Nor any drop to drink: Water relations of the mangrove *Avicennia marina* (Forssk.) Vierh

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ABSTRACT

Essential players within the planet's water cycle, plants are themselves sensitive to ongoing global change. Future shifts in water availability are predicted to change the face of the earth's forests. In the last decades there have been many advances in our understanding of water relations of plants and their response to environmental conditions. However, most of these studies are based on relatively few North-American and European tree species, with a notable underrepresentation of southern-hemisphere or tropical tree species. This kind of knowledge is needed, not only to reach a representative understanding of eco-physiological diversity of plant function, but also to establish the full spectrum of forest responses to changing environmental conditions.

In my project, I study the water relations of the grey mangrove, *Avicennia marina (Forssk.) Vierh.,* in the temperate mangrove forests of New Zealand. Mangroves are enigmatic species, which live in the harsh conditions imposed by brackish water and periodically flooded soils. Although they have elicited much scientific curiosity throughout history, much of our knowledge of mangrove water-related physiology is based on manipulative experiments with seedlings. Over the course of two years, I employed a diverse array of ecophysiological techniques to closely monitor different aspects of adult *A. marina* water relations in the stem and crown.

When investigating the seasonal and monthly courses of water-induced stem radial changes with a variety of stem cycle analysis techniques, I found that these were highly heterogeneous even within the same individual. Despite this heterogeneity however, it was still possible to correlate the amplitude of stem radius changes with environmental conditions. Presence or absence of precipitation events was an important driver of stem swelling periods, whilst atmospheric water availability indicators, such as VPD explained stem shrinkage periods. I found that *Avicennia marina* shows unusual daytime refilling of stem elastic water storage tissues, which deviates from most terrestrial trees and is hypothesized to be due to endogenous osmotic adjustment. This theory is reinforced by my finding that light sum, a proxy for photosynthesis, is a prominent driver of stem swelling amplitudes. In order to understand the "peristaltic" depletion of internally stored water within the tree stem, I studied the seasonal changes in the timing and time-lags of peak stem swelling at different tiers. I discovered an annual switch in the direction of the peristaltic water depletion wave along the stem, potentially related to seasonal changes crown photosynthesis and influence of non-structural carbohydrate dynamics on stem radial change caused by the onset of the growing season. Daytime stem swelling was studied further by performing measurements directly on xylem tissue, revealing the inner-bark turgor driven signal behind whole-stem daytime swelling. However, due to *A. marina's* unique wood structure, consisting of multiple phloem-xylem layers, the use of this methodology yielded heterogeneous and highly variable results. Lastly, I found that leaf turgor pressure-probes proved a reliable source of information on leaf turgor dynamics. The study of leaf turgor confirmed the importance of fresh water inputs for leaf hydration. My results also suggested that osmoregulation behind daytime stem swelling momentarily overrides the water demands of transpiring leaves, causing delayed recovery of leaf turgor in the evenings. The time-lags between stem water storage mobilization and leaf turgor recovery followed changes in atmospheric water demand, and also showed differences in the sensitivity of the upper and lower stem to leaf water demands. My work is a significant contribution not only to the understanding of mangrove ecophysiology in particular, but also adds to our increasingly complex map of plant physiological diversity which goes far beyond the established paradigms of plant-water relations.

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ATTESTATION OF AUTHORSHIP

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published by another person (except where explicitly defined), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Alicia May Donnelllan Barraclough

Alicia

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CO-AUTHOR CONTRIBUTIONS

Chapter 3 - Catching the Swell (Donnellan et al. 2018, Tree Physiology)

Author	Contribution	Total %	Signature of Authors
Alicia Donnellan	Experimental design Data collection and field work Statistical analysis Data interpretation Writing	80	
Roman Zweifel	Review/edit	6	
Jarrod Cusens	Data collection and field work	6	
Sebastian Leuzinger	Review/edit	8	

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"I would describe myself
like a landscape I've studied in detail
like a word I'm coming to understand;
like my mother's face
like a ship that carried me
when the waters raged."
Rilke's Book of Hours

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To my mother, and the forests too

E uhi tai uhi tai	*The tide flows*
E uhi tai uhi tai ē	*The tide flows in*
Kei uhi tai ana ko ngā	*The sea covers the estuaries up*
manga ki	*to Otawhiwhi*
Otāwhiwhi	*Feet may strike against the*
Kei tūtuki te waewae	*sharp young*
Ki te poro o te paiaka	*shoots of the mangrove*
Uhi tai uhi tai	*The tide flows on into the land*

Traditional story of the people of Tauranga Moana, Aotearoa (New Zealand)
(Complied by E. Stokes 1980)

Chapter 1 - Setting the route: General Introduction and thesis outline

"In the desert there were also other kinds of trees, one of which had foliage like that of the bay-tree, and grew in places washed by the waves of the sea. These trees were on ground which was left dry by the ebb-tide; but when the water advanced they looked as if they had grown in the sea"

The Campaigns of Alexander – Aristobulus

1.1 Literature review

1.1.1 The water relations of trees

a. *The basics of plant water transport theory*

Acquisition, transport and conservation of water are all essential for plant function. Water is not only the solvent and stadium of plant metabolic reactions, but also transports nutrients and carbon throughout the plant. Water also maintains cell turgor and is therefore indispensable for structural integrity, cell expansion, cell division, and thus growth (Hsiao and Acevedo 1974, Muller et al. 2011). The development of our understanding on how plants acquire and transport water has a long and rich history, and we are still far from fully understanding plant water relations as they are not easy to define or measure (Brown 2013). As early as 1896, H.H. Dixon proposed that the force pulling water up through trees is generated when water evaporates from plant leaves. This idea later became the basis of the Cohesion-Tension Theory (CTT). The CTT states that water's hydrogen bonds assure a high level of cohesion between molecules, which together with adhesion to a plant's conduit walls, leads to formation of continuous water columns (Tyree 1997). These continuous columns can be subjected to negative pressures, or tensions, generated by the evaporation of water from leaf surfaces through tiny pores called stomata. These negative tensions constitute the main force lifting the water through the plant's hydraulic system, like water that is sucked through a straw.

Later, in 1948, Van den Honert quantitatively described CTT water flux through plants as a catenary process, establishing parallels with Ohm's law, usually applied in electrical systems. Plants' roots, xylem and leaves, together with soil and leaf boundary layer, become resistances to water flow. Capacitances in our "plant circuit" are represented by the ability of soil and plant tissues to store water. The driving force of water flux in this model is water potential ψ_w, which is the chemical potential of water described in pressure units. Thus, water will always flow from higher to lower water potentials. The water potential in a particular compartment of a plant is the sum of four different components: (1) the osmotic potential, contributes negatively to water potential and depends on solute concentration and temperature, (2) the pressure potential, also called hydrostatic pressure, contributes positively (turgence) or negatively (suction) to water potential, (3) the matric potential arising from the forces caused by porous bodies, which contribute negatively to water potential, and (4) gravity, which depends on the height of the water column and contributes negatively to water potential (Lambers et al. 2008). Because the water potential of the atmosphere is an order of magnitude lower than that of the leaf's intercellular spaces, water loss (i.e. transpiration) becomes a physical necessity. In this manner, transpiration lowers the pressure potential component and negative water potential is transmitted through the xylem to the roots, where water uptake will take place, if the root water potential is lower than that of the soil. This idea of plant vessels as conduits connecting water absorbed by roots in the soil to atmospheric evaporative demands imposed on plant leaves is known as the Soil Plant Atmosphere Continuum (SPAC) (Philip 1966).

Starting at the roots, it is generally acknowledged that water uptake can only occur if the water potential of radicular intercellular spaces is below that of soil, despite alternative hypotheses (Wegner 2014). Thus, roots can take up water either (1) due to a drop in hydrostatic potential in a transpiring plant or (2) active or passive accumulation of solutes in the roots, which lowers osmotic potential (Steudle 2001). Once water is taken up by roots, the sap flows along either symplastic (within plant cells) or apoplastic (along cell walls) pathways along the vascular tissue responsible for its transport: the xylem. Xylem vessels are made up of dead cells called Traqueal Elements (vessels and tracheids). Vessel quantity, shape and size is the result of a trade-off between hydraulic conductivity (large vessels), hydraulic security (small vessels) and growth carbon-use efficiency (Holtta et al. 2011, Pfautsch 2016). Along these conduits, water, osmolytes and nutrients flow to meet the demand of various tissues and organs. Axial flow towards transpiring leaves provides them with water necessary for photosynthesis, turgor and growth (Philip 1966, Goldstein et al. 1998, Lambers et al. 1998, Meinzer et al. 2001, Steppe and Lemeur 2004). Maintenance of turgor and hydration will also affect the transport of photosynthates in the phloem, vascular tissue responsible for their distribution (Holtta et al. 2006, Jensen et al. 2011, De Schepper et al. 2013, Sevanto 2014, Knoblauch and Peters 2017). Further, maintenance of turgor and adequate viscosity in the phloem is important as sugar osmolytes are thought to play a role in embolism repair through the refilling of empty vessels, although it is under debate (De Baerdemaeker et al. 2017, Nardinia et al. 2011).

Embolisms occur when the pressure in a vessel drops below pure water vapour pressure (Pw = 0), causing the water column to be in an unstable metastable state and susceptible to rupture. So, when do these extremely low water potentials occur in the xylem? One possibility is due to lower water potential in soil, either because of a decrease of soil water content, an increase in soil salinity or increased soil compaction. A second possibility is the lowering of water potential in the atmosphere due, for example, to a decrease in relative humidity (RH). A third possibility is increased gravitational pull on the water column in extremely tall trees or increased resistances along the transport pathway. Cavitation was traditionally thought to render a xylem vessel useless, as redissolving the resulting air bubble, or embolism, requires Pw to be elevated to extreme values, a thermodynamically costly process (Zimmerman et al. 2004). However, recent studies have found that cavitation caused by drought occurs more frequently than initially thought and that the high redundancy of the vascular system and the refilling of embolized vessels allows certain maintenance of hydraulic conductance (Mayr et al. 2014), although this is still a matter of debate (Sperry 2013). Research also suggests that embolisms most commonly occur in the form of nanobubbles, which are relatively common and often harmless (Schenk et al. 2015). Although the initial formulation of CTT and SPAC might give the impression that plants are mere passive pipes of water transport between water potential gradients, plants have evolved a number of physiological and anatomical characteristics that allow them to guarantee water uptake and storage, avoiding catastrophic cavitation (Maseda and Fernandez 2006, McDowell et al. 2008, Pfautsch 2016)

b. *Facing the challenges of water supply and demand*

One of the ways that woody plants have risen to the challenge of facing the constant imbalances that occur between water supply and water demand, is by storing water in their tissues. Radial flux occurs when water flows from the xylem in and out of storage tissues, which expand or contract in times of supply or demand, respectively (Herzog et al. 1995, Zweifel et al. 2000, Zweifel and Hasler

2001, Deslauriers et al. 2003). Although water can be stored in a variety of tissues and organs, from roots to leaves, water storage in the elastic tissues of the stem has received a notable amount of attention in the last decades (Herzog et al. 1995, Zweifel et al. 2000, Zweifel and Hasler 2001, Deslauriers et al. 2003, Oliva Carrasco et al. 2014, Robert et al. 2014, Vandegehuchte et al. 2014a, De Swaef et al. 2015, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Mencuccini et al. 2017). Depending on environmental conditions, water reserves in the main stem can contribute 10-50% of total daily transpiration stream (Oliva Carrasco et al. 2014 and references therein). In the stem and branches, water is thought to be stored in outer parenchyma tissues, elastic sapwood (Scholz et al. 2008), phloem and also xylem (Zweifel et al. 2014, Pfautsch et al. 2015a, Pfautsch et al. 2015b), though the importance of each appears to be species dependent. Radial flow of water has been successfully modelled, in its most simple form, as the difference between the water potential of xylem and storage tissues, times the resistance to flow (Zweifel et al. 2000, Zweifel and Hasler 2001, Zweifel et al. 2001, Steppe et al. 2006, De Swaef et al. 2015).

These radial movements of water cause branches and trunks to significantly shrink and swell on a diel basis. This change in tissue volume under a certain change in water potential equals the tissue capacitance, and it describes the capacity of the storage tissue to mitigate changes in water potential caused by transpiration (De Swaef et al. 2015). The study and modelling of stem radius change (SRC) provides insights on how water storage and mobilization in elastic tissues varies with physiological and environmental conditions, and how water storage contributes to a tree’s “water economy”. Thus, the classic view of the two great transport systems in plants as separate, water in the xylem and sugar in the phloem is inaccurate (Pfautsch et al. 2015a, Pfautsch et al. 2015b, Pfautsch 2016). Not only are these systems intimately connected at the sites of sugar collection at sources and unloading at sinks, but they are also closely linked along the rest of the transport pathway, for example through ray parenchyma. Evidence suggests that radial movement of water can take an apoplastic (across cell walls) and/or a symplastic route (cell-to-cell) (Steppe et al. 2012, De Schepper et al. 2013, De Swaef et al. 2015, Pfautsch et al. 2015a, Pfautsch et al. 2015b). Symplastic pathways occur predominantly through parenchyma rays (Pfautsch et al. 2015a, Pfautsch et al. 2015b). An experiment by Pfaustch et al. (2015a) showed how water travelled from the phloem by entering ray parenchyma cells and travelling into the cambial zone until reaching the xylem. Transport through cell membranes is also influenced by aquaporin, and research suggests that changes in aquaporin expression can alter hydraulic conductance between xylem and water storage tissues (Steppe et al. 2012).

The key gatekeepers waiting at the end of water's pathway to the atmosphere are the stomata, where the bulk of CO_2 uptake and water loss takes place. In response to internal and environmental conditions, stomatal aperture can regulate transpirational water loss and thus plant water uptake, flux and storage. In this manner, stomata face a trade-off by which they must minimize water loss whilst guaranteeing carbon uptake (Buckley and Mott 2013). On the one hand, large timescale response of stomata to environmental conditions, such as light, CO_2 or water availability, are manifest through changes in stomatal density (number of stoma per leaf area). Hence, since the industrial revolution's increase in atmospheric CO_2, a clear decrease in stomatal density of certain species has been observed in herbarium records (Woodward 1987). On the other hand, the short term stomatal response to environmental signals occurs through changes in turgor of the cells that make up the stomatal pore, the guard and epithelial cells. These changes in aperture modify stomatal conductance (mmol m^{-2} s^{-1}) to CO_2 and water for a given boundary layer conductance (Buckley 2005). Stomatal guard and epithelial cells are known to respond predominantly to light, CO_2, temperature, water availability and wind speed. Though the metabolic mechanisms by which stomatal guard cells change turgor are relatively well described (Sirichandra et al. 2009) the complex response to multiple environmental and internal factors is not fully understood (Merilo et al. 2014). However, in trees it appears that the hydraulic signal dominates over photosynthetic signals (Aasamaa and Sober 2011, Merilo et al. 2014), prioritizing hydraulic security over carbon assimilation (although see Nikinmaa et al. 2013). In this manner, changes in soil water status are thought to lead to stomatal closure through Abscisic Acid signalling (ABA) or changes in xylem pH (Dodd 2005). Stomatal closure upon decreasing soil water potential is less well understood (Lambers et al. 1998) and thought to not rely primarily on long-distance ABA signalling, although the involvement of guard cell autonomously synthesized ABA has recently been discovered (Bauer et al. 2013). Stomatal sensitivity to leaf water potential, and consequent changes in stomatal conductance, has often led to plants being classified as: anisohydric (both leaf water potential and stomatal conductance decline with decreasing water availability) or isohydric (leaf water potential is kept within a narrow range through strict regulation of stomatal aperture during decreasing water availability) (Tardieu and Simonneau 1998). The level of stomatal control will determine water loss and risk of cavitation, nutrient uptake via promotion of mass flow, tolerance to toxic gases, regulation of leaf temperature, resistance to heat stress, and last but not least, carbon assimilation (Haworth et al. 2011).

As we have seen, water is an important limiting factor for plant growth and stomata play a key role in the trade-off plants face by which they must guarantee C assimilation while minimizing water loss. This ratio is termed Water Use Efficiency (WUE) and is one of the key parameters which links water and carbon cycles in plants. Drought induced stomatal closure leads to high WUE as the plant tries to conserve water. However, conservative water strategies are at the expense of carbon assimilation, eventually leading to dwindling carbon reserves (Woodruff et al. 2015). In this setting, the question arises of how trees die under drought, is it hydraulic failure, increased vulnerability to disease, or carbon starvation? Despite recent studies suggesting that "thirst beats hunger" (Hartmann et al. 2013) the question is far from resolved and this debate has been the object of enormous amount of research effort (Hsiao and Acevedo 1974, McDowell et al. 2008, Allen et al. 2010, Woodruff and Meinzer 2011, Anderegg et al. 2012, Hartmann et al. 2013, McDowell et al. 2013, Sperry and Love 2015, Pfautsch 2016). A deeper grasp of tree survival and mortality under water shortage will allow us to understand drought induced loss of ecosystem functions and services, and the role of forest within the globe’s water and carbon cycle.

c. *Additional pathways for water*

Additional to these well-accepted pathways of water within a plant, recent additions to the CTT and the SPAC propose other additional mechanisms of water supply and transport. Some of these additions were initially controversial, as they were accompanied by criticisms of the CTT (Zimmermann et al. 2004 and references therein). Zimmermann doubts the fact that land plants rely on the ascent of water in a metastable state and proposes that a combination of mechanisms guarantee water supply without the need to sustain very negative ψ_w. Despite the fact that xylem pressure probe measurements and other lines of evidence used by Zimmermann to disprove large negative water potentials in plants have been discredited (Angeles et al. 2004), many of the mechanisms in his Multi-Force theory of plant water supply are worthy of further investigation. Amongst the more widely acknowledged mechanisms additional to the traditional CTT and SPAC are foliar water uptake, reverse water fluxes and hydraulic redistribution (HR) (Nadezhdina et al. 2009, Nadezhdina et al. 2010). These are thought to occur when water flow reverses and can be directed in any direction along the ψ gradient. Goldsmith (2013) recently highlighted the importance of different kinds of HR and that, though the SPAC model is not incorrect, it is incomplete. There is compelling evidence to consider the existence of additional water pathways, which are the reverse of SPAC: the Atmosphere Plant Soil Continuum. One kind of HR occurs when the stem plays a role in redistributing water horizontally or vertically amongst roots at different depths and water availabilities. Another kind of HR is Foliar Uptake (FU), which distributes water taken up by leaves wetted in humid or foggy weather conditions and is thought to be an important source of water in tall trees (Burgess and Dawson 2004, Zimmermann et al. 2007, Eller et al. 2013). It is also thought to be an important source of water in arid ecosystems where small precipitation events do little to change soil water moisture but help maintain leaf hydration (Breshears et al. 2008). The importance of this source of water has been investigated in a variety of plant taxa and across ecosystems, including tropical montane cloud forests (Goldsmith et al. 2013); sub-tropical rain-forests (Yates and Hutley 1995); coastal redwood forests (Burgess and Dawson 2004, Limm et al. 2009); continental dry forests (Breshears et al. 2008); and, both angiosperms and gymnosperms.

1.1.2 Mangrove systems

Mangrove forests are a diverse biome that occupies the intertidal zones of tropical and subtropical latitudes around the world. They are a unique system of halophytic salt tolerant plants, which comprise approximately 54 species in 20 genera belonging to 16 families (Hogarth 1999). Mangrove ecosystems are characterized by zonation patterns based on tidal influence, salinity and aridity (Berlyn 1986, Tomlinson 1986, Ball 1988a, Ball and Pidsley 1995, Hogarth 1999, Morrisey et al. 2010). In New Zealand however, mangrove forests are composed exclusively of *Avicennia marina* (Forssk.) Vierh. (the grey mangrove, or *manawa* in New Zealand), which is one of the most widespread species with a latitudinal range from 25 °N in Japan to 38 °S in Australia (Berlyn 1986). Amongst mangroves, it has the highest tolerance to salinity, aridity, water temperature changes and frost frequency (Morrisey et al. 2010).

a. *Mangrove adaptations to their environment*

An intense debate still exists on the status of different mangrove species as obligate halophytes (Wang et al. 2011, Krauss and Ball 2013). A large body of research points towards the importance of adequate salinity in the early stages of mangrove development (Nguyen et al. 2015), but also to the importance of fresh water inputs for continued growth (Krauss et al. 2008). Mangroves present a series of unique characteristics which allow them to survive the extremely negative water potentials and waterlogging of intertidal soils. Not only must they surpass the dehydrating and toxic effects of salt, but also maintain water potentials within their hydraulic system that are negative enough to guarantee water uptake. These salt tolerant characteristics span a number of morphological, anatomical, physiological, biochemical and molecular attributes (Parida and Jha 2010), which are involved in the processes of salt exclusion, accumulation and excretion. Exclusion occurs in the hydrophobic and apoplastic barriers of the root endodermis, allowing for ultrafiltration of water and elimination of 97-99% of salt (Krishnamurthy et al. 2014). Excretion allows them to excrete salts at leaf level through specialized salt glands, in a process facilitated by abundant trichomes (hairs) on leaf abaxial surfaces. Salt accumulation in cell vacuoles is facilitated by compatible solutes in the cytoplasm (e.g. Parida et al. 2004). Accumulation of salt in leaves can lead to leaf succulence or accumulation in senescent leaves and subsequent abscission (Zheng et al. 1999).

A remarkable anatomical trait of *A. marina* is their multiple reticulate active cambia. This is a unique characteristic by which consecutive bands of xylem are interspersed with secondary internal phloem strands connected by a layer of parenchyma tissue (Schmitz et al. 2008). In a study comparing different wood growth rates, structure and densities in *Avicennia marina*, Santini et al. (2012) found that an increased quantity of phloem tissue was associated to higher salinity, lower wood density and lower growth rates. In this study they postulate the possible role of increased phloem percentage in refilling of embolized xylem, as starch polymerization is thought to be involved in this process (Nardinia et al. 2011). Robert et al. (2014) also found that this wood anatomical trait is associated to plants and trees which grow in water stressed environments, and thus must have a role in water storage and safety. Additional explanations may be found in the recent work of Oliva Carrasco et al. (2014), which revealed negative correlations between sapwood density and water storage capacity and capacitance of stems across multiple species. Thus, it may be that the low density of *A. marina* stems allows them to act as a “sponge”, storing water for times of demand in the elastic tissues of phloem and parenchyma (Pfautsch et al. 2015b).

