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Seedling xylem anatomy of two Banksia species relative to availability of groundwater

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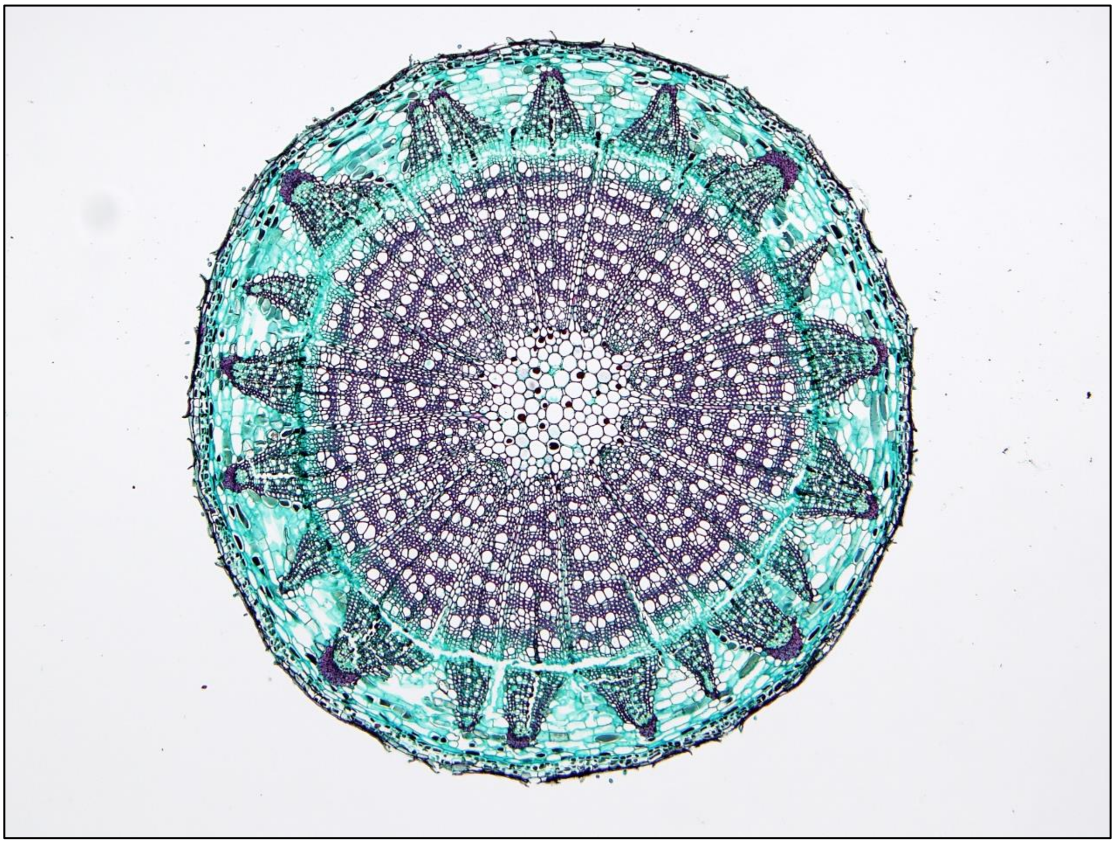
Seedling xylem anatomy of two *Banksia* species relative to availability of groundwater



This thesis is presented in partial fulfilment of the degree of
Master of Science (Biological Sciences)

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School of Science
2019

Abstract

Fluctuating environmental conditions place plants at risk of mortality if they cannot adapt, particularly to warmer temperatures and drier environments. Plants are known to modify their morphology, physiology and anatomy to thrive under these conditions, and xylem anatomy and hydraulic architecture are common traits studied to understand plant adaptations and responses to changeable water availability. However, little is known about the changes to the hydraulic architecture of groundwater dependent plants during their early establishment in water-limited environments. By exposing young seedlings of two *Banksia* species to contrasting groundwater availability treatments in a glasshouse experiment, it was possible to analyse the xylem vessel traits (vessel diameter (Vd), maximum vessel diameter (Dmax), vessel density (Dv) and vessel length (VL)) in root and stem tissue samples using ImageJ software. Analysis of vessel traits identified significant vascular tapering in both *Banksia attenuata and B. littoralis*, i.e. a low density of large diameter vessels found in roots, and a higher density of small diameter vessels found in the stem. Vessel traits of *B. attenuata* and *B. littoralis* seedlings with access to an artificial water table did not differ significantly to those with access to only unsaturated soil. This lack of significant difference was surprising as studies have shown variation in xylem anatomy of plants exposed to contrasting water availabilities, commonly referred to as the hydraulic efficiency and safety trade-off theory. However, the traits did vary between the two species, with significantly larger mean Vd and Dv in *B. attenuata* seedling roots, and significantly higher mean Dv in *B. littoralis* seedling roots. VL also differed, with *B. attenuata* having significantly longer vessels than *B. littoralis*. These differences may relate to the contrasting habitat requirements of the chosen species. *B. attenuata* occurs across varying gradients in depth to groundwater, while *B. littoralis* is found in low-lying swampy areas. This could suggest that *B. attenuata* may be more ‘flexible’ in its xylem development, whereas *B. littoralis* develops a more rigid anatomy, affording protection in case of disconnection from groundwater. The findings from this study provide a better insight into how the xylem anatomy of groundwater-dependent species varies in relation to groundwater availability, and how and why species of the same genus may develop significantly different vessel traits.

Declaration

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

i. *Incorporate, without acknowledgment, any material previously submitted for a degree or diploma in any institution of higher education;*

ii. *contain any material previously published or written by another person except where due reference is made in the text of this thesis; or*

iii. *contain any defamatory material.*

[redacted]

Melissa Karlinski

(June 2019)

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Glossary and Abbreviations

Capillary Fringe	The zone located above the water table where water fills the spaces under a pressure lower than the atmosphere and water is retained in the soil by surface tension
Cavitation	Formation of water vapour in the xylem pathway when water tensions exceed atmospheric pressure
Drought tolerance	A plants ability to continue normal production processes during dry/drought conditions
Embolism	A blockage caused by large gas bubbles in the xylem
Groundwater	Water in the subsurface in the zone of saturation below a water table
Groundwater Dependent Ecosystems (GDEs)	Ecosystems that rely entirely or partially on groundwater sources to meet their water requirements
Hydraulic architecture	The structure of the plant water conducting system
Mediterranean-type ecosystem (MTEs)	Ecosystem occurring in 5 global regions with characteristic and unique climatic regimes of mild, wet winters and warm, dry summers.
Mesic	A habitat with well-proportioned supply of water
Phreatophyte	Plants that have a strong connection with groundwater
Water table	The upper surface of the saturated zone of an aquifer. The zone between groundwater and the unsaturated zone

Xeric	A very dry habitat with minimal moisture supplies

Other abbreviations

Vessel diameter	Vd
Maximum vessel diameter	Dmax
Vessel density	Dv
Vessel length	VL
Hydraulically-weighted vessel diameter	Dh
Vessel area	V_A
Specific hydraulic conductivity	Ks
Theoretical hydraulic conductivity	Kth
Stem specific density	SSD
Volumetric water content	VWC

1. Introduction

1.1. Mediterranean-Type Ecosystems and a changing climate

Mediterranean-type ecosystems (MTEs) are characterised by high seasonal variability in rainfall and air temperature; with hot, dry summers and cold, wet winters presenting various limitations for plant species (Joffre, Rambal, & Damesin, 1999). Mediterranean-type climates occur across five regions of the globe and are well known for having high species richness, containing approximately 20% of all known vascular plants, with 50% of plant species being endemic to these ecosystems (Beltrán et al., 2014; Cowling et al., 2015; Onstein, Carter, Xing, Richardson, & Linder, 2015). The physiology, morphology, and anatomy of an individual plant species can provide insight to its capability to withstand the effects of drought, and assist with conservation and restoration (Martin-Benito et al., 2017). Trends of increasing average temperatures and decreasing average rainfall have become apparent in MTEs, resulting in incomplete groundwater recharge and exposing woody taxa to hotter and drier summers (Groom, Froend, Mattiske, & Gurner, 2001; He, D'Agui, Lim, Enright, & Luo, 2016). Understanding how individual species in MTEs will respond to these climatic shifts is of increasing importance so that appropriate actions can be made in regards to both ecosystem and groundwater management (Cowling et al., 2015; Onstein et al., 2015; Rosner, Heinze, Savi, & Dalla-Salda, 2018).

In Perth, Western Australia, the annual mean temperature has been increasing since the mid-1970s (Bureau of Meteorology [BoM], 2018). There has also been a decline in annual rainfall, which in turn has resulted in a reduction in aquifer recharge (BoM, 2018). Although MTEs have adapted to the distinctive seasonality of water availability (Joffre et al., 1999), changes in climatic conditions have resulted in reduced plant access to water sources (such as groundwater) during the critical summer period (Department of Water [DoW], 2016). This is also due to urban growth as it increases the demand for aquifer water supplies, and also land clearing as this can expose the soil to increased evaporation rates (DoW, 2016). If plant populations in MTEs cannot adapt to the changes in temperature and water availability, they risk being surpassed by invasive species that can better cope with harsh environmental conditions.

Plants have developed a number of methods to cope with the distinct climate of MTEs, many of which occur in the early stages of growth. Seedlings in MTEs tend to germinate in the cooler, wet winter periods and will continue to grow through to early-mid-summer (Richards, 1993). The ongoing survival and growth of seedlings in MTEs is dependent upon the early establishment and subsequent survival of the first summer drought (Canham, Froend, & Stock, 2015; Padilla & Pugnaire, 2007; Richards, 1993).

By developing deep roots, species may be able to avoid deleterious water stress during dry periods by accessing seasonally stable water supplies (Joffre et al., 1999; West et al., 2012).

In MTEs and habitats with prolonged dry periods, plants that extend their roots to establish a connection with the saturated zone of a soil profile (Canham et al., 2015; Orellana, Verma, Ii, & Daly, 2012; Thomas, 2014; Vonlanthen, Zhang, Bruelheide, 2010) are referred to as phreatophytes, these plants have developed deep roots that access water from the capillary fringe above a water table, or from the phreatic zone (zone of saturation) (Meinzer, 1926; Wang et al., 2018) to meet their water requirements. Phreatophytes commonly have a high density of shallow roots near the upper surface to allow for nutrient and water (from precipitation) acquisition, as well as deeper roots in the capillary zone, allowing the plant to utilise groundwater to meet water requirements during dry periods (Orellana et al., 2012). Although the density of roots tends to decrease with depth, deeper roots can still contribute significantly to water uptake due to their higher hydraulic conductance than the high-density shallow roots near the upper surface, as well as their ability to sustain water uptake when the upper surface soil layers dry out (Orellana et al., 2012).

The dependence of phreatophytes on groundwater varies considerably between species. Facultative phreatophytes, or temporary phreatophytes, are species that use groundwater opportunistically and tend to range from habitats with shallow groundwater tables to habitats with no water table present (Canham, Froend, & Stock, 2009; Thomas, 2014; Zencich, Froend, Turner, & Gailitis, 2002). These species have been shown to use groundwater and soil moisture interchangeably depending on the availability in a habitat (Zencich et al., 2002). Obligate phreatophytes, or permanent phreatophytes, are far more dependent on continuous access to groundwater and as a result, rapid declines in groundwater levels may drastically impact these plant species (Eamus, Zolfaghar, Villalobos-Vega, Cleverly, & Huete, 2015). Due to the higher reliability on access to groundwater, the distribution of obligate phreatophytes is confined to moist environments with persistent shallow water tables (i.e. damplands), while facultative phreatophytes have a wide-spread distribution (Zencich et al., 2002).

1.2. Plant adaptations to a changing climate

Although there is a general understanding that vertical root development towards a water table is rapid in phreatophyte seedlings, only a small number of studies have looked at the physiological and anatomical responses of phreatophytes in relation to water availability (Canham, 2011; Canham et al., 2015; Froend & Drake, 2006; McElrone, Pockman, Martinez-Vilalta, & Jackson, 2004). Researchers have analysed the morphology, physiology and anatomy of a range of species, including phreatophytic species to understand how plants respond to changes in water availability (Canham, 2011; Froend & Drake, 2006; McElrone et al., 2004; Sultan, 2000).

Modifications to plant physiological, biochemical or anatomical traits are required to tolerate the stress of changing environmental conditions, such as high soil salinity or drought (Gupta, Huang, Gupta, & Huang, 2014; Parida, Veerabathini, Kumari, & Agarwal, 2016). Examples of these modifications can be seen in aridity-adapted xerophytes that have altered leaf thickness, stomatal encryption and more to survive in dry environments (Carpenter, McLoughlin, Hill, McNamara, & Jordan, 2014; Jordan, Weston, Carpenter, Dillon, & Brodribb, 2008). Pronounced encrypted stomata are thought to be linked with reduced transpiration rates and dry climates (Jordan et al., 2008; Roth-Nebelsick, Hassiotou, & Veneklaas, 2009). Some plants have adapted to have amphistomatic leaf surfaces i.e., stomata on both surfaces, sunken or encrypted stomata, as well as vertical leaf growth which allows lower sun angles to be captured, reducing the risk of desiccation and harsh temperatures that are more prevalent when the sun is at a greater angle (Drake, Boer, Schymanski, & Veneklaas, in press). The adaptation of amphistomatic leaf surfaces means that there is an increase in pathways acting in parallel for CO_2 uptake and transportation, allowing for quicker transportation rates, and the increase in stomatal density also increases the gas exchange capacity between the leaves and the atmosphere (Drake et al., in press). Other plants, such as phreatophytes, have developed deeper roots to survive in drier environments, allowing them to access readily available groundwater that can be several metres below the soil surface (Dawson & Pate, 1996; Thomas, 2014). Plants exposed to drier conditions have developed controlled stomatal closure and leaves with a smaller surface area to minimise water loss during dry, hot periods (Addington et al., 2006; Domec, Warren, Meinzer, & Lachenbruch, 2009; Fonti, Heller, Cherubini, Rigling, & Arend, 2013; Nardini, Pedà, & Rocca, 2012). A study by Cochrane, Hoyle, Yates, Neeman, and Nicotra (2016) identified strong shifts in functional traits in *Banksia* species along a longitudinal climate gradient (decreasing soil moisture coupled with increasing temperature) in south-west Western Australia. Plants have also been shown to alter anatomical traits, such as xylem vessel traits, as a mechanism to cope with the effects of drought (Pfautsch et al., 2016; Rita, Borghetti, Todaro, & Saracino, 2016). The links between climate and plant functional traits are commonly used to predict a plants response to changing environmental conditions (i.e., nutrient availability, water availability) (Balachowski & Volaire, 2018; Moor, Hylander, & Norberg, 2015; Peppe et al., 2011).

1.3. Functional traits that govern plant response to drought

Xylem anatomy and hydraulic architecture encompass a range of functional traits commonly studied to better understand plant adaptations and responses to factors such as drought (Mouillot, Graham, Villéger, Mason, & Bellwood, 2013). The analysis of xylem traits allows assessment of functional properties, such as the hydraulic conductivity which can be used as a measure of plant hydraulic efficiency, and depends largely on vessel diameter and density (Durante, Maseda, & Fernandez, 2011). Large diameter vessels are commonly associated with increased hydraulic conductivity and therefore efficiency (Pfautsch et al., 2016; Sperry, Hacke, & Pittermann, 2006; Thomas, 2014; Tulyananda & Nilsen, 2017). This association is in accordance with the Hagen-Poiseuille law, stating that lamina flow through a cylinder is proportional to its radius, raised to the fourth power (Sperry et al., 2006). The flow through xylem, which is functionally analogous to a cylindrical pipe, is therefore highly sensitive to vessel diameter (Sperry et al., 2006). However, studies have suggested an increased vessel diameter may result in an increased risk of drought-induced cavitation (Borghetti, Gentilesca, Leonardi, van Noije, & Rita, 2017; Comas, Becker, Cruz, Byrne, & Dierig, 2013; Jaquish & Ewers, 2001; McElrone et al., 2004; Thomas, 2014; Tulyananda & Nilsen, 2017). Increasing hydraulic efficiency is therefore coupled with a decrease in hydraulic safety (McElrone et al., 2004). Plants with 'safer' hydraulic properties, for example, decreased vessel diameter, increased vessel density and thicker vessel walls, are less vulnerable to xylem cavitation (Burgess, Pittermann, & Dawson, 2006; Durante et al., 2011; Hacke, Sperry, Wheeler, & Castro, 2006; Pfautsch et al., 2016; Pratt, Jacobsen, Ewers, & Davis, 2007). However, hydraulic conductivity is significantly reduced as a result of this shift in traits (Burgess et al., 2006; Durante et al., 2011; Gleason et al., 2016; Goncalves et al., 2007; Pfautsch et al., 2016). As a result, there is a trade-off between hydraulic efficiency and safety.

The trade-off theory between hydraulic efficiency and safety suggests that plants develop a safer anatomy when growing in xeric environments as drought is more common, while plants in mesic environments rarely experience drought and develop a more efficient anatomy (Burgess et al., 2006; Durante et al., 2011; Pfautsch et al., 2016; Sperry et al., 2006; Tyree, Davis, & Cochard, 1994). Although the trade-off theory has gained a lot of attention, further research is required to provide stronger evidence of the theory (Froux, Huc, Ducrey, & Dreyer, 2002; Gleason et al., 2016; Hacke et al., 2006; Tulyananda & Nilsen, 2017).

Vessel diameter tends to be significantly larger in plants that can readily access water (Myburg & Sederoff, 2001), and mesic habitats, where water is plentiful, tend to support plants that grow faster and have a xylem network that can transport large amounts of water (Corcuera, Gil-Pelegrin, Notivol, & Tognetti, 2012). For species growing in mesic habitats, large diameter vessels are particularly advantageous as they increase the whole-plant efficiency for transporting water and nutrients (Borghetti et al., 2017; Mencuccini, Hölttä, Petit, & Magnani, 2007; Santini et al., 2015). Vessel lengths also contribute to efficiency, with longer vessels reducing the number of connections between vessels as well as the rate that water is required to flow through end walls with a high resistance (Lens et al., 2011). Therefore, plants found in mesic habitats are predicted to have a greater hydraulic efficiency of the xylem, with large diameter vessels and increasing vessel lengths (Olson et al., 2014; Pfautsch et al., 2016; Santini et al., 2015). Conversely, for species growing in xeric habitats, large diameter vessels are more likely to lead to hydraulic failure due to increased exposure to water deficits. Therefore, it may be beneficial for plants in drier environments to develop safer xylem traits such as an increased density of smaller diameter vessels (Borghetti et al., 2017; Myburg & Sederoff, 2001; Olson et al., 2014; Santini et al., 2015). This variation in xylem vessel anatomy in xeric habitats contributes to reduced growth but more conservative water usage and an improved avoidance of drought-induced cavitation (Borghetti et al., 2017; Corcuera et al., 2012). However, there are exceptions (Corcuera et al., 2012; Nicotra, Babicka, & Westoby, 2002).

Individual species can alter their anatomy when exposed to changing climatic conditions, particularly water deficits (Aref, Ahmed, Khan, El-Atta, & Iqbal, 2013; Beikircher & Mayr, 2009; Lovisolo et al., 1998). Studies which exposed plants to similar drought treatments discovered that those subject to drought developed xylem traits that were significantly different to plants in a control (well-watered) treatment (Aref et al., 2013; Beikircher & Mayr, 2009; Lovisolo et al., 1998). Aref et al., (2013) discovered that as drought intensity increased, *Acacia tortilis* subsp. *raddiana* developed a more drought tolerant anatomy with an increased density of small diameter vessels, alternatively, *A ehrenbergiana* developed fewer, larger diameter vessels. Beikircher & Mayr, (2009) identified similar results, with some plants responding to a drought treatment by decreasing the mean diameter of vessels and increasing vessel wall thickness, allowing them to tolerate drought. However, some plants developed larger diameter vessels, showing a reduced resistance to drought (Beikircher & Mayr, 2009). Douglas-fir seedlings from xeric environments developed root xylem that was less vulnerable to cavitation than seedlings in a mesic environment which had had vulnerable root and stem xylem (Kavanagh, Bond, Aitken, Gartner, & Knowe, 1999). These findings suggest that there is likely to be variation in the xylem traits within a species depending on the habitat water availability under which individual plants develop.

Xylem traits can also differ within an individual plant, with vessels in deeper roots typically having larger diameters than the vessels in shallow roots and stems (Hacke, Spicer, Schreiber, & Plavcová, 2017; McElrone et al., 2004; Pate, Jeschke, & Aylward, 1995). Studies on plant hydraulic architecture have shown that the xylem architecture within an individual plant generally alters in a basipetal direction from branches to roots, a process referred to as vessel tapering (Anfodillo, Carraro, Carrer, Fior, & Rossi, 2006; Choat, Lahr, Melcher, Zwieniecki, & Michele, 2005; Gričar et al., 2015; Marciszewska & Tulik, 2013; Tyree & Zimmermann, 2002). The literature suggests that vessel structure, and tapering, are responsible for the control of water distribution as path lengths increase with plant age and increased plant height (e.g., West, Brown and Enquist (WBE) model) (Lintunen & Kalliokoski, 2010; Olson et al., 2014; Pfautsch et al., 2016; West, Brown, & Enquist, 1999; Zimmermann, 1978). The tapering of vessels from the roots to branches allows plants to maintain a low hydraulic resistance while still increasing their height (Olson et al., 2014). However, this tapering is thought to limit the maximum height of individual trees, as once the maximum vessel diameter is reached, tapering becomes less efficient (Anfodillo et al., 2006; West, Brown, & Enquist, 1999). As a result, hydraulic resistance is no longer regulated and should increase as tree height increases (Anfodillo et al., 2006; West, Brown, & Enquist, 1999). In two *Banksia* species, sinker roots had longer and wider vessels than the shallow lateral roots and stems (Pate, Jeschke, & Aylward, 1995). Similarly, McElrone et al., (2004) revealed that deep roots supported larger diameter vessels than shallow roots and stems across four different species. This process of vessel tapering has also been shown to occur at the organ-level in plants, with roots having increasingly larger diameter vessels towards the tips (McElrone et al., 2004; Pate, Jeschke, & Aylward, 1995; Petit, Anfodillo, & De Zan, 2009). It is likely that larger diameter vessels are more common/suitable in deep roots as they facilitate more efficient water transport than a high density of narrow vessels (Gebauer & Volařík, 2013; Hacke et al., 2017). The exact nature of xylem taper in roots and stems is not, however, the focus of this study which is concerned with the anatomical plasticity of xylem in relation to water availability.

Within genera, species alter their xylem in relation to changes in environmental conditions, i.e. water availability (Aref et al., 2013; Barotto et al., 2017; Pfautsch et al., 2016). Pfautsch et al., (2016) found that vessels increased in diameter across *Eucalyptus* species from xeric environments towards mesic environments, showing that vessel diameter differed along an ecohydrological gradient as well as across species. Barotto et al., (2017) also analysed xylem traits across three different *Eucalyptus* species and found that select traits were significantly different amongst the species depending on their growth habit and habitat requirements. In another study, two different species of *Styrax* from contrasting habitats were analysed and it was discovered that the ‘savannah species’ had four times the number of vessels per mm^2, as well as shorter vessel lengths and smaller vessel diameters; anatomy more suited to a warm, dry environment (Silva et al., 2017). Willson & Jackson, (2006) assessed drought and freezing stress in *Juniperus* species and found that species located in xeric environments showed greater resistance to drought- and drought plus freezing-induced cavitation in comparison to species found in mesic environments. There are also differences in anatomy across different genera. A study by Choat et al., (2011) found that the xylem anatomy of various angiosperms differed significantly, with *Eucalypts* and *Acacia* species developing the largest mean vessel diameters (25-33 µm) while a *Hakea* species developed the smallest mean vessel diameter (~14 µm). Other species, such as *Banksia, Allocasuarina* and *Leptospermum* were in the middle with mean vessel diameters around 19 µm (Choat et al., 2011). These significant differences in anatomy across plant genera are due to a number of factors, including the adaptation to environmental conditions overtime (i.e., stress tolerance).

1.4. Significance of this study

The effects of climate change have heightened the need to understand how a plant's xylem anatomy may differ in relation to water availability in a drying landscape. Specifically, this study contributes to the understanding of how two MTE phreatophytes are likely to respond to changes in water availability. *Banksia* woodlands are the dominant vegetation type on the Swan Coastal Plain in Western Australia and due to a range of factors, including reductions in groundwater levels, the woodlands are rapidly declining. Only a limited number of studies have analysed xylem traits in phreatophyte species, and none have addressed the xylem traits of two dominant overstorey *Banksia* species, *B. attenuata* and *B. littoralis*, relative to their connection with a water table. Therefore, this study of phreatophytic xylem anatomy in relation to water availability highlights species differences in seedling vulnerability to drought during establishment, with an interest in the development of hydraulically safe or efficient xylem traits. This contribution towards a better understanding of how their growth and survival is likely to be influenced by changing groundwater levels will be of importance for population maintenance in a drying landscape, and the appropriate selection of species for revegetation purposes.

1.5. Aim, Objectives and Hypotheses

The following research aims to determine if phreatophyte seedlings develop different xylem traits during establishment relative to water availability. The focus of this study is to determine whether there is a trade-off between hydraulic efficiency and safety in root xylem of two *Banksia* species with contrasting habitat preferences in a Mediterranean-type environment.

With this aim in mind, the thesis is structured to address the following objectives and hypotheses:

Objective One – Compare the vessel traits of *Banksia* seedlings of different ages, and *Banksia* seedlings that have established (and connected) with a shallow water table with individuals that have no access to a water table.

H1: *There is no difference in the xylem traits of* Banksia *seedlings after 55 days of growth (Harvest 1) compared to seedlings after 174 days of growth (Harvest 2).*

H2: *There is no difference in the traits (anatomical or morphological) of* Banksia *seedlings of equivalent age, with or without access to (and use of) a water table*.

