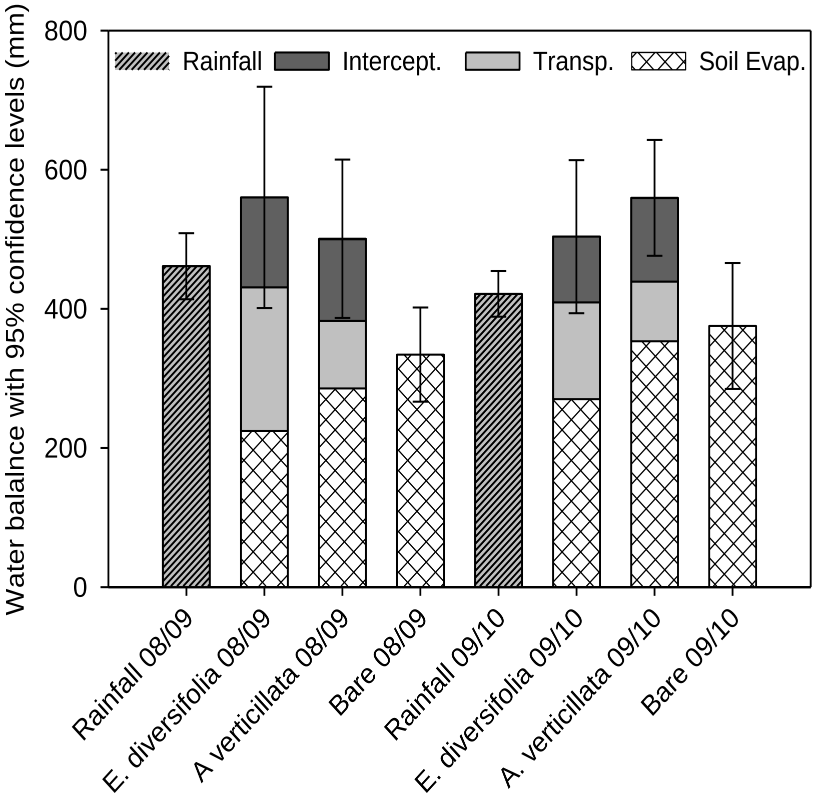

Figure 2.3.1 Components of *ET* (soil evaporation, interception and transpiration) and the volume of rainfall received from July 2008 until July 2010. Error margins indicate 95 % confidence levels.

ET at the *A. verticillata* site was estimated at 1060 ± 197 mm. This volume of water relates to 501 ± 114 mm in the first water balance year and 560 ± 83 mm in the second year. While Q_{wt} equalled – 39 mm and – 138 mm over each water balance year, again the volume of water lost through *ET* was not statistically different from P_g received based on the confidence intervals for both parameters (p = 0.36 and p = 0.24). These results indicate that the overall volume of precipitation received during each of the water balance years was not statistically different from the volume of water being lost through *ET* in each of the three measurement sites.

Table 2.3.1 Plot water balances between 17 July 2008 and 19 July 2010. Rainfall (P_g), potential evapotranspiration (ET_p) (FAO 56), actual evapotranspiration (ET), canopy interception (I), soil evaporation (E) and transpiration (T) residual flux (Q_{wt}). Values are means ± 95% confidence intervals (CI)

		mm							
		P_g	ET_p	ET	ET CI	I	E	T	Q_{wt}
	AVERAGE	**502**	**1524**						
Eucalyptus diversifolia	**TOTAL**	**883**	**3071**	**1064**	**±269**	**224**	**499**	**341**	**-181**
	WB Yr 1	461	1570	560	±159	129	229	202	-99
	WB Yr 2	422	1501	504	±110	95	270	139	-82
Allocasuarina verticillata	**TOTAL**	**883**	**3071**	**1060**	**±197**	**233**	**644**	**184**	**-177**
	WB Yr 1	461	1570	501	±114	113	291	97	-39
	WB Yr 2	422	1501	560	±83	120	353	87	-138
Bare	**TOTAL**	**883**	**3071**	**709**	**±158**	**0**	**709**	**0**	**174**
	WB Yr 1	461	1570	334	±68	0	334	0	127
	WB Yr 2	422	1501	375	±90	0	375	0	47

Statistically significant differences between the vegetated sites (*E. diversifolia* and *A. verticillata*) and the bare site were measured for ET ($p < 0.05$) over both water balance years, with the vegetated sites having higher overall ET than the bare site. However, there was no statistically significant difference in ET between the two tree species ($p = 0.83$) for either water balance period. Soil evaporation at the bare site was significantly higher than that of the *E. diversifolia* plot, but not the *A. verticillata* plot.

Figure 2.3.2 shows the partitioning of gross precipitation into canopy interception, soil evaporation, stemflow and throughfall and was used to estimate the volume of effective precipitation. Using the total volume of rainfall each month, *A. verticillata* canopy interception rates ranged from 9 to 20% of P_g (mean = 14 %; Std dev = 5 %); soil evaporation ranged from 25 to 61 % (mean = 43 %; Std dev = 13 %); stemflow ranged from 1.2 to 5.4 % of P_g (mean = 3.3 %; Std dev = 1.5

%) and throughfall ranged from 29 to 57 % of P_g (mean 39 %; Std dev = 10 %). The rate of canopy interception for *E. diversifolia* varied from 10 – 19 % of P_g (mean = 14%; Std dev = 3%), soil evaporation rates shifted between 25 and 47 % (mean = 39%; Std dev = 7%), stemflow volume was 1.1 – 2.6 % (mean = 1.7 %; Std dev = 0.5 %) and throughfall ranged from 36 to 58% of P_g (mean = 45 %; Std dev = 7 %). The residual volume of P_g after water loss through canopy interception and soil evaporation represents the volume of effective rainfall (P_e), that is, the water contained in the soil profile that is available for soil storage, transpiration or groundwater recharge. P_e was comparable at the *A. verticillata* plot (43% of P_g) and *E. diversifolia* plot (46 % of P_g) over the two year study period. This equates to around 378 mm entering the *A. verticillata* site as effective precipitation and 405 mm effectively entering the *E. diversifolia* site over the study period.

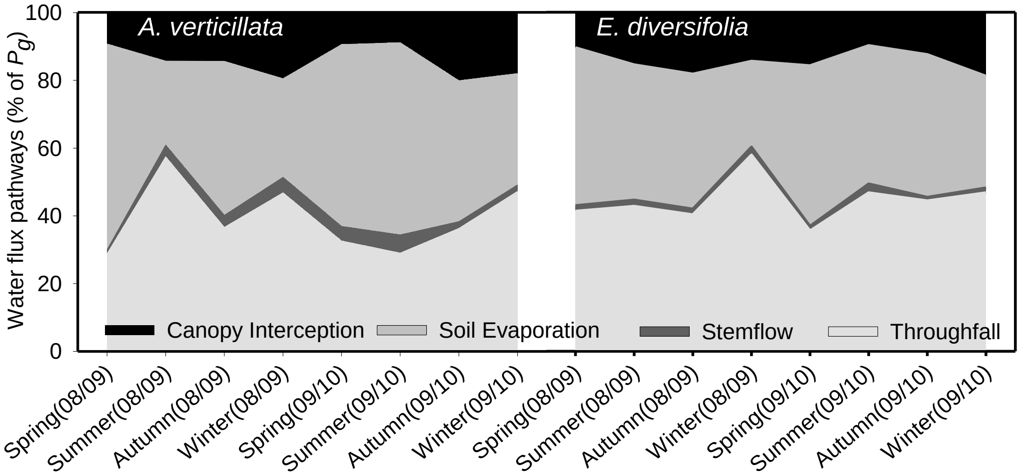

Figure 2.3.2 Relative contribution of canopy interception, soil evaporation, stemflow and throughfall to total water flux (%). The sum of stemflow and throughfall represents the volume of effective precipitation received in each of the vegetated sites.

Tree water sources

The stable isotope ratios of rainfall were highly variable across the two year measurement period (– 8.81 to 0.02 ‰ $\delta^{18}O$, – 60.40 to 10.70 ‰ $\delta^{2}H$), demonstrating the influence by meteorological characteristics and seasonal influences on the precipitation. These results were consistent with Evans (1997) who reported the isotopic signature of Uley Basin rainfall generally ranged between – 9.24 and – 0.28 ‰ $\delta^{18}O$, – 61.90 and – 0.40% $\delta^{2}H$. Groundwater samples show low variation (– 5.02 to – 4.41 ‰ $\delta^{18}O$, – 25.80 to – 24.30 ‰ $\delta^{2}H$) in isotopic values which lie within the majority of the cluster of the rainfall isotope samples and were consistent with winter dominated recharge of rainfall. The Adelaide local meteoric water line (MWL) reported by Evans (1997) also support a meteoric origin for the groundwater samples ($\delta^{2}H = 6.84\ \delta^{18}O + 6.05$).

Twig water samples (– 3.97 to – 1.90 ‰ $\delta^{18}O$, – 28.60 to – 15.90 ‰ $\delta^{2}H$) and surface soil water samples (0.69 to 7.85 ‰ $\delta^{18}O$, – 21.00 to 11.00 ‰ $\delta^{2}H$) were isotopically enriched relative to groundwater and the Adelaide MWL. Surface soils were sampled during late spring and summer, when evaporative demand was high and therefore likely to represent the end-member signature of potential tree water sources (Figure 2.3.3).

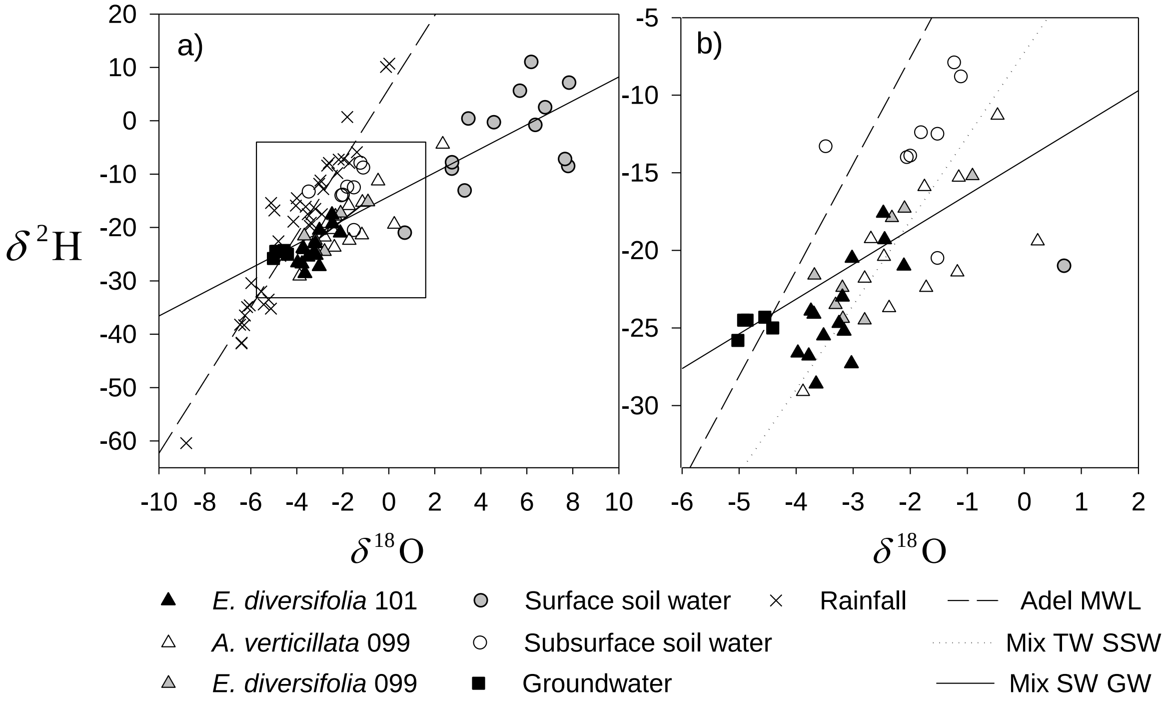

Figure 2.3.3 (a) Stable isotope ratios for all groundwater, surface soil water, subsurface soil water and twig water collected in March 2009, November 2009 and February 2010. Rainfall samples were collected monthly between May 2008 and July 2010. The Adelaide Meteoric Water Line (Adel MWL) is shown as well as a mixing line for twigs and subsurface soil water (Mix TW SSW) and a mixing line between surface soils and groundwater (Mix SW GW). (b) Insert of (a) magnifying the cluster of samples.

This was supported by soil matric potentials ranging from < – 5.0 to – 2.2 MPa. A mixing line described the linear pathway between mean surface soil isotope ratios and mean groundwater isotope values. Another mixing line plotted the linear distribution of twig isotope values for comparison to the soil groundwater mixing line. The twig mixing line suggests a soil water – groundwater mixing scenario. *E. diversifolia* twig water samples (mean ‰ = – 2.86 δ^{18}O, – 22.12 δ^2H) were significantly more depleted (negative) than *A. verticillata* twig water samples (mean ‰ = – 1.57 δ^{18}O, – 17.63 δ^{2}H) ($p = 0.002$).

The IsoSource results showed that in November 2009, the range of feasible source contributions for *E. diversifolia* (101) and *A. verticillata* (099) was not well constrained and, therefore, not particularly informative regarding tree water sources. Groundwater and subsurface soil water dominated twig water signatures for these twig samples. For *E. diversifolia* (101), the 25th and 75th percentiles for possible groundwater use ranged between 27 and 69 %, subsurface soil water was 17 – 61 % and surface soil water was 5 – 18 %. In the *A. verticillata* site (099), the 25th and 75th percentiles for possible groundwater use ranged between 32 and 65 %, possible subsurface soil water was 21 – 63 % and surface soil water was 5 – 13 %. However, for the *E. diversifolia* twig samples on the outside of the *A. verticillata* plot (*E. diversifolia* (099)), the possible combinations were more informative. The 25th and 75th percentiles for groundwater were estimated to be 66 – 85%, possible subsurface soil water was 9 – 30% and surface soil water was 2 – 6% (Figure 2.3.4).

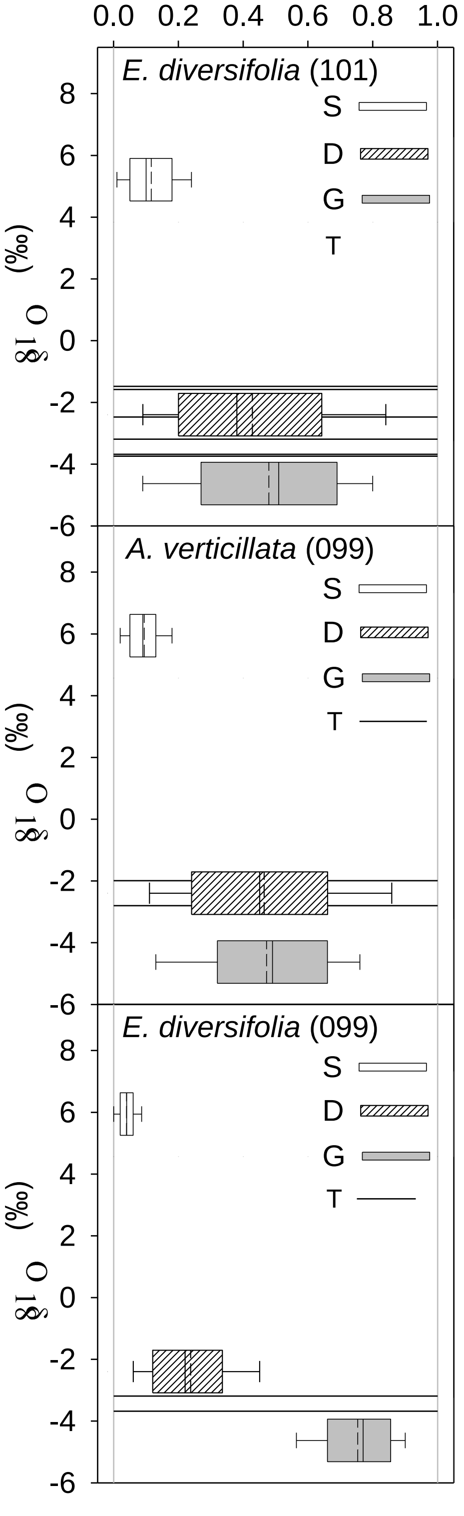

Figure 2.3.4 Feasible twig (*T*) water source combinations for *E. diversifolia* (101), *A verticillata* (099) and *E. diversifolia* (099), based on groundwater (*G*), subsurface soil water (*D*) and surface soil (*S*) samples collected in November 2009. Box plots show the 25th and 75th percentile and median values within. Whiskers show 10th and 90th percentile.

Eucalyptus diversifolia leaf water potentials remained high throughout the study period, recording a minimum Ψ_p of – 1.75 MPa (mean = – 0.94; Std dev = 0.27; n = 18) and Ψ_m of – 2.58 MPa (mean = – 2.06; Std dev = 0.24; n = 18). *A. verticillata* had lower minimum Ψ_p at – 2.35 MPa (mean = – 1.66; Std dev = 0.44; n = 17) and Ψ_m at – 4.03 MPa (mean = – 2.86; Std dev = 0.63; n = 17). The *E. diversifolia* trees located outside the *A. verticillata* plot (i.e. *E. diversifolia* 099) also remained high, with a minimum Ψ_p of – 0.88 MPa (mean = – 0.76; Std dev = 0.13; n= 9) and Ψ_m of – 2.13 MPa (mean = –1.64; Std dev = 0.28; n=9). *E. diversifolia* (099) Ψ_p and *E. diversifolia* (101) Ψ_p were not significantly different from each other; however, there was a significant difference in Ψ_p when compared to *A. verticillata* Ψ_p ($p < 0.05$). *E. diversifolia* (099), *A. verticillata* and *E. diversifolia* (101) all recorded significantly different Ψ_m (Figure 2.3.5).

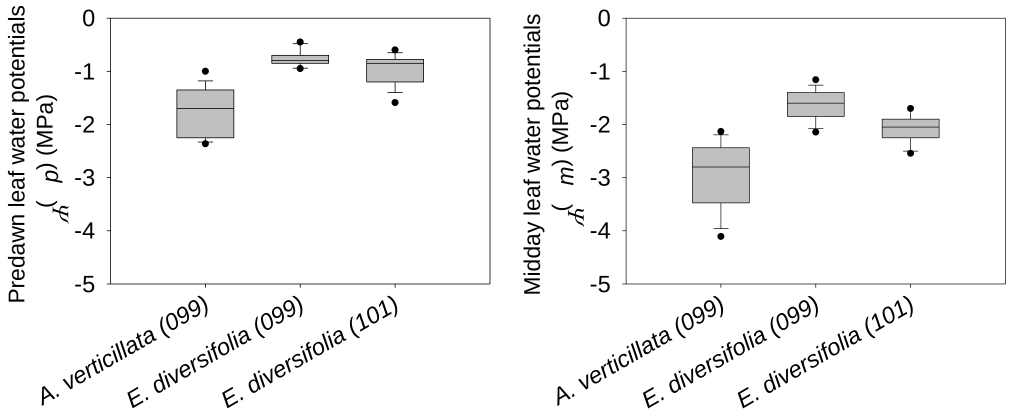

Figure 2.3.5 Leaf water potentials (Ψ) (MPa) for each tree species showing (a) predawn and (b) midday leaf water potentials measured during March 2009, November 2009 and February 2010. *E. diversifolia* (099) represents trees adjacent to the *A. verticillata* site. Box plots extend from the 25th to the 75th percentile with median values within. Whiskers show the 10th and 90th percentiles and symbols represent the 5th and 95th percentile.

Core samples of the karst unsaturated zone collected in November 2009 yielded bulk density values between 1.02 and 2.36 g cm^{-3} and large variation in relative water content (0.02 g g^{-1} to 0.38 g g^{-1}). Matric potentials ranged between – 0.1 and – 0.4 MPa; however, sample size was low (n = 4). Chloride sampling of the substrate confirmed the existence of a low salinity system, with chloride concentrations ranging between 76 and 326 Cl^{-} L^{-1}.

2.4. Discussion

To our knowledge, this is the first study to investigate the water use strategies of *E. diversifolia* and *A. verticillata* and consider the implications of this vegetation on the hydrological characteristics of a karst aquifer. This research combined two years of *ET* estimates with isotope sampling, leaf water potentials and unsaturated zone profiling to present an assessment of the ecohydrological characteristics of this environment.

Evapotranspiration

The components of *ET* – transpiration and evaporation from soil and plant surfaces – varied between the study sites. Soil evaporation contributed the largest volume of water loss for all three plots in this system, but *E* was highest in the bare site, where leaf litter and canopy shade were absent and the site was more exposed to the drivers of *E* (wind velocity, solar radiation, temperature, relative humidity). While the *A. verticillata* plot tended to have lower *E* than the bare site, the variance was not great enough to conclude the difference was statistically significant at the 95% confidence level.

Allocasuarina verticillata had significantly higher *E* compared to the *E. diversifolia* site. This reflected the more open habit of *A. verticillata* woodland (lower *LAI* and stand density than *E. diversifolia*), which would remain well coupled to the atmosphere and therefore influenced (to a larger extent) by atmospheric conditions.

As expected, the bare site had substantially lower *ET* rates for both years in comparison to *E. diversifolia* and *A. verticillata*, consistent with the well-established understanding that forested sites have higher *ET* losses compared to non-forested areas (Walsh *et al*., 1995, Zhang *et al*., 2001). Interestingly, despite the morphological and stand density differences between *E. diversifolia* and *A. verticillata*, the overall *ET* losses from these plots were remarkably similar and approached the estimated long-term average rainfall for the area. This suggested *ET* losses for both vegetated systems had evolved to a maximum threshold in this environment. Evolutionary convergence between *ET* and P_g is not a recently considered concept (Eagleson, 1978, Nemani and Running 1989, Ellis and Hatton 2008) and evident in a range of vegetation settings (Nemani and Running 1989, Zhang *et al*., 2001, Queretjeta *et al*., 2007, Zeppel *et al*., 2008, Ellis and Hatton, 2008, Mitchell *et al*., 2009). This consistency suggests a long-term equilibrium may also be present in Australian karstic environments, which has yet to be reported. These findings have improved confidence in the predictability of tree associations to evolve and adapt to the long-term climatic trends for an area, regardless of species or geological setting. The volume of *ET* reported here was also consistent with the relationships presented in Ellis and Hatton (2008) and O’Grady *et al*., (2011) for a similar volume of precipitation, potential *ET* and *LAI* values.

Convergence in overall ET still contained some interspecies differences within the components of *ET*, as volume of water transpired by *E. diversifolia* was almost double that of *A. verticillata*. Overall, the rate of transpiration for both species was low over the two year study period; averaging 170 mm year^{-1} (0.02 – 2.3 mm day^{-1}) for *E. diversifolia* and an average of 92 mm year^{-1} (0.01 – 1.7 mm day^{-1}) for *A. verticillata*. The rate of transpiration for *E. diversifolia* was comparable to that reported for other mallee eucalypts without access to groundwater (e.g. Wildy *et al*., (2004b), 2.4 mm day^{-1}; Carter and White (2009), 0.9 mm day^{-1}; and Mitchell *et al*., (2009), 0.2 – 0.8 mm day^{-1}). However, I have been unable to find similar reported transpiration rates for *A. verticillata*. Rates of *Casuarina* spp. transpiration appear variable, ranging from 1 mm day^{-1} (Walsh *et al*., 1995) to 3.0 mm day^{-1} (Cramer *et al*., 1999) by *Casuarina glauca*. Sun and Dickinson (1995) estimated *Casuarina cunninghamiana* transpiration at 11 mm day^{-1} in a tropical environment, albeit using only eight days of sap-flow measurements.

