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The response of Banksia roots to change in water table level in a Mediterranean-type environment

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THE RESPONSE OF *BANKSIA* ROOTS TO CHANGE IN WATER TABLE LEVEL IN A MEDITERRANEAN-TYPE ENVIRONMENT

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B. Sc. (Hons.)

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USE OF THESIS

Abstract

For phreatophytic plants to persist in a given habitat they need to maintain a functional connection to the water table, and the capacity for roots to respond to changes in the water table is a key aspect of this. If root growth is limited by season, plants may not be able to grow roots to adjust to changes in the water table at a particular time of the year. The redistribution of roots, particularly the capacity for roots to follow the water table down in summer and autumn months, is vital for phreatophytic plants to maintain a functional connection with the water table. Root activity by phreatophytic Banksia in south-west Western Australia was assessed using root in-growth bags, with above-ground plant phenological processes observed simultaneously. The root in-growth bag technique that was used showed that *Banksia* roots are able to grow, provided soil conditions are conducive and there are no endogenous limitations to root growth at different times of the year, such as a dormancy period. The ability to grow at any time in response to soil conditions might be an essential prerequisite for phreatophytes if they are to survive fluctuating water table conditions in seasonally water-limited environments.

Assuming that roots follow a seasonally dynamic watertable, a further study using chemical tracers assessed water uptake from the capillary fringe at contrasting times of the year. Lithium chloride was used in spring and ^{15}N in autumn, to avoid contamination between the two sampling periods. There was some indication that the phreatophytic *Banksia* study species were utilising groundwater. This was evident from observation of tracer uptake from the capillary fringe during spring, when the water table was at its highest level. However, water uptake from the capillary fringe by *Banksia* was not evident during the autumn sampling period. This is likely to be due to methodological constraints, as plants did not demonstrate any signs of water stress despite the low water content in the vadose zone. These physiological indicators suggest that plants were still accessing groundwater at this time of the year.

Quantifying the rate of root elongation for a particular species may give some indication of the rate of water table decline that they are able to survive. Root elongation rates and response to rapid water table decline were observed in rhizopods, allowing for the maintenance and manipulation of a water table under glasshouse conditions. The maximum rates of root elongation were found to be 36.7 mm day^{-1} and 18.2 mm day^{-1} for *Banksia attenuata* and *Banksia littoralis* respectively. These rates of root elongation may be regarded as rapid in comparison to the rates of water table decline that the species are likely to experience over summer and autumn water table decline. Such rapid rates of root elongation indicate that plants can potentially maintain contact with a water table decline at far greater rates than those that occur during a normal seasonal cycle of water table fluctuations.

Based on the rates of root elongation by phreatophytic *Banksia*, it was expected that plants would maintain a functional connection with a rapidly decreasing water table. However, despite plant-available water being present in the soil profile, there was very limited root extension following rapid water table drawdown. In addition to little root elongation response, seedlings demonstrated a rapid drought response to water table decline, quickly reducing stomatal conductance, transpiration and photosynthesis. It is likely that the lack of root elongation response to a declining water table by the phreatophytic *Banksia* study species was due to plants being unable to meet their water requirements once the water table fell below the rooting depth of the plants. It was found that the response of phreatophytic *Banksia* to rapid water table decline depends on the availability of other water sources, and the rate of water table decline.

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Date 23 May 2011

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TABLE OF CONTENTS

USE OF THESIS ii

ABSTRACT iii

DECLARATION v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

CHAPTER 1 General Introduction 1

CHAPTER 2 Study area and species descriptions 8

CHAPTER 3 Dynamics of *Banksia* root growth relative to a seasonally fluctuating water table. 19

Introduction 19

Materials and Methods 24

Results 31

Discussion 43

CHAPTER 4 Seasonal groundwater uptake by dominant canopy species in a *Banksia* woodland. 50

Introduction 50

Materials and Methods 53

Results 57

Discussion 62

CHAPTER 5 Seedling root elongation rates for two phreatophytic *Banksia* species with contrasting water requirements 66

Introduction 66

Materials and Methods 69

Results 77

Discussion 83

CHAPTER 6 Root response of phreatophytic *Banksia* exposed to rapid rates of water table decline. 86

Introduction 86

Materials and Methods 90

Results 95

Discussion 103

CHAPTER 7 General Discussion 109

REFERENCES 114

Chapter One

General Introduction

Groundwater accounts for more than 30% of the earth's fresh water (Shiklomanov 1998), and a variety of ecosystems have developed in the presence of groundwater (Eamus *et al*. 2006). Groundwater may be defined as part of the soil profile that has reached maximum water holding capacity, with all soil pores filled with water, also referred to as the zone of saturation (Freeze & Cherry 1979; Eamus *et al*. 2006; Fig. 1.1). Plants which use groundwater are referred to as phreatophytic, from the Greek 'phreatos' – a well, and 'phyte', denoting a plant having a particular characteristic (Meinzer 1923). Phreatophytes can access groundwater either directly from the water table (the upper surface of groundwater) or from the capillary fringe, which sits above the water table and contains groundwater held by capillary action (Freeze & Cherry 1979; Eamus *et al*. 2006). Phreatophytes are prevalent in water-limited environments, where annual evapotranspiration exceeds annual rainfall, resulting in a net deficit in water availability (Abrahams & Parsons 1994; Cui & Shao 2005). This water deficit is exacerbated in habitats with soils that have low water-holding capacity, such as coarse sands (Sperry *et al.* 2002). In such environments, water availability in the unsaturated (vadose) zone is often unreliable, and, as a result, many species develop deep root systems to access deeper water sources, such as groundwater (Stone & Kalisz 1991; Canadell *et al*. 1996). In water-limited environments, plants that have access to a water table often have higher rates of transpiration, with less regulation of stomatal conductance compared to species which utilise only vadic water (Gardner 1983). In addition, these phreatophytic plants may be more vulnerable to water stress, due to the physiology of xylem (Tyree *et al*. 1994; Pockman & Sperry 2000; Horton *et al.* 2001; Canham *et al*. 2009). It is the functional connection to groundwater, in terms of root contact with the capillary fringe or water table and use of groundwater, that allows phreatophytic plants to meet their higher water requirements.

Plants that are dependent on access to groundwater can be detrimentally impacted by changes in groundwater levels, which are dynamic, falling due to discharge and rising due to recharge. A decrease in rainfall can lead to reduced groundwater levels, particularly in environments where groundwater levels are tightly coupled with rainfall recharge (Allen 1976; Harrill & Prudic 1998). For example, the south west corner of Western Australia has experienced a drying trend, with a decline in annual rainfall since the mid 1970s (Bureau of Meteorology 2011) contributing to a gradual decline of groundwater levels over a landscape scale (Yesertener 2008). Groundwater levels can also be influenced by groundwater abstraction, which can result in a rapid decline of the water table. Changes in the water table can sever the functional connection between phreatophytic plants and the water table, which means plants are no longer able to meet their water requirements (Mahoney & Rood 1991; Naumburg *et al*. 2005). As such, groundwater decline has been associated with negative impacts on phreatophytic plants. Following changes in groundwater regime, phreatophytic plants have been found to exhibit signs of water stress, such as low predawn water potentials, reduced stomatal conductance, and eventually crown and plant death (Scott *et al*. 1999; Sperry & Hacke 2002; Cooper *et al*. 2003). Thus, for a phreatophytic species dependent on access to groundwater to persist in a given habitat, plants need to maintain a functional connection with a dynamic water table.

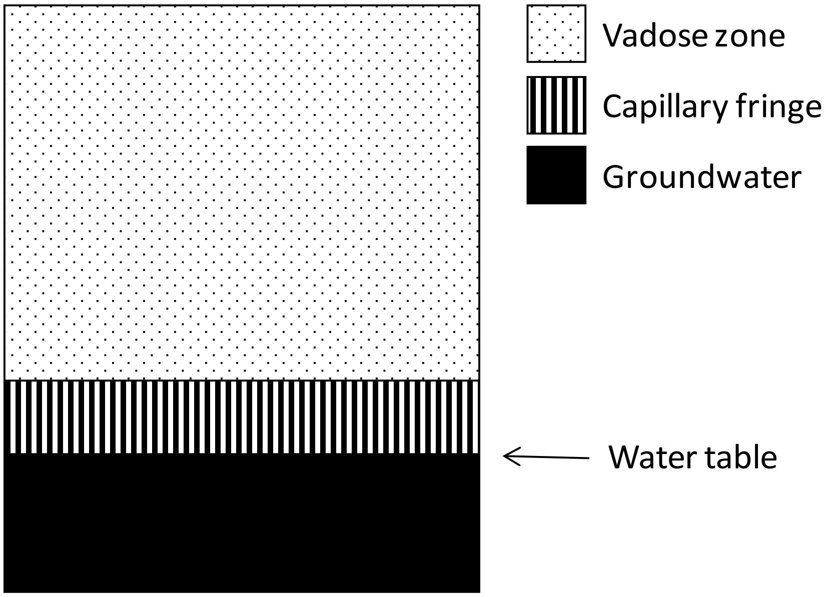


Figure 1.1. Schematic of potential plant water sources, with groundwater, the upper surface of which is the water table. Rising above the water table is the capillary fringe, with water drawn up by the capillary action, and the vadose zone is the unsaturated part of the soil profile. Adapted from Freeze & Cherry (1979).

Although phreatophytic plants can be impacted by changes in water table levels, there are indications that they have some capacity to adapt. Water table levels change seasonally, with these changes being greatest in environments which have marked wet and dry seasons (Gallart *et al.* 2002). Root growth in the zone just above the water table may be impacted by the change in soil moisture, for example, a rising water table can restrict root growth (Castelli *et al*. 2000). While flood tolerant species have structural and physiological adaptations to survive periods of saturation and anoxia (Kozlowski 1997; Mitsch & Gosselink 2000; Kozlowski 2002), the roots of most terrestrial plant species, including terrestrial phreatophytes, are less tolerant of anoxic conditions (Ganskopp 1986; Groom 2004b). Thus, a rising water table is likely to prevent root growth in the saturated part of the profile, with roots being redistributed to the unsaturated vadose zone and capillary fringe (Oosterbaan & Nabuurs 1991; Segelquist *et al*. 1993; Imada *et al*. 2010).

Following the rise of the water table due to winter recharge, water table levels decline if there is little recharge of the water table and ongoing discharge due to plant transpiration of groundwater, such as over a summer drought period. If phreatophytic plants are to maintain a functional connection with groundwater, they must be able to elongate their roots to follow the declining water table. This is particularly vital in seasonally water-limited environments, where vadic water is low over dry season months, and groundwater can be critical for plants to survive the drought period (Mooney *et al.* 1980). The notion that phreatophytes remain connected with the water table throughout the year is supported by observation of high transpiration rates and high predawn water potentials, despite low water content in the vadose zone (Nilsen *et al.* 1983; Scott *et al*. 2006). In addition, studies of plant water sources have indicated year-round groundwater use by phreatophytic plants in a Mediterranean-type environment, with a marked summer drought period (Zencich *et al.* 2002). This indicates that plants have been able to maintain a functional connection with groundwater as it has receded over summer months. From this, it may be inferred that some plants have the capacity to respond to the change in water availability, redistributing root growth in response to a rising water table inundating part of the root zone, and elongating roots to follow a declining water table.

For the roots of phreatophytic plants to be able to adapt to changes in the water table at different times of the year, roots need to be able to grow year round at the capillary fringe. Knowing when roots grow and die is critical if we are to understand whole-plant performance in water-limited environments. Root growth is controlled by both environmental and endogenous factors, and is therefore influenced by changes in soil temperature and water availability as well as endogenous constraints on carbon availability (Teskey & Hinckley 1981; Palacio & Montserrat-Martí 2007). Seasonal changes in environmental conditions impact the timing of plant growth, both above and below ground. The study of the timing of plant life events, such as flowering and vegetative growth is termed phenology (Lieth 1974). Throughout the year there can be large variations in water availability and temperature in highly seasonal environments, which may impact on the capacity for root growth (Teskey & Hinckley 1981). Additionally, root activity can be influenced by endogenous cues (Joslin *et al.* 2001). Root and shoot growth are intrinsically linked, with shoots dependent on roots for nutrient and water uptake, while continued root growth is reliant on photosynthates fixed in the leaves (Kramer & Boyer 1995). It is thus beneficial to observe root and shoot growth simultaneously. For two Mediterranean shrub species in Spain, *Echinospartum horridum* and *Salvia lavandulifolia,* vegetative growth occurs over a short period in spring, with warming air temperatures and high soil water availability, while root growth occurred in autumn (Palacio & Montserrat-Martí 2007). However, the relationship between root and shoot growth for most phreatophytic plants is less well known. Any restrictions on the timing of root growth may influence the ability for phreatophytes to respond to changes in the water table at a given time of the year.

The rate of water table decline can determine the ability of plants to survive changes in groundwater availability. In particular, maintenance of a functional connection with the water table is dependent on the initiation of root growth in response to changes in the water table and the plant's capacity for rapid root elongation. If water table decline occurs at a rate greater than the rate at which roots are able to elongate, it is likely that plants will become disconnected from groundwater. Rapid groundwater decline often results in signs of plant water stress, crown dieback and death (Sperry & Hacke 2002; Cooper *et al*. 2003). However, if roots are able to maintain contact with the declining water table, impacts on phreatophytic plants may be minor (Mahoney & Rood 1991;

Naumburg *et al.* 2005). Root response to water table decline has been investigated for riparian phreatophyte species, with studies often instigated by observation of plant decline due to changes in stream flow (Kranjcec *et al*. 1998; Horton & Clark 2001; Stave *et al*. 2005; Imada *et al*. 2008; Gonzalez *et al*. 2010). Many riparian species have been found to survive water table decline at a rate of 1 cm day^{-1}, including *Tamarix chinesis* (Horton & Clark 2001), *Populus alba* (Gonzalez *et al*. 2010) and the poplar hybrid *Populus deltoides* x *P. balsamifera* (Mahoney & Rood 1991). Furthermore, other species have survived faster rates of water table drawdown, with *Faidherbia albida* and *Acacia tortilis* following a declining water table at 5 cm day^{-1} (Stave *et al*. 2005). A range of phreatophytic plants have therefore demonstrated an ability to respond and elongate their roots to follow a rapidly declining water table. However, the majority of species studied occupy riparian type environments and there is a paucity of data for the root elongation response of phreatophytes from other ecosystems.

The Swan Coastal Plain, in the south west of Western Australia has large areas of phreatophytic vegetation, which access an unconfined, shallow aquifer. There are two groundwater mounds (Gnangara and Jandakot) where recharge rate exceeds discharge, resulting in a 'mound'. One of these, and the focus of this study, is the Gnangara Mound. This aquifer underlies a large area of the Swan Coastal Plain in the internationally recognised biodiversity hotspot of the south west corner of Western Australia (Myers *et al*. 2000). The hot, dry Mediterranean-type summers, in combination with the poor water-holding capacity of the sandy soils that are characteristic of the region (McArthur & Bettenay 1974), mean that water availability in the vadose zone can become limiting to plant survival in summer months. However, the shallow aquifer is accessible to plants that have sufficiently deep root systems to access it (Zencich *et al*. 2002; Groom 2004a). The Gnangara Mound supports large areas of phreatophytic vegetation, including a number of *Banksia* species. Previously large areas of *Banksia* have been adversely impacted by groundwater abstraction in combination with exceptionally hot, dry summers (Mattiske & Associates 1988; Groom *et al*. 2000), prompting research on *Banksia* ecophysiology (Pate *et al*. 1995; Groom 2002; Zencich *et al*. 2002; Veneklass & Poot 2003; Froend & Drake 2006; Canham *et al.* 2009). The connection between groundwater and phreatophytic plants has been implied from observation of year-round high water use, and high predawn shoot water potentials during periods of rainfall deficit. This implies that plants have access to deeper water sources, such as groundwater, since there is limited water availability in the shallow unsaturated parts of the soil profile. Furthermore, water source partitioning studies using isotopes of hydrogen and oxygen have identified year-round groundwater use by phreatophytic *Banksia*, although the proportion of groundwater used varies spatially and temporally (Dawson & Pate 1996; Zencich *et al*. 2002). Thus, *Banksia* on the Swan Coastal Plain which have a range of phreatophytic dependencies are an excellent group in which one can investigate the response of phreatophytes to changing water table levels.

The aim of this thesis is to investigate the responses of *Banksia* roots to changes in water table levels. To achieve this, a series of studies were undertaken to investigate the dynamics of root growth in relation to a water table and rates of change in the depths. I have also considered a range of environmental and endogenous factors that may influence the ability for plants to adapt to changes in a water table.

The specific aims of the study are;

1. **To assess the seasonality of root growth by phreatophytic *Banksia*, particularly the influence of environmental and endogenous cues**. If root growth is controlled by season-linked cues then plants may not be able to grow roots to adjust to changes in the water table at certain times of the year. Phreatophyte root activity in the zone above the water table is likely to be impacted by seasonal fluctuations in groundwater levels. In addition, there might be physiological constraints on the timing of root growth, due to the relationship between above-ground vegetative growth and flowering and root growth. The redistribution of roots, particularly the capacity for roots to follow the water table down in summer and autumn months, is vital for phreatophytic plants so that they can maintain a functional connection with the water table. The seasonality of root growth at the water table is assessed in Chapter 3. Chapter 4 provides supporting evidence of the year-round maintenance of a functional connection between phreatophytic plants and a water table.

2. **To assess the capacity for phreatophytic *Banksia* to follow a rapidly declining water table.** For phreatophytes to adapt to changes in water table levels, plants need to be able to meet their water requirements, which can be dependent on plants maintaining a functional connection with the water table. For plants to be able to follow a declining water table they need to have the capacity for rapid root elongation, as well as the ability to respond to changes in water availability. Quantifying the rate of root elongation for a particular species may give some indication of the rate of water table decline that they are able to adapt to for survival. The rate of root elongation for representative phreatophytic *Banksia* species is assessed in Chapter 5. Chapter 6 investigates the response of phreatophytic *Banksia* to water table decline at three different rates.

Chapter Two

Study area and species descriptions

The Swan Coastal Plain is situated on the west coast of south-western Australia, bounded to the east by the Gingin and Darling Scarps, the Indian Ocean to the west, the Collie-Naturalise Scarp to the south and the Hill River Scarp to the north (Fig. 2.1). The Swan Coastal Plain extends 400 km, from Jurien Bay in the north to Dunsborough in the south. The Plain is characterised by a series of ancient sand dune systems that run from north to south, parallel with the coast (Davidson 1995).

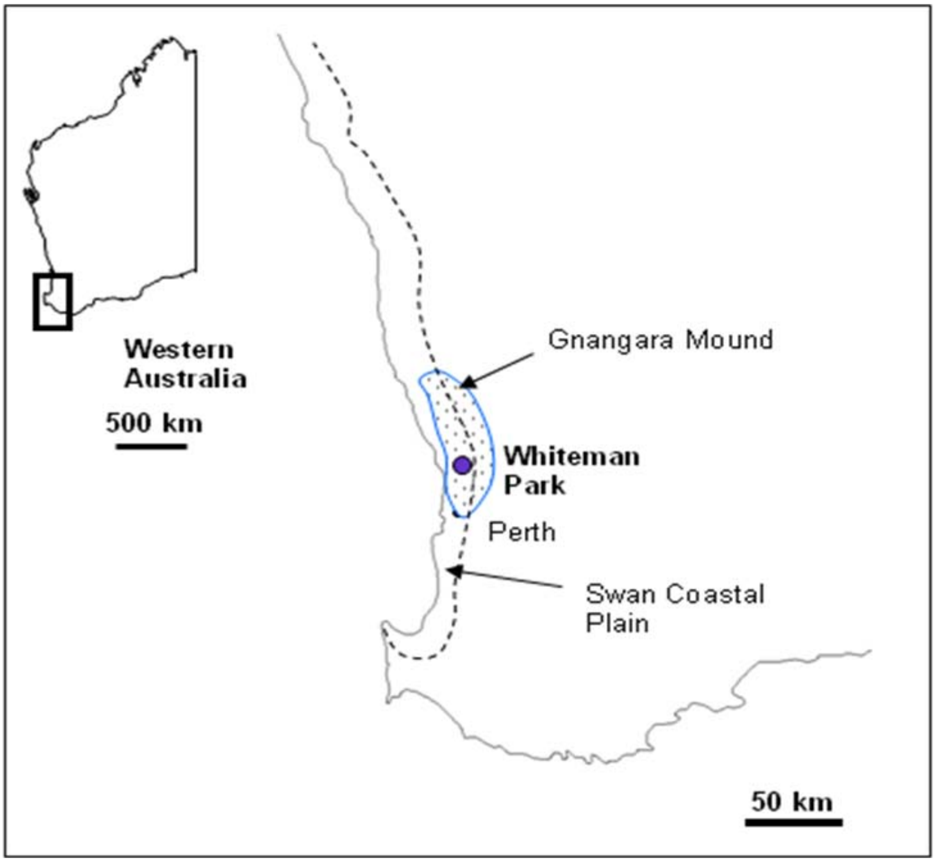


Figure 2.1. Location of the Swan Coastal Plain and the Gnangara Groundwater Mound. The specific study site, Whiteman Park, is also shown.

Description of the Swan Coastal Plain Environment

Climate

The climate of the Swan Coastal Plain is warm Mediterranean, with hot, dry summers and mild, wet winters (Bagnouls & Gaussen 1957; Gentilli 1972). The Mediterranean-type climate of the Plain has a marked dry season, with five to six dry months between approximately mid-October to the end of March. The long-term rainfall average for Perth from 1876 to 1975 is 881 mm (Bureau of Meteorology 2011). However, the region has experienced a reduction in average rainfall since 1975 and the mean annual rainfall for this period is 762 mm (average for years 1975-2010; Bureau of Meteorology 2011). Eighty-five percent of annual rainfall occurs between May and October and the winter period from June to August are the wettest months (Butcher 1986; Fig. 2.2). Rainfall generally only exceeds evaporation in June and August and mean annual evaporation is greater than mean annual rainfall. The average annual pan evaporation for Perth is 1890 mm compared to 762 mm (1975-2010 average) of annual rainfall, thus resulting in a rainfall deficit.

Mean maximum temperatures on the Swan Coastal Plain range from 30.2 ˚C in February and 17.5 ˚C in July (Fig. 2.2). Mean minimum temperatures range between 8.8 ˚C in July and 18.1 ˚C in February. The Swan Coastal Plain experiences a high proportion of cloudless days throughout the year, with 60% of clear days from December to March and 30% from mid-May to July. (Gentilli 1972). Day length varies from 10 hours to 14.3 hours over the year.

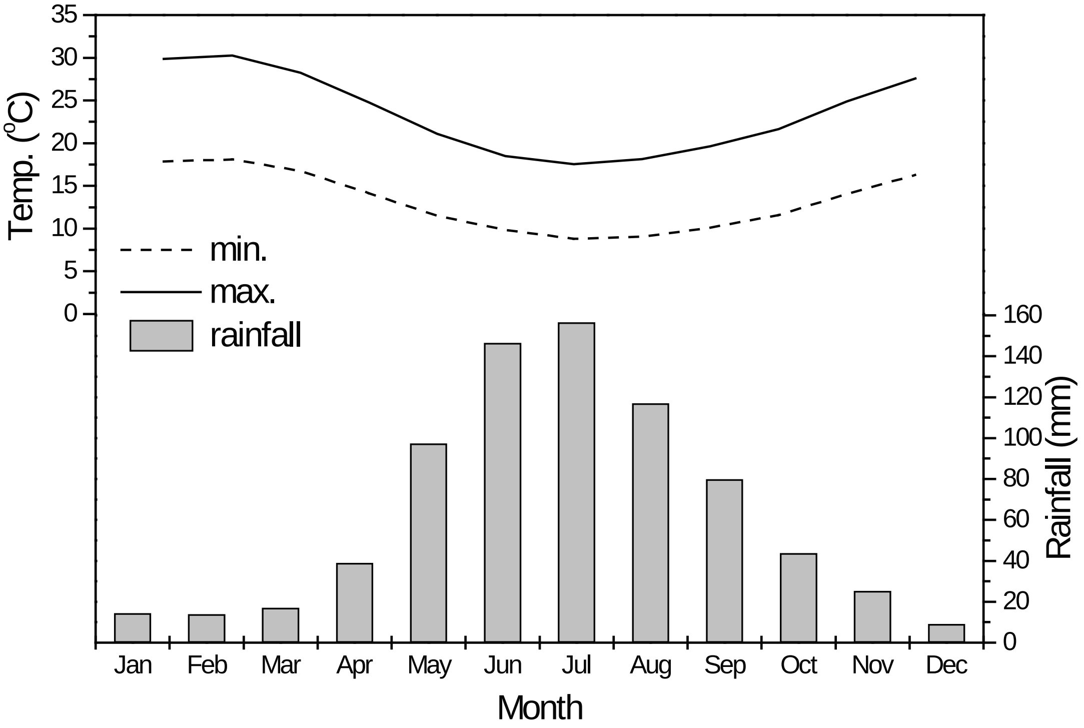


Figure 2.2. Mean monthly minimum and maximum temperatures and mean monthly rainfall for Perth, Western Australia. Data sourced from Bureau of Meteorology (2011).

Landforms and soils

The Swan Coastal Plain is comprised of a series of gently undulating aeolian sand dunes 20 km wide running north – south, parallel with the coast line (Davidson 1995). The region consists of a series of geomorphic units. Moving from the east, next to the Darling Scarp, through to the west which is bound by the Indian Ocean, they are; the Pinjarra Plain, and the Bassendean, Spearwood and Quindalup Dune Systems. All field work was carried out on the Bassendean Dune system, which dominates the centre of the Swan Coastal Plain. The dunes were formed during a period of higher sea levels, and are accumulations of marine deposits that originally consisted of a mixture of lime sand and quartz sand, with a minor fraction of fine-grained heavy-mineral concentrations. However, the sand dunes are between 115,000 and 429,000 years old and have been heavily weathered, with almost all carbonate material leached from the soil (McArthur & Bettenay 1974). The soil in the Bassendean Sand Dune system therefore consists almost entirely of quartz sand, with a low pH, very low nutrient availability and a poor water-holding capacity.

Hydrology

Groundwater is present in a series of aquifers underlying the unconfined Gnangara aquifer of the Swan Coastal Plain. There are two deep confined aquifers, which were formed at different times during geological history; the Yarragadee in the Jurassic period and the Leederville during the Cretaceous (Davidson 1995). The Gnangara superficial aquifer permeates the overlying sand and limestone formations and extends unconfined throughout the Swan Coastal Plain. The superficial aquifer covers an area of 2,140 km^2, from Gingin Brook to the north, the Swan River to the south, Gingin Scarp and Ellen Brook to the east and the Indian Ocean to the west (Fig. 2.1; Allen 1976). The maximum thickness of the aquifer is approximately 50 m, in the centre of the groundwater mounds, but ranges from 45 m in the north of the mound to 25 m in the south (Allen 1976). The upper surface of the superficial aquifer is the water table, which fluctuates seasonally by 0.5 to 3 metres, depending on the hydraulic conductivity of the soil and the direction of groundwater flow. The water table is at its highest level in September-October and lowest in April-May.

Influences on water table levels

There are a number of factors that influence the rate and magnitude of recharge and discharge of the superficial aquifer, which control the level of the water table. The superficial aquifer on the Swan Coastal Plain is primarily recharged by rainfall (Davidson 1995). The rate and magnitude of groundwater recharge by rainfall is dependent on rainfall patterns, land use cover and depth to the water table (Kite & Webster 1989). The processes of vertical rainfall infiltration and horizontal groundwater flow determine the level of the water table. When rainfall infiltration exceeds the flow of groundwater, as in winter months, the water table rises. Over the summer drought period the horizontal flow of groundwater exceeds the amount of recharge, thus the water table declines. In addition to horizontal flow, groundwater discharge also occurs through evapotranspiration of water by plants and groundwater abstraction in impacted areas. The influence of evapotranspiration on groundwater levels is greatest in habitats with a shallow depth to groundwater, and during periods when there is little recharge.

Specific Study site

Whiteman Park and Specific Study Site

Field studies were undertaken in Whiteman Park, a conservation reserve covering 2,600 hectares, approximately 20km north-east (31°48′ S, 115°56′ E) of Perth, Western Australia, and situated on the Bassendean Sand Dune system (Anon. 1989). The Gnangara Groundwater Mound underlies the Park, supporting a range of groundwater-dependent ecosystems, including large areas of phreatophytic vegetation. Vegetation ranges from eucalypt woodlands dominated by *Eucalyptus marginata* (Jarrah) and *Corymbia calophylla* (Marri), *Banksia* woodlands dominated by *B. attenuata*, *B. ilicifolia* and *B. menziesii*, to dampland environments dominated by wetland species such as *Melaleuca preissiana, Melaleuca rhaphiophylla* and *Banksia littoralis* (Anon. 1989). Deep-rooted plants, including *Banksia*, are often phreatophytic owing to the shallow water table throughout the Park.

A specific study site within Whiteman Park was selected for studies of plant phenology, as well as the functional connectivity between the phreatophytic *Banksia* and the water table (Chapters 3 & 4). A shallow water table was a primary factor in site selection, as it made the study of root growth at the water table logistically feasible. Depth to the water table was determined at the site prior to the start of the study, and was 3.8 m from February to June, before peaking at 3.0 m in September, in response to winter rainfall recharge (Fig. 2.3). The water table then receded again returning to 3.7 m by March 2008.

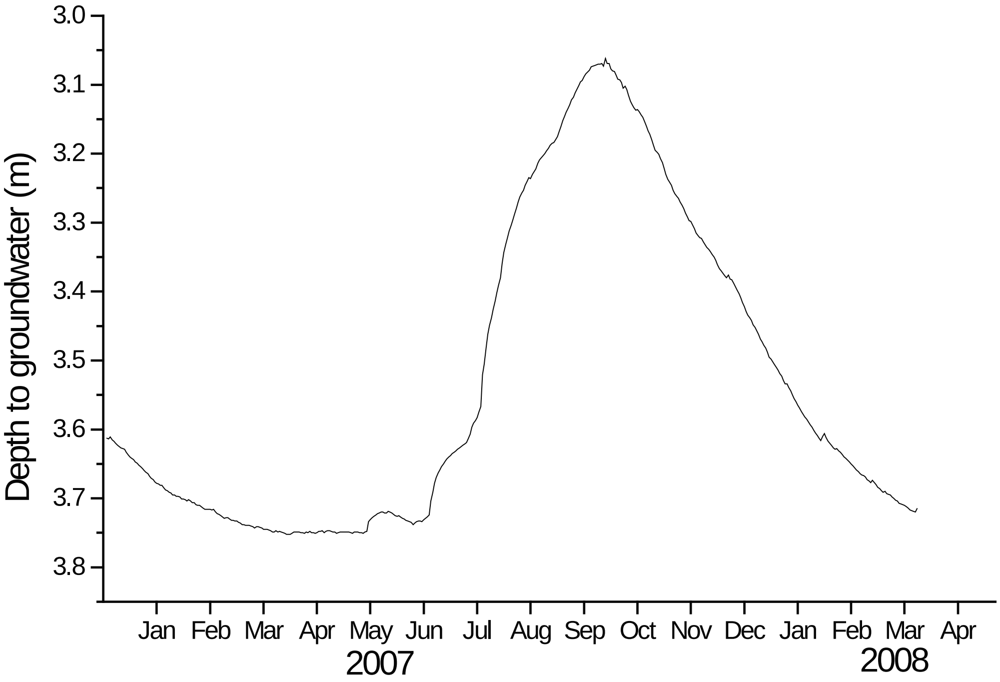


Figure 2.3. Seasonal cycle of water table levels from January 2007 to April 2008 at the Whiteman Park study site.