Lastly, to cope with water logged and oxygen deprived soils, *A. marina* possess pneumatophores, a form of aerial roots exposed to the air in periods of low tide (Tomlinson 1986). Pneumatophores contain numerous lenticels and extensive aerenchyma which facilitate the diffusion of oxygen. This takes place 1000 times faster through air than through water, diffusing down partial pressure gradients from the atmosphere to sites of respiration in the roots (Scholander et al. 1955)

b. *The study of mangrove ecophysiology*

The study of mangrove water relations has attracted significant interest for a long time, as scientists and naturalists have puzzled over the apparent resilience of this species (Scholander et al. 1955, Scholander et al. 1966, Scholander 1968, Ball 1988a, b). Thanks to advancements in available technology, a number of studies have now addressed the more dynamic aspects of mangrove water ecophysiology through high temporal resolution measurements that allow a glimpse into ever changing water fluxes and pools and their connection to growth. However, field experiments in adults are still rare, and seedling and sapling experiments dominate the mangrove literature.

Mangroves must maintain hydraulic safety at the same time as they guarantee carbon uptake. Thus, their environment makes them into obligate conservatives. Much evidence points towards higher salinities leading to a decrease of water uptake (Reef and Lovelock 2015), conductivity and transport

(López-Portillo et al. 2014), water use, transpiration and stomatal conductance (Ball 1988b, Parida and Jha 2010, Krauss et al. 2014, Reef et al. 2015, Santini et al. 2015), and an increased reliance on leaf and stem water storage (Lechthaler et al. 2016, Nguyen et al. 2017a). In the hydraulic system, increasing salinity is associated with increases in xylem tension, and changes in hydraulic conductance. These limitations to gas exchange and water supply have all been linked with reduced relative growth rates and leaf area ratios (Nguyen et al. 2015), with important implications for mangrove forest structure (Krauss et al. 2008). A significant amount of research also points towards the importance of freshwater inputs, as mangroves seem to preferably use freshwater sources when available (Reef and Lovelock 2015, Reef et al. 2015, Santini et al. 2015, Lovelock et al. 2017). Freshwater inputs not only come in the form of precipitation and fresh groundwater but recent evidence points towards the use of atmospheric water, at least at the foliar level (Lovelock et al. 2017, Nguyen et al. 2017a, Nguyen et al. 2017b).

Field studies on stem water storage in mangrove adults have provided backing for the idea of the importance of rainfall (Robert et al. 2014). However research remains inconclusive on the effects of natural changing salinity on stem radius change in adults (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b), although Lechthaler et al. (2016) showed an increased reliance on water stores in the hypocotyl of mangrove saplings in high soil salinity conditions. Although a large body of work has looked at the effects of different water availability and salinity regimes on mangrove growth, nutrient levels and water use in seedlings and saplings (Krauss et al. 2008, Nguyen et al. 2015), there is still little information on the dynamic elements of water fluxes and pools in mature mangrove trees living under fluctuating environmental conditions, water availability and natural salinity changes (Robert et al. 2014, Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b) .

Field studies of SRC in *A. marina* have shown that courses of water storage mobilization and refilling are highly heterogeneous, much more so than in other trees (Schmitz et al. 2008, Robert et al. 2011a, Robert et al. 2011b, Robert et al. 2014). Similar results exist for studies of sap flow density in *A. marina* stems, which have shown that sap flow varies radially within the stem, and different measurement points vary significantly in absolute values and responses to environmental conditions (Van de Wal 2011, Van de Wal et al. 2014). The reason for this heterogeneity is thought to lie in the reticulate wood structure of *A. marina*, which is a highly heterogeneous net of interconnected and highly branched web of internal secondary phloem and xylem patches (Robert et al. 2011a). Recent SRC studies on mangroves have shown the existence of daytime stem swelling, where stems continue expanding into the day, even after the start of transpiration and xylem water potential drop (Vandegehuchte et al. 2014a). This has been modelled successfully as a consequence of osmotic adjustment of storage tissues, where increased osmotic potential causes the flow of water into storage tissues in spite of xylem water potential drop (Vandegehuchte et al. 2014b). Although the aforementioned study showed osmotic adjustment was strongest in the study species *Rhizophora stylosa*, which presented more pronounced daytime swelling than *A. marina*, it is still to be established how prominent daytime swelling is in *A. marina*. Daytime stem swelling requires further research as it may have an important role in guaranteeing water storage for times of high demand and ensuring the turgor necessary for cell division, expansion and therefore growth.

1.2 Research justification

Since first encountering mangroves in 2012 during my work as a conservation practitioner in Madagascar, I was fascinated by this unusual and somewhat impenetrable biome. Why did some areas of our replantation succeed and others fail? What environmental conditions were playing a major role in mangrove reestablishment and growth? Understanding the ecophysiology of mangroves is not just a question of simple naturalist or scientific curiosity. Mangrove forests are recognized for their importance to humans, wildlife and the global carbon cycle, and suffer from degradation in many parts of the world (Kristensen et al. 2008, Walters et al. 2008, McLeod et al. 2011, Alongi 2014, Malik et al. 2015, Atwood et al. 2017). Furthering our ecophysiological knowledge of these unique forested wetlands can provide opportunities for researchers and planners to better understand them and improve their management (Daru and le Roux 2016).

Additionally, New Zealand mangroves constitute an ideal system for the pursuit of unanswered questions within forest water and carbon relations. Firstly, much of our water relations knowledge and classical paradigms are based on the study of a few European and North American tree species. Considering that the largest biomass and diversity of trees is found outside of these areas, notably in the tropics, it would not be unreasonable to say that our knowledge is likely skewed by a relative overrepresentation of certain highly studied species. As our understanding widens and our knowledge includes a greater variety of other species, we are coming to understand that the world of plant water and growth relations is much more diverse and complex than previously thought (Mencuccini et al. 2017). Secondly, due to water shortage and salinity exposure having comparable effects on plant physiology (Munns 2002), mangroves are a particularly interesting system for the study of the response and consequences of drought. Another appealing characteristic of New Zealand mangroves is their homogeneity, as they are solely composed of the grey mangrove tree, *A. marina*. In this manner, despite being a natural system, there is an obvious reduction in the variability introduced by heterogeneous vegetation composition and stand structure, present in most natural systems. The stand monospecificity also eliminates the possibility of interspecific competition, a confounding factor often found in water and carbon relations studies (Leuzinger and Hättenschwiler 2013).

Lastly, New Zealand mangroves, although they have their own unique challenges as a study system, allow us to circumvent some of the operational problems often associated with the study of forest stands. Mainly, access to tree canopies often involves deployment of costly specialized infrastructure and resources, such as cranes or tree climbers. In New Zealand, *A. marina* does not exceed 8 m and thus provides trouble-free access to the canopy. This also allows the study of adult individuals without the need to resort to logistically simpler seedling experiments. Mangrove seedling or young sapling experiments truly dominate mangrove research literature (Ball 1988a, Krauss et al. 2008, Nguyen et al. 2015). Not only are the latter markedly reductionist, one must also keep in mind that plant ecophysiological responses change throughout development, with carbon and water relations of adult individuals being different from that of seedlings.

1.3 Research Questions

My thesis is articulated around three main questions on the water relations of *Avicennia marina,* which are addressed in four experimental chapters (Figure 1.1). My main driving questions are as follows:

1. What are the main environmental and biological drivers of stem water storage dynamics of adult *Avicennia marina* under field conditions? Considering the previously recorded patchiness and heterogeneity of stem radial change in *A. marina*, what is the nature of the variability of different stem cycle elements, such as amplitudes and rates, and can they be related to seasonal changes and stem tier, or are they purely stochastic in nature?
2. How does daytime swelling relate to the rest of *A. marina* water relations? Does daytime stem swelling originate in the inner bark and is it caused by a phloem-generated turgor signal due to osmotic adjustment and carbohydrate dynamics? How does daytime stem swelling change along the stem and throughout the seasons?
3. What are the high resolution leaf turgor dynamics in *A. marina* and how are they affected by environmental conditions and freshwater inputs? How are leaf turgor dynamics and leaf hydration affected by the delayed onset of stem shrinking due to daytime stem swelling?

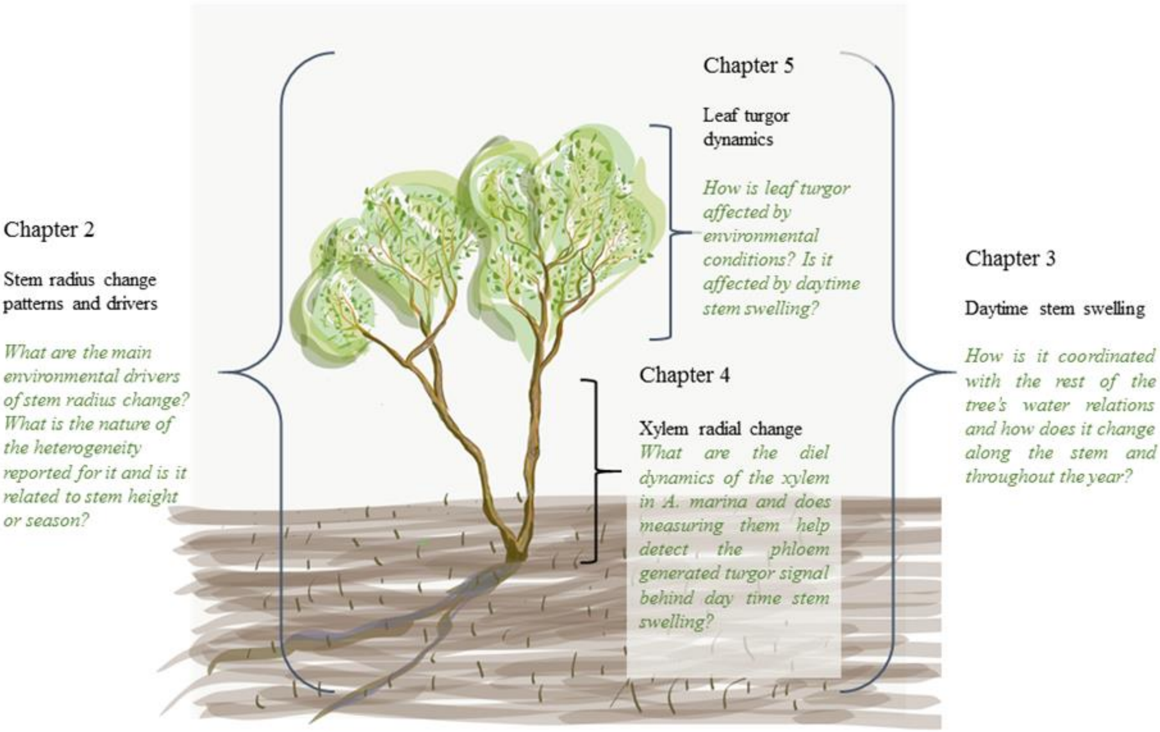


Figure 1.1 View of thesis chapters and the questions addressed in each in relation to the water relations of the mangrove *Avicennia marina*.

1.4 Thesis structure and rationale

In order to answer the questions established in the previous section, this thesis is structured into four experimental chapters (Chapter 2-5) and a general discussion (Chapter 6). The experimental chapters address the following questions:

Chapter 2 - Testing the Waters: Analysing the heterogeneous stem radial change dynamics of the mangrove *Avicennia marina (Forssk.) Vierh* looking for indicators of environmental influence on radial water storage

This chapter means to set the scene for my work. Interpreting stem radius changes in *A. marina* has been reported a challenge due to this species' unique wood structure causing heterogeneous patterns. In this chapter it is my intention to clarify the level of heterogeneity in water induced changes in SRC in *A. marina* through a stem cycle partitioning approach, and analyse if it is possible to discern the main environmental drivers of water induced shrinking and swelling of the stem.

Chapter 3 - Catching the swell: Daytime stem expansion and seasonal reversal in the peristaltic wave of stored water use along the stem of *Avicennia marina* (Forssk.) Vierh

This chapter means to lean in a little closer. I analyse diel water-related stem radius change in concert with other important measures of tree water relations, trying to make sense of daytime stem swelling. I will study how daytime stem swelling varies along the stem and throughout the year, and how it is affected by physiological and environmental changes.

Chapter 4 – Disentangling the net: Separating components of *Avicennia marina* (Forssk.) Vierh stem radial water dynamics by concomitant xylem and over-bark measurements

This chapter takes a step in the direction of understanding daytime stem swelling in *A. marina*. Firstly, I asses the utility of a method commonly used to ascertain the different contributions of stem tissues to stem radius change by performing whole stem and xylem radius change measurements. Secondly, I will assess if these measurements detect a phloem-generated turgor signal responsible for daytime stem swelling.

Chapter 5: No leaf is an island: using turgor pressure probes to study the relationship of leaf turgor to microclimate and water relations in *Avicennia marina (Forssk.) Vierh.*

In this chapter I take a closer look at leaf water relations, in order to understand the main environmental drivers of leaf turgor changes and how daytime stem swelling affects leaf turgor dynamics. I will give special attention to the effect of fresh water inputs through precipitation, to understand if they are important for maintaining leaf turgor.

And finally, a discussion on my results:

Chapter 6: In sight of land: Discussion, conclusions and way forward

1.5 Methods summary

> *“Rainwater poured off leaves all around them, and they turned their mouths up to the roof of the jungle and drank; but perhaps because the water came to them by way of sundri leaves and mangrove branches and nipa fronds, it acquired on its journey something of the insanity of the jungle, so that as they drank they fell deeper and deeper into the thraldom of that livid green world where the birds had voices like creaking wood and all the snakes were blind.”*
>
> — Salman Rushdie, Midnight's Children

Anyone who has worked in a mangrove system probably understands the kind of “mangrove fever” suffered in this passage by the character Saleem, which Salman Rushdie so genially puts to words. Despite its attractiveness, the mangrove is undoubtedly a challenging environment, for people and instruments! Many an instrument felt pray to rust unless constant maintenance was given, the salt and high humidity a fatal combination. The black mud and hidden holes always in cahoots with the merciless pneumatophores are an open challenge to the resilience of any footwear, and the footwear bearer too for that matter. Still, we succeeded to build a platform and even a (somewhat perilous) boardwalk through the mangroves in order to make the work viable. Although the methods appropriate to each question are described in detail in each chapter, here follows a brief overall summary.

1.5.1 Research site and station

The study site is part of the Mangawhai estuary, 100 km north of Auckland, New Zealand (36.097°S, 174.573°E). Mangawhai area has two main waterways running across it, the Tara Creek that drains to the north of the study area and Bob Creek that flows to the west. The surrounding wetlands are composed of mud and sand flats, marshes and approximately 80 ha of mangroves. The site was chosen because AUT University Institute of Applied Ecology New Zealand conducts a number of studies in this region (e.g. Tran 2014, Alfaro 2010). The study site receives the name of Jack Boyd and is situated at the upper tidal zone of Tara Creek, furthest from the sea shore with sandy substrate and short inundation times, twice every 24 hours. A channel bringing fresh water down from the mainland is on the eastern aspect of the site. The stand is monospecific and homogeneous, composed of *Avicennia marina* individuals 3-4 m in height. Within the stand, we picked an area at least 50 m from the channel to avoid edge effects related to light and proximity to the stream. Mature co-dominant trees were chosen for the study to avoid (1) heterogeneity arising from different tree ages (2) young trees in the exponential phase of their growth (3) differences in water use due to different canopy, root and stem sizes.

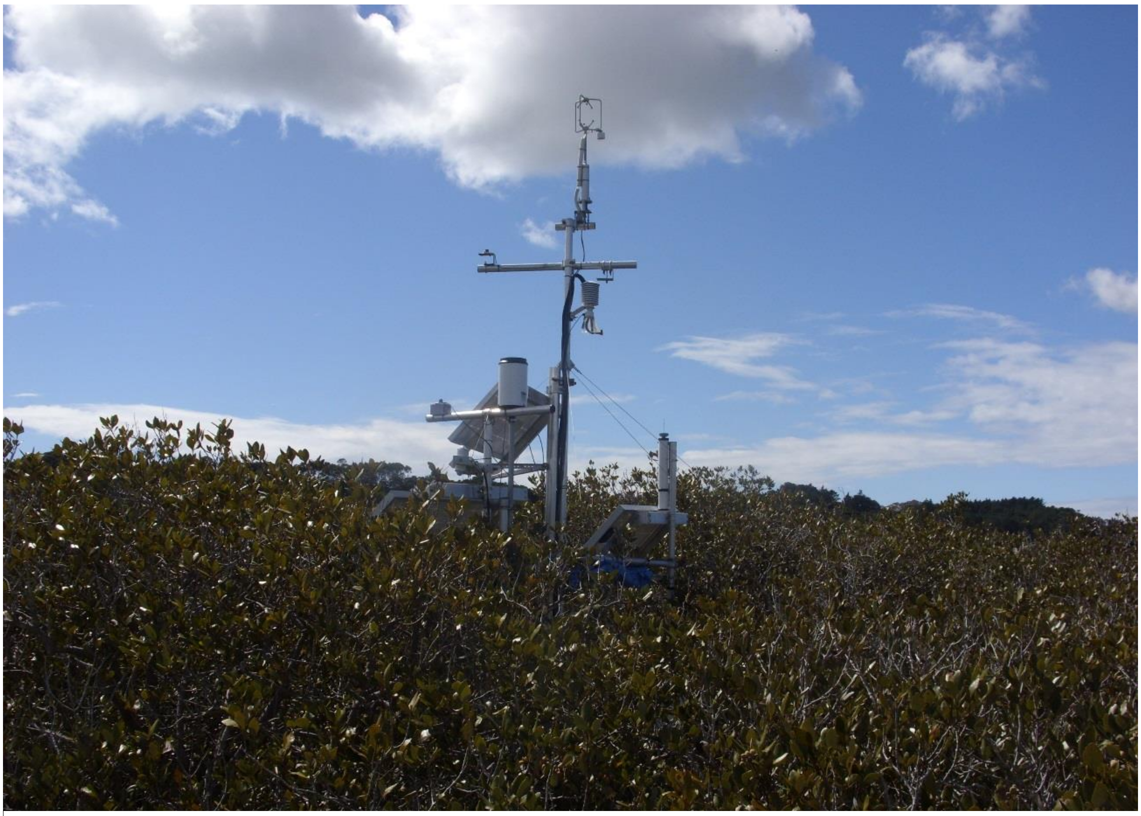


Figure 1.2 View from the mangrove canopy of the field station at Mangawhai estuary where microclimate instruments were installed.

To answer the proposed research questions a variety of environmental variables were measured on site. This is important, as research suggests that microclimatic conditions within or near the canopy explain plant physiological responses to a higher degree than those measured in near-by weather stations (Chelle 2005, Zweifel et al. 2007). Thus, a platform was set up in the canopy which recorded common microclimatic variables such as temperature, relative humidity, light, radiation balance and rainfall (Figure 2). Radiation balance was measured by a net radiometer (CNR1 Kipp & Zonen B.V.), which records incoming and reflected long- and short-wave radiation. Vapour Pressure Deficit (VPD) has been shown to be more indicative of atmospheric "dryness" experienced by plants than relative humidity. Thus, VPD is calculated from temperature and relative humidity data according to Buck (1981) measured with a temperature-relative humidity sensor (Sensiron, model SHT). Within the soil, a sensor which measures soil conductivity, temperature and tidal depth was deployed (CTD gage, Decagon Devices, Inc.). Soil water potential (MPa) was calculated from soil conductivity following McIntyre (1980) equation (7) which is as follows:

$$\log(\prod) = 1.09 \log k - 0.46$$

where $\prod$ is osmotic soil water potential in bar and k is conductivity in mS/cm.

In my work I employed a series of techniques for measuring water relations of *A. marina*. Some of these are point measurements, like those of leaf water potential or stomatal conductance. Others, like those measuring stem radial change, sap flow and leaf turgor, are continuous and were installed the study trees for the whole measurement period. The details on instrumentation and installation are described in each chapter.

Figure 1.3 Installation of ecophysiological sensors on one of the measured mangrove trees.

1.6 References

Aasamaa, K. and A. Sober. 2011. Stomatal sensitivities to changes in leaf water potential, air humidity, CO_2 concentration and light intensity, and the effect of abscisic acid on the sensitivities in six temperate deciduous tree species. Environmental and Experimental Botany **71**:72-78.

Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.-H. Lim, G. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management **259**:660-684.

Alongi, D. M. 2014. Carbon cycling and storage in mangrove forests. Annual Review of Marine Science **6**:195-219.

Anderegg, W. R. L., J. A. Berry, D. D. Smith, J. S. Sperry, L. D. L. Anderegg, and C. B. Field. 2012. The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. Proceedings of the National Academy of Sciences of the United States of America **109**:233-237.

Angeles, G., B. Bond, J. S. Boyer, T. Brodribb, J. R. Brooks, M. J. Burns, J. Cavender-Bares, M. Clearwater, H. Cochard, J. Comstock, S. D. Davis, J. C. Domec, L. Donovan, F. Ewers, B. Gartner, U. Hacke, T. Hinckley, N. M. Holbrook, H. G. Jones, K. Kavanagh, B. Law, J. Lopez-Portillo, C. Lovisolo, T. Martin, J. Martinez-Vilalta, S. Mayr, F. C. Meinzer, P. Melcher, M. Mencuccini, S. Mulkey, A. Nardini, H. S. Neufeld, J. Passioura, W. T. Pockman, R. B. Pratt, S. Rambal, H. Richter, L. Sack, S. Salleo, A. Schubert, P. Schulte, J. P. Sparks, J. Sperry, R. Teskey, and M. Tyree. 2004. The Cohesion-Tension theory. New Phytologist **163**:451-452.