The volume of water lost through canopy interception was similar between the tree species despite the variance in stand density and leaf morphology. This finding suggested that the meteorological characteristics associated with precipitation (for example rainfall intensity and duration, wind speed and temperature) had a larger influence on the volume of water intercepted by the trees than did the species-related morphological differences. It was surprising that the distinctive leaf and stand variability did not appear to affect the volume of water intercepted by canopy and stem surfaces; however, this was also consistent with previous studies (Staelens *et al*., 2008, Garcia-Estringana *et al*., 2010).

Water balance

Despite the lower than average rainfall experienced during the study period, Q_{wt} for the bare plot remained positive across both years, indicating that where trees are absent, annual groundwater recharge was likely to continue, including throughout drier than average rainfall periods. Where trees occur, however, annual groundwater recharge was more likely to be restricted to times of above average rainfall conditions, consistent with the findings of Zeppel *et al*., (2008), who reported that groundwater recharge was absent in dry years, but increased significantly during above average rainfall periods. Unfortunately, as both annual sampling periods received rainfall volumes less than the estimated long-term average for the site, I am unable to confirm the occurrence and magnitude of groundwater recharge under these vegetated systems during wetter years. More investigation encompassing the variability in P_g, and subsequent variability in ET, to elucidate any moderating effect the vegetation exerts on the water balance (similar to that reported by Zeppel *et al*., 2006) are required.

Annual Q_{wt} showed net water loss for both vegetated sites, *E. diversifolia* and *A. verticillata*, which may have been sourced from soil water and/or groundwater stores. It was difficult to partition the relative contribution of soil water and groundwater to Q_{wt}, due to the absence of soil moisture monitoring to measure changes to soil water storage. It is possible that the amount of water sourced from soil moisture stores can be reflective of the contributions of rainfall from previous years (Zhang *et al*., 2001), and therefore ET can exceed P_g, provided the water holding capacity of the soil profile is sufficient. If adequate stores in the soil profile do not persist over multiple years, additional water sources must be available for the system to be sustainable.

The water holding capacity of the core drilling samples were highly variable within the karst substrate; the material contained unconsolidated sands, cavities, hard calcrete, sandstone and clay which spanned a range of porosity values. It is plausible these characteristics could be capable of storing considerable volumes of water. Indeed, where porous substrates such as limestone occur, the substrate has been argued to serve a soil-like function by supporting additional volumes of water available to sustain vegetation during dry periods (e.g. Rose *et al*., 2003, Wilding, 1997). Queretjeta *et al*., (2006, 2007) also found trees were reliant on water stored within the unsaturated zone on the seasonally dry karstic Yucatan peninsula and argue the critical role of this water in sustaining tree transpiration requirements during dry periods.

Whether, and to what extent, groundwater may be contributing to moisture availability during drier periods and available for trees to transpire should be considered. Average daily T rates for both species show that the volume of water required to sustain transpiration is relatively low (< 200 mm year^{-1} for *E. diversifolia*, < 100 mm year^{-1} for *A. verticillata*), which is less than the estimated long-term effective rainfall (216 – 231 mm year^{-1}). This suggests two things: (1) that tree water requirements can be met with annual average rainfall, and therefore trees are not net users of groundwater and (2) that groundwater recharge is possible under these vegetation types in times of average or above average rainfall.

Regardless, neither of these scenarios preclude these trees from accessing groundwater over shorter than annual timeframes, notably in summer as soil moisture stores progressively dry out and the monthly Q_{wt} is negative, which can then be replenished in winter in times of excessive water.

Tree water sources

To consider the potential sources of twig water in more detail, examination of the isotope results was required. Generally speaking, the stem water isotope ratios did not present a clear separation of water sources between the species, although *A. verticillata* tended to use more enriched (positive) reserves than *E. diversifolia*. However, considering the nature of the karst substrate, it is unsurprising that the majority of twig water signatures were highly mixed, indicating a blend of water sources. The presence of overlapping twig isotope signatures was consistent with both species using similar, relatively well mixed water sources during the time of sampling.

The IsoSource model demonstrated a wide range of possible combinations of subsurface soil water and groundwater that could have explained the observed twig water signature for *E. diversifolia* (101) and *A. verticillata* (099), suggesting the twig water signatures for all the trees are most likely a mixture of subsurface soil water and groundwater, with very little of the highly fractionated surface soil water contributing to tree water sources. However, the model results were more constrained for *E. diversifolia* (099). These suggested that tree water was being sourced from different locations in the profile, considering *E. diversifolia* (099) grew adjacent, but outside, the *A. verticillata* (099) site.

The variability in isotope results was likely to be, at least in part, due to the variable nature of the karst substrate. The similarity between subsurface soil water and groundwater isotope signatures could be largely the result of high aquifer transmissivity – as the subsurface profile was highly permeable, rapid groundwater recharge often occurs after rain, particularly late in the wet season, such as was the time of sampling. When this occurred, the recent recharge water was essentially recent rainwater / subsurface soil water and would therefore retain a similar signature to water stored in the vadose zone. The variability in geology, and extent of subsurface soil moisture availability, was demonstrated by the drilling results where the karst was shown to contain both highly porous and hard substrates, and also contain perched water tables (one sample returned a relative water content of 0.38 g g^{-1}, prior to drilling into a cave pocket).

Despite generally similar twig water isotopic signatures between *E. diversifolia* (101) and *A. verticillata* (099), the tree species do exhibit different pre-dawn and midday leaf water potentials, and fall within the previously reported range for each genus (Myers and Neales 1984, Mitchell *et al*., 2008b, Carter and White 2009). *A. verticillata* recorded lower leaf water potentials than *E. diversifolia*, including the *E. diversifolia* trees growing just outside the *A. verticillata* plot. While the hydraulic architecture of each species will play a role in influencing leaf water potentials, pre-dawn leaf water potentials are often used as a proxy for soil moisture conditions (Ritchie and Hinckley 1975, Richter 1997). It is reasonable to presume that varying soil moisture gradients arise from varying root depths within a soil profile.

Tree root depth determines the volume from which plants are able to draw water and together with the hydraulic properties of the unsaturated zone determine the volume of plant-available water (Zhang *et al*., 2001).

Root zone separation, supported by the consistently higher pre-dawn leaf water potentials in *E. diversifolia* and different twig isotope results for both tree species associated with the ULE099 observation bore, was further suggested by the difference in transpiration rate during summer. *A. verticillata* showed seasonal *T* patterns, reaching a maxima during spring and declining to a minimum in autumn, presumably before rains replenished the soil profile. *E. diversifolia T* rates showed less seasonality, suggesting water sources were available within their root zone in summer, possibly due to greater depth. Sun and Dickinson (1995) found substantial root pattern variation between young *Eucalyptus camaldulensis* and *C. cunninghamiana* trees, where *C. cunninghamiana* had the majority of its roots within the upper 0.12 m of soil and *E. camaldulensis* reached depths of 1.10 m. In addition, Walsh *et al*., (1995) found the roots of *C. glauca* were distributed within the upper 2 m of the soil profile. By comparison, *Eucalyptus* roots have been known to reach considerable depths; Nulsen *et al*., (1986) reported root systems of mallee-form eucalypt have been found at a depth of 28 m. It may be reasonable to conclude that *A. verticillata*, due to its shallower root system, may be better adapted to accessing limited water stores, maintaining leaf turgor and avoiding xylem embolism compared to *E. diversifolia*.

Exploitation of water reserves by varying root depths of co-occurring trees was consistent with that reported by Schwinning (2008) where tree species exhibited distinct rooting and gas exchange patterns. Rose *et al*., (2003) also reported differences in functional rooting depth across forest tree species which was responsible for differences in stem isotopic signatures. Vertical partitioning of water sources, particularly in times of water deficit, appears to favour tree species co-existence. The depth to groundwater (~ 5 m) would not have precluded *E. diversifolia* from reaching groundwater if required, as mallee-form eucalypts are known to have deep, expansive root systems (Nulsen *et al.*, 1986, Wright and Ladiges 1997). Additional work characterising the root zone distributions of both *E. diversifolia* and *A. verticillata* would improve our ability to elucidate water sourcing characteristics.

2.5. Conclusion

The major driver for this work arose from the need to quantify the *ET* losses of co-occurring tree species growing in the recharge zone of this aquifer whose effect on water yield was not known. These results present the first quantitative assessment of *ET* for a karst – E. *diversifolia – A. verticillata* – bare system and highlight the consistency in overall *ET* for these trees in this system. In addition, the study demonstrates the necessity of considering the water storage capacity of porous geological substrates, and its response to inter- and intra-annual rainfall variability. Clarifying the extent of tree water access to groundwater would be improved by profiling soil moisture content towards the end of the dry season, as soil moisture stores become depleted.

Considering *ET* was significantly higher in both of the forested sites, in comparison to the bare site, the geographical extent of *E. diversifolia* and *A. verticillata* will influence the volume of annual groundwater recharge. Where the trees do not occur, groundwater recharge is likely to continue even during years that are drier than the long-term average. Conversely, where trees do persist in the landscape, average or above average rainfall conditions would be necessary to generate enough moisture for deep drainage. Therefore, the distinction between forested and non-forested vegetation types will be required when considering their effect on rainfall-induced recharge. However, our results show that differentiating between tree species to estimate annual *ET* losses is not warranted for this system. This information will improve the management of this regionally significant drinking water source.

3. Rainfall partitioning across two co-occurring, morphologically distinct tree species in a semi-arid environment[2]

3.1. Introduction

Redistribution of rainfall by vegetation canopies will increase the spatial variability of moisture stores in soil (Crockford and Richardson 1996, Johnson and Lehmann 2006, Liang *et al*., 2007) and can exert strong influence over the ecohydrological processes occurring in forested landscapes (Owens *et al*., 2006). Tree canopies partition precipitation in three ways: (i) interception, collected on plant surfaces and evaporated back into the atmosphere during and after rainfall; (ii) throughfall, which reaches the soil surface after passing through the canopy or dripping from plant surfaces; and (iii) stemflow, which collects on plant surfaces and flows to the ground via trunks and stems.

The relative contribution of each precipitation pathway has been reported to be a function of rainfall characteristics, meteorological conditions, stand and canopy characteristics and tree morphology (Nulsen *et al*., 1986, Crockford and Richardson 2000, Levia and Frost 2003, Levia and Frost 2006, Johnson and Lehmann 2006, Herbst *et al*., 2008). More specifically, abiotic factors related to precipitation intensity and duration (Carlyle-Moses 2004, Reid and Lewis 2009), wind, temperature and vapour pressure deficit (Dunkerley 2000), in combination with biotic factors such as stand density, canopy cover and leaf area index (Carlyle-Moses 2004, Liang *et al*., 2009), branch inclination, bark thickness and

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[2] Swaffer BA, Holland KL, Doody TM, Hutson J. 2014. Rainfall partitioning, tree form and measurement scale: a comparison of two co-occurring, morphologically distinct tree species in a semi-arid environment *Ecohydrology* DOI: 10.1002/eco.1461

leaf shape (Herwitz, 1986, Levia and Herwitz 2005) affect how trees redistribute rainfall.

Reviews attribute the difficulty in generalising rainfall partitioning pathways to the large number of tree and stand variables interacting with a range of meteorological influences at any given time (Crockford and Richardson 2000, Levia and Frost 2003) and the expense of collecting data over long timeframes (Levia and Frost 2006). Despite this, understanding the pathways of rainfall to soil remains an important consideration when quantifying the water balance of a system. Climate change is predicted to bring increasing uncertainty on future water availability (Hughes 2003), placing further pressure on sustainable water resource management, which in turn necessitates advances in decision making tools.

As the contribution of throughfall and stemflow is the main source of soil water for vegetated systems, the ability of trees and shrubs to effectively channel stemflow is particularly advantageous in xeric environments (Pressland 1973, Nulsen *et al*., 1986, Li *et al*., 2008, Wang *et al*., 2011), providing additional water resources (Nulsen *et al*., 1986, Levia and Frost 2003) and nutrients (Johnson and Lehmann 2006) to the plant and acting as a drought avoidance mechanism (Martinez-Meza and Whitford 1996, Li *et al*., 2008). However, the importance of stemflow has often been marginalized as it is volumetrically minor compared with throughfall (Levia and Frost 2003, Llorens and Domingo 2007, Li *et al*., 2008). Indeed, some studies have substituted an estimate of 2% of gross precipitation being channelled as stemflow (e.g. Benyon *et al*., 2006, Doody *et al*., 2009, Holland and Benyon 2010). However, this is in line with most single-stemmed tree and conifer related stemflow volumes being less than 5% of gross precipitation (Llorens and Domingo 2007, Li *et al*., 2009).

Rainfall partitioning studies tend to use a stemflow funnelling ratio as a means to quantify the contributing water from leaf canopy and branches to the trunk (Herwitz 1986) and has been used to measure the effectiveness of a tree's ability to generate stemflow (Carlyle-Moses *et al*., 2004, Li *et al*., 2008, Li *et al*., 2009, Wang *et al*., 2011). A funnelling ratio exceeding one indicates additional water being contributed to stemflow from the outlying portions of the tree canopy (Li *et al*., 2008). Herwitz (1986) reported funnelling ratios of up to two orders of magnitude larger than incident precipitation in a tropical rainforest. Indeed, reported ratios for stemflow funnelling can, in some cases, far exceed the volume of gross precipitation across a given area (i.e. 58, Navar 1993; 153, Li *et al*., 2008; 100, Levia *et al*., 2010).

In spite of the considerable and varied effort aimed at characterizing stemflow, driven largely by a desire to understand its hydrological influence on soils, the results remain highly variable (Figure 3.2.1).Furthermore, stemflow for single-stemmed trees tend to be reported from mesic environments, whereas multi-stemmed trees and shrubs are more prevalent in xeric environments (Table 3.2.1). Figure 3.2.1 also suggests that a measurement scale has an important influence on the final reported stemflow fraction – with studies scaled to individual trees tending to have higher values than studies at a plot scale. The influence of a measurement scale has, thus far, received little attention in the literature.

This study presents measurements of rainfall partitioning and stemflow funnelling for two morphologically distinct tree species growing across the recharge zone of a karst aquifer in a semi-arid environment.

The study had three main objectives: first, to compare the percentage of rainfall partitioned into interception, throughfall and stemflow for the co-occurring, but morphologically distinct, *Allocasuarina verticillata* and *Eucalyptus diversifolia* ssp. *diversifolia*; second, to determine each species' effectiveness at stemflow generation and infer its significance to the availability of soil water; and third, to test whether the influence of a measurement scale (individual canopy *versus* plot) influences the estimated stemflow fraction.

I hypothesised that rainfall partitioning would differ between species in the following ways: (i) the multiple-stemmed habit of *E. diversifolia* would be highly effective at stemflow funnelling and (ii) the rough bark of *A. verticillata* would limit its potential to generate stemflow and results in higher interception of gross precipitation (Figure 3.2.1).On the basis of the review of previous studies (Figure 3.2.1, Table 3.2.1), I also hypothesised that measurement of rainfall partitioning at the individual tree level will result in higher stemflow fractions than at the broader plot scale.

3.2. Methods

Study site

The study was undertaken at Uley South Water Reserve on southern Eyre Peninsula, Australia (34.79°S, 135.54°E). A more detailed site description is given in Swaffer *et al*., (2014). Uley South is an internally draining (Harrington *et* *al*., 2006) fresh groundwater lens with poor, shallow, nutrient deficient calcareous soils (Crocker 1946, Wen 2005, Harrington *et al*., 2006). The groundwater lens is an important drinking water source for the region.

Table 3.2.1 Global review of rainfall partitioning studies demonstrating the influence of tree morphology and measurement method on gross precipitation fractions into throughfall, stemflow and interception

							Annual average (mm)		% of Pg*			
#	**Community type**	**Form**[1]	**Method**[2]	**Length**[3]	**Species**	**Location**	P_g [4]	**PET**[5]	**TF**	**SF**	**I**	**Author(s)**
1	Mattoral shrubland	S	P; C	15 mo	*Acacia farnesianna* , *Calliandra eriophylla* , *Decatropis bicolor* , *Gymnosperma glutinosum* , *Tecoma stans* , *Quercus canbyi*	Mexico	635	*780*	83.3	8.5	8.2	Carlyle-Moses 2004
2	Deciduous woodland (leafed)	T_s	P; T	14 mo	*Quercus robur* and *Betula pubescens* overstorey, *Corylus avellana* understorey	UK	587	680	69.8	0.9	29.3	Herbst *et al* ., 2008
3	Native forest	T_s	P; T	1 - 8 y	*Fitzroya cupressoides*	Chile	3500	781	80.0	9.0	12.0	Huber and Iroumé 2001
		T_s			Mixed broadleaved		2400	981	66.6	1.9	31.5	
	Plantation	T_s		1 - 7 y	*Pseudotzunga menziesii*		2350	1126	60.0	6.0	34.0	
		T_c			*Pinus radiata*		2150	981	75.5	8.5	16.0	
		T_c			*Pinus radiata*		1540	1158	69.0	2.0	29.0	
		T_s			*Eucalyptus nitens*		1540	1158	67.3	3.2	29.5	
		T_c			*Pinus radiata*		1200	1122	74.0	4.0	22.0	
		T_c			*Pinus radiata*		1000	1200	62.5	4.3	33.2	
		T_c			*Pinus radiata*		1000	1211	70.5	2.0	27.5	
4	Woodland tree	T_m	I; T	3 y	*Juniperus asheii*	USA	600	1544	55.0	5.0	40.0	Owens *et al* ., 2006
		T_m			*Juniperus asheii*		900	1485	55.0	5.0	40.0	
5	Deciduous woodland	T_s	P; C, T	12 mo	*Quercus petraea*	Belgium	1044	*744*	-	1.0	-	Andre *et al* ., 2008
		T_s			*Fagus sylvatica*				-	0.3	-	
6	Mallee woodland	T_m	P; C	11 y	*Eucalyptus pileata*, *Eucalyptis eremophila*	Australia	376	1412	92.0	5.0	3.0	Nulsen *et al* ., 1986
		T_m	I; C		*Eucalyptus pileata*, *Eucalyptis eremophila*				60.0	25.0	15.0	
7	Semi-arid shrubs	S	P; C	14 mo	*Anthyllis cytisoides*	Spain	300	1121	40.0	20.0	40.0	Domingo *et al* ., 1998
		S			*Retama sphaerocarpa*				72.0	7.0	21.0	
8	Woodland tree	T_s	P; T	2 y	*Fagus sylvatica*	Belgium	755	701	71.0	8.0	21.0	Staelens *et al* ., 2008
9	Forest	T_s	P; C	3 y	*Sequoia sempervirens* , *Pseudotsunga menziesii*	USA	1285	937	75.1	2.5	22.4	Reid and Lewis 2009
10	Deciduous woodland	T_s	P; C	15 - 18 mo	*Fagus sylvatica*, *Tilia sp*, *Fraxinum excelsior*, *Acer*	Germany	544	742	70	2.2	27	Krämer and Hölscher 2009
11	Semi-arid shrubs	S	P; C	3 mo	*Diospyrus texana*	Mexico	740	*2200*	-	5.1	-	Navar 1993
		S			*Acacia farnesiana*				-	0.8	-	
		S			*Propsopsis laevigata*				-	1.0	-	
12	Desert shrub	S	I; S	-	*Flourensia cernua*	Mexico	283	1796	-	45	-	Mauchamp and Janeau 1993
		S			*Flourensia cernua*				-	4	-	
13	Subtropical open forest	T_m	I; C	16 mo	*Acacia aneura*	Australia	496	1668	69.0	18.0	13.0	Pressland 1973

#	Community type	Form^1	Method^2	Length^3	Species	Location	Annual average (mm)		% of Pg*			Author(s)
							P_g^{4}	PET^5	TF	SF	I	
14	Red oak forest	T_s	P; C	9 mo	*Quercus canbyi* , *Quercus cupreata*	Mexico	635	1581	84.2	-	-	Carlyle-Moses *et al* ., 2004
15	Arid to semi-arid shrubs	S	I; T	10 mo	*Tamarix ramosissima*	China	263	1099	-	2.2	-	Li *et al* ., 2008
		S			*Caragana korshinskii*				-	3.7	-	
		S			*Reaumuria soongorica*				-	7.2	-	
16	Semi-arid shrubs	S	I; C	12 mo	*Salix psammophila*	China	395	1046	-	6.3	-	Li *et al* ., 2009
		S			*Hedysarum scoparium*				-	3.4	-	
17	Semi-arid open woodland	T_s	P; C	18 mo	*Eucalyptus capillosa*	Australia	361	1435	-	3.2	-	Mitchell *et al* ., 2009
18	Arid shrubland	S	I; C	4 mo	*Caragana korshinkii*	China	191	1112	-	8.0	-	Wang *et al* ., 2011
19	Montane tropical forest	T_s	P; C	18 mo	*Balanops australiana*	Australia	6500	1392	-	0.11	-	Herwitz *et al.* , 1986
		T_s			*Cardwellia sublimis*				-	0.04	-	
		T_s			*Ceratopetalum virchowii*				-	0.13	-	
		T_s			*Elaeocarpus foveolatus*				-	0.26	-	
		T_s			*Elaeocarpus sp.*				-	0.04	-	
20	Tropical rainforest	SNR	P; C	5 y	Sedimentary plain	Columbia	3100	1623	87.2	0.85	11.8	Marin *et al* ., 2000
					High terrace				86.7	0.94	12.2	
					Low terrace				85.8	1.45	12.9	
					Floodplain				81.9	1.12	17.2	
21	Dry sclerophyll forest	T_s	P; C	7 y	*Eucalyptus rossii, Eucalyptus mannifera, Eucalyptus macrorhyncha, Eucalyptus meliodora*	Australia	650	1413	84.5	4.1	11.4	Crockford and Richardson 1990 a, b, c
	Plantation	T_c			*Pinus radiata*				72.8	8.9	18.3	
22	Desert shrubs	S	I; C	7 mo	*Larrea tridentata*	USA	230	*2290*	56.8	10.0	-	Martinez-Meza and Whitford 1996
					Prosopis glandulosa				62.8	5.4	-	
					Flourensia cernua				55.8	10.6	-	
23	Semi-arid shrubs	S	P; C	12 mo	*Acacia rigidula, Condalia hoockeri, Bumelia celastrina, Cordia boissieri, Pithecellobium pallens, Diospyrus palmeri, Zanthoxylum fragara, Prosopsis laevigata*	Mexico	805	*1150*	78.1	3.0	18.9	Návar *et al.* , 1999
24	Tropical rainforest	T_s	P; C, T	2 mo	*Orbignya phalerata*	Brazil	2300	1785	-	8.0	-	Germer *et al* ., 2010
25	Arid shrubland	S	I; T	6 mo	*Caragana korshinskii*	China	191	*2500*	-	8.8	-	Zhang *et al* ., 2013
					Artemisia ordosica				-	2.8	-	
26	Arid shrubland	S	I; S	-	*Atriplex vesicaria*	Australia	255	1441	-	-	5.8	Dunkerley and Booth 1999
		S			*Maireana sedifolia*				-	-	1.1	
27	Bamboo forest	T_s	P; C	12 mo	*Phyllostachys pubescens*	Japan	1790	1008	85.8	4.0	10.2	Shinohara *et al* ., 2013
28	Mountainous Mediterranean forest	T_s	P; T	3 y	*Quercus pubescens, Buxus sempervirens*	Spain	862	*823*	79.5	1.7	19.3	Mużyło *et al* ., 2012