Soils at the site are representative of those found on the Bassendean Dune system. The soil profile is deep medium to coarse grey sand down to a depth of approximately 3 m. At 3 – 3.25 m, sitting above the water table, there is a hard layer of cemented soil (colloquially referred to as 'coffee rock'), which contains varying amounts of aluminium, iron and organic matter. The coffee rock layer varies in thickness and has a patchy distribution throughout the site, and was not always encountered when digging boreholes. Below the coffee rock layer, from 3.25 – 6 m there is coarse to medium sand, which varies in colour from yellow-brown to white.

Figure 2.4. The study site, showing *Banksia ilicifolia* (top) and *Banksia attenuata* (bottom).

Study species

Banksia is a dominant genus on the Swan Coastal Plain, and groundwater use by a number of species has been described previously (Dawson & Pate 1996; Zencich *et al*. 2002). Over the past three decades large areas of *Banksia* woodland have been adversely impacted by groundwater abstraction in combination with exceptionally hot, dry summers (Mattiske & Associates 1988; Groom *et al*. 2000), prompting research on *Banksia* ecophysiology (Pate *et al.* 1995; Groom 2002; Zencich *et al*. 2002; Veneklass & Poot 2003; Froend & Drake 2006; Canham *et al.* 2009). Three species of *Banksia* were selected for study; *Banksia attenuata* R. Br., *Banksia ilicifolia* R. Br. and *Banksia littoralis* R. Br. The three study species are representative of canopy species that dominate *Banksia* woodland (Fig. 2.4).

The three study species differ in their distribution in relation to groundwater depth and dependency on groundwater resources (Fig. 2.5). The distribution of *B. littoralis* is confined to dampland environments with a shallow water table, which affords year-round access to groundwater, as has been previously identified by water source partitioning studies (Zencich *et al.* 2002). *Banksia ilicifolia* also maintains access with groundwater, with a wider distribution than *B. littoralis*, but still constrained to habitats where groundwater is within the maximum rooting depth of the species, approximately 8 m (Arrowsmith 1992). In contrast, *B. attenuata* as a species uses groundwater opportunistically. It has a wide distribution in relation to groundwater depth, co-occurring with more mesic phreatophytic *Banksia* in habitats with a shallow depth to groundwater, as well as occupying habitats with a deep water table, such as at dune crests. Groundwater can be a significant water source for *B. attenuata* plants in habitats with a shallow depth to groundwater. However, at sites with deep water tables, such as at dune crests where the water table can be 30 m below the soil surface, groundwater is not utilised by the species (Zencich *et al.* 2002). This is likely to be due to the water table being beyond the maximum rooting depth of the species, as well as plants being able to meet their water requirements from the vadic zone.

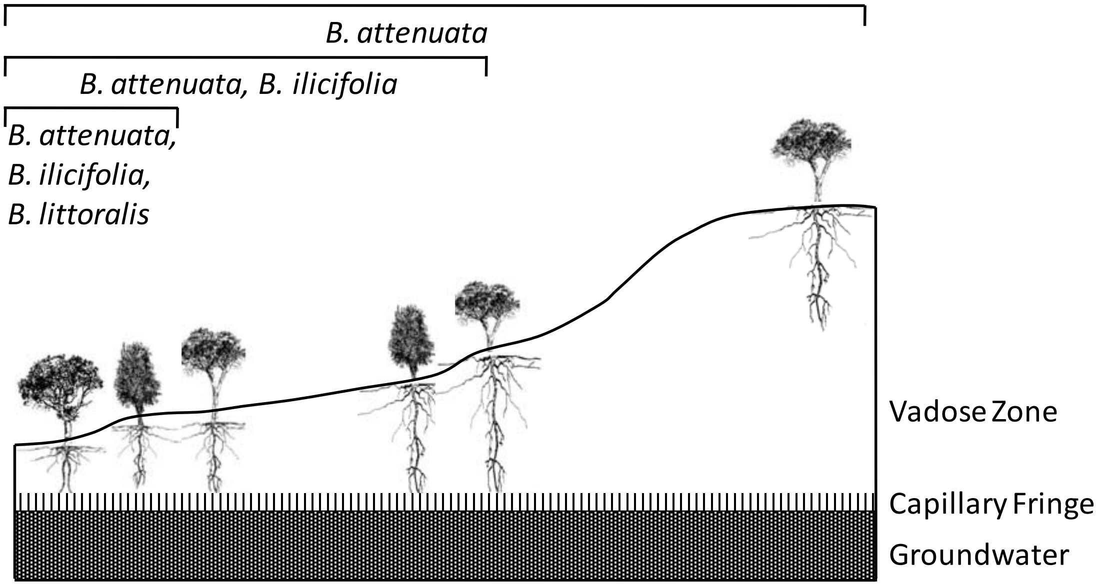


Figure 2.5. A representation of the distribution of *Banksia attenuata, Banksia ilicifolia* and *Banksia littoralis* along an ecohydrological gradient showing the water sources available.

Xeric phreatophytic species, such as *B. attenuata*, are physiologically adapted to survive the seasonal water deficit due to vadic water content decreasing over the summer drought period. One of these adaptations is increased resistance to xylem cavitation, which allows plants to be exposed to increased water stress before xylem embolism occurs (Tyree & Ewers 1991). *Banksia attenuata* has some plasticity in its vulnerability to xylem embolism, as plants in more xeric habitats, where groundwater is unavailable, are less vulnerable to water stress than plants that have year-round access to a shallow water table (Canham *et al.* 2009). The two more mesic study species, *B. ilicifolia* and *B. littoralis* do not have the same plasticity in the trait, and are thus more dependent on access to groundwater to avoid severe water stress. Therefore, the three study species represent a range of phreatophytic habit, with *B. littoralis* the most mesic species by virtue of its distribution and vulnerability to water stress, followed by *B. ilicifolia*, then *B. attenuata*, which is a more xeric phreatophyte which opportunistically utilises groundwater.

Banksia root systems

Terrestrial phreatophytic species typically have extensive root systems, allowing access to the water table which can be at great depth (Canadell *et al*. 1996; Eamus *et al.* 2006). Dimorphic root systems are a common rooting type in Mediterranean-type ecosystems, including *Banksia* species (Dodd *et al*. 1984; Lamont & Bergl 1991; Pate *et al*. 1998). Dimorphic root morphology refers to a root distribution that includes shallow lateral roots (primarily for nutrient acquisition) and deep sinker roots, which access stored-soil moisture or groundwater (Cannon 1949; Dodd *et al*. 1984; Dawson & Pate 1996). Dimorphic root morphology is particularly beneficial in the nutrient poor, water-limited environment of the Swan Coastal Plain, Western Australia and similar environments. In these environments many non-mycorrhizal species, such as *Banksia*, develop cluster roots, which are a plant adaptation to low nutrient environments. Cluster roots increase root surface area, which greatly increases the uptake of water and nutrients per unit of root length (Purnell 1960; Lamont 2003). Lateral roots also facilitate water uptake from shallow parts of the soil profile, optimising uptake of water during wet seasons (Zencich *et al*. 2002).

Studies on the Gnangara Mound have found that *Banksia* roots extend to depths of at least 9 m (Farrington *et al.* 1989) and are able to access stored soil moisture and groundwater if it is available (Zencich *et al*. 2002). In addition, the root architecture of *B. attenuata* has been described for individuals in Eneabba, south Western Australia (Lamont & Bergl 1991). Lateral roots were observed to extend up to 10 m from the bole of the tree, with sinker roots branching from the laterals, often reaching down to the water table. Although the root architecture of a number of *Banksia* species is well described (Table 2.1), few studies have observed the seasonality of root activity. One exception is a study by Lamont & Bergl (1991) who investigated the phenology of soil surface cluster root growth in conjunction with observations of plant-water relations and root and shoot architecture. Maximum cluster root development was observed following the first autumn rains, with cluster root growth continuing until late spring. However, observations of the phenology of root growth were restricted to cluster root development in the top 10 cm of the soil surface. This restriction gives a limited indication of overall root phenology for deep-rooted terrestrial phreatophytic *Banksia* species.

Table 2.1. Summary table of studies that have observed *Banksia* roots *in situ.*

Reference	**Spp. & Location**	**Method**	**Findings**
Low & Lamont 1990	• *B. attenuata, B. menziesii, B. hookeriana* • Eneabba	• Whole plant sampling of 12 year old Banksia	• Determined above and belowground phytomass
Lamont & Bergl 1991	• *B. attenuata, B. menziesii, B. hookeriana* • Eneabba	• Observed architecture of lateral roots • Seasonal observation of root mat (top 100 mm) for new rootlet growth	• Described architecture of lateral roots – extend up to 10 m from parent plant • Surface rootlet production commenced after rain (May) and ceased in late spring (October).
Enright & Lamont 1992	• *B .attenuata, B. leptphylla, B. hookeriana* • Eneabba	• Excavated 1 year old seedlings	• Described root morphology of seedlings
Pate *et al*. 1995	• *B. prionotes, B. ilicifolia* • Yanhcep & Shenton Park	• Excised trunk, lateral and sinker root material to determine hydraulic architecture	• Determined hydraulic conductivity, specific hydraulic conductance and xylem anatomy of trunk, lateral and sinker roots. • Examined mobile and non-mobile water in lateral and sinker roots.
Nicoski *et al*. 1997	• *B. ilicifolia, B. littoralis* • Gnangara Mound	• Excavated mature trees	• Described the dimorphic root morphology of the 2 species. • *B. littoralis* had fewer lateral and more dropper roots.
Pate *et al*. 1998	• *B. prionotes* • Yanchep & Shenton Park	• Whole plant harvest of seedlings every 3 months for 1 year • Max. depth: 2.5 m	• Described the dimorphic rooting morphology • Increased proportion in biomass at depth with increasing age • Winter proteoid root growth and increased nutrient uptake
Rokich et al. 2001	• *B. attenuata*, *B. menziesii* • Gnangara Mound	• Excavated 1 yr old seedlings	• Described root morphology of seedlings

Chapter Three

Dynamics of *Banksia* root growth relative to a seasonally fluctuating water table

Introduction

The response of roots to changes in the water table may be influenced by both endogenous and environmental cues. If root growth is limited by environmental factors or endogenous limitation of carbon availability, roots may not be able to respond to changes in water table levels. Root growth is influenced by changes in temperature and water availability (Teskey & Hinckley 1981). As such, seasonal environmental cues, such as changes in temperature and water availability, can influence the timing and intensity of root growth (Bevington & Castle 1985; Tierney *et al*. 2003). Phenological studies have typically observed the seasonal timing of above-ground traits, such as the commencement of leaf growth and flowering (Kummerow 1983; Cannell 1997; Koike *et al.* 2003; Rousi & Heinonen 2007), however, there is increasing interest in the phenology of root growth and the environmental parameters that influence it (Lamont & Bergl 1991; Joslin *et al.* 2001; Tierney *et al*. 2003; Palacio & Montserrat-Martí 2007; Janos *et al.* 2009). Studies in the Mediterranean-type ecosystems of Californian chaparral and Chilean matorral suggest that fine root growth in the top 20 cm begins after the first winter rains, reaching maximum production in spring and early summer (Kummerow *et al*. 1978; Montenegro *et al*. 1982). In contrast, Lamont and Bergl (1991) found that cluster root production under *Banksia* in Eneabba, 280 km north of Perth, occurred from May to September, which is out of phase with shoot growth. The dynamic relationship between root growth, shoot growth and other environmental variables is thus complex and species specific.

The environmental variables that influence root growth, such as temperature and water availability (Teskey & Hinckley 1981) change with soil depth. For example, soil temperature is more constant with depth (Voroney 2007), with changes in temperature at the soil surface generally having no influence below one metre (Popiel *et al.* 2001; Florides & Kalogira 2005). Water availability also changes with depth, influenced by hydrological attributes specific to the particular site. Water content in the vadose zone fluctuates seasonally, particularly in seasonally water-limited Mediterranean-type climates, where vadic water content is high during winter months, recharged by winter rainfall (Zencich *et al.* 2002). Water from the vadose zone may be used preferentially by the plant, as plant root density is highest in the shallower layers (Adiku *et al*. 2000; Schenk & Jackson 2002). However, Mediterranean-type ecosystems are characterised by a summer drought period, with negligible rainfall in summer. During this period water content in the vadose zone, particularly the shallow layers, becomes depleted, with evapotranspiration exceeding rainfall. Plants must therefore be adapted to survive water deficits or have root systems that access deeper water sources, such as groundwater. Thus, water sources utilised by phreatophytic vegetation can vary both spatially, down the soil profile, as well as temporally (Zencich *et al*. 2002). Root growth may also respond to seasonal changes in environmental parameters; however, previous investigations have been limited to observation of root growth in the very top portion (generally the top 20 cm) of the soil profile (Kummerow *et al.* 1978; Montenegro *et al.* 1982; Lamont & Bergl 1991), with few studies of root activity at depths greater than one metre. For deep rooted phreatophytes, observations limited to the upper portion of the soil profile provide a limited perspective of overall root dynamics, since the timing of root growth may change with depth.

Roots close to the water table are likely to be influenced by the rise and fall of the water table. Water table depths change seasonally, and root growth in the zone just above the water table may be impacted by the change in soil moisture, following groundwater as it declines over summer, and being restricted by the rising water table as recharge occurs (Castelli *et al*. 2000). While flood-tolerant species have structural and physiological adaptations to survive periods of saturation and anoxia (Kozlowski 1997; Mitsch & Gosselink 2000; Kozlowski 2002), the roots of most terrestrial plant species, including terrestrial phreatophytes, are less tolerant to anoxic conditions (Ganskopp 1986; Groom 2004b). Terrestrial phreatophytes are instead thought to access water from the capillary fringe, where water content is higher but the soil is not saturated, therefore sufficient oxygen is available for respiration (Mahoney & Rood 1998). As the water table and capillary fringe are seasonally dynamic, it is probable that root growth in this zone is influenced by rising and falling groundwater levels, as has been observed for riparian vegetation (Castelli *et al*. 2000; Martin & Chambers 2002). The notion that phreatophytes, particularly phreatophytic *Banksia*, remain connected with the water table throughout the year is supported by water source partitioning studies, which demonstrate year-round uptake of groundwater, particularly in summer months (Zencich *et al*. 2002). This indicates that plants must follow the water table as it reaches its deepest level in summer and autumn. The seasonality of root growth is thus implied, but it is rarely observed, particularly at depth. It is thus likely that roots redistribute in response to the seasonal changes in the water table depth.

Root activity can also be influenced by endogenous cues (Joslin *et al.* 2001). Root and shoot growth are intrinsically linked, with shoots dependent on roots for nutrient and water uptake, while continued root growth is reliant on photosynthates fixed in the leaves (Kramer & Boyer 1995). It is thus beneficial to observe root and shoot growth simultaneously. Plant growth is most readily perceived through observation of above-ground parameters, particularly leaf expansion and flowering (Baker *et al.* 1982; Orshan 1989; Tébar *et al*. 2004; Castro-Díez *et al*. 2005). In many ecosystems, plants have a spring flush of vegetative growth, in response to the warmer temperatures and longer day length, while there is still high soil moisture (Cannell 1997; Koike *et al.* 2003; Rousi & Heinonen 2007). This is true of most Mediterranean-type ecosystems, including Spain (Palacio & Montserrat-Martí 2007), southern France and California (Kummerow 1983). In contrast, the dominant species of the South African fynbos and the kwongan vegetation of south Western Australia are characterised by summer growth, despite this being a period of very little rainfall (Kummerow 1983). This climatically independent phenological pattern has been attributed to the deep root systems that are a feature of many of the dominant species in Mediterranean-type ecosystems (Mooney & Kummerow 1980; Pierce 1984; Groom 2004a). However, the phenology of root growth in relation to shoot growth has rarely been observed. A study by Palacio & Montserrat-Martí (2007) has investigated the above- and belowground phenology of four Mediterranean shrub species in Spain. They found that for *Echinospartum horridum* and *Salvia lavandulifolia* vegetative growth occurred over a short period in spring, corresponding with warming air temperatures and high soil water availability. However, root growth occurred independently, favouring autumn conditions. The relationship between root and shoot growth for most phreatophytic plants is not known, yet the timing of root growth in relation to water availability may be critical.

The impact of water table decline may be less severe, or negligible, if it occurs at a time of year when plants are not reliant on groundwater resources (Naumburg *et al.* 2005). Plant water and nutrient requirements are low during periods of dormancy, due to lower physiological demands (Harris & Campbell 1981; Smith & Nowak 1990). In addition, during wet winter months, plants are generally able to meet their requirements from soil moisture, with groundwater uptake significantly diminished (Zencich *et al*. 2002). If the water table declines during this period and plants are able to meet their water requirements from the vadose zone, there may be minimal impact on phreatophytes (Naumburg *et al.* 2005). However, to avoid severe water stress in drier months, when vadic water content has decreased, plants need to maintain a functional connection with the water table.

Studies of above-ground phenology were undertaken in a *Banksia* woodland from 1978-1981, with a number of species observed including *B. attenuata* (Bell & Stephens 1984). *Banksia attenuata* was found to have a summer growth pattern, as well as summer flowering. These observations of a summer growth pattern were supported by a later study of three *Banksia* species in Eneabba, including *B. attenuata*, with 90% of growth and leaf production occurring between November and March (Lamont & Bergl 1991). Plant growth in the hottest, driest part of the year implies year-round access to either stored soil moisture or groundwater. Groundwater use has been demonstrated quantitatively for both *B. attenuata* and *B. ilicifolia* in a water source partitioning study (Zencich *et al.* 2002). It was shown that, over summer months, groundwater is an important water source for plants, which implies that plant roots are able to follow the water table as it recedes. However, root growth at the capillary fringe and water table has rarely been observed, particularly for terrestrial phreatophytes.

The objective of this study is to determine how root growth by phreatophytes is affected by seasonal changes in environmental conditions. The primary focus was to assess the dynamics of root growth closest to the water table, which is influenced by seasonal changes in groundwater levels. Specifically, it was hypothesised that root growth would be limited by the rising water table in winter and that roots would recover and follow the water table as it declined over summer and autumn. In addition, environmental conditions change with soil depth; therefore it was hypothesised that patterns in root growth would change with depth. Finally, the interrelationship between above- and belowground growth activity was assessed, with the expectation that root growth in the deeper soil layers would allow for above-ground growth to extend into the summer drought period.

Materials and Methods

Measurements of root growth

Root growth by adult *Banksia* trees was investigated at the Whiteman Park study site, which was selected for having a shallow depth to groundwater (3.0 to 3.8 m) and a high number of individuals of the study species within close proximity. The two species studied were *Banksia attenuata* and *Banksia ilicifolia,* which differ in their water requirements, with *B. attenuata* considered more xeric in terms of distribution in relation to groundwater, as well as its physiological adaptation to drought stress. The roots of both species were likely to reach the water table at this site, given previous observations of groundwater uptake from a similar depth (Zencich *et al.* 2002). Seasonal observation of root growth at the water table thus required measurements of root growth at depths of up to 3.8 m.

Root growth and functioning is often implied through indirect means of observation. For example, water source partitioning studies for phreatophytes have previously indicated plant uptake of groundwater, which should indicate root activity in the vicinity of the water table (Busch *et al*. 1992; Zencich *et al.* 2002). Chemical tracers have also been used to the same effect and have inferred water uptake, thus root activity, from as deep as 76 m (Lubczynski & Obakeng 2007). While these are useful indicators of root growth at depth, there remains a paucity of data on root growth phenology at a water table, and how this relates to seasonal cues. Minirhizotrons are increasingly being used to observe root turnover and seasonality (Eamus *et al*. 2006), however, such studies are limited to the top 1 to 2 m of soil (Hendrick & Pregitzer 1997; Steinaker *et al.* 2010). While this is appropriate for some species, roots of most phreatophytes extend well beyond this zone. Alternative approaches, such as root in-growth bags have been shown to be effective in repeated observations of root activity (Bohm 1979). This method involves placing bags full of root-free soil at the required depth, then leaving them for root in-growth to occur. Bags are later retrieved and the contents analysed for root mass, giving an indication of root activity at various depths and at different seasons.

Root in-growth

Ten individuals of *B. attenuata* and *B. ilicifolia* were selected at the study site and tagged. To investigate belowground phenology, root in-growth bags were placed at four different depths around each plant. The depth of root bag placement was determined from observations of depths to groundwater from the previous year (Chapter 2), where it was seen that groundwater depth was greatest in April (3.75 m) and shallowest in October (3.00 m). To determine root growth at the water table/capillary fringe, root bags were placed at 3.50-3.75 m and 2.70-2.95 m. This meant that root bags placed at the 3.50–3.75 m level would be at the capillary fringe in April, then, as the water table rose to its highest level, the 2.60-2.85 m bags were at the capillary fringe and the 3.50 m bags submerged (see Fig. 3.1). Bags were also placed at 1.20-1.45 m and 0-0.25 m to observe root in-growth in the vadose zone, which dries out over summer and autumn. The sampling periods were selected to correspond with the spring peak and autumn low levels of the water table, and were, therefore; April to July 2008 (autumn), July to November 2008 (winter), November 2008 to January 2009 (spring) and January to April 2009 (summer). Due to a slight slope at the site, there was some variation in water table levels, with trees at the south end of the site tending to have a slightly deeper water table. However, all 3.75 m bags were saturated when collected for the July to November sampling period.

Bags were constructed out of aluminium flywire which was rolled into a cylinder (the same diameter as the PVC tube and 25 cm long), then sewn together down the side and bottom. Rope was cut to length, threaded through the bag then fastened at the bottom with a large knot behind a small plastic square, to prevent it being pulled back through the bag. Nutrients and water were added to the bags prior to being placed in the field, to encourage root in-growth. This was deemed necessary as it was thought that roots would not grow into the bag if it only contained sand and had lower soil water content than the surrounding soil. Bags were filled with a 1:6 mixture of peat and clean, white sand, then sewn shut at the top. Bags were placed into plastic trays and moistened to keep sand from falling through the mesh, then transported to the study site. To allow repeated access to these depths, access tubes were installed. This involved hand auguring to the required depth then sliding in a 90 mm PVC tube which was then capped. Bags were wet to field capacity before being placed in the access tube. Access tube length was checked to ensure that 25 cm was left uncovered at the bottom, to allow contact between the bag and the surrounding soil. Once the bags reached the bottom, the rope was secured at the top, to allow bags to be retrieved. No access pipe was required for the 0.25 m bags, as the hole tended to stay intact, with only a small amount of soil having to be removed to allow the bag to be covered again when it was replaced.

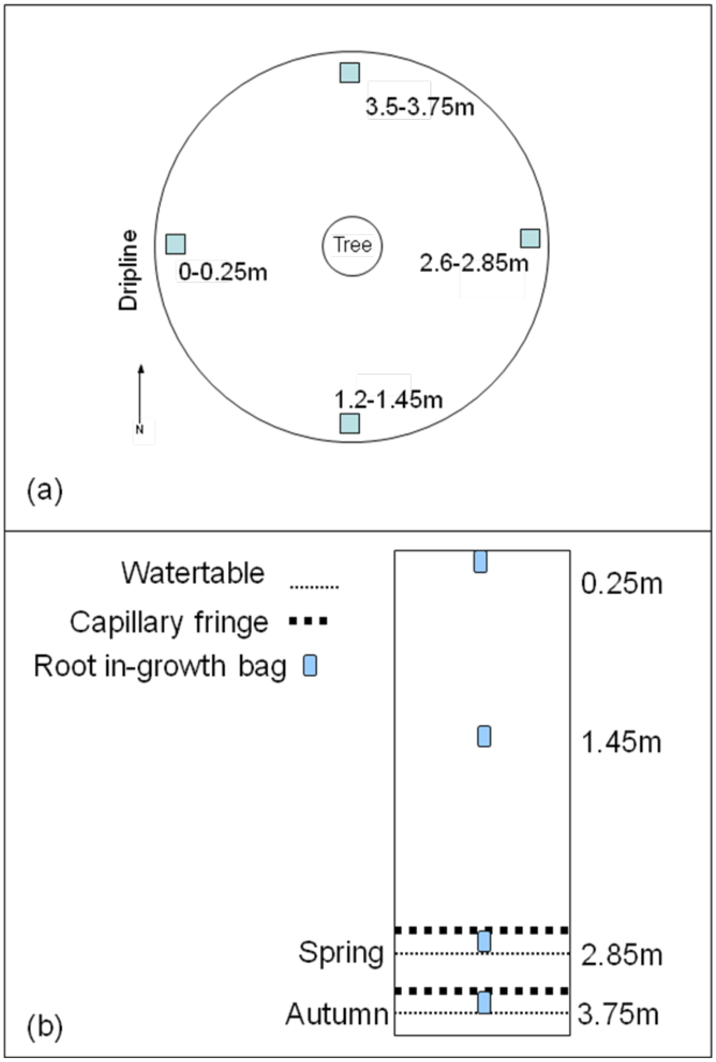


Figure 3.1. Schematic diagram of (a) sampling design and (b) root bag placement. Root access tubes were situated approximately halfway between the bole of the tree and the edge of the canopy drip line. The depths that bags were placed at were 0-0.25 m (cluster roots), 1.45 m (vadose zone), 2.85 m (spring capillary fringe), and 3.75 m (autumn capillary fringe).

Root bags were left for 12 weeks to allow for in-growth to occur. Bags were retrieved, wrapped in plastic and transported to the laboratory for sorting. As the study bags were retrieved, they were replaced with fresh bags, ready for the next period of root in-growth. In the laboratory, root bags were cut open and the material inside placed into a tray for sorting. Material was initially sorted by picking out the larger, more obvious roots by hand. Soil was then sieved to sort the finer roots, then all the material was sorted once again by hand, to ensure no root material was missed. Roots were washed by hand in water in small containers, ensuring all sand particles were removed. This was more problematic for the cluster roots, generally found in the 0.25 m samples. These often needed soaking to loosen the sand, which had become firmly attached due to exudates produced by the roots. These samples were soaked and agitated by hand until sand could be removed. It was possible that root in-growth had come from other species also growing at the site. Roots were thus sorted according to appearance, compared with roots collected from known samples of co-occurring species. For example, *B. attenuata* and *B. ilicifolia* roots were found to be red to brown in colour and were woodier than roots from *Allocasuarina fraseriana*, which had finer, dark brown to black roots. *Nuytsia floribunda* roots were also commonly found, and were easily identified as they are white, thick and very brittle. Root material that was most likely to be from the two study species was bagged in paper and dried at 60 °C until reaching a constant weight (approximately 48 hours). Any sand that remained was removed at this stage by agitating the roots whilst still in the bags. Roots were then weighed to 3 decimal places.

Above-ground Growth Phenology

Above-ground phenological phases were recorded over a two year period using the same ten individual trees from each species. The timing of vegetative growth and flowering was recorded fortnightly, with binoculars being used to see into the canopy. A score that best described the vegetative and reproductive stage, as defined and used by Bell and Stephens (1984), was assigned to each tree. For example, when leaf growth commenced, trees were given a value of 2 – leaves emerged, small and immature. When flowers were observed, they were recorded as 16 – flowers open (Fig. 3.2). In addition to phenophase observations, leaf fall was recorded. Three buckets (25 cm diameter) were placed, evenly spaced halfway between the bole and the edge of the tree canopy. Leaves of the species that the buckets were under were collected, placed in paper bags then dried at 60 °C. Leaf area was then determined using an area meter (Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK).

Figure 3.2. Examples of the phenophases of *Banksia attenuata* recorded and used for analysis. Anthesis, or phenophase 16 (top), leaves emerged, small, immature – phenophase 2 (middle) and leaves maturing, shoot growth continuing – phenophase 4 (bottom).

Changes in trunk diameter were determined, using dendrometers. These were made following the methods of Keeland & Young (n.d.). To install the dendrometers the bole of the tree was prepared. Tree bark was filed down, to remove any rough, uneven patches, thus allowing the dendrometer to sit flat against the tree. Dendrometers were constructed out of segments of stainless steel measuring tape as this was flexible enough to wrap around the tree and would not corrode after being left in the field over the two year observation period. The tape was fixed with a spring, allowing for the expansion and/or contraction of the tree trunk over time. Phenophase, leaf fall and trunk diameter were all recorded approximately fortnightly from week 18 of 2008 to week 18 of 2010. Leaf area index was measured using an LAI meter (LAI 2000, LiCor Biosciences) and was recorded every three months between week 9 of 2008 to week 20 of 2009. A 50 x 50 m plot was marked out with 10 m intervals. Measurements were taken across four transects within the plot, recording values every 10 m. Xylem pressure potentials were measured approximately every six weeks. To determine predawn potentials, plants were sampled prior to sunrise (approx. 4.30-6.30 am depending on sunrise time). Small twigs were cut from three individuals of each species and water potential immediately determined using a Scholander-type pressure chamber (Mk3005 Soil Moisture Equipment Co., Santa Barbara, CA., USA). Midday water potentials were also determined, with trees sampled between 12.00-2.00 pm.

Environmental variables

Meteorological data was recorded at a weather station (EnviroStation^{TM}, ICT International Armidale, NSW) situated approximately 2 km from the study site. Rainfall, wind and temperature were logged at half hourly intervals for the duration of the study period. Soil volumetric water content was determined for the site approximately every six weeks using a neutron moisture meter (Didcot Instrument Co., Abingdon, UK). Neutron counts were recorded then calibrated according to methods outlined by Greacen *et al*. (1981). To allow for calibration, soil samples were collected from boreholes drilled adjacent to the neutron moisture probe access tube at five different times over the study period, during various seasons. There were three soil horizons present in the soil profile (as determined by soil texture) therefore three calibration curves were used. The number of samples used for calibration varied with thickness of the horizon. Thus, soil samples were collected at 11, 2 and 6 points in each horizon of soil types 1, 2 and 3, respectively. Count ratios were plotted against known volumetric water content values determined gravimetrically from and a linear relationship was determined. The neutron count ratio was then converted to volumetric water content using the linear equation. Groundwater depth was recorded using a groundwater sensor and logger (miniTROLL, In Situ Inc., Colorado, USA) and was logged daily.

Statistical Analysis

Phenophase and environmental data were analysed using binary logistic regression. It was found difficult distinguishing between all the phenophases; this difficulty had also been encountered by Stephens (1985), therefore the most obvious, thus easiest to observe, were used for analysis. Phenophase data were converted to binary, with phenophases assigned to two groups. For vegetative phenophases these groups were defined as “no growth” (0) and “leaf growth” (1). The leaf growth group included phenophase numbers 2, 3 and 4, as defined by Bell and Stephens (1984; Fig. 3.5) with all remaining phenophases defined as “no growth” (0). Reproductive phenophases were defined as “flowers” (phenophase 16; 1), with the remainder termed “no flowers” (0). Binary logistic regressions were used to determine environmental predictors for the initiation of leaf growth and flowering. The environmental variables used were average temperature, total rainfall and daily wind run, which were averaged for the time period between observations. For example, phenophase observations for week 18 were paired with the mean average temperature observations for the fortnight between weeks 16 and 18. Data were entered into the model forward stepwise (likelihood ratio) and the model that predicted the highest proportion of observations was used. The timing of root in-growth was analysed using multiple linear regressions. Root weight at each depth was coupled with average temperature (°C), total rainfall (mm), daily wind run (km hr^{-1}) and average volumetric water content of the vadose zone (m^3 m^{-3}). Since root in-growth was measured every 12 weeks, environmental parameters were averaged for this time period and data were entered into the model using a forward stepwise procedure. All statistical analyses were performed using SPSS v. 17.