Atwood, T. B., R. M. Connolly, H. Almahasheer, P. E. Carnell, C. M. Duarte, C. J. E. Lewis, X. Irigoien, J. J. Kelleway, P. S. Lavery, P. I. Macreadie, O. Serrano, C. J. Sanders, I. Santos, A. D. L. Steven, and C. E. Lovelock. 2017. Global patterns in mangrove soil carbon stocks and losses. Nature Climate Change **7**:523-528

Ball, M. C. 1988a. Ecophysiology of mangroves. Trees **2**:129-142.

Ball, M. C. 1988b. Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I. Water use in relation to growth, carbon partitioning, and salt balance. Functional Plant Biology **15**:447-464.

Ball, M. C. and S. M. Pidsley. 1995. Growth reponses to salinity in relation to ditribution of two mangrove species, *Sonneratia* alba and *S. lanceolata*, in northern Australia. Functional Ecology **9**:77-85.

Bauer, H., P. Ache, S. Lautner, J. Fromm, W. Hartung, K. A. S. Al-Rasheid, S. Sonnewald, U. Sonnewald, S. Kneitz, N. Lachmann, R. R. Mendel, F.

Bittner, A. M. Hetherington, and R. Hedrich. 2013. The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. Current Biology **23**:53-57.

Berlyn, G. P. 1986. The botany of mangroves: Tomlinson, P. B. Science **234**:373-373.

Breshears, D. D., N. G. McDowell, K. L. Goddard, K. E. Dayem, S. N. Martens, C. W. Meyer, and K. M. Brown. 2008. Foliar absorption of intercepted rainfall improves woody plant water status most during drought. Ecology **89**:41-47.

Brown, H. R. 2013. The theory of the rise of sap in trees: Some historical and conceptual remarks. Physics in Perspective **15**:320-358.

Buck, A. L. 1981. New equations for computing vapor-pressure and enhancement factor. Pages 1527-1532 Journal of Applied Meteorology.

Buckley, T. N. 2005. The control of stomata by water balance. New Phytologist **168**:275-292.

Buckley, T. N. and K. A. Mott. 2013. Modelling stomatal conductance in response to environmental factors. Plant Cell and Environment **36**:1691-1699.

Burgess, S. S. O. and T. E. Dawson. 2004. The contribution of fog to the water relations of Sequoia sempervirens (D. Don): foliar uptake and prevention of dehydration. Plant Cell and Environment **27**:1023-1034.

Chelle, M. 2005. Phylloclimate or the climate perceived by individual plant organs: What is it? How to model it? What for? New Phytologist **166**:781-790.

Daru, B. H. and P. C. le Roux. 2016. Marine protected areas are insufficient to conserve global marine plant diversity. Global Ecology and Biogeography **25**:324-334.

De Baerdemaeker, N. J. F., R. L. Salomon, L. De Roo, and K. Steppe. 2017. Sugars from woody tissue photosynthesis reduce xylem vulnerability to cavitation. New Phytologist **216**:720-727.

De Schepper, V., T. De Swaef, I. Bauweraerts, and K. Steppe. 2013. Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany **64**:4839-4850.

De Swaef, T., V. De Schepper, M. W. Vandegehuchte, and K. Steppe. 2015. Stem diameter variations as a versatile research tool in ecophysiology. Tree Physiology **35**:1047-1061.

Deslauriers, A., H. Morin, C. Urbinati, and M. Carrer. 2003. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees **17**:477-484.

Dodd, I. C. 2005. Root-to-shoot signalling: Assessing the roles of 'up' in the up and down world of long-distance signalling in planta. Plant and Soil **274**:251-270.

Eller, C. B., A. L. Lima, and R. S. Oliveira. 2013. Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). New Phytologist **199**:151-162.

Goldsmith, G. R. 2013. Changing directions: the atmosphere-plant-soil continuum. New Phytologist **199**:4-6.

Goldsmith, G. R., N. J. Matzke, and T. E. Dawson. 2013. The incidence and implications of clouds for cloud forest plant water relations. Ecology Letters **16**:307-314.

Goldstein, G., J. L. Andrade, F. C. Meinzer, N. M. Holbrook, J. Cavelier, P. Jackson, and A. Celis. 1998. Stem water storage and diurnal patterns of water use in tropical forest canopy trees. Plant Cell and Environment **21**:397-406.

Hartmann, H., W. Ziegler, O. Kolle, and S. Trumbore. 2013. Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. New Phytologist **200**:340-349.

Haworth, M., C. Elliott-Kingston, and J. C. McElwain. 2011. Stomatal control as a driver of plant evolution. Journal of Experimental Botany **62**:2419-2423.

Herzog, K. M., R. Häsler, and R. Thum. 1995. Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees **10**:94-101.

Hogarth, P. J. 1999. The biology of mangroves. Oxford University Press (OUP), Oxford, UK.

Holtta, T., M. Mencuccini, and E. Nikinmaa. 2011. A carbon cost-gain model explains the observed patterns of xylem safety and efficiency. Plant Cell and Environment **34**:1819-1834.

Holtta, T., T. Vesala, S. Sevanto, M. Peramaki, and E. Nikinmaa. 2006. Modeling xylem and phloem water flows in trees according to cohesion theory and Munch hypothesis. Trees-Structure and Function **20**:67-78.

Hsiao, T. C. and E. Acevedo. 1974a. Plant responses to water deficits, water-use efficiency, and drought resistance. Agricultural Meteorology **14**:59-84.

Jensen, K. H., J. Lee, T. Bohr, H. Bruus, N. M. Holbrook, and M. A. Zwieniecki. 2011. Optimality of the Munch mechanism for translocation of sugars in plants. Journal of the Royal Society Interface **8**:1155-1165.

Knoblauch, M. and W. S. Peters. 2017. What actually is the Munch hypothesis? A short history of assimilate transport by mass flow. Journal of Integrative Plant Biology **59**:292-310.

Krauss, K. W. and M. C. Ball. 2013. On the halophytic nature of mangroves. Trees **27**:7-11.

Krauss, K. W., C. E. Lovelock, K. L. McKee, L. López-Hoffman, S. M. L. Ewe, and W. P. Sousa. 2008. Environmental drivers in mangrove establishment and early development: a review. Aquatic Botany **89**:105-127.

Krauss, K. W., K. L. McKee, C. E. Lovelock, D. R. Cahoon, N. Saintilan, R. Reef, and L. Z. Chen. 2014. How mangrove forests adjust to rising sea level. New Phytologist **202**:19-34.

Krishnamurthy, P., P. A. Jyothi-Prakash, L. Qin, J. He, Q. Lin, C. S. Loh, and P. P. Kumar. 2014. Role of root hydrophobic barriers in salt exclusion of a mangrove plant *Avicennia officinalis*. Plant Cell and Environment **37**:1656-1671.

Kristensen, E., S. Bouillon, T. Dittmar, and C. Marchand. 2008. Organic carbon dynamics in mangrove ecosystems: A review. Aquatic Botany **89**:201-219.

Lambers, H., F. S. Chapin, and T. L. Pons. 1998. Plant physiological ecology. Springer-Verlag New York Inc., New York,.

Lechthaler, S., E. M. R. Robert, N. Tonne, A. Prusova, E. Gerkema, H. Van As, N. Koedam, and C. W. Windt. 2016. Rhizophoraceae Mangrove Saplings Use Hypocotyl and Leaf Water Storage Capacity to Cope with Soil Water Salinity Changes. Frontiers in Plant Science **7**.

Leuzinger, S. and S. Hättenschwiler. 2013. Beyond global change: lessons from 25 years of CO2 research. Oecologia **171**:639-651.

Limm, E. B., K. A. Simonin, A. G. Bothman, and T. E. Dawson. 2009. Foliar water uptake: a common water acquisition strategy for plants of the redwood forest. Oecologia **161**:449-459.

López-Portillo, J., F. W. Ewers, R. Méndez-Alonzo, C. L. Paredes López, G. Angeles, A. L. Alarcón Jiménez, A. L. Lara-Domínguez, and M. d. C. Torres Barrera. 2014. Dynamic control of osmolality and ionic composition of the xylem sap in two mangrove species. American Journal of Botany.

Lovelock, C. E., R. Reef, and M. C. Ball. 2017. Isotopic signatures of stem water reveal differences in water sources accessed by mangrove tree species. Hydrobiologia **803**:133-145.

Malik, A., R. Fensholt, and O. Mertz. 2015. Mangrove exploitation effects on biodiversity and ecosystem services. Biodiversity and Conservation **24**:3543-3557.

Maseda, P. H. and R. J. Fernandez. 2006. Stay wet or else: three ways in which plants can adjust hydraulically to their environment. Journal of Experimental Botany **57**:3963-3977.

Mayr, S., P. Schmid, J. Laur, S. Rosner, K. Charra-Vaskou, B. Damon, and U. G. Hacke. 2014. Uptake of water via branches helps timberline conifers refill embolized xylem in late winter. Plant Physiology **164**:1731-1740.

McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D. G. Williams, and E. A. Yepez. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist **178**:719-739.

McDowell, N. G., R. A. Fisher, C. Xu, J. C. Domec, T. Holtta, D. S. Mackay, J. S. Sperry, A. Boutz, L. Dickman, N. Gehres, J. M. Limousin, A. Macalady, J. Martinez-Vilalta, M. Mencuccini, J. A. Plaut, J. Ogee, R. E. Pangle, D. P. Rasse, M. G. Ryan, S. Sevanto, R. H. Waring, A. P. Williams, E. A. Yepez, and W. T. Pockman. 2013. Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. New Phytologist **200**:304-321.

McLeod, E., G. L. Chmura, S. Bouillon, R. Salm, M. Björk, C. M. Duarte, C. E. Lovelock, W. H. Schlesinger, and B. R. Silliman. 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. Frontiers in Ecology and the Environment **9**:552-560.

Meinzer, F. C., M. J. Clearwater, and G. Goldstein. 2001. Water transport in trees: current perspectives, new insights and some controversies. Environmental and Experimental Botany **45**:239-262.

Mencuccini, M., Y. Salmon, P. Mitchell, T. Holtta, B. Choat, P. Meir, A. O'Grady, D. Tissue, R. Zweifel, S. Sevanto, and S. Pfautsch. 2017. An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant Cell Environ **40**:290-303.

Merilo, E., I. Joesaar, M. Brosche, and H. Kollist. 2014. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. New Phytologist **202**:499-508.

Morrisey, D. J., A. Swales, S. Dittman, M. A. Morrison, C. E. Lovelock, and C. M. Beard. 2010. The ecology and management of temperate mangroves. Oceanography and Marine Biology: An Annual Review **43**:43-160.

Muller, B., F. Pantin, M. Genard, O. Turc, S. Freixes, M. Piques, and Y. Gibon. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. Journal of Experimental Botany **62**:1715-1729.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell and Environment **25**:239-250.

Nadezhdina, N., T. S. David, J. S. David, M. I. Ferreira, M. Dohnal, M. Tesař, K. Gartner, E. Leitgeb, V. Nadezhdin, J. Cermak, M. S. Jimenez, and D. Morales. 2010. Trees never rest: the multiple facets of hydraulic redistribution. Ecohydrology **3**:431-444.

Nadezhdina, N., K. Steppe, D. J. De Pauw, R. Bequet, J. Cermak, and R. Ceulemans. 2009. Stem-mediated hydraulic redistribution in large roots on opposing sides of a Douglas-fir tree following localized irrigation. New Phytologist **184**:932-943.

Nardinia, A., M. A. L. Gullob, and S. Salle. 2011. Refilling embolized xylem conduits: Is it a matter of phloem unloading? Plant Science **180**:604-611.

Nguyen, H. T., P. Meir, L. Sack, J. Evans, R. S. Oliveira, and M. C. Ball. 2017a. Leaf water storage increases with salinity and aridity in the mangrove Avicennia marina: integration of leaf structure, osmotic adjustment and access to multiple water sources. Plant Cell and Environment **40**:1576-1591.

Nguyen, H. T., P. Meir, J. Wolfe, M. Mencuccini, and M. C. Ball. 2017b. Plumbing the depths: extracellular water storage in specialized leaf structures and its functional expression in a three-domain pressure-volume relationship. Plant Cell and Environment **40**:1021-1038.

Nguyen, H. T., D. E. Stanton, N. Schmitz, G. D. Farquhar, and M. C. Ball. 2015. Growth responses of the mangrove *Avicennia marina* to salinity: development and function of shoot hydraulic systems require saline conditions. Annals of Botany **115**:397-407.

Nikinmaa, E., T. Holtta, P. Hari, P. Kolari, A. Makela, S. Sevanto, and T. Vesala. 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. Plant Cell and Environment **36**:655-669.

Oliva Carrasco, L., S. J. Bucci, D. Di Francescantonio, O. A. Lezcano, P. I. Campanello, F. G. Scholz, S. Rodriguez, N. Madanes, P. M. Cristiano, G. Y. Hao, N. M. Holbrook, and G. Goldstein. 2014. Water storage dynamics in the main stem of subtropical tree species differing in wood density, growth rate and life history traits. Tree Physiology.

Parida, A. K., A. B. Das, Y. Sanada, and P. Mohanty. 2004. Effects of salinity on biochemical components of the mangrove, Aegiceras corniculatum. Aquatic Botany **80**:77-87.

Parida, A. K. and B. Jha. 2010. Salt tolerance mechanisms in mangroves: a review. Trees **24**:199-217.

Pfautsch, S. 2016. Hydraulic Anatomy and Function of Trees—Basics and Critical Developments. Current Forestry Reports **2**:236-248.

Pfautsch, S., T. Hölttä, and M. Mencuccini. 2015a. Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology **35**:706-722.

Pfautsch, S., J. Renard, M. G. Tjoelker, and A. Salih. 2015b. Phloem as Capacitor: Radial Transfer of Water into Xylem of Tree Stems Occurs via Symplastic Transport in Ray Parenchyma. Plant Physiology **167**:963-971.

Philip, J. R. 1966. Plant water relations: some physical aspects. Annual Review of Plant Physiology **17**:245-268.

Reef, R. and C. E. Lovelock. 2015. Regulation of water balance in mangroves. Annals of Botany **115**:385-395.

Reef, R., H. L. Markham, N. S. Santini, and C. E. Lovelock. 2015. The response of the mangrove Avicennia marina to heterogeneous salinity measured using a split-root approach. Plant and Soil **393**:297-305.

Robert, E. M. R., A. H. Jambia, N. Schmitz, D. J. R. De Ryck, J. De Mey, J. G. Kairo, F. Dahdouh-Guebas, H. Beeckman, and N. Koedam. 2014. How to catch the patch? A dendrometer study of the radial increment through successive cambia in the mangrove *Avicennia marina*. Annals of Botany **113**:741-752.

Robert, E. M. R., N. Schmitz, I. Boeren, T. Driessens, K. Herremans, J. De Mey, E. Van de Casteele, H. Beeckman, and N. Koedam. 2011a. Successive cambia: a developmental oddity or an adaptive structure? Plos One **6**:e16558.

Robert, E. M. R., N. Schmitz, J. A. Okello, I. Boeren, H. Beeckman, and N. Koedam. 2011b. Mangrove growth rings: fact or fiction? Trees **25**:49-58.

Santini, N. S., R. Reef, D. A. Lockington, and C. E. Lovelock. 2015. The use of fresh and saline water sources by the mangrove *Avicennia marina*. Hydrobiologia **745**:59-68.

Santini, N. S., N. Schmitz, and C. E. Lovelock. 2012. Variation in wood density and anatomy in a widespread mangrove species. Trees **26**:1555-1563.

Schenk, H. J., K. Steppe, and S. Jansen. 2015. Nanobubbles: a new paradigm for air-seeding in xylem. Trends in Plant Science **20**:199-205.

Schmitz, N., E. M. Robert, A. Verheyden, J. G. Kairo, H. Beeckman, and N. Koedam. 2008. A patchy growth via successive and simultaneous cambia: key to success of the most widespread mangrove species *Avicennia marina*? Annals of Botany **101**:49-58.

Scholander, P. F. 1968. How mangroves desalinate seawater. Physiologia Plantarum **21**:251-261.

Scholander, P. F., E. D. Bradstreet, H. T. Hammel and, and E. A. Hemmingsen. 1966. Sap concentrations in halophytes and some other plants. Plant Physiology **41**:529-532

Scholander, P. F., L. Vandam, and S. I. Scholander. 1955. Gas exchange in the roots of mangroves. American Journal of Botany **42**:92-98.

Scholz, F., S. Bucci, G. Goldstein, F. Meinzer, A. Franco, and F. Miralles-Wilhelm. 2008. Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water. Tree Physiology **28**:469-80.

Sevanto, S. 2014. Phloem transport and drought. Journal of Experimental Botany **65**:1751-1759.

Sirichandra, C., A. Wasilewska, F. Vlad, C. Valon, and J. Leung. 2009. The guard cell as a single-cell model towards understanding drought tolerance and abscisic acid action. Journal of Experimental Botany **60**:1439-1463.

Sperry, J. 2013. Cutting-edge research or cutting-edge artefact? An overdue control experiment complicates the xylem refilling story. Plant Cell and Environment **36**:1916-1918.

Sperry, J. S. and D. M. Love. 2015. What plant hydraulics can tell us about responses to climate-change droughts. New Phytol **207**:14-27.

Steppe, K., H. Cochard, A. Lacointe, and T. Ameglio. 2012. Could rapid diameter changes be facilitated by a variable hydraulic conductance? Plant Cell and Environment **35**:150-157.

Steppe, K., D. J. W. De Pauw, R. Lemeur, and P. A. Vanrolleghem. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257-273.

Steppe, K. and R. Lemeur. 2004. An experimental system for analysis of the dynamic sap-flow characteristics in young trees: results of a beech tree. Functional Plant Biology **31**:83-92.

Steudle, E. 2001. The cohesion-tension mechanism and the acquisition of water by plant roots. Annual Reviews in Plant Physiology and Plant Molecular Biology **52**:847-875.

Tardieu, F. and T. Simonneau. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. Journal of Experimental Botany **49**:419-432.

Tomlinson, P. B. 1986. The botany of mangroves. Cambridge University Press, New York, USA.

Tyree, M. T. 1997. The Cohesion-Tension theory of sap ascent: current controversies. Journal of Experimental Botany **48**:1753-1765.

Van de Wal, B. A. E. 2011. Ecophysiology of mangroves in Brisbane, Australia: Sap flow dynamics in *Avicennia marina*. Universiteit Gent, Gent, Belgium.

Van de Wal, B. A. E., A. Guyot, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014. Influence of temporospatial variation in sap flux density on estimates of whole-tree water use in *Avicennia marina*. Trees **29**:215-222.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, S. R. E. De Groote, N. J. F. De Baerdemaeker, M. Hayes, N. Welti, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014a. Long-term versus daily stem diameter variation in co-occurring mangrove species: environmental versus ecophysiological drivers. Agricultural and Forest Meteorology **192**:51-58.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, T. De Swaef, D. A. Lockington, and K. Steppe. 2014b. Modelling reveals endogenous osmotic adaptation of storage tissue water potential as an important driver determining different stem diameter variation patterns in the mangrove species *Avicennia marina* and *Rhizophora stylosa*. Annals of Botany.

Vandenhonert, T. H. 1948. Water transport in plants as a catenary process. **3**:146-153.

Walters, B. B., P. Ronnback, J. M. Kovacs, B. Crona, S. A. Hussain, R. Badola, J. H. Primavera, E. Barbier, and F. Dahdouh-Guebas. 2008. Ethnobiology, socio-economics and management of mangrove forests: A review. Aquatic Botany **89**:220-236.

Wang, W., Z. Yan, S. You, Y. Zhang, L. Chen, and G. Lin. 2011. Mangroves: obligate or facultative halophytes? A review. Trees **25**:953-963.

Wegner, L. H. 2014. Root pressure and beyond: energetically uphill water transport into xylem vessels? Journal of Experimental Botany **65**:381-393.

Woodruff, D. R. and F. C. Meinzer. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant, Cell & Environment **34**:1920-1930.

Woodruff, D. R., F. C. Meinzer, D. E. Marias, S. Sevanto, M. W. Jenkins, and N. G. McDowell. 2015. Linking nonstructural carbohydrate dynamics to gas exchange and leaf hydraulic behavior in *Pinus edulis* and *Juniperus monosperma*. New Phytologist **206**:411-421.

Woodward, F. I. 1987. Stomatal numbers are sensitive to increases in CO_2 from preindustrial levels. Nature **327**:617-618.

Yates, D. J. and L. B. Hutley. 1995. Foliar uptake of water by wet leaves of *Sloanea woollsii*, an australian subtropical rain-forest tree. Australian Journal of Botany **43**:157-167.

Zheng, W. J., W. Q. Wang, and P. Lin. 1999. Dynamics of element contents during the development of hypocotyles and leaves of certain mangrove species. Journal of Experimental Marine Biology and Ecology **233**:247-257.

Zimmermann, D., R. Reuss, M. Westhoff, P. Gessner, W. Bauer, E. Bamberg, F. W. Bentrup, and U. Zimmermann. 2008. A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. Journal of Experimental Botany **59**:3157-3167.

Zimmermann, D., M. Westhoff, G. Zimmermann, P. Gessner, A. Gessner, L. H. Wegner, M. Rokitta, P. Ache, H. Schneider, J. A. Vasquez, W. Kruck, S. Shirley, P. Jakob, R. Hedrich, F. W. Bentrup, E. Bamberg, and U. Zimmermann. 2007. Foliar water supply of tall trees: evidence for mucilage-facilitated moisture uptake from the atmosphere and the impact on pressure bomb measurements. Protoplasma **232**:11-34.