Objective Two – Investigate and confirm the presence of vessel tapering in individual plants, and characterise whole-plant (seedling) hydraulic architecture.

H3: *There is no conduit tapering in* Banksia *seedling root or hydraulic architecture*.

Objective Three – Investigate whether congeneric species of *Banksia* with different hydraulic requirements differ in their vessel traits.

H4: *There are no differences in the traits (anatomical and morphological) between* Banksia attenuata *and* B. littoralis *seedlings of equivalent age, with or without access to (and use of) a water table.*

2. Materials and Methods

2.1. Study species

Two *Banksia* species that are endemic to southwestern Australia, *Banksia attenuata* R.Br. and *Banksia littoralis* R.Br, were selected for this study due to their contrasting ecohydrological requirements. Both species form the overstorey of the vegetation type termed *Banksia* Woodland. In 2016 *Banksia* Woodlands were listed as an endangered ecological community under the *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC Act) (Department of the Environment and Energy, 2016). The Swan Coastal Plain consists of a sequence of dunes with varying depths to a relatively shallow superficial groundwater resource. This variation provides an ecohydrological gradient, supporting both xeric and mesic habitats (Canham, Froend, & Stock, 2009). Due to extensive clearing, a reduction in annual rainfall, over-abstraction of groundwater and other factors, *Banksia* Woodlands on the Swan Coastal Plain have undergone drastic changes to their structure and floristic composition, including a decline in geographical extent of approximately 60 percent (Brundrett et al., 2017; Williams, Yates, Saunders, Dawson, & Barrett, 2017). These changes have no doubt altered the services that these woodlands once provided. For example, *Banksia* Woodlands are an important food and habitat resource to various mammals and birds, particularly the nationally-threatened *Calyptorhynchus latirostris* (Carnaby's Cockatoo) (Brundrett et al., 2017; Williams et al., 2017). With a reduction in condition and geographical extent, it is unclear if the remaining *Banksia* woodlands can support these species.

Many *Banksia* Woodlands are groundwater-dependent ecosystems and both of the chosen plant species for this study are classified as phreatophytic when groundwater is near the surface (Orellana et al., 2012). *Banksia attenuata*, a facultative phreatophyte, was selected as it has the widest distribution of all Western *Banksia* species (Western Australian Herbarium, 1998). Although due to anthropogenic activities and the spread of disease (i.e., *Phytophthora*), the occurrence and overall health of *B. attenuata* in *Banksia* woodlands is deteriorating. Facultative phreatophytes access groundwater sources opportunistically, for example, during the drier months when surface water is at its lowest (Thomas, 2014). *Banksia attenuata* displays a lower vulnerability to drought in comparison to *B. littoralis* (Canham et al., 2009), and ranges from habitats with a shallow water table (<5 m) to habitats with water tables beyond the extent of roots (Zencich et al., 2002). The second study species, *B. littoralis*, is considered an obligate phreatophyte and has a distribution confined to moist environments with persistent shallow water tables (i.e., damplands) (Zencich et al., 2002). Obligate phreatophytes are referred to as 'permanent' phreatophytes, suggesting that they retain a permanent connection with a groundwater source (Thomas, 2014). *Banksia littoralis* was selected due to its restricted distribution along the ecohydrological gradient relative to *B. attenuata* (Canham, 2011) (Figure 2.1) and the resulting difference in water requirements (Zencich et al., 2002).

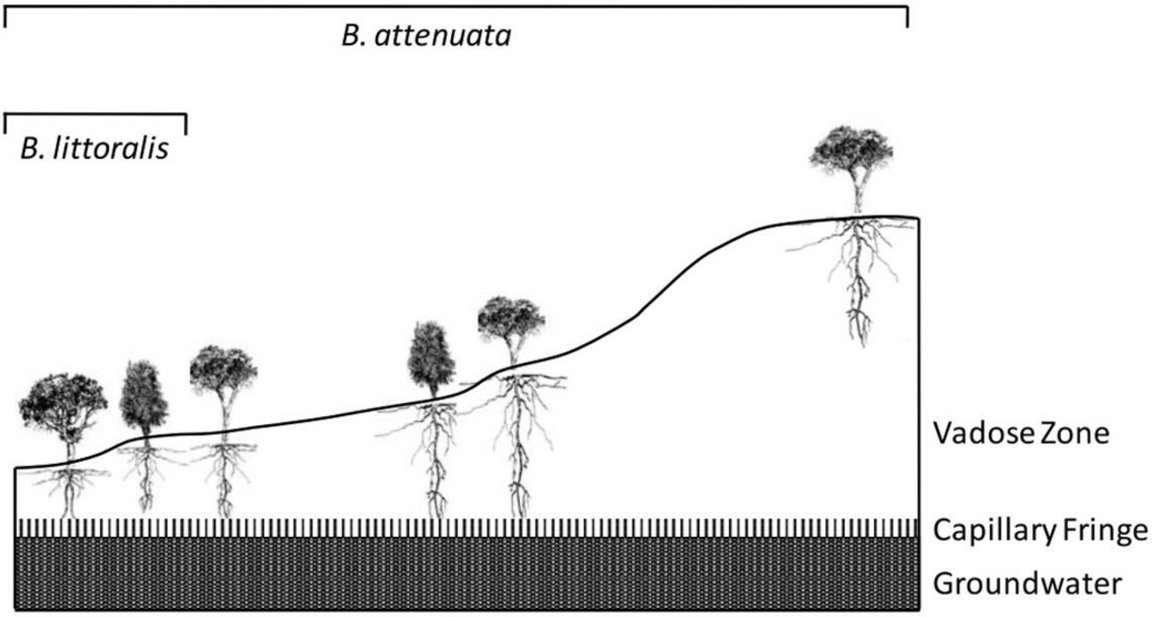


Figure 2.1 Ecohydrological gradient showing the differences in distribution of *Banksia attenuata* and *Banksia littoralis*. Figure modified from Canham (2011).

2.2. Experiment set-up and seed germination

Banksia attenuata and *B. littoralis* seedlings were grown in a glasshouse under contrasting artificial groundwater treatments. The experiment consisted of fourteen 195 L heavy duty plastic barrels, each containing six PVC tubes (each 90 mm dia. x 2 m) (Figure 2.2). Each tube was lined with a polypropylene film. The bottom end of each PVC tube was capped and filled with 10 cm of crushed granite to ensure drainage (particularly in the 'disconnected' treatment), and then filled to the top with steam sterilised white washed sand. The top 10 cm of sand was mixed with sterilised Scotts Osmocote

Professional Native Potting Mix to provide nutrients to the seedlings following germination. Seven of the barrels were filled to 50 cm with water to create an artificial pool of groundwater, referred to as an ‘artificial water table’ (Figure 2.2A), while the remaining seven barrels had holes drilled in the base to ensure complete drainage (Figure 2.2B). The seven barrels with the artificial water table are from here on in referred to as ‘connected’, and the remaining seven barrels are referred to as ‘disconnected’.

The glasshouse temperature was controlled and ranged between 15 and 30°C, with an average temperature of 23°C during the experiment. Relative humidity ranged between 20 and 100%, with an average of 63% (TGU-4500, Tinytag Ultra 2 Data Logger).

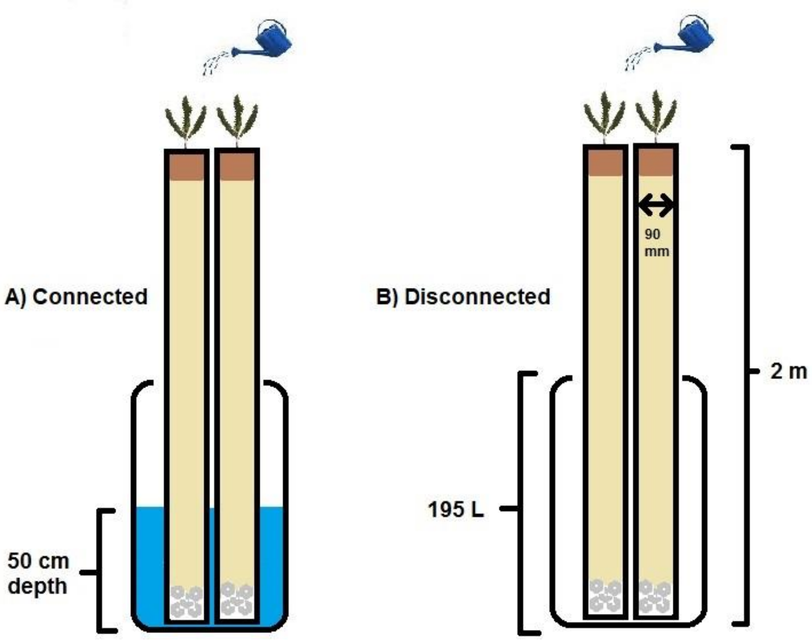


Figure 2.2 The two treatment types studied in a glasshouse experiment. **A)** ‘Connected’ represents the treatment that included an artificial water table (50 cm from the base). **B)** ‘Disconnected’ represents the treatment that did not have an artificial water table. Both treatments received 50 mL of surface water three days a week.

The seeds used for the study were sourced from within the Wanneroo province on the Swan Coastal Plain (*Nindethana Seed Company*). In late June 2017, half of the PVC tubes (n = 42) were sown with *B. attenuata*, and half with *B. littoralis* (n = 4 seeds per tube).

Each tube received up to 50 mL of surface water daily until the seeds had germinated. Once each tube had one dominant seedling presenting its first true leaves, the additional seedlings were removed (six weeks after sowing) leaving one seedling in each tube. Then seedlings received only 50 mL of surface water each, three times a week (Monday, Wednesday and Friday).

A subset of 12 tubes (6 from each treatment) were used to measure the volumetric water content (VWC) using a theta probe (Model MPKit-406, ICT International). Holes (8 mm diameter) were drilled into the selected tubes from the top to bottom at 20 cm intervals, making a total of 10 depths at which the VWC was measured. The theta probe was then inserted at each of the 20 cm depth intervals and a one off soil moisture level was recorded. The tubes from the ‘connected’ treatment remained immersed in the water up until the time of measurement. However, on the day of measurement, the plants had not received their 50 ml of surface water, therefore there was no increase in VWC at the surface. The VWC measurements were taken to represent a spatial average across the length of the probe at each depth.

2.3. Plant harvest

Two sampling events were included in this study to investigate whether xylem traits differed during seedling establishment, particularly before and after connection with an artificial water table. The first sampling event, referred to as Harvest 1, occurred after approximately 2 months of growth and before the roots of the seedlings in the connected treatment were in contact with the capillary fringe. There were eight seedlings from each species harvested (*n* = 8). The sampling time was calculated using information from a study that had a similar experimental design and looked at root elongation rates in the same species (Canham et al., 2015). This harvest was used as a representation of the seedlings prior to connection/contact with an artificial water table.

The second sampling event, referred to as Harvest 2, occurred after approximately 6 months of growth and 10 seedlings from each species were harvested (*n* = 10). Using the same study (Canham et al., 2015), it was estimated that seedlings growing in the treatment with the artificial water table would have reached the capillary fringe after ~3-4 months and would therefore have been utilising the groundwater source for over 2 months.

For both Harvest 1 and Harvest 2, the tubes were laid down horizontally and the end cap was removed. The sand and plant contents were pulled out of the tube in the polypropylene film to protect the roots. After removing the lining, the sand was washed away from the plant roots. The stem diameter was measured adjacent to the cotyledons or cotyledon scars and the root diameter was measured approximately 15 cm from the base using digital callipers. The above ground stem length, and below ground root length (main taproot) were also measured at this stage.

The methods for collecting plant segments differed slightly depending on the intended analysis. Approximately 30 cm of root (taken ~20 cm from the base of the plant) was collected and immediately placed into a plastic bag containing water, and then into a large black plastic bag to avoid desiccation. These samples were immediately transferred to the laboratory to measure root vessel length (see below). At the same time, approximately 3 cm of the root (from both distal and proximal locations), and 3 cm of the stem were collected and stored in 30 mL vials of formol acetic alcohol (FAA) fixative before being prepared for histological analysis. Another 3 cm of the stem was collected from each plant and stored in plastic bags with a moist paper towel. These segments were stored in a refrigerator and later used to measure the stem-specific density (SSD).

Samples collected for xylem trait analysis were collected from roots and stems from four main sample areas (Figure 2.3). Root 1, Root 2 and Stem 2 samples were used for analyses in Objectives One and Three, while the Stem 1 samples were only included in the tapering objective (Objective Two). The samples were collected from these four locations to enable a wider assessment of xylem anatomical traits within a plant.

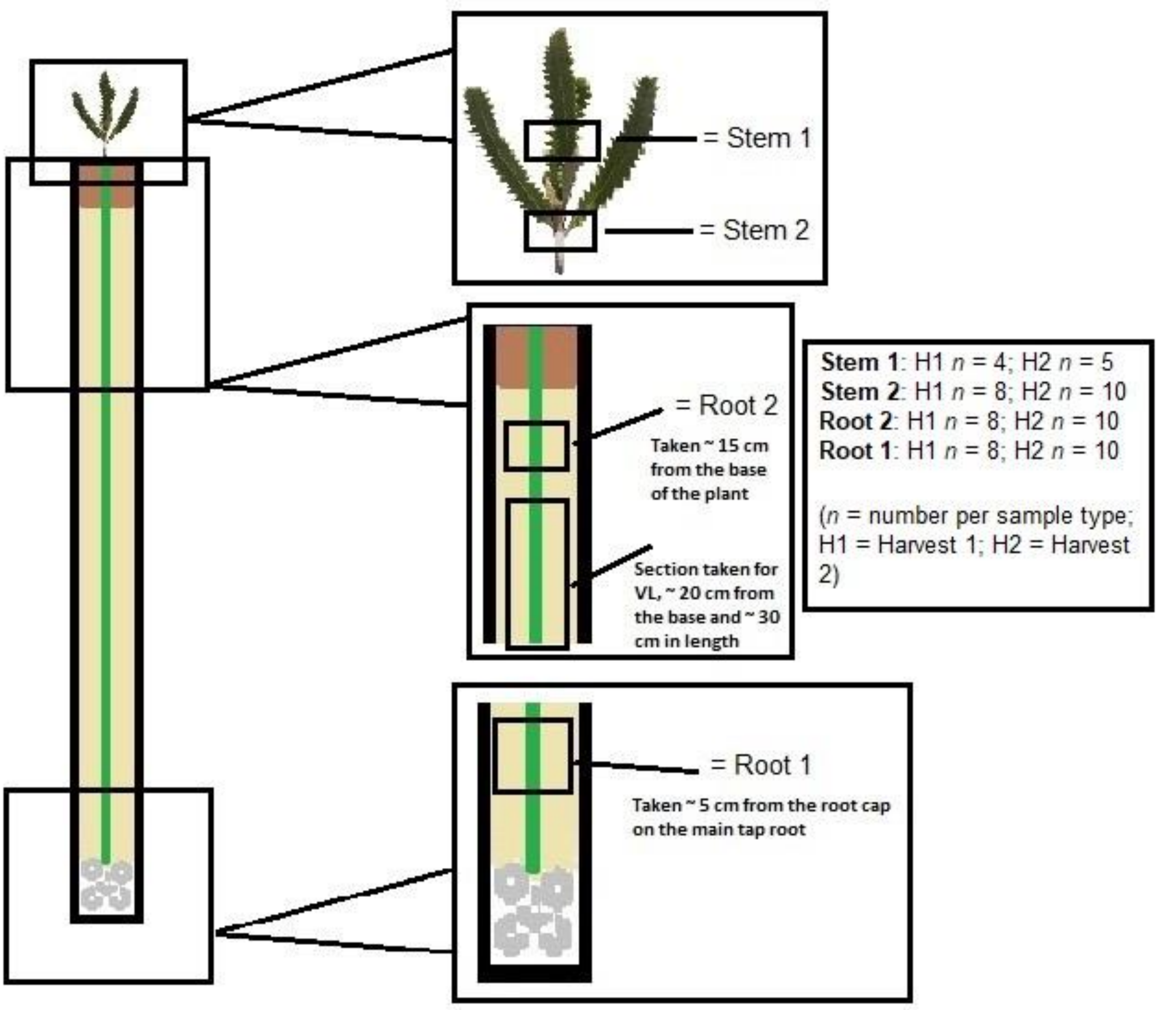


Figure 2.3 Three centimetre lengths of tissue samples were collected from four locations on the seedlings for analysis of xylem traits (Stem 1, Stem 2, Root 1, and Root 2). Another approximately 30 cm length of root was also collected for vessel length (VL) measurements (H1 *n* = 8; H2 *n* = 10).

2.4. Stem-specific Density (SSD)

The stem-specific density (SSD) (g cm^3) was measured following methods described by Pérez-Harguindeguy et al., (2013). The loose bark was scraped away from the 3 cm segments collected during the plant harvest. The stem volume (V) (cm^3) was calculated first using the dimensional method

(Equation 1). This method requires the total length (L) and diameter (D) from one or more places along a fresh stem segment.

$$V = (0.5D)^2 \times \pi \times L \qquad (1)$$

After the dimensions were recorded for the fresh segments, the samples were placed in an oven at 70$^{\circ C}$ for 72 h and then weighed immediately to get the oven-dried mass (g). The SSD was calculated by dividing the oven-dried mass by the volume (calculated in Equation 1 above) of the same stem segment.

2.5. Root xylem vessel length

The 30 cm sections of root were placed into water in a conical flask or beaker which were put into a large glass desiccator connected to a high vacuum rotary vane pump (model W2V10, WooSung Automa Co., Ltd). The samples were held under a strong vacuum for a minimum of 1 h. This approach served to remove any embolisms from xylem vessels (Figure 2.4) (Brodersen, Jansen, Choat, Rico, & Pittermann, 2014; Hacke et al., 2014; Scholz, Klepsch, Karimi, & Jansen, 2013).

Figure 2.4 Image showing the vacuum set-up used to remove xylem embolisms in preparation for vessel length analyses.

After a minimum of 1 h, the samples were removed from the vacuum and the ends were trimmed underwater using a sharp razor blade. The vessel length (VL) of roots was measured by the ‘Air Method’ described by Zimmermann and Jeje (1981). Parafilm™ was wrapped around the distal end of the root and secured with a rubber grommet to prevent leaks. The rubber grommet was connected to an air hose with injection at a pressure between 26 and 35 kPa. The proximal end of the root was placed under water and shortened by cutting until a stream of bubbles was visible, indicating an open vessel from the distal to proximal end. Root length (cm) and diameter (mm) were recorded. It is acknowledged that the actual maximum vessel length will likely differ from this estimate since each root sample comprises of a distribution of vessel lengths.

2.6. Flow meter and the conductive xylem area

Samples were prepared and placed under vacuum following the same method as the above section 2.5 '*Root vessel length'*. After the ends of the samples were trimmed underwater, each sample was connected to a rubber grommet that was then attached to a flow meter, similar to that described by Brodribb & Cochard (2009). Briefly, the flow meter was comprised on a section of (Peek™) capillary tubing, ordered in series with the sample, and a pressure transducer (PX26, OMEGA Engineering, CT, USA) connected to a data logger (CR1000, Campbell Scientific, UT, USA), recorded data at 1 second intervals. The capillary tubing was calibrated such that the relationship between the pressure gradient (ΔP, MPa) and flow rate (F, kg s^{-1}) was known. A head pressure of approximately 7 kPa was applied to the sample to drive flow. The samples were inserted in the flow meter and F recorded after reaching a stable value. Following measurements, the sample length (L, m) and conductive area (A_S, m^2) were determined.

To estimate A_s the proximal end of the sample was wrapped in Parafilm™ and attached to a mild vacuum. The distal end of the sample was immersed in a solution of toluidine blue (Figure 2.5).

Figure 2.5 Using toluidine blue to stain the conductive area of root tissue samples ($n = 5$).

After approximately 5 min the sample was removed from the apparatus and cross sections taken at the approximate mid-point of the sample using a razor blade. Each section was then mounted on a slide and imaged using a light microscope fitted with a camera (Leica, model M205C). The images were then analysed for the area of stained tissue in ImageJ (Schneider, Rasband, & Eliceiri, 2012), which was taken as A_S. The xylem specific hydraulic conductivity (K_S, kg s^{-1} MPa^{-1} m^{-1}) of the samples was determined as:

$$K_S = \frac{F}{\Delta P} \times \frac{L}{A_S} \tag{2}$$

2.7. Microscopy and image analysis

Tissue samples collected during the plant harvest (H1 $n = 8$; H2 $n = 10$) were immersed overnight in FAA at room temperature before being transferred to an automatic tissue processing machine (Leica, model TB1020). The tissue was dehydrated in 90% then 100% ethanol, cleared in a mixture of ethanol and chloroform and then again in 100% chloroform. The tissue samples were infiltrated with molten paraffin wax, followed by further infiltration with molten paraffin wax under vacuum and then embedded in fresh molten paraffin wax. Once cooled, each specimen block comprising of an individual tissue sample were sectioned at 8 to 10 µm using a rotary microtome (Leica, model RM2235) and the sections transferred to a heated water bath before being transferred onto glass slides. After drying at 60ºC, the slides were stained with Safranin and Fast Green following methods by Ruzin (1999). These stains are commonly used to stain lignified tissues (xylem) and fibrous cells in plant sections. Following staining, coverslips were mounted on the slides using a toluene based mounting medium and set aside to dry. Images were taken with a compound light microscope (Olympus, model BX51 fitted with an Olympus DP70 camera) at 100, 200 and 400x magnification.

Image analysis was completed using ImageJ (Schneider et al., 2012). The images were converted to an 8-bit greyscale format and then converted to a binary format (Figure 2.6). Following conversion to a binary image, the cross sectional area (V_A, μm^2) of vessels was measured. Occasional artefacts during image processing were corrected using the paintbrush tool in Image J (Figure 2.7).

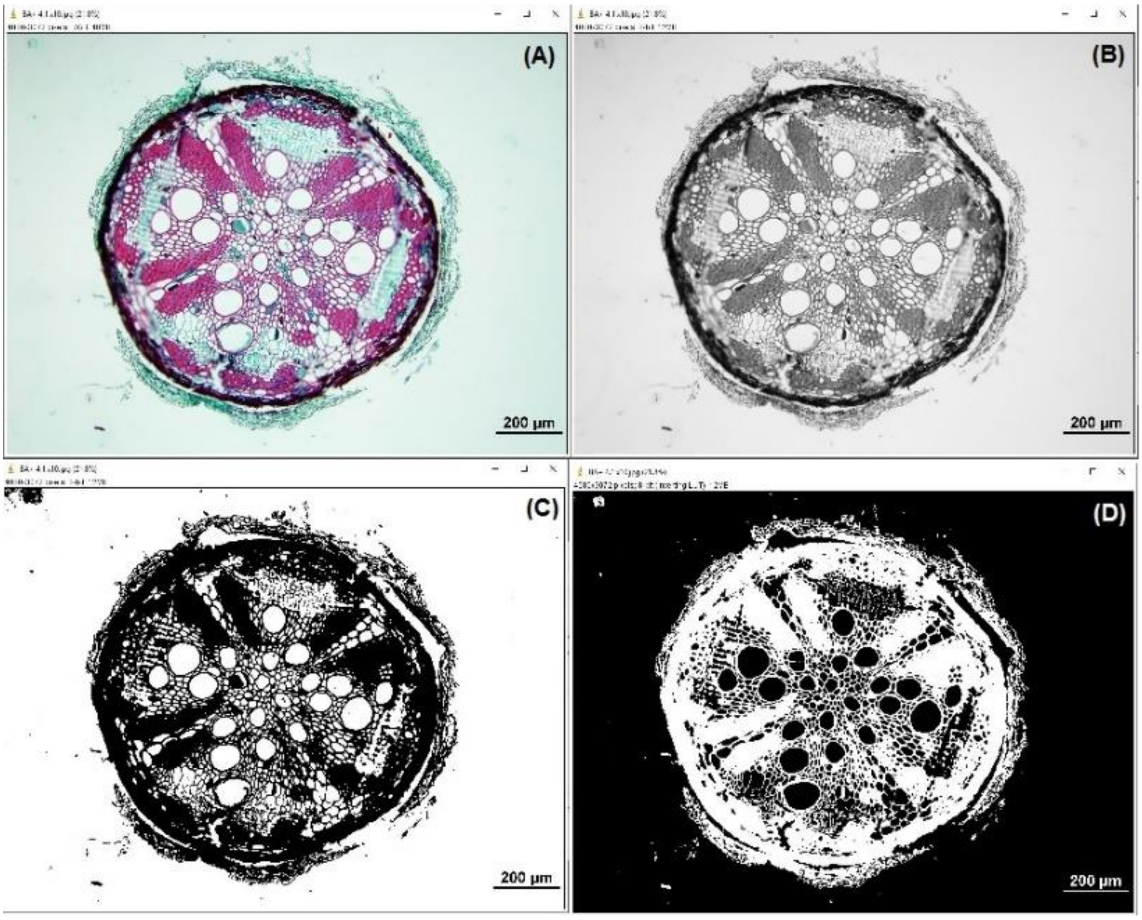


Figure 2.6. Steps to format JPEG image for image analysis in ImageJ program performed on a *Banksia attenuata* root tissue sample. (A) Original image, (B) 8-bit greyscale image, (C) contrast adjusted image, and (D) threshold adjusted image ready for analysis.