							Annual average (mm)		% of Pg*			
#	Community type	Form[1]	Method[2]	Length[3]	Species	Location	P_g[4]	PET[5]	TF	SF	I	Author(s)
29	Lower montane tropical forest	T_s	P, C	13 mo	Primary forest (e.g. *Hampea appendiculata* , *Virola sebifera*)	Panama	2250	1493	82.7	2.6	-	Macinnis-Ng *et al* ., 2012
		T_s			Secondary forest (e.g. *Hampea appendiculata* , *Virola sebifera*)				81.1	1.2	-	
30	Mediterranean shrubs	S	I; S	-	*Dorycnium pentaphyllum*	Spain	409*	1098*	-	21	-	Garcia-Estringana *et al* ., 2010
		S			*Medicago strasseri*				-	3.8	-	
		S			*Colutea arborescens*				-	17.3	-	
		S			*Retama sphaerocarpa*				-	9.7	-	
		S			*Cistus ladanifer*				-	5.6	-	
		S			*Cistus albidus*				-	20.8	-	
		S			*Rosmarinus officinalis*				-	23.2	-	
		S			*Lavandula latifolia*				-	25.7	-	
		S			*Cytisus zeelandia*				-	26.4	-	
31	Semi-arid, sub-tropical thornscrub	S	P; C	18 mo	*Acacia rigidula*	Mexico	740	1735	-	3.3	-	Návar 2011
		S			*Pithecellobium pallens*				-	3.4	-	
		T_m			*Diospyros texana*				-	2.7	-	
		S			*Cordia boissieri*				-	2.1	-	
		S			*Bernarida celastrina*				-	1.1	-	
		S			*Condalia hookeri*				-	1.0	-	
	Temperate pine-oak forest	T_s			*Quercus spp.*		640	*750*	-	0.8	-	
		T_c			*Pinus pseudostrobus*				-	0.3	-	

[1] T_s = single stemmed tree, T_m = multi-stemmed tree, T_c = conifer, S = shrub

[2] Method (spatial scale and temporal water flux): P = plot scale or I = individual plant measurements; T = tipping bucket, S = simulated rainfall or C = cumulative collectors

[3] Length of observation; y = years, mo = months

[4] Average if multiple study plots or periods presented for a given P_g

[5] Reported PET is noted using italics. Alternative values taken from Trambucco and Zomer (2009)

SNR = Species not reported, plotted as T_s

* Not reported, used data based on affiliation location

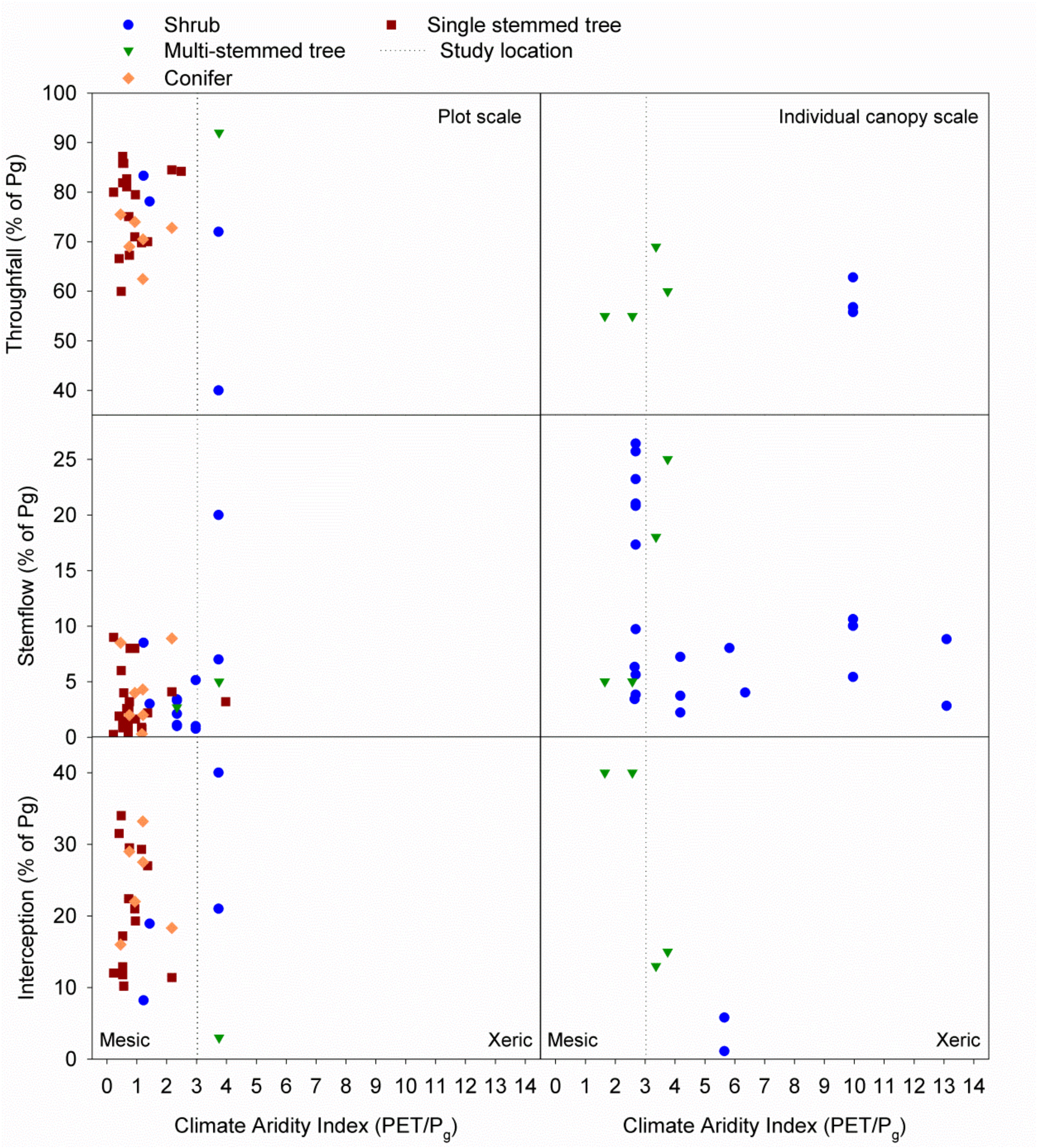

Figure 3.2.1 Collection of rainfall partitioning results from 31 studies across a global scale (data from Table 3.2.1) plotted along a climate aridity index. Tree form (single-stemmed tree, multi-stemmed tree, conifer, shrub) and scaling method (plot scale *versus* individual canopies) are shown.

Study species

Three 20 x 20 m plots were established to characterise rainfall partitioning of three different types of cover; (i) a stand of *E. diversifolia* ssp. *diversifolia* Bonpl.

(coastal white mallee); (ii) a stand of *A. verticillata* (Lam.) L. Johnson (drooping sheoak); and (iii) an open control area. Both species are common to the region, with *E. diversifolia* growing on coastal and sub-coastal areas across southern Australia, often on consolidated calcareous sands (Wright and Ladiges 1997). *A. verticillata* extends across south-eastern Australia and is associated with skeletal or shallow soils frequently derived from sandstone or limestone (Florabank 2011). The important morphological differences are *E. diversifolia* has a smooth bark, the multiple-stemmed habit characteristic of the mallee eucalypt and an umbrella-shaped canopy with true leaves. *A. verticillata* has a rough bark, single stemmed and its canopy comprises of long, thin, vertically hanging phyllodes (Figure 3.2.2).

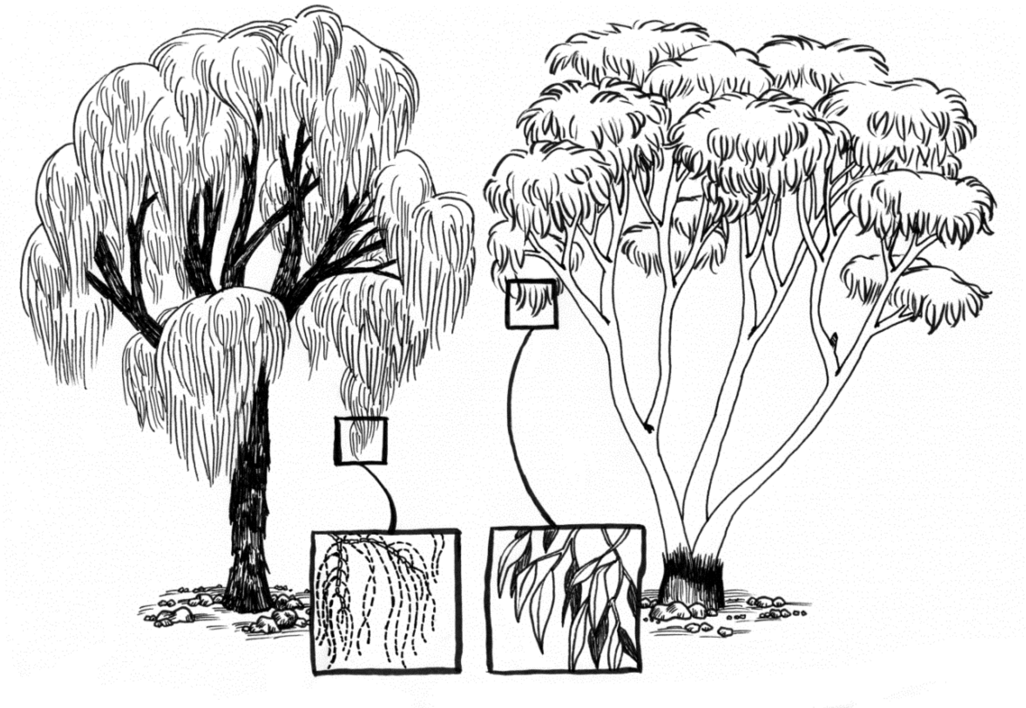

Figure 3.2.2 Line drawing demonstrating the morphological differences between *Allocasuarina verticillata*, which possesses vertical, thin phyllodes, rough bark and a single trunk (left), and *Eucalyptus diversifolia* ssp. *diversifolia*, which has smooth bark, true leaves and a multiple-stemmed habit (right).

The mass balance equation used to quantify rainfall partitioning volumes was:

$$P_g = I + TF + SF$$

Where

P_g = Gross precipitation, collected in an open adjacent area (mm),

I = Interception (mm),

TF = Throughfall (mm),

SF = Stemflow (mm).

Precipitation

Gross precipitation was measured by four randomly located rain gauges in the nearby open control plot. The volume of gross precipitation received over each measurement period was determined by averaging the four gauges. Net precipitation (P_n) was determined by summing the relative contribution of TF and SF to represent the volume of rainfall reaching the soil surface beneath the tree canopy. This indirect measurement of canopy interception was used as direct measurement of I is difficult and most often calculated as the difference between P_g and P_n (Huber and Iroumé 2001, Carlyle- Moses 2004, Staelens *et al*., 2008).

Throughfall

Throughfall was measured by eight randomly located galvanised steel troughs in each of the vegetated plots. Each trough had a horizontal projection area of 0.1716 m^2 (1.2 m x 0.143 m). Litres of TF collected were converted into millilitres of precipitation by dividing the litres (l) collected by the trough projection area (m^2). Plot throughfall was estimated by averaging the volumes from the eight *TF* troughs.

Stemflow

Stemflow was measured by splitting 16 mm internal diameter plastic tubing and winding it twice around larger tree size classes (DBH > 50 mm) and trimmed plastic funnels to < 20 mm for smaller-sized (DBH < 50 mm) trees. Approximately 10% of *E. diversifolia* stems in the plot (n = 9) and half of *A. verticillata* trees in the plot (n = 6) were instrumented for *SF*. Those stems instrumented for *SF* collection were selected by dividing all trees into three equal $BOAB_t$ size classes and randomly selecting an equal number of trees within each class. Stemflow instrumentation was sealed to the stem surface using staples and silicone, following Crockford *et al*., (1996). Plot *SF* (SF_p) was estimated using the volume of *SF* collected by each of the instrumented stems, then relating the basal area of instrumented stems to total plot basal area, following the upscaling method described by Mitchell *et al*., (2009). Individual tree *SF* (SF_i) was estimated by dividing the volume of water collected by an instrumented stem by its corresponding canopy cover.

Stemflow funnelling ratio

A stemflow funnelling ratio was used to quantify the ability of each stem instrumented with *SF* collectors to harvest precipitation falling on their canopies. The ratio determines the extent to which the outer lying branches spatially concentrate water to the tree bole (Herwitz 1986). The funnelling ratio (*F*) is calculated by

$$F = \frac{SF_v}{BAOB_t \times P_g}$$

Where

SF_v = Stemflow volume (L),

$BAOB_t$ = Basal area over bark for the tree (m^2), calculated from *DBH* measurements (cm),

P_g = Gross precipitation (mm).

The product of $BAOB_t$ and P_g is the volume of water that could be expected in a rain gauge occupying an area equivalent to the cross-sectional area of the instrumented stem. The funnelling ratio represents the volume of precipitation that has reached the ground compared to the volume if the tree was not present, therefore taking into account the influence of the canopy and branches. A funnelling ratio > 1 indicates that the canopy components are contributing to the volume of stemflow generated.

The contributing area of the canopy (C) (m^2) that has influenced the rate of stemflow generation can be calculated by

$$C = \frac{SF_v}{P_g}$$

This determines the effective contributing area (m^2) of each tree crown that has resulted in the generation of stemflow (Herwitz 1986).

Statistical analysis

Descriptive statistics characterised the volume of precipitation being partitioned into throughfall, stemflow and interception for the two vegetation types over the 2-year measurement period. Linear regressions compared gross precipitation volumes to each of the rainfall partitioning pathways. Statistically significant differences (at the $p < 0.05$ level) between both the tree species and the rainfall partitioning fractions were tested using t-tests, after ensuring assumptions for normality, homoscedacity and equal variance were observed. All analyses are performed using SigmaPlot 11.0 (Systat Software Inc., USA).

3.3. Results

Rainfall

During the 2-year measurement period, 883 mm of rainfall was recorded in the standard rain gauges located in the nearby open control plot. The majority of this precipitation was received during the cooler months, when evaporative demand was lowest (Figure 3.3.1).The volume of rainfall received during each year (462 mm and 421 mm respectively) was lower than the estimated annual long term rainfall (502 mm).

This was characteristic of the weather patterns received over much of southern Australia where severe drought conditions, attributed to El Niña-Southern Oscillation phenomena, saw major reductions in rainfall preceding, and during, the study period (Ummenhofer *et al*., 2011).

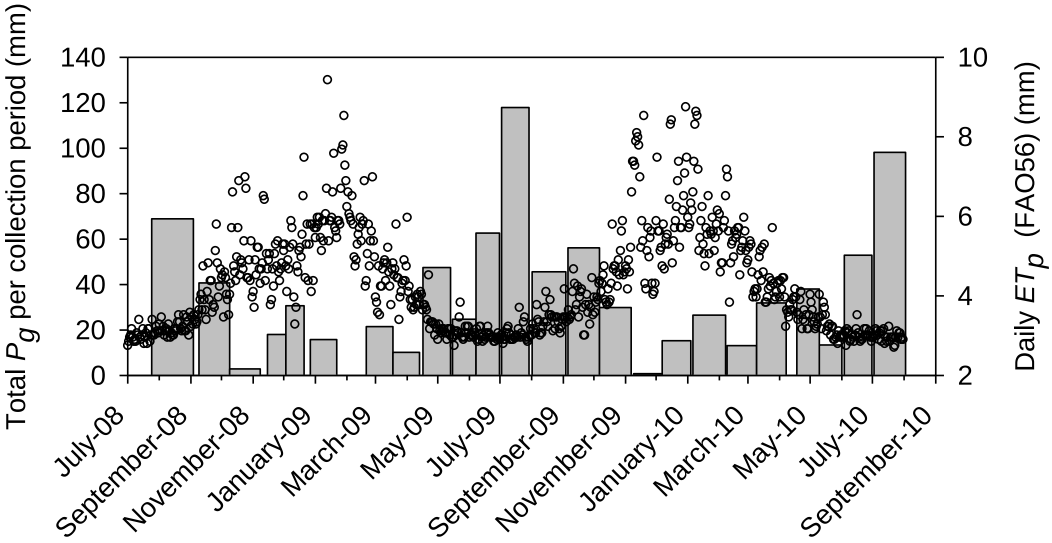

Figure 3.3.1 Total volume of rainfall received during each download period (approximately monthly) (P_g) (mm). Rainfall was measured using standard rain gauges (columns), and daily potential evapotranspiration (ET_p) (FAO56) (open circles) available through the Bureau of Meteorology (2010).

Tree Basal Area and Leaf Area Index

The stand of *E. diversifolia* contained more tree stems than the *A. verticillata* plot (83 *versus* 12 tree stems) however plot basal area, though statistically significant, was not remarkably different. Plot basal area over bark ($BAOB_p$) for *E. diversifolia* increased from 14.9 m^2 ha^{-1} to 15.8 m^2 ha^{-1}, and *A. verticillata* $BAOB_p$ increased from 11.3 m^2 ha^{-1} to 12.0 m^2 ha^{-1} over the 2-year study period.

The *A. verticillata* stand tended to be more open (average leaf area index = 0.48, std dev = 0.15, n = 7) than the *E. diversifolia* stand whose canopy was more dense (average leaf area index = 1.32, std dev = 0.17, n = 7). Tree basal area for both *E. diversifolia* and *A. verticillata* correlated strongly to projected canopy area

(Figure 3.3.2, $r^2 = 0.90$, std err = 4.52), confirming that $BAOB_t$ was an appropriate variable to substitute for canopy size when estimating stemflow generation.

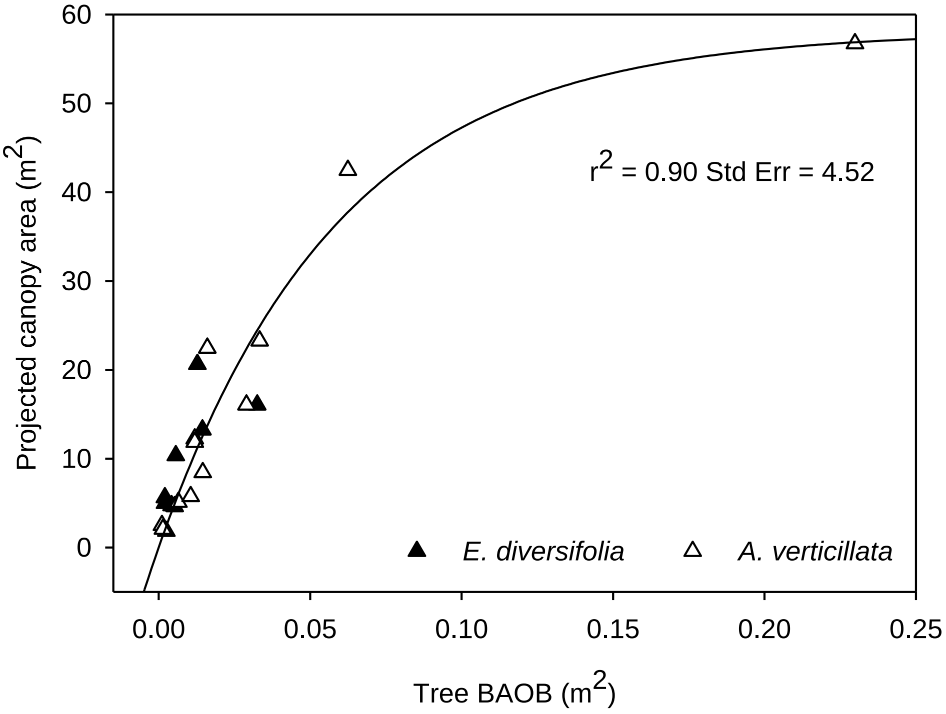

Figure 3.3.2 Relationship between tree basal area ($BAOB_t$) (m^2) and canopy projection area (m^2) for individual *Eucalyptus diversifolia* and *Allocasuarina verticillata* trees ($r^2 = 0.90$, standard error = 4.52).

Stemflow, Interception, Throughfall

Plot stemflow (SF_p), as expected, was volumetrically minor in comparison to the other two precipitation pathways; throughfall and interception. As a percentage of cumulative gross precipitation each month, SF_p ranged between 0 – 7.8 % (mean = 2.7 %, std err = 0.34) for *E. diversifolia* and 0 – 6.1 % (mean = 2.7 %, std err = 0.34) for *A. verticillata*. Throughfall was the largest and most consistent precipitation pathway for both tree species varying 4 – 84 % of P_g (mean = 66.4 %, std err = 4.2) for *E. diversifolia* and 16 – 93 % of P_g (mean = 65.9 %, std err =

3.8) for *A. verticillata*. Interception was the second largest water flux; the rate of P_g intercepted by canopies ranged between 11 – 95 % (mean = 30.9 %, std err = 4.3) for *E. diversifolia* and 3 – 84 % (mean = 31.4 %, std err = 3.9) for *A. verticillata* (Figure 3.3.3 a, c, e).

While variation in rainfall partitioning pathways between the trees was evident over shorter timeframes, Figure 3.3.3 a, c, e the pathways converge over 2 years of measurement. Throughfall median, 25th and 75th percentile values were similar for *E. diversifolia* (23, 10 and 37 mm) and *A. verticillata* (20, 9 and 37 mm) respectively (Figure 3.3.3 b). Similarly, interception median, 25th and 75th percentile values for *E. diversifolia* were 6, 4, and 11 mm and for *A. verticillata* were 8, 3 and 13 mm (Figure 3.3.3 d). Even SF_p volumes, which were the most variable within the stand for each measurement period, demonstrated little variation when all results were combined. *Eucalyptus diversifolia* median, 25th and 75th percentiles for stemflow were measured to be 0.8, 0.4 and 1.4 mm and for *A. verticillata* median, 25th and 75th values were 0.9, 0.4 and 1.4 mm (Figure 3.3.3 f).

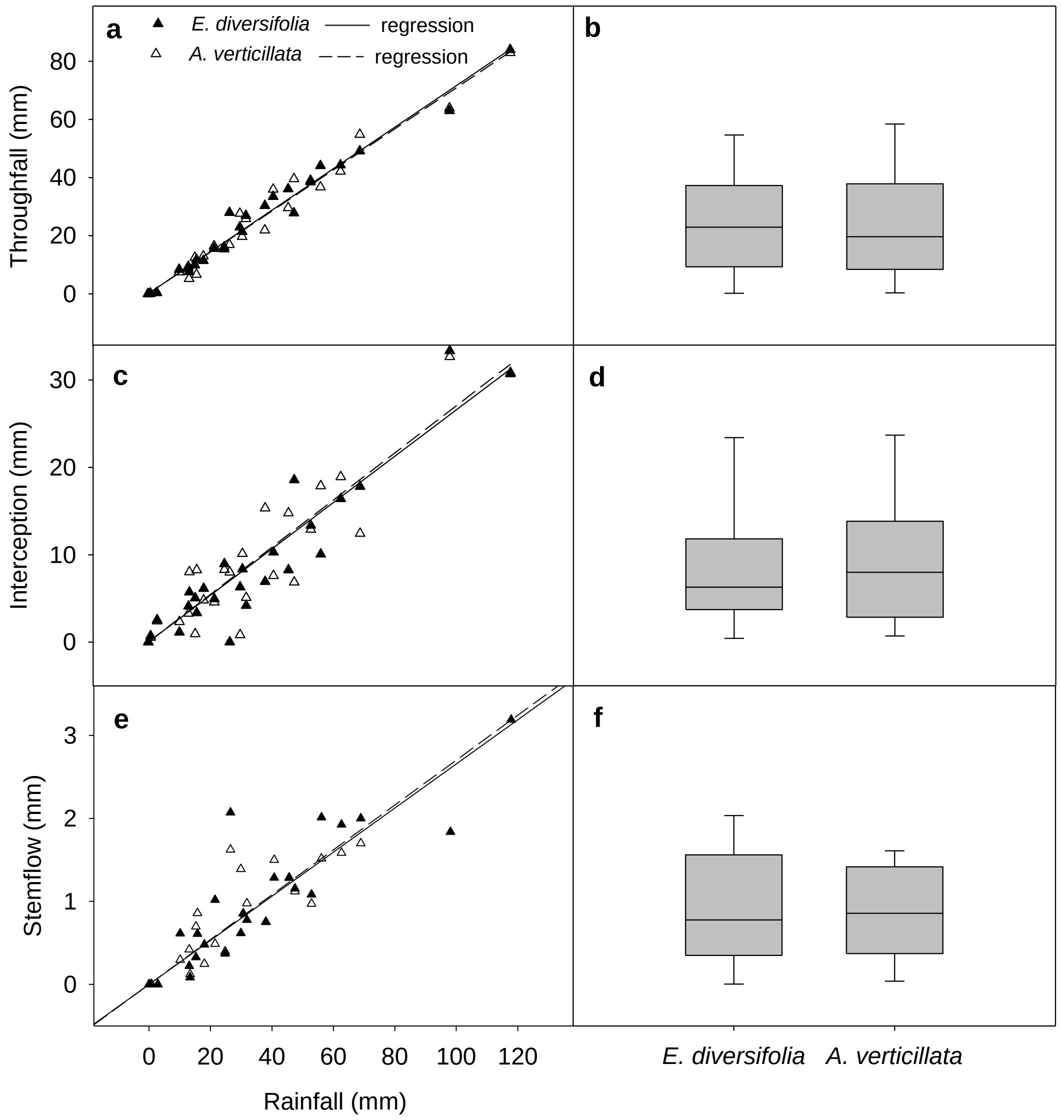

Figure 3.3.3 Relationship between ~ monthly total rainfall (mm) received and (a) total throughfall (mm), (c) total interception (mm) and (e) total stemflow (mm) for *E. diversifolia* and *A. verticillata* over the study period. Box plots depict median, 25th and 75th percentile comparisons for both tree species for (b) throughfall, (d) interception and (f) stemflow. Whiskers show 5th and 95th percentiles.