Zimmermann, U., H. Schneider, L. H. Wegner, and A. Haase. 2004. Water ascent in tall trees: does evolution of land plants rely on a highly metastable state? New Phytologist **162**:575-615.

Zweifel, R., D. M. Drew, F. Schweingruber, and G. M. Downes. 2014. Xylem as the main origin of stem radius changes in *Eucalyptus*. Functional Plant Biology **41**:520-534.

Zweifel, R. and R. Hasler. 2001. Dynamics of water storage in mature subalpine *Picea abies*: temporal and spatial patterns of change in stem radius. Tree Physiology **21**:561-569.

Zweifel, R., H. Item, and R. Hasler 2001. Link between diurnal stem radius changes and tree water relations. Tree Physiology **21**: 869–877.

Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees **15**:50-57.

Zweifel, R., K. Steppe, and F. J. Sterck. 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. Journal of Experimental Botany **58**:2113-2131.

Chapter 2 - Testing the Waters: Analysing the heterogeneous stem radial change dynamics of the mangrove *Avicennia marina* (Forssk.) Vierh looking for indicators of environmental influence on radial water storage

“Day after day, day after day,
We stuck, nor breath nor motion;
As idle as a painted ship
Upon a painted ocean.

Water, water, everywhere,
And all the boards did shrink;
Water, water, everywhere,
Nor any drop to drink”

Samuel T. Coleridge - The Rime of the Ancient Mariner (text of 1834)

2.1 Abstract

The dynamics of stem water storage can provide insights on a tree's capacity to face imbalances between water supply and demand, and thus maintain hydraulic function and growth under fluctuating environmental conditions. Recent work in the mangrove *Avicennia marina* (Forssk.) Vierh showed that stem radius change (SRC) is highly heterogeneous due to the structure of *A. marina* wood, composed of multiple reticulate cambia. The heterogeneity of short and long-term SRC in trees with reticulate cambia, common in dry environments, complicates the study of environmental drivers of stem water storage.

In order to find the environmental drivers of stem water storage and understand the nature of this heterogeneity we analysed high-resolution SRC of *A. marina* for one and a half years. Nine point dendrometers measured SRC in upper, lower and mid stem of three *A. marina* trees in northern New Zealand. Stem radius change was detrended for growth to obtain water-related shrinking and swelling only (ΔW).

Despite heterogeneity in SRC measurements, ΔW changes in *A. marina* still showed seasonal trends of high winter and low summer diel amplitudes and strong responses to mean diel environmental conditions. Environmental drivers of ΔW were more apparent once the data was standardized to show relative changes and segregated by upper and lower stem tiers. Moving window correlations showed stem contraction was correlated with atmospheric water demand and precipitation, whilst stem swelling was correlated with light sums more often than with measures of water availability. The correlation of stem water storage with light seen in our work provides backing for the hypothesized role of carbohydrates in the mechanism behind daytime stem swelling of *A. marina* water storage tissues. Our work highlights the importance of sensor locations and data analysis approaches if we are to use established SRC technologies in a larger diversity of environments and species.

2.2 Introduction

Plants live in a balance between water supply and water demand, and have developed a staggering array of anatomical, physiological and life history traits in order to keep themselves in a favourable water balance (Sperry and Love 2015, Pivovaroff, Pasquini et al. 2016). On a diel basis, the comparatively drier atmosphere drives the ascent of water up the stem's xylem to supply thirsty leaves, and as much as 99% of water taken up by roots is lost to transpiration (Lambers, Chapin III et al. 2008). When water demand is not compensated by water uptake at the roots, trees dip into their internal water stores, causing movement of water from storage tissues in stem, roots, leaves and fruits into the transpiration stream (Zweifel et al. 2001, Steppe et al. 2005, Turcotte et al. 2011, Nguyen et al. 2017). The capacity of trees to store water and supply it in times of high demand boosts their ability to maintain transpiration, photosynthesis, leaf hydration and, ultimately, growth (Deslauriers et al. 2003, Zweifel et al. 2006, Scholz et al. 2008, Zweifel et al. 2010, Deslauriers et al. 2014, Zielis et al. 2014, Oliva Carrasco et al. 2015, Steppe et al. 2015).

Radial movement of water to and from storage tissues in the stem causes measurable changes in stem diameter. These stem radius changes (SRC) can be measured with precision dendrometers, and are widely used to study high resolution radial stem water dynamics, tree seasonal growth, phenology, water deficits, climate-growth relationships and to parameterise water and sugar transport models (Steppe et al. 2005, Drew and Downes 2009, De Schepper and Steppe 2010, De Swaef et al. 2013). At an hourly to daily resolution, dendrometer measurements reflect radius changes in the stem mainly caused by water movement in and out of elastic water stores, reversible changes in xylem elements due to changes in water potential, and irreversible radial stem growth (Zweifel et al. 2001, Mencuccini et al. 2013, De Swaef et al. 2015, Pfautsch et al. 2015a, Steppe et al. 2015, Chan et al. 2016). At longer timescales, seasonal growth and long term water status can be observed, for example, through extended periods of stem shrinkage in times of water deficit or through periods of continuous stem radius increase during the growing season (Deslauriers et al. 2003, Zweifel et al. 2006, Deslauriers et al. 2014). Multiple approaches exist to calculate and separate the water and growth contributions to stem radius change, including the development of complex models (De Schepper and Steppe 2010, Deslauriers et al. 2011, Sevanto et al. 2011, Mencuccini et al. 2013, Deslauriers et al. 2014) and simpler fractionation techniques (Zweifel et al. 2006, Zweifel et al. 2016, Mencuccini et al. 2017). However, this has not proven a straightforward task and fully isolating the growth, osmotic and water signals from SRC measurements is still work in progress (Mencuccini et al. 2017).

Mangroves are halophytic salt tolerant plants which occupy the intertidal zones of tropical and subtropical latitudes. *Avicennia marina* (Forssk.) Vierh., the grey mangrove, is one of the most common species and has the widest latitudinal range (Quisthoudt, Schmitz et al. 2012). The task of maintaining a favourable water balance becomes much more challenging in a saline environment, and grey mangroves have developed a broad array of strategies to this end. These strategies include (i) exclusion of up to 95% of salts at the root level, avoiding the entry of salt into the transpiration stream, (ii) intracellular osmotic adjustment through controlled inorganic ion concentration in vacuoles or compatible solutes in the cytoplasm, and (iii) specialized cells for salt excretion at the leaf level (Ball 1988, Ball 1988, Flowers and Colmer 2008, Krauss and Ball 2013). It has recently been discovered that *Avicennia marina* also has an unusual wood anatomy by which multiple cambia result in consecutive bands of xylem interspersed with internal secondary phloem strands connected by parenchyma tissue (Schmitz et al. 2008, Robert et al. 2011a, Robert et al. 2011b, Robert et al. 2014). Robert *et al.* (2014) showed that of the 103 shrub and tree species with internal secondary phloem 84.9% occurred in dry or saline habitats, and thus this anatomy is thought to be an adaptive trait to cope with physiological drought, by contributing to internal water storage and security (Robert, Schmitz et al. 2011, Santini, Schmitz et al. 2012). The phloem not only has a vital role in water storage and transport of sugars, but is also thought to play a part in refilling of embolisms (Savage, Clearwater et al. 2016) and regeneration after wounding.

Although our knowledge of how *A. marina* takes up, stores, loses and regulates water balance has improved significantly in the last decade (Naidoo 2006, Krauss et al. 2008, Schmitz et al. 2008, Robert et al. 2011a, Van de Wal 2011, Wang et al. 2011, Krauss and Ball 2013, López-Portillo et al. 2014, Robert et al. 2014, Uddin 2014, Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b, Nguyen et al. 2017, Donnellan et al. *in press*), there are still many unknowns, especially around radial water flow and storage. The peculiar wood anatomy of the grey mangrove has been shown to make SRC studies in *A. marina* especially challenging but also particularly interesting. Studying growth in *A. marina*, Robert, Jambia et al. (2014) reported high heterogeneity and patchiness of point SRC measurements, which they explained with the patchy activation of growth within the network of multiple cambia. As we lack in-depth studies of water movements in and out of adult *A. marina* stems, it remains speculative to what extent the patchy response of radial water storage to the environment is determined by changes in the properties of elastic tissues, activation of cambium or the interplay of environmental conditions. Although the flow of water in and out of water stores is majorly determined by the difference in total water potential between the xylem and the bark, variability in the properties of elastic tissues can have a fundamental effect on SRC. For example, SRC is also determined by the radial hydraulic conductance to the flow of water, which can change through changes in aquaporin expression and abundance (Steppe, Cochard et al. 2012, Baert, De Schepper et al. 2015, Pfautsch, Hölttä et al. 2015). Additionally, volume changes caused by water movement also depend on the elastic modulus and the hydraulic capacitance of the storage tissues (De Swaef et al. 2015, Steppe et al. 2015). Thus, if species with reticulate cambia present variable hydraulic conductance, elasticity or capacitance along the stem due to the complexity and internal variability of their wood structure, it could hinder the interpretation of SRC measurements and our capacity to infer seasonal and absolute water storage and use. This is especially true if some of these wood properties cannot be easily measured in the field, a likely scenario in *A. marina* considering the high variability its tissue structure, which is known to change within the space of just a few micrometres (Robert et al. 2014).

Studying *A. marina's* SRC in more depth could increase knowledge of our capacity to measure and understand a greater diversity of radial water storage strategies potentially used by a more diverse array of tree species. It will also allow an evaluation of the tools and techniques available, and pinpoint where technical developments are needed. It is important to consider that SRC has largely been studied in comparatively few North-American and European slow-growing tree species, and recent research on a larger array of tree species is beginning to show that there is a greater diversity in elastic tissue water storage anatomy, patterns and mechanisms than previously thought, which has implications for studies of growth in ecosystems worldwide (Robert et al. 2011a, Robert et al. 2014, Zweifel et al. 2014, Pfautsch 2016, Mencuccini et al. 2017).

In this study, I extract the water-related component of several years of stem radius change measurements in *A. marina*. Our aim is to analyse the environmental drivers of diel and seasonal changes in the dynamics of water storage and depletion in the trunk of *A. marina,* and to understand the nature of the heterogeneity in stem radial water movements. Firstly, I ask if it is possible to study stem radius water-related changes in *A. marina* using point dendrometers, considering the previously recorded patchiness and heterogeneity of SRC in this species. Thus, I aim to describe the nature of the variability of different stem cycle elements, such as amplitudes and rates, and analyse if they can be related to seasonal changes and/or proximity to the crown, or are in fact, purely stochastic in nature, as suggested in other work. Secondly, I aim to pinpoint what are the environmental drivers of water-related stem radial swelling and shrinking patterns at different temporal scales, from diel to monthly means. Thus, I aim to provide the first exhaustive long-term analysis of the main environmental drivers from diel to seasonal scales of water storage and depletion in the stem of the mangrove *A. marina*.

2.3 Material and Methods

2.3.1 Site description

The study site is located in the Mangawhai estuary, northern New Zealand (36.097°S, 174.573°E). The estuary consists of two main waterways and approximately 80 ha of mangrove forest. The study site is located in the upper tidal zone of Tara Creek, furthest from the shore with sandy substrate and short inundation times twice every 24 hours. The stand is monospecific and homogeneous, composed of *A. marina* individuals 3-4 m in height. Within the stand, I picked an area at least 50 m from the channel to avoid edge effects. Annual weather trends during the study b). Daily mean relative humidity ranged from 38% to 100%, with a mean of 80.8%. Annual temperature extremes ranged from -1.5°C to 29.4°C with a seasonal mean of 15.4°C. Total precipitation sum was 1062.9 mm. November was the only month of the year 2015 with no precipitation, which is quite an unusual event in the climate of the area, where November usually receives an average of 68.9mm of rain, according to climate records of the last 10 years (Mangawhai Weather Station Records). Soil water potential (SWP) ranged over the whole measuring period from -5 MPa to -1.4 MPa, with a mean of -2.5 MPa. The salinity was measured on three occasions through the year with a refractometer (September 2014, December 2014 and March 2015) in soil pore water at a soil depth of 30cm and ranged from 20‰ to 39‰. Water table depth ranged from -12.8 cm below ground level to +64.4 cm above ground level during tidal inundation (Supplementary Figure 1).

2.3.2 Environmental data collection

A weather station was installed within the canopy in order to explain plant physiological responses to microclimate. A probe installed within the canopy logged temperature (T [°C]) and relative humidity (RH [%]) (Model SHT, Sensirion, Stäfa, Switzerland). Vapour pressure deficit (VPD [kPa]) was calculated from temperature and RH data according to Buck (1981). Solar irradiance (*I* [W m^{-2}], 380–1120 nm) was measured with a pyranometer (Model PYR, Decagon Devices, WA, USA). Rainfall (ppt, [mm]) was measured with a tipping-bucket rain gauge (Model 52202, R. M. Young Company, Traverse City, Michigan, USA). Pyranometer and rain-gauge were installed on a scaffolding platform so they rested just above canopy maximum height. Tidal inundation (WD [cm]), soil-water temperature (SWT [°C]) and soil-water electrical conductivity (EC [µS/cm]) were measured with a CTD sensor (CTD-10, Decagon Devices, WA, USA) fitted into a well dug into the sediment at a depth of 1m in tubing that allowed water flow. Osmotic potential (Ψ_{Π} [MPa]) of the soil and water was calculated using EC and WT following the procedure detailed elsewhere (McIntyre 1980). All instruments were logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland).

2.3.3 Stem radius change measurements

The study period went from September 2014 until December 2015, which was split into two different measurement periods. During measurement Period I (September 2014 until June 2015) three mature co-dominant trees (Tree A - C) of similar height were chosen for continuous physiological measurements. In June, Tree B and Tree C were discontinued from measurements as they were harvested in full for another experiment conducted at the research site. From July 2015 onwards (measurement Period II), there were two measurement trees, Tree A together with a new mature co-dominant tree, Tree D. Point dendrometers measured stem radius change (SRC) at a µm resolution. These are non-invasive small pistons softly pressed against the stem connected to a potentiometer, mounted on carbon fibre frames optimized for temperature insensitivity (ZN11-O-WP, Natkon, Oetwil am See, Switzerland). Three dendrometers were installed on each of the measured trees, evenly spaced along the stem, the first 90 cm above ground level and the third below branching of the crown (200-260 cm). These dendrometer positions are referred to as ‘Lower’, ‘Middle’ and ‘Upper’, respectively. Instruments logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland).

2.3.4 Calculating water induced stem cycle variation and other calculations

Stem radius measurements obtained from dendrometers were cleared of jumps and spikes. This was performed with a custom-written function which considered jumps as radius changes at a 10 minute scale which were larger than two times the monthly 95th percentile. Since SRC includes both growth of new cells and water related swelling and shrinking, the dendrometer data were detrended for growth according to Zweifel, Haeni et al. (2016). Briefly, this method assumes no growth during periods of stem shrinkage. The resulting values are a measure of tree water-deficit induced stem shrinkage (ΔW) and represent the difference in stem size relative to its size in fully hydrated conditions which is set as the reference point of zero. Thus, increasingly negative values indicate increasingly dehydrated stems.

By detrending the dendrometer data for growth, I assume the resulting time series of ΔW represents only water related shrinking and swelling of the stem, although the method has its limitations (Zweifel et al. 2016, Mencuccini et al. 2017). I then used a numerical approach to determine duration and amplitude of water related shrinking and swelling of storage tissues by implementing an approach similar to the one developed by Deslauriers, Rossi et al. (2011). I broke down ΔW stem circadian cycles of into shrinking periods between a radius maximum (R_{max}) and the following minimum (R_{min}) when stored water is depleted, and expansion periods between R_{min} and the next occurring R_{max} when water storage is being replenished (Donnellan et al. *in press*). This allowed the calculation of contraction amplitude (A_c), expansion amplitude (A_e), contraction duration (T_c) and expansion duration (T_e) in addition to the times of the day R_{max} and R_{min} occurred. Rates of expansion and contraction (R_e and R_c, respectively) were calculated by dividing each phase amplitude by its corresponding duration (Supplementary Figure 2). A custom function for this analysis was written in the software *R* (R Core Team 2017).

Additionally, due to the heterogeneity in ΔW amplitudes, possibly caused by changes in wood structure along stem height, I calculated standardized amplitudes of expansion and contraction (A_e* and A_c*, respectively). These were standardized by dividing amplitudes by the maximum recorded amplitude (95th percentile) for each individual measurement point for its corresponding measurement period (Period I and

Period II), resulting in amplitudes which went from 0-1 (no units) for all sensors and measurement points.

2.3.5 Statistical analysis

To assess the variation in the monthly mean amplitudes and rates of contraction (A_c, R_c) and expansion (A_e, R_e), I calculated the monthly mean for each measuring point and used those values to calculate the mean and standard deviation between dendrometer measurement points (n = 9 for September 2014 - June 2015, n = 6 for July 2015 - December 2015) for each month. This way, the standard deviations reflect monthly scale variations caused by heterogeneity between measuring points and not caused by environmental conditions. The similarity of seasonal trends between different stem measurement points within and between trees and tiers was assessed with Kendal tau-b correlations for daily and monthly mean A_e* and A_c*.

Environmental drivers of large-scale seasonal changes were analysed by calculating monthly means of expansion and shrinking properties (A_c, A_e, A_c*, A_e*, R_c, R_e, T_c, T_e) for (1) all measurement points pooled and (2) each measurement point and each stem tier, and then performing bootstrapped Kendall tau-b correlations with monthly mean of environmental variables. Correlations were considered significant if after 1000 iterations the correlation coefficient was at least twice the standard deviation and 95% confidence intervals did not include zero. As the effect of environmental factors on stem increment can be indirect through cycle and phase duration (Deslauriers et al. 2003, Deslauriers et al. 2011) I performed partial correlations using duration as a partial correlate to confirm these did not change (results not shown).

The cycle property with the largest and most significant correlation coefficients to environmental drivers and clearer seasonal trends was then chosen to perform smaller temporal scale correlations of 30-day moving windows over the whole 1.5 year period, using the values for each circadian cycle value and each individual measurement point. These smaller-scale environmental effects were assessed with moving window correlations by setting a moving window of a 30 day period iterated throughout each continuous measurement period (Period I & II). Moving window Kendal tau-b correlations were performed using the *rollapplyr* function from the R package *zoo.* All analyses were conducted using the free software package *R* (R Core Team, 2017).

2.4 Results

2.4.1 General yearly stem radius and ΔW courses

Both SRC and ΔW showed heterogeneous yearly courses between trees and stem tiers. During the first measurement period (Figure 2.1, Period I) three out of nine dendrometers showed stem growth: the lower position in Tree A and the lower and upper positions in Tree B. The initiation of growth happened in spring, between mid-September and mid-November 2014. Out of the other six measuring points which did not present stem growth, ΔW stayed within 0 to -200 µm during the whole measurement period, except for the lower position of Tree C, which reached minimum values of -450 µm. During the second measurement period from May to December 2015 (Figure 2.1, Period II), there was no stem growth in any of the measurement periods, and 5 out of 6 dendrometers showed stem shrinkage from October 2015.

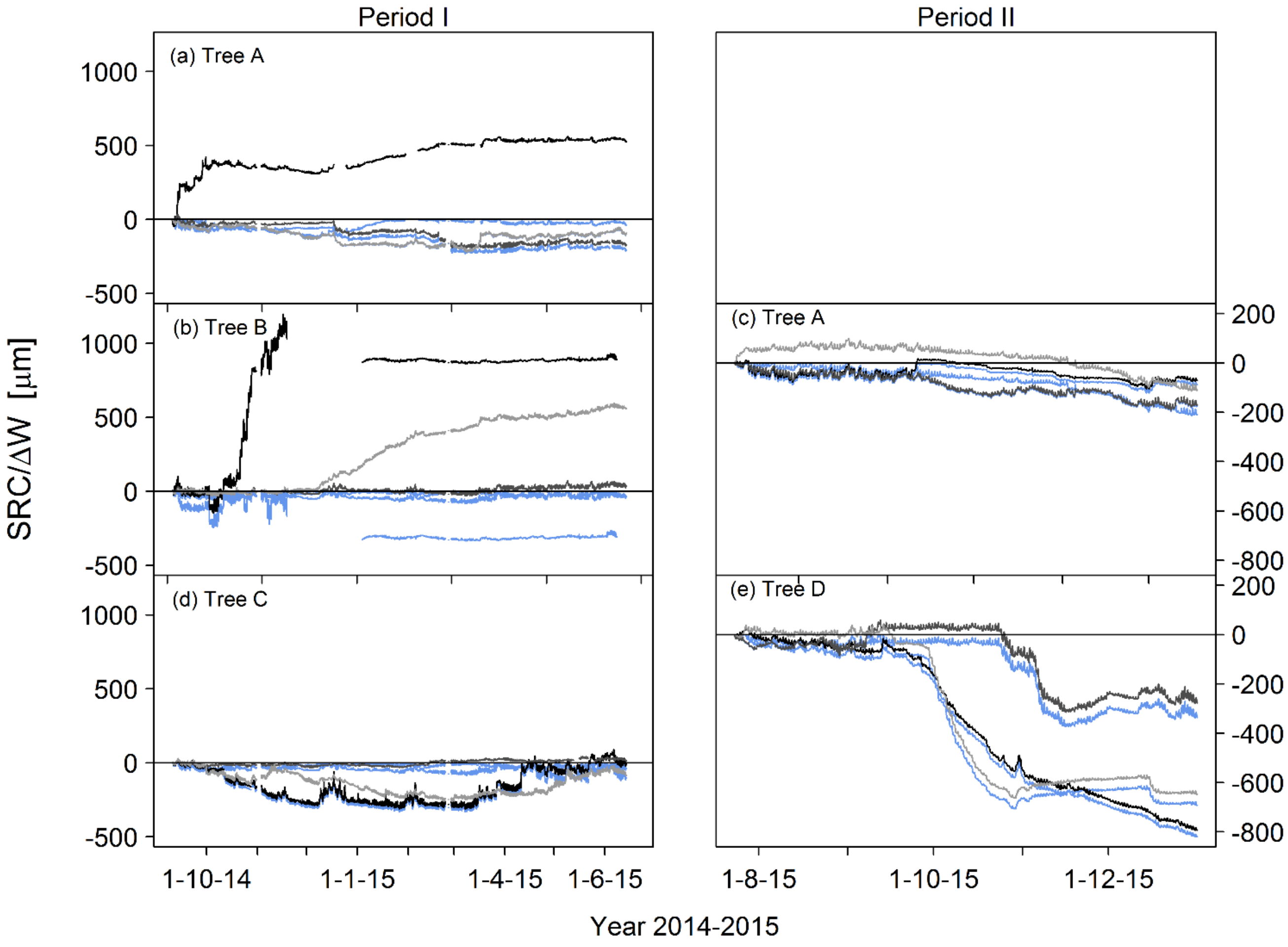


Figure 2.1 Annual courses in stem radius change (SRC) for upper (light gray), middle (dark gray) and lower (black) stem tiers together with stem water deficit (ΔW) (blue) for the first measurement period (Tree A-C, a,b,d) and the second measurement period (Tree A and Tree D, c,e).