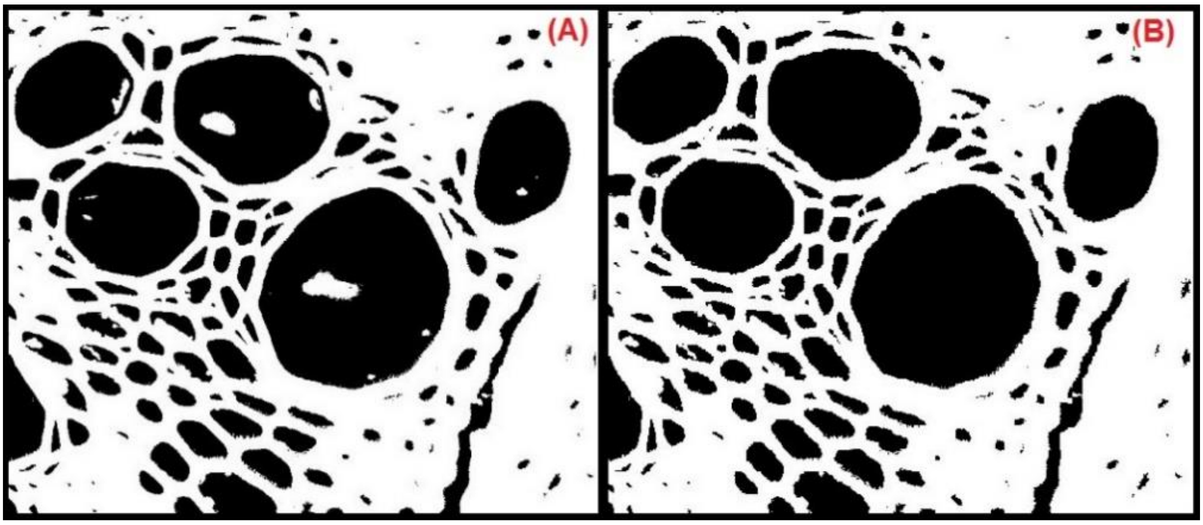
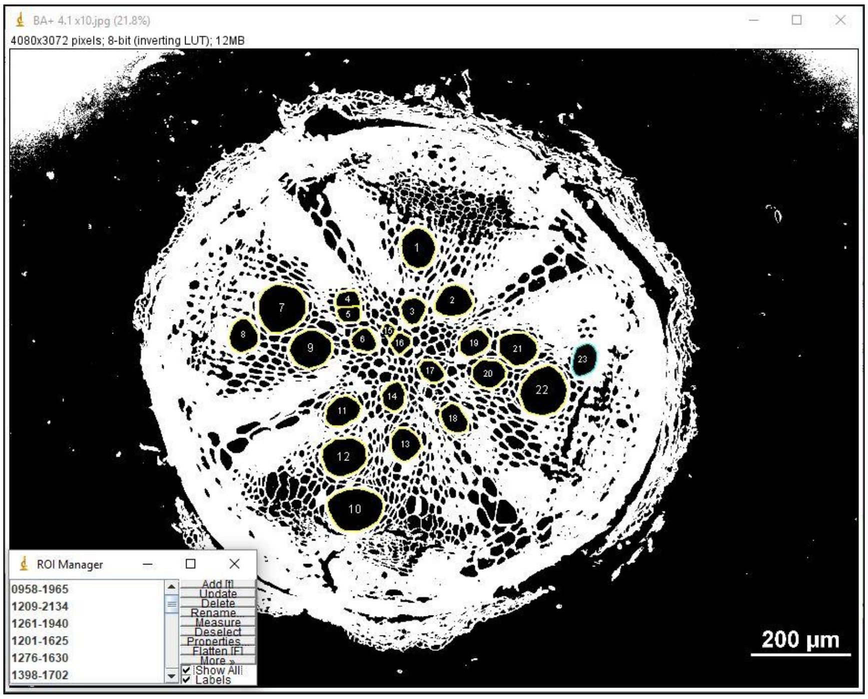


Figure 2.7 The ‘paintbrush tool’ in ImageJ was used if the threshold adjustments did not meet requirements for analysis. The edits presented above were performed on a *Banksia attenuata* root tissue sample. (A) areas within the vessels that did not fill out following threshold adjustments, (B) the final image after using the paintbrush tool to fill in holes.

2.8. Xylem traits

2.8.1. Vessel diameter

Vessel diameter was measured in ImageJ using the ‘Regions of Interest (ROI) Manager’ tool. which allows multiple selections and measurements to be made. For this study, the ROI tool was used to select all known xylem vessels and measure their cross-sectional area (V_A, µm^2) (Figure 2.8). The ROI tool performed better than the ‘Analyse Particles’ command, which was found to classify xylem vessels incorrectly.



BA+ 4.1 x10.jpg (21.8%)

4080x3072 pixels; 8-bit (inverting LUT); 12MB

ROI Manager

0958-1965
1209-2134
1261-1940
1201-1625
1276-1630
1398-1702

Figure 2.8 JPEG image in ImageJ showing selection of vessels in a root tissue sample of a *Banksia attenuata* seedling using the Wand (tracing) tool and ROI Manager tool to measure the cross sectional area (V_A, µm^2) of xylem vessels in order to calculate vessel diameter (*d*).

As suggested by James et al., (2003), only vessels that were more than half the diameter of the largest vessel in each sample were included in the calculation of mean vessel diameter. Although images analysed for this study are believed to be sufficiently accurate, this method was followed to avoid the inclusion of vessels that may have been tapering, and to avoid misclassifying parenchyma cells as xylem vessels, as the largest 50% of vessels will account for the bulk of the water transport.

The vessel diameter (*d*, µm), assuming a circular geometry, was calculated from the V_A of each vessel (Equation 2). For samples that had >100 vessels in total, a random number generator was used to select 100 vessels randomly.

$$d = \sqrt{\frac{4\,V_A}{\pi}} \qquad (3)$$

The mean vessel diameter (Vd, µm) and maximum vessel diameter (Dmax, µm) were then determined for each image.

The hydraulically-weighted mean vessel diameter (Tyree & Zimmermann, 2002) (Dh) was calculated as:

$$\mathrm{Dh} = \left(\frac{\sum d^4}{N}\right)^{\frac{1}{4}} \qquad (4)$$

where N is the number of vessels.

2.8.2. Vessel density

Xylem vessel density, the number of vessels per unit of cross-sectional area (Dv, mm^{-2}) was also determined from images of cross sections. For younger, and therefore smaller samples, the 'freehand selections' tool of ImageJ was used to select the area of interest, equivalent to the total cross-sectional area of sapwood, comprising of xylem (A, mm^2) and the ROI tool was used to measure this area. For stem samples, 5 vascular bundles were selected randomly, and the A measured for the individual bundles (Figure 2.9).

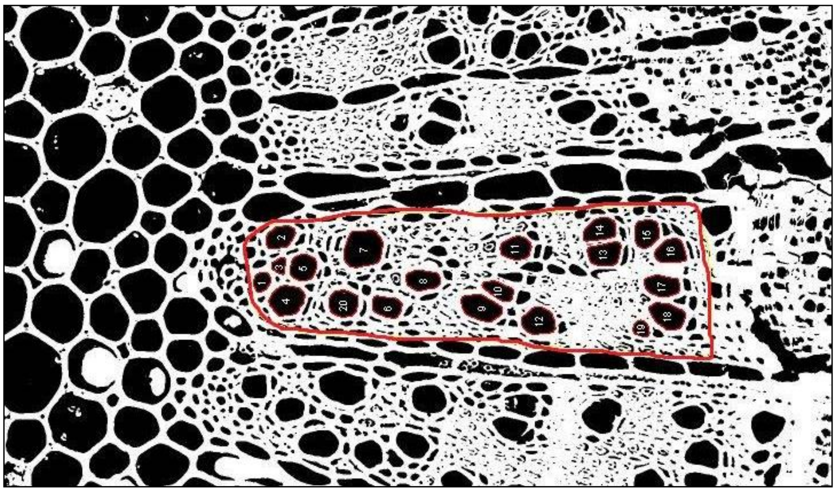


Figure 2.9 The approximate xylem area (A) was measured for each sample. The number of vessels within the A was recorded and later used to calculate the density of vessels for that sample. The above example was performed on stem tissue of a *Banksia attenuata* seedling.

All xylem vessels were counted within the measured A and Dv calculated as:

$$\mathrm{Dv} = \frac{N}{A} \qquad (5)$$

For the samples with 5 randomly measured vascular bundles, the mean *A* was taken and then the mean value of *A* and *N* were substituted into equation 5 to estimate Dv.

2.8.3. Theoretical hydraulic conductivity (K_{th})

Following the method in Pfautsch *et al.* (2016), the Hagen-Poiseuille equation was used to calculate the theoretical hydraulic conductivity (K_{th}, kg s^{-1} m^{-1} MPa^{-1}) of sapwood for each sample:

$$K_{\mathrm{th}} = \frac{\mathrm{D}_{\mathrm{h}}^{4}\pi}{128\eta} \times V_{\mathrm{D}} \times 1000 \qquad (6)$$

Where Dh is the hydraulically weighted diameter (converted to m), and η is the viscosity of water (1.002 x 10^{-9}, MPa s, at $20^{\circ C}$).

2.9. Data analysis

2.9.1. Objective One and Three

Objective one focussed on how the treatments (connected and disconnected) and sample locations (Root 1, Root 2, and Stem (sampled as ‘Stem 2’; Figure 2.2)) may have influenced differences in the development of xylem traits, particularly vessel diameter and vessel density. Objective three focussed on the interaction between the two species (*Banksia attenuata* and *B. littoralis*) and the sample locations (Root 1, Root 2, and Stem).

To statistically test objective one, Two-way ANOVAs were used to understand if there was an interaction between the two independent variables, treatment and sample location (Root 1, Root 2 and Stem)), on a range of dependent variables (mean vessel diameter, maximum vessel diameter, vessel density, hydraulically-weighted vessel diameter and theoretical hydraulic conductivity). Two-way ANOVAs were also used to test objective three, with the only difference being the replacement of the independent variable, treatment, with another independent variable, species. All analyses were carried out using the statistical software IBM SPSS Statistics (Version 24.0, IBM Corp., Armonk, NY, USA). performing these ANOVAs, preliminary tests were performed to assess whether the data met the required assumptions, in particular:

a) No outliers: Boxplots.

b) Normality: Shapiro-Wilk test of normality ($p > 0.05$).

c) Homogeneity of variances: Levene’s test for equality of variances ($p > 0.05$).

If outliers were present (data points that differed significantly to the main data points), the data was checked, and the outlier removed if deemed necessary. A Shapiro-Wilk test assessed normality, and transformations were applied if the assumption of normality was violated. The significance level was set at $\alpha = 0.05$ for the Two-way ANOVA and post-hoc tests. A Bonferroni adjustment for multiple comparisons was applied to all Two-way ANOVAs. If an analysis required a multiple comparison post-hoc test, the Tukey HSD was used.

Using SPSS, separate Independent-samples T-tests were performed on Root (Root 1 and Root 2 pooled) and Stem (Stem 2) samples for each species and treatment. The data was again assessed for outliers and normality.

To test the scaling relationship of vessel diameter and vessel density, a standardised major axis (SMA) regression was performed using the SMATR package in R (Warton, Duursma, Falster, & Taskinen, 2015).

2.9.2. *Objective Two*

Objective two used a One-way ANOVA and SMA regressions to analyse differences between sample locations in plants with relation to the tapering theory.

Preliminary tests were performed to assess whether the data met the following required assumptions.

a) No outliers: Boxplots

b) Normality: Shapiro-Wilk test of normality ($p > 0.05$).

c) Homogeneity of variances: Levene’s test for equality of variances ($p > 0.05$).

If outliers were present (data points that differed significantly to the main data points), the data was checked, and the outlier removed if deemed necessary. If outliers could not be rectified, a non-parametric Kruskal-Wallis H test was used. If data was found to be non-normal ($p < 0.05$), it was decided to proceed regardless of the violation, as one-way ANOVAs are fairly ‘robust’ (Lix, Keselman, & Keselman, 1996). A Levene’s test was used to check for homogeneity of variances. If the homogeneity of variances was violated ($p < 0.05$), there were no changes made to the data set, instead a Welch ANOVA was performed.

The significance level was set at $\alpha = 0.05$. When an analysis required a multiple comparison post-hoc test, the Tukey HSD was used for a standard one-way ANOVA, and a Games-Howell post-hoc test was used for a Welch ANOVA.

The boxplot diagram below (Figure 2.10) shows the breakdown of boxplots presented throughout the Results section of this report.

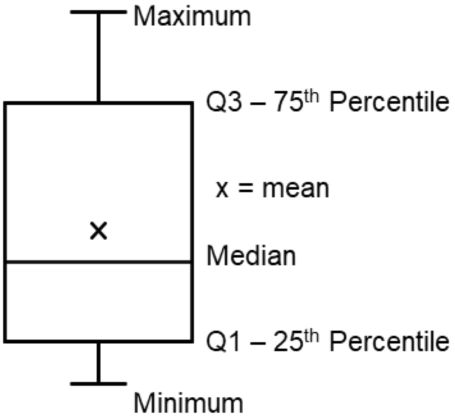


Figure 2.10 Legend for the boxplot format used for data presented throughout the Results section of this report. Each boxplot shows the median and mean observation, as well as the lower (Q1) and upper (Q3) quartiles.

3. Results

3.1. Objective 1

Compare the conduit traits of *Banksia* seedlings before and after establishment with a shallow water table with individuals that have no access to a water table

1. *H_0: There is no difference in the xylem traits of* Banksia *seedlings after 55 days of growth (Harvest 1) compared to seedlings after 174 days of growth (Harvest 2).*
2. *H_0: There is no difference in the traits (anatomical or morphological) of* Banksia *seedlings of equivalent age with or without access to (and use of) a water table.*

3.1.1. Comparison of harvests

H_0: There is no difference in the xylem traits of Banksia *seedlings after 55 days of growth (Harvest 1) compared to seedlings after 174 days of growth (Harvest 2).*

Stem height and root lengths increased with age in *Banksia attenuata* and *B. littoralis* seedlings. At the time of Harvest 1, the stem heights and root lengths were similar across both treatments. After letting the remaining seedlings grow for a further ~120 days, there were significant increases seen in both stem height and root lengths for both species (Appendix Table B.1 – B.4). *Banksia attenuata* seedlings indicated a faster root elongation rate than *B. littoralis* seedlings (Figure 3.1). Both species in the connected treatment had reached the zone of saturation at the time of Harvest 2, while those in the disconnected treatment continued to extend their roots towards the bottom of the PVC tubes (200 cm) in search of water.

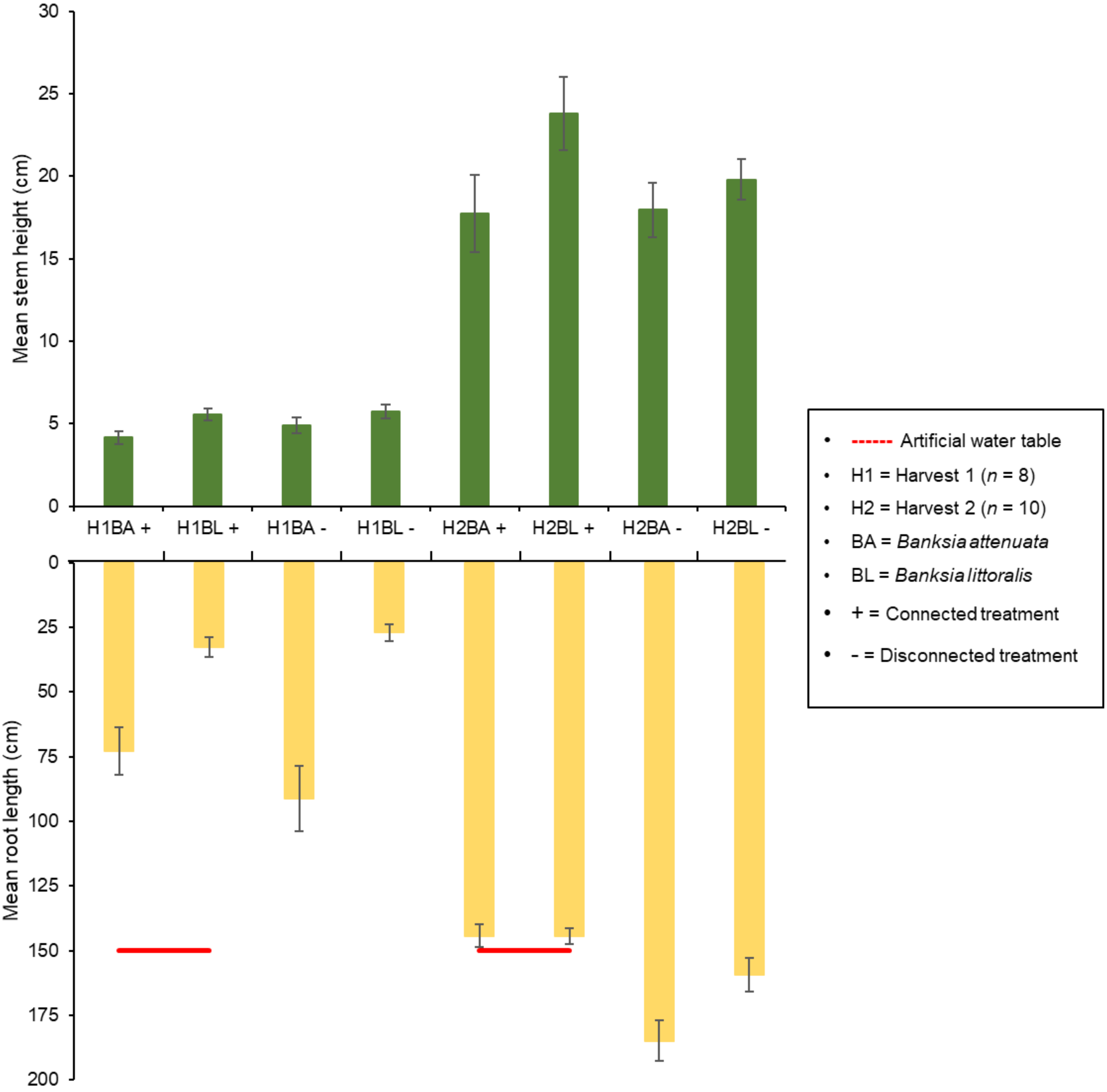


Figure 3.1 Mean above ground (stem height, cm) and below ground (root length, cm) heights of seedlings (BA = *Banksia attenuata*; BL = *Banksia littoralis*) from both harvests (H1 = Harvest 1; H2 = Harvest 2) and both treatments (+ = Connected; - = Disconnected). (Error bars represent the standard error of mean values).

After assessing the differences in morphological traits, we then wanted to determine if these changes with age (increased height and root length) would also be reflected at an anatomical level within the seedlings. To do this, Harvest 1 anatomical results were compared with Harvest 2 anatomical results for both species and both treatments.

Banksia attenuata – *Disconnected treatment*

The first comparison was made between *Banksia attenuata* seedlings at 55 days old and 174 days old, both growing without access to an artificial water table. There was a significant interaction between the age of seedlings (time of harvest) and sample location on vessel diameter (Vd) ($p < .001$, $f = 12.343$) and vessel density (Dv) ($p < .001$, $f = 22.081$) (Appendix Table A.1).

Vessel diameter was found to increase with age across all 3 sample locations, with the most significant increase seen in Root 1 and 2 samples. Age had an opposite effect on vessel density, with all sample locations decreasing the number of vessels per mm^2 in Harvest 2. The increase in Vd and decrease in Dv resulted in significant differences between Harvest 1 and Harvest 2 ($p < 0.05$) (Figure 3.2, Table 3.1). Stem and Root 1 samples from Harvest 1 had a significantly higher density of vessels when compared to Harvest 2 samples (Figure 3.2, Table 3.1). The maximum vessel diameter (Dmax) also increased across all sample locations with age, with the biggest increase in Dmax seen in Root 1 samples (36.84 µm in Harvest 1 to 94.83 µm in Harvest 2), and the smallest increase in Stem samples (21.31 µm in Harvest 1 to 23.07 µm in Harvest 2).

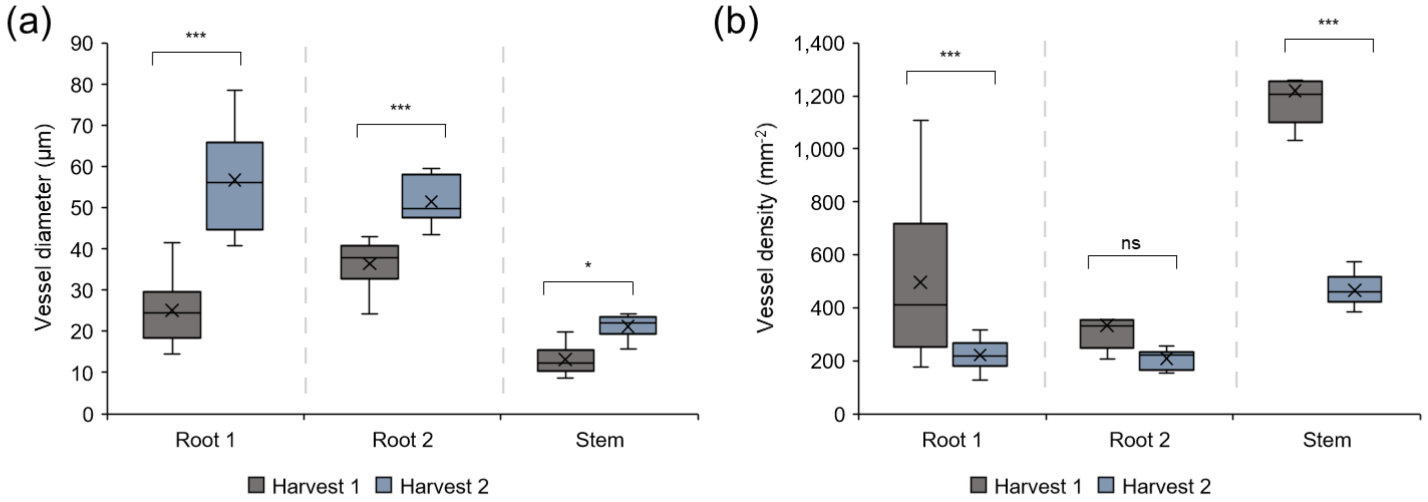


Figure 3.2 Comparison of xylem traits between Harvest 1 and Harvest 2 in *Banksia attenuata* seedlings in the disconnected treatment. **(a)** vessel diameter (Vd), **(b)** vessel density (Dv). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = a non-significant difference.

Table 3.1 Mean and standard deviation of xylem vessel diameter (Vd) and vessel density (Dv) in *Banksia attenuata* seedlings at 55 days old (Harvest 1, $n = 8$) compared to 174 days old (Harvest 2, $n = 10$) in the disconnected treatment. Different letters indicate a significant difference in a column ($p < 0.05$).

	Vd		
	Root 1	Root 2	Stem
Harvest 1	25.02 ± 8.44^{a}	36.44 ± 5.97^{a}	13.10 ± 3.55^{a}
Harvest 2	56.71 ± 12.27^{b}	51.53 ± 5.57^{b}	21.18 ± 2.80^{b}

	Dv		
	Root 1	Root 2	Stem
Harvest 1	495.88 ± 313.39^{a}	333.88 ± 104.39^{a}	1219.38 ± 169.80^{a}
Harvest 2	221.80 ± 55.25^{b}	208.30 ± 35.94^{a}	466.70 ± 60.48^{b}

Banksia attenuata – Connected treatment

The findings were similar for the connected treatment, with Vd increasing significanlty with age, and Dv again decreasing ($p < 0.05$) (Figure 3.3,

Table 3.2). There was a significant interaction between the age of seedlings (time of harvest) and sample location on Vd ($p < 0.05$, $f = 4.961$) and Dv ($p < .001$, $f = 60.243$) (Appendix Table A.2).

All three sample locations had a significantly higher density of vessels at the time of Harvest 1 when compared to Harvest 2 samples, with the greatest difference seen in Stem samples (Figure 3.3, Table 3.2). The maximum vessel diameter (Dmax) again increased across all sample locations with age, with the biggest increase in Dmax again seen in Root 1 samples (38.61 µm in Harvest 1 to 92.33 µm in Harvest 2), and the smallest increase in Stem samples (18.31 µm in Harvest 1 to 32.10 µm in Harvest 2).

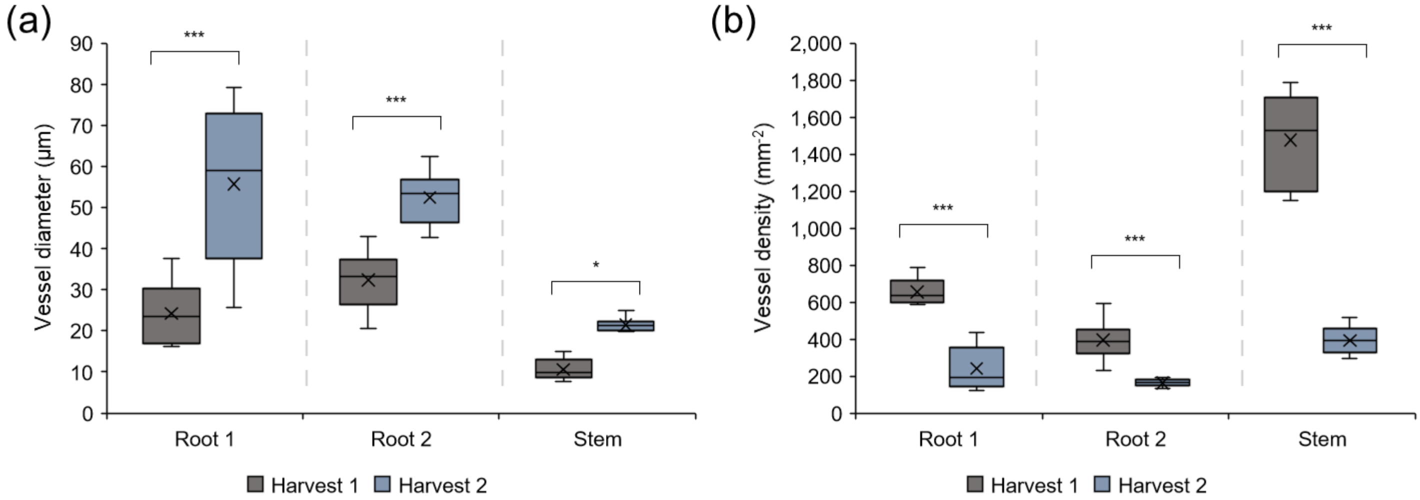


Figure 3.3 Comparison of xylem traits between Harvest 1 and Harvest 2 in *Banksia attenuata* seedlings in the connected treatment. **(a)** vessel diameter (Vd), **(b)** vessel density (Dv). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = a non-significant difference.