Results

Environmental variables

The 24 month study period was typical of the Mediterranean climate of the south west of Western Australia. The two summers were hot, with peaks in weekly average maximum temperatures of 36.7 °C in week 1 and 37.8 °C in week 3 in 2009 and 2010 respectively (Fig. 3.3). The summer of 2009-2010 was particularly hot and dry, with only 1 mm of rain in the 16 weeks between week 47, 2009 and week 11, 2010, combined with daily maximum temperatures of more than 29 °C. The winter months were mild, with average daily maximum temperatures ranging between 17 and 20 °C and minimum temperatures between 0 and 9 °C. Total daily wind run followed the same general pattern as temperatures, lower in winter and peaking in summer. There were, however, a number of occasions in the cooler months with high daily wind runs which were associated with storm fronts, such as week 26, in June 2009 (Fig. 3.3).

The majority of rain fell between May and October, although there was a significant amount of rain in November 2008. This contrasts with 2009, where there was little rainfall between August 2009 and March 2010 (Fig. 3.3c). Water table levels increased in response to precipitation, with a short lag between rainfall and groundwater recharge. Recharge of the vadose zone is evident in observations of volumetric water content. Wetting fronts are evident following precipitation events (Fig. 3.4) and increases in groundwater levels can be seen soon after the wetting front has percolated down to the water table. Following winter recharge, the water table rose at a rate of 0.28 cm day^{-1} between April and July and at a rate of 0.36 cm day^{-1} between July and October (average for the two sampling years). Water table levels peaked at 2.8 m in August, 2008 and September in 2009. The water table decreased over summer and autumn, declining at an average rate of 0.38 cm day^{-1} between October and January and a rate of 0.30 cm day^{-1} between January and April. At its deepest, the water table was at 3.76 m from the soil surface, which occurred in April in 2008, and in May 2009 (Fig. 3.5).

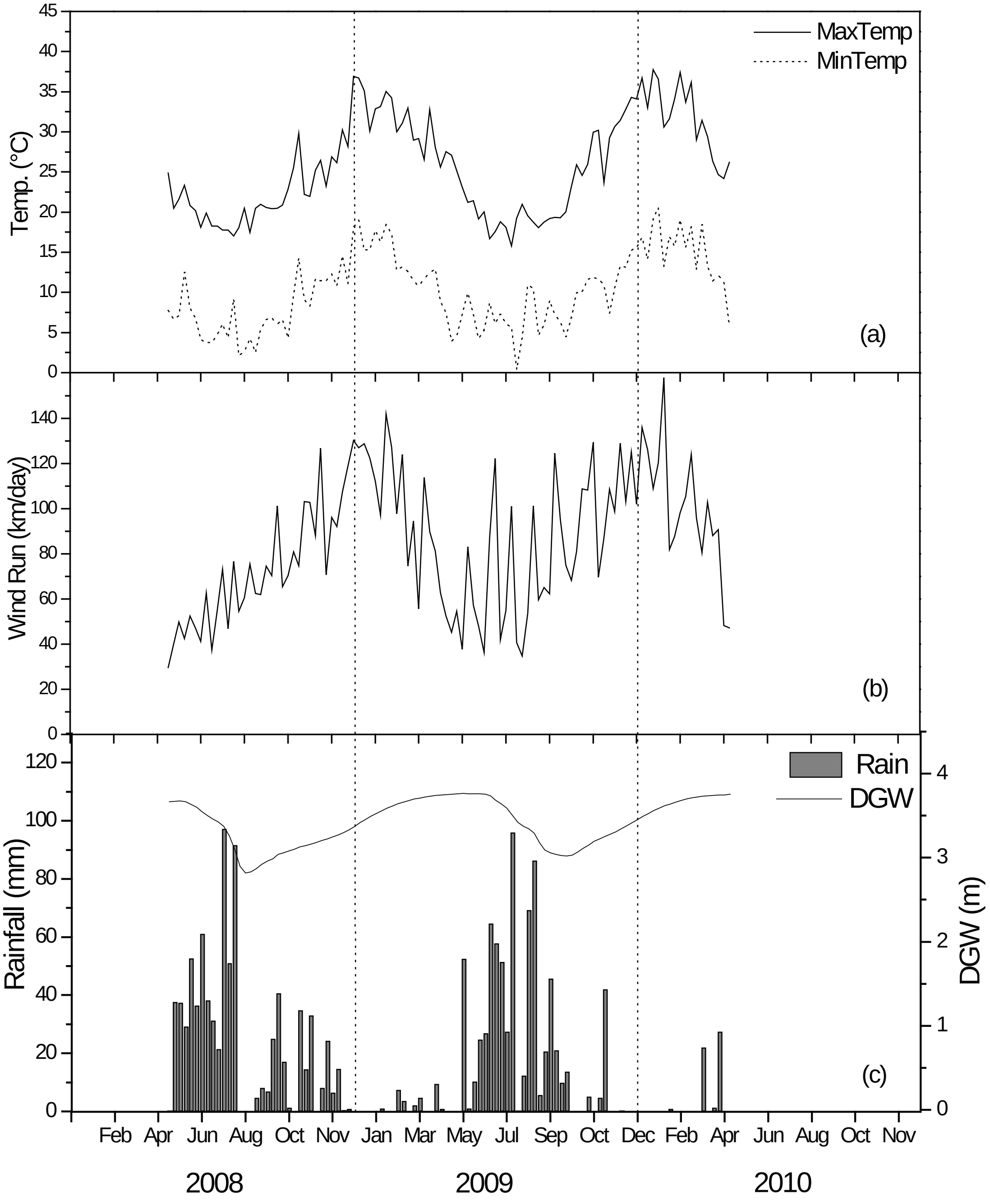


Figure 3.3. Environmental variables recorded over the study period at the study site. Variables shown are (a) minimum and maximum temperature, (b) daily wind run (c) total rainfall and depth to groundwater (DGW). Values are weekly averages.

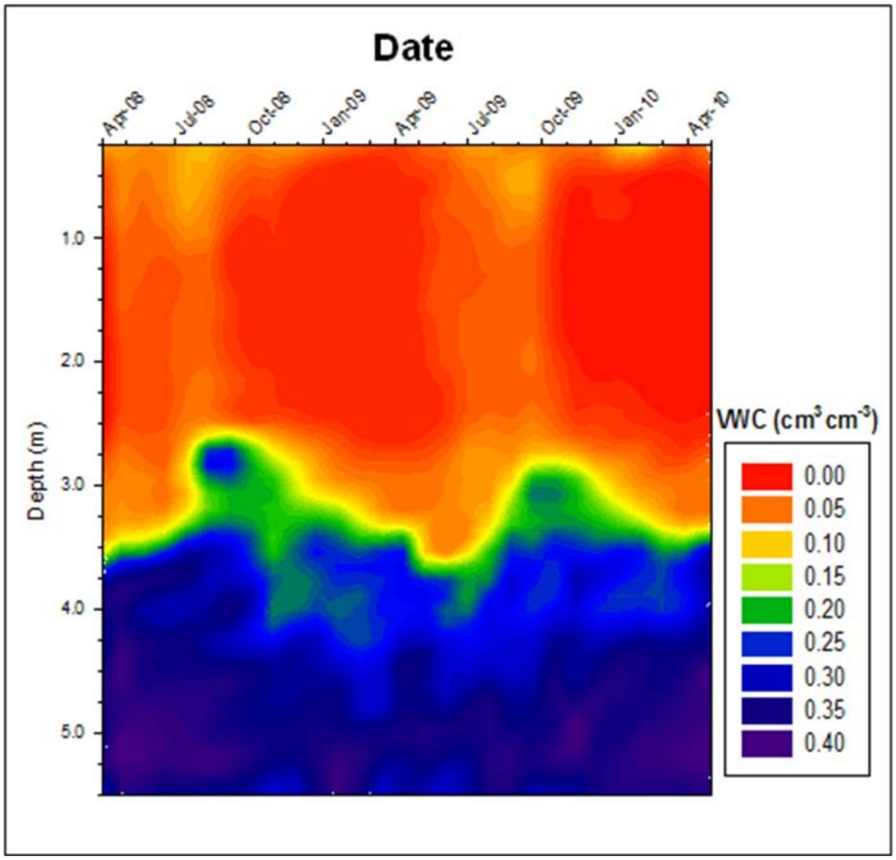


Figure 3.4. Volumetric water content (cm^3 cm^{-3}) at the study site from April 2008 to April 2010. Volumetric water content was determined at 25 cm increments.

Plant variables

Root in-growth at the water table/capillary fringe

The mass of root in-growth was generally lowest in the zone closest to the water table/capillary fringe (Fig. 3.5). The root zone at 2.75 m was not inundated by the water table at any point during the study, and constant root in-growth throughout the year reflects this. In contrast, at 3.7 m, roots were saturated from late autumn until the following summer. Root in-growth at this depth was negligible over the winter and spring sampling periods, when this zone was saturated by the rising water table. However, root in-growth was observed at this depth over autumn, when the water table was deepest, and again in summer, when the water table declined again.

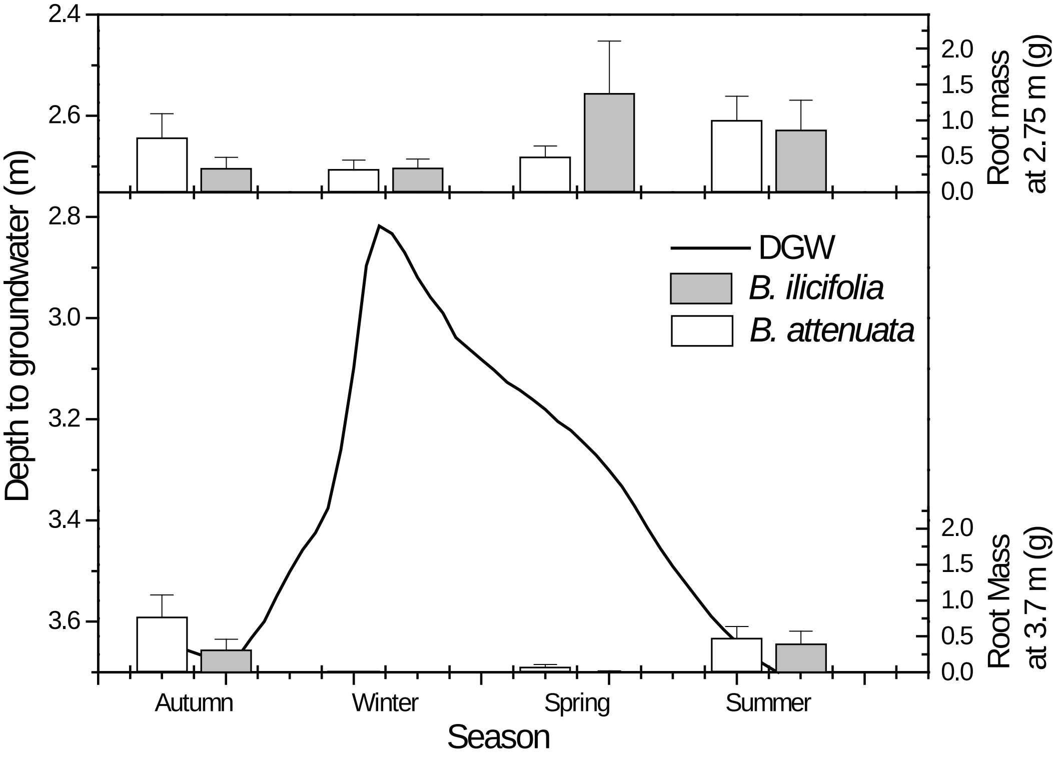


Figure 3.5. Depth to groundwater (DGW) and dry mass of root in-growth at 2.75 and 3.7 m for *Banksia attenuata* and *Banksia ilicifolia* over 12 months at the study site. Study period was from April 2008 to April 2009.

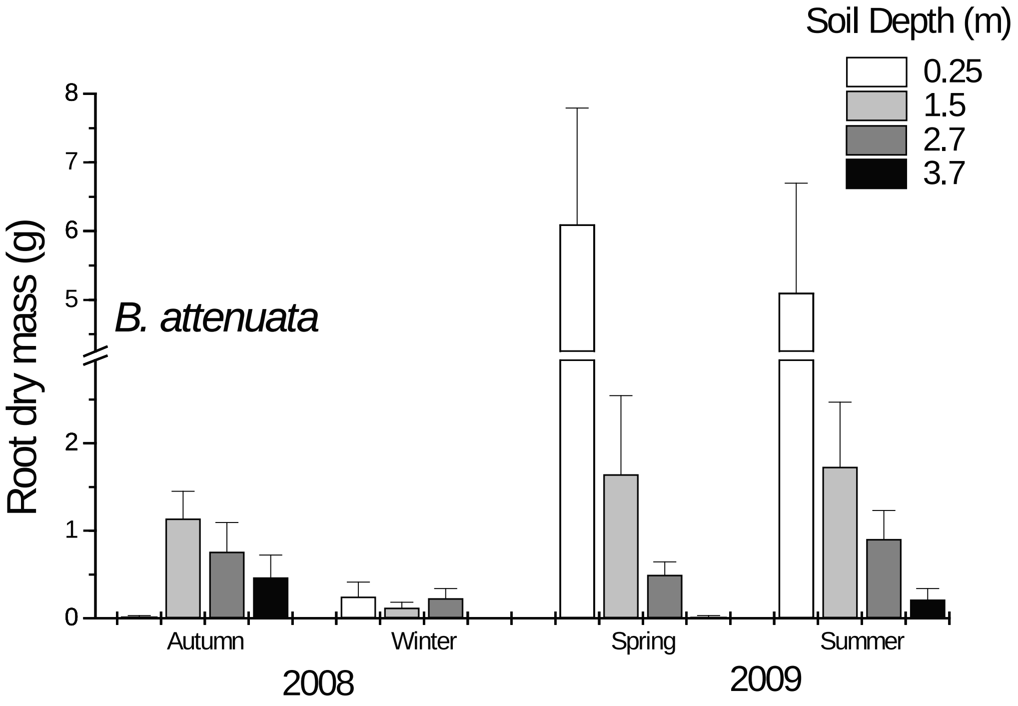
Root in-growth phenology down a soil profile

Patterns in root in-growth were similar between the two study species (Fig. 3.6). The overall pattern indicated that root growth was lowest in the winter sampling period. This coincides with high soil moisture content at the site and low plant growth activity (Fig. 3.6). As temperatures increased, and with the onset of plant growth in December, root activity increased, with overall root mass highest in the summer sampling period for both species. Maximum root growth (by in-growth mass) was found in the 0.25 m zone, and it consisted mainly of cluster roots. There was a distinct pattern in the seasonality of root growth in the top 0.25 m of the soil profile with little new cluster root growth recorded in the autumn and winter observation periods (i.e. April to November). Root growth peaked in the warmer sampling periods, from November until the following April. In *B. attenuata* root in-growth weight increment at 0.25 m for autumn was 0.02 ± 0.01 g, compared with 6.1 ± 1.69 g for the spring period. Average temperature was the best predictor of the timing of root growth at 0.25 m for both *B. attenuata* ($r^2 = 0.373$, $p < 0.05$) and *B. ilicifolia* ($r^2 = 0.195$, $p < 0.05$; Table 3.1). Deeper in the soil profile, at 1.5 m, it was found that soil moisture was the best predictor of root in-growth in bags placed under *B. attenuata* ($r^2 = 0.105$, $p < 0.042$). Root growth in this part of the soil profile was highest in the summer sampling period for both species, coinciding with the period of least soil moisture availability and period of highest plant activity.

There were no environmental predictors identified for root activity at the two deepest parts of the profile (Table 3.1). Root activity at 2.7 m was very similar between the summer and autumn sampling periods for *B. attenuata.* For *B. ilicifolia*, root in-growth at 2.7 m was greatest for the spring sampling period, although there was a large standard error. For *B. attenuata*, root in-growth was highest during the autumn sampling period, when the water table was deepest. There was very little root growth in this part of the soil profile during the winter and spring months. Root activity at 3.7 m increased from January to April when the water table receded. *Banksia ilicifolia* exhibited a similar pattern, with root growth at 3.7 m in the summer and autumn sampling periods.

Above-ground phenology

Above-ground plant activity was greatest between December and March (Figs 3.7 and 3.8). This pattern is strongest for *B. attenuata*, which had distinct growth and flowering periods and high synchrony between individuals. Leaf growth for *B. attenuata* was first observed in week 49 in 2008 and week 46 in 2009 with average temperatures during these weeks of 18.6 °C and 17.2 °C respectively. The peak period of leaf growth occurred in summer, from December through to late March. Flowering occurred over the same time period, with anthesis starting in week 47 in 2008 and week 46 in 2009, with corresponding average temperatures of 17.3 °C and 17.2 °C. Regression analyses indicated that temperature was the strongest predictor of both flowering and shallow (0.25 m) root growth for *B. attenuata* (Tables 3.1 & 3.2).



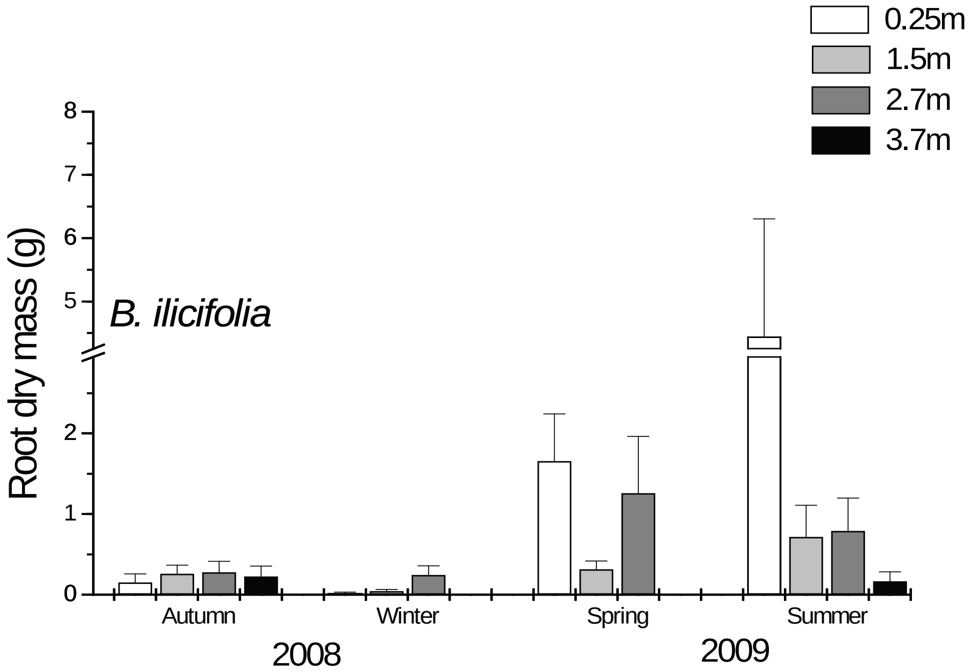


Figure 3.6. Root in-growth (as dry mass) for *Banksia attenuata* and *Banksia ilicifolia* recorded every 12 weeks over 12 months at four different soil depths (n = 10).

Table 3.1. Significant (<0.05) multiple linear regression models for root in-growth weights for *Banksia attenuata* and *Banksia ilicifolia* at four different soil depths; 0.25 m, 1.5 m, 2.5 m and 3.7 m. Predictor refers to the environmental parameter used in the model, as determined by entering data stepwise. n/a refers to instances where the model did not run due to none of the parameters fitting the model.

	B. attenuata				***B. ilicifolia***			
	0.25 m	1.5 m	2.7 m	3.7 m	0.25 m	1.5 m	2.7 m	3.7 m
Predictor(s)	Temp	SM	n/a	n/a	Temp	n/a	n/a	n/a
r^2	0.373	0.105	-	-	0.195	-	-	-
F (1, 38)	22.065	4.443	-	-	9.233	-	-	-
p	<0.000	0.042	-	-	0.004	-	-	-

Leaf growth for *B. ilicifolia* began and finished slightly earlier than that for *B. attenuata*. It did not extend as far into the hot months, with leaf growth finished by February in both study years (Fig. 3.7). Both soil moisture and solar radiation were found to be significant predictors of the timing of leaf growth for *B. ilicifolia*, but no environmental predictors were identified for the timing of flowering. Flowering occurred over a longer period, with less synchrony between individual plants compared to *B. attenuata* and there were often singular study trees with flowers open. The flowering periods were generally the same over the two year study, starting in July and finishing in April, but the peak period (i.e. >50% of individuals) occurred later in 2009 compared to 2008.

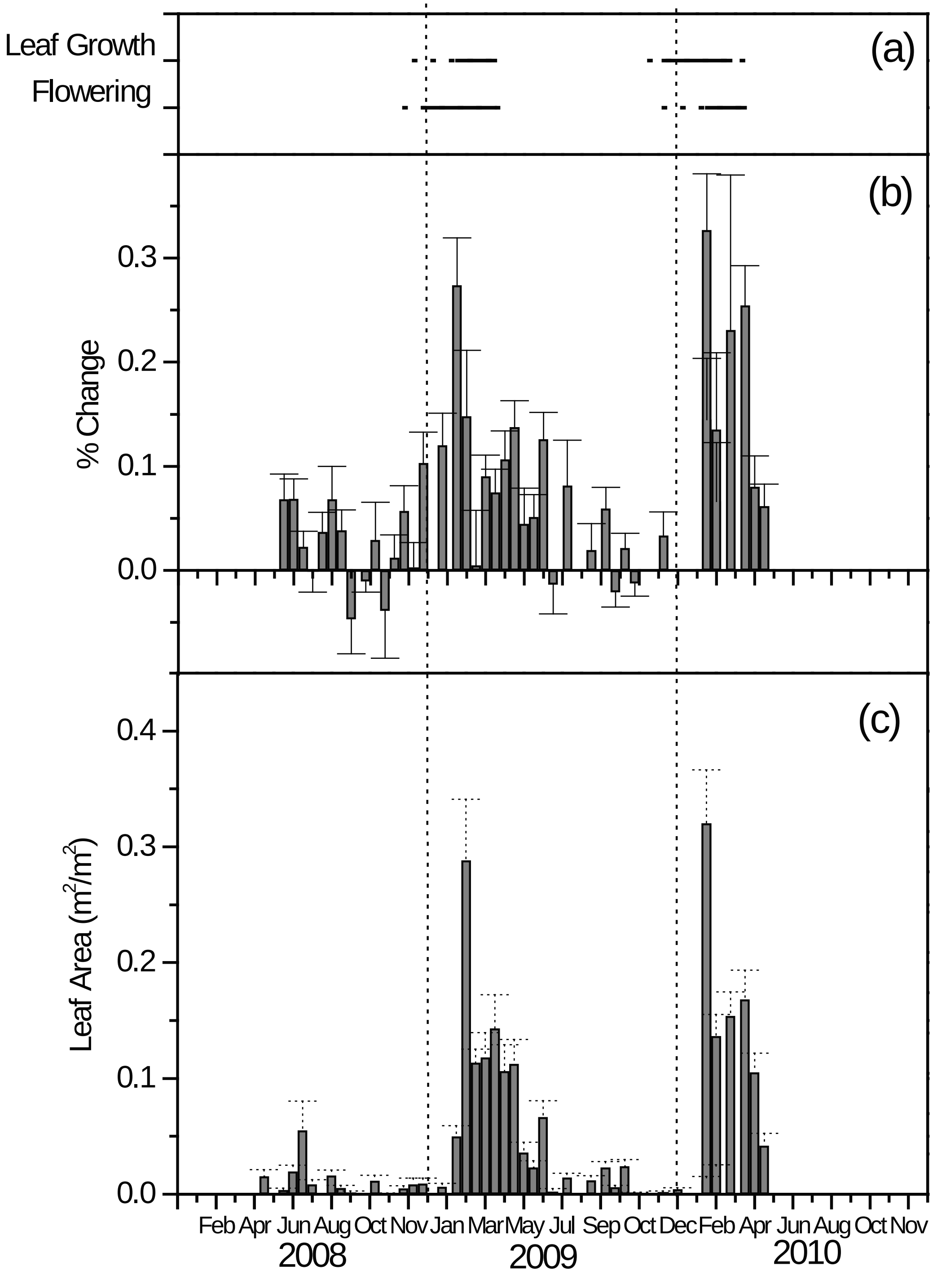


Figure 3.7. Above-ground phenology data for *Banksia attenuata* over a 24 month period, showing (a) timing of leaf growth and anthesis, (b) change in bole diameter as a percentage of starting bole size, and (c) leaf area of fallen leaves collected in litter traps, presented as leaf area m^{-2} (n =10).

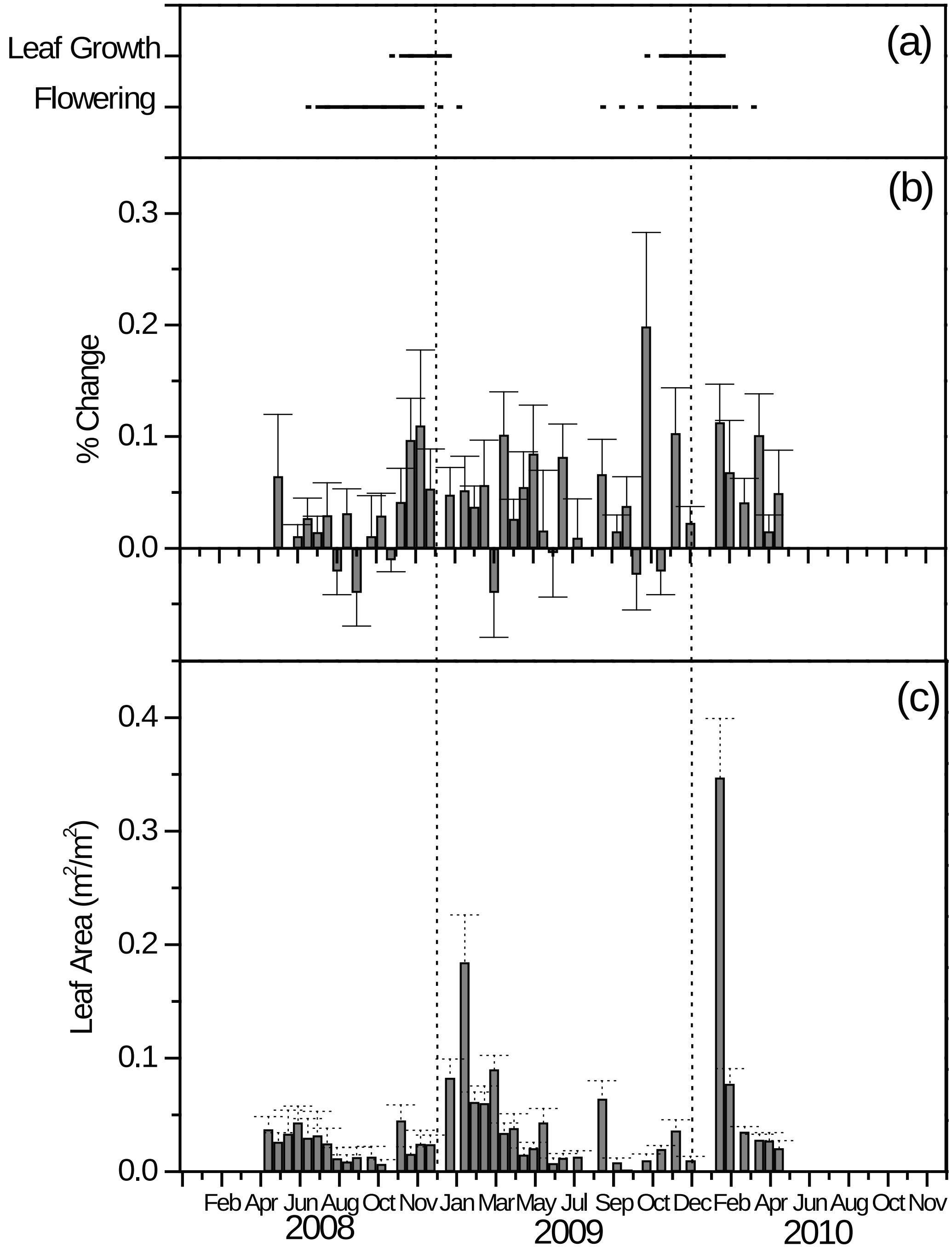


Figure 3.8. Above-ground phenology data for *Banksia ilicifolia* over a 24 month period, showing (a) timing of leaf growth and anthesis, (b) change in bole diameter as a percentage of starting bole size, and (c) leaf area of fallen leaves collected in litter traps, presented as leaf area/m^2 (n =10).

Leaf fall peaked in summer in association with high winds, high temperatures and low soil moisture conditions (Fig. 3.8c). The peak in leaf fall is more pronounced for *B. attenuata* than *B. ilicifolia* (0.3 m^2 m^{-2} c.f. 0.2 m^2 m^{-2}). *B. attenuata* leaf fall was greatest in late January, coinciding with high temperatures and high winds, and these two parameters emerged as the best predictors of leaf fall ($r^2 = 0.387$, $p < 0.05$) (Table 3.3). In contrast, *B. ilicifolia* lost leaves throughout the year, but had a peak in late January. Overall change in bole diameter was very low, 20.5 ± 3.0 mm yr^{-1} and 8.5 ± 1.6 mm yr^{-1} for *B. attenuata* and *B. ilicifolia* respectively. *Banksia attenuata* showed highest bole growth in the summer months, corresponding with new leaf growth and the loss of old leaves. Temperature was the best predictor of change in bole diameter ($r^2 = 0.129$, $p < 0.05$). In contrast, *B. ilicifolia* showed more variable changes in stem diameter throughout the year (Fig. 3.8b) with temperature as the best predictor of growth (Table 3.3).

Table 3.2. Binary logistic regression results for vegetative and reproductive phenophases in *Banksia attenuate* and *Banksia ilicifolia*. ‘Flowers’ refers to phenophase number 16 and ‘NoFl’ refers to all other phenophases. ‘Growth’ refers to phenophases 2, 3 and 4, ‘NoGr’ to remaining phenophases. *Banksia ilicifolia* flowering data did not fit the model, and are not presented. Predictor is the environmental parameter that predicted the highest percentage of observation, as determined by entering data forward stepwise regression (likelihood ratio). ***indicates significance value of <0.001.

	B. attenuata				*B. ilicfolia*	
	NoFl	Flowers	NoGr	Growth	NoGr	Growth
% Predicted	93.8	78.2	95.7	80.6	98.0	75.9
Overall % Predicted	90.7		92.5		93.9	
Predictor(s)	Av. Temperature		Av. Temperature		SM & Solar Rad.	
P	<0.000		<0.000		SM = 0.000 SR = 0.015	
B (SE)	0.600 (0.062)		0.695 (0.71)		SM = -88.487(13.453) SR = 0.146(0.060)	
Wald (df)	95.092 (1)		95.816 (1)		SM = 43.262 (1) SR = 5.913 (1)	

Predawn shoot water potential (Ψ_{PD}) did not suggest plant water deficit was occurring, with the majority of observations above -0.5 MPa for both species (Fig. 3.9a & b). There was a tendency for the more mesic phreatophyte, *B. ilicifolia* to have higher Ψ_{PD}, with the majority close to 0 MPa, except for the July 2009 sampling period where Ψ_{PD} was -0.58 ± 0.06 MPa. This sampling event also saw a low Ψ_{PD} for *B. attenuata* (-0.55 ± 0.08 MPa). Midday shoot water potentials (Ψ_{MD}) varied throughout the seasons and were lower for the more xeric phreatophyte, *B. attenuata*. Higher predawn shoot water potentials were observed in the cooler, wetter winter and autumn months (Fig. 3.9a). Leaf area index for the site stayed relatively stable at approximately 1 through the different seasons (Fig. 3.9c).