2.4.2 Seasonal courses in ΔW amplitudes and rates: magnitudes and variation.

Annual changes of water-related stem expansion and contraction amplitude showed an overall trend of decreasing mean monthly amplitudes towards summer months of late 2014, and increasing amplitudes towards winter months of 2015 (Table 2.1, Figure 2.2 & 2.3). This trend of decreasing amplitudes continued into the second summer, when the rainless month of November 2015 coincided with lowered amplitudes, which then recovered after the rain event at the end of the measuring period in December 2015. Rates of water-related expansion and contraction showed the same trends as expansion and contraction amplitudes, respectively (Table 2.1). There was a slight decrease in rate for both expansion and contraction phases during peak summer months (Dec 2014 – Mar 2015) and during the months of low precipitation (Oct - Nov 2015), whilst there was an increase in rates during winter (Table 2.1). Mean time of maximum water-related stem swelling T_{max} also followed a similar seasonal shift, with earlier peaks during summer months and later peaks during winter months (Table 2.1).

Table 2.1 Variation in the monthly mean amplitudes and rates of contraction (Ac, Rc), expansion (Ae, Re), and timing of peak swelling Tmax ± standard deviation between dendrometer measurement points (n=9 for Sep 2014- June 2015, n=6 for July 2015-Dec 2015) showing for each month during the September 2014-December 2015 measurement period.

Date	Ac (µm)	Ae (µm)	Rc (µm/min)	Re (µm/min)	Tmax (time)
9/14	15.70 ± 5.26	13.86 ± 6.72	0.021 ± 0.007	0.018 ± 0.009	10:15 ± 2.5
10/14	11.74 ± 7.13	12.22 ± 9.61	0.018 ± 0.014	0.017 ± 0.012	11:30 ± 2.5
11/14	13.31 ± 13.3	12.41 ± 14.43	0.024 ± 0.03	0.024 ± 0.039	11:30 ± 1.9
12/14	8.65 ± 6.15	7.93 ± 4.88	0.012 ± 0.008	0.010 ± 0.007	10:30 ± 1.3
1/15	9.27 ± 5.73	9.15 ± 4.75	0.013 ± 0.008	0.012 ± 0.007	9:45 ± 1.7
2/15	10.23 ± 6.84	8.51 ± 3.97	0.014 ± 0.009	0.011 ± 0.005	9:00 ± 1.9
3/15	11.26 ± 6.21	11.75 ± 6.63	0.016 ± 0.008	0.015 ± 0.009	8:15 ± 1.5
4/15	12.73 ± 6.29	12.43 ± 5.14	0.019 ± 0.008	0.016 ± 0.008	9:00 ± 1.5
5/15	13.03 ± 6.88	13.34 ± 5.76	0.019 ± 0.011	0.016 ± 0.008	10:00 ± 1.4
6/15	14.80 ± 6.95	15.07 ± 5.08	0.022 ± 0.01	0.021 ± 0.009	10:00 ± 1.2
7/15	14.36 ± 4.05	10.13 ± 3	0.019 ± 0.006	0.015 ± 0.004	10:00 ± 1.7
8/15	13.83 ± 2.84	14.28 ± 3.28	0.018 ± 0.003	0.018 ± 0.004	11:00 ± 1.9
9/15	14.04 ± 2.31	12.14 ± 3.34	0.017 ± 0.003	0.015 ± 0.005	10:30 ± 1.9
10/15	13.36 ± 7.05	7.85 ± 5.53	0.015 ± 0.008	0.010 ± 0.008	9:00 ± 2.5
11/15	10.82 ± 7.30	9.11 ± 5.24	0.014 ± 0.009	0.012 ± 0.008	10:30 ± 2
12/15	11.4 ± 3.53	9.53 ± 3.37	0.015 ± 0.006	0.016 ± 0.005	11:15± 1

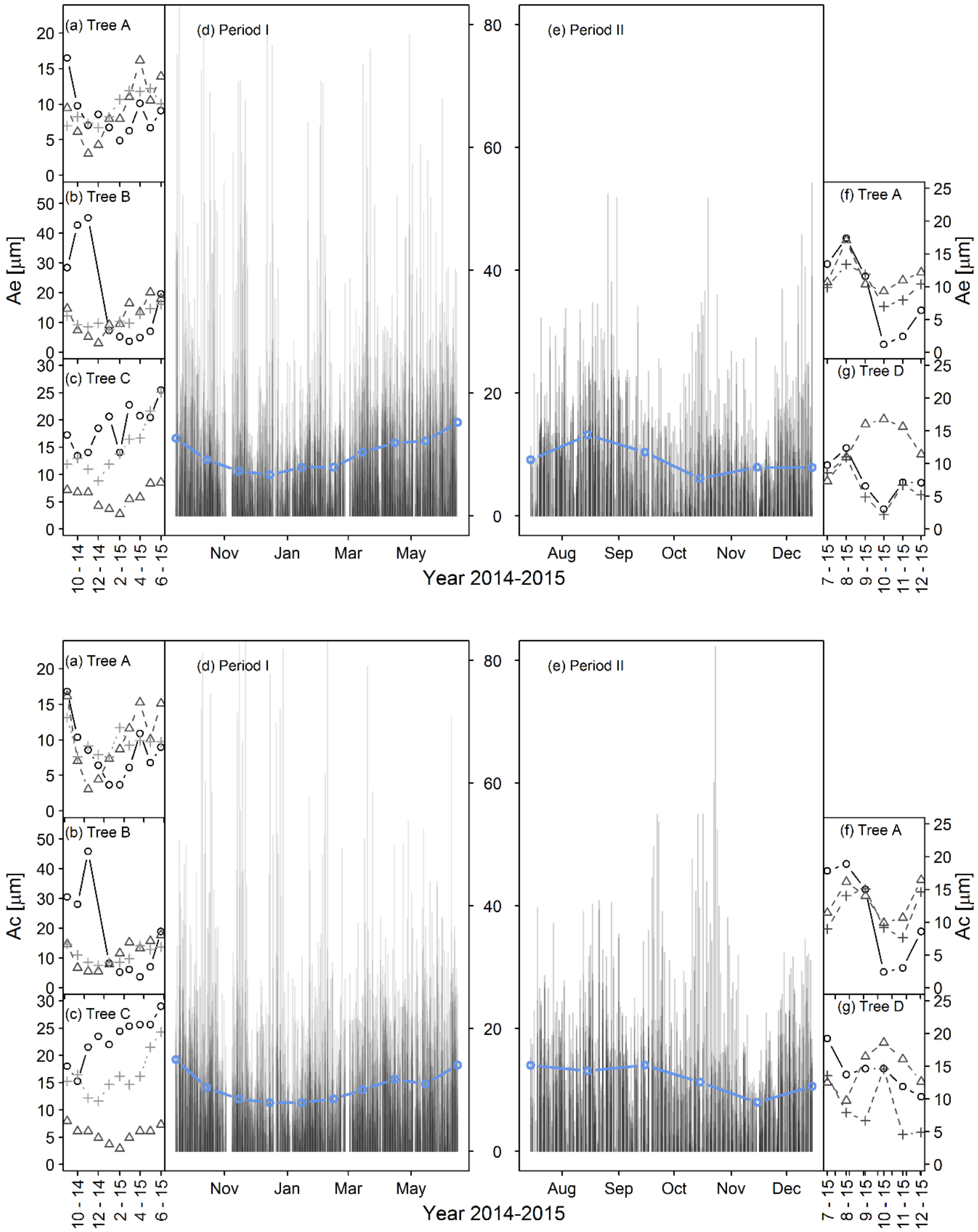


Figure 2.2 Changes in expansion amplitude (Ae) and in contraction amplitude (Ac) of Period I, from September 2014 to June 2015 showing monthly means for different stem heights for Tree A (a) Tree B (b) and Tree C (c) and daily values for all trees and all measurement points (d) and the expansion and contraction amplitudes for measurement Period II from July 2015 – December 2015, with the daily values for all trees and all measurement points (e) and the monthly means for measuring points at different heights on Tree A (f) and Tree D (g). Blue lines and points in (d) and (e) indicate monthly mean for all trees and tiers. In (a-c, f-g) symbols indicate upper (crosses), middle (triangles) and lower (circles) stem tiers.

Seasonal differences between summer and winter water related stem radius change (Figure 2.4) were weak as there was large variance in the absolute magnitude of daily and monthly mean amplitudes of ΔW between measuring points, heights and trees, which was sometimes more than half the value of the mean (Table 2.1, Figure 2.2). The highest variance recorded was at the start of the growing season, in October-November 2014. The lowest variation between measuring points occurred during mid-winter months of July and August 2015. Although seasonal trends were obscured by a high variation in the magnitude of contraction and expansion (Table 2.1, Figure 2.2 & 2.3), the seasonal trends became more apparent when amplitudes standardized by each measurement point maxima, A_c* and A_e*, were used (Figure 2.4). Standardized amplitudes also showed contraction and expansion trends had a higher similarity between sensors at the same stem height in different trees than within the same tree at different heights (Figure 2.4).

Notably, during Period I the upper and middle stem tier measurement points were more highly correlated to each other than the lower tier, which was much more heterogeneous (Upper to upper: $R^2 = 0.4$, Upper to other: $R^2 = 0.2$, Middle to Middle R^2

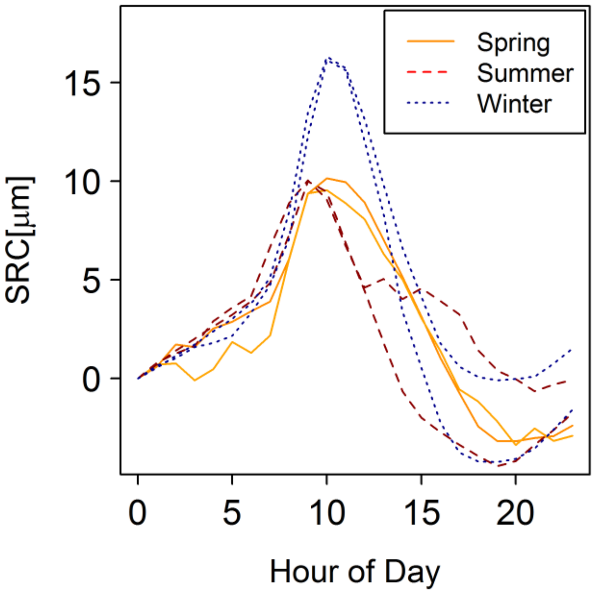


Figure 2.3 Monthly mean hourly course of water-related stem radius change (SRC) de-trended of growth, showing higher winter amplitudes. Lines show monthly average across three trees (Tree A-C, n=3) and three measuring points per tree (n=3). For graph clarity, alternating months were chosen and no error bars shown (see Table 1) for autumn 2014 (September and November 2014, orange lines), summer 2015 (December and February 2015, dashed red line) and winter 2015 (June-August 2015)

= 0.4, Middle to other $R^2 = 0.27$, Lower to Lower $R^2 = 0.2$, Lower to other $R^2 = 0.1$) (Figure 2.4). During Period II, upper, middle and lower tier were all similar, except upper tier Tree C (Figure 2.4).

2.4.3 Strength of the seasonal correlation between monthly mean environmental variables and different ΔW stem cycle properties

Monthly means water-related stem expansion and contraction both showed a significant positive correlation with monthly mean humidity, and a negative correlation with light and VPD (Table 2.2). Expansion was also negatively correlated to soil water potential and contraction was negatively correlated to temperature (Table 2.2). In all cases, correlation coefficients increased when standardized amplitudes (Ae* and Ac*) were used, in some cases resulting in significant relationships to environmental drivers which were not present in non-normalized amplitudes or rates (Table 2.2).

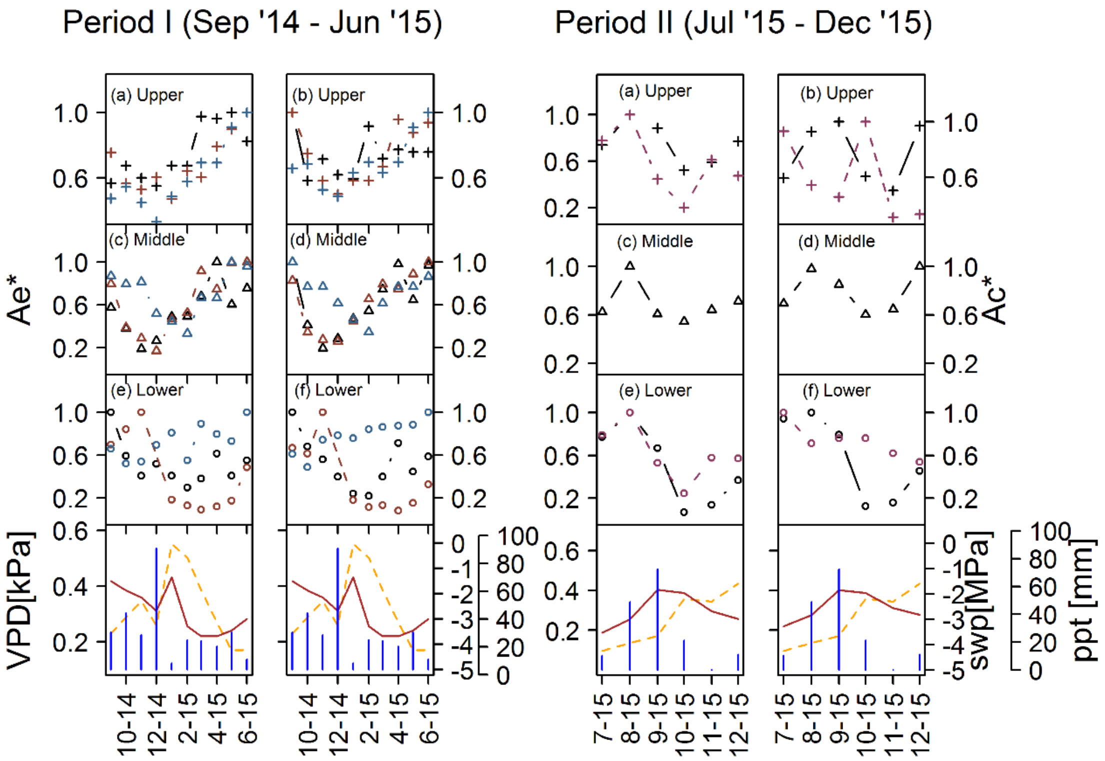


Figure 2.4 Seasonal changes in amplitudes standardized by measurement point maxima for expansion (Ae*) and contraction (Ac*) for the measurement periods of 2014-2015 (Period I left, Period II right) for upper stem tier (a-b), middle stem tier (c-d) and lower stem tier (e-f), together with monthly mean courses of VPD (dashed yellow line) and soil water potential (swp, brown line) and precipitation sums (ppt, blue bars). Different shade symbols indicate Tree A (black), Tree B or Tree D (both dark gray)

There was a marked difference in the environmental correlates of expansion and contraction between different stem tiers. Upper and mid-stem expansion and contraction were both significantly correlated, positively with humidity and negatively with light and VPD.

Table 2.2 Correlations between monthly mean ΔW cycle properties for all trees combined and monthly mean environmental variables, for all measured periods pooled (September 2014-December 2015). Stem cycle properties are expansion and contraction amplitude (Ae and Ac), expansion and contraction amplitude standardized by each measurement point maxima (Ae* and Ac*) expansion and contraction rate (Re and Rc) and duration of expansion and contraction (Te and Tc). Star (*) indicates a correlation significant at $p \leq 0.05$ and a point (•) at $p \leq 0.1$.

	Ae	Ae*	Re	Te	Ac	Ac*	Rc	Tc
Humidity	0.47•	0.57*	0.48•	0.27	0.3	0.43•	0.35	-0.1
temperature	-0.22	-0.32	-0.33	0.08	-0.42	-0.55*	-0.40	-0.24
Depth	-0.28	-0.28	-0.20	-0.27	-0.18	-0.25	-0.17	-0.27
Light	-0.55*	-0.58*	-0.63*	-0.28	-0.65*	-0.72*	-0.63*	-0.06
VPD	-0.33	-0.43•	-0.38	-0.17	-0.3	-0.50*	-0.28	-0.03
Swp	-0.35	-0.45•	-0.23	-0.31	-0.22	-0.05	-0.23	-0.44•
Ppt	-0.03	-0.10	-0.12	0.03	-0.03	0.13	-0.12	-0.02

Water depth and soil water potential were both negatively correlated with expansion only in the upper tiers. Temperature was significantly correlated with contraction in the middle and upper tiers, but only to expansion in the middle tier (Table 2.3). In both the upper and the middle stem, light always had the highest correlation coefficient with both normalized, non-normalized amplitudes and rates. Lower stem tiers did not show a significant correlation between monthly mean contraction and expansion values and any of the measured environmental variables. In all cases, normalized contraction and expansion amplitudes again showed higher and more significant correlations with environmental correlates than non-normalized values.

Table 2.3 Kendal tau correlation coefficients between monthly mean environmental variables and monthly mean ΔW cycle properties for September 2014-December 2015 for upper, lower and middle stem tiers. Stem cycle properties are expansion and contraction amplitude (Ae and Ac), expansion and contraction amplitude standardized by each measurement point maxima (Ae* and Ac*) expansion and contraction rate (Re and Rc) and duration of expansion and contraction (Te and Tc). Star indicates a correlation significant at (**) p ≤ 0.01 or (*) p ≤ 0.05 and a point (•) at p ≤ 0.1.

		Parameter					
Tier	**Variable**	**Ae**	**Ae***	**Ac**	**Ac***	**Re**	**Rc**
Upper Stem	Humidity	0.29	0.42*	0.18	0.31•	0.19	0.22
	Temperature	-0.24	-0.29	-0.23	-0.38*	-0.23	-0.26
	Water depth	-0.35•	-0.39*	-0.16	-0.19	0.21	-0.2
	Light	-0.47**	-0.59**	-0.34•	-0.53**	-0.33•	-0.4*
	VPD	-0.22	-0.32•	-0.19	-0.33•	-0.17	-0.23
	Swp	-0.33•	-0.41*	-0.13	-0.16	-0.19	-0.15
	ppt	-0.21	-0.28	0.09	-0.15	-0.13	-0.13
Middle Stem	Humidity	0.24	0.35•	0.28	0.38*	0.25	0.33•
	Temperature	-0.28	-0.37*	-0.32•	-0.41*	-0.3	-0.26
	Water depth	-0.24	-0.27	0.18	-0.13	-0.24	-0.17
	Light	-0.44*	-0.54**	-0.47**	-0.75**	-0.46**	-0.49**
	VPD	-0.22	-0.34•	-0.29	-0.38*	-0.24	-0.29
	Swp	-0.19	-0.24	-0.13	-0.13	-0.2	-0.18
	ppt	-0.24	-0.21	-0.20	-0.15	-0.23	-0.21
Lower Stem	Humidity	0.1	0.09	0.12	0.09	0.07	0.09
	Temperature	-0.23	-0.17	-0.25	-0.15	-0.23	-0.23
	Water depth	0.18	0.15	0.12	0.08	0.15	0.1
	Light	-0.07	-0.06	-0.09	-0.06	-0.06	-0.07
	VPD	-0.16	-0.14	-0.18	0.13	-0.13	-0.14
	Swp	-0.19	0.19	0.15	0.1	0.18	0.15
	ppt	0.11	0.12	0.12	0.11	0.09	0.11

2.4.4 Correlation between diel mean environmental variables and ΔW at different stem heights

Moving window correlations performed on standardized amplitudes showed that monthly mean correlations did not always maintain the direction of the relationship at smaller scales of 30 day windows, although highly correlated variables were still light sum, relative humidity and VPD. Light was again the most highly correlated environmental driver of expansion and contraction in both measurement periods, but the correlation with light when