Table 3.2 Mean and standard deviation of xylem vessel diameter (Vd) and vessel density (Dv) in *Banksia attenuata* seedlings at 55 days old (Harvest 1, $n = 8$) compared to 174 days old (Harvest 2, $n = 10$) in the connected treatment. Different letters indicate a significant difference in a column ($p < 0.05$).

	Vd		
	Root 1	Root 2	Stem
Harvest 1	24.24 ± 7.64^{a}	32.38 ± 7.10^{a}	10.54 ± 2.62^{a}
Harvest 2	55.76 ± 19.25^{b}	52.49 ± 6.70^{b}	21.47 ± 1.56^{b}

	Dv		
	Root 1	Root 2	Stem
Harvest 1	655.00 ± 79.30^{a}	394.00 ± 108.15^{a}	1477.38 ± 244.83^{a}
Harvest 2	241.30 ± 117.53^{b}	163.20 ± 18.18^{b}	392.40 ± 75.24^{b}

Banksia littoralis – Disconnected treatment

There was a significant interaction found between the age of the seedlings (time of harvest) and sample location on Vd ($p < 0.05$, $f = 4.12$) and Dv ($p < .001$, $f = 26.690$) (Appendix Table A.3). Age contributed to significant differences in the vessel diameter of Root 1, Root 2 and Stem samples of *Banksia littoralis* seedlings. Vessel density was also significantly different with age in Root 1 and Root 2 samples ($p < 0.05$) (Figure 3.4, Table 3.3). There were no differences found in the Dv of Stem samples from Harvest 1 to Harvest 2.

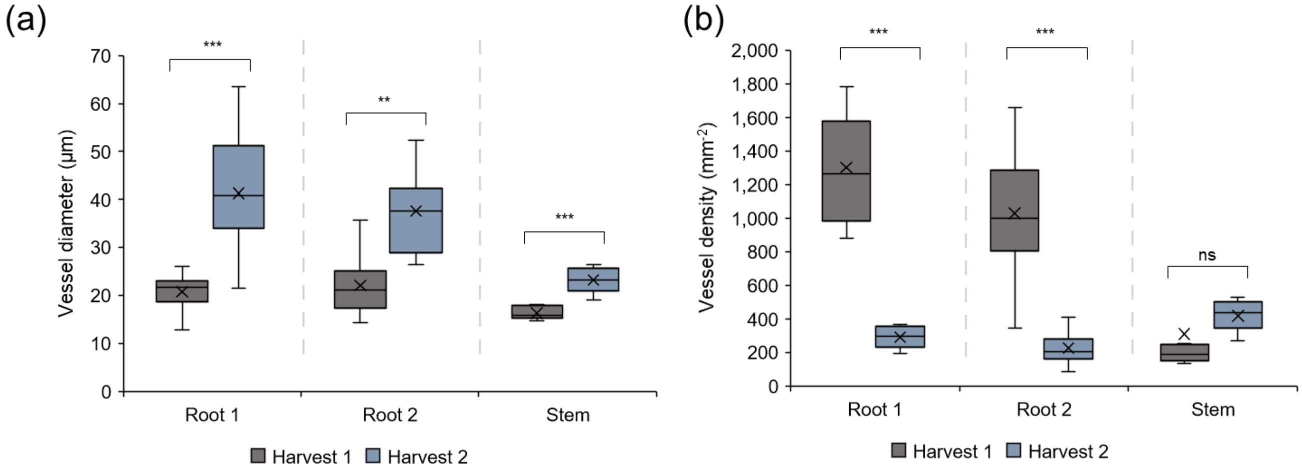


Figure 3.4 Comparison of xylem traits between Harvest 1 and Harvest 2 in *Banksia littoralis* seedlings in the disconnected treatment. **(a)** vessel diameter (Vd), **(b)** vessel density (Dv). * = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = a non-significant difference.

Table 3.3 Mean and standard deviation of xylem vessel diameter (Vd) and vessel density (Dv) in *Banksia littoralis* seedlings at 55 days old (Harvest 1) compared to 174 days old (Harvest 2) in the disconnected treatment. Different letters indicate a significant difference in a column ($p < 0.05$).

	Vd		
	Root 1	Root 2	Stem
Harvest 1	20.75 ± 3.89^{a}	22.02 ± 6.59^{a}	16.27 ± 1.38^{a}
Harvest 2	41.31 ± 12.30^{b}	37.57 ± 8.40^{b}	23.17 ± 2.46^{b}

	Dv		
	Root 1	Root 2	Stem
Harvest 1	1300.63 ± 320.72^{a}	1028.75 ± 390.08^{a}	307.75 ± 352.84^{a}
Harvest 2	290.10 ± 63.86^{b}	223.60 ± 92.43^{b}	417.10 ± 88.67^{a}

Banksia littoralis – Connected treatment

There were more significant differences with age in the connected treatment for *B. littoralis* seedlings. Vessel diameter increased significanlty with age across all three sample locations, and Dv again decreased in Root 1 and Root 2 samples ($p < 0.05$) (Figure 3.5, Table 3.4). There was also a significant difference in the Dv of Stem samples, with Harvest 2 having a higher mean Dv than Harvest 1, a result that was inconsistent with the patterns of the other sample locations (decline in Dv with age) (Figure 3.5, Table 3.4).

Both Root 1 and Root 2 samples had a significantly higher density of vessels at the time of Harvest 1 when compared to Harvest 2 samples, while Stem samples had a lower Dv in Harvest 1 compared to Harvest 2 (Figure 3.5, Table 3.4). The maximum vessel diameter (Dmax) increased across all sample locations with age, with the biggest increase in Dmax seen in Root 1 samples (30.87 µm in Harvest 1 to 60.62 µm in Harvest 2), and the smallest increase in Stem samples (26.52 µm in Harvest 1 to 35.02 µm in Harvest 2).

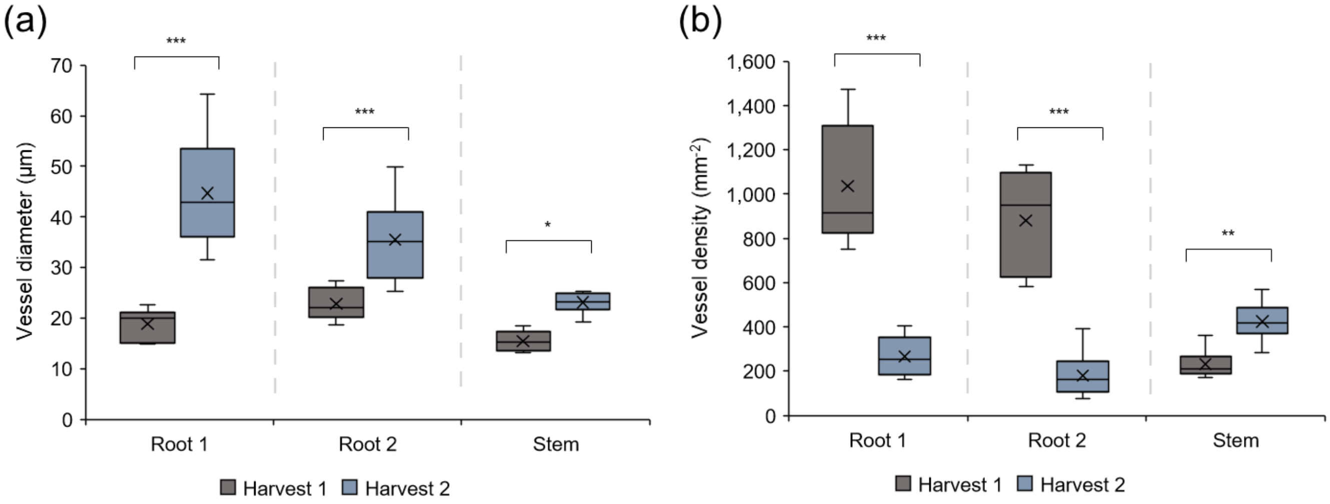


Figure 3.5 Comparison of xylem traits between Harvest 1 and Harvest 2 in *Banksia littoralis* seedlings in the connected treatment. **(a)** vessel diameter (Vd), **(b)** vessel density (Dv). * = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = a non-significant difference.

Table 3.4 Mean and standard deviation of xylem vessel diameter (Vd) and vessel density (Dv) in *Banksia littoralis* seedlings at 55 days old (Harvest 1) compared to 174 days old (Harvest 2) in the connected treatment. Different letters indicate a significant difference in a column ($p < 0.05$).

	Vd		
	Root 1	Root 2	Stem
Harvest 1	18.85 ± 3.20^{a}	22.81 ± 3.08^{a}	15.48 ± 2.19^{a}
Harvest 2	44.66 ± 10.87^{b}	35.51 ± 7.99^{b}	23.12 ± 2.00^{b}

	Dv		
	Root 1	Root 2	Stem
Harvest 1	1035.80 ± 282.33^{a}	880.75 ± 223.99^{a}	232.13 ± 61.77^{a}
Harvest 2	265.40 ± 83.83^{b}	180.20 ± 94.32^{b}	424.22 ± 84.89^{b}

3.1.2. Comparison of treatments (connected Vs disconnected)

H_0: There is no difference in the traits (anatomical or morphological) of Banksia *seedlings of equivalent age with or without access to (and use of) a water table.*

The second part of Objective one was looking at whether the treatment influenced the development of the seedlings, particularly whether the seedlings anatomical traits varied when growing with access to an artificial water table.

Harvest 1

<u>Anatomical traits</u>

Xylem vessel diameter and vessel density

Banksia attenuata

At the time of Harvest 1, it was predicted that the roots of the seedlings in the connected treatment would not yet have reached the artificial water table, and therefore significant differences between the two treatments were not expected. Figure 3.6 shows that the roots of both *B. attenuata* and *B. littoralis* seedlings were not yet reaching the capillary fringe of the artificial water table.

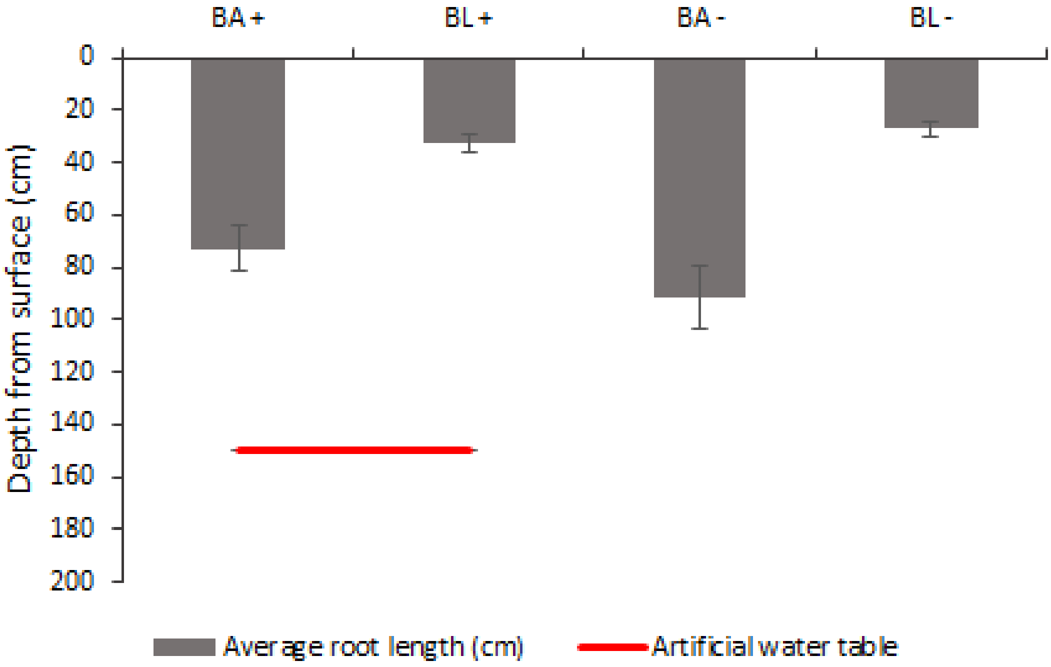


Figure 3.6 Average rooting depth (cm) of *Banksia attenuata* and *Banksia littoralis* seedlings sampled at 55 days old, showing that seedlings in the connected (+) treatment were not yet in contact with the artificial water table at the time of the first harvest. (- = disconnected treatment, or no artificial water table). BA = *Banksia attenuata*, BL = *Banksia littoralis.* (Error bars represent the standard error of mean values).

The findings supported the null hypothesis with no significant interaction found between treatment and sample location on vessel diameter (Vd) (*p* = 0.761, *f* = 0.274) and vessel density (Dv) (*p* = 0.345, *f* = 1.093) of *B. attenuata* seedlings (Figure 3.7, Appendix Table A.5). This meant that the treatments did not have an influence on the development of xylem traits in the seedlings.

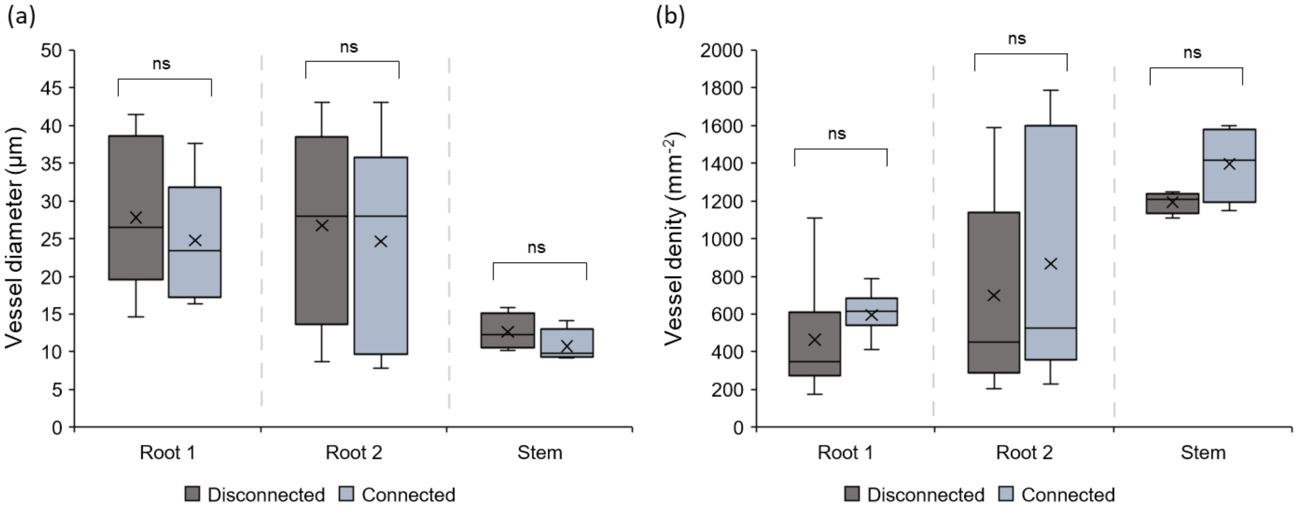


Figure 3.7 Comparison of xylem anatomical traits between connected and disconnected treatments in *Banksia attenuata* seedlings from Harvest 1. **(a)** vessel diameter (Vd) and **(b)** vessel density (Dv). ns = a non-significant difference between the two treatments on individual sample locations (*p* > 0.05).

Irrespective of the treatment, it was found that both Vd and Dv measurements were significantly different across the three sample locations ($p < .001$) (Table 3.5).

Table 3.5 Mean and standard error of xylem vessel diameter (Vd) and vessel density (Dv) in different plant organs sampled from *Banksia attenuata* and *Banksia littoralis* seedlings at 55 days old. Different letters indicate a significant difference in a column (p < 0.05).

Banksia attenuata			*Banksia littoralis*		
Plant Organ	Vd	Dv	Plant Organ	Vd	Dv
Root 1	24.63 ± 1.57^{a}	575.41 ± 47.30^{a}	Root 1	19.80 ± 1.02^{a}	1168.22 ± 83.59^{a}
Root 2	34.41 ± 1.57^{b}	364.02 ± 47.30^{b}	Root 2	22.41 ± .947^{a}	954.62 ± 73.31^{a}
Stem	11.82 ± 1.57^{c}	1348.47 ± 47.30^{c}	Stem	15.88 ± .947^{b}	269.98 ± 73.31^{b}

A Tukey post-hoc test was used to determine where the differences lay. Focussing first on Vd, it was found that all three sample locations in *B. attenuata* seedlings were significantly different to each other, with the largest Vd in Root 2 samples, followed by Root 1 and Stem samples respectively ($p < 0.05$) (Figure 3.8b). The Dv was significantly larger in Stem samples of *B. attenuata* compared to both Root 1 and Root 2 samples ($p < 0.05$). The Dv of Root 1 and Root 2 samples were not significantly different from one another ($p > 0.05$) (Figure 3.8b).

A standardised major axis (SMA) regression analysis was used to assess the scaling relationship between Vd and Dv (Figure 3.8a). There was a correlation between the Vd and Dv of all groups, as well as Stem and Root 2 samples (Figure 3.8a). There was no scaling relationship between Vd and Dv in Root 1 samples.

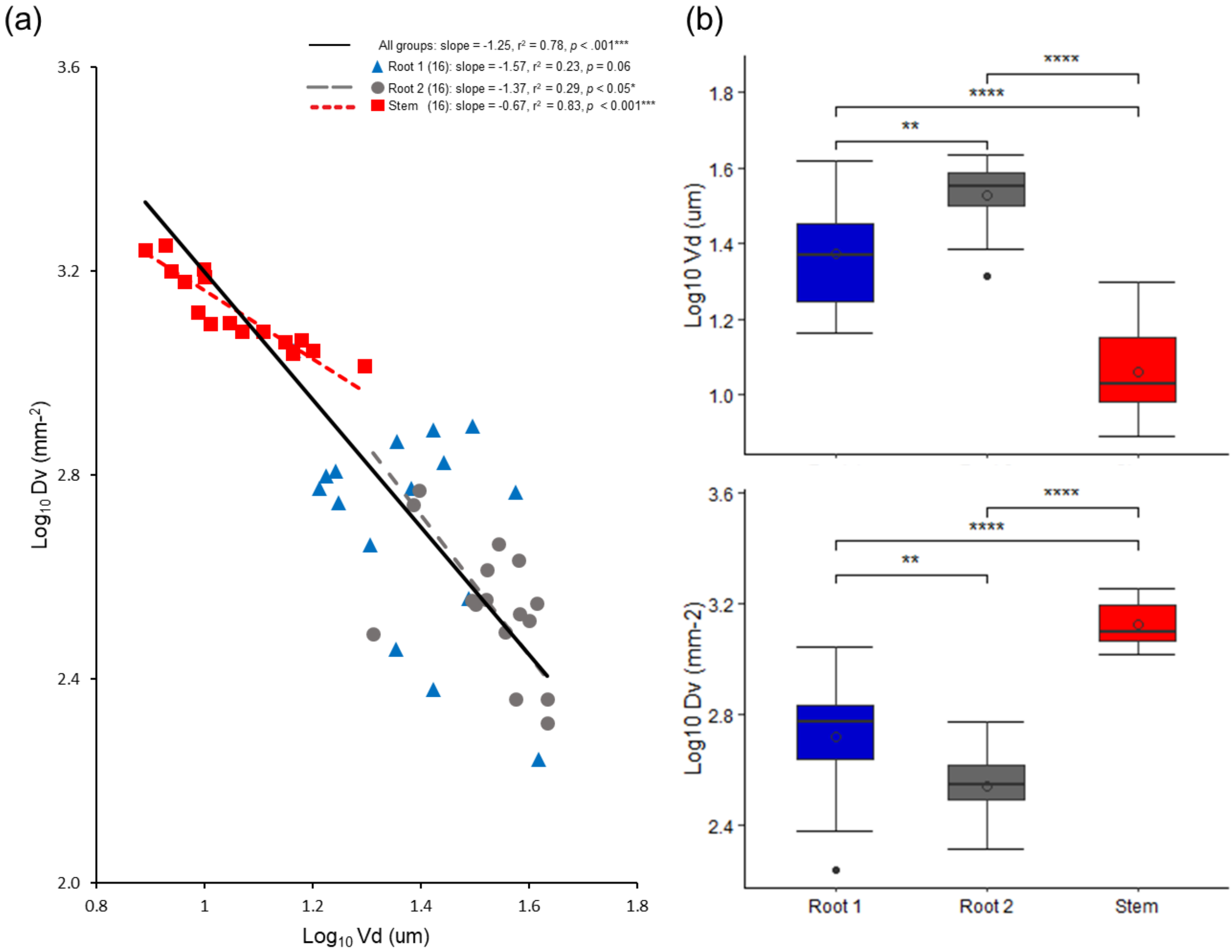


Figure 3.8 **(a)** Standaradised major axis (SMA) relationship between mean vessel diameter (Vd) and mean vessel density (Dv) in *Banksia attenuata* seedlings, **(b)** boxplots showing differences in Vd (top) and Dv (bottom) across sample locations. Log10 transformations have been applied to all data.

Banksia littoralis

The findings from *Banksia littoralis* seedlings in Harvest 1 also supported the null hypothesis with no significant interaction found between treatment and sample location on mean Vd ($p = 0.625$, $f = 0.476$) and Dv ($p = 0.698$, $f = 0.363$) (Figure 3.9, Appendix Table A.6). This was again supporting that the treatments were not influencing the development of xylem traits in the seedlings (Figure 3.6).

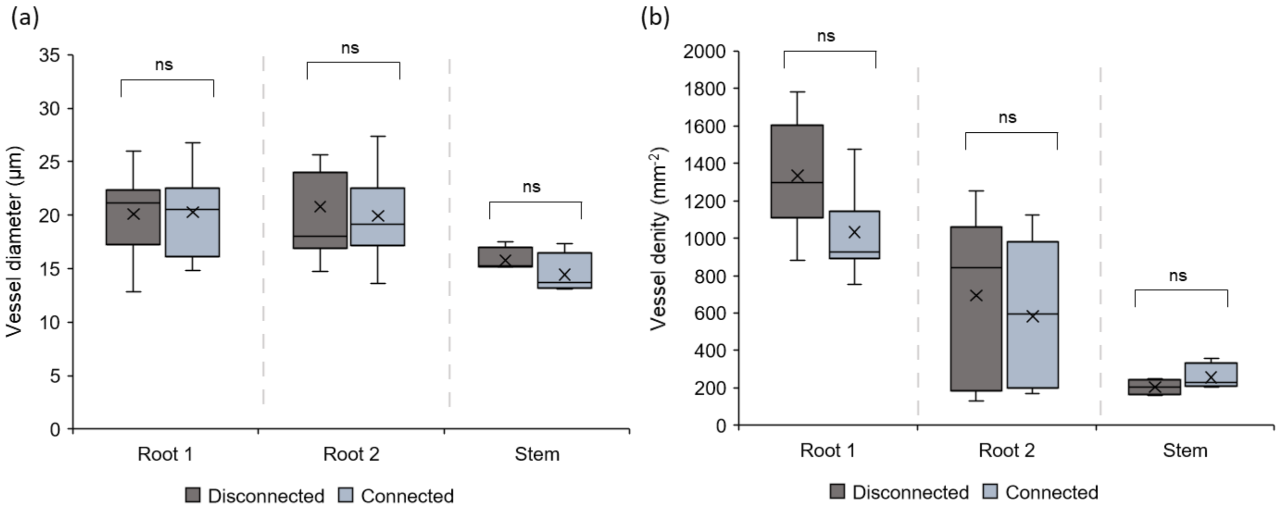


Figure 3.9 Comparison of xylem anatomical traits between connected and disconnected treatments in *Banksia littoralis* seedlings from Harvest 1. **(a)** vessel diameter (Vd) and **(b)** vessel density (Dv). ns = a non-significant difference between the two treatments on individual sample locations ($p > 0.05$).

Irrespective of the treatment, it was found that both Vd and Dv measurements were significantly different across the three sample locations ($p < .001$) (Table 3.5).

A Tukey post-hoc test was used to determine where the differences lay. The mean Vd was significantly different between Root 1 and Stem samples, and Root 2 and Stem samples ($p < 0.05$) (Figure 3.10b). Root 1 and Root 2 samples were not significantly different from one another ($p > 0.05$). The Dv was significantly lower in Stem samples compared to both Root 1 and Root 2 samples ($p < 0.05$). Root 1 and Root 2 samples were not significant ($p > 0.05$) (Figure 3.10b).

An SMA regression analysis found correlations between Vd and Dv for xylem in all groups, as well as across all 3 sample locations (Figure 3.10a).