Table 3.3. Significant ($p < 0.05$) multiple linear regression models for leaf fall (mm tree^{-1}) and percentage change in bole diameter for *Banksia attenuata* and *Banksia ilicifolia*. Predictors are environmental variables used in the model that gave the highest r^2, and were entered stepwise (likelihood ratio).

Species	***B. attenuata***		***B. ilicifolia***	
	Leaves	Bole Change	Leaves	Bole Change
Predictor(s)	Temp., Wind Run	Temp.	Temp., Rain, Wind Run	Temp.
r^2	0.387	0.129	0.442	0.114
F (df)	137.874 (2, 437)	63.158 (1, 438)	35.308 (3, 346)	5.653 (1, 438)

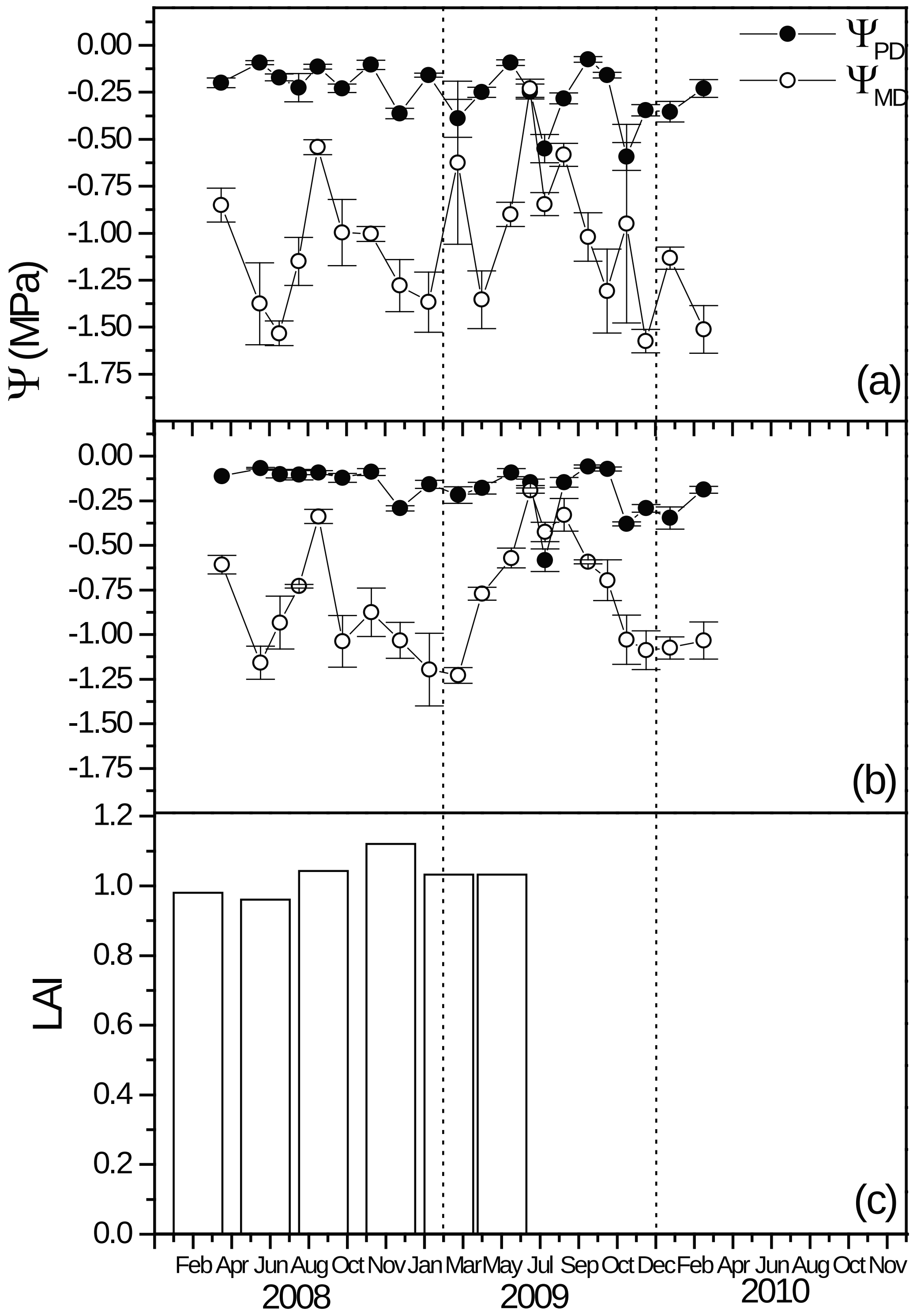


Figure 3.9. Pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}) shoot water potential data (Ψ) for *Banksia attenuata* (a) and *Banksia ilicifolia* (b); (n = 3 for both species), and leaf area index data (c) for trees at the study site.

Discussion

The root in-growth bag technique that was used, showed *Banksia* roots at the capillary fringe are able to grow all year round, provided soil conditions are conducive, and that there are no endogenous limitations to root growth at different times of the year, such as a dormancy period. This is demonstrated most clearly at 2.7 m depth, where the mass of root in-growth was consistent between seasons. The ability to grow at any time in response to soil conditions might be an essential prerequisite for phreatophytes, if they are to survive fluctuating water table conditions in seasonally water-limited environments. This study highlights the differences in root phenology with depth in a dimorphic root system. There was an overall trend of reduced root in-growth mass with depth, which follows the distribution of roots generally (Specht & Rayson 1957; Schenk & Jackson 2002). The timing of root growth differed with depth and this may be associated with different environmental parameters having more influence at certain depths compared to others. Roots in the top 0.25 m of the soil profile appear to be most influenced by seasonal changes in temperature. Roots found at 0.25 m were primarily cluster roots (Purnell 1960) which are typical of the O and A soil horizons in *Banksia* woodlands (Lamont 2003). There was little root growth at this soil depth during the cool, wet winter season (i.e. between April and October) which is contrary to observations made by Lamont & Bergl (1991), who found that cluster root growth was initiated by the first rain events of winter. However, studies of chaparral in southern California and Chilean matorral suggest that fine root growth in the top 20 cm begins after winter rain, reaching maximum production in spring and early summer (Kummerow *et al*. 1978; Montenegro *et al*. 1982). Root growth in the shallow parts of the soil profile in Mediterranean-type ecosystems is likely to be opportunistic, influenced be the large seasonal variations in temperature, as well as responding to changes in water and nutrient availability.

In contrast to the high seasonality of environmental variables and root growth at the soil surface, conditions are stable at deeper soil depths. The magnitude of diurnal and seasonal changes in soil temperature decreases with depth (Voroney 2007), and generally do not have an influence below 1 m (Popiel *et al.* 2001; Florides & Kalogira 2005). Instead, water availability appears to be a primary driver of root growth at the water table/capillary fringe interface, with root in-growth at 3.7 m most impacted by seasonal changes in the water table and root growth did not occur when this area became saturated. Root in-growth at 2.75 m occurred throughout the seasons, corresponding with the relatively stable conditions. Deep root systems enable access to deeper soil layers, which are generally more stable in terms of soil moisture and temperature. For phreatophytic plants in water-limited environments, this allows plants to survive and meet their water requirements throughout the year.

Seasonal fluctuations of the water table routinely saturate part of the root zone, which can impact on root activity (Kozlowski *et al*. 1997; Mahoney & Rood 1998; Castelli *et al*. 2000). In this current study root in-growth was initially observed at 3.7 m in autumn, when this zone was high in moisture, but not saturated (i.e. the capillary fringe). However, as the water table rose in winter and early spring the zone became saturated, and negligible root growth was observed (Fig. 3.8). In contrast, at 2.7 m, which was never saturated, root growth was similar for each sampling period, indicating that roots are able to grow at these depths throughout the year. This implies that the root growth by phreatophytic *Banksia* is restricted by the saturated conditions of the water table. This is supported by an investigation of the flooding tolerance of *Banksia prionotes* and *Banksia littoralis* seedlings, where it was found that both species are intolerant to long periods (>100 days) of flooding (Groom 2004b). Of the two species, *B. littoralis* was more tolerant of saturated conditions, and this was attributed to the distribution of *B. littoralis*, which is restricted to wetland fringes and damplands on the Swan Coastal Plain. Groom (2004b) hypothesised that the roots of *B. littoralis* may be able to form aerenchyma tissue, which would allow respiration under flooded conditions. In contrast, all seedlings of *B. prionotes*, a more xeric phreatophyte, died after 54 days of submergence. The species in this current study, *B. attenuata* and *B. ilicifolia*, are not distributed in areas that are exposed to flooding and it is likely that they are not physiologically adapted to survive inundation, as demonstrated by the lack of root growth when saturated.

Winter water table rise may inhibit root growth at the capillary fringe; however, the declining water table in summer and autumn provides a high moisture environment suitable for root growth. Root growth following a declining water table has been observed for a wide range of species, including phreatophytic plants. Under glasshouse conditions, roots of phreatophytic seedlings have been observed to elongate in response to a declining water table (Mahoney & Rood 1991; Kranjcec *et al*. 1998; Horton & Clark 2001; Stave *et al*. 2005). However, *in situ* observations of root elongation following a water table decline are fewer. In this current study, the patterns of root in-growth at the capillary fringe-water table interface indicate that roots elongate, following the water table as it declines in summer and autumn. The winter/spring increase in water table levels inhibited root growth at 3.7 m, but when the water table fell below the root bags over summer/autumn, root in-growth was observed at this soil depth. This indicates that roots respond to the change in water availability and follow the seasonally declining water table.

The pattern of root activity close to the water table is indicative of a seasonal cycle of root trimming in response to water table rise, and subsequent recovery as the water table falls again. Root redistribution can occur as phreatophytic plants adjust root growth in response to a rising and declining water table (Naumburg *et al*. 2005). As the rising water table inhibits root growth, root growth may continue and be encouraged in the unsaturated layers just above the water table (Castelli *et al.* 2000; Martin & Chambers 2002). Imada *et al*. (2010) described the proliferation of roots of *Populus alba* cuttings in the zone above a dynamic water table. Cuttings with a fluctuating water table had a higher dead fine root biomass than those with a static water table. However, the live fine root biomass was similar between the control and the treatments, suggesting that plants maintain total fine root biomass, redistributing growth in response to inundation of part of the root zone (Imada *et al*. 2010). Observation of root in-growth by phreatophytic *Banksia* with a shallow water table, as observed in this current study, indicate that roots close to the water table undergo a cycle of root trimming and recovery in response to seasonal fluctuations in groundwater levels.

For phreatophytic *Banksia*, year-round root growth at the water table/capillary fringe may impact on above-ground phenological processes. Although the volume of roots at depth may be smaller, water uptake by deep roots can account for up to 60% of the total plant water balance (Canadell *et al.* 1996). Root activity and water uptake at depth may therefore be influential in allowing plants to maintain leaf growth, flowering and secondary growth such as increasing bole diameter. Both *B. attenuata* and *B. ilicifolia* demonstrate restricted above-ground growth periods, with leaf growth occurring in late spring and early summer, a pattern observed both in this current study and in Bell and Stephens (1984). As new leaves develop, older leaves are shed. The synchrony between the loss of old leaves and new growth results in little overall change in canopy cover, with leaf area index remaining consistent throughout the year under *Banksia* woodland, as seen in this current study and observed by others (Farrington *et al.* 1989; Veneklass & Poot 2003). In addition, secondary growth, as observed using changes in bole diameter, indicates that growth is more pronounced in summer and autumn. Thus, all above-ground growth occurred between late spring and autumn, when water availability in the vadose zone is depleted due to the summer drought period of the Mediterranean-type climate. Therefore, this pattern of growth may be facilitated by root growth and water uptake from deeper soil moisture sources and the capillary fringe.

In addition to testing the stated primary hypotheses, this study also provides an opportunity to compare phenological observations made on *B. attenuata* over two study periods spaced approximately 30 years apart (i.e. Bell & Stephens 1984). There was generally strong agreement between the timing of phenophases and the associated temperatures observed in this current study of *B. attenuata* and the previous assessment by Bell & Stephens (1984; Table 3.5). In particular, the timing and the mean daily temperature when anthesis occurred was consistent between the study periods. Stephens (1985) found that an average air temperature of 17.8 °C initiated flowering in *B. attenuata* with only 3% variation over a four year study period. In the current study, anthesis was first observed in week 48 in 2008 and week 44 in 2009 and the average temperatures for these weeks were 17.1 °C and 18.5 °C respectively (although it should be noted that in 2009 the mean temperature in the preceding week was 17.5 °C and flowering could have started then). Rising temperatures in late spring thus trigger anthesis in *B. attenuata*, with its flowering period occurring over late spring and early summer (Bell & Stephens 1984; George 1984). The initiation of flowering by *Banksia* species is likely to be linked to changes in temperature. This allows closely related co-occurring *Banksia* species to maintain their genetic identity through flowering at different times of the year (Lewis & Bell 1981).

Table 3.5. Comparison of the timing of anthesis and beginning of leaf growth of *Banksia attenuata* in Stephens 1985 (^{1}) and the current study (^{2}) and the mean values of daily average temperature (°C) and daily total solar radiation (MJ m^{-2}).

		1978-1979^1	**1979-1980^1**	**1980-1981^1**	**1981-1982^1**	**2008^2**	**2009^2**	**2010^2**
Anthesis	**Av. Temp.**	17.8 (3% variation between years)				17.3	18.5	
	Solar Rad.	20.5 (31% variation between years)				22.7	17.2	
	Yr Week	45 →	5 42 →	10 45 →	4 38	48 →	16 44 →	11
Leaf Growth	**Av. Temp.**	16.5 (13% variation between years)				17.5	18.5	
	Solar Rad.	18.4 (28% variation between years)				14.8	17.2	
	Yr Week	41 →	6 43 →	9 40 →	2 41	43 →	6 44 →	11

Vegetative growth was restricted to late spring and early summer for both species, a pattern previously observed in Mediterranean species in the south west of Western Australia (Specht *et al.* 1983; Bell & Stephens 1984). Temperature was again a significant factor in triggering leaf growth, although there was some variation between the study years (2008 and 2009). Also, the temperature at which leaf growth was initiated varied between this study and that by Bell & Stephens (1984; 18 ˚C c.f. 16.5 ˚C). Bell and Stephens (1984) showed that the temperature that triggers leaf growth varies between years, with 13% variation between years. The variations in temperatures that trigger leaf growth between years may be related to temperature not being the only significant variable that controls the timing of leaf growth. The dynamics between other factors, such as soil moisture, day length and endogenous cues may make it difficult to identify a single controlling parameter.

The primary focus of this study was to investigate root activity at the water table/capillary fringe interface, and the methodology was designed to meet this aim. Root in-growth bags thus contained some slow release nutrients and were wet to field capacity prior to installation, as it was thought that root in-growth would otherwise not occur if the surrounding wet soil environment was more favourable. This may have allowed some root growth to be independent of soil conditions at the time of placement. This is likely to have had the most influence at the 0.25 m sampling depth, which dried out over the summer and autumn sampling periods, and the addition of water to the root bags may have made them particularly attractive for root growth. This may have been less influential in winter and spring when there was higher water content in the soil surrounding the root bags. However, the small amount of water added should not have had a prolonged effect over the three months the bag was in the field. The addition of water and nutrients appeared to have less influence on root activity at depth (i.e. 1.5-3.75 m), with root growth consistent between seasons. In addition, cluster root growth has been shown to be stimulated by the addition of nutrients at depth and at times of the year when they would not otherwise develop (Pate & Watt 2002). However, nutrient addition did not enhance cluster root formation at depth (found in < 4% of bags below 1.5 m) with most confined to the more nutrient-rich top 0.25 m of the soil profile. At 0.25 m the effect of the addition of water and nutrients is more pronounced. At 0.25 m the methodology may have influenced results by allowing some unseasonal cluster root production, with the timing of cluster root development in this study differing from that described by Lamont & Bergl (1991).

Conclusion

Growth by *B. attenuata* and *B. ilicifolia*, both above- and below ground, is greatest in spring and summer months, with leaf growth restricted to late spring and early summer. However, there did not appear to be any endogenous limitations on root growth, with root growth occurring year-round, as demonstrated by year-round growth at the capillary fringe. The influence of different environmental variables on root growth by phreatophytic *Banksia* changes with depth. In the shallow parts of the vadic zone, root growth is associated with temperature and water availability. Soil moisture can be more constant deep in the soil profile, and root growth is able to occur at different times of the year. However, deep roots that are growing near the water table can be influenced by the seasonal rise and fall of groundwater levels. Root growth by phreatophytic *Banksia* is restricted when the water table inundates the root zone in winter and spring. However, root growth was observed in the capillary fringe when the water table had receded to its deepest level in autumn. This indicates that roots have the capacity to respond to seasonal changes in groundwater levels.

Chapter Four

Seasonal groundwater uptake by dominant canopy species in a *Banksia* woodland

Introduction

The connection between phreatophytic plants and a water table is implied through the observation of above-ground parameters that can show if plants have access to a consistent water supply. Such indicators include observation of year-round vegetative growth and maintenance of high shoot water potentials and transpiration rates, despite a rainfall deficit (Noy-Meir 1973; Fernandez & Caldwell 1975; Poole & Miller 1975; Canadell *et al*. 1996). However, while these ecophysiological observations indicate that plants have adequate access to water, they do not directly demonstrate the water source utilised by the plant. Plants preferentially use water from areas with the highest water potential, and often the highest root density (Adiku *et al.* 2000). Root density typically changes with depth, with highest root density in the shallow soil layers, decreasing with depth (Schenk & Jackson 2002). This is true of plants with dimorphic root systems, including *Banksia* (Dodd *et al*. 1984), which have an extensive lateral root system, with sinker roots accessing deeper water sources (Dawson & Pate 1996; Zencich *et al.* 2002). Although the volume of roots at depth may be smaller, water uptake by deep roots can account for more than 60% of evapotranspiration in the dry season, as was found in an Amazonian rainforest (Nepstad *et al.* 1994). Water uptake from depth is often from plants accessing a water table; however, the water table is seasonally dynamic in Mediterranean-type ecosystems, rising in winter months to reach highest levels in spring, before declining over the summer months (Castelli *et al*. 2000; Martin & Chambers 2002). Changes in water table levels mean roots have to redistribute to maintain connection with the water table (Naumburg *et al*. 2005; Chapter 3). Roots can redistribute in response to a seasonally fluctuating water table, thus maintaining a functional connection with the water table. If roots are maintaining a connection with the water table, uptake of groundwater should be evident throughout the year.

Phreatophytic *Banksia* in areas with a shallow water table are likely to maintain year-round contact with the capillary fringe (Zencich *et al*. 2002). This can be inferred from observation of phreatophytic *Banksia* in habitats with a shallow depth to groundwater maintaining year-round high transpiration rates and high predawn shoot water potentials (Dodd & Bell 1993), demonstrating that they have access to sufficient water to avoid water deficits. This is particularly significant over summer and autumn months, when there is little rainfall in the Mediterranean-type climate of the Swan Coastal Plain, and water content in the vadose zone is low. Plants that are able to maintain high transpiration rates and predawn shoot water potential throughout the drought period are likely to be accessing groundwater. Groundwater uptake by phreatophytic *Banksia*, including *B. attenuata* and *B. ilicifolia*, has been previously investigated in a water source partitioning study using the natural abundance of hydrogen isotopes (Zencich *et al*. 2002). Although plants preferentially use water from the vadose zone, groundwater uptake was detected all year round, particularly in habitats with a shallow water table. Observation of year-round groundwater uptake is supported by root in-growth studies (Chapter 3), which indicate that roots close to the water table redistribute according to seasonally changing water table levels. A study that focuses on water uptake from the capillary fringe, where root growth has previously been observed, would further support these observations of year-round connection with a seasonally dynamic water table.

Root activity and use of water from the water table can be observed by applying chemical tracers to the water table and observing their uptake. Chemical tracers, such as lithium and strontium have been used successfully to assess root activity, particularly for crop species (Sayre & Morris 1940; Fox & Lipps 1964; Martin *et al.* 1982; Tofinga & Snaydon 1992; Haase *et al.* 1996; Obakeng 2007). Although many studies have utilised tracers to observe root activity of shallow-rooted species (Martin *et al.* 1982; Tofinga & Snaydon 1992; Simmons 1993), the method is being used increasingly to investigate water uptake by deep-rooted phreatophytes (Haase *et al.* 1996; Obakeng 2007). For example, Haase *et al*. (1996) used lithium chloride to investigate the rooting depth of a shrub species, *Retama sphaerocarpa*, in a semi-arid environment in Spain. Uptake of the tracer by the plants was observed from depths of up to 28 m. Similarly, Obakeng (2007) observed lithium uptake by seven different tree species in the Kalahari from depths of up to 73 m. In addition, isotopes of different elements can also be used as tracers, including the ^{15}N nitrogen isotope. ^{15}N is an effective tracer as its natural abundance in plants lies between -5% and +8%, thus only a small addition of highly enriched ^{15}N is required for uptake and distribution to be observed in a plant (Fry 1991; Nadelhoffer & Fry 1994). The uptake of ^{15}N can be rapidly detected in plant shoots, with one glasshouse experiment demonstrating the major amino acids in the shoots of Norway spruce were enriched with ^{15}N within four hours to one day (Geneger *et al*. 2003). In addition, ^{15}N has been observed to concentrate in plant shoots (Aarnes *et al.* 1995). The rapid uptake and accumulation of ^{15}N, in combination with the low natural abundance of the isotope in plants, makes it an effective tracer for observation of groundwater uptake by phreatophytic plants. Thus, there are a number of different chemicals that can be used effectively to observe root activity and groundwater uptake from a deep water table.

This study aims to investigate water uptake from the capillary fringe by phreatophytic *Banksia* throughout the year, to support observations in Chapter 3 of year-round root activity at the capillary fringe. It was hypothesised that if roots follow a seasonally dynamic water table there should be water uptake from the capillary fringe at contrasting times of the year. Two chemical tracers were used to assess water uptake by plants from the capillary fringe at contrasting times of the year. Lithium chloride was used in spring and ^{15}N in autumn, to avoid contamination between the two sampling periods. Additionally, although the dominant genus in a *Banksia* woodland, *Banksia* often occur in mixed stands with other trees, which are also deep rooted and likely to be phreatophytic. Thus, co-occurring trees species were also sampled, to determine if they were also utilising groundwater.

Materials and Methods

Study site and species

Groundwater uptake in contrasting seasons (spring and autumn) was assessed for four canopy species dominant in the *Banksia* woodland at the Whiteman Park study site. The study used the same site as Chapter 3, and existing infrastructure was used to monitor environmental variables during the course of the experiment. Meteorological parameters (temperature and rainfall) were recorded at a weather station (EnviroStation^{TM}, ICT International Armidale, NSW) approximately 1 km from the study site. Water table depth at the study site was recorded daily, using a groundwater sensor and logger (miniTROLL, In Situ Inc., Colorado, USA) placed in a monitoring bore at the site. Soil volumetric water content was recorded using a neutron moisture meter (Didcot Instrument Co., Abingdon, UK), with observations made five days prior to autumn sampling and on the second last day of spring sampling. Chemical tracers were applied using existing boreholes (see details in Chapter 3) to access the capillary fringe/water table. Two different chemical tracers were used: lithium chloride in spring and ^{15}N in autumn. This was to avoid contamination between the sampling periods and to ensure tracer uptake occurred during the observation period.

Four species, representing the dominant canopy species at the site, were selected for study. They were *B. attenuata* (five replicates), *B. ilicifolia* (five replicates), *Allocasuarina fraseriana* Miq. (three replicates), and a hemi-parasitic tree *Nuytsia floribunda* (Labill.) Fenzl (three replicates). While *B. attenuata* and *B. ilicifolia* are the most common species, *A. fraseriana* and *N. floribunda* trees co-occur at the site. These two species were therefore also sampled for tracer uptake, to determine if they are also accessing the water table. *Allocasuarina fraseriana* is a deep rooted tree species, with needle-like cladodes. *Nuytsia floribunda* is a hemi-parasitic species with an extensive root system capable of attaching itself to the roots of host plants to gain access to their water and nutrients (Herbert 1919; Weber 1980).

Spring Sampling – Lithium Tracer

The spring sampling period was selected to represent the period when the water table is highest as well as being the period of greatest water availability in the vadose zone. Spring sampling took place between the 19th and the 28th of October 2009. A 2.7 m borehole used to access root in-growth bags in Chapter 3 was used for the application of the lithium tracer. Using a hole at this depth ensured the tracer was applied to the capillary fringe, where root growth had been observed in the previous season (Chapter 3). Trees closest to the tracer injection well were sampled for analysis, ranging from 1.5 m to 19 m from the site of tracer application (Fig. 4.1). Plants were sampled prior to tracer application as a control. Sunlit leaves from the current year, (using the budscar ageing method of Lamont 1985), were sampled from the canopy, using a pole pruner. Two small bunches of leaves (approximately 5-10 leaves per bunch) were picked from each sample tree and placed into paper bags. Leaves were then transported to the laboratory and dried at 60 ˚C until a constant weight was attained (approximately 48 hours). A solution of lithium chloride (100 g diluted in 300 ml DI water) was poured into the hole. The solution was then washed out into the surrounding soil profile with 20 L of water. Following lithium injection, leaves were collected daily for the next ten days. Dry leaf material was ground and digested in 15.8 mol L^{-1} nitric acid and 11.6 mol L^{-1} perchloric acid. Lithium concentration in the extract was measured using ICP-OES (CCD Simultaneous Inductively Coupled Plasma Optical Emission Spectrometry radial torch, Varian Australia).

Autumn sampling – ^{15}N tracer

Autumn sampling took place between the 9th and the 19th of March 2010, representing the period when water table is deepest and soil water storage is diminished. Previous observation of groundwater depth at the site identified the depth to be approximately 3.85 m at this time of the year (Chapter 3). The tracer was applied to three 3.7 m deep wells, in an approximate triangle formation and trees were sampled from inside the triangle (Fig. 4.2). The nitrate tracer solution consisted of 1 g of labelled ^{15}N diluted with 99 g of ammonium nitrate in 900 ml DI water. This solution was divided into three equal parts then placed down the three boreholes. The same species and sampling protocol used for the lithium tracer was followed with leaves collected for ten days. Dried samples were encapsulated in a tin capsule and combusted in elemental analyser (Europa Scientific, Crewe, UK) to CO_2, N_2 and H_2O. H_2O was removed by magnesium percholate (SerCon Crewe, UK) and CO_2 was removed by Carbosorb (SerCon, Crewe, UK). The N_2 was purified by gas chromatography before being analysed by a 20-20 continuous flow isotope ratio mass spectrometer (CF-IRMS, Europa Scientific, Crewe, UK). Raw data was corrected for instrument drift. A standard weighed at variable weights, was used to correct for source pressure effects and IAEA-N-1 and IAEA-N-2 were used to normalise the results to the nitrogen in AIR scale, such that IAEA-N-1 = 0.43% and IAEA-N-2 = 20.32%.

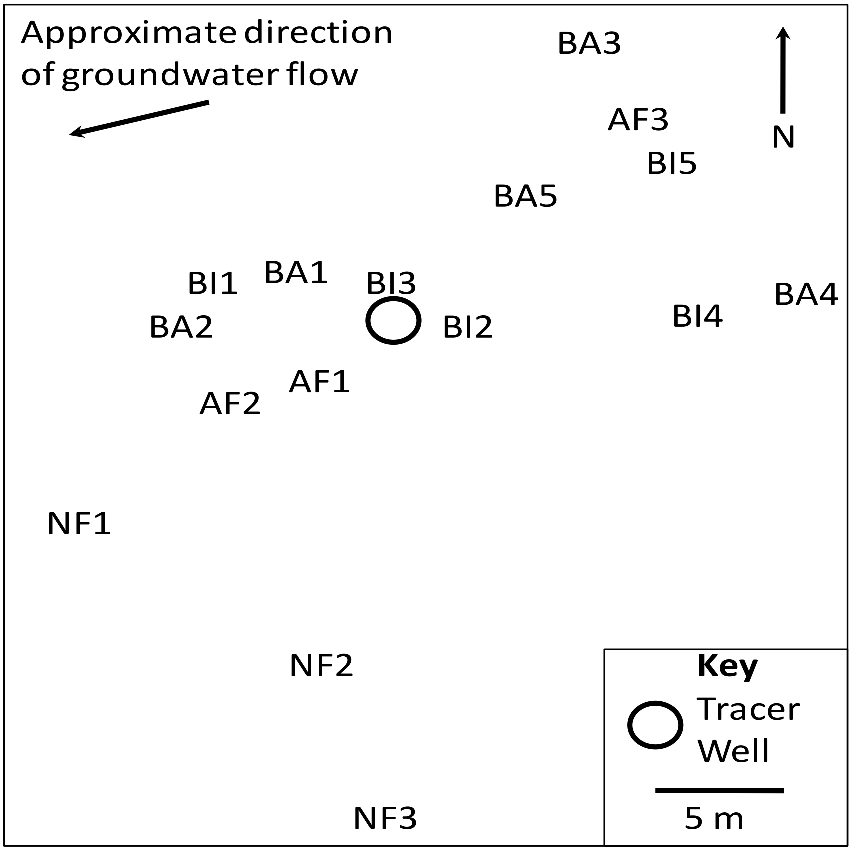


Figure 4.1. Study site layout for spring sampling period showing plant replicate locations for *Banksia attenuata* (BA), *Banksia ilicifolia* (BI), *Allocasuarina fraseriana* (AF) and *Nuytsia floribunda* (NF). The lithium chloride tracer was added to a central well that was 2.7 m deep, corresponding with the spring capillary fringe.

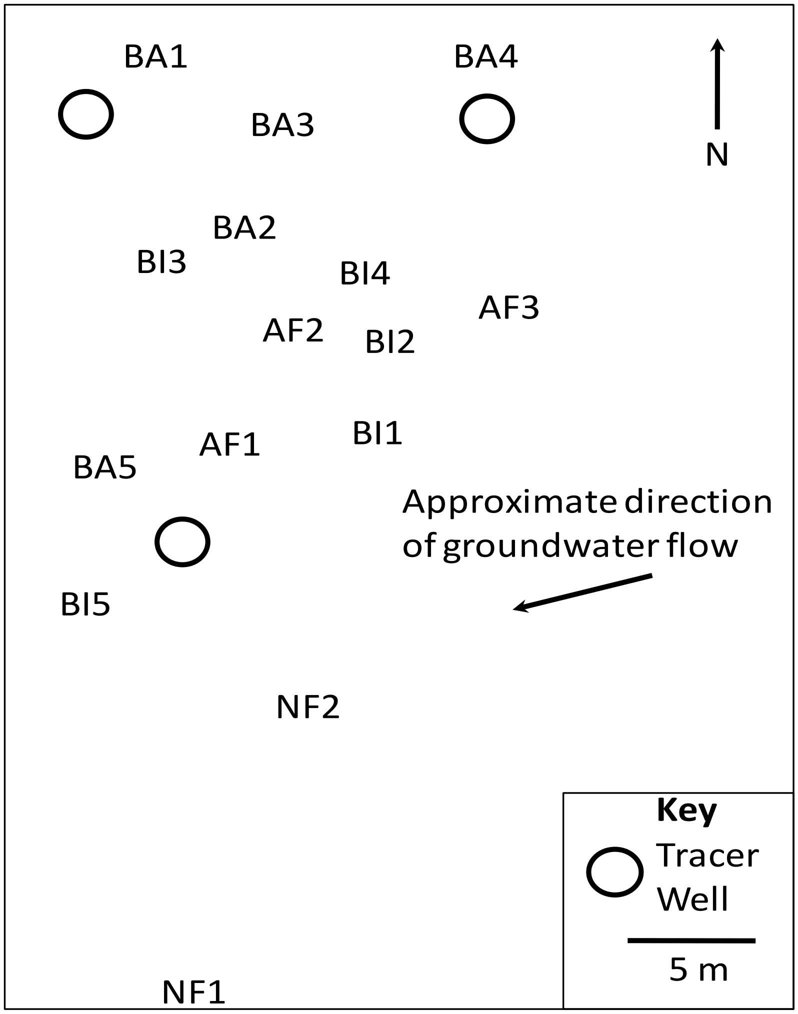


Figure 4.2. Study site layout for autumn sampling period showing plant replicate locations for *Banksia attenuata* (BA), *Banksia ilicifolia* (BI), *Allocasuarina fraseriana* (AF) and *Nuytsia floribunda* (NF). The N^{15} tracer was added to a central well that was 3.7 m deep, corresponding with the summer capillary fringe.