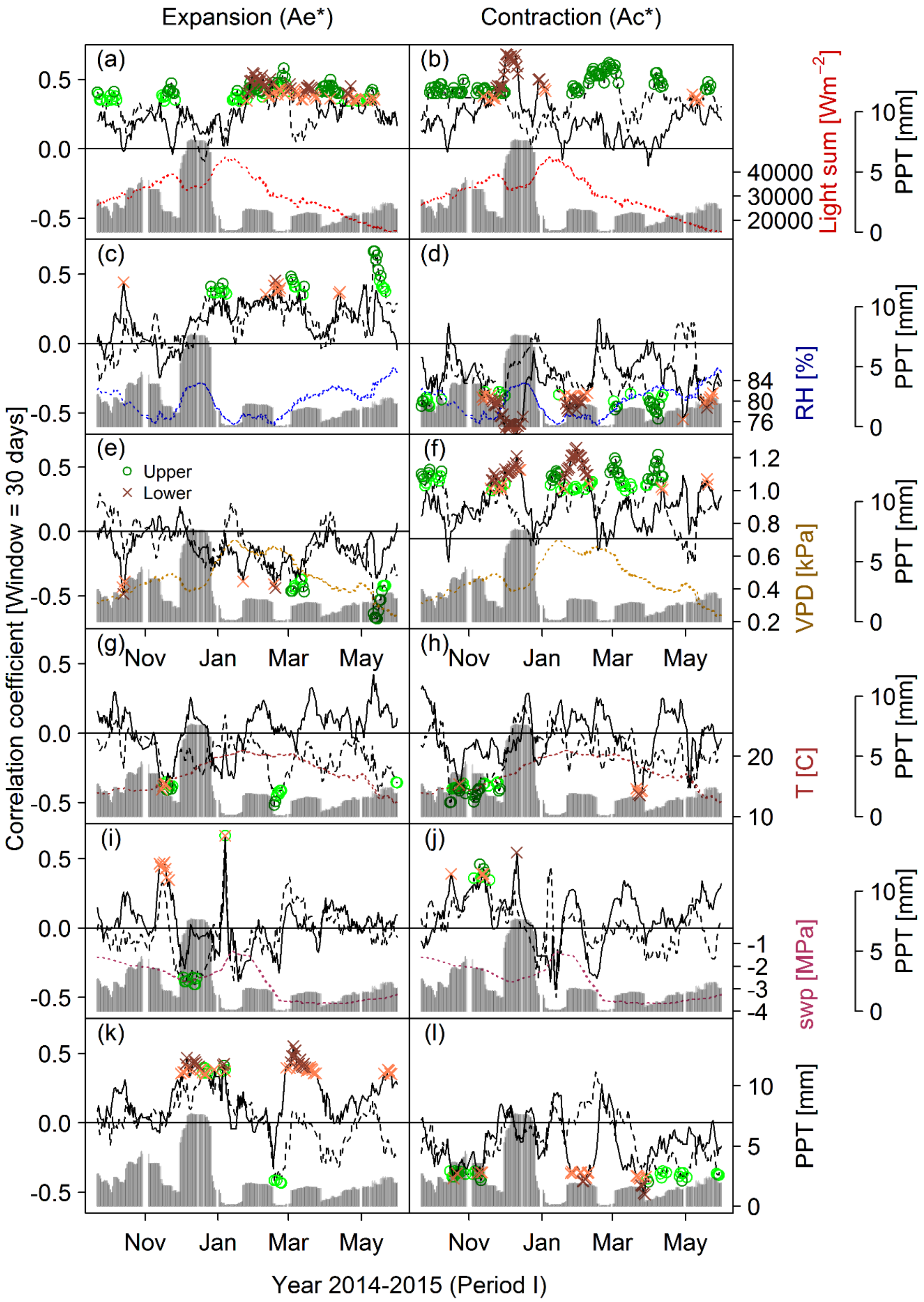


Figure 2.5 Moving 30 day window Kendal correlations for Upper (green circles) and Lower stem (dark crosses) of all three measured trees (n=3) of Period I (September 2014-June 2015) standardized expansion amplitude (Ae*) and contraction amplitude (Ac*) for all trees to 30 day moving averages of daily light sum (red line, a-b), daily mean relative humidity (RH, blue line, c-d), daily mean vapour pressure deficit (VPD, ochre line, e-f), daily mean temperature (T, brown line, g-h), daily mean soil water potential (swp, dark pink, i-j) and daily precipitation sum (PPT, gray bars, k-l). Grey bars indicate daily sum of precipitation (PPT). Light colour indicates significant correlation at p-value < 0.1 and dark colour indicates significant correlation at p-value < 0.05

significant was positive in both cases, instead of negative (Figure 2.5 & 2.6, a-b). The correlation with light was strong all year round and its drop often coincided with a significant effect on amplitudes of precipitation (Figure 2.5 & 2.6), both in the big precipitation event in December 2014 and the absence of precipitation in November 2015. Expansion and contraction amplitudes were also significantly correlated with relative humidity. Expansion was significantly correlated with humidity predominantly in cases when the correlation of expansion with light was low (Figures 2.5 & 2.6).

Contraction amplitude was positively correlated with VPD, and this correlation was more often significant than correlations with relative humidity during the summer in Period I, but similar during winter of Period II (Figure 2.5 & 2.6, e-f). The rainless month of November 2015 during Period II coincided with a high correlation of contraction amplitude in lower tiers with RH and VPD (Figure 2.6, e-f). This month also had a strong effect on contraction amplitudes in lower tiers (Appendix Figure 2.3), although moving correlations could not detect this due to all the 0 value recordings of precipitation during that time period.

During measurement Period I moving correlations upper tiers were more often correlated with light, relative humidity and VPD than lower tiers (Figure 2.5). There were also notable differences between upper and lower tiers in Period I in the cases of temperature, soil water potential and precipitation (Figure 2.5). Upper tier contraction and expansion were negatively correlated with temperature on more occasions than the lower tier. In turn, lower tier expansion amplitude was positively correlated to precipitation during more instances than the upper tier, although both tiers were affected by the large precipitation event in December 2014. After a large precipitation event, less negative soil water potential became negatively correlated to contraction amplitude in both tiers, although the correlation was stronger in the lower tier. In a similar fashion during Period II lower stem tiers were highly correlated to RH and VPD when soil water potential was low, but ceased to be so when it was high (close to 0 MPa) (Figure 2.6). In the rest of cases, correlation of ΔW to soil water potential changed sign on several occasions. Correlation of stem cycle amplitudes with water depth was not significant in any of the measured periods (data not shown).

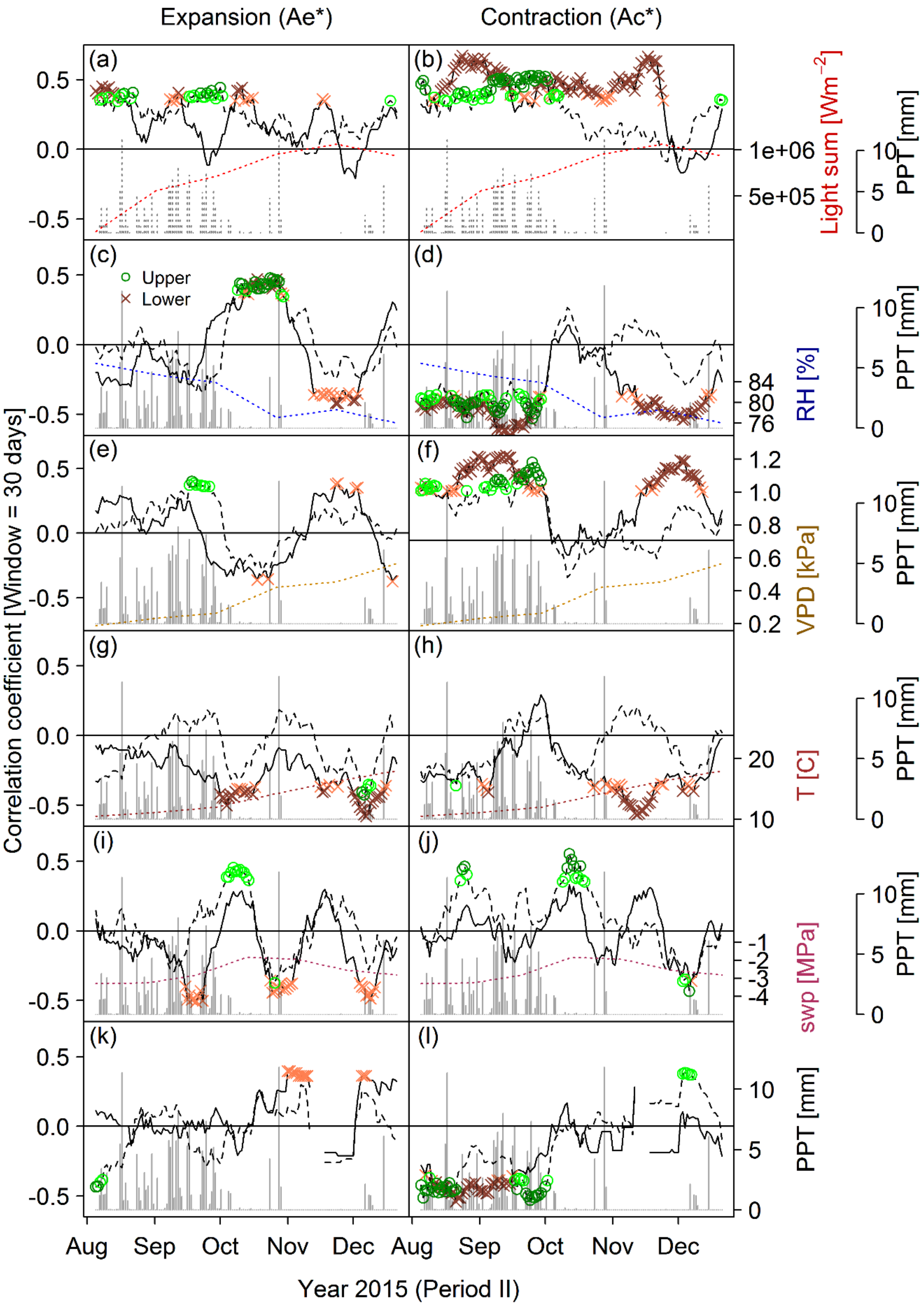


Figure 2.6 Moving 30 day window Kendal correlations for Upper (green circles) and Lower (dark crosses) stem for standardized expansion amplitude (Ae*) and contraction amplitude (Ac*) for all two trees of Period II (July 2015 - December 2015) with 30 day moving averages of daily light sum (red line, a-b), daily mean relative humidity (RH, blue line, c-d), daily mean vapour pressure deficit (VPD , ochre line, e-f), daily mean temperature (T, brown line, g-h), daily mean soil water potential (swp, dark pink, i-j) and daily precipitation sum (PPT, gray bars, k-l). Grey bars indicate daily sum of precipitation (PPT). Light colour indicates significant correlation at p-value < 0.1 and dark colour indicates significant correlation at p-value < 0.05

2.4.5 Importance of light: scale in assessing environmental relationships

Light was always the strongest correlate of expansion and contraction amplitudes, both in correlations of monthly mean expansion amplitude with mean daily light sum and in moving window correlations of 30 days over the whole year. However, despite the fact that other environmental drivers maintained the direction of the correlation with A_e^* and A_c^* across temporal scales (Table 2.3, Figure 2.5 & 2.6) the correlation with light changed from negative to positive when going from a seasonal to a diel time scale (Figure 2.7). Thus, monthly means showed there was a strong negative correlation, where smaller amplitudes in summer were associated with high light levels, whilst large amplitudes in winter months were correlated to lower light levels (Table 2.2). However, when each month was assessed independently in moving window correlations, higher light levels were associated with higher amplitudes (Figure 2.7). When the moving window correlation was enlarged from 30 days to 200, correlation coefficients between expansion

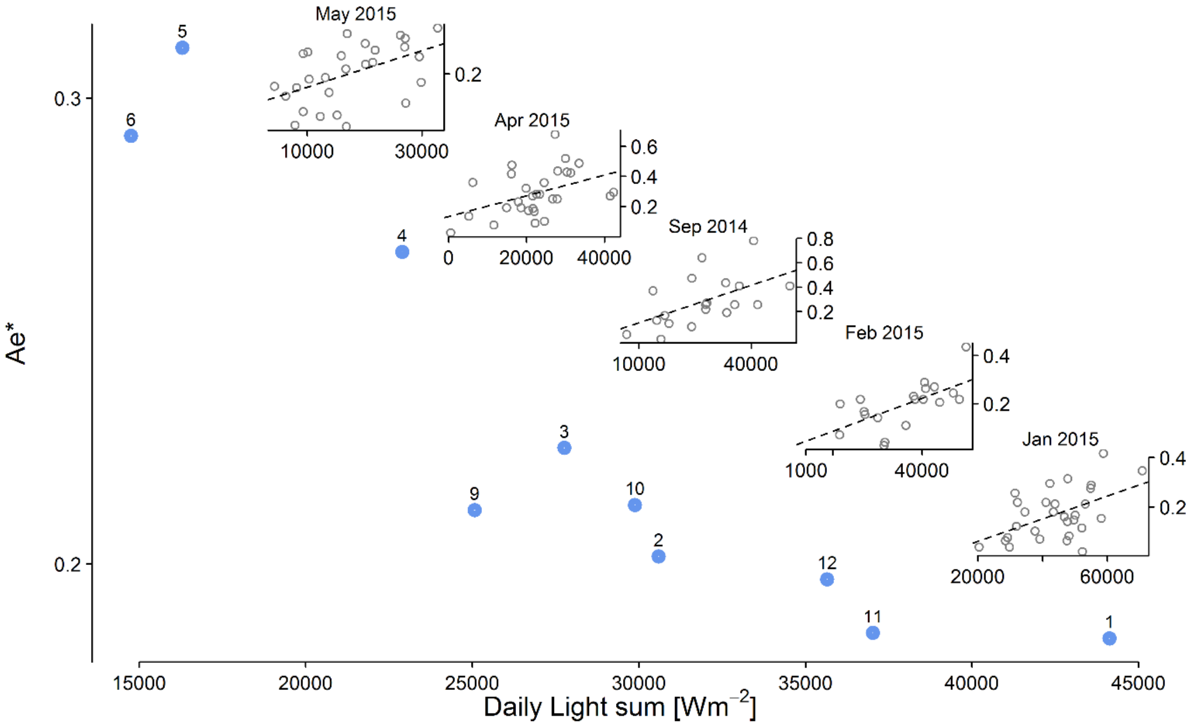


Figure 2.7 Relationship between monthly mean of daily light sum and monthly mean standarized expansion amplitude (Ae*) (blue dots) where numbers indicate each month of the measurement Period I, from September 2014 (month 9) until June 2015 (month 6). By each month there is a small inset showing the relationship between light sum (x axis) and and standardized expansion (Ae*, y axis) were each dot represents a day of the month for all measured trees (n=3 or n=2). Note: insets with daily values for each month show a positive correlation between Ae and light, whilst monthly means show a negative correlation. There was no difference between Ae* and Ac*, so Ac* is omitted for simplicity.

amplitudes and light became less positive and less significant, and correlations with relative humidity became more significant (Supplementary Figure 2.3).

2.5 Discussion

My results show the existence of seasonal and monthly patterns in water-related stem expansion and contraction, with large amplitudes in times of high water availability and strong correlations with environmental variables at both seasonal and monthly scales. My results confirm that long term measurements of SRC are heterogeneous (Robert et al. 2014), more so than other studied species (e.g. Zweifel, Zimmermann et al. 2005, Drew and Downes 2009, Drew, Richards et al. 2011). However, I show that stem cycle components of ΔW are not simply random or only influenced by underlying heterogeneous growth patterns along the stem, but can be related to environmental change. Once stem relative amplitude trends were analysed by tier, seasonal trends and diel correlations with environmental variables became stronger. The correlations with environmental conditions showed that whilst contraction was strongly influenced by environmental indicators of atmospheric water demand or soil water supply, periods of expansion were strongly driven by light. This is an unusual finding as light is often found to correlate negatively with stem expansion, as stem expansion usually occurs during late afternoon and night in conditions of high water availability. The importance of light found in my work provides more evidence for the theories that sugars play a role in the osmotic adjustment behind unusual daytime stem swelling present in *A. marina* (Figure 2.4) (Vandegehuchte, Guyot et al. 2014, Vandegehuchte, Guyot et al. 2014), since light sum is a proxy of photosynthesis and the resulting carbohydrates are the primary resource for osmoregulation (Hartmann et al. 2016).

2.5.1 The relationship between water-related stem cycle properties and environmental variables at mean diel scales

Further evidence for the valuable information contained in *A. marina* storage tissue contraction and expansion was that they were correlated, very strongly in some cases, with both seasonal and diel changes in environmental conditions. Standardized values of daily mean stem expansion and contraction showed especially strong relationships to changes in light as well as traditional indicators of atmospheric water demand (RH, VPD) and soil water supply (SWP and PPT) (Herzog et al. 1995, Zweifel et al. 2000, Deslauriers et al. 2003, Zweifel et al. 2005, Steppe et al. 2006, Devine and Harrington 2011, Turcotte et al. 2011, King et al. 2013, Urrutia-Jalabert et al. 2015). Therefore, despite the fact that stem responses have previously been reported as patchy (Robert et al. 2014), I found that these responses were far from random, and standardization and segregation by tiers helped shed light on both seasonal and diel mean scale trends.

Light sum was by far the most significant positive correlate of expansion and contraction. In the case of expansion, this is a surprising finding, as light is often negatively correlated with expansion amplitude, as increased transpiration and water loss hinder cell turgor maintenance and water store refill (Herzog et al. 1995, Zweifel et al. 2000, Deslauriers et al. 2003, Zweifel et al. 2005, Steppe et al. 2006, Devine and Harrington 2011, Turcotte et al. 2011, King et al. 2013, Urrutia-Jalabert et al. 2015). An explanation for this must lie in the peculiar daytime swelling present in *A. marina* (Figure 2.3), a pattern observed in another mangrove species, *Rhizophora stylosa* Griff., by Vandegehuchte, Guyot et al. (2014). Delayed onset of stem shrinking was successfully modelled as the result of osmotic adjustment of storage tissues, and soluble sugars are thought to play a role in this osmotic adjustment (De Swaef et al. 2013, Mencuccini et al. 2013, Vandegehuchte et al. 2014b, De Swaef et al. 2015, Chan et al. 2016, Mencuccini et al. 2017). Sugar loading into the phloem increases its osmotic potential and creates what is known as the “phloem generated turgor signal”, which can have an important role in elastic tissue radial change (Sevanto et al. 2011, Mencuccini et al. 2013, Pfautsch et al. 2015a, Chan et al. 2016). Thus, the positive effect of diel light sums on mean diel expansion amplitude in *A. marina* found in this study can be explained if photosynthates play a role in osmotic adjustment responsible for daytime swelling, as photosynthetic active radiation in light is a proxy of photosynthesis (Mencuccini et al. 2013, Chan et al. 2016). Therefore, stem expansion lasting into the late morning caused by loading of photosynthates into the phloem would be positively affected by increased light sums, which would increase photosynthesis, sugar production and hence available carbohydrates for osmoregulation of storage tissues (Donnellan et al. 2018).

Moving window correlations of diel means revealed that higher values of atmospheric humidity were correlated to higher amplitudes of expansion, whilst higher values of vapour pressure deficit were more important for determining higher contraction. This is a common finding, as higher atmospheric humidity can help relieve leaf and xylem water potentials necessary for refilling water stores (Cermak, Kucera et al. 2007, Scholz, Bucci et al. 2007, Turcotte, Rossi et al. 2011, Köcher, Horna et al. 2012, Sallo, Sanches et al. 2017), whilst warmer and drier days with high VPD cause increased use of stored water in transpiration and thus increase stem shrinkage (Zweifel, Item et al. 2000, Zweifel, Zimmermann et al. 2005, Steppe, De Pauw et al. 2006, Devine and Harrington 2011). The sensitivity to VPD found in this study can also be explained by the changes in soil water potential. For example, the relatively high (less negative) soil water potentials recorded during early summer resulted in stem water storage dynamics becoming correlated with VPD, a common response if soil water supply is abundant (Devine and Harrington 2011, Belien, Rossi et al. 2014, Sallo, Sanches et al. 2017).

My results also highlight the importance of freshwater inputs in the form of precipitation, with increased expansion occurring during times of high precipitation and with times of precipitation lack leading to reduced water store expansion (Supplementary Figure 2.2). Extended periods with no precipitation like the measured month of November 2015 are uncommon in the North Island of New Zealand, although they are likely to become more frequent due to climate change (NIWA 2017). Thus, these results confirm for the first time in the field the importance of precipitation in helping mangroves maintain a favourable water balance (Uddin 2014, Reef and Lovelock 2015, Reef et al. 2015, Santini et al. 2015, Lovelock et al. 2017, Nguyen et al. 2017) and highlight their potential vulnerability to a global increase in drought frequency (IPCC 2013, Krauss, McKee et al. 2014, Lovelock, Krauss et al. 2016).

Surprisingly my results showed that the correlation of stem expansion and contraction with soil water potential was not often significant, and it changed direction at different times of the year (Figure 2.5 & 2.6). Predominantly, the relationship of soil water potential to contraction and expansion was negative, implying larger use of water stores and higher expansion at times of high salinity. Higher contraction at times of high salinity coincides with the work of Lechthaler, Robert et al. (2016) which showed a higher reliance on water stores in mangrove saplings in times of high salinity. In the present study, higher expansion in times of higher salinity could be due perhaps to higher osmoregulation of storage tissues. Responses of radial water storage to dry or saline soil can be manifest either by increased amplitudes as the tree depletes its internal water stores (Zweifel, Zimmermann et al. 2005, Cermak, Kucera et al. 2007) or by decreased amplitudes as the tree closes stomata and downregulates transpiration (e.g. Belien 2014). However, the changing nature of the correlation of soil water potential with stem cycle amplitudes in this study makes interpretation difficult. Studies which examine the effect of changing salinity on radial stem water dynamics in mangrove are still rare (Vandegehuchte et al. 2014). Salinity in mangrove systems is a complex interplay between tides, fresh water inputs, evaporation, transpiration and tree distribution (Krauss 2014). It is important to keep in mind that in mangroves systems higher transpiration and water uptake can cause increase of salinity due to increased root filtration of water leaving salts behind (Ball 1988b, Marchand et al. 2004, Schmitz et al. 2012, Krauss & Ball 2013, Krauss et al. 2015, Reef and Lovelock 2015, Jiang et al. 2017). Further work should analyse the interaction between soil water potential, VPD, stomatal control, osmoregulation and radial water storage in mangrove systems.