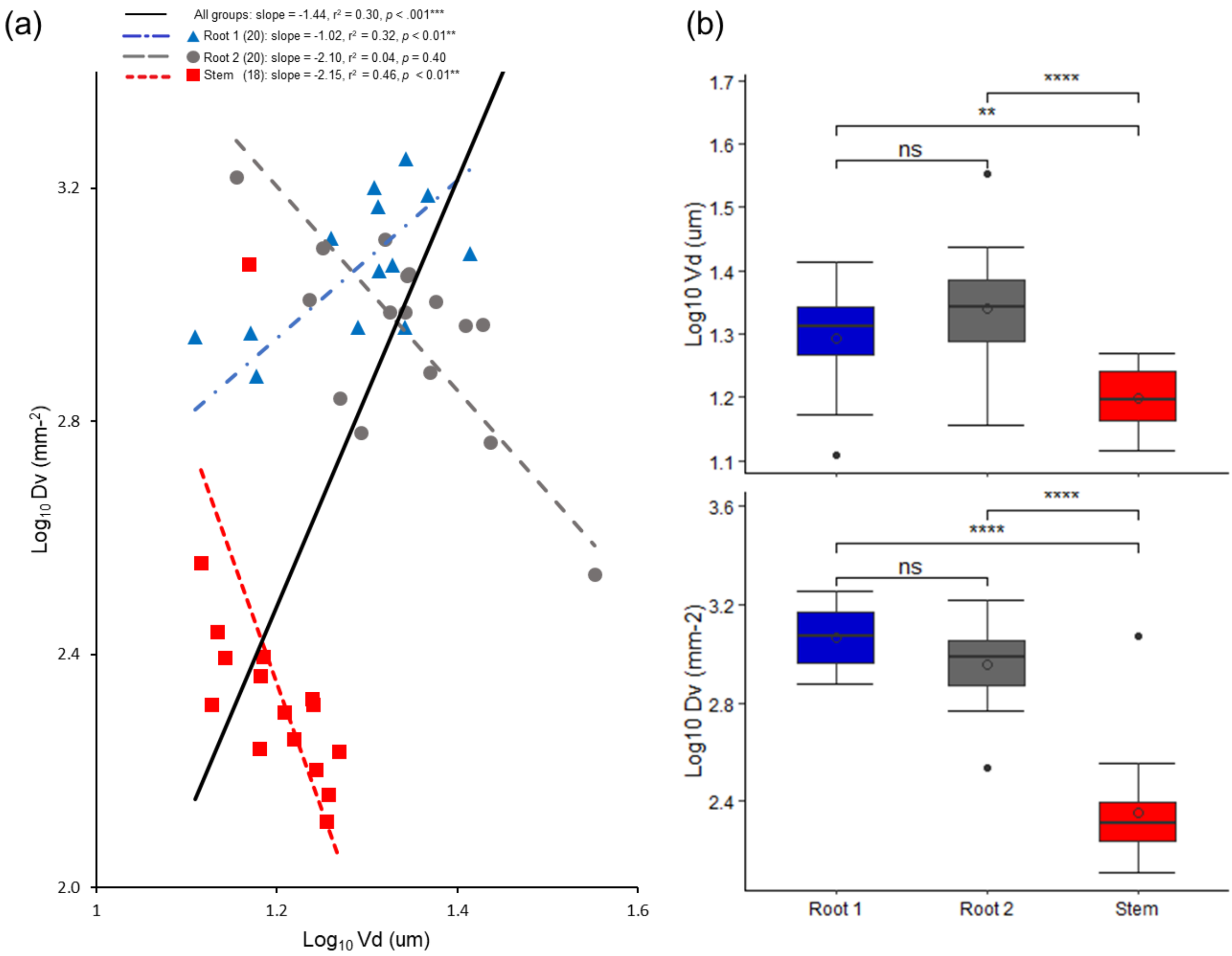


Figure 3.10 **(a)** Standaradised major axis (SMA) relationship between mean vessel diameter (Vd) and mean vessel density (Dv) in *Banksia littoralis* seedlings, **(b)** boxplots showing differences in Vd (top) and Dv (bottom) across sample locations. Log10 transformations have been applied to all data.

Differences in maximum vessel diameter (Dmax), hydraulically-weighted vessel diameter (Dh) and theoretical hydraulic conductivity (Kth) were also assessed between treatments and sample locations, results from separate Two-way ANOVAs for *B. attenuata* and *B. littoralis* can be found in Appendix A.

Root vessel length

The final trait measured, root vessel length, did not present any significant differences between the connected and disconnected treatments for *B. attenuata* seedlings ($p = 0.236$, $f = 1.593$) (Appendix B.5). *B. littoralis* root vessel lengths were all recorded as less than 1 cm in length, and therefore statistical analyses could not be performed (Appendix Table B.5).

Harvest 2

Harvest 2 was timed so that the roots of seedlings growing in the connected treatment should have been in contact with the capillary fringe for an extended period of growth. Figure 3.11 shows that the roots of both *B. attenuata* and *B. littoralis* seedlings in the connected treatment had reached the capillary fringe of the artificial water table, while those in the disconnected treatment grew beyond this mark, searching for water further down the tube. Figure 3.12 shows the difference in volumetric water content (VWC) between the connected and disconnected treatments. VWC was significantly higher in the connected treatment at both 160 cm and 180 cm.

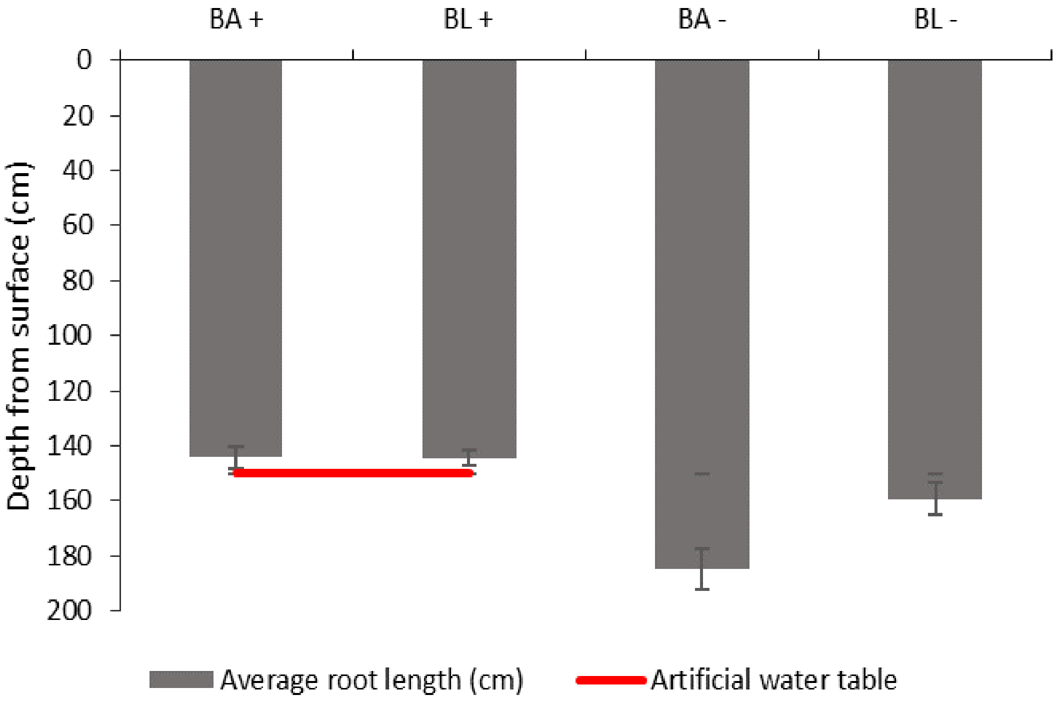


Figure 3.11 Average rooting depth (cm) of seedlings sampled at 174 days old, showing that seedlings in the connected (+) treatment were now in contact with the capillary zone of the artificial water table at the time of the second harvest. (- = disconnected treatment, or no artificial water table). BA = *Banksia attenuata*, BL = *Banksia littoralis.* (Error bars represent the standard error of mean values).

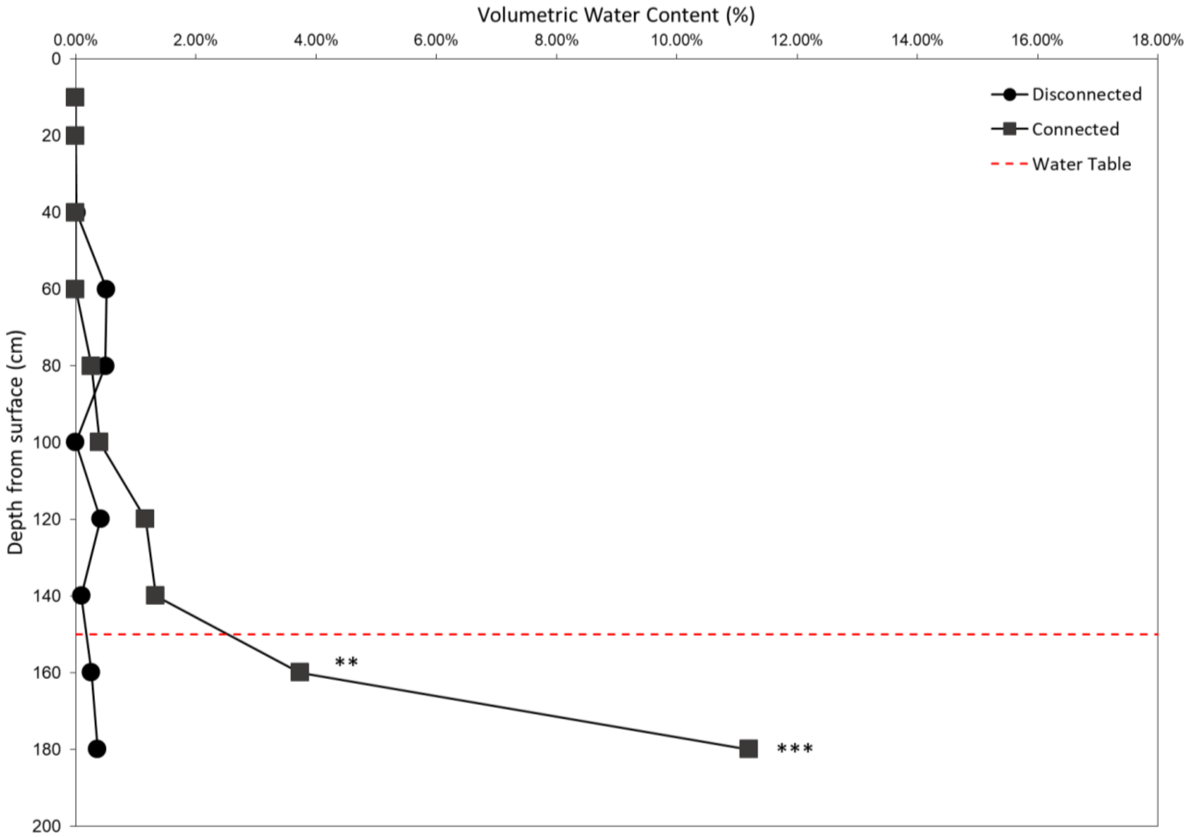


Figure 3.12 Comparison of mean volumetric water content (VWC %) in the connected and disconnected treatments at different depths. ** = p < 0.01, *** = p < 0.001. (*n* = 6).

Anatomical traits

Xylem vessel diameter and vessel density

Banksia attenuata:

Vessel diameter and density were similar between the two treatments. The treatments and sample locations presented a non-significant interaction for mean Vd (*p* = 0.954, *f* = 0.047) and Dv (*p* = 0.093, *f* = 2.486) of *B. attenuata* seedlings (Figure 3.13; Appendix Table A.7).

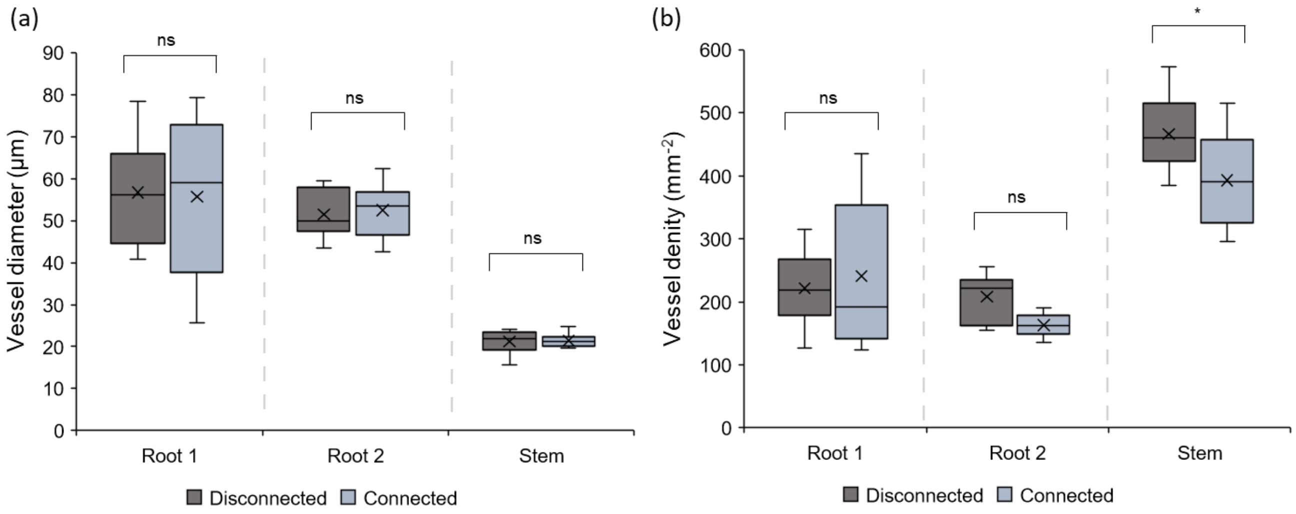


Figure 3.13 Comparison between connected and disconnected treatments in *Banksia attenuata* seedlings from Harvest 2. **(a)** Vessel diameter (Vd) **(b)** Vessel density (Dv). * = a significant difference between the two treatments (p <0.05), ns = a non-significant difference between the two treatments on individual sample location (p > 0.05).

Irrespective of the treatment, it was found that both Vd and Dv measurements were significantly different across the three sample locations (p < .001) (Table 3.6).

Table 3.6 Mean and standard error of xylem vessel diameter (Vd) and vessel density (Dv) in different plant organs sampled from *Banksia attenuata* and *Banksia littoralis* seedlings at 174 days old. Different letters indicate a significant difference in a column (p < 0.05).

Banksia attenuata			*Banksia littoralis*		
Plant Organ	Vd	Dv	Plant Organ	Vd	Dv
Root 1	56.23 ± 2.25^a	231.55 ± 15.22^a	Root 1	42.99 ± 1.87^a	277.75 ± 19.07^a
Root 2	52.01 ± 2.25^a	185.75 ± 15.22^a	Root 2	36.54 ± 1.87^b	201.90 ± 19.07^b
Stem	21.32 ± 2.25^b	429.55 ± 15.22^b	Stem	23.14 ± 1.87^c	420.66 ± 19.07^c

A Tukey post-hoc test was used to determine where the differences lay. Root 1 and Root 2 had significantly larger Vd measurements than samples collected from the Stem sample (p < 0.05) (Figure 3.14b). The difference in Vd between the root samples was not significant (p > 0.05). Dv was significantly larger in Stem samples of *B. attenuata* compared to both Root 1 and Root 2 samples (p < 0.05). The difference in Dv between Root 1 and Root 2 samples was not significant (p > 0.05) (Figure 3.14b).

A SMA regression analysis found correlations between Vd and Dv for xylem in all groups, as well as in both root sample locations (Root 1 and Root 2) (Figure 3.14a).

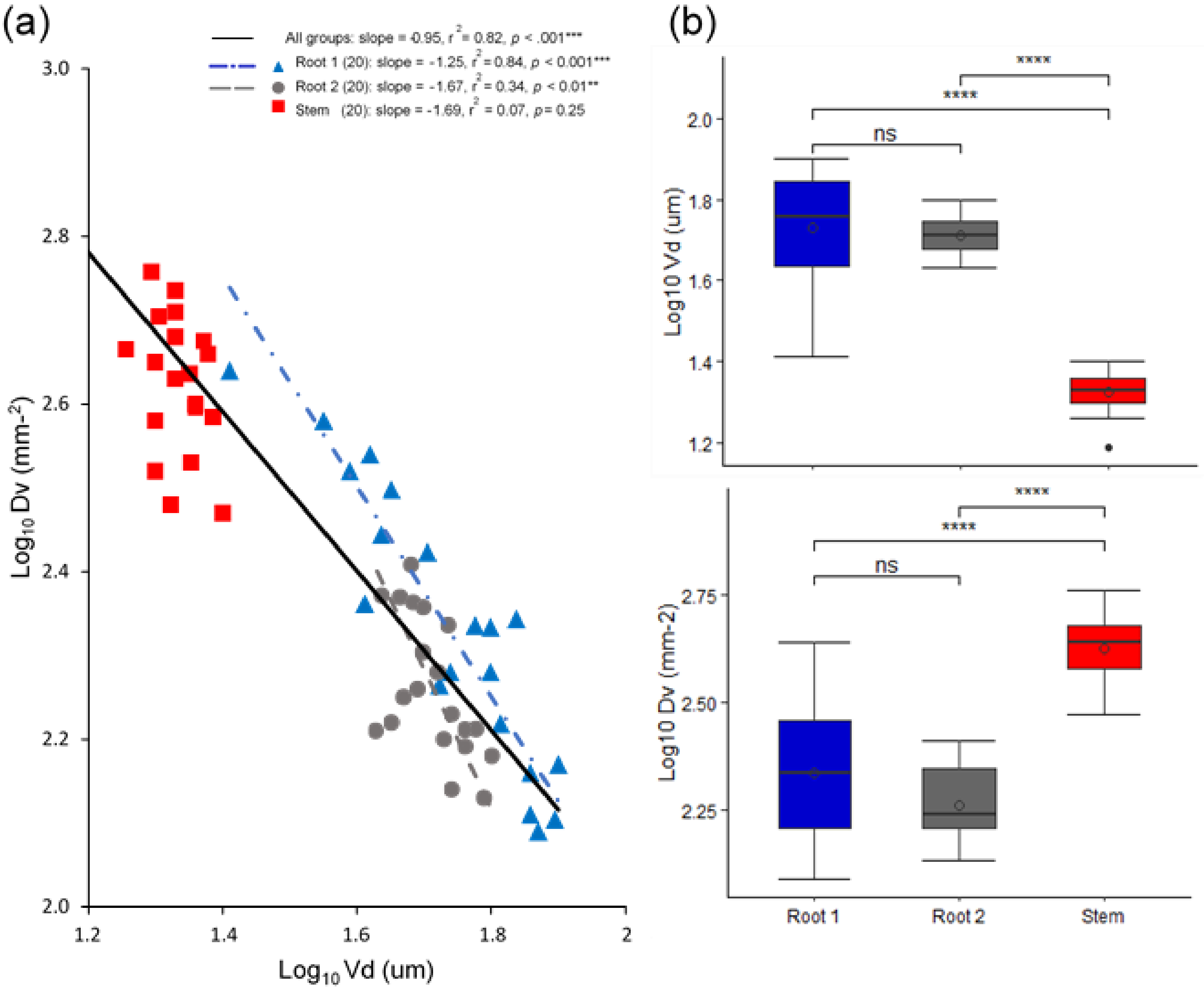


Figure 3.14 **(a)** Standaradised major axis (SMA) relationship between mean vessel diameter (Vd) and mean vessel density (Dv) in *Banksia attenuata* seedlings, **(b)** boxplots showing differences in Vd (top) and Dv (bottom) across sample locations. Log10 transformations have been applied to all data.

There were distinct shifts between the three sample locations and the diameter of vessels in both species (Figure 3.15). All sample locations for both species had positively skewed distributions (Figure 3.15).

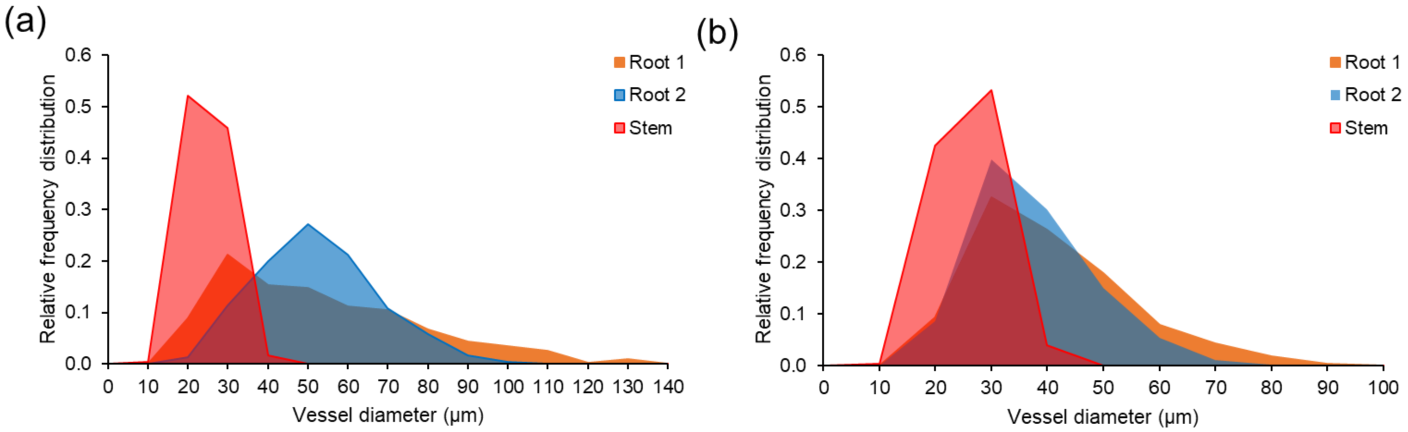


Figure 3.15 Relative frequency distribution of xylem vessel diameters. **(a)** Vessel diameters of *Banksia attenuata* seedlings; data were binned into the three sample locations, *Root 1* (n = 432), *Root 2* (n = 808) and *Stem* (n = 7011), and **(b)** *B. littoralis* seedlings; *Root 1* (n = 601), *Root 2* (n = 1334) and *Stem* (n = 6053).

Banksia littoralis:

Anatomical traits were again found to be similar between the two treatments. There was no significant interaction between treatment and sample location on Vd (p = 0.590, f = 0.534) and Dv (p = 0.650, f = 0.435) of *B. littoralis* seedlings (Figure 3.16; Appendix Table A.8). Further inspection found that Vd and Dv differed between sample locations within seedlings, irrespective of the treatment (p <.001) (Table 3.6).

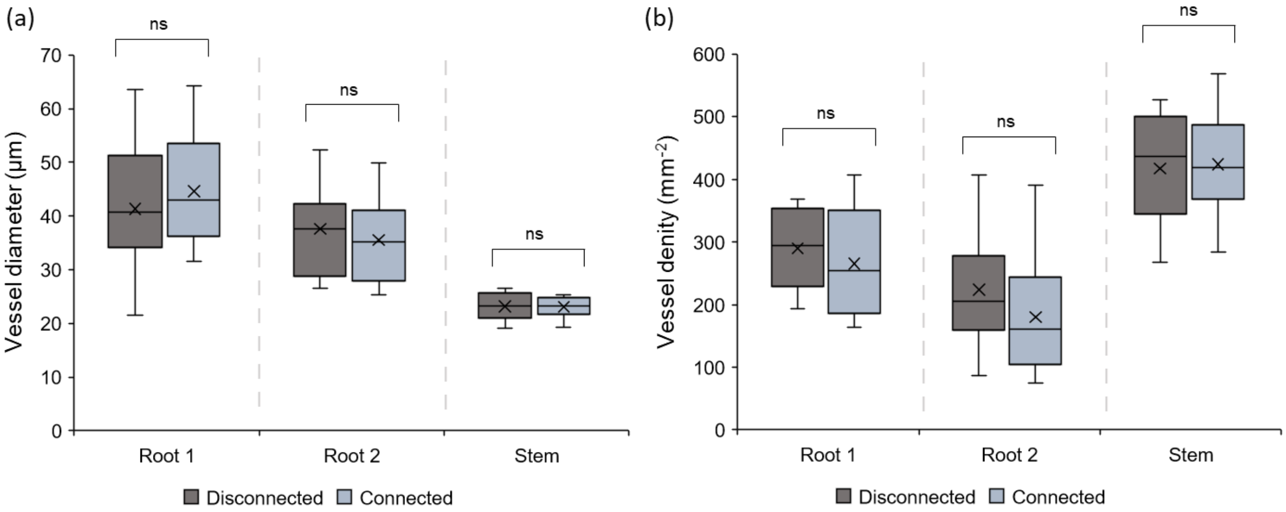


Figure 3.16 Comparison between connected and disconnected treatments in *Banksia littoralis* seedlings from Harvest 2. **(a)** Vessel diameter (Vd) **(b)** Vessel density (Dv). ns = a non-significant difference between the two treatments on individual sample location (p > 0.05).

A Tukey post-hoc test was used to determine where the differences lay. The mean Vd of *B. littoralis* seedlings followed a similar pattern to that of *B. attenuata* seedlings, with Root 1 samples having the largest mean Vd, followed by Root 2 and Stem respectively. The differences in mean Vd were all significantly different across the three sample locations ($p < 0.05$) (Figure 3.17b). The mean Dv of was also significantly different across all three sample locations ($p < 0.05$). The Stem samples had the largest mean Dv, followed by Root 1 and Root 2 samples respectively (Figure 3.17b).

An SMA regression analysis found correlations between Vd and Dv for xylem in all groups, as well as Root 1 and Stem sample locations (Figure 3.17a).