Results

Spring tracer uptake

Environmental conditions during the study period were typical of spring weather for the Mediterranean-type ecosystem on the Swan Coastal Plain. Maximum temperatures ranged between 20.4 and 33.4 ˚C, and cool nights, with minimum temperatures ranging between 5.6 and 15.6 ˚C. Depth to groundwater was approximately 3.11 m below the soil surface and appeared to be beginning to decline (Fig. 4.3). Only a very small amount of rainfall occurred during the ten day study period, with 3.6 mm recorded on day eight. The vadose zone indicated recharge from winter precipitation, with volumetric soil water content between 0.02 cm^3 cm^{-3} and 0.06 cm^3 cm^{-3}. Soil water content increased rapidly towards the water table, and was 0.11 cm^3 cm^{-3} at 2.75 m (Fig. 4.4).

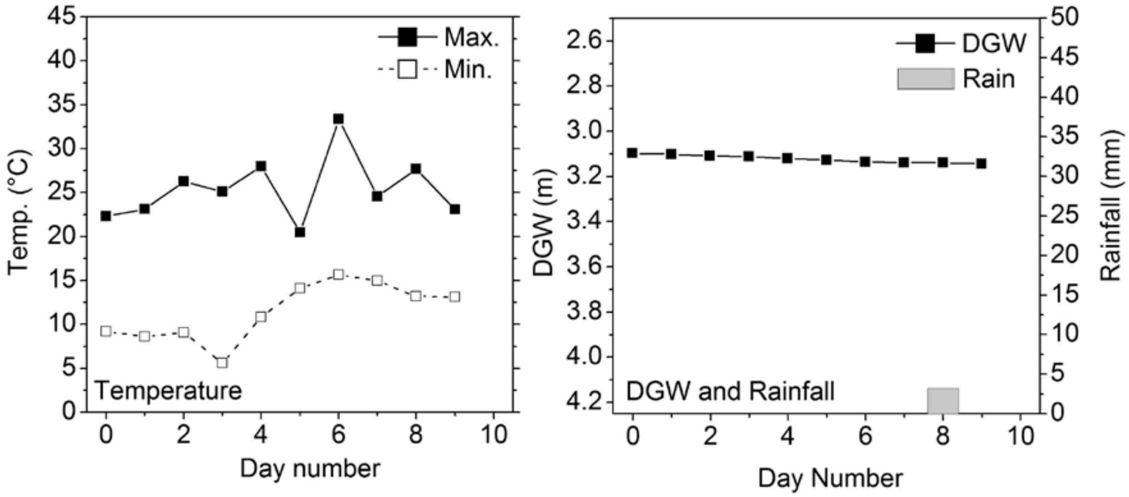


Figure 4.3. Temperature, rainfall and depth to groundwater for the study site at the spring sampling periods.

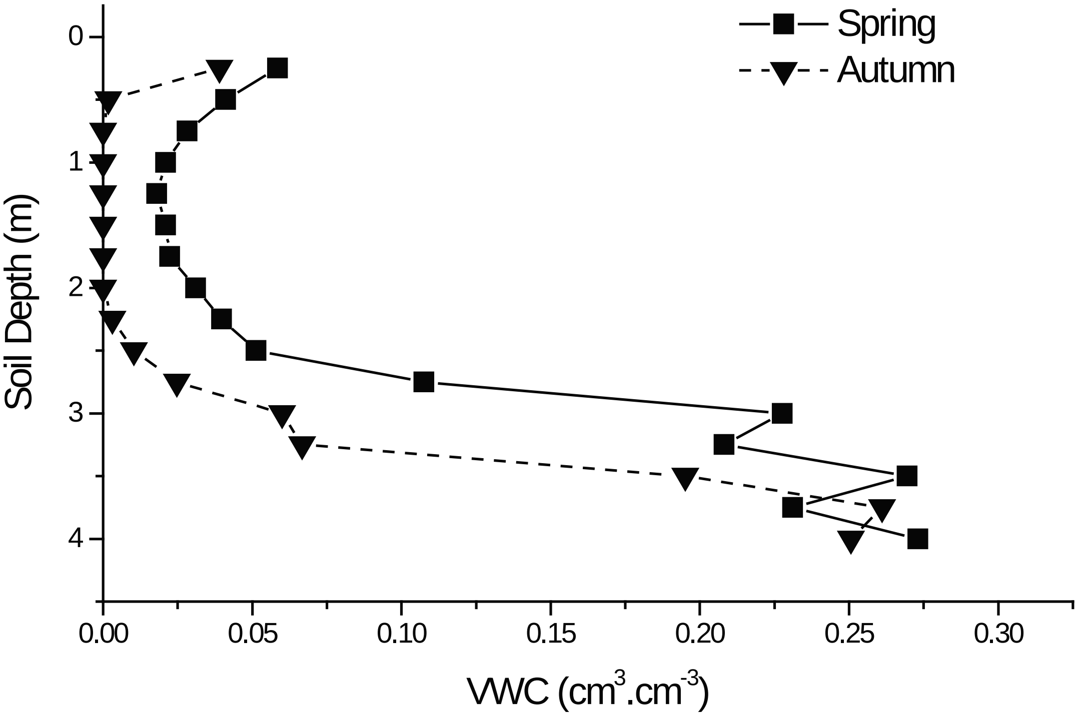


Figure 4.4. Volumetric water content (VWC) down the soil profile during spring (squares) and autumn (triangles).

Generally, the signal of lithium uptake in the leaves over the 10 day study period was inconsistent, with high variation both between individuals of the same species and between days from the same plant (Fig. 4.5). The concentration of lithium in the leaves was low, but there did appear to be a pattern of uptake for some individuals and this was strongest for replicates of *B. ilicifolia* and *N. floribunda*. The peak in lithium concentration for these two species was between seven and nine days after tracer injection. Lithium concentrations were highest for *N. floribunda*, reaching a peak of 1.9 µg g^{-1} compared with the much lower concentrations observed in *B. attenuata* leaves (highest value of 0.3 µg g^{-1}). There was little evidence of tracer uptake by *B. attenuata* and *A. fraseriana*. Both species demonstrated low concentrations of lithium in general and there was no indication of increased levels following tracer injection. One possible exception to this is a *B. attenuata* replicate, which had a very low starting concentration of lithium (0.1 µg g^{-1}). Six days after the tracer injection, lithium concentration had increased to 0.3 µg g^{-1}), however this value is still very low and concentrations dropped again over subsequent sampling days (Fig. 4.5).

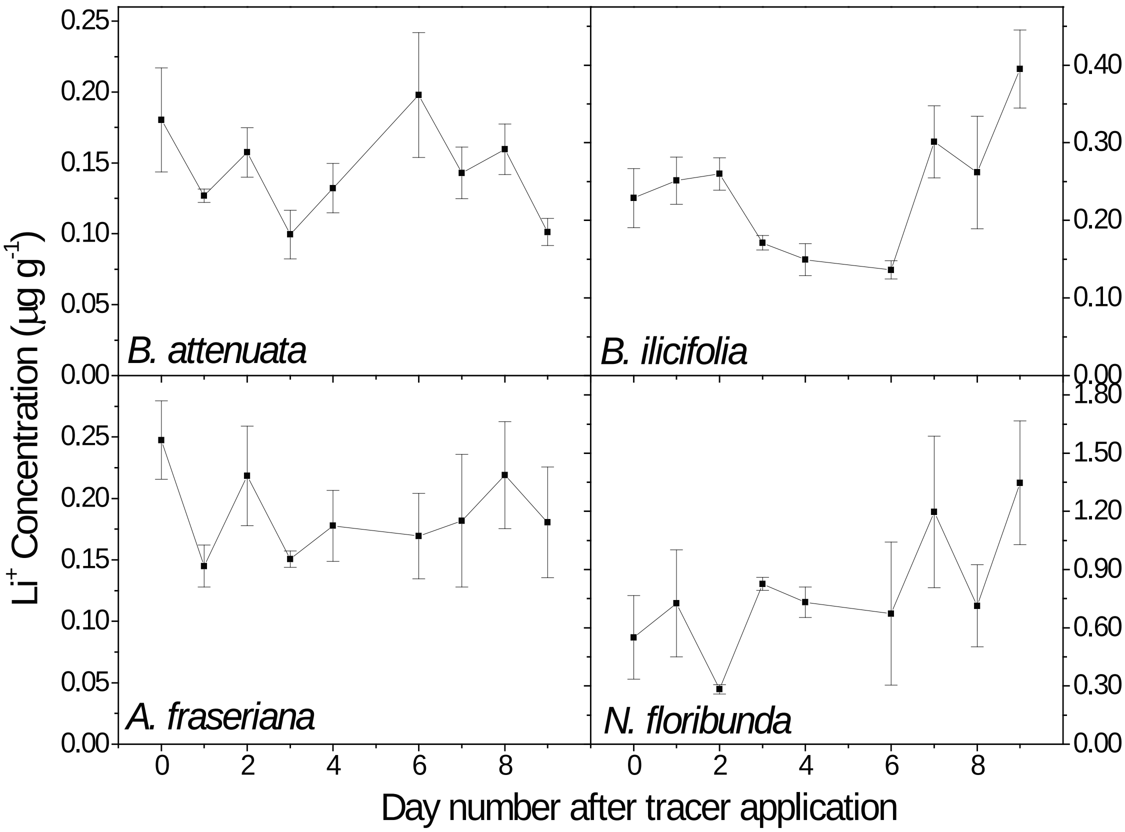


Figure 4.5. Lithium concentration in *Banksia attenuata*, *Banksia ilicifolia*, *Allocasuarina fraseriana* and *Nuytsia floribunda* leaves sampled following injection of LiCl solution into the spring capillary fringe (DGW approximately 3.1 m).

Autumn tracer uptake

Conditions during the sampling period were hot and dry, representative of Mediterranean-type summer on the Swan Coastal Plain. However, the sampling period was particularly hot for March, with maximum temperatures ranging between 24.7 and 42.9 ˚C. Minimum temperatures were between 10.1 and 20.0 ˚C (Fig. 4.6). The summer of 2009-2010 was particularly hot and dry, with only 1 mm of rain in the 16 weeks between week 47, 2009 and week 11, 2010, combined with daily maximum temperatures of more than 29 °C. This is reflected in the limited amount of soil moisture in the vadose zone over the study period (Fig. 4.4) with volumetric water content ranging between 0 and 0.06 cm^3 cm^{-3} down to 3 m below the soil surface. In addition, water content was approximately 0 cm^3 cm^{-3} between 0.75 and 2 m, indicative of the lack of recharge and the poor water-holding capacity of the Bassendean type sands. Depth to groundwater declined over the summer months and was at 3.75 m at the time of sampling.

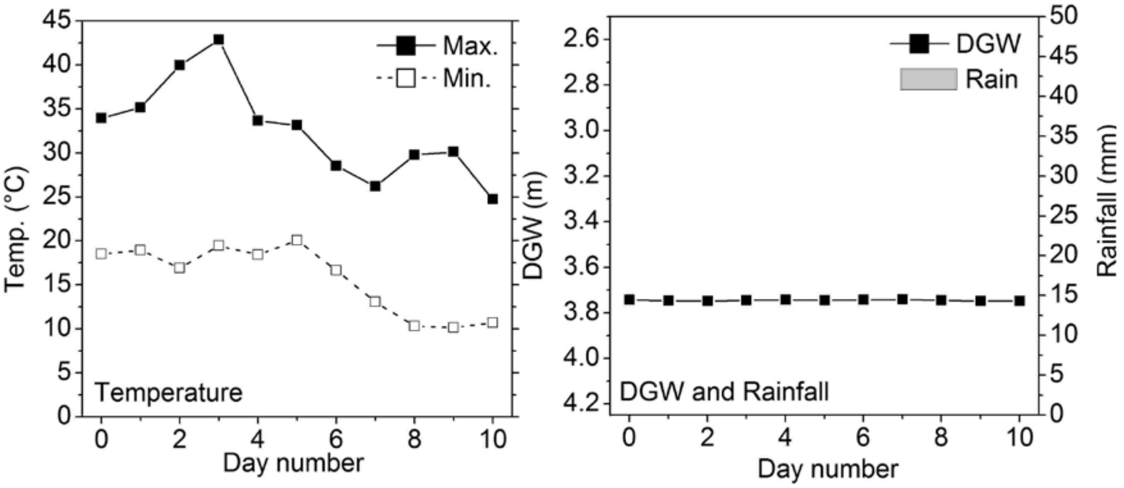


Figure 4.6. Temperature, depth to groundwater (DGW) at the study site over the autumn sampling period. Note that no rainfall was recorded during this period.

The concentration of tracer present in the leaves following injection was again very inconsistent between sampling days and between individual trees. There is generally little evidence of change in δ^{15}N abundance after the application of ^{15}N tracer (Fig. 4.7). One day after tracer injection, two replicates of *A. fraseriana* (AF1 & AF3) and one replicate of *N. floribunda* (NF2) had a spike in δ^{15}N abundance. However, abundance returned to similar values as those before tracer application by the following day and remained relatively stable for the rest of the sampling period. There was little variation in δ^{15}N abundance for *B. attenuata* and *B. ilicifolia* following tracer injection and there was no evidence of an increase in values (Fig. 4.7).

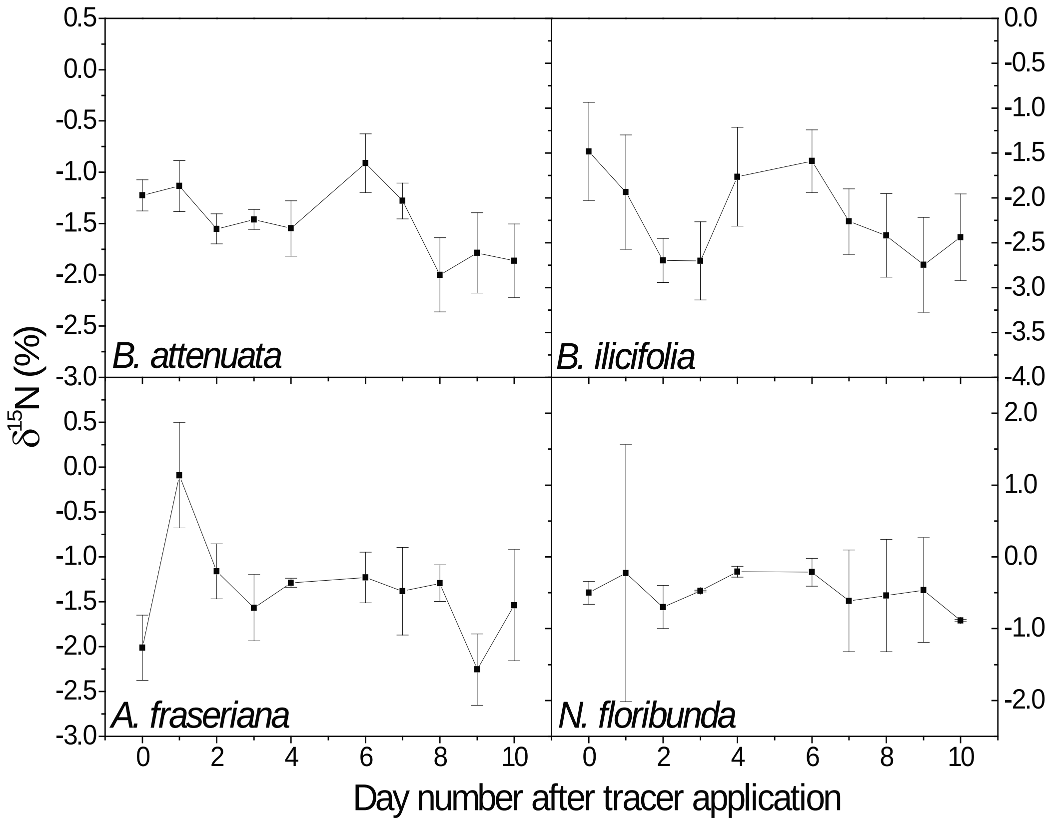


Figure 4.7. Nitrate abundance ($\delta^{15}N$) in *Banksia attenuata*, *Banksia ilicifolia*, *Allocasuarina fraseriana* and *Nuytsia floribunda* leaves sampled following injection of a ^{15}N labelled nitrate solution into the autumn capillary fringe (DGW approximately 3.75 m).

Shoot water potentials were similar across the two contrasting seasons (Table 4.1). Predawn shoot water potential for both *B. attenuata* and *B. ilicifolia* was close to zero during both the spring and autumn sampling periods, indicating that plants were not water stressed. Midday shoot water potentials were lower for the more xeric phreatophyte, *B. attenuata*, but values were once again similar between the two sampling periods.

Table 4.1. Predawn (Ψ_{PD}) and midday (Ψ_{MD}) shoot water potentials (MPa) for *Banksia attenuata* and *Banksia ilicifolia* sampled in spring and autumn. Values shown are means with standard errors (*n* = 3).

	B. attenuata		***B. ilicifolia***	
	Ψ_{PD}	**Ψ_{MD}**	**Ψ_{PD}**	**Ψ_{MD}**
Spring	-0.16 ± 0□0□	-1□31 ± 0.22	-0.07 ± 0.01	-0.70 ± 0.11
Autumn	-0.23 ± 0.05	-1.51 ± 0.13	-0.19 ± 0.02	-1.03 ± 0.10

Discussion

It was hypothesised that water uptake from the capillary fringe would be evident in phreatophytic *Banksia* in contrasting seasons. This study provides evidence of root activity at the capillary fringe in early spring, but does not indicate tracer uptake by either *Banksia* study species in autumn. Water uptake in spring, as indicated by an increase in lithium concentration in plant leaves, was most evident in the more mesic phreatophyte species *B. ilicifolia*, with all individuals showing an increase in lithium concentration by the end of sampling (Fig. 4.5). It is possible that *B. ilicifolia* utilise water from the capillary fringe in spring, despite there being water available in the vadose zone, as was also observed by Zencich *et al.* (2002). This may be related to the higher water requirements of this more mesic phreatophyte (Canham *et al*. 2009). In contrast, *B. attenuata* did not show a clear pattern of uptake of the lithium tracer from the water table in spring. This may be partly attributed to the extensive root system of the species, which explores large volumes of the sandy soil profile for water (Zencich *et al*. 2002; Groom 2004a). As plant water uptake is a function of soil water potential and rooting density (Adiku *et al*. 2000), it may be that *B. attenuata* was able to meet its water demands from the vadose zone during spring, when water content in the was high. However, it is likely that roots were in contact with the capillary fringe, when the water table was at its highest level. If plants were preferentially using vadic water, water uptake from the capillary fringe may have been too low to provide a tracer uptake signal.

Similarly, there did not appear to be any tracer uptake by either *B. attenuata* or *B. ilicifolia* in the autumn sampling period. The concentration of $\delta^{15}N$ fell well within natural abundance levels and no real increase detected following the injection of the tracer. It may be that the soil containing the tracer was too saturated for root growth by the species in the study. If the sampling period were increased this may allow for more root growth in the zone containing the tracer, as water content decreased. Although uptake of the tracer was not detected in the leaves, it appears that plants maintain connection with deeper water sources over the summer and autumn period. Water availability was severely diminished in the vadose zone over summer and autumn, and when sampling took place there was limited water available in the top 2.8 m of the soil profile. Despite this, plants still maintained high predawn water potentials, indicating that there was sufficient water available. Given the low water-holding capacity of the soils, in combination with an extended drought period, it is likely that plants were accessing water from the capillary fringe.

The other canopy species present at the site, *N. floribunda* and *A. fraseriana*, also showed water uptake from the capillary fringe, both in the spring and autumn sampling periods. *Nuytsia floribunda* had the strongest signal of increasing concentration of lithium, and all three replicates demonstrated tracer uptake during the spring sampling period. It is likely that the parasitic rooting trait of *N. floribunda* allowed the study plants to access the roots of nearby plants that also absorbed tracer, increasing the load of tracer taken up by the *N. floribunda* plants. In addition, *N. floribunda,* has an extensive root system, which stretches well beyond the canopy of the tree. This was noted in Chapter 3, with *N. floribunda* roots evident in root in-growth bags up to 20 m from the nearest individual of that species. This also could have contributed to the higher tracer values evident for *N. floribunda*. Rapid uptake of the tracer by both *N. floribunda* and *A. fraseriana* was also evident in autumn, with abundance of $\delta^{15}N$ peaking one day after the tracer was applied to the capillary fringe, although $\delta^{15}N$ abundance quickly returned to values similar to those observed prior to the addition of the tracer. When the water table was at its deepest and there was low water availability in the vadose zone both *N. floribunda* and *A. fraseriana* showed rapid uptake of the ^{15}N tracer. It is therefore probable that other deep-rooted species that co-occur in *Banksia* woodland, particularly *N. floribunda* and *A. fraseriana*, also utilise water from the capillary fringe.

Few studies that have investigated groundwater uptake using chemical tracers have assessed the seasonality of groundwater use. In this study, two tracers were used, to avoid cross-contamination of the site between the two seasons. However, the use of the two different tracers may have contributed to some difficulty in interpreting the results. While it was expected that water uptake from the capillary fringe by *B. attenuata* and *B. ilicifolia* could occur all year round, there was little tracer uptake in autumn. There are a number of possible reasons why uptake of the ^{15}N tracer was not evident in the autumn sampling period. It is possible that the lack of tracer uptake over that period may have been due to roots being inactive in the capillary fringe during this time. This may indicate that plants have access to other water sources, although considered unlikely given the low water content in the vadose zone at the time of sampling. However, *Banksia* have extensive root systems, which extend laterally from the plant and roots may have been active in the capillary fringe in an area where the tracer was not present, given that it was applied in only one central well. In addition, the concentration of the tracer used may also have been too low for the autumn sampling period using the ^{15}N isotope. However, as natural levels are so low, only a small amount of the tracer should be required to get a signal of tracer uptake. It may be the case that sampling of leaf material after tracer injection did not occur for long enough. Although previous studies of ^{15}N uptake by plants indicate that a signal is quickly observed in the shoots, these observations were based on shallow rooted plants under glasshouse condition (Geneger *et al.* 2003). Plants *in situ* may utilise water sources other than that part of the capillary fringe containing the tracer, which may dilute the signal. In addition, a longer sampling period may be necessary to observe water uptake by deeper-rooted species.

Although the lithium tracer generally gave a better signal of uptake, the concentration of lithium in the leaves was low overall and varied considerably, both between days and between individuals of the same species. The low concentrations of lithium following tracer application may be due to insufficient lithium being added to the water table to get a reliable signal in the leaves of the study trees. The methodology used in this study followed that outlined by Haase *et al*. (1996) who used 100 g of lithium chloride to observe water uptake from depths of 26 m and reported uptake in trees within one day of tracer application, peaking after seven days. However, Obakeng (2007) undertook a similar study using Kalahari tree species and used far greater quantities of lithium chloride, applying 4,950 g of lithium chloride to the water table. The small amount of tracer used in the current study may not be enough lithium chloride for a clear signal of water uptake by plants in the spring sampling period. The inconsistency between sampling periods evident in the results was also reported by Haase *et al*. (1996) and Obakeng (2007) who found that lithium concentrations were highly variable between sampling periods. Although that could be attributed to the environmental conditions over the sampling period, it is unlikely in this study as only negligible rainfall and stable temperatures were observed within each sampling period.

The variation in tracer concentrations between each sampling day may also be due to different sets of leaves being sampled each time. An effort was made to be consistent with the leaves sampled, collecting the current year's growth from the same side of the tree. However, there were unavoidable variations in the locations from which the leaves were collected each day and this may, in part, account for the variability in the results. This was also considered to be a factor in the variation between treatments reported by Obakeng (2007).

Conclusion

The chemical tracers used in this study give some indication that the phreatophytic *Banksia* study species were utilising groundwater, thus maintaining a functional connection with the water table. This is evident from observation of tracer uptake from the capillary fringe during spring, when the water table was at its highest level. However, water uptake from the capillary fringe by *Banksia* was not evident during the autumn sampling period. This is likely to be due to methodological reasons, as plants did not demonstrate any signs of water stress despite the low water content in the vadose zone. These physiological indicators suggest that plants were still accessing groundwater at this time of the year. Thus, it is likely that plants maintain a physiological connection with the water table, following it as it recedes over summer and autumn.

Chapter Five

Seedling root elongation rates for two phreatophytic *Banksia* species with contrasting water requirements

Introduction

Phreatophytic plants often have high water requirements and therefore need to be able to access a reliable water source, such as groundwater. The connection between groundwater and phreatophytic plants is dependent on plant roots being able to maintain functional contact with the capillary fringe. In environments where the water table is seasonally dynamic, plant roots are likely to redistribute in response to fluctuations in water table levels (Castelli *et al*. 2000; Naumburg *et al.* 2005; Chapter 3). This implies that, under normal seasonal conditions, the roots of phreatophytic plants elongate to follow a declining water table. In Chapter 3, roots appeared to adjust to water table decline at an average rate of 0.38 cm day^{-1} over the summer study period and 0.3 cm day^{-1} over autumn. The presence of roots suggests that roots were able to elongate and follow the water table that decline at these rates. However, these observations only infer the rate of root elongation; the actual root elongation rates for *Banksia* species have not been demonstrated. In addition, the rate of water table decline that roots are able to match was at a 'natural' rate; that is, the rate that the water table declines over summer drought due to evapotranspiration and the horizontal discharge of groundwater. Water table decline can occur at more rapid rates, impacted by reduced rainfall recharge and groundwater abstraction (Kite & Webster 1989; Scott *et al.* 2000). The rate of water table decline to which phreatophytic plants are able to adapt, may be dependent on the plant's capacity for rapid root elongation. For phreatophytic plants to avoid detrimental impacts from groundwater decline, root elongation must be adequate to maintain functional contact with the water table (Mahoney & Rood 1991). Thus, the ability for phreatophytic plants to survive changes in water table levels can depend on the root elongation rate of which plants are capable. Identifying the rate of root elongation by phreatophytic plants can assist our understanding of the adaptability of phreatophytes to changing water table levels.

Root elongation rates are species dependent and may differ between species that occupy different ecohydrological habitats. There is a wide range in the root elongation rates that have been previously described. For example, riparian poplar species have been found to have modest root elongation rates and are able to survive water table decline rates of between 1 – 4 cm day^{-1} (Mahoney & Rood 1991; Kranjcec *et al.* 1998; Gonzalez *et al*. 2010). Similarly, Hughes *et al*. (1997) found that in *Alnus incana,* a riparian phreatophytic tree species, plants grew well at a rate of decline of 1 cm day^{-1}, but they could also survive a drawdown rate of 3 cm day^{-1}. Riparian tree species in semi-arid Africa have much greater capacity to adapt to high rates of water decline since they are able to elongate roots at 5 cm day^{-1} (*Faidherbia albida)* to 10 cm day^{-1} (*Acacia tortilis)* (Stave *et al*. 2005). Additionally, there is some evidence that root development differs between mesic and more xeric species. In particular, root elongation rates are reported to be slower for more mesic species that occupy habitats with high degrees of water availability (Booth *et al*. 1990; Richards *et al.* 1995; Stave *et al*. 2005). Slower root elongation by more mesic plants may be due to these species occupying areas where water availability is more reliable, thus slower root elongation rates are sufficient (Kranjcec *et al.* 1998). However, there are few studies comparing seedling root elongation rates between phreatophyte species. For more xeric species, roots that are quick to penetrate to deeper soil layers may be advantageous, allowing plants to follow the water table when shallower soil layers dry out (Leishman & Westoby 1994). In contrast, mesic phreatophytes distributed in habitats with a shallow depth to groundwater do not require roots to penetrate as far, before they are able to access water from the capillary fringe. Thus, rapid root elongation may be less advantageous for mesic phreatophytes, as water availability is higher at shallower depths.

Phreatophytic *Banksia* on the Swan Coastal Plain of south Western Australia range in their dependency on groundwater, as determined through their distribution in relation to groundwater availability and physiological traits such as vulnerability to xylem cavitation (Zencich *et al*. 2002; Canham *et al.* 2009). The root elongation rates of these phreatophytic *Banksia* have not been previously documented, but this is a vital parameter in further understanding the adaptability of phreatophytic *Banksia* in response to changes in water table levels. The current study investigates the capacity for root elongation, and the rate at which root elongation occurs for two phreatophytic *Banksia* species. Quantifying the rate of root elongation of which phreatophytic *Banksia* are capable, may give some indication of the rate of water table decline that they are able to withstand. It was expected that the roots would be able to elongate at a rate of water table decline at least as fast as that which occurs over summer, which was found to be 0.38 cm day^{-1} in Chapter 3. Furthermore, it is thought that the rate will be faster, based on previous observation of rapid root development by *Banksia* seedlings (Bowen 1991; Rokich *et al.* 2001). In addition, the two study species, *B. attenuata* and *B. littoralis*, represent phreatophytes that range in their dependency on groundwater, as demonstrated by their distribution across the ecohydrological landscape and physiological traits, such as vulnerability to xylem cavitation. *Banksia attenuata* is a widespread species, and can tolerate greater water stress than *B. littoralis*, which occurs in dampland habitats. It was hypothesised that the mesic phreatophyte (*B. littoralis*) would have a slower rate of root elongation than the more xeric phreatophyte (*B. attenuata*). Root elongation rates were determined in a glasshouse experiment using rhizopods (Mahoney & Rood 1991). Other plant traits, including root and shoot weights, seed weight, cotyledon leaf condition and predawn leaf water potentials, were also observed to support the interpretation of root elongation.