2.5.2 Seasonal relationships between water-related stem cycle properties and environmental variables at mean monthly scales

The environmental drivers of seasonal monthly mean trends are more difficult to interpret than the diel mean relationships. It is evidently surprising that high correlation to light at diel scales does not lead to higher amplitudes during summer months, which must be due to water limitation during this time and the consequent stomatal closure, reducing water loss and thus stem shrinkage. My analysis showed an apparent switch between RH and light as main drivers of expansion amplitude when expanding the time-scale of moving window correlations (Figure 2.5 & 2.6). Water shortages during summer months or months with low precipitation must overcome any positive effect of light on expansion amplitudes by limiting the water available for refilling storage tissues (e.g. Drew 2011). Therefore, at a certain water stress level, osmoregulation seems not to be able to compensate (or overcompensate) for the drop in xylem water potentials due to transpiration (Donnellan et al. 2018, *in press*). These kind of switches between different drivers of ΔW are common, such as the well-known change between VPD and soil water potential (Zweifel 2005), although such switches occurring between water and light have not been recorded before to my knowledge. Evidently, the seasonal correlation results in this study where light was a significant negative correlate of expansion and contraction amplitude must not be directly causal and more related to rain shortages during summer months than to changes in light. Considering the positive relationship of light to expansion during almost every individual month, increasing the integration time must, in this sense, decouple the trends from the mechanism acting on daytime stem swelling at shorter time-scales. These kind of spurious correlations are abundant in the literature, especially in studies involving time series of many highly correlated environmental variables (Zuur *et al.*, 2010) and are a reminder to be cautious in interpreting single correlation results and considering different integration periods.

Although some work considers that Maximum Daily Shrinkage (MDS) is an indicator of plant water stress (Goldhamer and Fereres 2001, Deslauriers et al. 2003, Fereres and Goldhamer 2003, Fernandez and Cuevas 2010, Belien et al. 2014, Robert et al. 2014), my results don't seem to support this for my study environment. In this study, amplitudes of both expansion and contraction diminished in conditions of water shortage and high atmospheric demand. Thus, as pointed out by other authors, the MDS approach may only be true when looking at short time-scale responses in relatively wet environments e.g. orchards, but not hold in ecological research of trees under part time dry conditions (Fernandez & Cuevas, 2010, Zweifel pers. comm.). The reduced amplitudes found in this study could indicate reduced storage when freshwater supply is reduced due to low precipitation in summer, and strong stomatal regulation during times of high vapour pressure deficit, minimizing transpiration and protecting tissues from dangerously low water potential levels and embolism (Cochard *et al.*, 1996). The idea of strong stomatal regulation as an explanation for lowered rates of both contraction and expansion seen in my data is backed by measurements in this site which show lower stomatal conductance in summer and higher stomatal conductance in winter (Donnellan et al. 2018).

It seems important to mention that the recorded times of reduced expansion and contraction in summer occurred in temporal proximity to times when soil water potential was high (closer to 1 MPa) in December 2014 after a large precipitation event just at the start of summer. Thus, it is possible that water potential causes the seasonal change of lower amplitudes, as increased soil water availability could reduce the reliance on water stores. However, it seems unlikely that less negative soil water potential would not be accompanied by refilling of water stores and thus larger expansion amplitudes. Additionally, the notion that favourable conditions caused by higher availability of water in soil result in decreased amplitudes of expansion and contraction seems incompatible with the clear effect that decreased water supply had during precipitation absence in November 2015 (Supplementary Figure 2.3).

2.5.3 Differences in water-related stem radius change between stem tiers

My results show differences between stem tiers in their response to environmental drivers, a phenomenon rarely observed before. Lower stem tiers seemed much more sensitive to soil water potential changes and precipitation than upper tiers. This was especially notable when both contraction and expansion amplitudes were dramatically reduced during the month of no precipitation in November 2015. It seems logical that areas of the stem closest to the water source are more sensitive to changes in water supply in the soil. In turn, upper stem tiers were more strongly correlated to light. Considering the proposed role of osmoregulation in stem expansion, proximity to sugar sources in the crown, as well changes in source sink activity, might be responsible for the different sensitivity of the stem to water availability. Thus, stem areas closer to the crown are more influenced by a carbohydrate induced phloem turgor signal (Mencuccini et al. 2013, Chan et al. 2016). Further research should explore the potential semi-autonomy of tree-branches in other species with multiple reticulate cambia, and the influence of osmoregulation along the stem (Donnellan et al. 2018).

2.5.4 Seasonal courses in amplitudes and rates of ΔW are noisy but not nonsense

There was notable heterogeneity in long terms trends of SRC and ΔW, and differences in the response magnitude and variation across measurement points. The heterogeneous nature of stem cycle components and long term stem radius courses found in this study has been reported previously for mangroves by Robert et al. (2014). However, although daily amplitudes showed different absolute magnitudes of expansion or contraction between measurement points under the same conditions, once each point was standardized by its own maximum, the relative changes revealed much clearer seasonal trends and increased correlation coefficients with environmental variables.

The heterogeneity of *A. marina* wood structure, formed by successive multiple cambia in which bands of xylem are connected to multiple bands of internal secondary phloem in an irregular fishnet structure, is thought to be the reason for the variability both of stem radius change and sap flow measurements (Robert et al. 2014, Van de Wal et al. 2014). This network of wood patches creates a new arrangement of water conducting and water storing compartments in the stem which affects the water flow and storage dynamics, and very little is known about how reticulate wood structure affects stem radial change (e.g. Zweifel et al 2014). Changes in wood structure along the stem must also cause differences is wood properties which influence the dynamics of radial water storage in elastic tissues, like hydraulic resistance to water flow, elasticity, xylem resistance and stem capacitance (Sevanto et al. 2011, Steppe et al. 2012, Baert et al. 2015, De Swaef et al. 2015, Pfautsch et al. 2015a, Steppe et al. 2015, Pfautsch 2016). Variation in storage tissue physical properties, such as elasticity, could be responsible for the variation caused by different absolute values of stem expansion or contraction. In these cases, standardization by maxima helped increase correlation coefficients as it showed the relative changes of all measurement points. Mixed models approaches or *in situ* measurements of wood properties should be explored in future studies in order to use the values of these parameter to understand their contribution to the water storage and depletion dynamics (Steppe 2006, 2008). However this might prove difficult, firstly due to the high spatial variation of tissue structure reported by Robert et al. (2014) which can vary in just a few millimetres and secondly because of temporal variations in tissue structure caused by changes from the start to the end of the experimental period.

This procedure of standardization also made evident that amplitude trends, if not absolute values, were more similar at the same height in different trees than trends within the same tree at different heights. The effect of stem tier on radial change could be caused by different responses to environmental conditions depending on the proximity to water supply (soil) and to transpirational water loss (leaves) (Zweifel et al., 2000; Zweifel et al., 2001) or proximity to carbon sources and sinks as seen in correlation results (Sevanto et al. 2002, 2003, Mencuccini et al. 2013), especially if sugars are involved in osmoregulation (Vandegehuchte et al., 2014a). Additionally, studies on A. marina wood anatomy found the branching of the xylem and the phloem increased significantly with height, together with phloem to xylem ratio (Robert et al. 2011a).

Finally, the variability between measuring points was higher during the start of the growing season in period I than during the winter months of period I & II, which poses the question of the influence of the patchy activation of growth on the heterogeneity in ΔW courses (Schmitz et al. 2008, Robert et al. 2011a, Robert et al. 2011b, Robert et al. 2014). Even if I assume that the procedure for obtaining ΔW excluded all growth signal in dendrometer measurements (Zweifel et al. 2016, Mencuccini et al. 2017), growth processes could still affect radial water dynamics indirectly (Sevanto et al. 2003, Holtta et al. 2006, De Schepper and Steppe 2010, Sevanto et al. 2011, De Schepper et al. 2013, Mencuccini et al. 2013, Pfautsch et al. 2015b, Savage et al. 2016, Paljakka et al. 2017). However, growth cannot be the driving force of this heterogeneity as upper stem tier trends were relatively homogeneous between trees despite one of these measuring points showing growth (Figure 2.1 & 2.2).

2.6 Conclusion

The results of the present study show that stem radius change provides a wealth of information on the diel and seasonal changes and environmental drivers of stem water storage dynamics in species with multiple reticulate cambia, like the mangrove *A. marina*. Reduced amplitudes during summer months point towards potential importance of water availability and atmospheric demand in limiting stem water storage in mangroves. However, although indicators of water supply and demand were important in determining stem radial water dynamics, light was the strongest correlate of stem expansion. These results, together with the differences in the lower and upper stem sensitivity to light could be due to the effect of carbohydrate-related osmoregulation along the stem. Therefore, the results of this study provide additional backing for the theory that osmoregulation and carbohydrate dynamics in the phloem has an important role in stem water dynamics of *A. marina*.

2.7 References

Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.-H. Lim, G. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management **259**:660-684.

Baert, A., V. De Schepper, and K. Steppe. 2015. Variable hydraulic resistances and their impact on plant drought response modelling. Tree Physiology **35**:439-449.

Ball, M. C. 1988a. Ecophysiology of mangroves. Trees **2**:129-142.

Ball, M. C. 1988b. Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I. Water use in relation to growth, carbon partitioning, and salt balance. Functional Plant Biology **15**:447-464.

Belien, E., S. Rossi, H. Morin, and A. Deslauriers. 2014. High-resolution analysis of stem radius variations in black spruce *Picea mariana* (Mill.) BSP subjected to rain exclusion for three summers. Trees-Structure and Function **28**:1257-1265.

Boisvenue, C. and S. W. Running. 2006. Impacts of climate change on natural forest productivity: evidence since the middle of the 20th century. Global Change Biology **12**:862-882.

Buck, A. L. 1981. New equations for computing vapor-pressure and enhancement factor. Journal of Applied Meteorology. **20**:1527-1532

Cermak, J., J. Kucera, W. L. Bauerle, N. Phillips, and T. M. Hinckley. 2007. Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiology **27**:181-198.

Chan, T., T. Holtta, F. Berninger, H. Makinen, P. Nojd, M. Mencuccini, and E. Nikinmaa. 2016. Separating water-potential induced swelling and shrinking from measured radial stem variations reveals a cambial growth and osmotic concentration signal. Plant Cell and Environment **39**:233-244.

Cochard, H., N. Breda, and A. Granier. 1996. Whole tree hydraulic conductance and water loss regulation in Quercus during drought: Evidence for stomatal control of embolism? Annales Des Sciences Forestieres **53**:197-206.

De Schepper, V., T. De Swaef, I. Bauweraerts, and K. Steppe. 2013. Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany **64**:4839-4850.

De Schepper, V. and K. Steppe. 2010. Development and verification of a water and sugar transport model using measured stem diameter variations. Journal of Experimental Botany **61**:2083-2099.

De Swaef, T., V. De Schepper, M. W. Vandegehuchte, and K. Steppe. 2015a. Stem diameter variations as a versatile research tool in ecophysiology. Tree Physiol **35**:1047-1061.

De Swaef, T., S. M. Driever, L. Van Meulebroek, L. Vanhaecke, L. F. Marcelis, and K. Steppe. 2013. Understanding the effect of carbon status on stem diameter variations. Ann Bot **111**:31-46.

Deslauriers, A., M. Beaulieu, L. Balducci, A. Giovannelli, M. J. Gagnon, and S. Rossi. 2014. Impact of warming and drought on carbon balance related to wood formation in black spruce. Ann Bot **114**:335-345.

Deslauriers, A., H. Morin, C. Urbinati, and M. Carrer. 2003. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Quebec (Canada). Trees - Structure and Function **17**:477-484.

Deslauriers, A., S. Rossi, A. Turcotte, H. Morin, and C. Krause. 2011. A three-step procedure in SAS to analyze the time series from automatic dendrometers. Dendrochronologia **29**:151-161.

Devine, W. D. and C. A. Harrington. 2011. Factors affecting diurnal stem contraction in young Douglas-fir. Agricultural and Forest Meteorology **151**:414-419.

Donnellan, A., Zweifel, R., Cusens, J., Leuzinger, S. 2018. Daytime stem swelling and seasonal reversal in the peristaltic depletion of stored water along the stem of *Avicennia marina* (Forssk.) Vierh. Tree Physiology **00**:1-4

Drew, D. M. and G. M. Downes. 2009. The use of precision dendrometers in research on daily stem size and wood property variation: a review. Dendrochronologia **27**:159-172.

Drew, D. M., A. E. Richards, G. M. Downes, G. D. Cook, and P. Baker. 2011. The development of seasonal tree water deficit in *Callitris intratropica*. Tree Physiology **31**:953-964.

Fereres, E. and D. A. Goldhamer. 2003. Suitability of stem diameter variations and water potential as indicators for irrigation scheduling of almond trees. Journal of Horticultural Science & Biotechnology **78**:139-144.

Fernandez, J. E. and M. V. Cuevas. 2010. Irrigation scheduling from stem diameter variations: A review. Agricultural and Forest Meteorology **150**:135-151.

Flowers, T. J. and T. D. Colmer. 2008. Salinity tolerance in halophytes*. New Phytologist **179**:945-963.

Goldhamer, D. A. and E. Fereres. 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrigation Science **20**:115-125.

Hartmann H, Trumbore S. 2016. Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. New Phytologist 211(2): 386-403.

Herzog, K. M., R. Häsler, and R. Thum. 1995. Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees **10**:94-101.

Holtta, T., T. Vesala, S. Sevanto, M. Peramaki, and E. Nikinmaa. 2006. Modeling xylem and phloem water flows in trees according to cohesion theory and Munch hypothesis. Trees-Structure and Function **20**:67-78.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA.

Jasechko, S., Z. D. Sharp, J. J. Gibson, S. J. Birks, Y. Yi, and P. J. Fawcett. 2013. Terrestrial water fluxes dominated by transpiration. Nature **496**:347-350.

Jiang, G. F., U. M. Goodale, Y. Y. Liu, G. Y. Hao, and K. F. Cao. 2017. Salt management strategy defines the stem and leaf hydraulic characteristics of six mangrove tree species. Tree Physiology **37**:389-401.

King, G., P. Fonti, D. Nievergelt, U. Büntgen, and D. Frank. 2013. Climatic drivers of hourly to yearly tree radius variations along a 6°C natural warming gradient. Agricultural and Forest Meteorology **168**:36-46.

Köcher, P., V. Horna, and C. Leuschner. 2012. Environmental control of daily stem growth patterns in five temperate broad-leaved tree species. Tree Physiology. **32**:1021-1032.

Krauss, K. W. and M. C. Ball. 2013. On the halophytic nature of mangroves. Trees **27**:7-11.

Krauss, K. W., J. G. Barr, V. Engel, J. D. Fuentes, and H. Q. Wang. 2015. Approximations of stand water use versus evapotranspiration from three mangrove forests in southwest Florida, USA. Agricultural and Forest Meteorology **213**:291-303.

Krauss, K. W., C. E. Lovelock, K. L. McKee, L. López-Hoffman, S. M. L. Ewe, and W. P. Sousa. 2008. Environmental drivers in mangrove establishment and early development: a review. Aquatic Botany **89**:105-127.

Krauss, K. W., K. L. McKee, C. E. Lovelock, D. R. Cahoon, N. Saintilan, R. Reef, and L. Z. Chen. 2014. How mangrove forests adjust to rising sea level. New Phytologist **202**:19-34.

Lambers, H., F. S. Chapin III, and T. L. Pons. 2008. Plant Physical Ecology. Second edition. Springer Science+Business Media, New York.

Lechthaler, S., E. M. R. Robert, N. Tonne, A. Prusova, E. Gerkema, H. Van As, N. Koedam, and C. W. Windt. 2016. Rhizophoraceae mangrove saplings use hypocotyl and leaf water storage capacity to cope with soil water salinity changes. Frontiers in Plant Science **7**.

López-Portillo, J., F. W. Ewers, R. Méndez-Alonzo, C. L. Paredes López, G. Angeles, A. L. Alarcón Jiménez, A. L. Lara-Domínguez, and M. d. C. Torres Barrera. 2014. Dynamic control of osmolality and ionic composition of the xylem sap in two mangrove species. American Journal of Botany. **1**:1013-1022

Lovelock, C. E., K. W. Krauss, M. J. Osland, R. Reef, and M. C. Ball. 2016. The physiology of mangrove trees with changing climate. Pages 149-179 *in* G. Goldstein and L. S. Santiago, editors. Tropical tree physiology: adaptations and responses in a changing environment. Springer, Dordrecht.

Lovelock, C. E., R. Reef, and M. C. Ball. 2017. Isotopic signatures of stem water reveal differences in water sources accessed by mangrove tree species. Hydrobiologia **803**:133-145.

Marchand, C., F. Baltzer, E. Lallier-Verges, and P. Alberic. 2004. Pore-water chemistry in mangrove sediments: relationship with species composition and developmental stages (French Guiana). Marine Geology **208**:361-381.

McIntyre, D. S. 1980. Basic relationships for salinity evaluation from measurements on soil solution. Autralian Journal of Soil Research **18**:199-206.

Mencuccini, M., T. Holtta, S. Sevanto, and E. Nikinmaa. 2013. Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytologist **198**:1143-1154.

Mencuccini, M., Y. Salmon, P. Mitchell, T. Holtta, B. Choat, P. Meir, A. O'Grady, D. Tissue, R. Zweifel, S. Sevanto, and S. Pfautsch. 2017. An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant Cell and Environment **40**:290-303.

Naidoo, G. 2006. Factors contributing to dwarfing in the mangrove *Avicennia marina*. Annals of Botany **97**:1095-1101.

Nguyen, H. T., P. Meir, L. Sack, J. Evans, R. S. Oliveira, and M. C. Ball. 2017. Leaf water storage increases with salinity and aridity in the mangrove *Avicennia marina*: integration of leaf structure, osmotic adjustment and access to multiple water sources. Plant Cell and Environment **40**:1576-1591.

NIWA. 2017. NIWA Annual Report New Zealand

Oliva Carrasco, L., S. J. Bucci, D. Di Francescantonio, O. A. Lezcano, P. I. Campanello, F. G. Scholz, S. Rodriguez, N. Madanes, P. M. Cristiano, G. Y. Hao, N. M. Holbrook, and G. Goldstein. 2015. Water storage dynamics in the main stem of subtropical tree species differing in wood density, growth rate and life history traits. Tree Physiol **35**:354-365.

Paljakka, T., T. Jyske, A. Lintunen, H. Aaltonen, E. Nikinmaa, and T. Holtta. 2017. Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst). Plant Cell and Environment **40**:2160-2173.

Pfautsch, S. 2016. Hydraulic anatomy and function of trees—basics and critical developments. Current Forestry Reports **2**:236-248.

Pfautsch, S., T. Hölttä, and M. Mencuccini. 2015a. Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology **35**:706-722.

Pfautsch, S., J. Renard, M. G. Tjoelker, and A. Salih. 2015b. Phloem as capacitor: radial transfer of water into xylem of tree stems occurs via symplastic transport in ray parenchyma. Plant Physiology **167**:963-971.

Pivovaroff, A. L., S. C. Pasquini, M. E. De Guzman, K. P. Alstad, J. S. Stemke, L. S. Santiago, and K. Field. 2016. Multiple strategies for drought survival among woody plant species. Functional Ecology **30**:517-526.

Quisthoudt, K., N. Schmitz, C. F. Randin, F. Dahdouh-Guebas, E. M. R. Robert, and N. Koedam. 2012. Temperature variation among mangrove latitudinal range limits worldwide. Trees **26**:1919-1931.

Reef, R. and C. E. Lovelock. 2015. Regulation of water balance in mangroves. Annals of Botany **115**:385-395.

Reef, R., H. L. Markham, N. S. Santini, and C. E. Lovelock. 2015. The response of the mangrove *Avicennia marina* to heterogeneous salinity measured using a split-root approach. Plant and Soil **393**:297-305.

Reef, R., N. Schmitz, B. A. Rogers, M. C. Ball, and C. E. Lovelock. 2012. Differential responses of the mangrove *Avicennia marina* to salinity and abscisic acid. Functional Plant Biology **39**:1038-1046.

Robert, E. M. R., A. H. Jambia, N. Schmitz, D. J. R. De Ryck, J. De Mey, J. G. Kairo, F. Dahdouh-Guebas, H. Beeckman, and N. Koedam. 2014. How to catch the patch? A dendrometer study of the radial increment through successive cambia in the mangrove *Avicennia marina*. Annals of Botany **113**:741-752.

Robert, E. M. R., N. Schmitz, I. Boeren, T. Driessens, K. Herremans, J. De Mey, E. Van de Casteele, H. Beeckman, and N. Koedam. 2011a. Successive cambia: a developmental oddity or an adaptive structure? Plos One **6**:e16558.

Robert, E. M. R., N. Schmitz, J. A. Okello, I. Boeren, H. Beeckman, and N. Koedam. 2011b. Mangrove growth rings: fact or fiction? Trees **25**:49-58.

Sallo, F. D., L. Sanches, V. R. D. Dias, R. D. Palacios, and J. D. Nogueira. 2017. Stem water storage dynamics of *Vochysia divergens* in a seasonally flooded environment. Agricultural and Forest Meteorology **232**:566-575.

Santini, N. S., R. Reef, D. A. Lockington, and C. E. Lovelock. 2015. The use of fresh and saline water sources by the mangrove *Avicennia marina*. Hydrobiologia **745**:59-68.

Santini, N. S., N. Schmitz, and C. E. Lovelock. 2012. Variation in wood density and anatomy in a widespread mangrove species. Trees **26**:1555-1563.

Savage, J. A., M. J. Clearwater, D. F. Haines, T. Klein, M. Mencuccini, S. Sevanto, R. Turgeon, and C. Zhang. 2016. Allocation, stress tolerance and carbon transport in plants: how does phloem physiology affect plant ecology? Plant Cell and Environment **39**:709-725.

Schmitz, N., E. M. Robert, A. Verheyden, J. G. Kairo, H. Beeckman, and N. Koedam. 2008. A patchy growth via successive and simultaneous cambia: key to success of the most widespread mangrove species Avicennia marina? Ann Bot **101**:49-58.