Stemflow funnelling ratio

Stemflow funnelling ratios were variable within both the *E. diversifolia* and *A. verticillata* plots, suggesting that some trees were more effective at channelling stemflow than others (Table 3.3.1). Average stemflow funnelling ratio for *E. diversifolia* stems ranged between 2 and 74, resulting in 51 and 133 L of water delivered to the tree boles respectively. In the *A. verticillata* stand, the stem funnelling ratios ranged between 10 and 147 which represented 497 and 179 L of channelled water, respectively, demonstrating that the tree or stem with the highest funnelling ratio did not necessarily channel the greatest volume of water. Tree basal area was an important variable influencing the ability to funnel precipitation effectively, with larger instrumented stems tending to be less effective at funnelling water down tree trunks (Figure 3.3.4).The *E. diversifolia* relationship between $BAOB_t$ and stemflow funnelling ratio was weaker ($r^2 = 0.55$) than the relationship for *A. verticillata* ($r^2 = 0.98$).

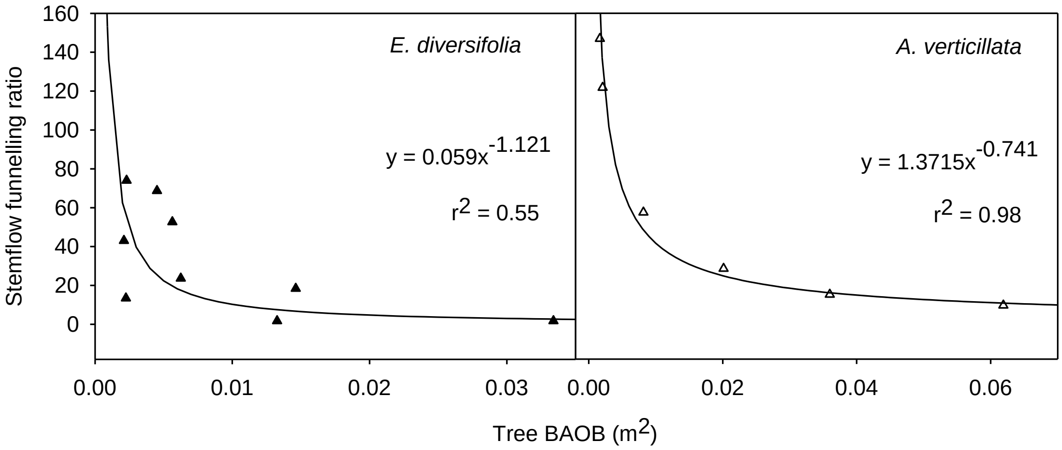

Figure 3.3.4 Relationship between tree size and average stemflow funnelling ratio for *Eucalyptus diversifolia* and *Allocasuarina verticillata* using total rainfall (mm), stemflow volume (L) and tree size (m^2) over the study period.

Table 3.3.1 Total rainfall received over the two year study period, with total stemflow volume (l) and overall funnelling ratio (F) for each of the instrumented stems, including corresponding stem basal area (m^2) and contributing canopy area (m^2)

	Eucalyptus diversifolia subsp. *diversifolia*									*Allocasuarina verticillata*					
TreeID	**T33**	**T45**	**T57**	**T11**	**T71**	**T62**	**T65**	**T4**	**T27**	**T12**	**T10**	**T9**	**T1**	**T7**	**T8**
Contributing area (m^2x10^{-2})	5.8	26.9	17.0	31.0	9.0	29.6	3.0	14.8	2.3	54.6	59.5	46.9	57.4	24.5	25.4
Rainfall (mm)	883	883	785	883	883	883	853	838	838	782	835	817	817	730	817
Stemflow (L)	51	238	133	274	80	262	26	124	19	427	497	383	469	179	207
Funnelling ratio	**2**	**18**	**74**	**69**	**43**	**53**	**13**	**24**	**2**	**15**	**10**	**57**	**29**	**147**	**122**
n	23	23	22	23	23	23	22	22	22	20	21	20	20	19	20
BAOB (m^2x10^{-2}) Aug 2008	3.31	1.47	0.20	0.41	0.19	0.54	0.22	0.54	1.28	3.41	5.84	0.72	1.76	0.12	0.17
BAOB (m^2x10^{-2}) July 2010	3.37	1.45	0.26	0.49	0.23	0.59	0.23	0.71	1.37	3.78	6.54	0.92	2.27	0.21	0.25

* The volume of rainfall (mm) and number of measurements (*n*) varies due to exclusion of data when collection containers overflowed

These relationships were used to estimate the volume of P_g that could have been expected to be channelled as *SF* for all of the stems within each plot (Table 3.3.2). Overall, a volume of 8542 L is estimated to have travelled as stemflow into the *E. diversifolia* site over the duration of the study. I note that the estimated stemflow funnelling ratio maximum is high for *E. diversifolia* (701), and far exceeds the maximum measured ratio of 74. This is due to the size of the smallest stem in the plot ($BAOB_t$ < 3 cm^2) and the nature of the exponential decay regression shown in Figure 3.3.4. A total volume of 5377 L was estimated to have entered the plot as *SF* for the *A. verticillata* site over the duration of the study period for all 12 stems within the plot.

Table 3.3.2 Estimated stemflow funnelling ratio and volume of stemflow generated in the study site scaled to a plot estimate using the regression from Figure 3.3.4 and volume of rainfall received.

Species	P_g	**Stem**	$BAOB_t$		F		***SF***	***SF***
	mm	n	$m^2 \times 10^{-2}$	$m^2 \times 10^{-2}$	Ratio		L	kL ha^{-1}
			min	max	min	max		
E. diversifolia	883	84	0.02	7.74	1	701	8542	214
A. verticillata	883	12	0.19	22.64	4	141	5377	134

Scaling stemflow

The effect of using either individual plant canopies or plot basal area (SF_i *versus* SF_p) to estimate annual *SF* % is evident in Figure 3.3.5. The annual SF_p fraction

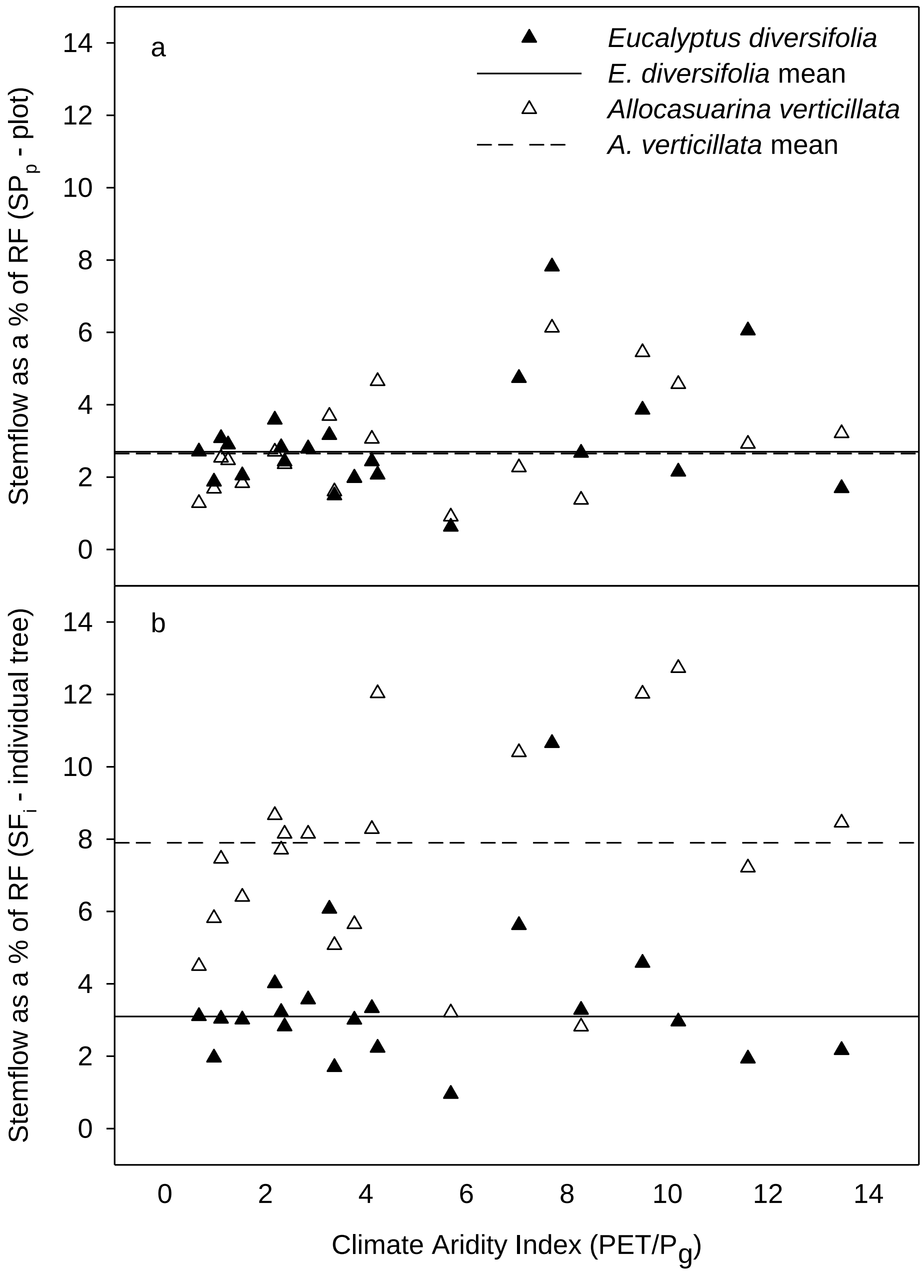

Figure 3.3.5 Difference in annual SF % of P_g when calculated using (a) a plot scale or (b) individual plant canopies. Plot scale (400 m^2) results in both species averaging 2.7 % of P_g as *SF*. Individual plant canopies increase *Eucalyptus diversifolia SF* average to 3.1 % and *Allocasuarina verticillata* to 7.9 %

of 2.7% is derived using a plot scale of $400m^2$ (20 x 20 m). However, when the volume of SF_i (l) is compared with the volume of rainfall falling upon a tree canopy (m^2), the percentage channelled as stemflow was higher. *Eucalyptus* *diversifolia* SF_i fraction was 3.1%, and for *A. verticillata*, the average SF_i fraction was 7.9%. This suggests that *SF* % will be higher when reported using individual canopy studies, in comparison with reporting at a plot scale.

3.4. Discussion

Partitioning of rainfall into interception, throughfall and stemflow continues to receive attention at a global scale, which is likely to continue as pressure on water resources intensifies. Our review of rainfall partitioning studies highlighted the importance of climate, tree form and experimental design on the distribution of rainfall partitioning fractions. I tested this observation by measuring the pathways of water flow across two common, co-occurring but morphologically distinct tree species in a semi-arid karstic environment. Furthermore, the sensitivity of spatial scale when converting volumetric *SF* to millimetre using two spatial extents (individual plant canopy *versus* plot) was assessed. I hypothesised the rainfall partitioning results would reflect the variance in stand and tree characteristics, with the multiple-stemmed habit and smooth bark of *E. diversifolia* being highly effective at stemflow funnelling. I also expected that converting volumetric stemflow using a plot scale would result in lower overall *SF* % than would be reported at the individual tree level.

I found that, contrary to expectation, the relative contributions of throughfall, stemflow and interception were less variable than expected between the two tree species. This is consistent with the review of Levia and Frost (2006) who note that inter-event variability tended to be larger than that between stands or vegetation types. Over the measurement period at the plot scale, *E. diversifolia* and *A. verticillata* recorded rainfall partitioning results that were close to parity (and consistent with Marin *et al*., 2000, Krämer and Hölscher, 2009), despite the morphological and stand differences between the species. Increased bark thickness and roughness did not appear to influence stemflow volumes, despite evidence suggesting that high bark storage capacities will limit stemflow generation (Levia and Herwitz, 2005). I suggest this finding may be due to the lower stand density and open canopy of *A. verticillata*, which would increase tree and trunk exposure to rain and result in more rapid saturation, in comparison with the denser *E. diversifolia* stand.

The stemflow funnelling ratios demonstrate that stemflow was capable of providing significant volumes of water to an individual tree or stem, which has important implications for tree water sources, biogeochemical cycling and ecosystem processes (Levia and Frost, 2003). Although the relationship was weaker for *E. diversifolia*, the results indicate that it is possible to estimate stemflow funnelling ratios based on stem diameter. I attribute this variability in the *E. diversifolia* relationship to the recurrence of bark shedding, creating drip points and irregular trunk surfaces.

The ability of these trees to funnel substantial water volumes to tree boles is likely to exert a positive effect on the moisture stores available within the porous karst substrate. This storage would assist to prolong tree transpiration into the drier summer months, with water infiltration at least partly depending on rooting depth (Allison and Hughes, 1983, Nulsen *et al*., 1986, Wang *et al*., 2011) and is likely to be a key component of the hydrologic processes in this environment.

Karst is a geological formation where highly soluble materials dissolve to form a complex network of cracks, sinkholes, caves and dissolution features

(Schwinning, 2008). The highly heterogeneous nature of karst systems is reported to be capable of storing considerable quantities of water (Einsiedl, 2005) that could sustain tree transpiration requirements (Nulsen *et al*., 1986, Querejeta *et al*., 2007, Wang *et al*., 2011). Numerous studies have suggested stemflow as a means by which plants are able to uncouple themselves from short-term climatic variability (Specht, 1957, Martinez-Meza and Whitford, 1996, Carlyle-Moses 2004).

Although the percentage of gross precipitation channelled as stemflow appears to be hydrologically minor, and exerts little influence on the regional water balance, its ecological importance at the tree scale, particularly in karst environments, should not be overlooked. Our findings support the assumption that stemflow contributions are generally low (in the order of 2 – 3% of precipitation). Although measured stemflow data are required when considering the ecohydrological significance of, for example, stemflow funnelling ratios or nutrient influx, this study demonstrates that substituting 2 – 3% of gross precipitation may be appropriate for many woodland environments.

The data has improved our ability to predict the pathways of water flow at the stand level and enhanced our understanding of the role of stemflow in providing moisture to individual trees (Garcia-Estingana *et al*., 2010). Although variation over shorter timeframes, i.e. monthly was evident, the similarities in annual pathways of water flow across these morphologically distinct species increase our confidence in predicting rainfall redistribution across broader spatial and temporal scales.

This study showed that the calculation of *SF* % in low density vegetation is highly sensitive to the upscaling method with higher rates of *SF* reported when using individual canopy cover, rather than plot area. This is consistent with that reported by Nulsen *et al*., (1986), who report *SF* of 25 % at the individual tree level and 5 % at the plot scale. Despite the significance of this early observation, rainfall partitioning studies have not used a standard method for calculating stemflow fractions.

I suggest this is, at least in part, likely to have arisen from differing objectives: catchment hydrology/water balance studies, which need plot scale measurements, whereas ecological studies of nutrient cycling/biogeochemical processes are adequately undertaken using individual tree canopies. Interestingly, the ecological studies of individual canopies were in more mesic environments. However, distinguishing between stemflow scaling methods has important implications when comparing *SF* results across the literature, applying the values to particular climatic conditions or scaling findings to a landscape level.

3.5. Conclusion

Understanding the manner in which precipitation is redistributed across vegetation canopies provides valuable information for regional groundwater management. Although partitioning variability occurred at finer temporal and spatial resolution (individual tree level, monthly precipitation volume), after two years of measurement, the rainfall partitioning fractions converge. The similarity in annual pathways of water flow between these morphologically distinct species improves confidence in applying these findings at a regional scale.

Stemflow, although highly variable and volumetrically minor compared with its counterpart throughfall, is capable of providing significant volumes of water to tree boles. This is likely to have a positive effect on the availability of soil moisture stores within the porous karst substrate and, presumably, tree water availability during drier months. However, the calculation of *SF* % is sensitive to the scale that *SF* volume is converted to millimetres; using individual plant canopies will result in higher reported *SF* fractions than would be reported using a plot scale. I suggest that plot scale is preferable, to improve comparability across biomes and ease of scaling results to the landscape level.

4. Comparing ecophysiological traits and evapotranspiration of an invasive exotic, *Pinus halepensis* in native woodland overlying a karst aquifer[3]

4.1. Introduction

Exotic plant invasions have the potential to adversely affect natural systems (Wilcox 2002), driving the need to understand the impact of their presence. This is particularly critical in water-limited environments, where invasive plants may exacerbate water scarcity by increasing stand density, transpiration, canopy interception, or altering soil moisture flux (Sala *et al*., 1996, Nagler *et al*., 2008, Cavaleri and Sack 2010, Doody and Benyon 2011, Doody *et al*., 2011, Rascher *et al*., 2011). Variability between plant species' water use capacity has been recognised (Calder and Dye 2001, Asbjornsen *et al*., 2007, Owens and Moore 2007) with ecosystems dominated by invasive tree species capable of consuming a larger volume of water per unit area compared to systems dominated by native species (Caveleri and Sack, 2010). This has been attributed to differences in invasive plant density, total leaf area and relative sapwood area (Sala *et al*., 1996) and suggests that plant invasions can substantially impact the local water balance.

Recognising this, resource managers have sought to remove invasive species as a means of returning water to the system (Wilcox 2002, Shafroth *et al*., 2005). The concept of enhancing water availability through invasive species' control is not new (introduced in the USA in the 1930's) (Chew 2009), and has recently been quantified under Australian conditions (Doody and Benyon, 2011).

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[3] Swaffer BA, Holland KL. 2014. Comparing ecophysiological traits and evapotranspiration of an invasive exotic, *Pinus halepensis* in native woodland overlying a karst aquifer. *Ecohydrology* DOI: 10.1002/eco.1502

Several attempts to increase water availability through vegetation control have been carried out in the USA, with most of the research effort focussed on *Tamarix* spp. (Sala *et al*., 1996, Owens and Moore 2007, Nagler *et al*., 2008, Hultine *et al*., 2010, Moore and Owens 2012). Generally, these suggest widespread *Tamarix* spp. control appears capable of delivering only modest water savings (Glenn and Nagler 2005, Doody *et al*., 2011, Moore *et al*., 2012). Nagler *et al*., (2008) calculated approximately 0.2 ML yr^{-1} ha^{-1} of additional water after *Tamarix* spp. control (assuming the *Tamarix spp*. monoculture was replaced by a mixture of native species), which was in the order of 0.2 % of mean annual river flow.

Doody and Benyon (2011) examined potential water savings from *Salix.* spp. control in Australian riparian zones and propose an average saving of 5.5 ML yr^{-1} ha^{-1} of crown projected area could be made, provided individuals situated in-stream were controlled, rather than trees on the stream bank. Similar to the findings of Nagler *et al*., (2008), they conclude that control of stream bank willows would be unlikely to result in net water savings, as the native species would replace willows to re-attain maximum canopy coverage. This finding suggests that considering niche occupancy can be a critical factor when assessing the potential for successful water salvage.

In addition to niche occupancy, ecophysiological characteristics related to hydraulic conductivity could also explain variability in patterns of water use between invasive and native species, assuming water is not a limiting factor.

Physiological variation in xylem characteristics (density of vessel elements, tracheids) may influence the volume of water capable of being processed by a species, as hydraulic conductance of a plant is strongly correlated to maximum transpiration (Bucci *et al*., 2004, Eamus *et al*., 2006, O'Grady *et al*., 2008). Therefore, the density of sapwood can serve to compare the potential effect of invasive species to their native counterparts.

Due to the multitude of ecosystem impacts attributed to invasive species, literature will often focus on biological, ecological or environmental changes rather than the hydrological influences of invasive plants. This is somewhat surprising considering global freshwater shortages (Vorosmarty *et al*., 2000), especially in Australia, with its dry climate and recent severe drought. So far, examining the influence of invasive plants has been restricted largely to riparian zones (Doody and Benyon 2011), with systems characterised by fresh groundwater resources receiving little attention, despite their importance in many areas across Australia. One notable exception was the use of karstic groundwater resources by plantation *Pinus radiata* (Benyon *et al*., 2006), where up to 72 % of transpiration requirements were met by groundwater, with negligible recharge to the karstic aquifer once the tree canopy had closed.

Karst is a geological formation where recharge and discharge processes are particularly variable across a small spatial scale (Schwinning 2008). Highly soluble rock is eroded to form a complex pathway of dissolution features, caves and sinkholes, causing preferential water movement and resulting in diverse recharge pathways to the water table (Herczeg *et al*., 1997, White 2002).

Concern regarding the potential detrimental influence of the invasive *Pinus halepensis* on recharge processes of a karstic aquifer was the driver for this work. *Pinus halepensis* has naturalised across many regions of southern Australia (Virtue and Melland 2003) and commonly invades the dry, rocky limestone soils that are often associated with karstic systems. The species has the ability to form dense monocultures and out-compete the native woodland areas (Virtue and Melland 2003, Quarmby 2004). An understanding of the comparative water use characteristics of the invasive and native species in this geological setting is required to inform resource management of *P. halepensis*.

I hypothesised that the invasive *P. halepensis* would possess traits that would facilitate higher rates of water use per unit sapwood area compared to the native tree and shrub species. Furthermore, I suggested that this increased water use would correspond to higher actual evapotranspiration (ET_a) in areas that have been invaded by *P. halepensis* in comparison to areas occupied only by native species. I tested these hypotheses in two ways: 1) I compared ecophysiological traits related to tree water use (sap flux, sapwood density, leaf and soil water potentials) of the invasive and native species at the plot scale; and 2) used satellite-derived estimates of ET_a to estimate net recharge under different vegetation types, including areas of *P. halepensis* invasion and removal. Satellite-derived ET_a estimates are becoming increasingly popular due to the need to understand water flux patterns across a broader spatial and temporal span than are usually available with more traditional ET_a methods (Glenn *et al*., 2011). Here, it has enabled a water balance to be estimated at the basin scale and for different vegetation types, from 2001 to 2010.