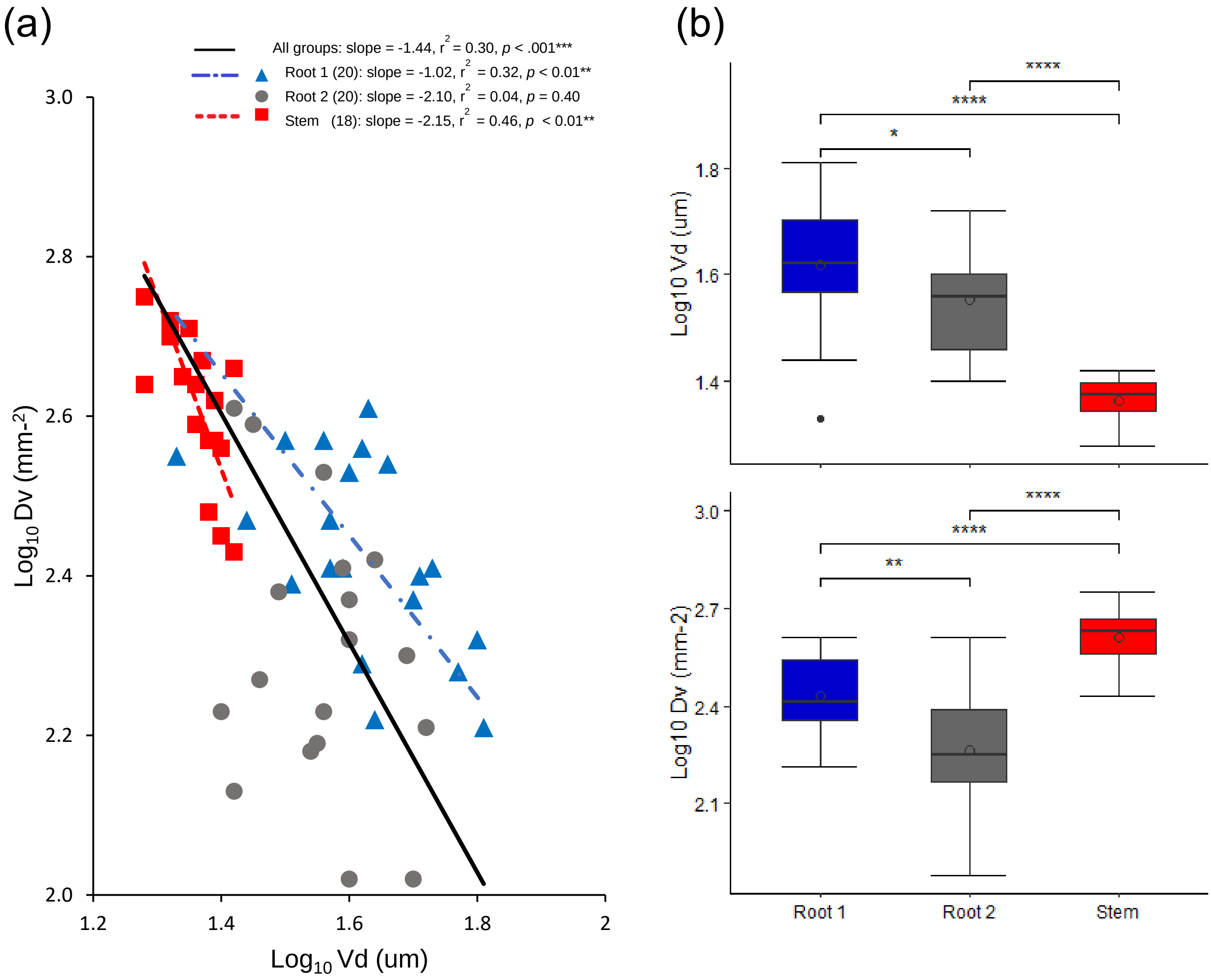


Figure 3.17 **(a)** Standaradised major axis (SMA) relationship between mean vessel diameter (Vd) and mean vessel density (Dv) in *Banksia littoralis* seedlings, **(b)** boxplots showing differences in Vd (top) and Dv (bottom) across sample locations. Log10 transformations have been applied to all data.

Differences in maximum vessel diameter (Dmax), hydraulically-weighted vessel diameter (Dh) and theoretical hydraulic conductivity (Kth) were also assessed between treatments and sample locations, results from separate Two-way ANOVAs for *B. attenuata* and *B. littoralis* can be found in Appendix A.

Root vessel length

Root vessel length in both the connected and disconnected treatments was not significantly different for both *B. attenuata* and *B. littoralis* respectively (Appendix Table B.8).

Morphological traits

Although the anatomical traits did not present any significant differences between the two treatments, there were some differences found in morphological traits.

Stem and root length

B. attenuata root length was found to be significantly larger in the disconnected treatment compared to the connected treatment ($p < .001$, $f = 19.069$). The stem length of *B. attenuata* seedlings however did not show any significant differences between the two treatments ($p = 0.945$, $f = 0.005$) (Appendix Table B.9). Alternatively, *B. littoralis* seedlings did not show any significant differences in root and stem lengths between the two treatments, ($p = 0.053$, $f = 4.276$; $p = 0.134$, $f = 2.469$) (Appendix Table B.10).

Stem-specific density

Stem-specific density (SSD) of *Banksia attenuata* was not significantly different between the connected and disconnected treatments, ($p = 0.199$, $f = 2.709$) (Appendix Table B.9). The same results were found for SSD of *B. littoralis* samples, ($p = 0.354$, $f = 0.905$) (Appendix Table B.10).

Independent-samples t-test

Although there were no significant differences found in vessel diameter (Vd) and vessels density (Dv) between the treatments for both species, it was decided to test whether these two traits differed between the pooled sample locations. To do this, an independent-samples t-test was conducted for both connected (+) and disconnected (-) treatments to determine if there were differences in Vd and Dv between Root (Root 1 and Root 2 pooled) and Stem (Stem 2) samples. Vessel diameter measurements were larger in Root samples across both species and both treatments (Table 3.7), while vessel density measurements were higher in Stem samples across both species and both treatments (Table 3.7), all significant differences ($p < .001$).

Table 3.7 Independent-samples T-test results comparing Root samples (Root 1 and Root 2 pooled) against Stem samples for $\log_{10}$ transformed vessel diameter (Vd) and vessel density (Dv) measurements in *Banksia attenuata* (BA) and *Banksia littoralis* (BL) seedlings. '+' = connected treatment; '-' = disconnected treatment. Data is presented as mean ± s.d.

		Log_{10} Vessel Diameter (Vd)				Log_{10} Vessel Density (Dv)			
		M	SD	*t*	*p*	M	SD	*t*	*p*
BA -	Root	1.727	0.074	14.842	< .001	2.323	0.097	-10.343	< .001
	Stem	1.322	0.061			2.666	0.056		
BA +	Root	1.718	0.122	9.789	< .001	2.273	0.162	-5.703	< .001
	Stem	1.331	0.031			2.587	0.084		
BL -	Root	1.581	0.117	5.668	<.001	2.384	0.163	-4.026	<.001
	Stem	1.363	0.047			2.611	0.100		
BL +	Root	1.590	0.110	5.983	<.001	2.306	0.202	-4.434	<.001
	Stem	1.363	0.039			2.620	0.089		

3.2. Objective 2

Investigate and confirm the presence of conduit tapering in individual plants and characterise whole-plant (seedling) hydraulic architecture.

1. *H_0: There is no conduit tapering in Banksia seedling root or hydraulic architecture.*

3.2.1. Harvest 1

As confirmed by addressing objective 1, there was no significant interaction between treatment and sample locations. Thus, in the following section on conduit taper analyses pertain to pooled data from both the connected and disconnected treatments.

Banksia attenuata

A tapering pattern was confirmed in *Banksia attenuata* seedlings with significant differences found in the vessel diameters (Vd) across sample locations ($p < .001$, $f = 52.563$). A subsequent Games-Howell post-hoc test found that the Vd of each sample location was significantly different to one another, excluding the two stem samples, Stem 1 and Stem 2, where there was no significant difference (Figure 3.18a). Although not significant, there was a slight decrease in Vd from Stem 1 to Stem 2. This result is the opposite to what is expected and is possibly related to where the sample was taken on the plants, or the health of the plants.

Vessel density (Dv) measurements also differed significantly across the sample locations (*p* < .001, *f* = 71.614), with Root 2 samples found to be significantly different to all other sample locations and Stem 2 samples significantly different to both Root 1 and Root 2 samples (Figure 3.18b).

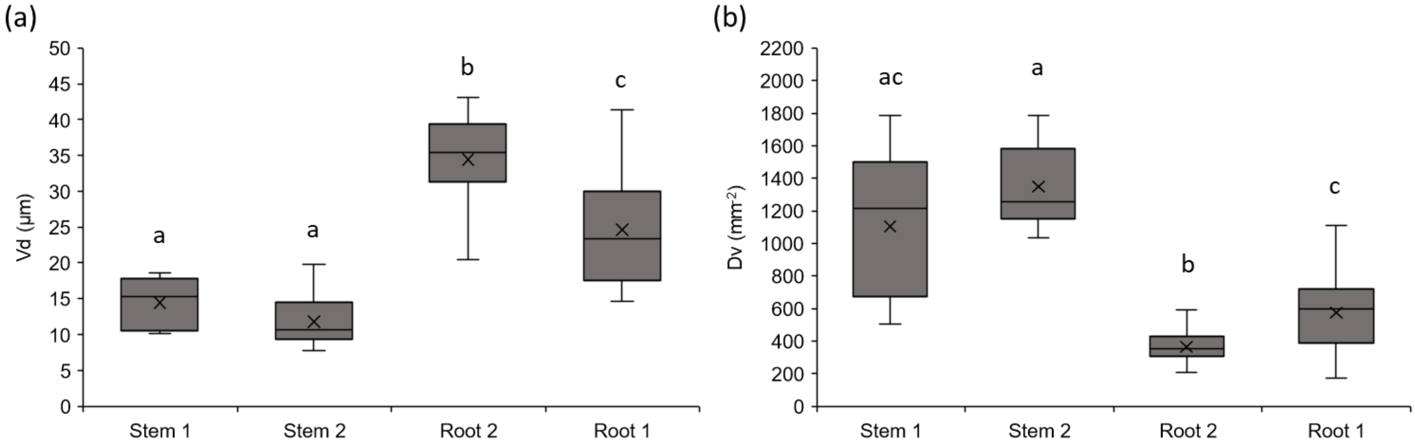


Figure 3.18 Presence of tapering basipetally in *Banksia attenuata* seedlings after 55 days of growth using mean values. (a) vessel diameter (Vd) across 4 sample locations (Stem 1, Stem 2, Root 2 and Root 1), (b) vessel density (Dv) across the same 4 sample locations. Different letters indicate a significant difference between sample locations (p < 0.05).

Banksia littoralis

A tapering pattern was also confirmed in *B. littoralis* seedlings. Vessel diameter (Vd) was found to differ significantly across sample locations (*p* < .001, *f* = 15.460), with Stem 2 samples being significantly different to both Root 1 and Root 2 samples, but not different to Stem 1 samples. Stem 1 samples were also significantly different to both Root 1 and Root 2 samples (Figure 3.19a). Root 1 and Root 2 samples were not significantly different from one another.

Significant differences were also found in the vessel density (Dv) measurements across sample locations (*p* < .001, *f* = 101.575). A follow-up Games-Howell post-hoc test found that both Stem 1 and Stem 2 samples were significantly different to all other sample locations, while Root 1 and Root 2 were not significantly different to one another (Figure 3.19b).

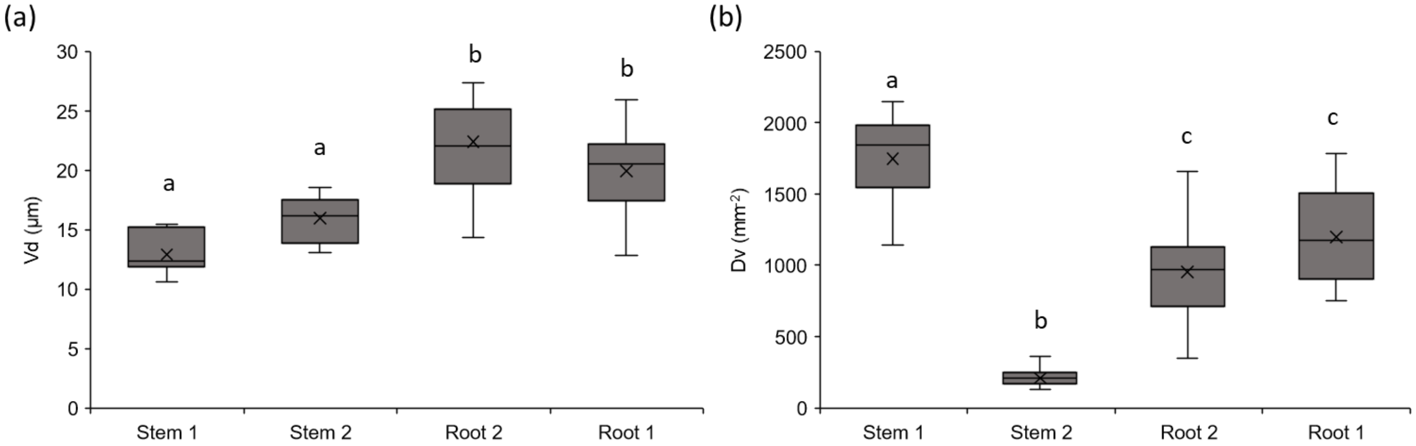


Figure 3.19 Presence of tapering basipetally in *Banksia littoralis* seedlings after 55 days of growth using mean values. (a) vessel diameter (Vd) across 4 sample locations (Stem 1, Stem 2, Root 2 and Root 1), (b) vessel density (Dv) across the same 4 sample locations. Different letters indicate a significant difference between sample locations ($p < 0.05$).

A standardised major axis (SMA) regression analysis was used to assess the scaling relationship between Vd and Dv in *B. attenuata* and *B.littoralis* seedlings (Figure 3.20). In *B. attenuata* seedlings there was a correlation between the Vd and Dv of all groups, as well as Stem 1, Stem 2 and Root 2 samples (Figure 3.20a). There was no scaling relationship between Vd and Dv in Root 1 samples. In *B. littoralis* seedlings there was a correlation between the Vd and Dv of Stem 2, Root 2 and Root 1 samples, but not Stem 1 or all groups (Figure 3.20b). The Root 1 relationship in Figure 3.20b is somewhat unusual as all other results are showing an opposite pattern (increasing Vd with decreasing Dv). This result was only apparent in the *B. littoralis* seedlings from Harvest 1, and may be due to the fact that some plants appeared more stressed than others, or there may have been an error with sampling/analysis.

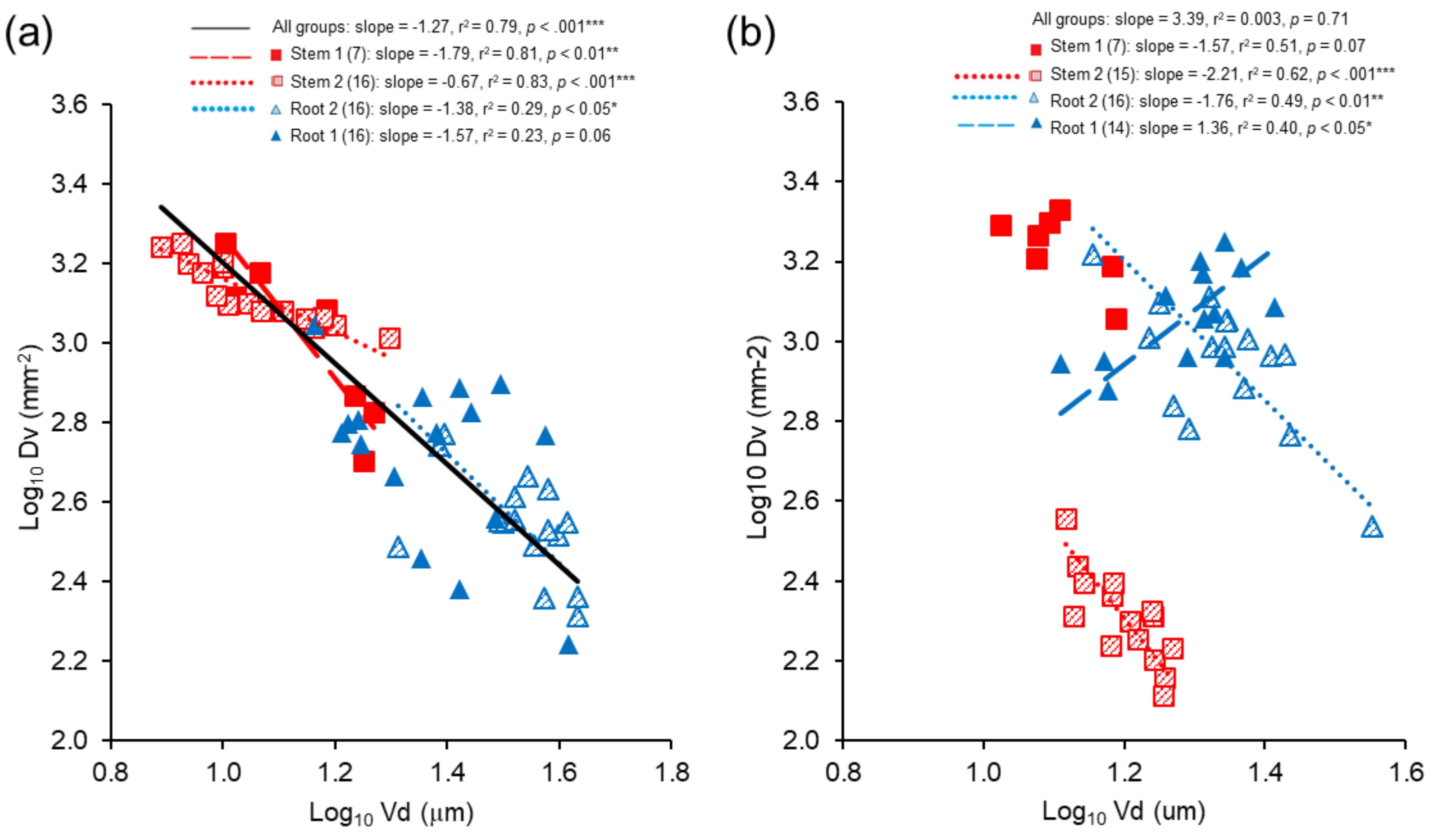


Figure 3.20 Standardised Major Axis (SMA) relationship between mean vessel diameter (Vd) and mean vessel density (Dv) across 4 sample locations (Stem 1, Stem 2, Root 2 and Root 1) from Harvest 1 (55 days of growth). **(a)** SMA relationship between Vd and Dv in *Banksia attenuata* seedlings, **(b)** SMA relationship between Vd and Dv in *B. littoralis* seedlings. Log10 transformations have been applied to all data.

3.2.2. Harvest 2

Banksia attenuata

Vessel diameter (Vd) was found to differ significantly across sample locations in *B. attenuata* seedlings ($p < .001$, $f = 180.178$). A Games-Howell post-hoc test found Root 1 was significantly different to both Stem 1 and Stem 2 samples, and Root 2 was significantly different to Stem 1 and Stem 2 samples (Figure 3.21a). Root 1 and Root 2 Vd measurements were not significantly different from one another, neither was Stem 1 and Stem 2 ($p > 0.05$). Tapering was visible (increasing Vd with depth), with Stem 2 having the smallest Vd, followed by an increase in Root 2, and then the largest Vd was recorded in Root 1 samples (the youngest root samples).

Vessel density (Dv) measurements were found to differ significantly across sample locations ($p < .001$, $f = 98.411$). A Games-Howell post-hoc test presented significant differences in the Dv of each sample location, excluding the two root samples, Root 1 and Root 2, as the difference between the two was not significant (Figure 3.21b). Again, tapering was visible (decreasing Dv with depth). Stem 1 was found to have the greatest Dv, and Root 2 had the lowest.

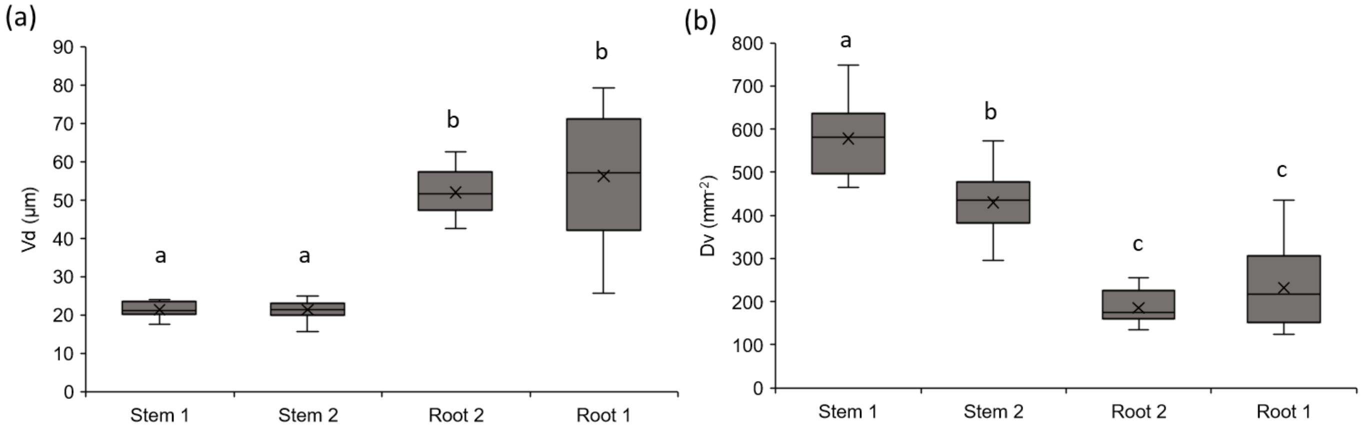


Figure 3.21 Presence of tapering basipetally in *Banksia attenuata* seedlings after 174 days of growth using mean values. (a) vessel diameter (Vd) across 4 sample locations (Stem 1, Stem 2, Root 2 and Root 1), (b) vessel density (Dv) across the same 4 sample locations. Different letters indicate a significant difference between sample locations (p < 0.05).

Banksia littoralis

A significant difference was found in Vd across sample locations in *B. littoralis* seedlings ($p < .001$, $f = 34.016$). A Games-Howell post-hoc test found that all sample locations were significantly different to one another, excluding the two root samples, Root 1 and Root 2, which were not significantly different to one another (Figure 3.22a). The same tapering pattern that was visible in *B. attenuata* samples was also present for *B. littoralis*.

A significant difference was found in Dv across sample locations in *B. littoralis* seedlings ($p < .001$, $f = 61.393$). A follow-up Tukey post-hoc test found that all sample locations were significantly different to one another (Figure 3.22b).

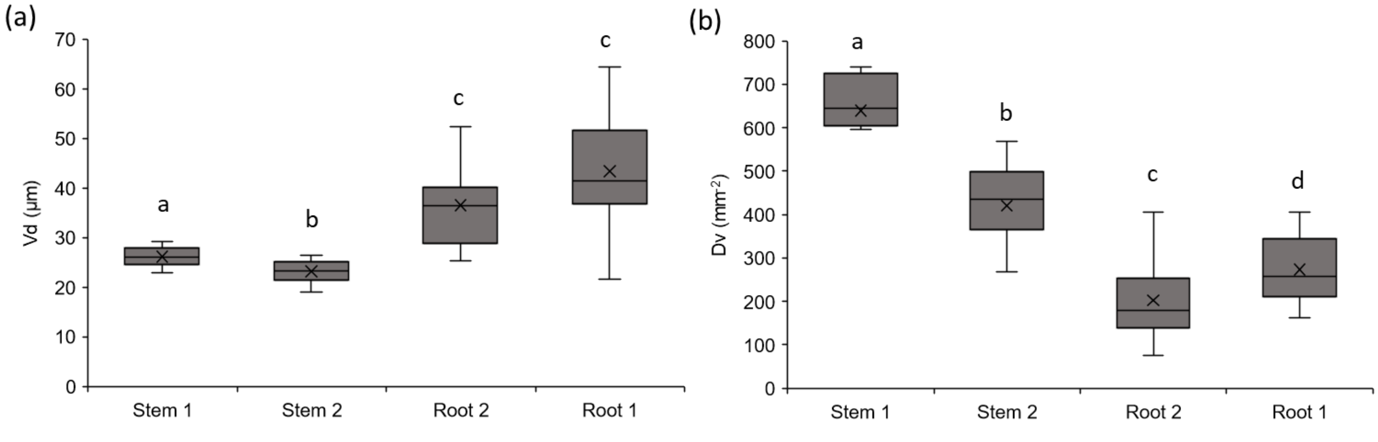


Figure 3.22 Presence of tapering basipetally in *Banksia littoralis* seedlings after 174 days of growth using mean values. (a) vessel diameter (Vd) across 4 sample locations (Stem 1, Stem 2, Root 2 and Root 1), (b) vessel density (Dv) across the same 4 sample locations. Different letters indicate a significant difference between sample locations (p < 0.05).

A standardised major axis (SMA) regression analysis was used to assess the scaling relationship between Vd and Dv in *B. attenuata* and *B.littoralis* seedlings (Figure 3.23). In *B. attenuata* seedlings there was a correlation between the Vd and Dv of all groups, as well as Root 1 and Root 2 samples (Figure 3.23a). There was no scaling relationship between Vd and Dv in Stem 1 or Stem 2 samples. In *B. littoralis* seedlings there was a correlation between the Vd and Dv of all groups, as well as Stem 2 and Root 1 samples, but not Stem 1 or Root 2 samples (Figure 3.23b).