Materials and Methods

Glasshouse Experimental Design

Root studies are often difficult, due to the logistical constraints of observing roots and this difficulty is compounded when the study species are deep rooted, as are many phreatophytes. Mahoney and Rood (1991) used a 'rhizopod' to investigate root elongation rates of phreatophytes in response to a declining water table in an experimental setting. The rhizopod is an array of tube ‘pots’ connected to a reservoir, which allows for the maintenance and manipulation of a water table. Such an apparatus has appeared in a number of studies since its inception, sometimes undergoing minor variations (Hughes *et al*. 1997; Horton & Clark 2001; Stave *et al*. 2005). The rhizopod design is useful for the study of phreatophyte root elongation, although it does not allow for direct observation of roots. Instead, previous studies have investigated root elongation rates by destructively sampling seedlings (Mahoney & Rood 1991). This technique is useful as it allows for root and shoot biomass to be determined, in addition to root lengths, but it does not necessarily provide detailed data on root elongation rates. Root windows allow for regular observation of root growth and elongation (Bohm 1979). Rhizotrons, used in the field to investigate root growth, can have transparent walls through which roots may be mapped (Soileau *et al.* 1974). Root windows have also been used in glasshouse studies, cut into the sides of growing containers, which allows regular monitoring of root growth (BassiriRad & Caldwell 1992; Gallardo *et al.* 1994). Root windows allow for detailed observation of root growth, which is vital to quantifying plant root elongation rates. Rhizopod apparatus may be combined with root windows to allow detailed observation of the growth and root elongation of phreatophytic species.

Rhizopods were constructed using a similar design to that of Mahoney and Rood (1991). They consisted of 2 m high 90 mm diameter PVC pipe attached via 20 mm pipe to a 150 cm tall reservoir. The reservoir is to maintain and manipulate water table depths (Fig. 5.1). To avoid psuedoreplication (*sensu* Hurlbert 1984) there were just three plant tubes for each reservoir, meaning there was one replicate per species per reservoir plus a spare plant. There were 34 sets of three, with 30 of these used for destructive sampling and measurement of plants. The remaining four sets had windows installed, to allow for *in situ* observation of root growth (Fig. 5.2). To provide dark conditions for root growth, the windows were covered with a layer of black plastic and this in turn was covered with a thin reflective layer.

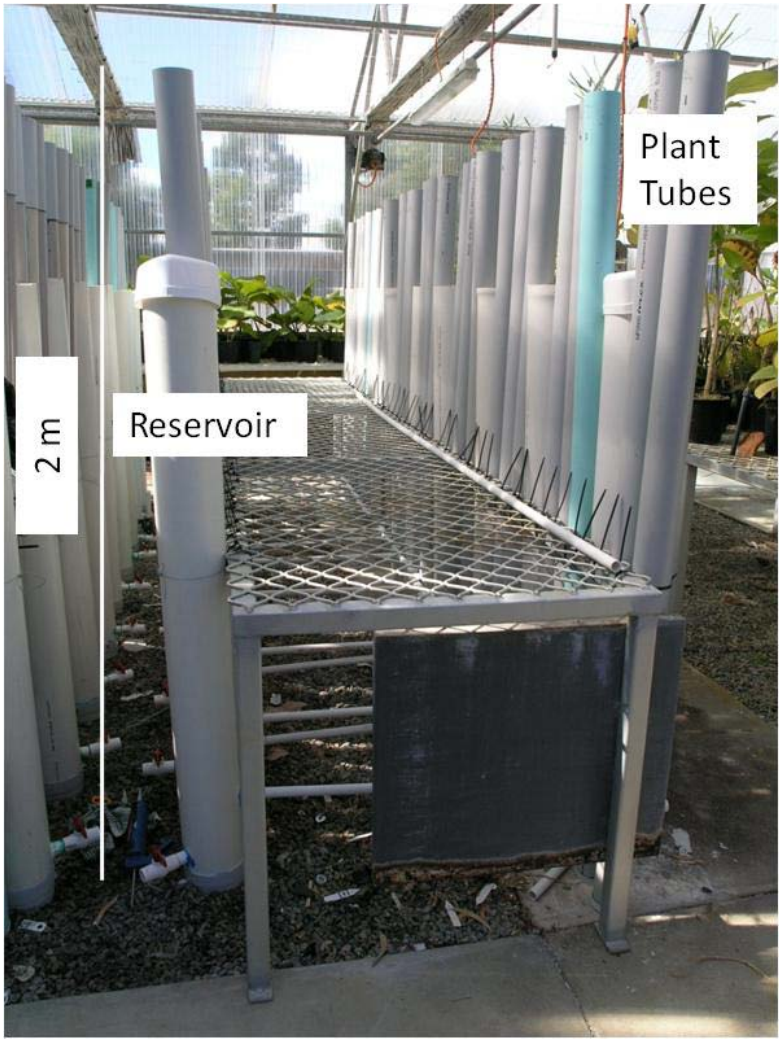


Figure 5.1. Rhizopods in the glasshouse on campus. Seedlings were grown in 2 m tall plant tubes attached to reservoirs. Reservoirs were used to maintain the water table at 1 m below the soil depth.

Figure 5.2. Root windows with roots showing *Banksia attenuata* root growth towards water table.

Rhizopods were filled with a medium to coarse sand, similar to that of the Bassendean Sand Dune system. The soil was predominately medium sand, with fine sand only 3% of the soil fraction, and silt and clay less than 1% (Table 5.1). The soil had a very low water-holding capacity, as determined from an analysis of the water retention properties of the soil (Table 5.2).

Table 5.1. Particle size distribution for soil media used in rhizopods

Sand Fraction (µm)	**% of Sample**
Coarse Sand (2000–600)	14.02
Medium Sand (600–200)	81.94
Fine Sand (200–75)	3.09
Silt and Clay (< 75)	0.95

Table 5.2. Water retention data for soil media used in rhizopods

Matric Potential (kPa)	**VWC (%)**
0	31.83
10	0.36
33	0.18
100	0.10
1500	0.04

Germination and seedling transplantation

Two phreatophytic *Banksia* species were selected for study. *Banksia attenuata* is considered a more xeric phreatophyte, with a wide distribution across the ecohydrological landscape and is relatively more resistant to xylem cavitation. In contrast, the distribution of *B. littoralis* is restricted to mesic environments with a shallow depth to groundwater and the species is more susceptible to xylem cavitation (Canham *et al*. 2009). Seeds of *B. attenuata* and *B. littoralis* were sown in January 2008. The study species normally germinate in winter/spring and because they were being germinated in summer they were given a hot/cold treatment prior to sowing. Seedlings were placed on filter paper in a petri-dish and dampened. They were refrigerated at approximately 4 °C for eight hours, and then taken outside to be exposed to 30-32 °C for six hours. This temperature oscillation was repeated twice more. Seeds were then sown in root trainer pits filled with white sand then watered daily. Seedlings were transplanted in March, eight weeks after sowing and all seedlings still had green cotyledon leaves (Fig. 5.3). A slow release fertiliser (8 g of Osmocote© Native Plants) was applied to each tube and plants were watered with 200 ml water every three days for 33 days after transplantation to avoid desiccation. After this period, seedlings were no longer watered, with the water table the only water source available once the unsaturated part of the profile had dried out. The glasshouse temperature was controlled and ranged from 18 to 25 ºC. There were five days when temperatures reached 37 ºC due to a malfunction of the coolers. Relative humidity ranged between 25 and 90%, and averaged 50% across the experimental period.

Figure 5.3. Example of a *Banksia attenuata* seedling transplanted into a rhizopod.

Destructive Sampling

Destructive sampling took place on the 33rd, 49th, 65th and 92nd day after transplanting. After leaves had been collected for water potential measurements, the reservoirs were drained. The plant tube was cut using a reciprocating saw to approximately 30 cm below the water table to allow for any root growth beyond the water table. The tube containing the plant was laid on its side and the soil was washed away from the plant roots. Intact plants were laid out and total root and shoot lengths were recorded immediately, before desiccation could occur. Plants were photographed, then shoots removed and placed into plastic bags. Roots were cut into 20 cm increments and placed into paper bags and dried at 40 °C until they reached a constant weight (approx. 48 hours). Shoots were returned to the laboratory on the same day to have their leaves removed and leaf area determined using a leaf area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK.) Following this, the leaves were placed in a paper bag with the rest of the shoot portion and dried along with the roots. The plant material was weighed to determine total root mass, total shoot mass and root:shoot ratios. In addition, whole seed weight was determined by weighing 20 individual seeds of each species.

Leaf Water Potential

On the same days as the destructive sampling, pre-dawn leaf water potential (n = 3) was determined using a dewpoint potentiometer (WP4, Decagon Services). Leaves were excised from the plant, with sampling taking place between 6 and 7 am. Following the methods recommended by Decagon (Decagon application note n.d.) leaves were scoured using 600 grit sand paper and DI water to remove the cuticle and allow for faster equilibration. Leaves were patted dry using a lint-free tissue (Kimwipe^{TM}), then excised from the plant and placed in a plastic bag with a piece of moist paper to minimise desiccation. Leaves were taken into the laboratory and cut to a size that covered the bottom of the WP4 sample container. Subsequently, the sample was placed into the drawer of the WP4 and water potential measurements were logged for up to an hour. It appeared that after 40 minutes the majority of replicates had equilibrated, so values recorded at 40 minutes were used for analysis.

Soil Moisture

Soil moisture content was determined by collecting soil samples at each sampling period. The day before plants were sampled soil samples were taken from a randomly selected tube (n = 1 per species). A 25 mm diameter hole was drilled and a horizontal soil core taken. This was repeated every 5 cm from the top of the tube to the water table (100 cm). Each core of sand was placed in a plastic sample jar, sealed and kept refrigerated until analysed. Soil water potential was determined using the WP4 dewpoint potentiometer. Samples were shaken, to ensure even distribution of water, and a small sample placed in the bottom of the WP4 sample cup. The sample was placed in the WP4 and water potentials were logged for up to 20 minutes. The value recorded closest to 15 minutes was used for analysis as samples appeared to have equilibrated sufficiently in that time.

Gravimetric water content was also determined. Wet weight of samples was recorded and samples placed in the oven at 105 °C until a constant weight was reached (approx. 48 hours). Dry sample weight was recorded and gravimetric water content (θ_{dw}) was determined as thus;

$$\theta_{dw} = \frac{weight\ of\ wetsoil}{weight\ of\ drysoil}$$

To determine bulk density pieces of the sand filled pipe (*n* = 3) were cut, keeping the sand inside the pipe. The volume of pipe was determined and the weight of the sand from that volume of pipe was dried, before being weighed. Bulk density (BD) was then determined as:

$$BD = \frac{weight\ of\ drysoil}{volume\ of\ soil}$$

Volumetric water content (VWC) was then determined using the gravimetric water content and the bulk density:

$$VWC(\%) = (BD \times Gravimetric\ Water) \times 100$$

Root elongation rates (RER) were determined for both the destructively sampled seedlings and the seedling roots observed in rhizopod windows. For the sampled RER, the mean root length was used to determine the elongation rate of individuals at the next sampling event. This may be shown as:

$$RER = \frac{x_i - \bar{x}_{i-1}}{n_i - n_{i-1}}$$

where n = number of days, x = sample root length, $\bar{x}$ = mean root length.

Root elongation rates for the seedlings in the window tubes were calculated by determining the difference in root length since the previous observation and then dividing by the number of days between observations. The maximum root elongation rate was determined for each individual and this value was used for statistical analysis. The capillary fringe was determined to be 20 cm above the water table, thus at 80 cm depth. Window observations of roots were used to determine the number of days it took for each individual to reach 80 cm depth, the capillary fringe.

Data Analysis

Independent t-tests were performed on each parameter at each sampling period to see if there were significant differences between the two study species. Where data did not meet normality requirements, a log transformation was used. Leaf area and root:shoot ratio data could not be transformed to a normal distribution so non-parametric Kruskal-Wallis tests were used to test for significant differences. All analyses were done using SPSS v. 17 software (SPSS Inc.).

Results

Soil moisture

At day 33, when initial watering ceased, volumetric water content was between 1 and 5% throughout the soil profile (Fig. 5.4a & 5.4c). After this, the profile began to dry out from the top down and by the conclusion of the experiment 75% of the profile had less than 1% volumetric water content (VWC) for *B. attenuata* (Fig. 5.4a). In contrast, soil moisture in the rhizopods containing *B. littoralis* was higher, with only 40% of the soil profile having less than 1% VWC (Fig. 5.4c). Whilst the profile dried from the top down, the water table was maintained at 1 m, providing a constant water source if the roots could reach it. The capillary fringe was approximately 20 cm above the water table and remained constant for the duration of the experiment.

Root Elongation

Seedlings of both study species were observed to have roots extending to the capillary fringe by the end of the experiment (92 days after transplanting; Fig. 5.4b & 5.4d) The total root length reached by the end of the study was not significantly different between species, though the more mesic phreatophyte, *B. littoralis*, had a greater total root length at 1,018 ± 25 mm compared to 950 ± 44 mm for *B. attenuata* (t (10) = -1.33 p = 0.212 (Table 5.5). Root elongation was rapid immediately after watering from the top ceased, but slowed again as roots got closer to the capillary fringe. The pattern in root elongation rates differed between the two study species. The more xeric phreatophyte, *B. attenuata*, had a sharp peak in rate of root elongation post-watering, followed by a rapid decrease as roots reached the capillary fringe (Fig. 5.5 a & b). This contrasts with *B. littoralis* which showed a flatter response, with a lower peak and a more gradual slowing of root elongation rate as roots approached the capillary fringe. Peak elongation rates were found on day 41 for both species, eight days after the cessation of water from the top.

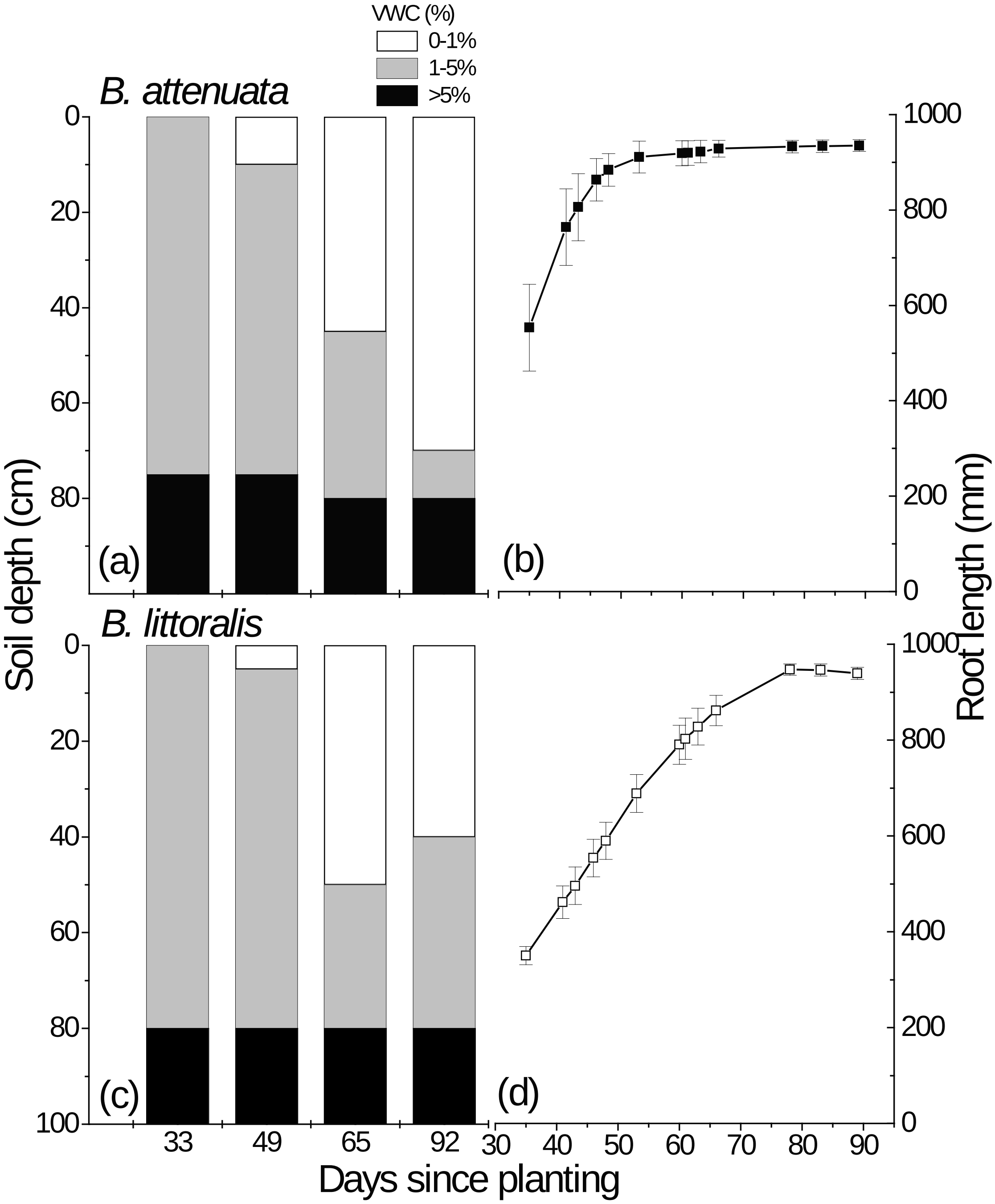


Figure 5.4. Volumetric water content (VWC) during the study period for (a) *Banksia attenuata* and (c) *Banksia littoralis* (n = 1 for both species). Volumetric water content is categorised as 0-1%, 1 to 5% and more than 5% VWC. Root lengths for (b) *B. attenuata* and (d) *B. littoralis*, as recorded through observation windows (mean ± SE; *n* = 5).

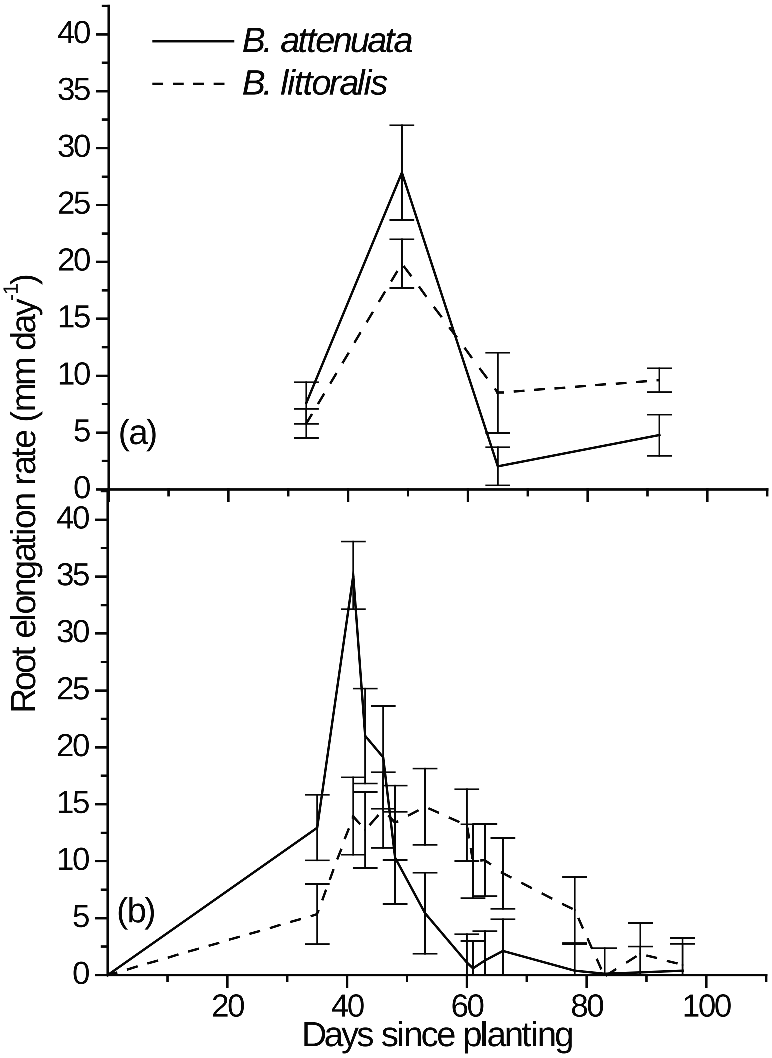


Figure 5.5. Root elongation rates for *Banksia attenuata* (solid line) and *Banksia littoralis* (dashed line) as determined by (a) destructive sampling and by (b) observation windows.

Maximum RER was significantly higher for the more xeric phreatophyte, peaking at 37 ± 4 mm day^{-1} for *B. attenuata* compared to 18 ± 3 mm day^{-1} for *B. littoralis* (t (8) = 3.783 p = 0.005; Table 5.3). The difference in RER is reflected in the number of days it took for seedling roots to reach the capillary fringe. *B. attenuata* roots reached the capillary fringe by day 46 ± 4, whilst the mesic phreatophyte, *B. littoralis* took significantly longer at 69 ± 5 days (t (8) = -4.437 p = 0.009). There was strong agreement in root elongation rates as determined from root length observations from destructive sampling and root window observations.

Table 5.3. Mean and standard errors for seed weights (n = 20) and root elongation rates (n = 5) for *Banksia attenuata* and *Banksia littoralis* as observed through root windows. Max. RER refers to the highest root elongation rate observed for each individual and average RER – peak period is the mean RER after cessation of watering and when the roots reached the capillary fringe. Symbols indicate significant differences between species (*t tests, and † Kruskal-Wallis, p <0.05).

	Seed Weight (mg)	**Max. RER (mm day^{-1})**	**Average RER– Peak Period (mm day^{-1})**
B. attenuata	108.12 ± 3.94†	37 ± 4*	19 ± 5
B. littoralis	21.42 ± 1.25†	18 ± 3*	15 ± 2

Seed and Seedling Size

Banksia attenuata had significantly larger seeds than the more mesic phreatophyte, *B. littoralis* (Table 5.3), and *B. attenuata* also had larger seedlings overall. Although there were no significant differences in total root length identified, there was a significant difference in total root mass, particularly at the final sampling period. *Banksia attenuata* had a much larger root mass than *B. littoralis* (t (10) = 4.298 p = 0.002; Table 5.5). *B. attenuata* also had a higher shoot mass than *B. littoralis* at day 92 (t (10) = 5.423 p <0.001). *Banksia attenuata* seedlings increased root mass, then shoot mass, at a faster rate than the mesic phreatophyte, and leaf area increased accordingly. By the conclusion of the experiment, *B. attenuata* had twice the leaf area of *B. littoralis* (Kruskal-Wallis, χ^2 (1 N = 12) = 5.769 p = 0.016). This corresponds with observations that *B. attenuata* seedlings were twice the size of *B. littoralis* seedlings by day 92, both in terms of root and shoot mass. Cotyledon leaves were larger for *B. attenuata* and remained green for longer (Table 5.4). By the end of the experiment, the majority of *B. littoralis* seedlings (87%) had brown, shrivelled cotyledon leaves. This contrasts with *B. attenuata*, where the majority of seedlings had chlorotic cotyledon leaves but 33% still had green cotyledon leaves.

Table 5.4. Condition of cotyledon leaves observed on harvesting days 33, 49, 65 and 92. Values are a percentage of total plant numbers for *Banksia attenuata* (BA) and *Banksia littoralis* (BL) and n = 6 for each species.

Cotyledon Condition		**Day 33**	**Day 49**	**Day 65**	**Day 92**
Green	BA	100	83	17	33
	BL	100	50	17	0
Yellow	BA	0	17	83	67
	BL	0	50	33	17
Brown	BA	0	0	0	0
	BL	0	0	50	83

Root:shoot ratios (R:S) varied for each species as seedlings became established. At day 33 for *B. attenuata*, the R:S was relatively low at 0.3 ± 0.0 differing to *B. littoralis* at 0.6 ± 0.0 (Kruskal-Wallis, χ^2 (1 N = 12) = 8.308 p = 0.004; Table 5.5). These had reversed by day 49, with *B. attenuata* seedlings investing more in roots and R:S ratio increased to 0.9 ± 0.2 compared with 0.6 ± 0.1 for *B. littoralis*. At day 65 *B. littoralis* had a significantly higher R:S ratio, at 1.2 ± 0.2, though this had decreased to 0.9 ± 0.1 by day 92. After the initial increase in R:S between days 33 and 49 *B. attenuata* remained relatively constant. There were no significant interspecific differences in leaf water potential, and values were consistent at each sampling period, although it was highest at day 33, before the soil profile started to dry from the top.

Table 5.5. Summary of plant parameters measured on destructively sampled seedlings. All data are mean and standard errors (n = 6, except for Ψ leaf where n = 3). * indicates a significant difference detected between *Banksia attenuata* (BA) and *Bankisa littoralis* (BL) at that sampling period as determined using t tests and † indicates a significant difference detected using Kruskal-Wallis test, as data did not meet the normality of distribution requirements for parametric analysis.

Parameter	**Spp.**	**Day 33**	**Day 49**	**Day 65**	**Day 92**
Root length (mm)	BA	350.3 ± 54.9	795.8 ± 66.4*	838.7 ± 35.4	953.5 ± 43.6
	BL	291.5 ± 38.5	609.2 ± 34.1*	787.5 ± 74.3	1018.2 ± 25.1
Root weight (mg)	BA	50.5 ± 7.4*	289.1 ± 65.7	333.5 ± 30.1	600.4 ± 22.9*
	BL	91.3 ± 17.2*	149.5 ± 18.7	353.0 ± 31.7	321.1 ± 39.7*
Shoot weight (mg)	BA	168.0 ± 19.2	334.3 ± 33.0	546.3 ± 50.9*	866.7 ± 72.3*
	BL	156.7 ± 26.8	243.2 ± 36.1	324.5 ± 28.0*	360.0 ± 59.2*
Root:Shoot	BA	0.3 ± 0.0†	0.9 ± 0.2	0.6 ± 0.0†	0.7 ± 0.1
	BL	0.6 ± 0.0†	0.6 ± 0.1	1.2 ± 0.2†	0.9 ± 0.1
Leaf area (cm^2)	BA	9.5 ± 2.2	22.3 ± 1.4	34.4 ± 4.5	54.0 ± 5.7†
	BL	13.7 ± 1.9	21.6 ± 3.1	26.0 ± 2.6	25.6 ± 5.6†
Pre-dawn Ψ_{leaf} (MPa)	BA	-0.6 ± 0.1	-1.1 ± 0.2	-1.4 ± 0.3	-1.2 ± 0.0
	BL	-0.8 ± 0.1	-1.4 ± 0.2	-1.7 ± 0.3	-1.4 ± 0.1

Discussion

The root elongation rates observed in the current study greatly exceed those found in field observations of root growth in response to seasonal changes in water table levels (Chapter 3). The maximum rates of root elongation were found to be 36.7 mm day^{-1} and 18.2 mm day^{-1} for *B. attenuata* and *B. littoralis* respectively. These rates of root elongation may be regarded as rapid in comparison to the rates of water table decline that the species are likely to experience over summer and autumn water table decline (approximately 3.8 mm day^{-1}; Chapter 3). However, the root elongation rates fall within the rates that have been recorded in studies of root elongation by phreatophytic plants in response to different rates of water table decline. It has been suggested that phreatophytic plants are capable of root elongation rates between 10 and 40 mm day^{-1}, as shown for a number of riparian tree species (Mahoney & Rood 1991; Hughes *et al*. 1997; Kranjcec *et al.* 1998 Gonzalez *et al*. 2010) and possibly even up to 100 mm day^{-1} (Stave *et al*. 2005). In addition, previous researchers have commented on the rapid development of tap and sinker roots by *Banksia* seedlings. Enright & Lamont (1992) found that one year old seedlings of *B. attenuata, Banksia hookeriana* and *Banksia leptophylla* at a dune crest site in Eneabba (280 km north of Perth) had tap roots exceeding 2 m in length. Bowen (1991) found roots of 16 month old *B. attenuata* and *B. prionotes* extending down to a 2.2 m water table at a site on the Swan Coastal Plain. Similarly, Rokich *et al*. (2001) described tap roots of up to 1.5 m in length for one year old *B. attenuata* and *Banksia menziesii* growing in *Banksia* woodland, also on the Swan Coastal Plain. Thus, *Banksia* are capable of rapid root elongation during development.

Root elongation may be encouraged by decreased water availability, as plants are prompted to increase their rooting volume, thus increasing absorptive surfaces for water uptake (Hutchings & de Kroon 1994). In this current study, root elongation rates peaked soon after surface watering had stopped, as roots followed the drying front through the sandy soil in the rhizopod. It is likely that the root elongation rate increased as roots responded to decreasing soil water potential. Teskey and Hinckley (1981) reported a root growth response to decreased soil water potential for white oak (*Quercus alba*), with root elongation and the number of growing roots increasing with soil water deficits between -0.4 to -0.8 MPa. This response means plants increase their total rooting volume and the volume of soil from which water can be accessed, which is a critical factor that allows phreatophytic plants to respond to changes in water table levels.

Differences in root elongation rate by the two phreatophytic *Banksia* species may be related in part to the ecohydrological habitats in which the species are distributed. For phreatophytes that occupy more xeric environments with deep sandy profiles, a rapid rate of root elongation may be necessary for plants to access deeper water stores quickly. For species that are limited to more mesic environments, such as *B. littoralis*, access to groundwater is still vital, with the distribution of these species often limited to habitats with a shallow depth to groundwater (Busch *et al.* 1992; Zencich *et al.* 2002). Therefore, roots do not have to penetrate as deeply to access the capillary fringe and water table, and less time and fewer resources are required to access a reliable water source. High water availability at shallow soil depth may mean there is less selection pressure for newly germinated seedlings to be capable of rapid root elongation. A rapid rate of root elongation may be unnecessary for the establishment and persistence of mesic species, since plants can have a slower rate of root elongation and still be able to access sufficient water (Kranjcec *et al*. 1998). This is supported by observation of species from more mesic environments having slower root elongation rates compared to more xeric species (Matsuda & McBride 1986; Richards & Lamont 1996; Milberg & Lamont 1996). The root elongation rates observed in this current study support the hypothesis that the more xeric phreatophyte, *B. attenuata*, has a faster elongation rate and can reach the capillary fringe before the mesic phreatophyte, *B. littoralis*. It is more beneficial for *B. attenuata* to invest in more extensive root growth than *B. littoralis*, since it is a xeric phreatophyte with a distribution that includes habitats of deep sands with no available groundwater. *Banksia attenuata* therefore requires an extensive root system exploiting a large volume of soil. Conversely, the narrow distribution of *B. littoralis* means that this species is confined to habitats that afford year-round access to a shallow water table (Zencich *et al.* 2002).

The use of rhizopods in the current study necessitated the use of seedlings to investigate root elongation rates by phreatophytic *Banksia*, and this may have influenced the rates observed. The young seedlings still had cotyledons intact and it is likely that seed size had an influence on the rates of seedling growth and interspecific differences in root elongation rates. Seedling root elongation rate, in part, may be determined by seed size, as large seeds produce larger initial seedlings than small seeds (Marshall 1986; Jurado & Westoby 1992; Osunkoya *et al.* 1993; Leishman & Westoby 1994). Additionally, there is evidence that larger seeds are advantageous in xeric habitats (Baker 1972; Stock *et al*. 1990; Stromberg & Patten 1990; Leishman & Westoby 1994; Richards & Lamont 1996; Milberg & Lamont 1997), which is significant when contrasting root elongation rates between phreatophytes with different water requirements. The more xeric species, *B. attenuata* had significantly larger seeds than the mesic species, *B. littoralis*. Observations in the current study of *Banksia* seedling growth in the first few months following germination, support previous studies that found that larger seeds produce larger seedlings in this genus (Stock *et al*. 1990). Leishman and Westoby (1994) observed a similar trend for a range of semi-arid species, noting that overall seedling size was related to root development, with large seedlings developing large roots capable of accessing deeper soil water. The faster root elongation rates observed for the xeric phreatophyte are most likely associated with the large seed size of *B. attenuata* in comparison to *B. littoralis*. It may be argued that it is less of an imperative for *B. littoralis* seedlings to invest energy into producing larger seeds in order to produce larger seedlings with extensive root systems. The distribution of *B. littoralis* ensures it is in habitats with greater and more constant water availability, and rapid development of a deep root system may not be as strongly selected for, compared to species that occur in more xeric environments.