4.2. Methods

Study site and plant species

The study was located in Uley Wanilla water reserve, southern Australia (34.62 °S, 135.63 °E). Groundwater from the reserve has been an important historical drinking water source for the region (Department for Water 2012a), supplying ~ 50 % of the reticulated groundwater supply (circa 1970s) (Department for Water 2012b). Since 1985, groundwater levels have declined, on average 4 m (± 1 m std dev), despite reduced extractions beginning in the early 1990s. Postulated causes include; reduced rainfall, historical groundwater extraction, interception of rainfall and direct use of groundwater by vegetation, including the invasive *P. halepensis* (Department for Water 2012a) (Figure 4.2.1).

Average potential evapotranspiration (FAO56) was 1081 mm year^{-1} and average annual rainfall was 564 mm year^{-1} (station # 18017) (1900 – 2010) (Bureau of Meteorology 2010). Soils in the area were generally very shallow and typically comprise of skeletal calcareous or shallow sandy and clayey loams (Harrington 2006). A sheet limestone sub-stratum was often present, visible on local topographic rises, with soil pockets occupying local depressions.

Open woodlands and shrublands consisting of trees such as *Eucalyptus diversifolia* subsp. *diversifolia* Bonpl. (coastal white mallee), *Allocasuarina verticillata* (Lam.) L.A.S. Johnson (drooping she-oak), *Melaleuca lanceolata* Otto (dryland tea tree) and shrubs comprising of *Acacia longifolia* var. *sophorae* Labill. Court (coastal wattle), *Pittosporum angustifolium* Lodd., G. Lodd. & W. Lodd. (native apricot) and *Leucopogon parviflorus* (Andrews) Lindl. (coastal beard heath) dominate the landscape across the Uley Wanilla groundwater lens.

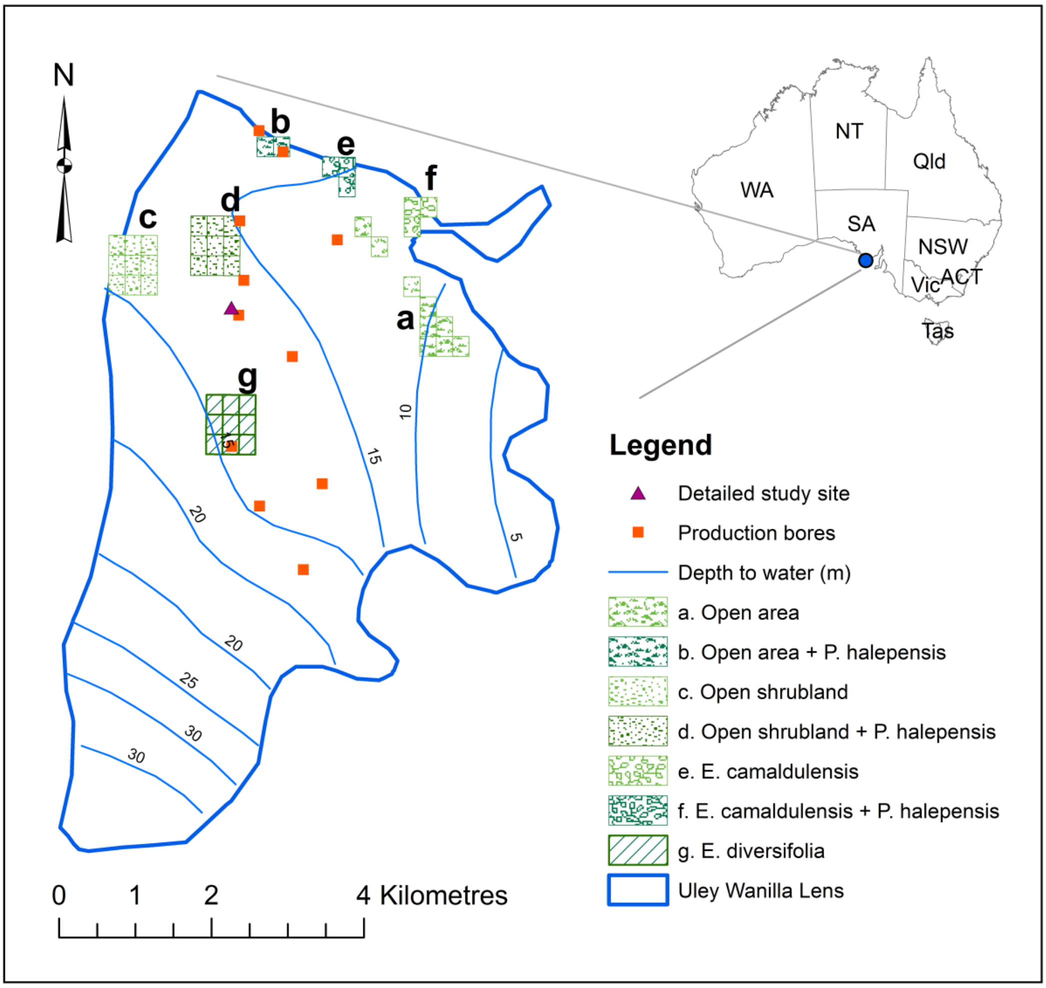

Figure 4.2.1 Location of Uley Wanilla groundwater, showing production bores used for public drinking water supply, location of detailed study site, and 250 m grid locations for reference vegetation classes used for ET_a comparisons. Depth to water contours modified from Harrington *et al*., 2006.

Isolated stands of *Eucalyptus camaldulensis* Denh. (river red gum) were present across the northern section of the reserve, where the water table was relatively shallow. *Pinus halepensis* Mill. (Aleppo pine), introduced in the early 1800's (Way 2006), has invaded across the region, with density estimates ranging from isolated plants to canopy coverage of 70 – 100% (Quarmby, 2004).

A 900 m^2 plot was used for detailed sampling of a mixed stand containing each of the species described above, with the exception of *E. camaldulensis*. Species basal area within the plot was calculated by summing tree and shrub diameter at breast height (DBH) measurements. Leaf Area Index (LAI) was calculated from digital photographs using Matlab R2011b and the Image Processing Toolbox (The Mathworks Inc., MA, USA). Five digital photos were taken at 2 m intervals along seven transects at 10 m intervals, generating thirty-five images to calculate plot LAI, following Macfarlane *et al*., (2007) and Fuentes *et al*., (2008). The gap criterion was set to 0.75 and an extinction coefficient of 0.5 was used (appropriate for eucalypt woodlands), following Macfarlane *et al*., (2007).

Tree water use

Tree transpiration (*T*) was estimated from heat pulse measurements of sap flow velocity on seventeen stems every 30 minutes for 50 days between 28th April and 17th June 2010 (Table 4.3.1).The technique was based on the compensation method originally proposed by Huber and Schmidt (1937) and later refined (Marshall 1958, Swanson and Whitfield 1981). Sap flow velocity was measured using thermistor probes attached to a logger (SF 300 probes, Greenspan Technology, Warwick, Queensland) which can be used to accurately estimate sap flow provided appropriate calibration procedures (sapwood wood and water fractions, probe separation distance and species-specific wound-widths) are followed (Thorburn *et al*., 1993b, Vertessey *et al*., 1997, Cramer *et al*., 1999, Bleby *et al*., 2004).

Sapwood wood and water fractions were determined using 5-mm diameter cores, collected at the commencement of sap flow measurements (Benyon *et al*., 1999).

Probe separation values were checked by inserting blank probes at equivalent depth to the sensor and measuring variation from 10 mm above and 5 mm below the heater probe. Drillholes were rejected where variance was larger than 1 mm and the holes redrilled. Species-specific wound widths of 0.34 mm for *E. diversifolia*, 0.31 mm for *A. verticillata*, 0.34 mm for *M. lanceolata*, 0.31 mm for *P. angustifolium*, 0.37 mm for *P. halepensis* and 0.34 mm for *A. longifolia* were determined following Swanson and Whitfield (1981).

Depth of conducting wood for the instrumented trees was determined by incrementally adjusting probe depth on a sunny day until the measured heat pulse velocity was lower than the minimum reading taken between midnight and 5 AM the previous night (Benyon 1999) and accounting for bark thickness. A linear regression between tree basal area (using DBH) and conducting wood area allowed species-specific mean sap flow velocity to be scaled to daily transpiration at the stand level following Hatton *et al*., (1990).

Sapwood density

The density of sapwood as an indicator of potential conductance was determined following O’Grady *et al*., (2009) using cuttings from 66 stems from the seven study species located within the plot. First, sapwood cross-sectional area was measured using manual callipers and then bark and phloem layers were removed with a sharp knife. Samples were placed in distilled water for 24 hours to fully saturate. Using the Archimedes principle, an object immersed in fluid has a volume equal to the weight of the fluid displaced by the object.

Therefore density was calculated by dividing the dry weight of the object (g) by the weight of the displaced water volume (equivalent to cm^3). The dry weight of the sapwood was determined by oven-drying at 105 ºC for 24 hours.

Tree water sources

Analyses of the stable isotopes of water ($\delta^{18}O$ and $\delta^{2}H$) have become standard tools for tracing plant water sources (Ehleringer and Dawson 1992, Dawson *et al.*, 2002). Matching twig isotopic signatures to their potential sources found throughout the environment is possible as plants do not alter the isotopic composition of water during uptake by roots (Thornburn *et al.*, 1993a, b, Cramer *et al.*, 1999). However, the method relies on each water source having a distinctive isotopic signature. Twig cuttings were collected from trees instrumented with sap flow loggers, ensuring twigs were selected where heartwood was yet to develop. Approximately 200 mm of twig sample was collected, immediately cut into pieces and immersed in airtight 150 ml glass jars filled with kerosene to minimise isotopic fractionation. Surface soil samples were taken within 2 m of the base of four sample trees by hand.

The shallow nature of the soil and the hardness of sheet limestone prevented the use of an auger to obtain deeper samples. Soil samples were placed into 500 ml glass jars and sealed for later analysis of both isotopic composition and matric potential. Groundwater samples were obtained by first purging three well volumes, to ensure the sample was representative of the aquifer (Barber and Davies, 1987).

Five rainfall isotope samples collected approximately 15 km away were also used in this isotopic comparison (Swaffer *et al.*, 2014). All water samples were analysed by the CSIRO Isotope Analysis Service, Adelaide. All isotopic concentrations are reported in standard delta notation in units per mil (‰) relative to the Vienna-Standard Mean Oceanic Water (V-SMOW).

A mixing line was used to estimate the proportion of twig water being used from each of the potential tree water sources, by placement of the twig isotope data along the distance of the source water mixing line, compared with the length of the line (Thorburn *et al*., 1993b).

Leaf and soil water potentials

Leaf water potential (Ψ) (MPa) (a measure of the suction gradient and an indicator of plant water stress) was measured on three replicate twigs for each tree instrumented with sap flow loggers using a PMS 1000 pressure chamber (PMS Instrument Company, Albany, Oregon) (Scholander *et al*., 1965). Twig samples were collected, bagged and placed into a humidified chamber to minimise water loss (Turner, 1988). Time between sample collection and measurement was < 1 minute. Measurements commenced before dawn (Ψ_p) (0400 – 0600 h), when the plant was assumed to be in equilibrium with the water potential of its water source. Fresh twig samples from the same trees were collected and measured every two hours (approximately) therein until dusk (1730 – 1830 h). Midday leaf water potentials (Ψ_m) (1130 – 1330 h) were used to measure plant water status when evaporative demands on the vegetation would be expected to be strongest.

Soil water potentials (Ψ) (MPa) represent the sum of matric (dryness) and osmotic (saltiness) potential, where a water potential of 0 MPa is equivalent to free water. Osmotic potential was assumed to exert minimal influence due to the fresh nature of the karst system (salinity 480 – 620 mg L^{-1}, DEWNR 2012). Soil matric potentials were determined using the filter paper technique (Greacen *et al.*, 1989), where three replicate filter papers were equilibrated in contact with the soil for seven days in a temperature controlled environment. The papers were removed, weighed immediately after surplus soil was removed, oven-dried at 105 ºC for 24 hours, and reweighed. Using known calibrations, the matric potential was estimated from the water content of the filter papers. Reported values were the average of the three replicates.

Water balance using satellite derived estimates of evapotranspiration

A water balance (Q_{wt}) (mm) was calculated using the difference between rainfall (P) (mm) and actual evapotranspiration (ET_a), (mm) assuming negligible runoff and changes in soil moisture content.

$$Q_{wt} = P - ET_a$$

Groundwater discharge was assumed where Q_{wt} was negative; conversely, water available for deep drainage and groundwater recharge was represented by positive Q_{wt}. Where Q_{wt} results are in dimensionless units, the result has been normalised by rainfall received for the corresponding period (Q_{wt} [mm] / P [mm]). Normalising Q_{wt} by rainfall provided an water budget estimate relative to zero, where moisture surplus or deficit was presented as a multiplier of water input (in this case annual rainfall).

Where Q_{wt} is presented in mm year^{-1}, error margins are provided based on the standard error of the data. Estimates of ET_a were derived using CSIRO's MODIS Reflectance-based Scaling EvapoTranspiration (CMRSET) algorithm (developed by Guerschman *et al.*, 2009, and assessed against a range of *ET*-estimate algorithms by Glenn *et al*., 2011, King *et al*., 2011). Briefly, the method uses eight-day aggregated Enhanced Vegetation Index (EVI) and Global Vegetation Moisture Index (GVMI) data to scale ET_a estimates from potential evapotranspiration (ET_o) on a 250 m resolution grid. Data were available from 2001 – 2010. The rainfall and ET_o data used in this assessment was measured by the aforementioned weather station. Reference vegetation classes were assigned to specific cells to examine the differences in ET_a attributable to vegetation types that were selected based on the location of uniform, continuous stands of each vegetation association. Each reference association was initially identified using aerial photography and ground-truthed in February 2014 (Figure 4.2.2).

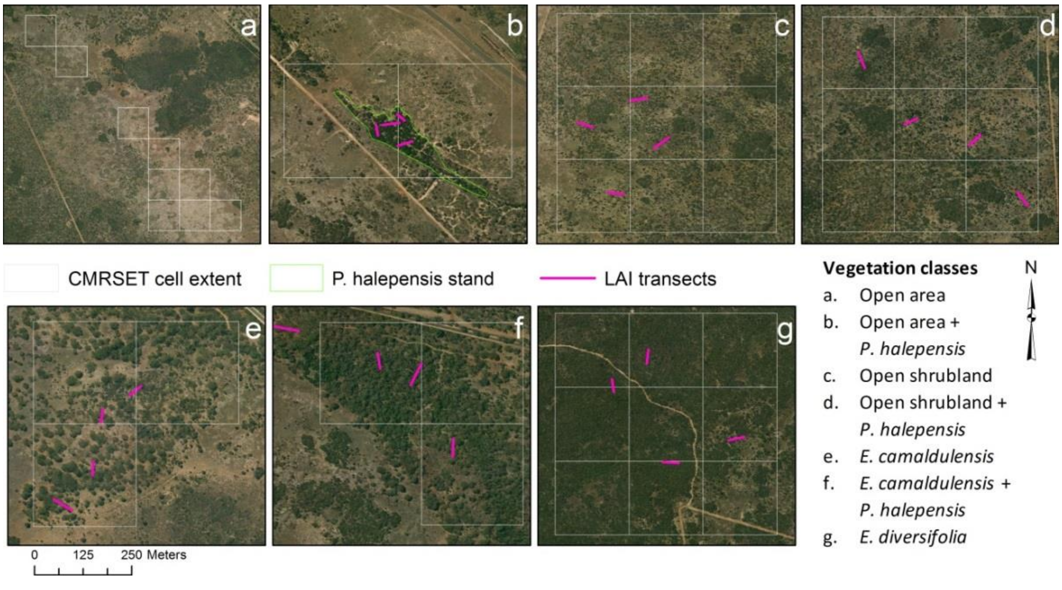

Figure 4.2.2 Aerial imagery of the grids for each reference vegetation class used to compare ET_a and Q_wt from 2001 – 2010. Site codes are: (a) Open area; (b) open area + *P. halepensis*; (c) open shrubland; (d) open shrubland *P. halepensis*; (e) *E. camaldulensis*; (f) *E. camaldulensis* + *P. halepensis*; (g) *E. diversifolia*. Photo: 2008

The extent of the uniform, continuous vegetation stand was mapped, and the corresponding CMRSET cell reference assigned using the coordinates of the mapped vegetation coverage. Only CMRSET cells that were completely within the mapped vegetation stand were assigned a reference vegetation class.

Reference vegetation classes included;

- an open area covered by bare soil, exposed calcrete and annual grasses;
- an open area containing 10 % coverage of a dense stand of *P. halepensis*;
- open shrubland dominated by *L. parviflorus* and *M. lanceolata*
- open shrubland dominated by *L. parviflorus* , *M. lanceolata* and *P. halepensis*;
- *E. camaldulensis* woodland;
- *E. camaldulensis* woodland, where removal of a *P. halepensis* infestation took place at the end of 2008; and
- *E. diversifolia* woodland (Table 4.3.1)

LAI transects were randomly located within the reference vegetation classes, digital images were taken every 2 m along a 50 m transect (n = 104 per reference class) and LAI estimated following Macfarlane *et al*., (2007) and Fuentes *et al*., (2008).

Due to one stand of *P. halepensis* failing to completely cover the required 250 m cell size, ET_a for this site (open area + 10 % *P. halepensis*) was estimated by scaling up the CMRSET data. This adjusted for the tree coverage extending across only 10 % of the cell (rather than 100 %, as for the other vegetation reference sites) and delivered an ET_a estimate consistent with a CMRSET cell covered entirely by *P. halepensis*.

Statistical analyses

Comparisons of sap flow velocity were made using one-way analysis of variance on ranks (ANOVA), followed by Dunn's method for pairwise comparisons. Sapwood density comparisons were made using a one way ANOVA, followed by Tukey Tests for pairwise comparisons. Comparisons of reference vegetation ET_a estimates derived from satellite imagery were performed using a repeated measures ANOVA. Differences in ET_a before and after removal of *P. halepensis* were assessed using t-tests. All statistical analyses were conducted using SigmaPlot version 11.0 (Systat Software Inc. USA). All spatial analyses were performed using ArcGIS version 10.0 (ESRI Inc. USA).

4.3. Results

Plot characteristics

Seven tree and shrub species were recorded within the plot; composition, basal area and location were measured on 28th April 2010. *Leucopogon parviflorus* was the most commonly recorded species, but contributed only 7 % of the total plot basal area. The stems were too narrow for the installation of sap flow probes; therefore *T* was not measured for this species. *Melaleuca lanceolata* contributed 36 % of plot basal area, while *E. diversifolia* contributed 23 % of the plot basal area.

Twenty-two individual *P. halepensis* stems were present, comprising 15 % of the plot basal area. Three large *A. verticillata* were present (17 % plot basal area). *Acacia longifolia* (< 1 %) and *P. angustifoium* (2 %) were minor contributors to plot basal area. Overall, the plot contained 287 plant stems, and 13.37 m^2 ha^{-1} of basal area.

Tree water use

Sap flow was measured over 50 consecutive days during mid-autumn and early winter. Over this time, 72 mm of precipitation fell across the site, the majority in the latter half of the measurement period. The increased moisture resulted in a 45 % increase in sap flow velocity for *P. halepensis*, and 35 % increase for *E. diversifolia.* In contrast, the remaining tree and shrub species did not respond (Figure 4.3.1). Median sap flow velocity of *P. halepensis* (5.5 ± 2.4 cm hr^{-1}) was significantly higher ($p < 0.05$) than the median velocity for *E. diversifolia* (3.5 ± 3.6 cm hr^{-1}), *A. verticillata* (3.4 ± 0.9 cm hr^{-1}), *M. lanceolata* (3.2 ± 1.7 cm hr^{-1}), *P. angustifolium* (1.2 ± 0.3 cm hr^{-1}) and *A. longifolia* (1.0 ± 0.3 cm hr^{-1}), indicating faster transport of water per unit sapwood area compared to the native species (Table 4.3.1) (Figure 4.3.2). The volume of water transpired by the vegetation in the plot was 32 mm over the 50 day study period, which corresponds to an average of 0.63 mm day^{-1}. Approximately 32 % of daily transpired water was attributed to *P. halepensis* (0.20 mm day^{-1}), comprising only 15 % of plot basal area. However, the majority (38 %) of transpired water originated from *M. lanceolata* (0.24 mm day^{-1}), attributed to its larger proportion of basal area (36 %) within the plot (Table 4.3.1, Figure 4.3.2).

Table 4.3.1 Plot and sapflow tree site details, including wood and water fractions (%) and number of sap flow probes installed in each species, used to calculate median sap flow velocity (cm hr^{-1}) (referring to per unit of sapwood area) and species water use (mm day^{-1}) (incorporating basal area per hectare) . Significant pairwise comparisons ($p < 0.05$) are differentiated by ^{a}, ^{b}, ^{c}, ^{d}.

						Transpiration parameters					
	Form	**Stem**	**Basal Area**	**Basal Area**	**Sapwood density**	**Probes**	**Wood frac.**	**Water frac**	**Transpiration**		
	T = tree S = shrub	**n**	**m^2 ha^{-1}**	**%**	**g cm^{-3}**	**n**	**%**	**%**	**cm hr^{-1}**	**mm day^{-1}**	**%**
P. halepensis	**T**	**22**	**1.98**	**15**	**0.513^a**	**8**	**22**	**36**	**5.5^a**	**0.2**	**32**
E. diversifolia	T	70	3.12	23	0.606^b	6	26	25	3.5^b	0.15	24
A. verticillata	T	3	2.25	17	0.709^b	4	40	36	3.4^b	0.04	6
M. lanceolata	T	72	4.85	36	0.618^b	8	30	34	3.2^c	0.24	38
A. longifolia	S	5	0.05	<1	0.600^b	1	23	31	1.2^d	<0.01	<1
P. angustifolium	S	13	0.23	2	0.681^b	3	34	28	1.0^d	<0.01	<1
L. parviflorus	S	102	0.88	7	0.719^b						
TOTAL		**287**	**13.37**	**100**		**30**				**0.63**	**100**

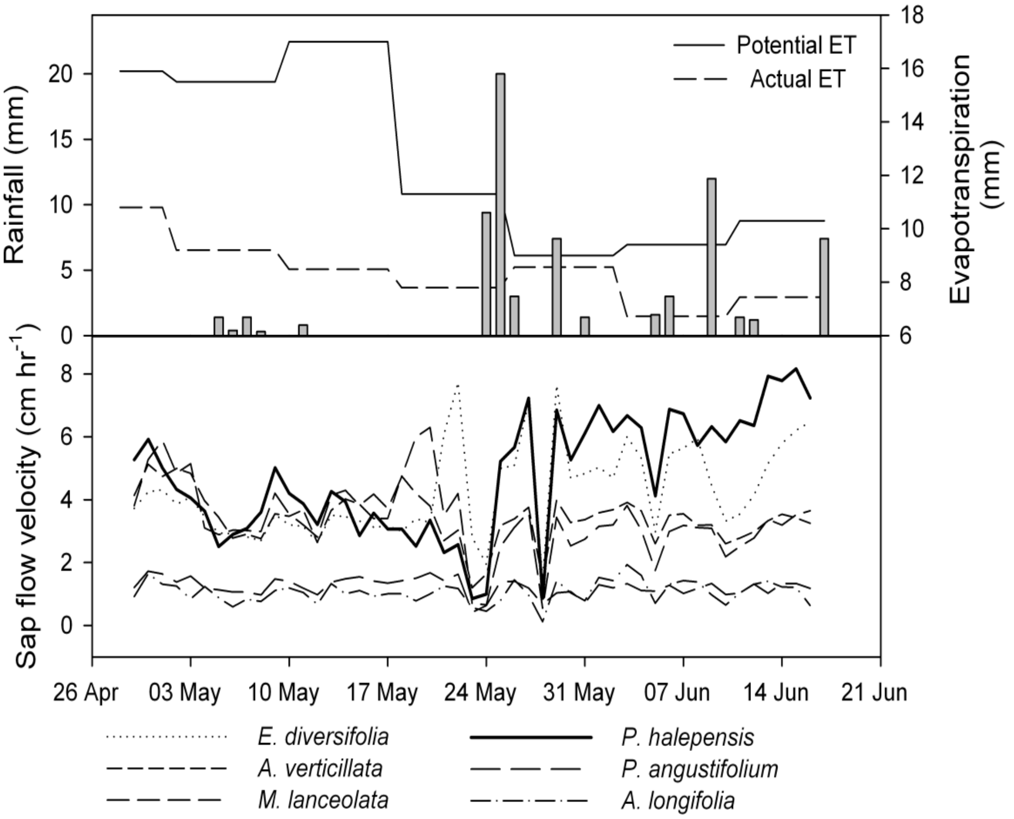

Figure 4.3.1 Project timeline showing daily rainfall (mm) and average sap flow velocity (cm hr^{-1}) for the six species instrumented with sap flow loggers. Potential evapotranspiration (FAO56) and actual evapotranspiration (ET_a, mm) are presented as 8-day composites.