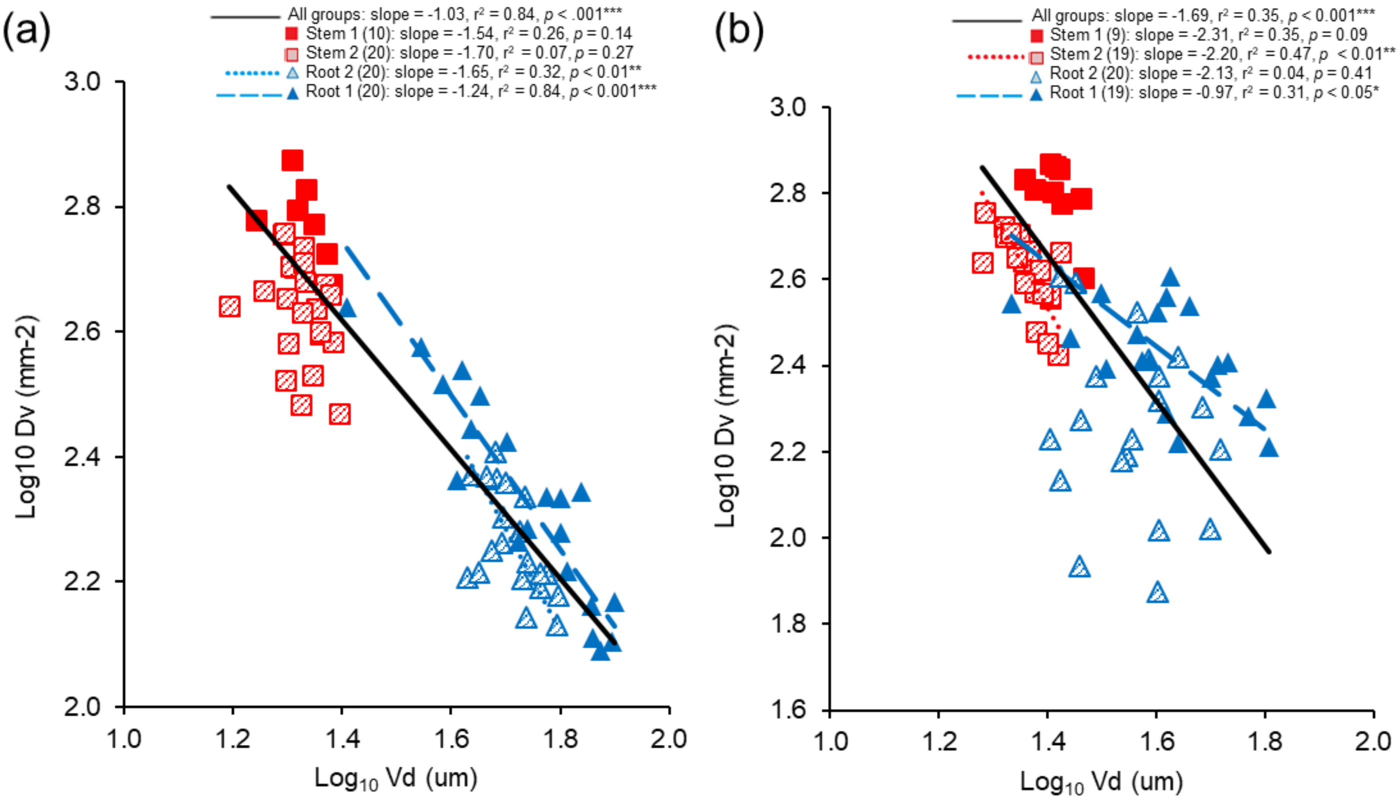


Figure 3.23 Standardised Major Axis (SMA) relationship between mean vessel diameter (Vd) and mean vessel density (Dv) across 4 sample locations (Stem 1, Stem 2, Root 2 and Root 1) from Harvest 2 (174 days of growth). **(a)** SMA relationship between Vd and Dv in *Banksia attenuata* seedlings, **(b)** SMA relationship between Vd and Dv in *B. littoralis* seedlings. Log10 transformations have been applied to all data.

3.3. Objective 3

Investigate whether congeneric species of *Banksia* with different hydrological habitat requirements differ in their conduit traits.

1. *H_0: There are no differences in the traits (anatomical and morphological) between Banksia attenuata and B. littoralis seedlings of equivalent age with or without access to (and use of) a water table.*

3.3.1. Comparison of species

Owing to the observation that there was no significant interaction between treatment and sample locations (objective 1), the following analyses are applied to pooled data from both the connected and disconnected treatments.

Due to the two species contrasting habitat requirements, it was decided that comparisons of *B. attenuata* and *B. littoralis* should be made to determine if their distributions are somewhat reflected in their anatomical and/or morphological traits.

Anatomical traits

Harvest 1

Anatomical traits were found to differ in Harvest 1 with a significant interaction between species and sample location on vessel diameter (Vd) ($p < .001$, $f = 19.510$) and vessel density (Dv) ($p < .001$, $f = 116.223$). A Tukey post-hoc test found significant differences in both Vd and Dv measurements between *B.* attenuata and *B. littoralis* for all three sample locations ($p < 0.05$) (Figure 3.24). Vessel density measurements were significantly higher in *B. littoralis* Root 1 and Root 2 samples in comparison to *B. attenuata*.

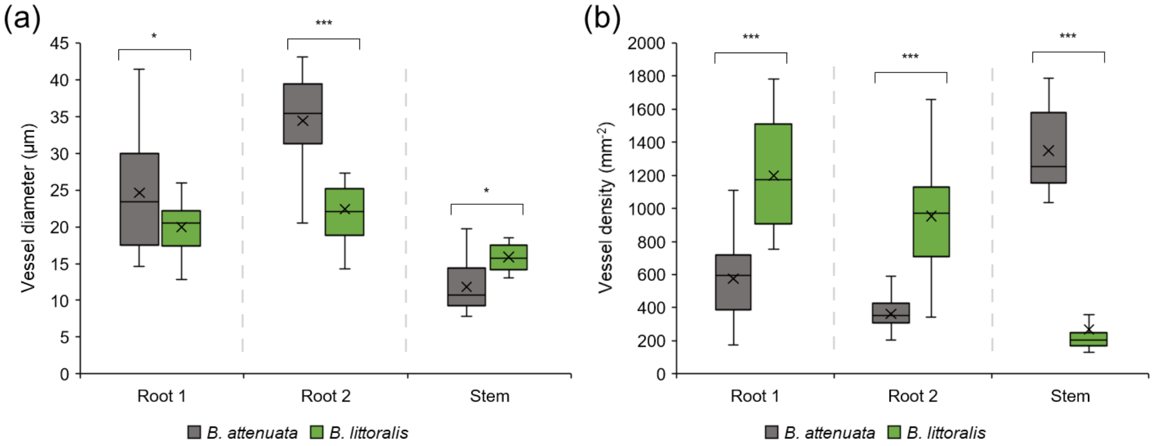


Figure 3.24 Comparison of xylem traits in *Banksia attenuata* seedlings with xylem traits in *B. littoralis* seedlings. (A) comparison of mean Vd across sample locations, (B) comparison of mean Dv across sample locations. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = a non-significant difference.

Harvest 2

There was also significant interaction at the time of Harvest 2 between species and sample location on Vd ($p < .001$, $f = 10.597$), but not Dv ($p = 0.293$, $f = 1.243$). A Tukey post-hoc test found significant differences between species and sample locations for Vd ($p < 0.05$) (Figure 3.25).

Vessel diameter was significantly larger in Root 1 and Root 2 samples of *B. attenuata* seedlings compared to *B. littoralis* seedlings. The vessel diameter of Stem samples was not significantly different between the two species.

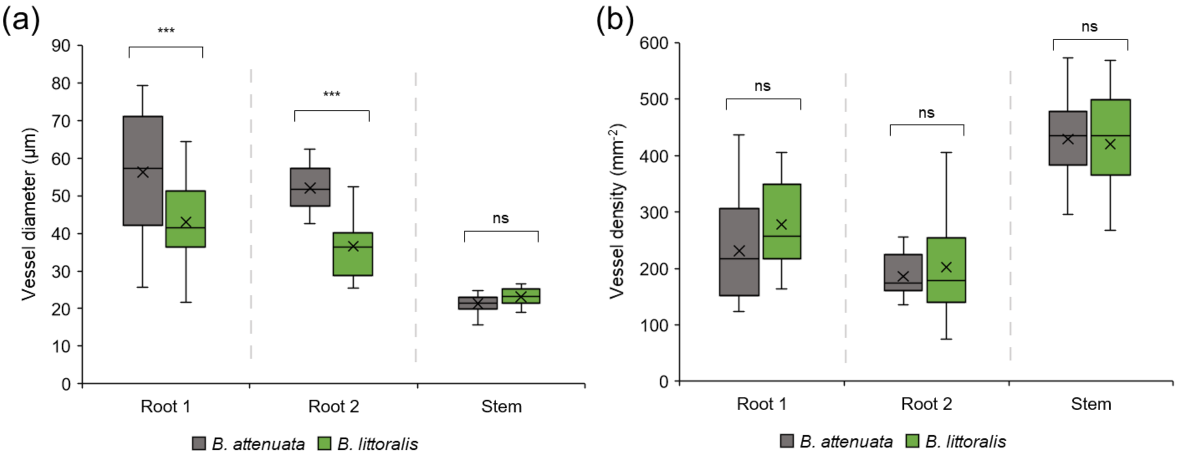


Figure 3.25 Comparison of xylem traits in *Banksia attenuata* seedlings with xylem traits in *B. littoralis* seedlings. (A) comparison of mean Vd across sample locations, (B) comparison of mean Dv across sample locations. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = a non-significant difference.

Vessel length

Vessel length (VL) also differed between the two species, with the VL in *B. attenuata* roots found to be significantly longer than the those in *B. littoralis* roots *(p* < .001) (Appendix Table C.1). A statistical comparison for VL in Harvest 1 could not be made as all vessels measured for *B. littoralis* were less than 1 cm in length.

Theoretical hydraulic conductivity

Harvest 1

Banksia attenuata had a significantly higher K_{th} in Root 2 samples when compared to *B. littoralis* seedlings of Harvest 1, while the K_{th} in Root 1 and Stem samples was not significantly different between the two species (Figure 3.26).

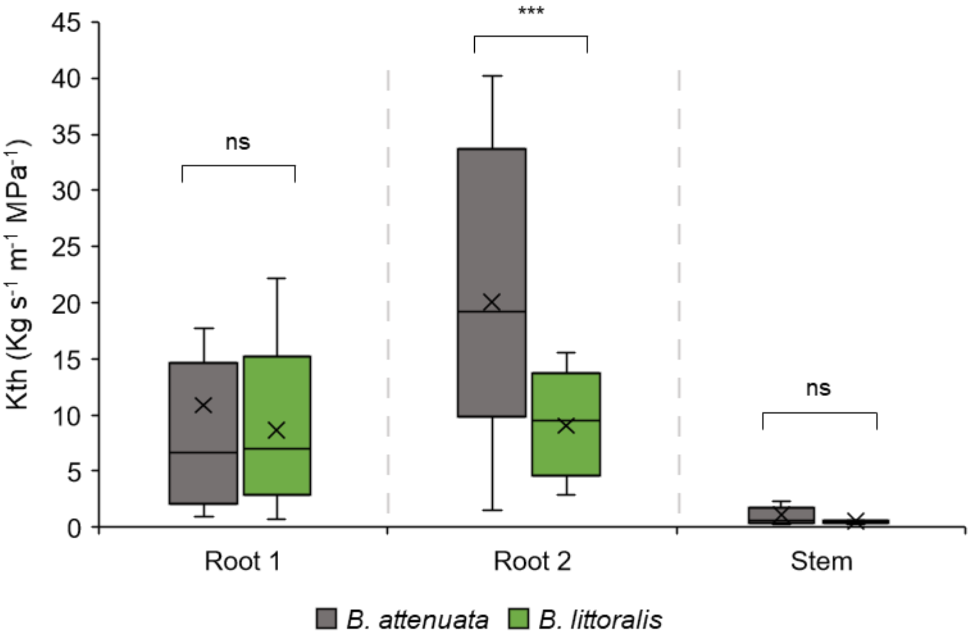


Figure 3.26 Comparison of the mean theoretical hydraulic conductivity (K_{th}) of vessels sampled at three different sample locations in *B. attenuata* and *B. littoralis* seedlings from Harvest 1. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = a non-significant difference.

Harvest 2

Banksia attenuata seedlings from Harvest 2 had a significantly higher K_{th} in Root 1 and Root 2 samples when compared to *B. littoralis* seedlings (Figure 3.27). The K_{th} in the Stem samples was not significantly different between the two species (Figure 3.27).

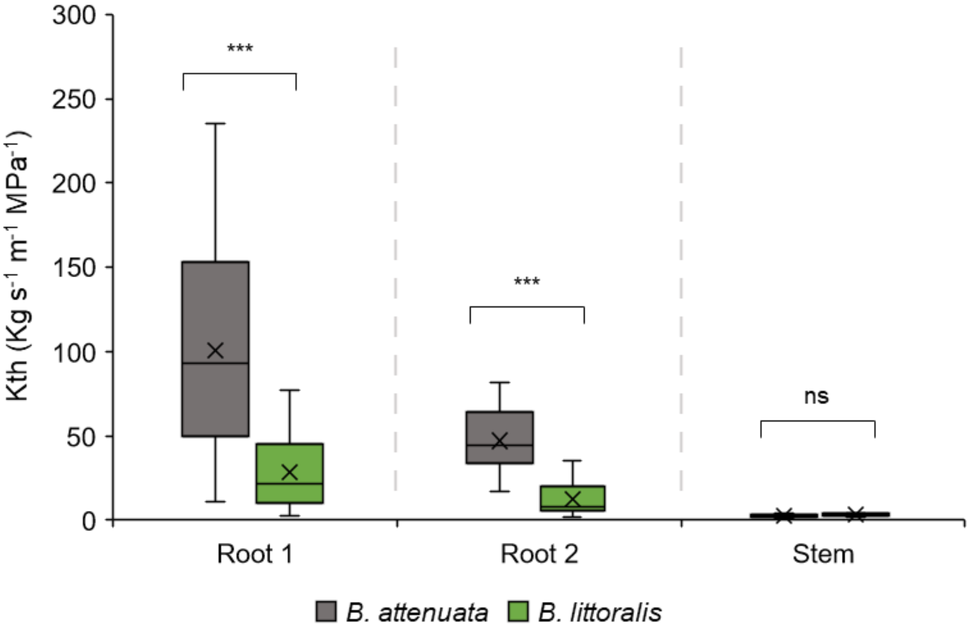


Figure 3.27 Comparison of the mean theoretical hydraulic conductivity (K_{th}) of vessels sampled at three different sample locations in *Banksia attenuata* and *Banksia littoralis* seedlings from Harvest 2. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = a non-significant difference.

It should be noted that conductive area (A_s) was analysed, however there were no significant differences found in A_s between species and/or treatments. This trait was measured to increase the accuracy of the Kth measurements and is therefore not reported on in the results.

Specific hydraulic conductivity

The mean root-specific hydraulic conductivity (Ks) of *B. attenuata* roots was significantly higher than the Ks of *B. littoralis* roots ($p < 0.05$) (33.57 ± 36.36; 2.00 ± 12.37).

Morphological traits

Stem specific density

The mean stem specific density (SSD) of *Banksia attenuata* was significantly larger than that of *B. littoralis* ($p < 0.05$) (Appendix Table C.3).

Stem and root length

The mean stem length of *B. littoralis* seedlings in Harvest 1 was significantly larger than *B. attenuata* seedling stem lengths ($p < 0.05$) (Appendix Table C.2). The mean stem length from Harvest 2 was not significantly different between the two *Banksia* species ($p > 0.05$) (Appendix Table C.3).

The mean root length of *B. attenuata* seedlings in Harvest 1 was significantly larger than *B. littoralis* seedling root length ($p < 0.05$) (Appendix Table C.2). The mean root length in Harvest 2 was also significantly larger in *B. attenuata* seedlings compared to *B. littoralis* seedlings ($p < 0.05$) (Appendix Table C.3).

4. Discussion

The results from this study found that xylem traits of *Banksia* seedlings differed as the seedlings aged. The same traits did not differ between the connected and disconnected treatments, though xylem traits did differ across organs (roots and stems) within individual seedlings. This study also discovered that the two congeneric *Banksia* species had significant differences in their xylem development (irrespective of treatment). These findings will be discussed in further detail below.

Plant age and height are believed to have a greater influence on the development of xylem traits than climate alone (Olson et al., 2014; Pfautsch et al., 2016). This study first looked at how traits of xylem in seedlings varied with age and increasing height by comparing seedlings of approximately 2 months of age with seedlings of approximately 6 months of age. Vessel diameter increased as the plant aged, while vessel density decreased. The height of individual seedlings also increased with age, and according to the literature, the hydraulic path length would also increase as a consequence of increasing stature (Olson et al., 2014; Prendin, Mayr, Beikircher, Von Arx, & Petit, 2018). The significant differences between Harvest 1 and Harvest 2 of both *Banksia attenuata* and *B. littoralis* meant that the null hypothesis, "*There is no difference in the xylem traits of* Banksia *seedlings after 55 days of growth (Harvest 1) compared to seedlings after 174 days of growth (Harvest 2)"* was not supported as the results showed that conduit traits do differ with ontogeny.

Vessel tapering plays an important role in maintaining water flow through vessels, with studies suggesting that tapering is related to plant size, rather than age (Petit, Anfodillo, & Mencuccini, 2008). When they analysed trees of different ages, but of similar heights, only the environment or the size of the tree was shown to be responsible for variations in xylem anatomical traits (Petit et al., 2008). Both of the analysed *Banksia* species, *B. attenuata* and *B. littoralis*, followed a similar pattern in the variation of conduit traits from Harvest 1 to Harvest 2, i.e., increased vessel diameter and decreased vessel density.

After comparing the two species at different ages, a comparison was then made between conduit traits of seedlings growing without an artificial water table, with seedlings of the same species growing with an artificial water table. It was hypothesised that plants growing in the connected treatment would develop traits that characterised hydraulic efficiency, with significantly larger diameter and lengths of vessels than plants growing in the disconnected treatment, particularly in the deeper roots (McElrone et al., 2004; Rajaei et al., 2013). Those growing in the disconnected treatment were expected to have a higher density of smaller diameter vessels, representative of a hydraulically safe anatomy (Durante et al., 2011). However, the analysed traits did not differ significantly between the connected and disconnected treatments, supporting the null hypothesis '*There is no difference in the traits (anatomical or morphological) of Banksia seedlings of equivalent age with or without access to (and use of) a water table*'.

Drought has been shown to influence changes in individuals, with plants shifting their anatomy to increase their chances of survival and avoid hydraulic failure (Martin-Benito et al., 2017). By altering xylem traits such as the diameter and frequency of vessels, as well as vessel wall thickness and inter-vessel pit membrane thickness, plants are able to reduce the risk of drought-induced cavitation and avoid deleterious water stress (Aref et al., 2013; Guijarro-Real et al., 2014). Plants commonly display traits that are characteristic of either a hydraulically-efficient or -safe anatomy depending on the type of environment (Durante et al., 2011). Studies have shown plants can alter the diameter and density of vessels when exposed to xeric environments during development, by producing a greater number of smaller diameter vessels and fewer large diameter vessels (De Micco, Aronne, & Baas, 2008; Durante et al., 2011; El Aou-ouad et al., 2017; Pfautsch et al., 2016). The opposite has been shown in mesic environments, with plants producing a lower density of vessels, but with much larger diameters and longer vessel lengths (De Micco et al., 2008; Durante et al., 2011; El Aou-ouad et al., 2017; Pfautsch et al., 2016; Santini et al., 2015). Pit membranes have also been shown to differ in relation to water availability (Aref et al., 2013). Pit membranes play an important role in the hydraulic resistance in xylem, and in the avoidance of embolism (Aref et al., 2013; Hacke et al., 2006; Wheeler, Sperry, Hacke, & Hoang, 2005). Thicker pit membranes are less likely to encounter the spread of embolism and have been found in plants that are experiencing water deficit (Aref et al., 2013). In support of the trade-off theory, a number of studies have shown both hydraulically efficient and safe anatomy in plants, depending on the environmental conditions the plants are exposed to (mesic or xeric) (Martínez-Vilalta, Prat, Oliveras, & Piñol, 2002; McElrone et al., 2004; Santini et al., 2015). A lower density of large diameter vessels were predicted in *Banksia* seedlings growing in the connected treatment, as large diameter vessels allow for rapid and efficient transport of readily available water (Burgess et al., 2006; Durante et al., 2011; McElrone et al., 2004; Pfautsch et al., 2016; Santini et al., 2015). Seedlings that were only receiving surface water (disconnected treatment) were expected to develop a safer anatomy to avoid the increased risk of drought-induced cavitation and hydraulic failure (Burgess, Adams, Turner, White, & Ong, 2001; Durante et al., 2011; Hacke et al., 2006; Pfautsch et al., 2016).

Although many studies have shown that xylem anatomy can differ between mesic and xeric ecosystems, there are a number of potential reasons to support why we may not have seen significant differences in this study.

Plants have been shown to alter traits in relation to cambial age and height, with studies finding an increase in vessel diameter as cambial age and path length (height) increase (Domec & Gartner, 2002; Nielsen et al., 2017; Rita et al., 2016; Zhao, 2016). This glasshouse study ran for 174 days in total; therefore, the seedlings were still very young, and a comparison of cambial age was not possible. The findings of this study may suggest that the length of the experiment was too short to see any significant variation in xylem traits as both plant age and height have been shown to influence the development of xylem in plants (Zhao, 2016). Changes in xylem anatomy, particularly vessel diameter, are thought to be closely linked to tree stature, rather than climatic conditions (Olson et al., 2014). It has also been suggested that the anatomical structure of young seedlings may not be capable of avoiding cavitation and embolism and that the xylem anatomy of young seedlings is likely not a useful representation of mature individuals (Klein et al., 2018; Miller & Johnson, 2017).

Variations in specific hydraulic conductivity have also been found to strongly depend on the cambial age of plants (Rita et al., 2016). Alternatively, some processes, such as vessel tapering, have been shown to be more substantial with increased height (rather than age) (Anfodillo et al., 2006; Petit, Pfautsch, Anfodillo, & Adams, 2010; Pittermann & Olson, 2018). This study found that the height of the seedlings between the two treatments was not significantly different. As already discussed, there is supporting literature that links variations in xylem anatomy to both the path length (height) and age of the plants. With the seedlings being the same age and of similar heights, we would expect their anatomy to be the same, or similar. Plants exposed to water deficits are more prone to reduced growth (Hopkins & Huner, 2009; Merchant, Callister, Arndt, Tausz, & Adams, 2007), therefore, it is likely that if the seedlings were older and exposed to the two treatments for a longer period, we may have seen a significant increase in the height of those with access to an artificial water table and eventually, larger differences in the xylem traits measured.

Banksia woodland vegetation is affected by fluctuations in water availability, particularly rapid declines in groundwater levels (Broun, 2004; Canham, 2011; Groom, 2002). Groom, (2002) exposed seven-month-old *Banksia littoralis* seedlings to two contrasting conditions in a glasshouse experiment, well-watered and drought, and it was found that the seedlings did not recover well from short term drought exposure, but they did show a high desiccation tolerance (ability to withstand extreme drought). Similar responses were expected for this study, however the lack of significant differences between the two treatments may suggest that the disconnected treatment was not dry enough. The seedlings in this study were quick to show responses to stress (leaf yellowing and wilting), and for that reason, each seedling received surface water (50 mL) three times a week throughout the experiment. Due to the regular watering, the seedlings in the glasshouse were receiving at least twice the amount of water than they would if they were growing under the natural rainfall regimes of south-west Western Australia (BOM, 2018). Therefore, it is possible that the disconnected treatment was not an accurate representation of a drought event, thus resulting in no significant differences between the two treatments. Alternatively, the results may be suggesting that although the treatment was sufficiently dry, the experiment did not run long enough, and therefore there was not yet a need for the plants to adapt.

Although xylem vessel dimensions often reflect prevailing water availability, this is not always the case. Corcuera *et al.,* (2012) found that xylem vessel dimensions in *Pinus pinaster* seedlings were not significantly different between drought and control treatments, and therefore could not be used to explain changes in the hydraulic conductivity in plants. Another study that analysed 34 different species from both low rainfall and high rainfall areas found that there was a lower density of xylem vessels in species of low rainfall areas compared to those in high rainfall areas (opposite to what would be expected) (Nicotra et al., 2002). The same study also looked at whether vessel size, in particular, differed between the low and high rainfall areas, as vessel diameter limits flow (more so than vessel density), and found that were no consistent patterns to suggest the plants were responding differently to the variations in rainfall (Nicotra et al., 2002).

A comparison of traits of *Banksia attenuata* and *B. littoralis* revealed that they developed significantly different anatomical and morphological traits when exposed to the same environmental conditions and sampled at the same age. The null hypothesis for this objective '*There are no differences in the traits (anatomical and morphological) between Banksia attenuata and B. littoralis seedlings of equivalent age with or without access to (and use of) a water table'* was not supported, as the results show significant differences between the two *Banksia* species. A number of studies have found similar results, showing that species of the same genus differ in their anatomy, physiology or morphology and that this may provide reasoning for their contrasting habitat distributions (Barotto et al., 2017; Silva et al., 2017; Willson & Jackson, 2006).

Banksia attenuata seedlings displayed a more rapid root elongation rate following germination. Faster root growth in *B. attenuata* can be supported by a study that analysed the root elongation rates of both *B. attenuata* and *B. littoralis* seedlings and found that *B. attenuata* had a faster root elongation rate than *B. littoralis* (Canham et al., 2015). Rapid root elongation is thought to be an advantage to facultative phreatophyte seedlings growing in ecosystems with increasing depths to available soil moisture, particularly in their first year of growth when establishment and survival of the first summer drought are critical (Canham et al., 2015; Thomas, 2014). Plants growing in seasonally arid environments are at greater risk of surface soils drying out and therefore require fast access to deeper soil moisture stores in the dry seasons (Canham et al., 2015). The demand for *B. littoralis* seedlings to rapidly elongate roots in search of available water would be far less common as they favour locations with a shallow water table that provides access to moisture year-round (Canham et al., 2015; Zencich et al., 2002). The height and age of individual plants has been linked to traits such as increased vessel diameter, and this could support the finding of larger vessels in *B. attenuata* roots in comparison to *B. littoralis* roots (Olson et al., 2018), as *B. attenuata* seedlings roots were significantly longer than *B. littoralis* seedlings roots in both the connected and disconnected treatments. The idea that path length is responsible for variations in xylem traits (i.e., tapering) may be supported by the traits analysed in the stems of both species from Harvest 2. A study by Petit et al., (2008) found that xylem traits did not vary in small trees (of similar height), irrespective of different ages and growing conditions. In this study, *B. attenuata* and *B. littoralis* stem lengths did not differ significantly to one another, and neither did the anatomical traits that were measured in the stems.