Conclusion

To maintain a functional connection with a rapidly declining water table, phreatophytic plants need to have the capacity for root elongation rates that allow plants to follow the water table. The rates of 1.8 cm day^{-1} for *B. littoralis* and 3.7 cm day^{-1} for *B. attenuata* that were observed in this current study fall within the range of root elongation rates observed for phreatophytes in similar glasshouse experiments. Such rapid rates of root elongation indicate that plants can potentially maintain contact with a water table decline at rates far greater than those that occur during a normal seasonal cycle of water table fluctuations.

Chapter Six

Root response of phreatophytic *Banksia* exposed to rapid water table decline

Introduction

Plants that develop in the presence of a shallow water table can become dependent on access to groundwater as a water source. While phreatophytes often preferentially utilise water from the vadose zone when it is available (Snyder & Williams 2000; Zencich *et al*. 2002), groundwater uptake allows phreatophytes to not only survive summer drought, but maintain high water potentials and continue photosynthesis and leaf growth (Fernandez & Caldwell 1975; Poole & Miller 1975; Canadell *et al*. 1996; Chapter 3). Many phreatophytes are physiologically adapted to a high level of water availability, with comparatively high water demands (Horton *et al*. 2001) and increased vulnerability to water stress (Hacke *et al.* 2000; Pockman & Sperry 2000; Canham *et al.* 2009). Phreatophytes are therefore vulnerable to water table decline and their response is often observed as physiological water stress, reduced photosynthesis and transpiration, branch dieback and eventually, plant death (Scott *et al*. 1999; Sperry & Hacke 2002; Cooper *et al*. 2003). These canopy level processes can indicate disconnection from the water table. However, phreatophytic plants may avoid severe water stress if they have a root elongation response to declining groundwater levels and are able to maintain contact with the water table and capillary fringe (Naumburg *et al*. 2005).

The adaptability of phreatophytic *Banksia* to changes in groundwater availability is influenced by the capacity for roots to respond to a declining water table. Root growth responds to changes in soil moisture, limited by either a deficiency or an excess of soil water (Kramer & Boyer 1995). Root growth can be limited by reduced water availability. For example, root growth by loblolly and Scotch pine seedlings was reduced to about 25% of the rate at field capacity when soil water potential was reduced to -0.6 or -0.7 MPa (Kaufmann 1968). Conversely, root elongation may be encouraged by decreased water availability, since plants need to increase their rooting volume, thus increasing absorptive surfaces for water uptake (Hutchings & de Kroon 1994). Teskey and Hinckley (1981) observed a root growth response to decreased soil water potential for white oak (*Quercus alba*). They reported that root growth, including root elongation and the number of growing roots, increased with soil water deficits between -0.4 to -0.8 MPa. The capacity for plants to detect decreased soil water availability, and invest in root growth as water availability decreases, allows them to extend deeper into the soil profile and maximise the volume of soil that is able to be searched for moisture. Root growth response to decreased water availability may allow phreatophytic plants to maintain contact with a declining water table.

Roots of phreatophytic plants may respond to a declining water table; however, the rate of water table decline and the capacity for root growth will determine whether plants are able to continue to meet their water requirements. Under normal seasonal conditions, water table rise and fall occurs at relatively low rates. For example, the shallow unconfined aquifers in the sandy soils on the Swan Coastal Plain, Western Australia, decline at a rate of approximately 0.38 cm day^{-1} over summer months (Chapter 3). However, decreased recharge of the aquifer, due to reduced rainfall, in addition to groundwater abstraction, can increase the rate of water table decline that phreatophytic plants are likely to encounter. Changes in ecosystem hydrology have prompted investigations of maximum rate of water table decline that phreatophytic plants are able to adapt to, specifically examining the root growth and elongation in relation to water table decline. For example, Mahoney and Rood (1991) found that a water table drop of 1 cm day^{-1} best matched the potential for root elongation in the natural poplar hybrid *Populus deltoides x P. balsamifera*. Others have also found that certain phreatophyte species respond best to a 1 cm day^{-1} rate of water table decline rather than faster rates (Krancjec *et al*. 1998; Horton & Clark 2001). Stave *et al*. (2005) found that *Faidherbia albida* seedling root elongation could maintain contact with a water table decreasing at a rate as high as 5 cm day^{-1}. In Chapter 5 of this study it was found that two phreatophytic *Banksia* species had the capacity for relatively rapid rates of root elongation, with rates of 1.8 cm day^{-1} for *B. littoralis* and 3.6 cm day^{-1} for *B. attenuata*. Thus, as phreatophytic *Banksia* are capable of rapid rates of root elongation to establish a connection to a water table, they may also be able to elongate roots in response to a rapidly declining water table.

Root elongation response (including rate of root elongation) can differ between species and may differ according to the distribution of a species in an ecohydrological landscape. It has been suggested that there is a general trend for more mesic species to have slower root extension than more xeric species (Matsuda & McBride 1986; Richards *et al.* 1995). For example, Horton & Clark (2001) compared *Salix gooddingii*, a mesic phreatophyte which inhabits river edges, with *Tamarix chinesis*, an exotic, more xeric phreatophyte. It was found that *S. gooddingii* had slower root elongation rates in response to the declining water table than *T. chinesis*. For *Banksia* species, it was shown in Chapter 5 that germinants of *B. attenuata*, a xeric phreatophyte, have faster root elongation rates than the smaller-seeded mesic phreatophyte *B. littoralis*.

Plant water status indicators, such as predawn shoot water potential, stomatal conductance, transpiration and photosynthetic rate, indicate if plants are experiencing a water deficit (Cooper *et al*. 2005). Regulation of stomatal conductance can reduce transpirational water loss and is one way plants can maintain their water status in response to decreased water availability. However, this is at the expense of photosynthesis and, over time, may limit the availability of carbohydrates necessary for further growth, including root extension (Hughes *et al.* 1997; Martinez-Vilalta *et al*. 2002). Additionally, plants can suffer irreversible xylem cavitation if water deficits become too great (Zimmermann 1983; Sperry *et al.* 1988). To avoid this, roots of phreatophytic plants need to elongate in response to the declining water table, to ensure plants maintain access to a sufficient water supply. Observation of stomatal conductance, transpiration and photosynthetic rate can indicate if plants have access to sufficient water. Phreatophytic plants may be considered to have responded sufficiently to water table drawdown if there is little change in plant water status. Thus, a functional connection to the capillary fringe may be inferred from measurements of plant water status, such as shoot water potential, transpiration and gas exchange.

The current study investigates the response of phreatophytic *Banksia* to three different rates of water table decline. It was expected that phreatophytic *Banksia* would demonstrate a root elongation response to a declining water table. Based on previous observation of the rate of root elongation by phreatophytic *Banksia* (Chapter 5), as well as other phreatophytic species (Krancjec *et al.* 1998; Stave *et al*. 2005), it is also expected that these species are able to maintain a functional connection with a rapidly decreasing water table. Differences in plant response between phreatophytic *Banksia* species that have differing distribution along an ecohydrological gradient, and associated physiological traits were also investigated. It was hypothesised that a more xeric species, such as *B. attenuata*, would exhibit a stronger root elongation response than mesic species, like *B. ilicifolia* and *B. littoralis.*

Materials and Methods

Seed germination and plant establishment

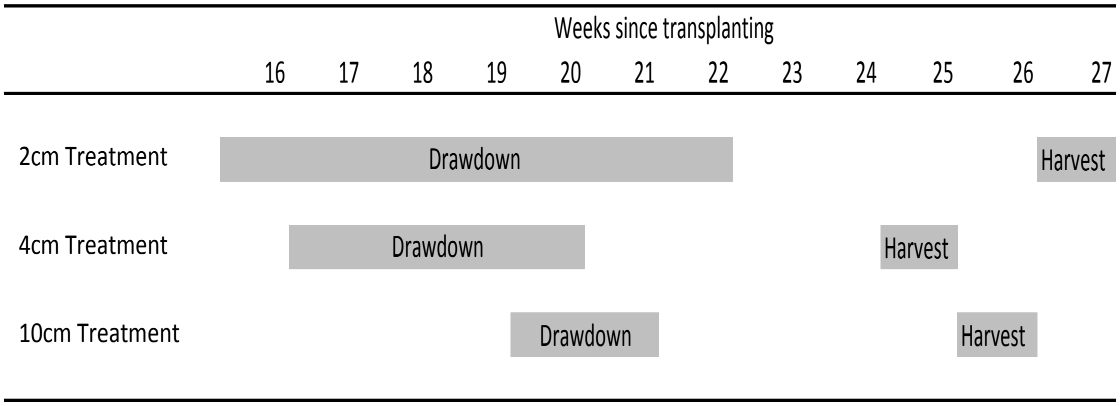
Three phreatophytic *Banksia* species were selected for study. *Banksia attenuata* is considered a more xeric phreatophyte, with a wide distribution across the ecohydrological landscape and is relatively more resistant to xylem cavitation. In contrast, the distribution of *B. littoralis* is restricted to mesic environments with a shallow depth to groundwater and the species is more susceptible to xylem cavitation. *Banksia ilicifolia* represents an intermediate species, with a wider distribution than *B. littoralis*, but with a distribution still limited to habitats where groundwater is within the maximum rooting depth of the species (Canham *et al*. 2009). The experiment took place from September 2008 to May 2009. Seeds were germinated in September 2008, with a total of 200 seeds sown to get 36 healthy seedlings for experimentation. Seeds were sown in pots filled with the same sand substrate used in the rhizopods (for soil water-holding capacity analysis see Chapter 5). Seedlings were transplanted in the beginning of November, eight weeks after sowing. The healthiest seedlings, with cotyledons still intact and no more than two fully extended leaves were selected and transplanted. The soil was prepared by adding slow release fertiliser (Osmocote© Native Plants) and wetting the soil profile to field capacity. Seedlings were again fertilised at three and six weeks after transplanting, and watered (200 ml per growing tube) every second day for eight weeks, to ensure root contact with the water table, which was maintained at 1 m below soil depth. After this period, watering from the top ceased and the unsaturated part of the profile dried out, with the water table remaining as the only plant water source. Plants were left to establish at the water table until the commencement of drawdown treatments.

Drawdown rates and sampling design

Plants were grown in rhizopods (Chapter 5) to allow for the manipulation of the water table at different rates. Tubes were filled with a medium to coarse white sand, representative of that found on the Bassendean Dune System, and water table levels were controlled and monitored using reservoirs. Three different drawdown rates were selected to investigate the maximum rate of water table drawdown tolerable to phreatophytic *Banksia*. Chapter 5 indicates that the study species do not extend their roots into the saturated zone; therefore, prior to drawdown, root distribution is assumed to be limited to the part of the soil profile above the water table and including the capillary fringe. Rhizopods require a large amount of glasshouse space, which was a limiting factor for the number of replicates that were feasible. To allow for three drawdown rates to be investigated, a control of 0 cm day^{-1} rate was not used, as it was assumed that root elongation would not occur beyond the water table. The previous study (Chapter 5) shows that recently germinated *B. attenuata* seedlings had a maximum root elongation rate of 3.6 cm day^{-1} and an average rate of 1.8 cm day^{-1}. It was thus assumed that seedlings would be able to match water table decline treatments of 2 cm day^{-1} and 4 cm day^{-1}, but would become disconnected at 10 cm day^{-1}. There were seven replicates of each species and each treatment for plant harvesting, and three replicates per species per treatment for soil moisture sampling.

The start of each treatment was staggered so that all would finish within three weeks of each other. This was to ensure the plants were all of a similar age at the time of harvesting, to try and limit differences in biomass due to plant age. The water table started at 100 cm and was drawn down by 80 cm to 180 cm below the soil surface. The 2 cm day^{-1} treatment started 16 weeks after transplanting and the drawdown took 40 days. The 4 cm day^{-1} treatment started 17 weeks after transplanting and lasted 20 days, and the 10 cm day^{-1} treatment started 20 weeks after transplanting and took eight days (Table 6.1). Seedlings were left in the rhizopods for a further 30 days to allow plants to adjust to the new water table depth. Water table levels were lowered using a tap at the bottom of the reservoir and drawdown was regulated in a step-wise manner on a daily basis. Glasshouse temperatures were controlled, generally ranging between 18 °C and 31 °C. Temperatures were highest during the establishment phase, when seedlings were being watered every second day. Maximum temperatures during this period averaged approximately 31 °C. This period was during the hottest part of the year (November to March). After watering had finished and plants were establishing at the water table, glasshouse temperatures ranged between 18 °C and 25 °C. Relative humidity ranged between 20% and 90% and was approximately 50% on average.

Table 6.1. Timing of drawdown treatments and plant harvesting.



Plant water relations

Gas exchange was measured to investigate plant response to the declining water table using an infra-red gas analyser (IRGA; Model Li 6400, Li-cor Inc., Lincoln Nebraska). Measurements were recorded at time 0 (i.e. the day that water table decline commenced), then every 10 days until plants were harvested. Transpiration, stomatal conductance and photosynthetic rates were recorded within a two hour period over the middle of the day. PPFD was controlled at 1400 µmol m^{-2}, CO_2 at 385 µmol mol^{-1} and chamber temperature was set at 25 °C. Prior to harvesting, predawn plant water potentials were determined using a Scholander-type pressure chamber as a measure of hydraulic connection to the capillary fringe (Model 3005, PMS instruments, Oregon, USA). Seedlings were small enough to fit into the chamber; therefore, water potentials were determined using the whole plant and were taken in the hour before sunrise.

Plant harvesting

After predawn water potentials had been determined, shoots were placed into plastic bags and taken to the laboratory. Leaves were removed and leaf area was determined using an area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK). Leaves and shoots were then placed into paper bags and dried. In order to sample plant roots, the water table was drained completely from the rhizopods. Tubes were cut into 20 cm sections containing plant roots and soil, which were bagged and transported to the laboratory. Plant roots were sorted and cleaned, then dried, along with the shoots, at 50 °C until a constant weight was reached (approx. 48 hours). Following this, they were weighed, thus determining root weights for 20 cm increments. Total root weight, shoot weight and root to shoot ratios were all determined.

Soil sampling

Soil was sampled on three occasions for each treatment; the day that drawdown treatments began, the day that drawdown ceased and the day before plants were harvested, with one replicate per species (except for *B. attenuata* at drawdown end for the 2 cm day^{-1} treatment as there were not enough replicates). Holes 25 mm in diameter were drilled into the side of the tube and a soil core taken, which was then placed into a plastic jar and sealed tightly. This was repeated along the tube every 5 cm. Gravimetric water content was then determined. Wet weight of samples was recorded and samples placed in the oven at 110 °C until a constant weight was reached (approx. 48 hours). Dry sample weight was recorded and gravimetric water content was determined as thus;

$$\theta_{dw} = \frac{weight\ of\ wet\ soil}{weight\ of\ dry\ soil}$$

Bulk density in the tubes had been previously determined as 1.474g cm^{-3}, which was used to determine volumetric water content:

$$VWC\ (\%) = (BD \times Gravimetric\ Water) \times 100$$

Statistical analysis

Two-way analysis of variance (ANOVA) was used to statistically analyse total root dry weights, total shoot dry weight and leaf area. A square-root transformation was used on non-normally distributed data. Where significant differences were detected, a Tukey *post hoc* test was used. Root:shoot ratios and predawn pressure potentials were not normally distributed and could not be transformed to meet the assumptions required for ANOVA. These parameters were therefore analysed using non-parametric Kruskal Wallis and Mann-Whitney-U tests. The relationship between leaf area and predawn xylem pressure potential was examined using a Spearman's rank order correlation. All analyses were carried out using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Soil water availability

The sandy soil has limited water-holding capacity, with soil moisture in the capillary fringe and saturated zone rapidly depleted as water table drawdown occurs. This is evident in the similarities in volumetric water content between the treatments at the end of drawdown (Fig. 6.1). At the end of drawdown, the 2 cm day^{-1} treatment had similar volumetric water content in the top 120 cm of the soil profile as the 4 cm day^{-1} and 10 cm day^{-1} treatments. This is despite the 2 cm day^{-1} drawdown taking 40 days to complete, while the 10 cm day^{-1} treatment took just eight days. However, the rate of water table drawdown was more important in determining the volume of water available in the vadose zone at any given time. After ten days of drawdown treatment, there was approximately 330 ± 34 ml of water in the top 100 cm of the soil profile for the 2 cm day^{-1} treatment compared with 246 ± 26 and 35 ± 10 ml for the 4 and 10 cm day^{-1} treatments respectively (Fig. 6.2).

Plant response to water drawdown

As the soil water store decreased, transpiration and photosynthetic rates decreased (Fig. 6.2). Photosynthetic rates were generally less than 5 µmol m^{-2} s^{-1} when total volumetric soil water in the top 1 m was reduced to less than 150 ml. Transpiration rates reduced similarly in response to decreased water availability, as they declined to less than 2 mmol m^{-2} s^{-1} when total volumetric soil water in the top 1 m was reduced to less than 150 ml. Over the first 20 days, photosynthetic rates declined slowest for the 2 cm day^{-1} treatment for all three species (Fig. 6.3). However, at 30 days there was a small increase in the photosynthetic rate for the 4 cm day^{-1} treatment.

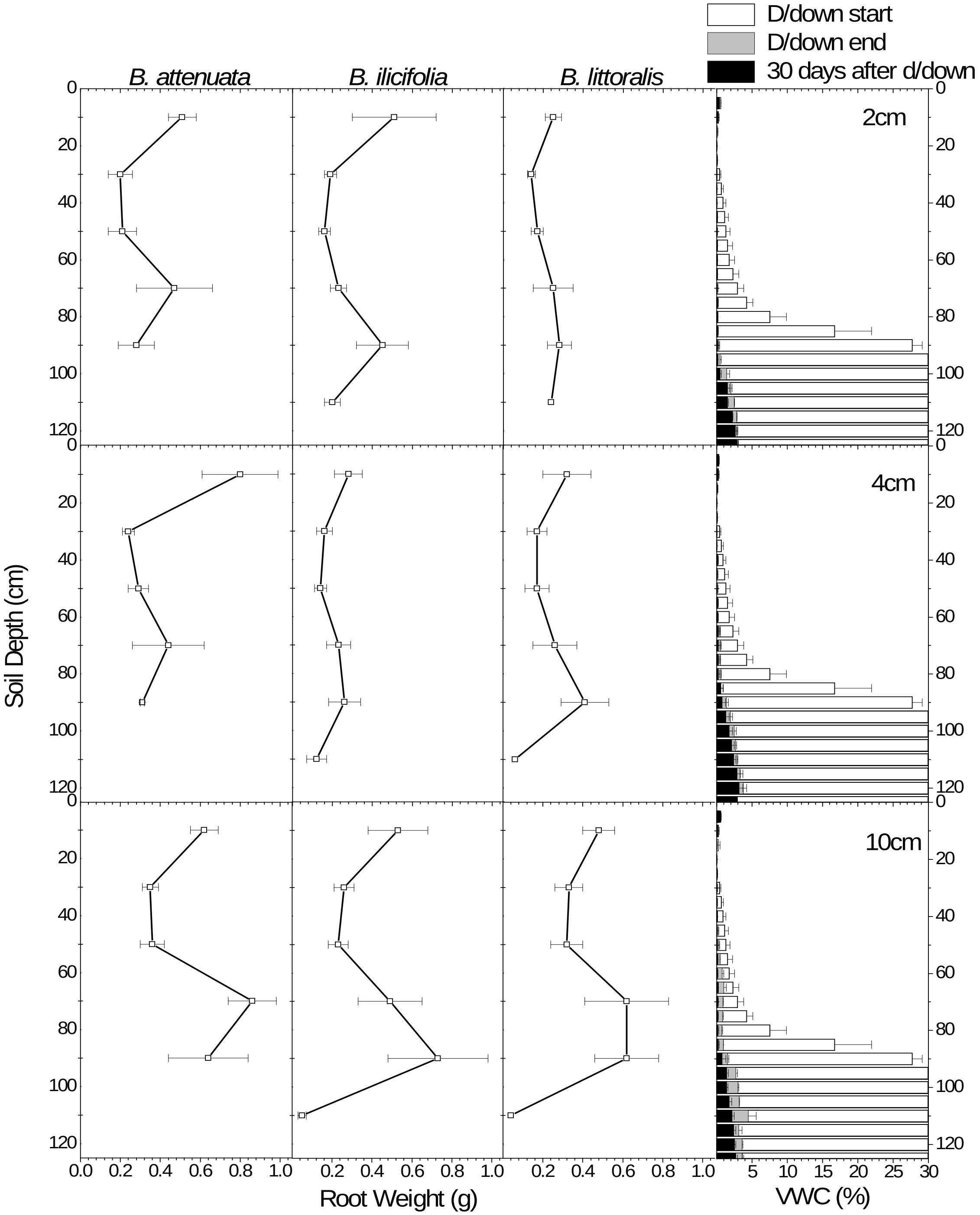


Figure 6.1. Rhizopod profiles. Line graphs depict root dry weights at 20 cm increments for *Banksia attenuata* (n= 4 for 2 cm day^{-1} and 4 cm day^{-1} treatments, 7 for 10 cm day^{-1}), *Banksia ilicifolia* (n = 6) and *Banksia littoralis* (n = 5 for 2 cm day^{-1} treatment and 6 for the 4 cm day^{-1} and 10 cm day^{-1}) harvested 30 days after drawdown treatments at differing rates. Values are mean +/- 1SE (n=7). Bar graphs show changes in volumetric water content (VWC) before drawdown start, at drawdown end and 30 days after drawdown finished.

By 30 days after the start of drawdown, all treatments and all species appear to have been disconnected from the water table/capillary fringe, with very low photosynthetic rates observed. Stomatal conductance follows a similar trend, with the gradient in decline being steepest for the 10 cm day^{-1} drawdown treatment. The 2 cm day^{-1} treatment appeared to take the longest to decline in stomatal conductance. *Banksia attenuata* showed a faster rate of decline in photosynthetic rate compared with the other two study species, with a sharp drop observed for both the 4 and 10 cm day^{-1} treatments, taking 10 days to reach less than 6 µmol m^{-2} s^{-1}. The decline was slower for the 2 cm day^{-1} treatment, taking 30 days to reach a similar value.

There was limited evidence of a root elongation response following the declining water table, with no roots observed beyond 120 cm below the soil surface. Root elongation did not appear to initiate in response to the change in water availability for *B. attenuata*. This can be seen by roots only being found down to the 80 to 100 cm portion of the soil profile for all drawdown rate treatments, which corresponds with the position of the capillary fringe prior to drawdown (Fig. 6.1). In contrast, the mesic phreatophytic species, *B. ilicifolia* and *B. littoralis*, showed some response to water table decline for all three treatments. For these more mesic species, roots extended beyond the pre-drawdown capillary zone, with roots recorded at 100-120 cm deep for all treatments.

Total root weight was significantly higher for the three study species at the 10 cm day^{-1} treatment (d.f. = 2, f. = 4.997 p = 0.011), with the largest root mass recorded for *B. attenuata* (2.73 ± 0.37 g. Fig. 6.4). This contrasts with the root weight for the same species with the 2 cm day^{-1} treatment (1.67 ± 0.46 g). Root mass was generally greatest at 80-100 cm, particularly for the 10 cm day^{-1} treatments, once again corresponding with the presence of the capillary fringe during the three month establishment phase (Fig. 6.1).

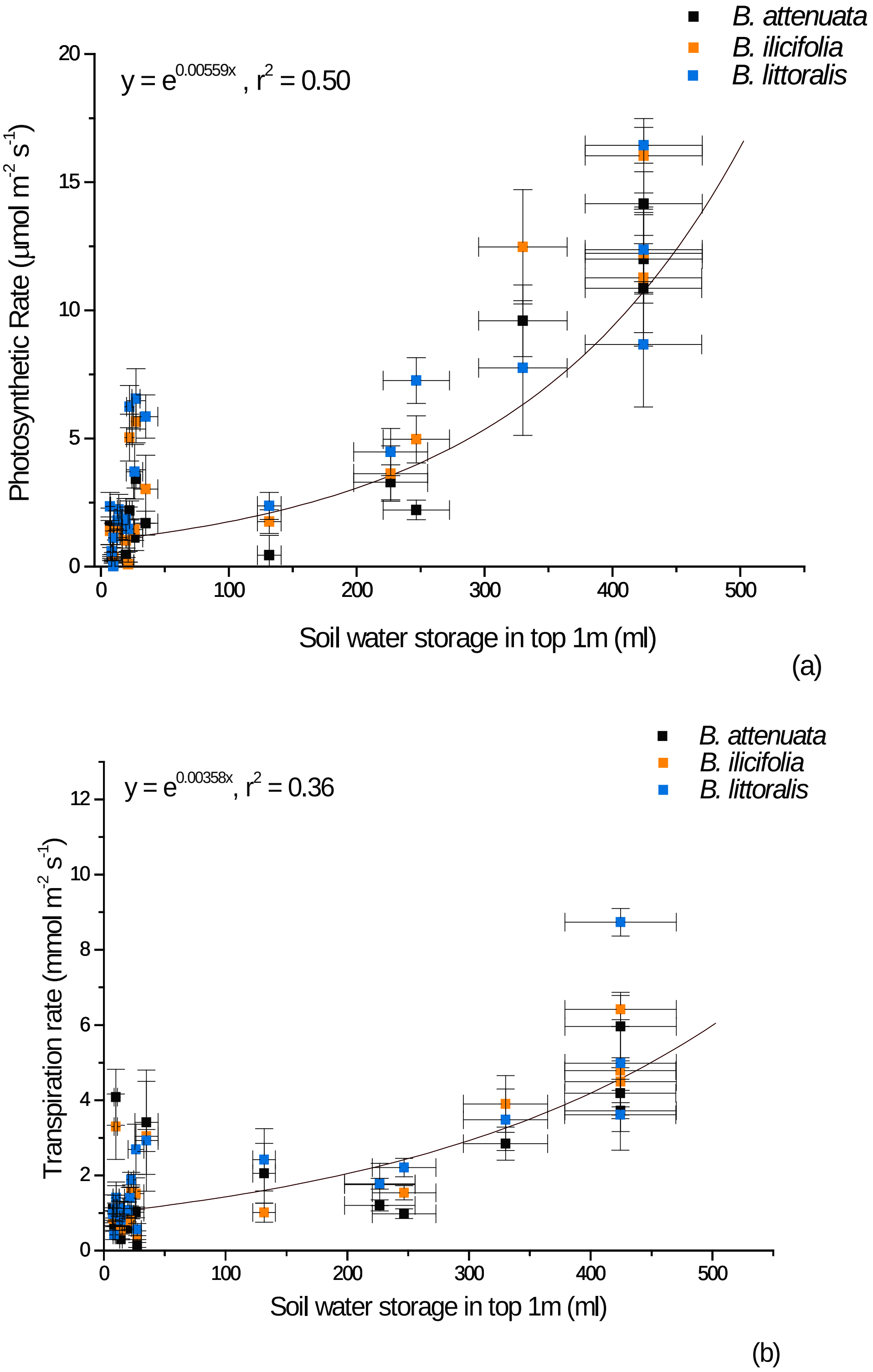


Figure 6.2. Relationship between soil water storage in the top 1 m of the rhizopod and photosynthetic (a) and transpiration (b) rates. *n* = 399.

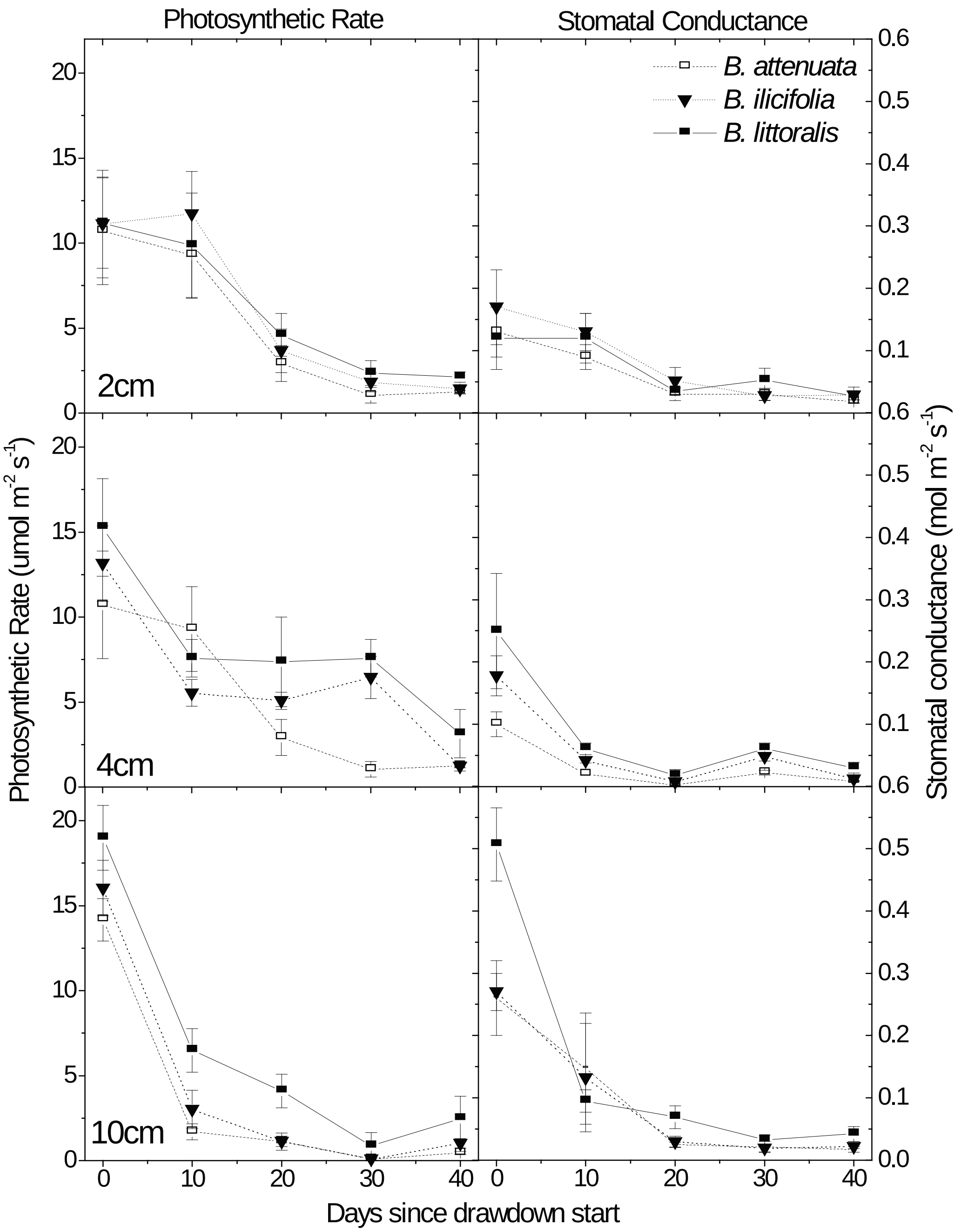


Figure 6.3. Photosynthetic rate and stomatal conductance (mean +/-1 SE) for *Banksia attenuata* (n= 4 for 2 cm day^{-1} and 4 cm day^{-1} treatments, 7 for 10 cm day^{-1}), *Banksia ilicifolia* (n = 6) and *Banksia littoralis* (n = 5 for 2 cm day^{-1} treatment and 6 for the 4 cm day^{-1} and 10 cm day^{-1}) seedlings exposed to different water table drawdown rates.