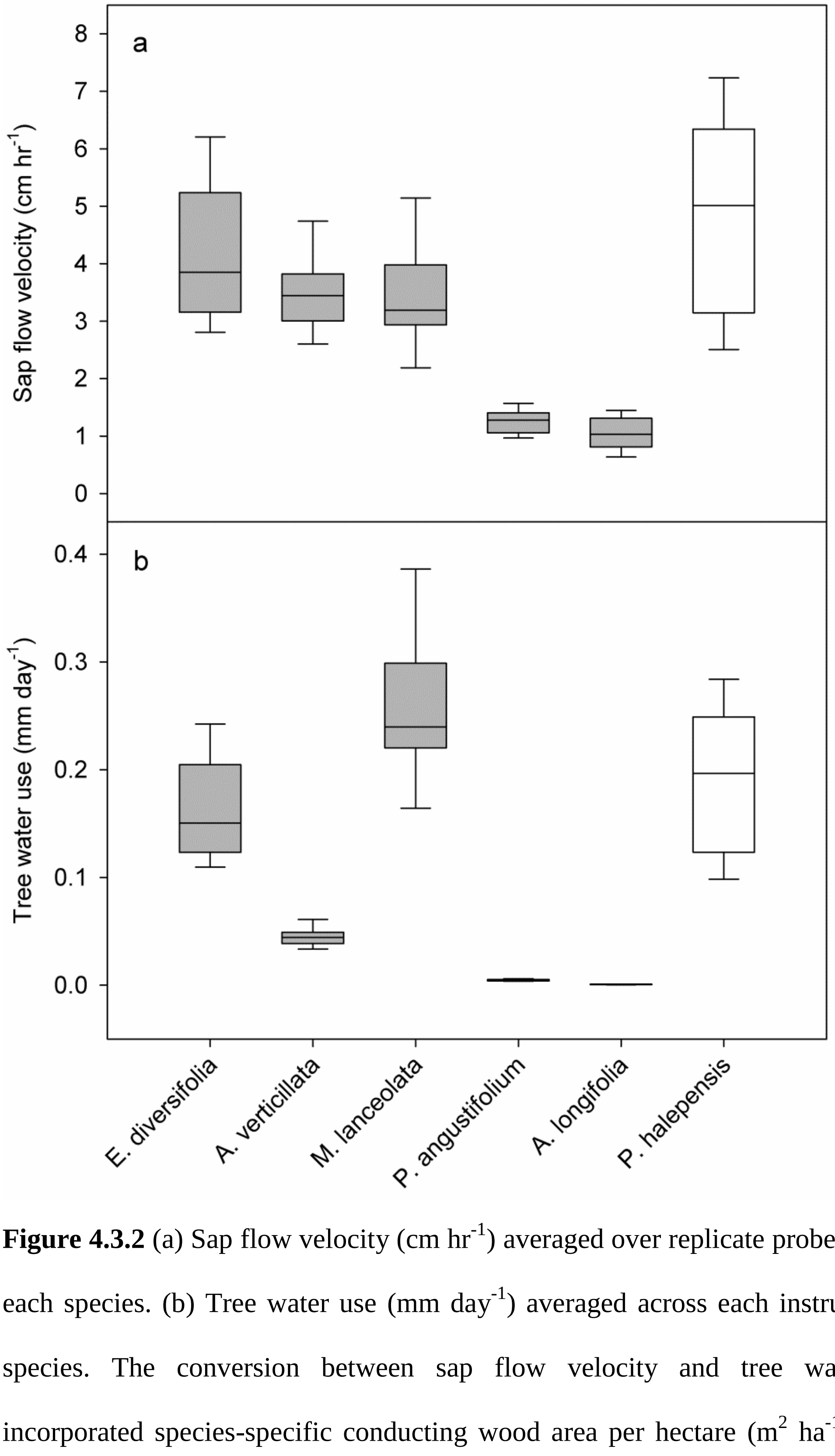

Figure 4.3.2 (a) Sap flow velocity (cm hr^{-1}) averaged over replicate probes within each species. (b) Tree water use (mm day^{-1}) averaged across each instrumented species. The conversion between sap flow velocity and tree water use incorporated species-specific conducting wood area per hectare (m^{2} ha^{-1}) found within the plot. Box plots extend from the 25th to 75th percentile, median values within. Whiskers show 10th and 90th percentile. All data n = 49.

Sapwood density

Sapwood density, used here as a proxy for hydraulic conductivity was lowest in *P. halepensis*, averaging 0.513 ± 0.035 g cm^{-3} (*n* = 13), moderate in *A. longifolia* (0.600 ± 0.013 g cm^{-3}, *n* = 7), *E. diversifolia* (0.606 ± 0.024 g cm^{-3}, *n* = 6), *M. lanceolata* (0.618 ± 0.031 g cm^{-3}, *n* = 12) and *P. angustifolium* (0.681 ± 0.032 g cm^{-3}, *n* = 10) and highest in *A. verticillata* (0.709 ± 0.008 g cm^{-3}, *n* = 6) and *L. parviflorus* (0.719 ± 0.030 g cm^{-3}, *n* = 12) (Figure 4.3.3). *Pinus halepensis* sapwood density was significantly less than the native species' sapwood density (F-value = 61.964, $p < 0.001$) (Table 4.3.1).

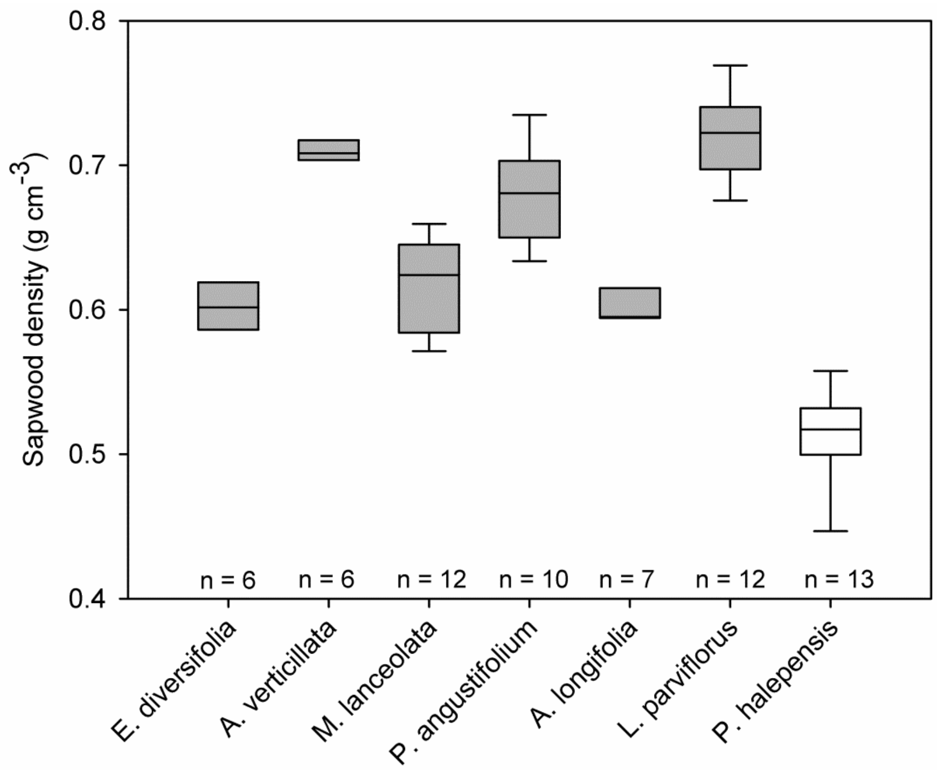

Figure 4.3.3 Sapwood density of replicate twigs (g cm^{-3}) from multiple individuals of each of the species within the plot. Box plots extend from the 25th to 75th percentile, median values within. Whiskers show 10th and 90th percentile.

Tree water sources

The isotopic composition of rainfall was variable, and ranged between – 1.81 to – 6.26 ‰ δ^{18}O and 0.7 to – 36.5 ‰ δ^{2}H. Groundwater samples from two bores (~ 500 m apart) within 350 m of the study site averaged – 4.55 ‰ δ^{18}O and – 26.0 ‰ δ^{2}H. Surface soil water samples were enriched relative to rainfall, consistent with evaporative demands and likely represent the end-member for isotopic enrichment at the time of sampling. Twig water samples were variable, but differences between the native and non-native species were not marked (Figure 4.3.4). Native species twig isotope ratios ranged between – 2.90 to – 0.01 ‰ for δ^{18}O and – 20.2 to – 7.2 ‰ for δ^{2}H. *Pinus halepensis* twig isotope ratios varied between – 3.47 to – 0.64 ‰ for δ^{18}O and – 23.4 to – 12.8 ‰ for δ^{2}H.

Twig water samples fell along a mixing line between surface soil and ground water end-members (Figure 4.3.4). The third source of water, subsurface soil water, was not sampled due to the hardness of the karst material. There was no obvious match between the twig isotope ratios and two of its potential sources (surface soil water or groundwater). This ambiguity may be the result of simultaneous uptake from surface soil and groundwater, or that subsurface soil water may represent a mixture of evaporatively enriched surface soil water and depleted rainfall (similar to the groundwater signature).

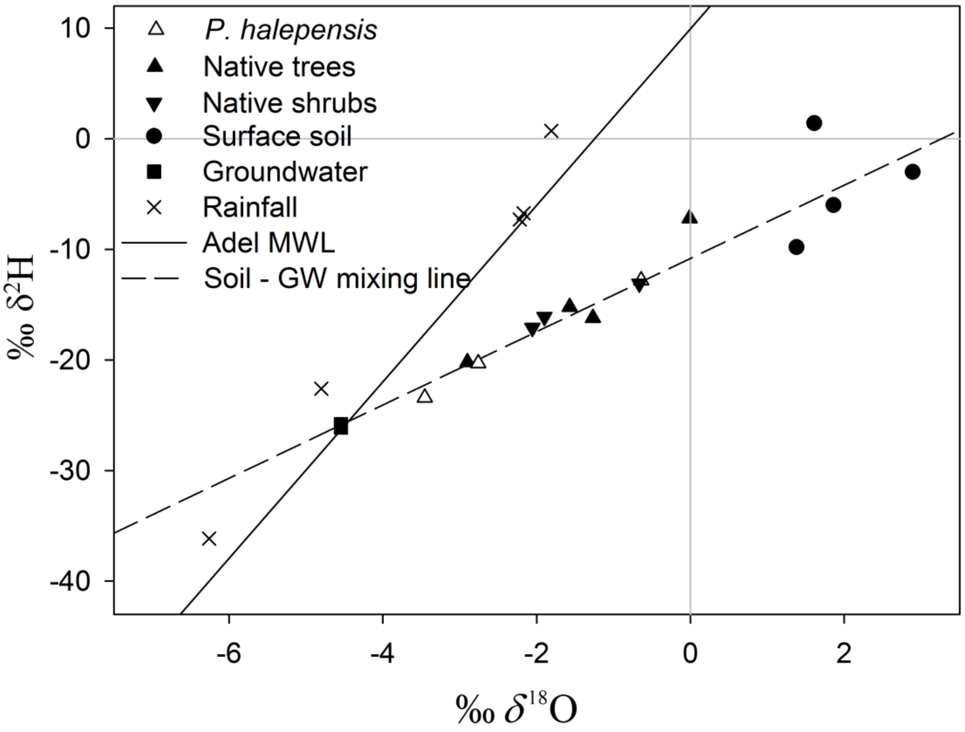

Figure 4.3.4 Plots of δ^{18}O (‰) *versus* δ^{2}H (‰) for groundwater (■), surface soils (●), *Pinus halepensis* (Δ), native tree (▲) and native shrubs (▼) collected on 28th April 2010. Rainfall samples are cumulative monthly samples collected from March to July 2010.The solid line is the Adelaide Meteoric Water Line and the dashed line represents an evaporative series inferred from the position of the groundwater and surface soil water samples on the plot.

Leaf and soil water potentials

Native species predawn water potentials were variable (– 2.1 to – 0.9 MPa), suggesting species- or location-specific variability was present within the site. The relatively low predawn *Ψ* values suggest these individuals had limited access to water reserves at the time of sampling. The native species predawn *Ψ* were not significantly different to *P. halepensis* predawn *Ψ* values (– 1.5 to – 1.3 MPa) ($p > 0.05$). Midday leaf water potentials also lacked statistical differences between native species (– 3.3 to – 1.9 MPa) and *P. halepensis* (– 2.6 to – 2.3 MPa). Surface soil matric potentials ranged between – 5 and – 1 MPa (n = 4) (Figure 4.3.5).

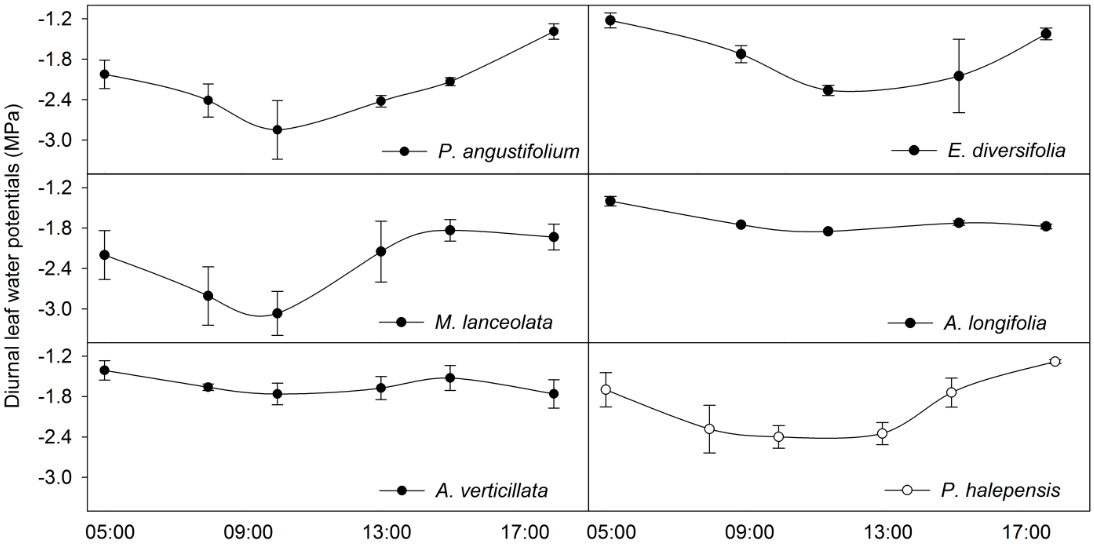

Figure 4.3.5 Diurnal pattern of leaf water potential (Ψ, MPa, mean ± std dev) for native species (●) and *P. halepensis* (○) measured on 28th April between 5 AM and 5 PM. Multiple trees per species were sampled, except *P. angustifolium* and *A. longifolia*, where only one individual was sampled. Three twig samples were measured per tree per time and averaged.

Water balance using satellite derived estimates of evapotranspiration

Evapotranspiration from the Uley Wanilla groundwater basin exceeded the volume of rainfall received from 2001 – 2010, indicating a total net discharge of 9 % of the rainfall received (5,532 mm). A discharging basin is consistent with the region experiencing what is commonly referred to as the 'Millennium Drought' (2001 and 2009) (Van Dijk *et al*., 2013), amplified by the extremely low rainfall years of 2002, 2006 and 2008 (Figure 4.3.6) (Table 4.3.2).

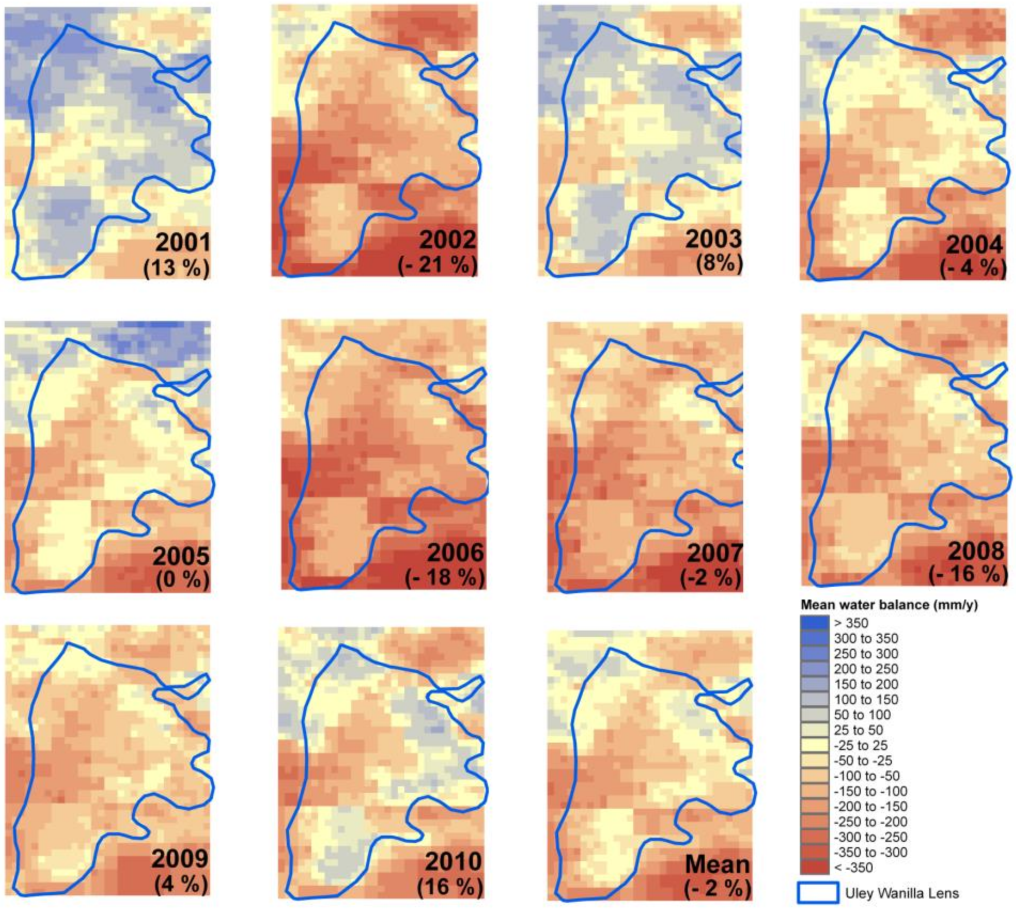

Figure 4.3.6 Annual net recharge (mm year^{-1}) for each year from 2001 to 2010. Received rainfall relative to the long term average of 564 mm year^{-1} is shown in brackets.

Q_{wt} was spatially variable across the basin for all water balance years (2001 – 2010) (Figure 4.3.6). There were detectable ET_a differences between the reference vegetation classes relative to the volume of rainfall received. Median Q_{wt} was positive (recharging) for open areas (0.16) largely devoid of vegetation. Median Q_{wt} was also positive in shrublands, regardless of the presence (0.02) or absence (0.09) of *P. halepensis*. However, median Q_{wt} was negative (discharging) for regions covered by *P. halepensis* (– 0.03), *E. camaldulensis* with (– 0.21) and without (– 0.11) *P. halepensis* and *E. diversifolia* woodland (– 0.18) (Table 4.3.2, Figure 4.3.7).

Table 4.3.2 Net recharge (Qwt) across the Uley Wanilla basin and the seven reference vegetation classes, shown for each year as (i) a proportion of received rainfall, and (ii) as cumulative net recharge spanning 2001 – 2010

		Invaded	Cells	LAI[1]		Annual net recharge as a proportion of rainfall				Cumulative net recharge (2001 – 2010)	
			n	mean	SE	median	min	max	SE	mm	% rainfall[2]
a	Open area		9			0.16	– 0.18	0.35	0.013	826	15%
b	Open area + *P. halepensis* [3]	x	2	2.24	0.13	– 0.03	– 0.40	0.24	0.043	– 151	– 3 %
c	Open shrubland		9	0.44	0.09	0.09	– 0.21	0.28	0.013	458	8%
d	Open shrubland + *P. halepensis*	x	9	1.09	0.14	0.02	– 0.32	0.2	0.014	135	2%
e	*E. camaldulensis*			1.2	0.07						
	2001 – 2008		3			– 0.16	– 0.46	0.16	0.032	– 566	– 13 %
	2009 – 2010		3			0.02	– 0.07	0.12	0.027	– 8	– 0.2 %
f	*E. camaldulensis* + *P. halepensis*			0.83	0.05						
	2001 – 2008	x	3			– 0.27	– 0.52	0.01	0.03	– 1067	– 25 %
	2009 – 2010		3			– 0.01	– 0.12	0.07	0.032	– 34	– 1 %
g	*E. diversifolia*		9	1.15	0.07	– 0.18	– 0.66	0.03	0.02	– 1213	– 22 %
	Uley Wanilla basin	x	703			– 0.08	– 1.04	0.35	0.003	– 482	– 9 %

[1] LAI measured 19th February 2014

[2] Total rainfall (2001 – 2010) = 5532 mm

[3] Estimates have been scaled based on the ratio of *P. halepensis* stand area (12,283 m^2) and CMRSET cell areas (125,000 m^2)

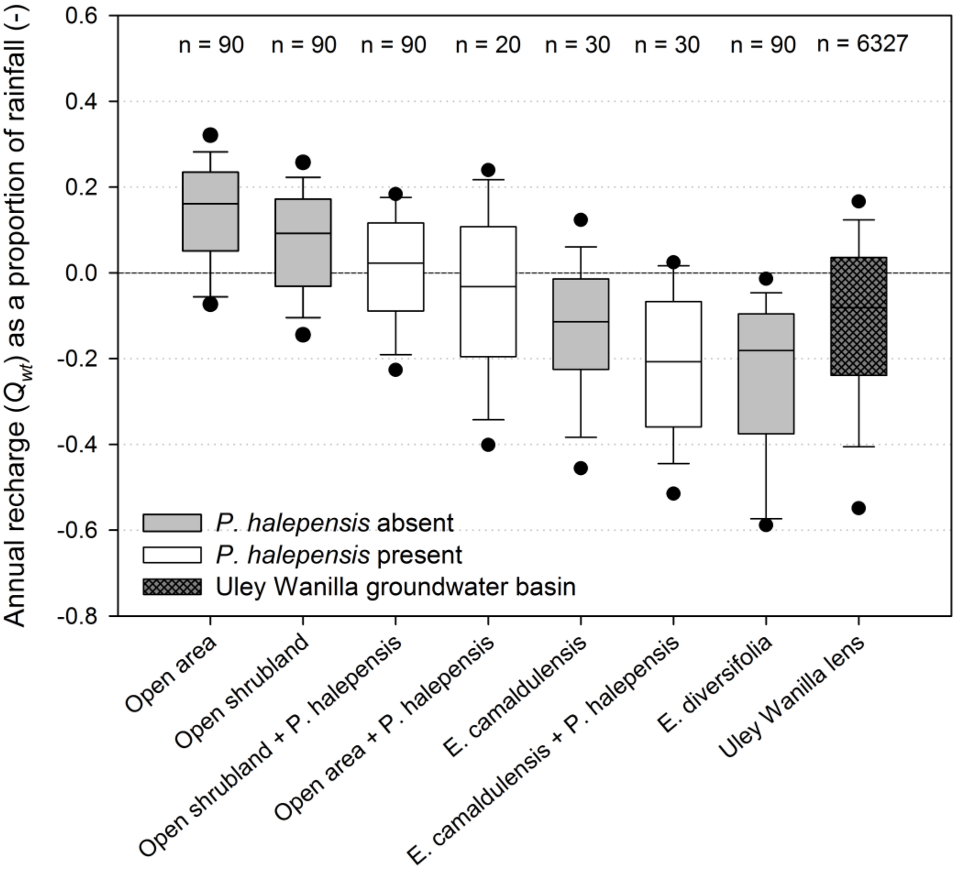

Figure 4.3.7 Annual net recharge over the period of 2001 to 2010, normalised by rainfall for each vegetation class and the extent of the Uley Wanilla groundwater basin. Box plots extend from the 25th to 75th percentile, median values within. Whiskers show 10th and 90th percentile.