The findings from this study suggest that the rate of root elongation for *B. attenuata* and *B. littoralis* may reflect their contrasting habitat preferences (Canham et al., 2015). There is increasing support behind the idea that species from the same genus may develop different traits in relation to their spatial distribution, or that these distributions may be directly related to the plant's ability to develop and alter traits, particularly anatomical traits (Silva et al., 2017; Willson & Jackson, 2006). Xylem anatomical traits are commonly used to determine a plants ability to survive in a given environment, tying in with the hydraulic efficiency and safety trade-off theory (Durante et al., 2011). Plants typically have xylem traits that are suitable for the climatic conditions they are exposed to, i.e., fewer, large diameter vessels in individuals found in mesic environments, and many small diameter vessels in xeric environments (Durante et al., 2011; Santini et al., 2015). *Banksia littoralis*, commonly known as the 'swamp *Banksia*', prefers low lying swampy areas with a shallow water table that provides constant access/connection to water (mesic environment). Due to this species habitat preference, it was hypothesised that the seedlings would have greater hydraulic efficiency when compared to *B. attenuata* seedlings. However, *B. littoralis* seedlings developed a higher density of smaller diameter vessels, suggesting a more hydraulically-safe xylem anatomy. This finding was unexpected and in order to better understand this result it is suggested that the study be replicated for a longer period of time under controlled conditions. A field study would also provide valuable information about the chosen *Banksia* species.

Another focus of this study was the variations in xylem traits at the intra-plant level, with studies showing that both xylem vessel diameter and vessel density can differ in an individual plant (Anfodillo et al., 2006; McElrone et al., 2004; Petit et al., 2009). Changes in xylem vessel traits are often in response to increased hydraulic resistance and decreased conductivity within a plant, caused by increasing plant height (Petit et al., 2010). Conduit tapering was confirmed in both *Banksia attenuata* and *B. littoralis* seedlings at 55 days old, and again at 174 days old, thus the null hypothesis '*There is no conduit tapering in Banksia seedling root or hydraulic architecture*' was not supported. Tapering patterns were then analysed in the two species of *Banksia* to determine if the findings were consistent with previous research. Numerous studies have led to our in-depth understanding of vessel tapering in aboveground organs; however the study of roots and root xylem anatomy is still uncommon (Brunner, Herzog, Dawes, Arend, & Sperisen, 2015; Kirfel, Leuschner, Hertel, & Schuldt, 2017; Petit et al., 2009). A number of authors have confirmed the presence of vessel tapering in various plants, showing that vessels increase in diameter in a basipetal direction (from branches to the stem base) (Anfodillo et al., 2006; McElrone et al., 2004; Petit et al., 2009). With a number of researchers now also supporting that tapering continues belowground (McElrone et al., 2004; Petit et al., 2009).

The largest diameter vessels were recorded in the root samples of both species. We found consistent patterns of vessel tapering in both *B. attenuata* and *B. littoralis* that complemented the findings from previous studies (Lintunen & Kalliokoski, 2010; McElrone et al., 2004; Pate, Jeschke, Aylward, 1995; Petit et al., 2009). Vessel tapering allows plants to maintain a hydraulically efficient anatomy while bypassing the implications of increasing path lengths, i.e., increased hydraulic resistance (Choat et al., 2005; Petit et al., 2009, 2010; Pfautsch et al., 2016).

Numerous studies have found that tapering in a variety of tree species follows a consistent pattern, i.e., larger diameter vessels in the roots that decrease in diameter towards the stem, branches and then leaves (Domec et al., 2009; Goncalves et al., 2007; Kotowska et al., 2015; Lintunen & Kalliokoski, 2010; McElrone et al., 2004; Tyree & Zimmermann, 2002). This tapering appears to be a common functional trait among all tree hydraulic systems (Anfodillo et al., 2006; Carvalho et al., 2015). It may be closely linked to the hydraulic efficiency and safety trade-off theory, with the degree of tapering largely dependent on plant height/age, but also surrounding environmental conditions (Kotowska et al., 2015). As vessels taper from the roots towards the branches and leaves (larger to smaller diameter), there is a shift in the hydraulic efficiency of vessels. Organs such as leaves are exposed to greater negative pressure potentials as well as more severe water deficits, and as a result, they generally have a safer hydraulic architecture (higher density of small diameter vessels) (Couvreur et al., 2018; Johnson et al., 2016). Roots, on the other hand, experience much lower (less negative) pressure potentials and less severe water deficits, allowing them to support a hydraulically efficient architecture (Couvreur et al., 2018; Johnson et al., 2016). This shift between hydraulic efficiency and safety at the intra-plant level is possible because of tapering.

This study found that both species of *Banksia* altered their anatomy and morphology with ontogeny. As a plant grows, the xylem anatomy also needs to evolve to ensure that the transport of water and vital nutrients is not impaired. Changes in anatomy with age are important, as they allow the plant to maintain efficiency, or to develop safer anatomy, depending on the given environment. The findings from this study showed that as *B. attenuata* and *B. littoralis* seedlings grew taller stems and deeper roots, anatomical traits within the stems and roots (xylem vessel diameter and density) also changed. A clear pattern was found that showed vessel diameters to increase as the plants aged, while vessel density typically decreased with age. These traits are also known to alter during growth and development when plants are exposed to different environmental conditions. It was predicted that seedlings growing with access to an artificial water table would develop fewer, larger xylem vessels than those growing without access. Although this study did not find significant differences between the connected and disconnected treatments, it does not mean that these differences do not occur. There is potential for further investigation surrounding this topic i.e., a longer duration glasshouse experiment as well as the possibility of a field experiment. If the glasshouse experiment was to be replicated, it is suggested that the plants be exposed to a more severe drought treatment, as well as running the experiment for a longer duration. The seedlings were very young in this experiment (~6 months old) and this is thought to have contributed to the similarity between treatments. Running the experiment for a longer duration, combined with a more severe drought treatment is more likely to challenge the seedlings, thereby encouraging different functional and structural responses. There are also a number of measurements/analyses that were not included in this study due to time and cost constraints that may help with comparisons between treatments and species if the experiment was to be repeated. These include vulnerability to cavitation, whole plant (leaf, stem and root) water potentials, xylem water potential, pit membrane measurements, intervessel wall thickness, theoretical vessel implosion resistance, photosynthetic rate and stomatal conductance. A field aspect would also contribute significantly to this research area and allow for a more in-depth understanding of the xylem anatomy of mature *Banksia attenuata* and *B. littoralis* plants regarding groundwater access.

Although there were no significant differences found between the two treatments, there were differences identified at the intra-plant level, including the presence of conduit tapering. Tapering in both *Banksia* species followed the general tapering pattern often reported on in plant anatomical studies (Lintunen & Kalliokoski, 2010; McElrone, Pockman, Martinez-Vilalta, & Jackson, 2004; Pate, J; Jeschke, W; Aylward, 1995). For a more accurate representation of tapering within individual plants, it is suggested that the plants be grown for a longer duration and that samples are collected from more locations along individual plants.

The discovery that the two *Banksia* species develop significantly different anatomical and morphological traits is an interesting and important finding. The differences in each species anatomy and morphology are believed to play a role in their distinct habitat preferences and may help provide a better understanding into MTE functionality, as well as providing a guide to improve restoration efforts. *Banksia attenuata* seedlings showed a faster root elongation rate than *B. littoralis* seedlings, which supports their ability to thrive in ecosystems with varying depths to groundwater sources. Understanding the response of *Banksias* to fluctuating groundwater levels and drought is vital in Western Australia, particularly for restoration projects and groundwater management. Water stress in MTEs is a significant contributor to seedling mortality, and while *B. littoralis* seedlings are likely to be strongly affected by changes in water availability (i.e., declining groundwater levels), *B. attenuata* seedlings anatomy and morphology suggests that they could potentially adapt/cope with changes. This idea could be better supported if the study was replicated and the previously mentioned additional analyses were included. More evidence is required to confirm whether the non-significant differences between the connected and disconnected treatments was due to the length of the experiment/age of the seedlings, or to confirm that plasticity does not occur within these species.

Irrespective of the need to further investigate, the findings from this study provide new insight into the water transport architecture of these two significant Western Australia natives, *B. attenuata* and *B. littoralis*. *Banksia attenuata* appears to be a flexible species that have good survival strategies for hot, dry environments, as well as environments that have cooler temperatures and shallow water tables. Although *B. littoralis* grew successfully under both treatments, the results from this study and others (i.e., Canham et al., 2015), as well as the species habitat distribution, suggest that it may not be able to survive long periods of drought. *Banksia littoralis* may gradually be replaced by species with a more widespread ecohydrological distribution (i.e., *B. attenuata*) if groundwater levels continue to decline (Canham, 2011). The possible replacement of dominant overstorey species could over time see different plant genera become the dominant overstorey species and gradually lead to a shift in vegetation type.

5. References

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6. Appendices

Appendix A

Objective 1: Two-way ANOVA results

Table A.1 Results from separate Two-way ANOVAs testing for differences in various traits across harvest (Harvest 1 (55 days growth) and Harvest 2 (174 days growth)) and sample locations (Root 1, Root 2 and Stem) in *Banksia attenuata* seedlings (disconnected treatment).

Vessel trait	d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)				
Harvest	1	4456.79	84.22	**< 0.0001**
Sample Location	2	7665.02	72.43	**< 0.0001**
Harvest x Location	2	1306.31	12.34	**< 0.0001**
Max vessel diameter (Dmax)				
Harvest	1	11340.32	90.74	**< 0.0001**
Sample Location	2	19842.59	79.38	**< 0.0001**
Harvest x Location	2	5682.55	22.73	**< 0.0001**
Vessel density (Dv)				
Harvest	1	1967189.49	91.00	**< 0.0001**
Sample Location	2	3373490.40	78.03	**< 0.0001**
Harvest x Location	2	954614.25	22.08	**< 0.0001**
Hyd-weighted Diameter (Dh)				
Harvest	1	5559.37	100.73	**< 0.0001**
Sample Location	2	10529.70	95.39	**< 0.0001**
Harvest x Location	2	2060.11	18.66	**< 0.0001**
Theoretical Cond (Kth)				
Harvest	1	6698.28	22.02	**< 0.0001**
Sample Location	2	10751.63	17.67	**< 0.0001**
Harvest x Location	2	6578.29	10.81	**< 0.001**

Table A.2 Results from separate Two-way ANOVAs testing for differences in various traits across harvest (Harvest 1 (55 days growth) and Harvest 2 (174 days growth)) and sample locations (Root 1, Root 2 and Stem) in *Banksia attenuata* seedlings (connected treatment).

Vessel trait		d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)					
	Harvest	1	5797.00	60.85	**< 0.0001**
	Sample Location	2	7588.26	39.83	**< 0.0001**
	Harvest x Location	2	945.28	4.96	**< 0.01**
Max vessel diameter (Dmax)					
	Harvest	1	11156.50	44.27	**< 0.0001**
	Sample Location	2	18567.54	36.84	**< 0.0001**
	Harvest x Location	2	4163.10	8.26	**< 0.001**
Vessel density (Dv)					
	Harvest	1	4431235.22	296.94	**< 0.0001**
	Sample Location	2	4126660.02	138.26	**< 0.0001**
	Harvest x Location	2	1798040.69	60.24	**< 0.0001**
Hyd-weighted Diameter (Dh)					
	Harvest	1	7161.38	61.84	**< 0.0001**
	Sample Location	2	9476.56	40.91	**< 0.0001**
	Harvest x Location	2	1601.13	6.91	**< 0.01**
Theoretical Cond (Kth)					
	Harvest	1	5609.67	11.39	**< 0.01**
	Sample Location	2	11550.37	11.73	**< 0.0001**
	Harvest x Location	2	4614.00	4.68	**< 0.05**

Table A.3 Results from separate Two-way ANOVAs testing for differences in various traits across harvest (Harvest 1 (55 days growth) and Harvest 2 (174 days growth)) and sample locations (Root 1, Root 2 and Stem) in *Banksia littoralis* seedlings (disconnected treatment).

Vessel trait		d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)					
	Harvest	1	2740.33	53.17	**< 0.0001**
	Sample Location	2	1368.65	13.28	**< 0.0001**
	Harvest x Location	2	424.50	4.12	**< 0.05**
Max vessel diameter (Dmax)					
	Harvest	1	3938.08	46.90	**< 0.0001**
	Sample Location	2	2420.48	14.41	**< 0.0001**
	Harvest x Location	2	927.86	5.53	**< 0.01**
Vessel density (Dv)					
	Harvest	1	2148785.38	105.32	**< 0.0001**
	Sample Location	2	816377.47	20.01	**< 0.0001**
	Harvest x Location	2	2343266.58	57.43	**< 0.0001**
Hyd-weighted Diameter (Dh)					
	Harvest	1	2677.62	65.78	**< 0.0001**
	Sample Location	2	1791.67	22.01	**< 0.0001**
	Harvest x Location	2	703.01	8.64	**< 0.01**
Theoretical Cond (Kth)					
	Harvest	1	719.81	6.39	**< 0.05**
	Sample Location	2	1955.05	8.67	**< 0.001**
	Harvest x Location	2	1340.08	5.94	**< 0.01**

Table A.4 Results from separate Two-way ANOVAs testing for differences in various traits across harvest (Harvest 1 (55 days growth) and Harvest 2 (174 days growth)) and sample locations (Root 1, Root 2 and Stem) in *Banksia littoralis* seedlings (connected treatment).

Vessel trait	d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)				
Harvest	1	234.93	4.07	**< 0.05**
Sample Location	2	1325.40	11.47	**< 0.0001**
Harvest x Location	2	460.19	3.98	**< 0.05**
Max vessel diameter (Dmax)				
Harvest	1	4050.46	54.57	**< 0.0001**
Sample Location	2	1960.08	13.21	**< 0.0001**
Harvest x Location	2	789.06	5.32	**< 0.01**
Vessel density (Dv)				
Harvest	1	4313400.01	72.88	**< 0.0001**
Sample Location	2	1692582.82	14.30	**< 0.0001**
Harvest x Location	2	3159420.97	26.69	**< 0.0001**
Hyd-weighted Diameter (Dh)				
Harvest	1	2650.52	50.58	**< 0.0001**
Sample Location	2	1585.44	15.13	**< 0.0001**
Harvest x Location	2	473.07	4.51	**< 0.05**
Theoretical Cond (Kth)				
Harvest	1	852.14	5.84	**< 0.05**
Sample Location	2	2498.62	8.56	**< 0.001**
Harvest x Location	2	535.47	1.84	0.1706

Table A.5 Results from separate Two-way ANOVAs testing for differences in various traits across treatment (connected and disconnected) and sample locations (Root 1, Root 2 and Stem) in *Banksia attenuata* seedlings (55 days old).

Vessel trait	d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)				
Treatment	1	73.16	1.87	0.1791
Sample Location	2	4108.90	52.42	**< 0.0001**
Treatment x Location	2	21.50	0.27	0.7614
Max vessel diameter (Dmax)				
Treatment	1	58.26	0.43	0.5182
Sample Location	2	11136.00	40.58	**< 0.0001**
Treatment x Location	2	106.29	0.39	0.6813
Vessel density (Dv)				
Treatment	1	304024.63	8.49	**0.0057**
Sample Location	2	8594262.46	120.04	**< 0.0001**
Treatment x Location	2	78260.91	1.09	0.3445
Hyd-weighted Diameter (Dh)				
Treatment	1	152.40	2.64	0.1116
Sample Location	2	4905.68	42.51	**< 0.0001**
Treatment x Location	2	65.40	0.57	0.5717
Theoretical Cond (Kth) (Log_{10})				
Treatment	1	0.30	1.54	0.2221
Sample Location	2	13.95	35.28	**< 0.0001**
Treatment x Location	2	0.41	1.02	0.3680

Table A.6 Results from separate Two-way ANOVAs testing for differences in various traits across treatment (connected and disconnected) and sample locations (Root 1, Root 2 and Stem) in *Banksia littoralis* seedlings (55 days old).

Vessel trait	d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)				
Treatment	1	4.58	0.32	0.5756
Sample Location	2	345.71	12.03	**< 0.0001**
Treatment x Location	2	13.67	0.48	0.6249
Max vessel diameter (Dmax)				
Treatment	1	38.90	1.35	0.2526
Sample Location	2	571.46	9.90	**< 0.0001**
Treatment x Location	2	67.67	1.17	0.3201
Vessel density (Dv)				
Treatment	1	288817.06	3.36	0.0745
Sample Location	2	6496986.79	37.78	**< 0.0001**
Treatment x Location	2	62358.27	0.36	0.6982
Hyd-weighted Diameter (Dh)				
Treatment	1	0.09	0.01	0.9369
Sample Location	2	478.75	16.27	**< 0.0001**
Treatment x Location	2	15.92	0.54	0.5862
Theoretical Cond (Kth) (Log$_{10}$)				
Treatment	1	0.06	0.64	0.4295
Sample Location	2	13.82	74.06	**< 0.0001**
Treatment x Location	2	0.19	0.99	0.3798

Table A.7 Results from separate Two-way ANOVAs testing for differences in various traits across treatment (connected and disconnected) and sample locations (Root 1, Root 2 and Stem) in *Banksia attenuata* seedlings (174 days old).

Vessel trait	d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)				
Treatment	1	0.15	0.00	0.9691
Sample Location	2	14520.95	71.72	**< 0.0001**
Treatment x Location	2	9.45	0.05	0.9544
Max vessel diameter (Dmax)				
Treatment	1	89.35	0.39	0.5343
Sample Location	2	40175.36	87.97	**< 0.0001**
Treatment x Location	2	59.52	0.13	0.8781
Vessel density (Dv)				
Treatment	1	16633.35	3.59	0.0635
Sample Location	2	671600.53	72.45	**< 0.0001**
Treatment x Location	2	23040.40	2.49	0.0928
Hyd-weighted Diameter (Dh)				
Treatment	1	9.79	0.09	0.7636
Sample Location	2	20403.39	95.23	**< 0.0001**
Treatment x Location	2	8.69	0.04	0.9603
Theoretical Cond (Kth)				
Treatment	1	46.40	0.07	0.7867
Sample Location	2	33720.58	26.86	**< 0.0001**
Treatment x Location	2	33.00	0.03	0.9741

Table A.8 Results from separate Two-way ANOVAs testing for differences in various traits across treatment (connected and disconnected) and sample locations (Root 1, Root 2 and Stem) in *Banksia littoralis* seedlings (174 days old).

Vessel trait	d.f.	SS	*F*-value	*P*-value
Mean vessel diameter (Vd)				
Treatment	1	2.54	0.04	0.8497
Sample Location	2	3966.77	28.26	**< 0.0001**
Treatment x Location	2	74.90	0.43	0.5897
Max vessel diameter (Dmax)				
Treatment	1	75.59	0.65	0.4246
Sample Location	2	6047.31	25.91	**< 0.0001**
Treatment x Location	2	105.08	0.45	0.6400
Vessel density (Dv)				
Treatment	1	6084.47	0.84	0.3644
Sample Location	2	477982.64	32.87	**< 0.0001**
Treatment x Location	2	6322.93	0.44	0.6496
Hyd-weighted Diameter (Dh)				
Treatment	1	4.06	0.81	0.8119
Sample Location	2	4512.49	31.82	**< 0.0001**
Treatment x Location	2	108.84	0.77	0.4693
Theoretical Cond (Kth)				
Treatment	1	12.28	0.06	0.8103
Sample Location	2	6633.42	15.72	**< 0.0001**
Treatment x Location	2	151.46	0.36	0.7001

Appendix B

Objective 1: One-way ANOVA results

Harvest comparison

Table B.1 Mean and standard error from One-way ANOVAs comparing morphological traits of *Banksia attenuata* seedlings in the connected treatment. Different letters indicate a significant difference in a column ($p < 0.001$).

	Stem length (cm)	Root length (cm)
Harvest 1	4.14 ± 0.373 ^{a}	72.86 ± 9.30 ^{a}
Harvest 2	17.67 ± 2.63 ^{b}	144.22 ± 4.41 ^{b}

Table B.2 Mean and standard error from One-way ANOVAs comparing morphological traits of *Banksia attenuata* seedlings in the disconnected treatment. Different letters indicate a significant difference in a column ($p < 0.001$).

	Stem length (cm)	Root length (cm)
Harvest 1	4.88 ± 0.479 ^{a}	91.19 ± 12.77 ^{a}
Harvest 2	17.95 ± 1.64 ^{b}	184.80 ± 7.86 ^{b}

Table B.3 Mean and standard error from One-way ANOVAs comparing morphological traits of *Banksia littoralis* seedlings in the connected treatment. Different letters indicate a significant difference in a column ($p < 0.001$).

	Stem length (cm)	Root length (cm)
Harvest 1	5.57 ± 0.352 ^{a}	32.86 ± 3.83 ^{a}
Harvest 2	23.80 ± 2.23 ^{b}	144.40 ± 3.20 ^{b}

Table B.4 Mean and standard error from One-way ANOVAs comparing morphological traits of *Banksia littoralis* seedlings in the disconnected treatment. Different letters indicate a significant difference in a column ($p < 0.001$).

	Stem length (cm)	Root length (cm)
Harvest 1	5.75 ± 0.423 ^{a}	27.13 ± 3.28 ^{a}
Harvest 2	19.80 ± 1.24 ^{b}	159.20 ± 6.40 ^{b}

Treatment comparison

Harvest 1

Table B.5 One-way ANOVA results showing mean and standard error of vessel length measurements in *Banksia attenuata* and *B. littoralis* seedlings for both connected and disconnected treatments. ns = a non-significant difference found in a column (*p* > 0.05).

	Banksia attenuata	*Banksia littoralis*
Connected Treatment	8.260 ± 1.494 ^{ns}	All < 1 cm
Disconnected Treatment	5.729 ± 1.316 ^{ns}	All < 1 cm

Table B.6 Mean and standard error of morphological traits of *Banksia attenuata* seedlings in both connected and disconnected treatments. ns = a non-significant difference found in a column (*p* > 0.05).

	Stem length (cm)	Root length (cm)
Connected Treatment	4.143 ± .373 ^{ns}	78.833 ± 8.428 ^{ns}
Disconnected Treatment	4.875 ± .479 ^{ns}	80.214 ± 7.551 ^{ns}

Table B.7 Mean and standard error of morphological traits of *Banksia littoralis* seedlings in both connected and disconnected treatments. ns = a non-significant difference found in a column (*p* > 0.05).

	Stem length (cm)	Root length (cm)
Connected Treatment	5.571 ± .352 ^{ns}	32.857 ± 3.826 ^{ns}
Disconnected Treatment	5.570 ± .423 ^{ns}	27.125 ± 3.280 ^{ns}

Harvest 2

Table B.8 Mean and standard error of vessel length measurements in *Banksia attenuata* and *B. littoralis* seedlings for both connected and disconnected treatments. ns = a non-significant difference found in a column ($p > 0.05$).

	Banksia attenuata	*Banksia littoralis*
Connected Treatment	17.900 ± .676 ^{ns}	8.920 ± 1.261 ^{ns}
Disconnected Treatment	19.340 ± .671 ^{ns}	9.533 ± .844 ^{ns}

Table B.9 Mean and standard error of morphological traits of *Banksia attenuata* seedlings in both connected and disconnected treatments. Different letters indicate a significant difference in a column ($p < 0.05$), ns = a non-significant difference found in a column ($p > 0.05$).

	Stem specific density	Stem length (cm)	Root length (cm)
Connected Treatment	0.325 ± .008 ^{ns}	17.75 ± 2.350 ^{ns}	144.22 ± 4.409 ^{a}
Disconnected Treatment	0.307 ± .007 ^{ns}	17.95 ± 1.644 ^{ns}	184.80 ± 7.855 ^{b}

Table B.10 Mean and standard error of morphological traits of *Banksia littoralis* seedlings in both connected and disconnected treatments. ns = a non-significant difference found in a column ($p > 0.05$).

	Stem specific density	Stem length (cm)	Root length (cm)
Connected Treatment	0.262 ± .008 ^{ns}	23.80 ± 2.225 ^{ns}	144.40 ± 3.198 ^{ns}
Disconnected Treatment	0.274 ± .009 ^{ns}	19.80 ± 1.236 ^{ns}	159.20 ± 6.403 ^{ns}

Appendix C

Objective 3: One-way ANOVA results

Table C.1 Mean and standard deviation of vessel length (VL) measurements in *Banksia attenuata* and *Banksia littoralis* seedlings for both Harvest 1 and Harvest 2. Different letters indicate a significant difference in a column ($p < .001$).

	Harvest 1	Harvest 2
Banksia attenuata	6.783 ± 3.517	18.658 ± 2.150 ^{a}
Banksia littoralis	All < 1 cm	9.150 ± 3.327 ^{b}

Table C.2 Mean and standard deviation of morphological traits of *Banksia attenuata* and *Banksia littoralis* in Harvest 1. Different letters indicate a significant difference in a column ($p < 0.05$).

	Stem length (cm)	Root length (cm)
Banksia attenuata	4.577 ± 1.305 ^{a}	79.578 ± 19.433 ^{a}
Banksia littoralis	5.607 ± 1.059 ^{b}	28.679 ± 9.097 ^{b}

Table C.3 Mean and standard deviation of morphological traits of *Banksia attenuata* and *Banksia littoralis* in Harvest 2. Different letters indicate a significant difference in a column ($p < 0.05$). ns = no significant difference found in a column ($p > 0.05$).

	Stem specific density	Stem length (cm)	Root length (cm)
Banksia attenuata	0.316 ± 0.024 ^{a}	17.850 ± 6.243 ^{ns}	165.579 ± 28.628 ^{ns}
Banksia littoralis	0.268 ± 0.027 ^{b}	21.800 ± 5.908 ^{ns}	151.800 ± 17.328 ^{ns}