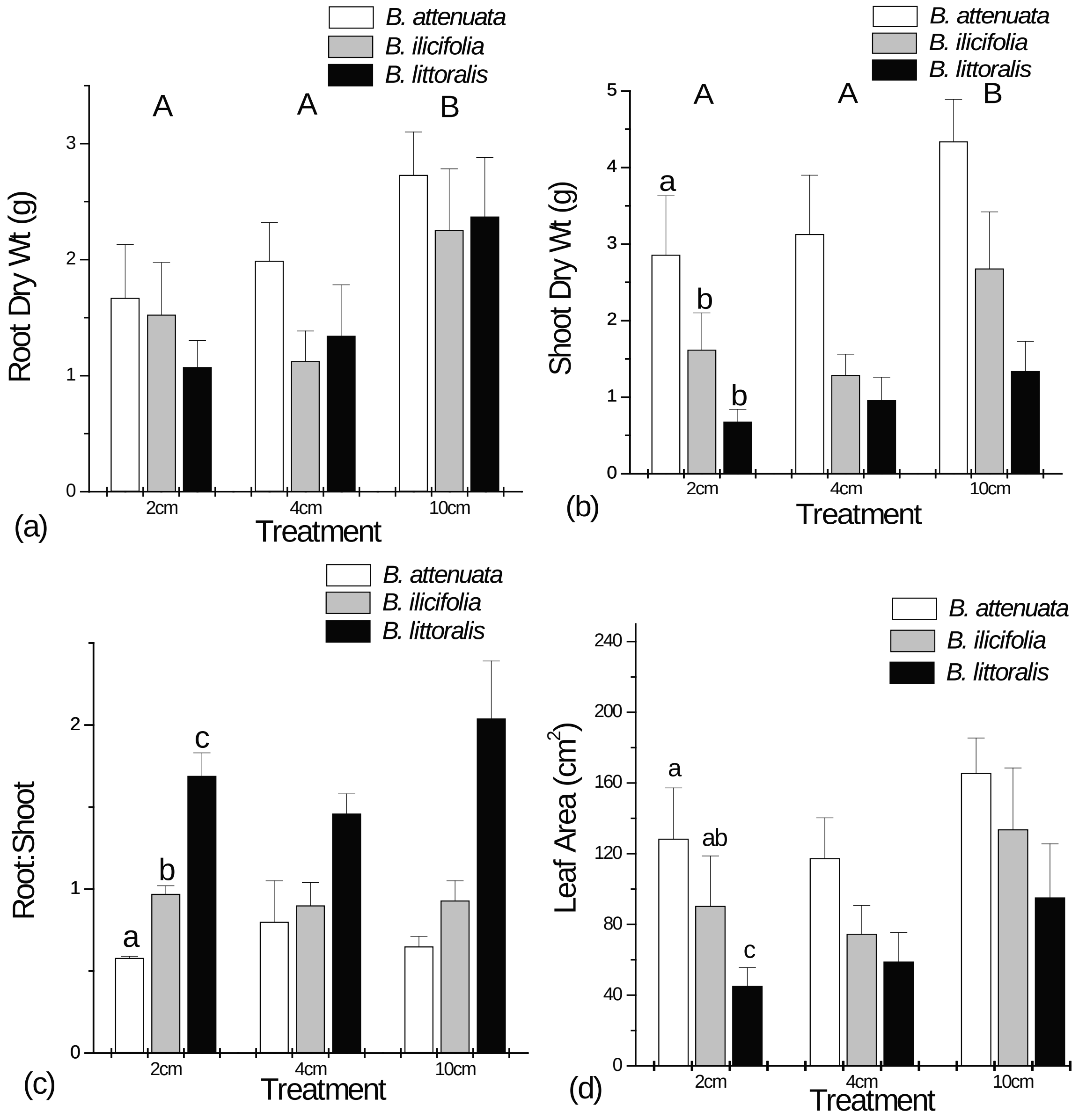


Figure 6.4. Mean (± 1 S.E) values for (a) total root dry weight, (b) shoot dry weight, (c) root:shoot ratios, and (d) leaf area for *Banksia attenuata* (n= 4 for 2 cm day^{-1} and 4 cm day^{-1} treatments, 7 for 10 cm day^{-1}), *Banksia ilicifolia* (n = 6) and *Banksia littoralis* (n = 5 for 2 cm day^{-1} treatment and 6 for the 4 cm day^{-1} and 10 cm day^{-1}) seedlings 30 days after cessation of water table drawdown at different rates. Different capital letters indicate significant differences between treatments and different lowercase letters indicate significant differences between species for; (a) according to ANOVA, d.f. = 2, f. = 4.997 p = 0.011; (b).Differing capital letters indicate significant differences between treatments (ANOVA, d.f. = 2, f = 3.513, p = 0.039). Differing lowercase letters indicate significant differences between species (ANOVA, d.f. = 2, f = 15.44, p = <0.000); (c) Kruskal-Wallis, d.f. = 2, χ^2 = 33.058, sig. = <0.000; and (d) ANOVA, d.f. = 2, f = 6.341, p = 0.004.

Shoot weights differed significantly both at species and treatment levels (Fig. 6.4a). For example, for the 2 cm day^{-1} treatment shoot weight for the more xeric phreatophyte, *B. attenuata*, was larger than the two mesic phreatophyte species (2.86 ± 0.77 g, c. f, 1.62 ± 0.48 g for *B. ilicifolia* and 0.68 ± 0.16 g for *B. littoralis*). As with root weight, shoot weights were also found to be larger for the 10 cm day^{-1} treatment (Fig. 6.4b). Root:shoot ratios (R:S) differed between the three species (Fig. 6.4c). The more xeric phreatophyte, *B. attenuata*, was found to have the lowest R:S and the most mesic species, *B. littoralis*, had the highest (eg. 0.58 ± 0.01 c.f. 1.69 ± 0.14 for the 2 cm day^{-1} treatment). Root:shoot ratios were very similar across the treatments for each of the species.

Leaf area was largest for *B. attenuata*, corresponding with the larger shoot weights observed for this species, and leaf area was significantly lower for the mesic phreatophyte, *B. littoralis* (Fig. 6.4d). Predawn water potentials were significantly lower for *B. attenuata* (Fig. 6.5a*).* This reflects the higher number of mortalities of this species, with three out of the seven *B. attenuata* replicates dying with the 2 and 4 cm day^{-1} treatments (Fig. 6.5b), although there were no mortalities for *B. attenuata* with the 10cm day^{-1} treatment, however xylem pressure potentials were low (-2.23 ± 0.44 MPa) indicating plants were water-stressed. There was a significant correlation between final leaf area and predawn xylem pressure potentials ($r = 0.636$, $p < 0.001$, $n=49$), with individuals with larger leaves tending to be more water-stressed by the end of the experiment. *Banksia attenuata*, which had a larger leaf area, tended to have lower xylem pressure potentials than the other two species, although there is a large spread in the data (Fig. 6.6).

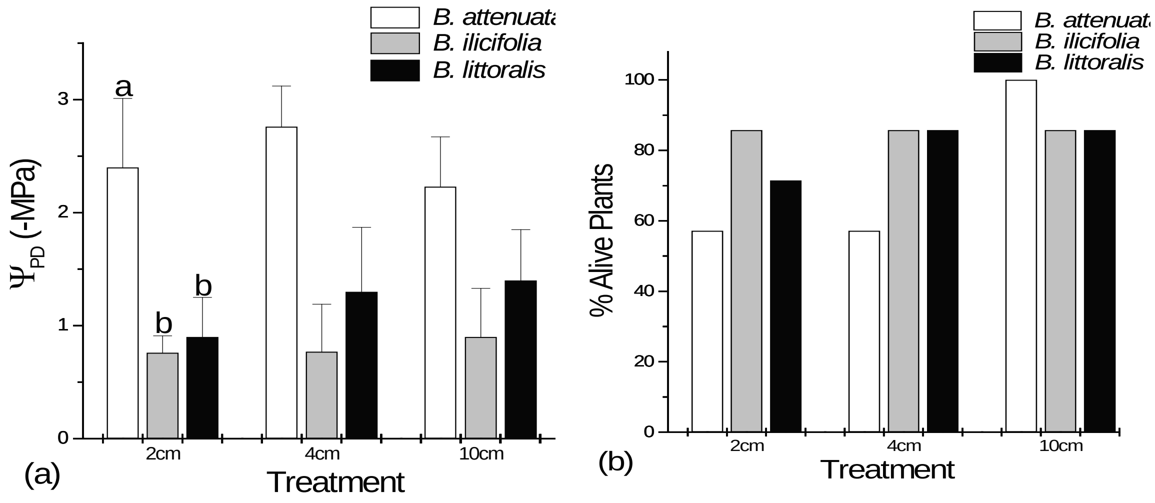


Figure 6.5. Mean (± 1 S.E) values of (a) predawn xylem pressure potential (Ψ_{PD}) for *Banksia attenuata* (n= 4 for 2 cm day^{-1} and 4 cm day^{-1} treatments, 7 for 10 cm day^{-1}), *Banksia ilicifolia* (n = 6) and *Banksia littoralis* (n = 5 for 2 cm day^{-1} treatment and 6 for the 4 cm day^{-1} and 10 cm day^{-1}) seedlings 30 days after cessation of water table drawdown at different rates. Different lowercase letters indicate significant differences between species (Kruskal-Wallis, d.f. = 2, χ^2 = 17.024, p = <0.000). Seedling mortality (b) expressed as a percentage of replicates alive at experiment end (n = 7).

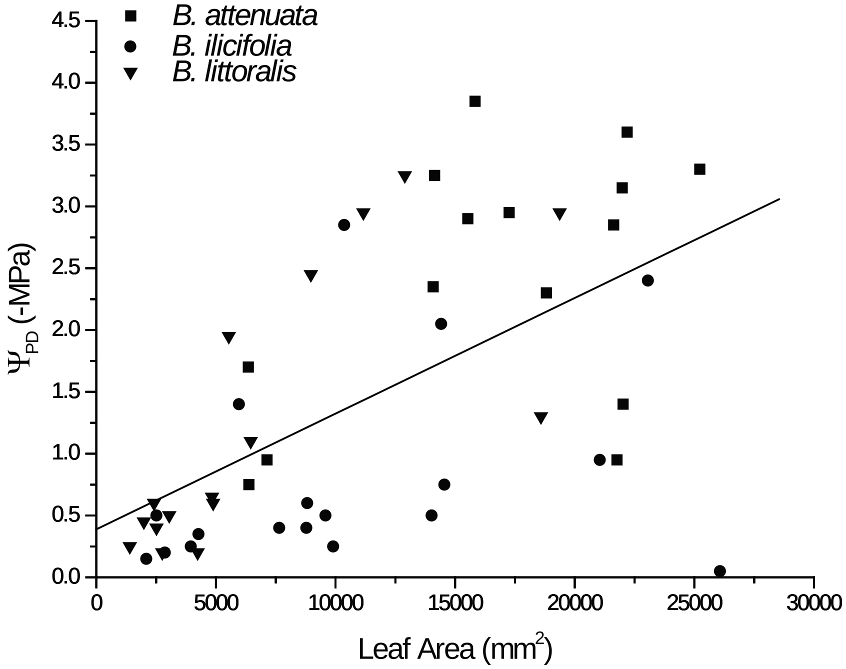


Figure 6.6. Leaf area and pre-dawn plant water potentials (Ψ_{PD}) for all treatments. y = -0.0000935x + 0.388. Spearman's rank order correlation results are: r (49) = 0.636, p < 0.000.

Discussion

It was expected that the roots of phreatophytic *Banksia* would demonstrate root growth in response to a declining water table. However, despite plant-available water being present in the soil profile, there was very limited root extension following water table decline. In addition to a poor root elongation response, seedlings demonstrated a rapid drought response to water table decline, quickly reducing stomatal conductance, transpiration and photosynthesis (Fig. 6.2). It is likely that the phreatophytic *Banksia* in this current study experienced hydraulic failure, due to a rapid disconnection from the water table. This is indicated by the rapid reduction in transpiration and gas exchange, as well as the low predawn xylem pressure potential observed during the experiment. In the event of a sudden decrease in water availability, such as that caused by a rapidly declining water table, it is likely that hydraulic failure is the primary cause of plant death (Sparks & Black 1999; McDowell *et al.* 2008). If the water potential becomes more negative than what can be sustained by the plant, runaway embolisms can occur, leading to the complete desiccation of the plant and subsequently to cellular death (McDowell *et al*. 2008). If onset of a water-deficit response is less acute but more prolonged, plants may become ‘carbon starved’ (McDowell *et al*. 2008). In this scenario, plants respond to water stress by closing their stomata to reduce transpirational loss; however, if water stress is chronic and plants do not photosynthesise sufficiently, metabolic demands may exceed available carbohydrates (Martínez-Vilalta *et al*. 2002). This will impact on the capacity of the plant to invest in further root growth to find soil moisture, thereby exacerbating the problem. Thus, if roots become disconnected from the water table/capillary fringe, plants become water-stressed and unable to grow either roots or shoots. Similar responses to water table decline have been observed for poplars, with reports that although root elongation was stimulated by a water table decline of 4 cm day^{-1}, plants became water-stressed and were unable to extend their roots when water table drawdown occurred at a rate of 10 cm day^{-1} (Kranjcec *et al.* 1998). It is likely that the lack of root elongation response to a declining water table by the phreatophytic *Banksia* study species was due to plants being unable to meet their water requirements once the water table fell below the rooting depth of the plants.

It was hypothesised that the roots of the phreatophytic *Banksia* study species have the capacity to elongate roots at a rapid rate of water table decline, specifically 2 or 4 cm day^{-1}, based on the root elongation rates observed in Chapter 5, where it is observed that *B. attenuata* had a maximum root elongation rate of 3.7 cm day^{-1}. The notion that phreatophyte roots are able to extend at such a rate is supported by Stave *et al*. (2005), who found that *Faidherbia albida* seedlings survived drawdown rates of 5 cm day^{-1}. Similarly, Kranjcec *et al*. (1998) reported that *Populus balsamifera* is able to maintain contact with a water table declining at 4 cm day^{-1}. The same study also indicated a root growth response following a 10 cm day^{-1} water table decline, although plant water relations data indicated that this rate was too rapid for roots to maintain water table contact. Despite there being some evidence indicating that phreatophytes are able to survive water table declines of more than 4 cm day^{-1}, all the rates used in this current experiment (2, 4 and 10 cm day^{-1}) appear to be too fast for the phreatophytic *Banksia* seedlings to survive. It has been shown previously that a drawdown rate of 1 cm day^{-1} best matches potential for root elongation for a number of phreatophytic species (Mahoney & Rood 1991; Krancjec *et al.* 1998; Horton & Clark 2001). This study has shown a conservative response amongst juvenile *Banksia* plants to water table declines with the drawdown rates tested (more than 2 cm day^{-1}) all leading to plant death. Thus, phreatophytic *Banksia* are likely to elongate their roots to maintain a functional connection with a water table declining at a rate of less than 2 cm day^{-1}.

It was hypothesised that the more xeric phreatophyte, *B. attenuata,* would exhibit a stronger root elongation response to the rapidly declining water table, based on previous observations of rapid root elongation rates. However, the species did not initiate root growth in response to the declining water table, with roots not extending beyond the pre-drawdown capillary fringe. As a consequence of this functional disconnection from the capillary fringe, *B. attenuata* also had the most negative predawn water potentials and the highest mortality rate of the three study species. The lack of root growth response may be due to the rapid reduction in the photosynthetic rate by *B. attenuata* in contrast to the other two study species. A drought response to limit photosynthesis and stomatal conductance to conserve water loss will further limit plant growth through reduced carbohydrates available for growth (Pedersen 1998).

Thus, the lack of root growth response may have been due, in part, to the feedback between water deficits inducing a leaf-level drought response, exacerbating the problem of reduced water uptake as roots are unable to grow to follow the declining water table.

In contrast, the two more mesic phreatophyte study species, *B. littoralis* and *B. ilicifolia*, both showed a root elongation response to the declining water table. Both species extended their roots beyond the depth of the capillary fringe at the start of the experiment, with roots recorded in the 100-120 cm zone across all treatments. The expectation that the more mesic phreatophytes would have a slower root elongation response to water table drawdown was based on previously observed root elongation rates (Chapter 5). However, an alternative hypothesis could suggest that a root elongation response to water table drawdown is more beneficial for species dependent on access to groundwater, such as the two mesic study species. Both *B. ilicifolia* and *B. littoralis* have been shown previously to be more vulnerable to drought stress than the xeric phreatophyte (Canham *et al*. 2009). They are species that require contact with the water table and capillary fringe in order to meet high water requirements and to avoid xylem cavitation. Therefore, root elongation in response to a water deficit may be greater for the more mesic phreatophyte species, in contrast to the more xeric species.

In addition to interspecific differences in root length and the subsequent disconnection from the water table, species also differed in overall plant size, particularly shoot weights. Root:shoot ratios (R:S) were shown to differ between the species, with *B. attenuata* shown to have the smallest R:S, *B. littoralis* the largest and *B. ilicifolia* intermediary, which follows the pattern in the water requirements of the three species. The R:S ratios in the study are similar to those in Chapter 5, with *B. littoralis* having the highest proportion of roots to shoots in both studies. However, these differences are more pronounced in this current study, which may be due to the mesic phreatophytes increasing their roots in proportion to shoot mass as the water table declined. In contrast, *B. attenuata* shows considerable shoot growth during the establishment phase, which may be attributed to the large seed size and larger seedlings of this species, as demonstrated in Chapter 5. As *B. attenuata* did not extend its roots during the drawdown, its R:S did not differ significantly compared to pre-drawdown. This may account for the low proportion of root mass to shoot mass compared to the other two study species. In addition, the larger shoot mass and leaf area of *B. attenuata* suggest that individuals of this species had a higher transpirational demand than the two mesic phreatophyte species. This may help explain why the more xeric phreatophyte was more water-stressed than the mesic phreatophyte species, with more water required to meet plant-water demands.

There are some methodological considerations that should be taken into account in the interpretation of results. The majority of previous rhizopod studies focussed on riparian species found in very shallow groundwater habitats, and the methodology was to plant either seeds or germinants with cotyledons still intact into rhizopods with the water table just below the soil surface (Mahoney & Rood 1991; Stave *et a*l. 2005; Gonzalez *et al*. 2010). For example, Stave *et al.* (2005) planted six day old seedlings in rhizopods with a water table established 5 cm below the soil surface, corresponding with the mean rooting depth of the seedlings. Therefore, seedlings were in contact with the water table almost immediately after germination. This is beneficial for interpreting how seedlings which grow in habitats with a very shallow water table may establish contact with a declining water table, but may not represent the post-cotyledon response of terrestrial species to drawdown of deeper water tables. The species in this current study invest in rapid root development to reach a water table (Chapter 5). Once at the water table, individual plants become dependent upon access to the reliable water source, as indicated by year-round groundwater usage (Zencich *et al.* 2002) and plasticity in vulnerability to water stress (Canham *et al.* 2009). The plants in this current study had exhausted their cotyledon reserves which may have limited the capacity for rapid root elongation. It may be hypothesised that juvenile plants do not have sufficient nutrient reserves to support rapid root development to meet increased transpirational demands. This phenomenon has previously been reported for glasshouse experiments by Sparks and Black (1999), who attributed it to seedling leaf area and thus transpirational demand being particularly high under glasshouse conditions compared to the rooting volume available to pot-grown plants. Thus, the age of the seedlings used may account for the lack of root elongation response to water table drawdown, with older plants having fewer resources and higher transpirational demands than recently germinated seedlings.

There may also be differences in plant response between rhizopod glasshouse studies and adult trees *in situ*, particularly in relation to the water sources available to plants and the rates of water table decline that are likely to occur. The rates of the decline used in this study were designed to investigate the maximum rate of water table decline that phreatophytic *Banksia* can survive. However, it is unlikely that adult plants in the field would be exposed to such rapid rates of water table decline. Seasonal changes in water table levels at study sites in Whiteman Park are approximately 0.38 cm day^{-1}; a slow rate that plants are able to accommodate for the redistribution of their roots (Chapter 3). However, altered hydrological regimes influenced by factors such as reduced rainfall recharge and groundwater abstraction, can impact water table depths. *In situ* winter groundwater drawdown experiments have induced water table decline in winter at rates of 1.76 cm day^{-1} over a period of 34 days in 2009 and 1.76 cm day^{-1} over 77 days in 2010 (Pers. Obsv.). *Banksia* at the sites exposed to this rate of drawdown did not indicate any signs of water stress, as determined by predawn shoot water potential associated with the declining water table (Froend *et al*. 2010). This suggests that there is some ability for *Banksia* to tolerate groundwater drawdown at relatively rapid rates, although it should be noted these water table declines occurred over winter months when vadic water was also available to plants. Adult plants with extensive root systems can access a large volume of both vadic and capillary fringe water, and may respond differently from seedlings in a glasshouse. The design of the glasshouse studies restricts horizontal root growth to the diameter of the growth tubes, limiting root volume. This influences the availability of water sources to seedlings, particularly as the study species have dimorphic root systems, which extend considerable distances horizontally from the plant (Lamont & Bergl 1991; Pate *et al.* 1995). Thus, the rhizopod design limits rooting volume, which may limit comparisons between plant response to water drawdown in rhizopods and in the field.

Conclusion

The response of phreatophytic *Banksia* to rapid water table decline depends on the availability of other water sources and the rate of water table decline. Despite previously demonstrating the capacity for rapid root elongation rates of up to 3.7 cm day^{-1}, plants in this current water table drawdown experiment overall demonstrated little root elongation response. Soon after water table drawdown had started, transpiration rates decreased, indicating a functional disconnection from the water table. The lack of root elongation may be due to the strong drought response, with plants reducing stomatal conductance and photosynthesis. Contrary to the initial hypothesis, based on previous observation of root elongation rates, *B. attenuata* was the least responsive to the declining water table. *Banksia attenuata* may have a stronger drought response, limiting stomatal conductance and photosynthesis, which limits the capacity for further root growth. In contrast, the more mesic phreatophyte species, *B. ilicifolia* and *B. littoralis,* showed some root elongation response to the declining water table. There may be greater selection pressure for these species to maintain connection to groundwater, due to higher water demands and increased vulnerability to water stress.

Chapter 7

General Discussion

For phreatophytic plants to persist in a given habitat they need to be able to adapt to the environmental conditions they are exposed to, including the seasonal hydrological cycle. For the phreatophytic *Banksia* study species at a site on the Gnangara Mound it was found that root growth by phreatophytic *Banksia* at the capillary fringe could occur the whole year round, with no endogenous limitations to root growth at different times of the year (Chapter 3). Roots of phreatophytic *Banksia* were found to redistribute in response to seasonal fluctuations of the water table. The root zone closest to the water table becomes saturated in winter and spring, which limits root growth in that part of the soil profile. This was evident in the seasonal pattern of root activity observed, with a lack of root activity by the study species in the saturated soil profile (Chapter 3). Thus, the two terrestrial phreatophyte study species demonstrated root trimming in response to the rising water table, with negligible root activity observed when this part of the root zone became saturated. However, plants are likely to redistribute roots in response to the rising water table, maintaining functional connection with the capillary fringe, as was demonstrated with uptake of water from the capillary fringe in spring (Chapter 4). When the water table was at its highest level, root activity in the capillary fringe was evident, from both observation of root in-growth and water uptake from the capillary fringe (Chapters 3 & 4). In addition to demonstrating the connection between phreatophytes and a dynamic water table, this supports the concept of phreatophytes accessing water from the unsaturated capillary fringe rather than directly from the water table (Mahoney & Rood 1998).

Following the spring peak of water table levels, the water table declines over summer and autumn, and roots must respond to the change in water table levels if they are to maintain a functional connection with the water table. The rate of water table decline that plants were found to be exposed to, in a normal year, was 3.8 mm day^{-1}. The slowly declining water table leaves behind an unsaturated zone of high moisture content suitable for root growth (Imada *et al*. 2008). When the water table was at its lowest level in autumn, root activity was observed in the previously saturated zone, suggesting that the phreatophyte roots had followed the declining water table. Plant water status measurements, as determined from predawn shoot water potentials provide further evidence of *Banksia* maintaining a connection with groundwater (Chapter 3). Root growth at the capillary fringe and year-round high predawn water potential observed in this study, supports previous observations of phreatophytic *Banksia* maintaining year-round functional connection with the water table (Zencich *et al*. 2002). This connection with the water table is enabled by roots responding to the seasonal changes in water table levels. Thus, it is likely that the phreatophytic *Banksia* study species have the capacity to respond to changes in water availability, including elongating roots to maintain a functional connection with a seasonal decline of the water table.

Although phreatophytic plants are able to adjust to the seasonal changes in water table levels, water table declines can also occur at faster rates, impacted by reduced rainfall recharge and groundwater abstraction. For phreatophytic plants to avoid detrimental impacts from groundwater decline, root elongation must be adequate to maintain functional contact with the water table. The rate of water table decline that phreatophytic plants are able to adapt to may be dependent on the plant's capacity for rapid root elongation (Mahoney & Rood 1991; Naumburg *et al*. 2005). Both phreatophytic *Banksia* species have demonstrated a capacity for rapid root elongation, under experimental conditions. *Banksia littoralis* had a maximum root elongation rate of 1.8 cm day^{-1}, and the more xeric phreatophyte, *B. attenuata* demonstrated root elongation at twice this rate, at 3.7 cm day^{-1} (Chapter 5). These rapid rates of root elongation suggest plants have the capacity to maintain contact with a rapidly declining water table. However, when this was tested under glasshouse conditions, seedlings showed limited root elongation response to rapidly declining water tables at rates of more than 2 cm day^{-1} (Chapter 6), which is further discussed below. The rapid rates of root elongation shown in Chapter 5 may be typical of the rapid root development by *Banksia* seedlings in the first year, with roots extending deep into the vadose zone to maximise rooting volume and to access deeper soil water stores (Stone & Kalisz 1991; Rokich *et al*. 2001). In addition, differences in the root elongation rate by the two phreatophytic *Banksia* species may be related, in part, to the ecohydrological habitats in which the species are distributed. It is more beneficial for *B. attenuata* to invest in root growth than *B. littoralis*, as *B. attenuata* is a more xeric phreatophyte with a distribution that includes habitats of deep sands with no available groundwater. *Banksia attenuata* therefore requires an extensive root system exploiting a large volume of soil. Conversely, the narrow distribution of *B. littoralis* means that this species is confined to habitats that afford year-round access to groundwater (Zencich *et al.* 2002).

While the rapid rate of root elongation suggests that plants have the capacity to follow a rapidly receding water table, it does not specifically measure plant response to water table decline. Plant response to a declining water table may be limited, depending on the capacity for plants to continue to meet their water requirements. If water table decline occurs at a rate too fast for root growth to respond, plants are likely to experience water stress. Findings from Chapter 6 suggest that the rate of water table decline the *Banksia* study species are able to survive is likely to be less than 2 cm day^{-1}, as rates greater than this resulted in severe plant water stress. Additionally, the availability of other water sources, particularly vadic water, may impact on plant drought response and the ability for plants to adjust to a declining water table. If water table decline occurs when vadic water availability is high, plants may be able to continue to meet their water requirements. This may allow plants to continue to grow roots, and thus follow the water table. In contrast, if vadic water content is very low phreatophytic plants are likely to be dependent on accessing water from the capillary fringe to be able to meet water requirements. If the water table falls at a rate faster than plants are able to elongate roots to maintain a functional connection with it, plants will no longer be able to meet their water requirements. This can initiate a drought response, with the plant closing stomata to limit water loss, but also limiting photosynthesis and thus the availability of carbohydrates for continued plant growth. This may limit the capacity for further root growth, exacerbating the problem by plants being unable to elongate roots to follow the water table. Plant response to water table decline may therefore be dependent on the rate of water table decline and the availability of other water sources, such as vadic soil moisture.

Consequences of hydrological change and implications for groundwater management

Like many superficial aquifers worldwide, the Gnangara Mound is in decline, and this is likely to continue as the south west of Western Australia continues to experience less rainfall, in addition to abstraction from the aquifer for urban water supply. However, there may be some capacity for continued use of groundwater as a resource. If abstraction is timed to occur when groundwater usage by plants is low, then phreatophytes may be able to adapt to the change in the water table level. In winter, groundwater use by phreatophytes is diminished as water availability in the vadose zone is high, and plants preferentially use this soil moisture (Zencich *et al.* 2002). If the water table were to remain stable or even decline in winter, plants are likely to continue to be able to meet their water requirements from vadic water. However, for plants to continue to meet their water requirements in the following summer months when vadic water availability decreases, plants must maintain a functional connection with the water table. It is likely that roots have the capacity to follow a declining water table in winter, as immediate hydraulic failure is avoided due to plant-water demand being met from the vadic water source. In addition, plant root growth is not limited by season, thus roots should be able to redistribute in response to the change in water table levels in winter. Groundwater abstraction may therefore be scheduled to occur at a time of the year that is more sympathetic with the demands and physiology of phreatophytic plants.

A groundwater abstraction regime that mitigates impacts on phreatophytic *Banksia* needs to account for the rate of water table decline to which plants are able to adjust. The adaptability of phreatophytic plants to a declining water table is dependent on the rate of decline and the capacity for plants to elongate roots at a sufficient rate to maintain functional contact. For phreatophytic *Banksia* that have established in a habitat with a shallow water table, acceptable rates of water table decline are likely to be less than 2 cm day^{-1}. Faster rates are likely to induce hydraulic failure and plant death, although this may be dependent on the availability of other plant-water sources. However, slower rates would allow plants to respond to the change in water availability, and elongate roots to follow the declining water table, as demonstrated in response to normal seasonal groundwater fluctuations (Chapter 3) and from preliminary results of a drawdown trial (Froend *et al.* 2010). Therefore, roots of phreatophytic *Banksia* may be able to adapt to a water table decline at an acceptable rate, which is less than 2 cm day^{-1}. However, there may be a limit to the depth which plants are able to follow a declining water table. The extent to which roots are able to elongate is yet to be demonstrated; however, maximum rooting depths for the *Banksia* study species are thought to be approximately 8m (Farrington *et al*. 1989; Arrowsmith 1992). Further research could identify the limits of root growth for phreatophytic *Banksia*, and the maximum depth to which they are able to maintain contact with a declining water table.

If groundwater decline on the Gnangara Mound continues, it is likely that more mesic species, such as *B. littoralis*, will be replaced by more xeric species, such as *B. attenuata*. Under drier conditions the more xeric phreatophyte is at an advantage, starting from the recruitment stage. The larger seed size of *B. attenuata* enables rapid plant growth (Chapter 5). This includes rapid root growth, both mass and length, with roots rapidly penetrating down to either deep-stored soil moisture or a water table. *Banksia attenuata* seedlings often establish in xeric habitats, where groundwater is unavailable and plants are reliant instead on deep, stored soil moisture (Zencich *et al*. 2002). Seedlings rapidly develop extensive root systems that are quick to penetrate to deeper soil layers, increasing the volume of soil explored for moisture. In contrast, the more mesic phreatophyte, *B. littoralis*, has smaller seedlings, lower overall root mass and a slower rate of root elongation. *Banksia littoralis* are able to persist due to their distribution being restricted to mesic environments. The slower root extension is tolerable as the water table is shallower, thus there is less of an imperative for rapid root growth. However, if the Gnangara Groundwater Mound continues to decline, the recruitment success of mesic species like *B. littoralis* may be impacted. The difference in seed size and the resulting growth rates of seedlings can play a role in the replacement of mesic phreatophyte species with more xeric species in the event of long-term water table decline.

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