The hydrological effect of *P. halepensis* infestation was particularly evident upon closer examination of the two *E. camaldulensis* stands. Removal of *Pinus halepensis* took place in 2008; prior to this, median net recharge relative to rainfall for the stand was – 0.27. In the two years after clearance, median net recharge increased to – 0.01 (gain of 0.26 relative to rainfall).

A comparison stand of *E. camaldulensis* without *P. halepensis* invasion demonstrates a similar trend; during 2001 to 2008 net recharge for the intact site was – 0.16, which increased to 0.02 during 2009 and 2010 (gain of 0.18 relative to rainfall). After accounting for the corresponding increase in ET_a in the intact site in 2009 and 2010 (0.26 – 0.18), the pine removal equated to a reduction in ET_a of 50 (±11 SE) mm year^{-1} over the two years of measurement in the invaded site.

Net recharge was significantly lower in the invaded *E. camaldulensis* stand during 2001 – 2008, compared to the intact *E. camaldulensis* site (F-value = 37.249, p = <0.001). However, importantly, there was no significant difference in net recharge between the intact and invaded sites post-clearance (2009 and 2010) (F-value = 1.589, p = 0.263). Within each site but comparing across the two periods (pre- and post-clearance), net recharge was significantly lower in 2001 – 2008, in comparison to 2009 – 2010 for both vegetation types (*E. camaldulensis* with *P. halepensis*, – p = <0.001; *E. camaldulensis* without *P. halepensis* – $p < 0.05$), consistent with the timing of the end of the drought (Figure 4.3.8).

Based on the median net recharge estimates in Table 4.3.2 and adopting the average rainfall of 564 mm year^{-1}, it is possible to postulate the changes to annual ET_a with future removal of *P. halepensis*. For example, removing *P. halepensis* in the open shrubland site may result in a reduction of ET_a by 39 (± 1 SE) mm year^{-1}. Removing a dense, closed canopy *P. halepensis* stand and replacing it with open grassland may reduce ET_a losses by 109 (± 17 SE) mm year^{-1}. Allowing encroachment of open shrubland by dense *E. diversifolia* woodland would increase ET_a by 50 (± 19 SE) mm year^{-1}.

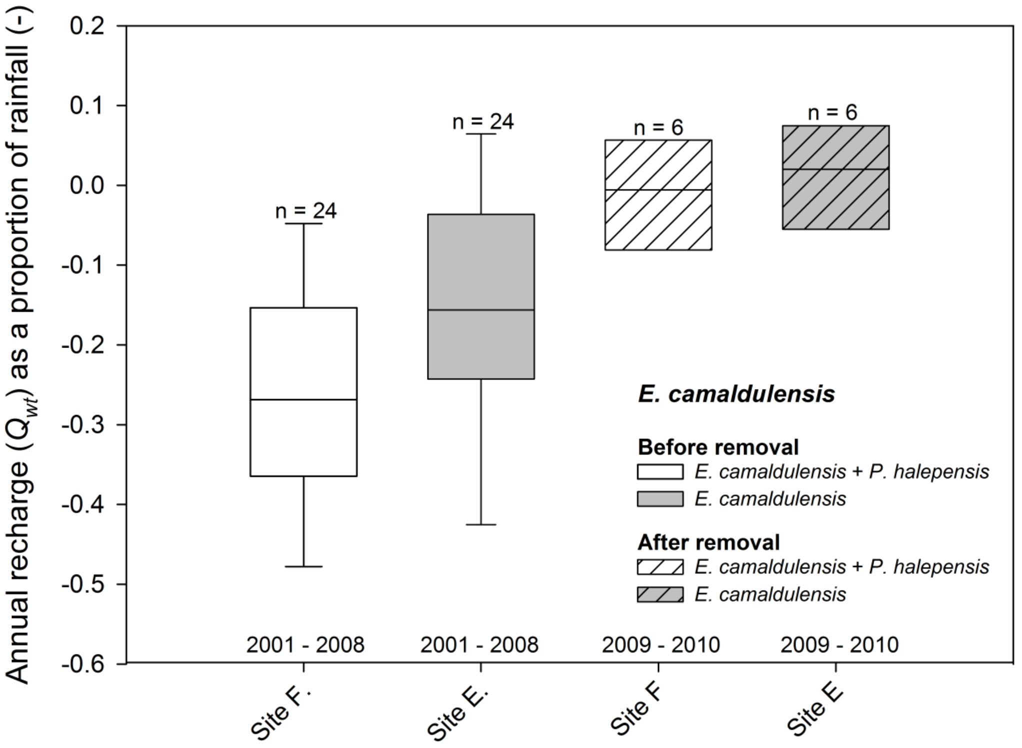

Figure 4.3.8 Annual net recharge (Q_{wt}) as a proportion of rainfall received before (2001 – 2008) and after (2009 – 2010) removal of *P. halepensis* within an *E. camaldulensis* stand (Site F). A comparative *E. camaldulensis* stand with no history of *P. halepensis* infestation (Site E) is shown for comparative purposes for both periods (filled boxes).

Caution is recommended when considering these figures, as other location-specific variables may influence ET_a besides vegetation cover (depth to groundwater, soil depth, etc., and were not considered in this work). Perhaps the most reliable water saving estimate was evident for the *E. camaldulensis* stand, before and after removal within a single site, where measured ET_a was reduced by 50 (± 11 SE) mm year^{-1} once *P. halepensis* was removed.

4.4. Discussion

Ecophysiological traits

The declining status of a freshwater aquifer prompted this investigation into tree water use and ET_a losses associated with the invasive pine species. Differences between the ecophysiological traits and ET_a of *P. halepensis* and the native tree and shrub species were detected. *P. halepensis* had the highest median sap flux rate and its transpiration rate (0.20 mm day^{-1}) was comparable to previously reported values (0.15 – 2.1 mm day^{-1}; Schiller and Cohen, 1998; Yaseef *et al.*, 2010). The higher sap flow velocity was consistent with the lower sapwood density of *P. halepensis*. Wood density can be used as a predictor of a range of parameters that relate to plant water use (O’Grady *et al.*, 2009, McCulloh *et al.*, 2011), with sapwood density negatively correlated with sapwood conductivity (Stratton *et al.*, 2000, Bucci *et al.*, 2004) and positively correlated to specific leaf area (O’Grady *et al.*, 2009). This indicates that removal of *P. halepensis* and replacement by native species (with higher sapwood density and lower transpiration rates), may reduce the volume of water lost as transpiration. I note however, species-specific transpiration differences may be compensated by variable soil evaporation or canopy interception rates for different trees, and result in similar net ET_a values (Swaffer *et al.*, 2014).

Water balance using remotely sensed $\mathrm{ET_a}$

I examined the water balance of the aquifer system and above ground vegetation using 10 years of ET_a data derived using the CMRSET algorithm.

When all years were combined, the data suggested net discharge was occurring for the Uley Wanilla groundwater basin, which is consistent with the observed water level trends. It is important to note that the region was in severe drought during most of the ten years when ET_a data were available, which was likely to have increased the amount of discharge relative to historical levels. Regardless, higher ET_a, than can be attributed to the presence of *P. halepensis*, was detected by comparisons between the open shrubland and *E. camaldulensis* vegetation associations with and without *P. halepensis*.

I compared ET_a from two *E. camaldulensis* sites: one where *P. halepensis* was removed at the end of 2008 and another where there was no history of *P. halepensis* invasion. I showed that removal of *P. halepensis* reduced ET_a by 50 (± 11) mm year^{-1}. Considering the reported annual recharge rate for the Uley Wanilla groundwater reserve has ranged between 11 – 16 mm yr^{-1} (2001/02 – 2011/12) (Government Gazette 2012), this additional water has the potential to increase groundwater recharge. Invasive species have been reported to negatively impact system hydrology elsewhere (Elkington 2009, Rascher *et al.*, 2011, Boyce *et al.*, 2012). Our results suggest there is a linkage between water use by the invasive pine and a reduction in recharge to this karst aquifer. However, I consider that more than two years of post-clearance ET_a data are required to confidently assess the value of these water savings.

Groundwater use by vegetation

It is difficult to separate the decline in water levels from reduced recharge or increased discharge (groundwater access) by *P. halepensis*. Not all negative Q_{wt}

values indicate groundwater use, as excess soil moisture can be present in the subsurface profile from the previous year's rainfall. However, five consecutive years (2004 – 2008) were at or below the long term average of 564 mm year^{-1}. It seems reasonable then, in the absence of surface water expressions, that discharging sites during 2008 contain vegetation that was using groundwater to maintain transpiration.

Groundwater use, however, was not clearly demonstrated by the tree water source data (isotopes and leaf and soil water potentials) collected in 2010. The observed response *P. halepensis* transpiration response to rainfall on 24th May 2010 suggested the trees were previously water limited. The isotope signatures do, however, suggest different species access difference water sources within the karst profile, as has been reported in similar environments (Schwinning 2008). The *P. halepensis* isotope values were more depleted, most likely indicating deeper, water stores in the profile or groundwater compared to most of the native tree and shrub twig water signatures.

Benyon *et al*., (2006) observed groundwater uptake by plantation pines in a karst environment in areas where the water table was < 8 m below the soil surface. Groundwater depth at our detailed study site was ~15 metres, which exceeds Benyon's *et al*., (2006) threshold in a similar geological setting. A review of groundwater discharge studies in Australia and global reviews of plant rooting distributions concluded that the maximum depth of groundwater discharge was 5−10 m (O'Grady *et al*., 2010).

Groundwater use, of all the vegetation classes examined here, appears most likely for the *E. diversifolia* woodland. Here, the CMRSET data suggested these areas had negative Q_{wt}, therefore additional water was being lost than can be met by rainfall. Following this, it appears that groundwater recharge will be adversely affected not only by the presence of *P. halepensis*, but also by encroachment of some native vegetation associations. Annual ET_a was less than rainfall in open and shrubland regions, suggesting that this additional water would facilitate deep drainage and groundwater recharge. The Q_{wt} rates in this study, normalised for rainfall received, are comparable with the two years of detailed plot scale measurements in Swaffer *et al.*, (2014), and provide confidence in the CMRSET application to this location. In a similar karstic setting, open area Q_{wt} was 0.28 and 0.11, similar to the 0.16 reported here and the *E. diversifolia* Q_{wt} was – 0.21 and – 0.19, which is comparable to the – 0.18 calculated in this study for this vegetation type.

Our results indicate that the presence of *P. halepensis* infestations and expansion of *E. diversifolia* would reduce recharge rates. In addition, it is important to recognise that much of the ET_a data used in this analysis was obtained during one of the most severe droughts to have affected south-eastern Australia; therefore it is unsurprising to have recorded few positive (draining) Q_{wt} areas. It would be beneficial to expand the CMRSET ET_a analysis to more recent years (2011 – 2013), with higher rainfall totals; however the data were unavailable at the time of this analysis.

4.5. Conclusion

This study demonstrated that *Pinus halepensis* was capable of transpiring a larger volume of water per unit of conducting wood compared to the native tree and shrub species. I also showed that actual evapotranspiration was higher from sites where *P. halepensis* had invaded *E. camaldulensis* woodland systems, and that removal of the trees can reduce ET_a by 50 mm year^{-1}. However, our analysis also showed that encroachment by other native vegetation associations (*E. diversifolia*) would also reduce groundwater recharge. Groundwater discharge was consistently observed in some areas, which was most likely due to groundwater use by vegetation during the below average rainfall conditions. Our findings link the ecophysiology of *P. halepensis* and the hydrology of the system to provide data to support the active management of the invasive pine and increase the volume of water available for groundwater recharge.

5. Final remarks

5.1. Major contributions to knowledge

This thesis demonstrates the ecohydrological links between vegetation and the shallow, karstic groundwater resources on Eyre Peninsula, South Australia. It considered whether the declining status of the fresh water resources could be attributed to the evapotranspiration from native and non-native trees encroaching across groundwater recharge areas. Ultimately, the goal of this work is to address important knowledge gaps to improve the management of these regionally significant groundwater supplies, with the results expected to be used to inform water resource policy.

Upon reflection of the objectives stated in section 1.3, I have synthesised the major findings of this thesis into sub-sections, which are outlined below.

Evapotranspiration

Annual evapotranspiration for two native tree species were similar to the estimated long term rainfall for the region, suggesting evolutionary convergence with available precipitation. This demonstrated the ability for both *E. diversifolia* and *A. verticillata* to maximise the use of available precipitation, irrespective of stand and morphological differences, and has been reported elsewhere (Eagleson, 1978, Nemani and Running, 1989, Ellis and Hatton, 2008).

Furthermore, while some divergence within the components of *ET* was observed; i.e. *E. diversifolia* had transpired more water whereas *A. verticillata* demonstrated higher soil evaporation, at the annual timestep each pathway compensated for the other, resulting in convergence in overall *ET*. This offset further supported the conclusion that vegetation water use has evolved along with the average climatic characteristics.

Similar to the precipitation and *ET* losses, rainfall partitioning fractions between the two native tree species were also remarkably similar. This was despite the often cited morphological differences meant to result in divergent rainfall pathways, such as bark thickness, leaf angle, etc. Throughfall, as expected, represented the largest pathway for rainfall followed by canopy interception and finally stemflow. Further to this, I suggest that stemflow, while volumetrically minor compared to throughfall and interception, is likely to play a significant ecohydrological role in enhancing the volume of soil moisture available to trees during times of water deficit.

I also identified discrepancies in the available literature regarding stemflow studies, where the magnitude of stemflow fractions reported was dependent on the methodology (scale) applied to obtain the data. I believe that this divergence is likely to have arisen because of, at least in part, differing discipline objectives: catchment hydrology/water balance studies require plot scale measurements, whereas ecological studies of nutrient recycling/biogeochemical processes are more suited to individual tree canopies.

Groundwater use by trees

Groundwater access by trees was not clearly demonstrated using stable isotopes of water. Results, as discussed throughout Chapters 2 and 4, were ambiguous, which was attributed to the heterogeneity of the karst material and high degree of mixing of unsaturated zone profiles, paired with the calcrete capping most likely preventing, or at least reducing, the establishment of an evaporative front. I have explored the suitability of using the stable isotopes of water as environmental tracers in karst systems (see section 5.2.3), where I have recognised the limitations of the approach and suggested future approaches which may improve the interpretability of results.

Beyond the use of the stable isotopes of water, the water balance information derived from remotely sensed *ET* data was also useful for assessing the potential for groundwater use by trees. Once normalised for rainfall, *ET* was similar between the two years of detailed plot scale measurements described in Chapter 2 and the water balance assessments presented in Chapter 4. For example, Chapter 2 showed that the normalised water balance of the open area in Uley South was 0.28 and 0.11, which is close to the 0.16 estimated in Uley Wanilla and indicated deep drainage was occurring. Further to this, where areas were dominated by *E. diversifolia*, the normalised water balance was – 0.21 and – 0.19 (Uley South, obtained through detailed plot scale measurements), which, again, was similar to the normalised water balance of – 0.18 (Uley Wanilla, delivered using remote sensing data), suggesting discharge occurred under this vegetation. This comparability across methods provides confidence to the application of CMRSET data to the broader Eyre Peninsula region.

Implication of invasive tree species

Beyond native vegetation, the exotic *Pinus halepensis* was found to have higher rates of sap flux per unit conducting wood area compared to its native co-occurring counterparts. Chapter 4 suggested this higher rate of sap flux is facilitated by higher sapwood hydraulic conductivity, demonstrated by the significantly lower sapwood density of *P. halepensis*. Therefore, depending on the density of the invaded areas by *P. halepensis*, higher rates of tree water use might be expected, which is likely to have exacerbated the water level decline of the Uley Wanilla groundwater lens. I went further than tree water use alone, by comparing evapotranspiration rates before and after removal of *P. halepensis*, and suggested the control program resulted in a reduction of ~ 50 mm year^{-1} ET_a, demonstrating that active management of this species should improve the volume of soil moisture available for deep drainage and presumably, groundwater recharge.

I do note however, that *Pinus halepensis* is unlikely to be the only tree species capable of contributing to reduced recharge levels; high rates of *ET* were also attributed to *E. diversifolia* and *E. camaldulensis*, suggesting encroachment of these native species beyond their current range will have a similarly negative effect on long term sustainability of the groundwater system.

5.2. Trees and the Southern Basins – what else have we learnt?

5.2.1. Groundwater recharge – will it still occur?

This assessment of the ecohydrological characteristics of common tree species growing across the basin has yielded useful information for resource managers. Consistent with the literature, the presence of woody tree species increased *ET* in comparison to non-vegetated areas, reduced soil moisture contents and consequently groundwater recharge rates.

Despite the shallow nature of the groundwater lens, the data did not provide strong evidence for direct groundwater extraction by trees. Rather, the native tree species evapotranspired a similar volume of water to the estimated long term rainfall for the area, which led to the conclusion the trees had evolved to maximise their use of available precipitation. It is important to note the estimated long term average rainfall of 500 mm year^{-1} was derived using long term records from a station ~15 km away and applying a regression established from an on-site weather station with a relatively short data span. The installation of gauges across Uley South reserve in 2009 is now establishing a measured rainfall record, which in the future can be compared to the estimated 500 mm year^{-1} cited in this research.

Chapter 2 concluded that above average rainfall conditions will be required to facilitate groundwater recharge under vegetated areas, based on the convergence between annual *ET* and the estimated long-term average rainfall for the sites. It should be noted however, that karst systems, with the characteristic presence preferential flow pathways, has the ability to rapidly transfer rainfall from surface soils to groundwater and bypass the subsurface profile containing tree root systems. This mechanism results in the ability of shorter, more intense rainfall periods to generate groundwater recharge, if occurring in quick succession, which while wasn't noted in Chapter 2, is recognised as a valid recharge mechanism for this system.

This thesis has demonstrated the ecohydrological impact of two dominant native tree species, and while at an annual timestep they require little distinction, the temporal dynamics in transpiration were different. *Allocasuarina verticillata* showed seasonal variation in transpiration, with negligible amounts of water being used in summer and early autumn. Therefore, the timing of recharge rates may be influenced by tree species if intense, summer recharge is important to the aquifer. In its dormant state, *A. verticillata* would be less efficient at using water delivered during summer storms compared to *E. diversifolia*, which showed little seasonal variability in tree water use. In addition, *P. halepensis* also demonstrated a transpirational response to rainfall received in May 2010, providing further evidence for the reliance of trees on soil moisture, rather than groundwater (Figure 5.2.1.1).

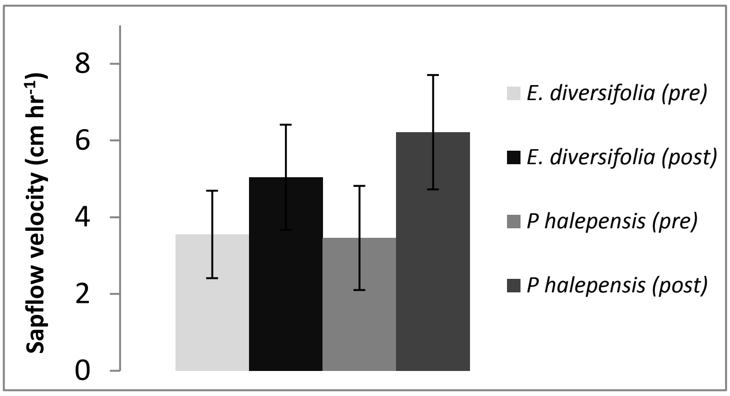

Figure 5.2.1.1 *Eucalyptus diversifolia* and *P. halepensis* sap flow velocity response to rainfall received on 25th May 2010, as discussed in Chapter 4.

5.2.2. Scaling sap flux data – from xylem to stand

The inclusion of tree water use measurements are often necessary to accurately estimate a catchment water balance, especially if one of the objectives is to partition the components of *ET*. One of the primary methods through which transpiration estimates are generated measures the rate of sap flow through tree stems (Hatton *et al*., 1995). Such techniques can be used to obtain accurate point measurements of rates of water flow through trees, which are then scaled to the level of whole trees and/or stands (e.g. Schaeffer *et al*., 2000, Tateishi *et al*., 2010).

For such scaled measurements to be accurate, the sampling must adequately encompass the spatial and temporal variation in sap velocity (e.g. Tsuruta *et al*., 2010). To do so requires parameters that are relatively simple to measure, and by applying a conversion factor related to the areal coverage of the trees (i.e. basal area, trunk diameter, sapwood area etc.) (Thorburn *et al*., 1993a, Hatton *et al*., 1995, Vertessey *et al*., 1995, Caterina *et al*., 2014) tree or stand water use can be estimated. However, failure to follow the procedures carefully can lead to significant margins of error in water use estimates.

The uncertainty associated with the use of heat pulse techniques to estimate stand transpiration are primarily connected to the calculation of sap flow velocity (using sapwood wounding, wood and water fractions, resolution of probe spacing), integration from sap flow point estimates to stem water use (due to variable sap flow velocity across the radial profile) and conversion of stem to stand transpiration using a scalar for areal coverage (Hatton *et al*., 1995). These errors have been shown to be minimised by stratifying the sensors the sensors within a given stem by depth into conducting wood and aspect, and by stratifying the selection of trees within the stand by size class (Hatton *et al*., 1995). These error minimisation techniques were applied during sap flux investigations outlined in this thesis; aspect was varied, placement of sensors within the sapwood was staged across the radial profile, and stems were assigned to one of three tree size classes (*E. diversifolia* DBH: 0 – 10 cm, 10.1 – 20 cm, 20.1 – 30 cm. *A. verticillata* DBH: 0 – 15 cm, 15.1 – 30 cm, 30.1 – 60 cm) with trees selected for instrumentation randomly chosen within each size class.

In the studies outlined in this thesis, each probe was installed below the height of the lowest live branch, to ensure installation in sapwood which supported the whole trees' water use requirements. This height therefore varied, depending on the tree to be instrumented, but was generally between 0.5 and 1.5 m above the ground level. Sap flow velocity for a particular stem was calculated by averaging the sensors installed within the stem which, due to their staged depth and varying aspect (which also controlled for the effect of solar radiation), accounted for the radial variability in velocity rate. From this, each average stem sap flow velocity was again averaged across the rest of the instrumented stems in the plot, further reducing the influence of a single sensor or stem on the final sap flow velocity calculation. This delivered an estimate which accounted for both sap wood and stem variability, and was therefore more likely to represent an ‘average’ sap flow velocity that could be considered representative of the stems within the stand.

The dataset was also corrected for minimum sap flow velocity following Benyon (1999), where the threshold for ‘no flows’ was determined on nights when the relative humidity was high (and therefore sap flow unlikely to be occurring). This threshold was estimated by averaging the sensor readings between midnight and 5AM over several suitable night time periods, and calculating the upper 99 % confidence intervals based on the standard deviation. Subsequently, any sensor reading below this defined threshold was assumed to represent no flow and hence, sap flow velocity was converted to zero.

In addition to the calibration steps described above, some studies have corrected transpiration estimates using the water use of cut trees inserted into water reservoirs (i.e. Caterina *et al*., 2014) at the conclusion of sensor deployment. These calibration procedures have demonstrated significant underestimation of sap flow velocity generated using sensors (Caterina *et al*., 2014), highlighting the value of such procedures. While this process wasn’t used in this thesis, it is acknowledged future works deploying sap flow sensors could incorporate additional calibration protocols, such as cut trees, into the experimental design.

5.2.3. Use of isotope tracers in karst systems – can it be done?

Groundwater use, as aforementioned, could not be excluded from the interpretations described throughout the thesis; however evidence that the trees were reliant on groundwater to sustain the populations was not strong. Isotope data suggested the subsurface profile contained a mixture of water of differing isotopic signatures, which is consistent with the behaviour of karst systems. It is possible that groundwater from the capillary fringe was mixing with deeper soil moisture stores, and that trees (most likely *E. diversifolia*) were using this water in times of water deficit.

Mixing lines were presented in Chapter 2 and 4 (Figure 2.3.3 and Figure 4.3.4), (following Thorburn *et al.*, 1993a) based on the premise that ambiguity in matching sources to twig water signatures could be, at least partially, explained by combining simultaneous uptake from more than one water source. The lines were created by building a linear regression between the two likely isotopic end-members (in this case surface soil and groundwater), and the relative contribution of each source is estimated by the placement of the twig water signature along the mixing line. The technique assumes source signatures which fall closest to that of the mixture provide the greatest contribution. However, it should be noted the approach has attracted some criticism, as certain combinations of distant sources could explain the signature, with little or no contribution from the proximate sources (Phillips and Gregg, 2003). Regardless, mixing lines provide one tool through which twig water signatures and various tree water sources can be explored. Those that are presented in this thesis serve primarily an illustrative purpose, rather than suggesting a definitive solution to the sources of water being accessed by the tree species studied.

A more sophisticated approach from mixing lines uses simple linear mixing (SLM) models to calculate the relative contribution of each potential source to a sample result (Ogle *et al*., 2014). Examples include IsoError (Phillips and Gregg, 2001), IsoSource (Phillips and Gregg, 2003), IsoConc (Phillips and Koch, 2002), MixSIR (Moore and Semmens, 2008) and SIAR (Parnell *et al*., 2010). While these models are useful, and have certainly improved our ability to use stable isotopes to partition environmental processes (Ogle *et al*., 2014), their application remains constrained by several limitations. One of the more important is their inability to incorporate additional information to reflect the ecological processes which are influencing source contributions (Ogle *et al*., 2014). For example, the relative contribution of soil moisture to plant water uptake can be characterised using SLM models, but because root uptake cannot, in itself, be incorporated, they are unable to predict how soil water contributions may change through space and time or in response to certain events i.e. rainfall.

The SLM model used in this Chapter 2 of this thesis (IsoSource) has been successfully used to characterise vegetation water use patterns along tidal creeks (Wei *et al*., 2013), above shallow groundwater (Li et al., 2013) and cloud immersion (Carter Berry *et al*., 2014), however, it too, has important limitations regarding its suitability. The selection of a particular mass balance tolerance level can affect the median values when source contributions become less constrained (Phillips and Gregg 2003), the tool has been argued to lack a solid statistical foundation (Ogle *et al*., 2014) and is incapable of differentiating multiple sources with very similar isotopic signatures (Phillips and Gregg, 2003). In response to some of these limitations, Phillips and Gregg (2003) recommended reporting the range or distribution of feasible solutions, as was presented in Chapter 2, rather than focussing on a single value such as the mean or median.

In hindsight, and as discussed in Chapter 2, the rapid recharge tendency of karst systems, where rainfall can reach the water table very quickly, combined with the degree of mixing through preferential and diffuse recharge pathways would blur the distinction between water source signatures. While isotopic profiling in karst systems should not necessarily be discounted, substantial attention should be given during the experimental design phase of any potential application, in light of the level of ambiguity in signatures that should be expected. The work presented through this thesis underestimated the similarity between subsurface soil moisture and groundwater, and did not include sufficient subsurface profiling of the unsaturated zone (due to logistical constraints, primarily cost), to characterise the water available for tree transpiration.

5.2.4. Constraints upon evapotranspiration

Recognising that actual *ET* remained well below (one third of) potential *ET,* a significant constraint is evident on the system (which may not be water-related i.e. nutrient). The volume of effective precipitation was also enough to sustain the amount of water transpired each year, so theoretically, adequate supplies of water should be present in the subsurface profile to sustain both of *E. diversifolia* and *A. verticillata* associations.

Understanding how climate regulates tree water use is important, as it can reflect the environmental conditions which drive evaporative processes, including tree water use. Primarily, the leaf-to-air vapour pressure difference is the measure of evaporative demand, commonly referred to as the vapour pressure deficit (VPD) (Eamus *et al.*, 2006). VPD delivers the required gradient to 'pull' water through the trees, out of the leaves and into the atmosphere, providing stomata remain open and plant water uptake can keep pace with atmospheric demands (Glenn *et al.*, 2013). Quantifying VPD can be useful for elucidating tree water behaviour, both at times where sap flow rates could be expected to be limited or optimal, depending on water availability i.e. if actual *ET* approaches potential *ET*, it can suggest the system is driven by climate (VPD) rather than resource (water) availability. This research found, as stated above, actual ET remained well below potential ET, suggesting water availability is limited, and providing another line of evidence which suggests reliance on groundwater was not strong at these sites.

Another restriction on tree water use (which received little attention in this thesis) relates to the ability of salts to restrict the movement of free water molecules, thereby reducing water availability (Eamus *et al.*, 2006) and potentially transpiration rates (Doody *et al.*, 2009). This regulatory mechanism is commonly reported in dryland environments, particularly where infrequent flooding leads to salt accumulation in the soil profile surrounding tree root systems, for example across the River Murray floodplain (Holland et al., 2006, Overton *et al.*, 2006, Doody *et al.*, 2009). Referred to as osmotic or solute potential and recognised as one component of the overall soil water potential, the attention I directed towards quantifying this parameter was limited. This was a deliberate decision informed by the salinity data available for both the Uley Wanilla and Uley South groundwater system lens, averaging ~ 1000 EC (DEWNR 2011).The highly responsive nature of the water table to rainfall-derived recharge, supported by low salinity groundwater and confirmed using the subsurface soil samples described in Chapter 2 led to the determination that the osmotic water potential was negligible, and unlikely to be capable of limiting tree water use in this geological setting.

5.3. Roots and rocks – a perplexing association

While this research has yielded much insight into the system, to evolve our ecohydrological understanding, an increased focus on the change in water storage in the rocky unsaturated zone is required, and most importantly, how much of this stored water is accessible to the trees?

For over a century, scientists have been aware of plants extending their roots into a fractured rock surface (Cannon 1911), presumably in search of water. More recent work has demonstrated the importance of water held within weathered rock substrates to sustain transpiration (Rose *et al*., 2003, Querejeta *et al*., 2006); with some profiles storing a similar amount of water as a coarse textured soil (Schwinning 2010).

Recognising the potential for water stores within weathered rock, a critical question remains regarding the volume of water present that is available for plants. In typical soils and with a tendency of referring to crops, it is assumed the volume of plant available water is the water stored from the soil surface to the maximum root depth, between field capacity and permanent wilting point (– 1.5 MPa). It should be noted that this classical definition of plant available water does not translate well to Australian native plant species, which are known to extract water well below – 1.5 MPa (i.e. Cramer *et al*., 1999, Holland *et al*., 2006, Doody *et al*., 2009). Regardless of the extraction potential of the tree species of interest, assuming no physical barriers are in place preventing root expansion, roots should extend evenly to deplete soil water contents relatively uniformly (Schwinning 2010).

This assumption of even soil water depletion may not be valid for a substrate that is derived from rock, where impermeable layers or barriers remain, and pathways for root extension remain limited. Thus, the fundamental assumption that storage capacity and water availability is proportional to root depth may not hold true for roots in rocks.

Regardless, the presence of a rock-derived substrate does not come without advantages. Certainly, while access to water may be more complex than within a regular soil, that same water is also stored below rock layers that are protected from evaporative losses, and therefore is available for slower rates of extraction. I suggest that this is one factor that facilitates the persistence of trees with low rates of transpiration, such as those measured in this research. Despite the lack of an evaporative front in the unsaturated zone, is in interesting to reiterate that *A. verticillata* remained coupled to the season, showing dormant behaviour towards the end of summer. This further suggests that the root zone of *A. verticillata* is constrained to the upper parts of the profile that despite limited evaporation, the rate of drainage and transpiration was sufficient to dry the soil profile.

High temporal resolution monitoring of soil water changes within the root zone (with, for example, neutron moisture meters), would provide further insight into the ability of the unsaturated zone to sustain tree transpiration. Notably, due to the karstic nature of the profile, spatial coverage of such probes should also be high, to gain certainty that the data spans the environmental variability expected with this geological formation. Regardless this monitoring approach, in addition to tree root trenching (for example, following the approach of Querejeta *et al*., 2007), would assist elucidating tree water use and the partitioning between soil moisture and possible reliance on groundwater.

5.4. Potential for encroachment over the recharge zone

Woody plant encroachment into regions historically void of trees is occurring worldwide (Huxman *et al*., 2005, Heilman *et al*., 2009), and has resulted in ongoing debate regarding the subsequent hydrological impact (Doody *et al*., 2011). Understanding the implications from such encroachment is particularly critical on water-limited karst systems used for drinking water supplies (Schwinning 2008), where climate change predictions suggest hotter and drier scenarios are likely (CSIRO 2007) and resulting recharge rates are increasingly uncertain (Ward *et al*., 2009).

There is a common perception that encroachment by woody trees over grassland has a significant negative effect on the yield of fresh water resources (Heilman *et al*., 2009), and certainly, it is well accepted that forested *ET* will be higher than that of grassland *ET* (Zhang *et al*., 2001). This study has shown that, in the Uley South lens, encroachment by trees over grassland areas will increase *ET*, and should be recognised by water resource managers.

Another facet deserving consideration is the obvious historical extent of *A. verticillata* over the recharge zone of Uley South, demonstrated by the remnant trunks. With the almost complete absence of this association in the modern era, what successional phases are occurring to occupy this empty niche? Is encroachment by an alternative species, albeit slow, occurring?

Figure 5.4.1 Remnant *Allocasuarina verticillata* trunks lying across the low lying recharge zone of Uley South groundwater reserve. Insert: *E. diversifolia* adjacent remnant *A. verticillata* trunks

The mechanisms which may facilitate woody plant encroachment include mechanistic (root zone separation), land use change (grazing), ecological (predation) and/or climatological (increased atmospheric CO_2 favours a shift from C_3 grasses to C_4 shrubs). For this system, the most obvious mechanistic influence is from grazing, where high numbers of kangaroos and rabbits preferentially graze grasses and the highly palatable *A. verticillata* seedlings, opening up a pathway for the spread of *E. diversifolia* and coastal shrubs such as *L. parviflorus*.

Identifying spatial changes in vegetation extent through time can be investigated using satellite imagery and aerial photographs. Alternatively, quantifying tree ages through dendrochronology (examining tree rings) may provide a simpler approach to understanding stand age, particularly for comparing the age of trees growing within a stand. This would be especially interesting where *E. diversifolia* is growing amongst remnant *A. verticillata* trunks, e.g. the *E. diversifolia* site (ULE101) studied here (Figure 5.4.1).

The formation of annual tree rings is the result of cambial activity (Kozlowski 1971); the initiation and development of which is affected by physiological and environmental conditions (Fritts 1976). Therefore, this seemingly straightforward approach to estimating tree age is affected by the circumstances present in a given year; unfavourable situations may stimulate only partial cambial activity, and conversely in years of favourable growth conditions, intra-annual rings may form (von Platen 2008). *Eucalyptus* spp. often lack a true dormancy period compared to other genera (i.e. conifers) resulting in the boundary between early wood and latewood (signifying the completion of one growth cycle and the onset of another) difficult to distinguish (von Platen 2008). This can be further exacerbated if attempting to determine the age of sub-dominant or suppressed trees (Rayner 1992). These anomalies led to the belief that *Eucalyptus* spp. do not develop reliable rings, as they are particularly susceptible to the formation of intra-annual bands, false or missing rings (Argent, 1995, Pearson and Searson, 2002), and subsequently dendrochronology methods focussed on *Eucalyptus* spp. have received limited deployment in Australia (Pearson and Searson, 2002). However, recent methodological advances (i.e. use of atmospheric and cellulose C^{14} by Akeroyd *et al*., (2002), radiocarbon dating by Pearson *et al*., (2001)) have been successfully applied to resolve some of this uncertainty, leading to calls to revisit the application of dendrochronology in Australia (Pearson and Searson, 2002).

The absence and lack of regeneration of *A. verticillata* has been suggested, in this thesis, to be the result of continual heavy grazing pressure by rabbits and kangaroos. This should place *E. diversifolia*, much less palatable, in an advantageous position to colonise the area. Energy is not a limiting factor, with potential *ET* rates in the order of 1500 mm year^{-1}, and if access to groundwater is possible, water would not be a limiting factor either. The fact measured *ET* was 1/3 of potential *ET* provides strong evidence of a significant limitation on this system.

An alternative explanation, and one not explored in this thesis, is nutrient availability. *Allocasuarina verticillata*, as a nitrogen-fixing species, may have historically been capable of colonising the karst landscapes of western Eyre Peninsula because of the provision of nutrients through a symbiotic association with nitrogen fixing bacteria, *Frankia* spp. The lack of similar access to nitrogen for *E. diversifolia* may have significantly hampered its ability to spread across the shallow soils above the groundwater systems, and constrained its occurrence to isolated area where geomorphological differences facilitated small stands of trees establishing.

The link between stomatal conductance, foliar nitrogen and carbon assimilation was, to my knowledge, first explored by Taylor and Eamus (2008). They examined these relationships for seven tree species across a significant rainfall gradient and reported that trees with reduced water transport capacity were able to support a higher photosynthetic rate by increasing investment in foliar nitrogen. This finding appears consistent with the ability of *A. verticillata*, despite having lower transpiration rates compared to *E. diversifolia*, to establish itself across much of western Eyre Peninsula. Unfortunately, this concept does not appear to have been re-tested in the literature since Taylor and Eamus (2008) so it is difficult to state whether these findings may translate to other tree species and ecosystems.

The benefit of symbiotic relationships between plants and nitrogen-fixing bacteria are, however, well understood (see reviews by Benson and Silvester, 1993, Schwencke and Carú, 2001). Considering the largely hostile environment of Eyre Peninsula (with its episodic and erratic rainfall and poor soils), I suggest the competitive advantage gained using nitrogen-fixing bacteria could be a key strategy which allowed such an expanse of *A. verticillata*. European settlement brought about such dramatic land use changes (primarily clearing for agriculture), which paired with the introduction of rabbits and increase in kangaroo populations, explains the demise in *A. verticillata* populations and ability for other species (i.e. *E. diversifolia*) to colonise the region.

5.5. Comparing groundwater flux using Australian studies

The ability to sustainably manage the groundwater resources of Eyre Peninsula requires an accurate water balance, which in turn necessities the quantification of groundwater flux. The findings presented thus far in this thesis are compared to a range of alternative groundwater recharge and discharge studies, to evaluate the consistency or dissimilarity of the Southern Basins groundwater resources to other characterised systems.

This section presents a review of studies where the links between vegetation and groundwater have been empirically estimated, in an attempt to identify parameters associated with climate, soils, landuse, geology and vegetation that collectively influence groundwater dynamics. Groundwater recharge, however, can be quantified using a variety of methods each with their own limitations and uncertainties, which make it difficult to directly compare recharge results (Crosbie *et al*., 2010). As identified by Scanlon *et al*., (2002) and Crosbie *et al*., (2010) depending on the method applied, recharge estimates may relate to flux of water within the unsaturated zone (i.e. deep drainage), flux to a water table (i.e. gross recharge), or net change in level across a water table (i.e. net charge). Recognising this, the studies reviewed in comparison to the results of this thesis are restricted to deep drainage estimates, as they are comparable to the results obtained using the water balance method. An exception to this is the inclusion of Ordens *et al*., (2012), as it was specifically focussed on recharge to the Uley South groundwater lens. It should be noted that the majority of groundwater recharge studies tend to use chemical methods to estimate recharge, similar to Ordens *et al*., (2012), and substantially fewer focus on quantifying the water balance. A recent review into groundwater recharge estimates suggests that 78 % of studies used a steady-state chloride mass balance approach, whereby the remaining studies used water table fluctuation, water balance and environmental tracer approaches (Crosbie *et al,*. 2010).

Table 5.5.1 Comparison of studies linking vegetation and groundwater flux dynamics to the water balance results presented in Chapter 2 (Swaffer *et al*., 2014a)

Climate	P_g[4]	ET_p	ET_a	Q_{wt}	Geology/Soils	Landuse	Vegetation	LAI	Reference
Semi-arid	**502 (442[6])**	**1524**	**532[5]**	**- 91[6]**	**Skeletal Aeolian soil overlaying karstic limestone**	**Native woodland**	***Eucalyptus diversifolia***	**1.32**	**Swaffer *et al*., 2014a**
			531[6]	**- 89[6]**			***Allocasuarina verticillata***	**0.48**	
			355[6]	**87**			**Grassland**	**-**	
Tropical	1723 (-)	3000 (Open class A)	1100	200	Bathurst Island formation	Native woodland	*Eucalyptus miniata*, *Eucalptus tetradonta*, *Melaleuca viridiflora*	1.0	Cook *et al*., 1998
Tropical	1208 (1129)	1098	900	250	Clay-loam transitioning to clay at 1.2 – 1.5 m	Native woodland	Mixed species eucalypt forest	1.05 to 1.95	Mitchell *et al*., 2012
Semi-arid	404 (314)	1800	2410	-	Dense clay	Riparian woodland	*Salix babylonica* (in-stream)	-	Doody and Benyon 2011
			563	-			*Salix babylonica* (bank)	-	
			553	-			*Eucalyptus camaldulensis* (bank)	-	
Semi-arid	268 (97)	1900	305	- 248	Coonambidgal Clay	Native floodplain	*Eucalyptus camaldulensis*, *Eucalyptus largiflorens*, *Acacia stenophylla*	-	Doody *et al*., 2009
			143	- 46				-	
			129	- 32				-	
-	630 (846)	1385	598	0	Yellow sodosols, lithosolic, clastic rudosols	Agriculture	*Casuarina cumminghamiana*, *Acacia mearnsii*, *Callistemon* sp., *Eucalyptus* sp.	-	Crosbie *et al*., 2008
			1399	- 692				-	
Semi-arid	300	-	-	< 1	Sandy overlaying Murray Group Limestone	Agriculture	*Atriplex nummularia*, *Acacia salinga*	0.66	Knight *et al*., 2002
Temperate	711 (630)	980	1090	- 435	Sandy	Forestry	*Pinus radiata* and *Eucalyptus globulus* (< 6 m groundwater)	3 - 4	Benyon *et al*., 2006
Semi-arid	659	1070	560	0	Clay		*Eucalyptus grandis* (< 3 m groundwater)	2 - 3	

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[4] Annual precipitation provided as the long term average (mm), with received annual totals (mm) depicted in brackets

[5] Average over two years of measurement

Climate	P_g[4]	ET_p	ET_a	Q_{wt}	**Geology/Soils**	**Landuse**	**Vegetation**	**LAI**	**Reference**
Climate	**Location**		P_g	ET_p	**Method**	**Recharge**			**Reference**
Semi-arid	Uley South groundwater		560	1547	Chloride mass balance	52 - 63			Ordens *et al*., 2012
					Water table fluctuation	47 - 129			

The studies described in Table 5.5.1 highlight the variety of environments in which water balance studies have been used to estimate groundwater flux. The purpose of the cited research similarly varied, driven (primarily) by soil salinisation (e.g. Knight *et al*., 2002, Crosbie *et al*., 2008), water resource management (e.g. Benyon and Doody 2004) or ecological health (Doody *et al*., 2009). For these reasons, direct comparison of the published literature to the work presented in Chapters 2 – 4 of this thesis are difficult, and presumably, is a key reason for each Chapter being accepted for publication in an international peer-reviewed journal.

The novelty of the findings presented in this thesis resides within its focus on karstic groundwater resources, semi-arid dryland environments and the tree species studied. The importance of the work is aligned to the reliance on these water resources to support the economic and social requirements of the region. I have clearly demonstrated the reduction in groundwater recharge that would be expected to occur under areas vegetated with woody tree species. However, as acknowledged, some ambiguity resides concerning groundwater extraction by trees. Generalising relationships between trees and groundwater abstraction was also found to be problematic in the recent review by O'Grady et al., (2010), where the often cited influence of depth to water table, groundwater salinity, soil type and tree morphology did not result in a convincing correlation. However, I believe future work using the CMRSET ET_a data (used in this thesis for Uley

Wanilla) and assessing the water balance for all of the Southern Basins from 2001 to 2010 would go far in elucidating the role of groundwater reliance by trees in this environment.

Areas where trees were absent (i.e. grassland areas), not surprisingly, had the lowest ET_a overall. The volume of water estimated to have passed as deep drainage was in the order of 130 mm (year 1) and 50 mm (year 2). This is comparable to estimates gazetted by the Government of 130 – 140 mm between 2007/08 and 2009/10 (Government Gazette 2012)[6] and the range suggested by Ordens *et al*., (2012). This excess water will therefore infiltrate deep into the soil profile and theoretically become available for groundwater recharge (presuming no impeding layers exist under root systems). These high recharge zones are critical to the maintenance of groundwater recharge rates, and therefore, the long term sustainability of the Eyre Peninsula groundwater systems.

5.6. Conclusion

This thesis presented the first water balance assessment for a range of vegetation associations growing above karstic groundwater systems in a semi-arid region: an important geological formation which represents a disproportionate fraction of drinking water sources globally.

By combining a range of methodologies related to the ecophysiological and ecohydrological characteristics of the vegetation, I have demonstrated the hydrological influence of woody tree species on recharge and discharge processes. Certainly, the areas above the groundwater lens which are devoid of

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[6] This research is presented calendar years; however government gazetted recharge rates are in financial years.

trees represent important recharge zones that need to be preserved, should resource managers be concerned about groundwater sustainability.

Further to this, the research presented here has clearly demonstrated a need for continuing investment for the removal of *Pinus halepensis* invasions. While first principles promote the removal of weed species where they are present, to my knowledge, this research provides the first (quantified) evidence of their hydrological influence.

I believe the results of this research will be used to govern water resource policy for the Southern Basins Prescribed Wells Area on Eyre Peninsula, as competition for fresh water increases, and intensified by reduced certainty of availability due to climate change predictions.

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