Scholz, F. C., S. J. Bucci, G. Goldstein, F. C. Meinzer, A. C. Franco, and F. Miralles-Wilhelm. 2008. Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water. Tree Physiology **28**:469-480.

Scholz, F. G., S. J. Bucci, G. Goldstein, F. C. Meinzer, A. C. Franco, and F. Miralles-Wilhelm. 2007. Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. Plant Cell and Environment **30**:236-248.

Sevanto, S., T. Holtta, and N. M. Holbrook. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant Cell and Environment **34**:690-703.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2002. Time lags for xylem and stem diameter variations in a Scots pine tree. Plant Cell and Environment **25**:1071-1077.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2003. Sugar transport together with environmental conditions controls time lags between xylem and stem diameter changes. Plant Cell and Environment **26**:1257-1265.

Sitch, S., C. Huntingford, N. Gedney, P. E. Levy, M. Lomas, S. L. Piao, R. Betts, P. Ciais, P. Cox, P. Friedlingstein, C. D. Jones, I. C. Prentice, and F. I. Woodward. 2008. Evaluation of the terrestrial carbon cycle, future plant geography and climate-carbon cycle feedbacks using five Dynamic Global Vegetation Models (DGVMs). Global Change Biology **14**:2015-2039.

Sperry, J. S. and D. M. Love. 2015. What plant hydraulics can tell us about responses to climate-change droughts. New Phytologist **207**:14-27.

Steppe, K., H. Cochard, A. Lacointe, and T. Ameglio. 2012. Could rapid diameter changes be facilitated by a variable hydraulic conductance? Plant Cell and Environment **35**:150-157.

Steppe, K., D. De Pauw, R. Lemeur, and P. Vanrollegheim. 2005. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257–273.

Steppe, K., D. J. W. De Pauw, R. Lemeur, and P. A. Vanrolleghem. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257-273.

Steppe, K., F. Sterck, and A. Deslauriers. 2015. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends in Plant Science **20**:335-343.

Trenberth, K. E., A. Dai, G. van der Schrier, P. D. Jones, J. Barichivich, K. R. Briffa, and J. Sheffield. 2013. Global warming and changes in drought. Nature Climate Change **4**:17-22.

Turcotte, A., S. Rossi, A. Deslauriers, C. Krause, and H. Morin. 2011. Dynamics of depletion and replenishment of water storage in stem and roots of black spruce measured by dendrometers. Frontiers in Plant Science **2**:21.

Uddin, S. 2014. Canopy water uptake: An important survival mechanism of mangroves. Ghent University and Vrije Universiteit Brussel, Belgium.

Urrutia-Jalabert, R., S. Rossi, A. Deslauriers, Y. Malhi, and A. Lara. 2015. Environmental correlates of stem radius change in the endangered Fitzroya cupressoides forests of southern Chile. Agricultural and Forest Meteorology **200**:209-221.

Van de Wal, B. A. E. 2011. Ecophysiology of mangroves in Brisbane, Australia: Sap flow dynamics in *Avicennia marina*. Universiteit Gent, Gent, Belgium.

Van de Wal, B. A. E., A. Guyot, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014. Influence of temporospatial variation in sap flux density on estimates of whole-tree water use in *Avicennia marina*. Trees **29**:215-222.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, S. R. E. De Groote, N. J. F. De Baerdemaeker, M. Hayes, N. Welti, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014a. Long-term versus daily stem diameter variation in co-occurring mangrove species: environmental versus ecophysiological drivers. Agricultural and Forest Meteorology **192**:51-58.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, T. De Swaef, D. A. Lockington, and K. Steppe. 2014b. Modelling reveals endogenous osmotic adaptation of storage tissue water potential as an important driver determining different stem diameter variation patterns in the mangrove species *Avicennia marina* and *Rhizophora stylosa*. Annals of Botany. **114**:667-676.

Wang, W., Z. Yan, S. You, Y. Zhang, L. Chen, and G. Lin. 2011. Mangroves: obligate or facultative halophytes? A review. Trees **25**:953-963.

Zielis, S., S. Etzold, R. Zweifel, W. Eugster, M. Haeni, and N. Buchmann. 2014. NEP of a Swiss subalpine forest is significantly driven not only by current but also by previous year's weather. Biogeosciences **11**:1627-1635.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.

Zweifel, R., D. M. Drew, F. Schweingruber, and G. M. Downes. 2014. Xylem as the main origin of stem radius changes in *Eucalyptus*. Functional Plant Biology **41**:520-534.

Zweifel, R., W. Eugster, S. Etzold, M. Dobbertin, N. Buchmann, and R. Hasler. 2010. Link between continuous stem radius changes and net ecosystem productivity of a subalpine Norway spruce forest in the Swiss Alps. New Phytologist **187**:819-830.

Zweifel, R., M. Häni, N. Buchmann, and W. Eugster. 2016. Are trees able to grow in periods of stem shrinkage? New Phytologist **211**:839-849.

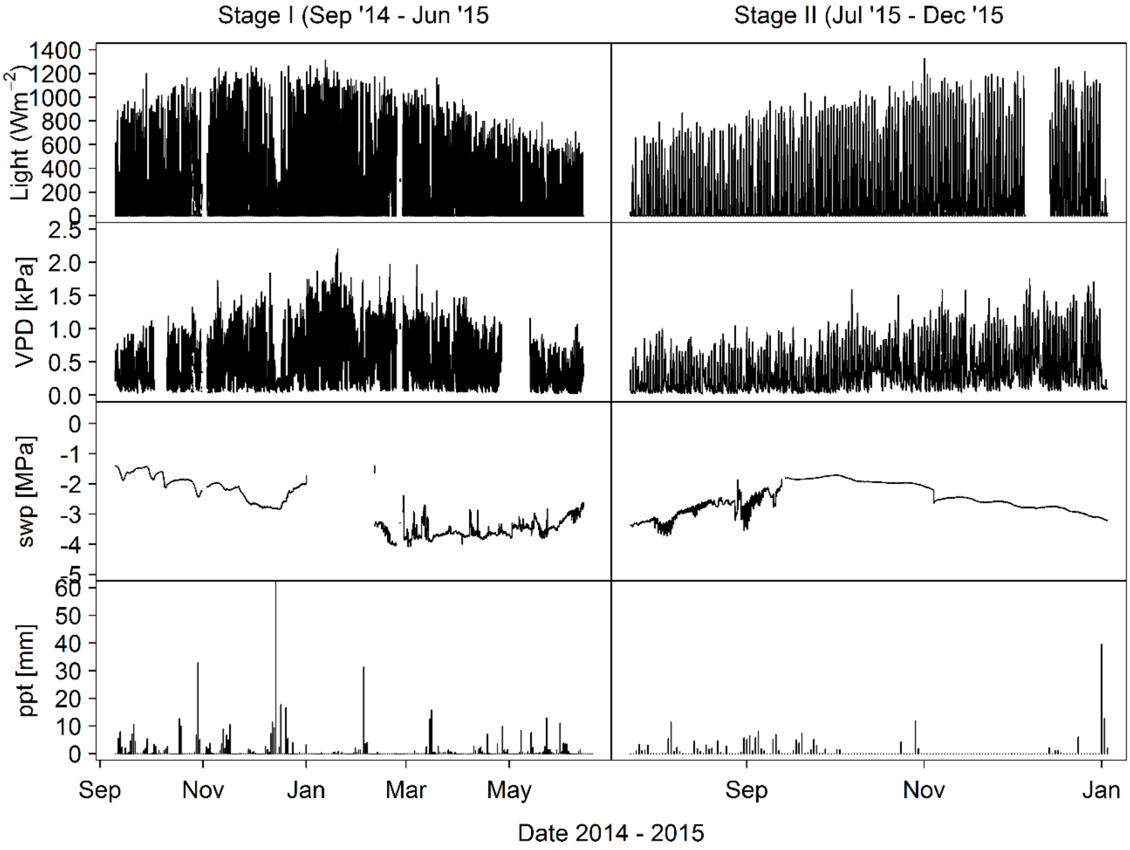
Zweifel, R., H. Item, and R. Hasler 2001. Link between diurnal stem radius changes and tree water relations. Tree Physiology **21**: 869–877.

Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees **15**:50-57.

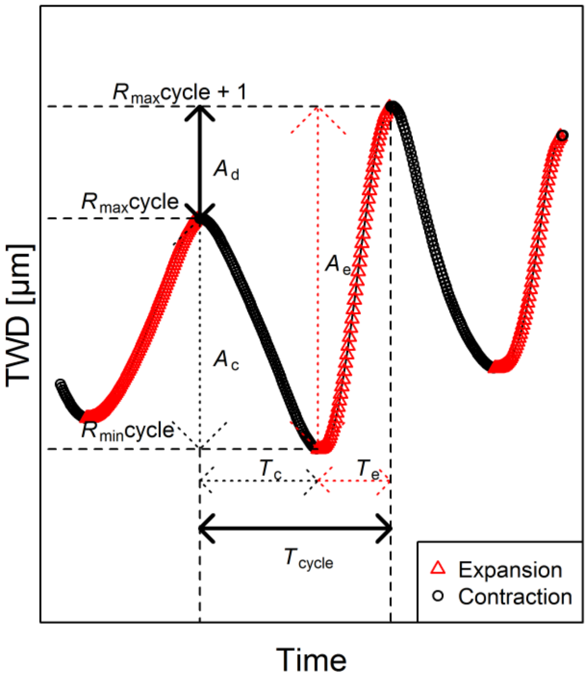
Zweifel, R., L. Zimmermann, and D. M. Newbery. 2005. Modeling tree water deficit from microclimate: an approach to quantifying drought stress. Tree Physiology **25**:147-156.

Zweifel, R., L. Zimmermann, F. Zeugin, and D. M. Newbery. 2006. Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. Journal of Experimental Botany **57**:1445-1459.

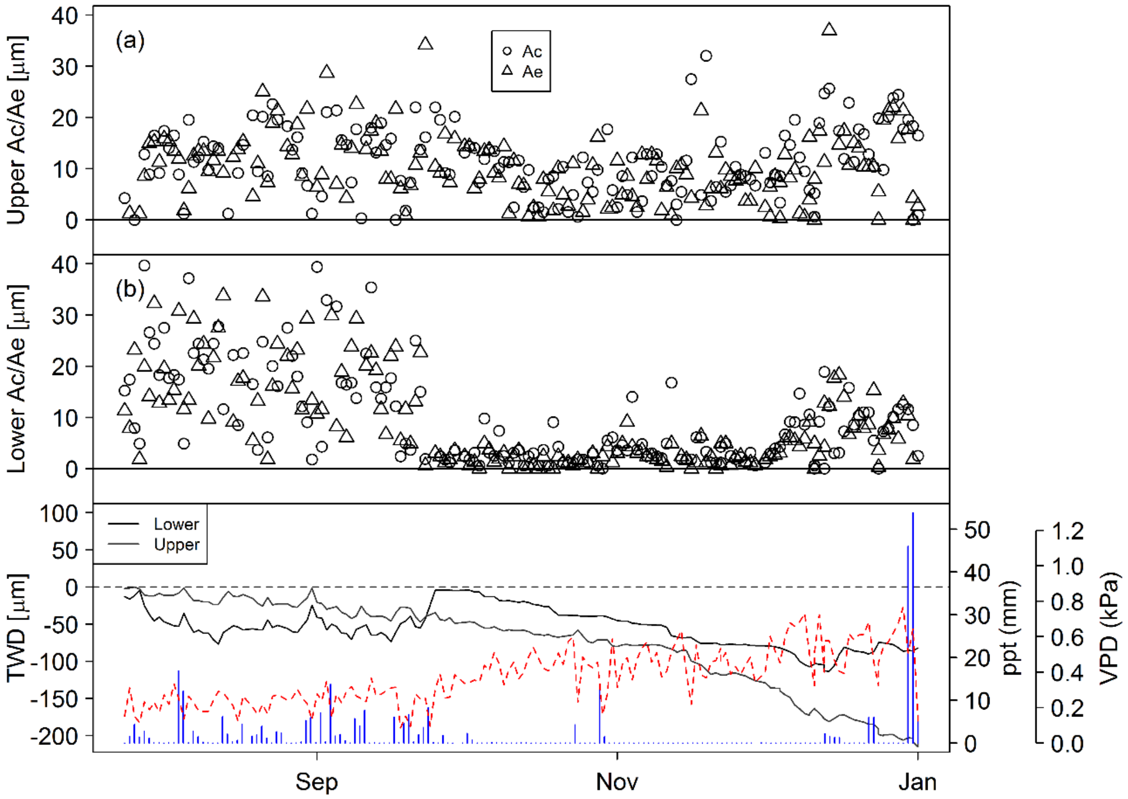
2.8 Supplementary Information



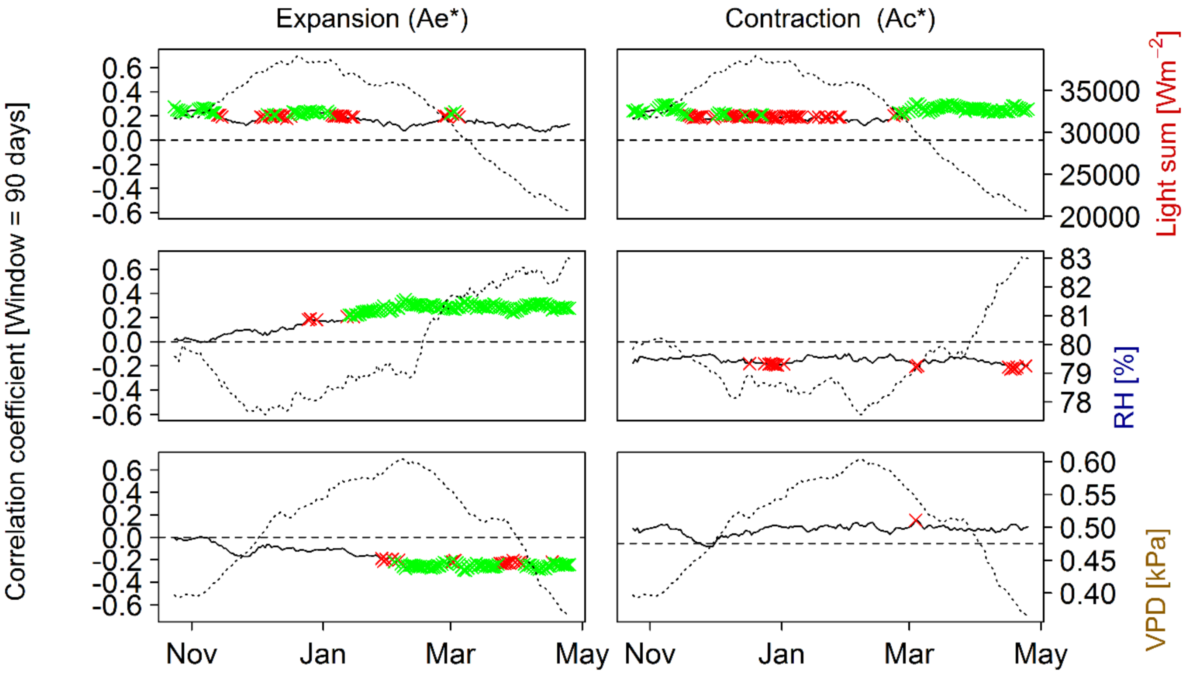
Supplementary Figure 2.1 Weather conditions for study Period I (Stage I, September 2014 – June 2015) and study Period II (Stage II, July 2015- December 2015) for 10-minute resolution light, vapour pressure deficit (VPD), soil water potential (swp) and daily precipitation sums (ppt).



Supplementary Figure 2.2 Parameters of water related stem radius changes at 10 minute intervals detrended for growth signal. Each cycle is defined as the time between a cycle maximum (R_{max}) and the subsequent maximum. Cycles are separated into a contraction phase (in black), which goes from a R_{max} to the next R_{min} and expansion phase (in red), which goes from R_{min} to the next R_{max}. Each cycle is characterized by a contraction amplitude (Ac), expansion amplitude (A_e), contraction duration (Tc), expansion duration (T_e) and total cycle duration (Tcycle). Total Daily change (A_d) is the difference between a cycle maximum and the subsequent maximum (data not shown)



Supplementary Figure 2.3 Effect of precipitation absence during part of October and all of November 2015 on example Tree A upper (a) and lower (b) water-related diel stem expansion (Ae, triangles) and contraction (Ac, circles) amplitudes, and the corresponding water related stem radius courses (TWD) for upper (light grey) and lower stem (bark), daily precipitation sum and vapour pressure deficit mean.



Supplementary Figure 2.4 Moving 90 day window Kendal correlations for standardized expansion amplitude (Ae*) and contraction amplitude (Ac*) for all tiers in all three trees of Period I (July 2015 - December 2015) to 90 day moving averages of daily light sum (red line, a-b), daily mean relative humidity (RH, blue line, c-d) and daily mean vapour pressure deficit (VPD , ochre line, e-f). Red indicates significant correlation at p-value<0.1 and green indicates significant correlation at p-value<0.05

Chapter 3 - Catching the swell: Daytime stem expansion and seasonal reversal in the peristaltic wave of stored water use along the stem of *Avicennia marina* (Forssk.) Vierh

"The western wave was all a-flame.
The day was well-nigh done!
Almost upon the western wave
Rested the broad bright Sun;
When that strange shape drove suddenly
Betwixt us and the Sun"
Samuel T. Coleridge - The Rime of the Ancient Mariner (text of 1834)

An adapted version of this Chapter resulted in the following publication:

Donnellan, A., Zweifel, R., Cusens, J., Leuzinger, S. 2018. Daytime stem swelling and seasonal reversal in the peristaltic depletion of stored water along the stem of *Avicennia marina* (Forssk.) Vierh. Tree Physiology **00**:1-4

3.1 Abstract

Diurnal courses of stem radial water dynamics represent the sum of all internal and external conditions affecting tree water relations. Changes in stem radius due to early morning water depletion and night time refilling of storage tissues are generally well documented. This study seeks to understand the unusual daytime refilling of stem elastic storage tissues present in the mangrove species *Avicennia marina* (Forssk.) Vierh, which deviates from our traditional understanding of stem hydraulics in terrestrial trees. I explored the relationship of this pattern with other water-related physiological processes and environmental variables, and investigated the seasonal changes in the timing and time lags of peak swelling at different stem heights, in order to understand the "peristaltic" depletion of internally stored water. Our findings show that daytime stem swelling occurs year-round, even on days when leaf water potentials dropped to values lower than -4 MPa. The diel courses of stem swelling were positively correlated with light more often than with measures of water availability in air and soil. There was also a clear seasonal reversal in the timing and direction of the "peristaltic" depletion of water along the stem, with an earlier onset of shrinking in the upper (median = 10:00 h) than in the lower stem (median = 12:00 h) in winter, but an earlier onset of shrinking in the lower (median=08:00 h) than in the upper stem (median=11:00 h) in summer. This time lag was closely correlated with daily temperature and VPD, with a clear switch in the direction of peristaltic stem shrinking at the start of the growing season. I propose that sugar loading/unloading and changes in source-sink activity play a role in the endogenous osmotic adjustment responsible for daytime stem swelling and the seasonal switch in the direction of peristaltic water storage depletion in *A. marina*.

3.2 Introduction

Plants are in a permanent battle against water loss, fighting to maintain their tissues in an adequate state of hydration. Water comprises anything between 70 to 90% of the mass of non-woody tissues, such as leaves and fruits. Keeping a positive water balance is essential both at the cellular level, for maintaining metabolic function, cell turgor and cell growth, and at the whole plant level, for maintaining structural integrity and the transport of solutes (Lambers et al. 2008).

The water status of plants is a fine balance between soil water supply and atmospheric demand, an exchange which is tightly regulated in order to maintain a positive water balance between pools and fluxes (Hsiao and Acevedo 1974, Tilman 1982). One of the main water fluxes in plants is vertical sap flow, where water travels from the roots to the leaves and other organs through the xylem (Steppe et al. 2015b). Only about 1% of water taken up by roots is incorporated into plant biomass, and the rest is eventually lost to the atmosphere in the form of transpiration (Lambers et al. 2008). On an average sunny day, as the sun comes up, plants begin to lose water from leaves and sap-flow starts. This characteristic loss of water from leaves and initiation of transpiration can be observed through a drop in water potential in leaves and xylem, where water potential is a measure of the chemical potential of the water in those compartments in reference to pure water. Water loss through leaves is controlled in part through the stomata, which can actively regulate transpirational water loss and thus plant water uptake, flux and storage (Buckley 2005). Regulation of stomatal aperture can help protect from cavitation and maintain tissue and leaf turgor (Zweifel et al. 2007, Schachtman and Goodger 2008, Dodd 2013, Pantin et al. 2013, Merilo et al. 2014).

Another important flux of water in the plant is the radial movement of water to and from storage tissues in the stem, which can help relieve excessive hydrostatic tension in the xylem and leaves in times of high transpiration demand or low soil water availability (Zweifel et al. 2001, Steppe et al. 2006, De Swaef et al. 2015). These elastic storage tissues are usually comprised of the outer parenchyma tissues, elastic phloem tissues of the bark and elastic sapwood such as newly formed xylem (De Schepper et al. 2012, Zweifel et al. 2014, Pfautsch et al. 2015a). Depending on evaporative demand, soil water availability and the state of internal stores, stored water can contribute significantly to the daily transpiration stream (Herzog et al. 1995, Zweifel et al. 2000, Zweifel et al. 2001, Zweifel et al. 2005, Steppe et al. 2006, Cermak et al. 2007, Devine and Harrington 2011, De Schepper et al. 2012, Belien et al. 2014, Oliva Carrasco et al. 2015, Urrutia-Jalabert et al. 2015, Sallo et al. 2017). These movements of water cause volume changes in the stem which can be measured with automated point dendrometers (Drew and Downes 2009, De Swaef et al. 2015). Common patterns of stem radius change (SRC) recorded in the literature show stem shrinkage during the day, which results from water moving out of elastic water stores and into the xylem, a movement caused by the drop in xylem water potential at the initiation of transpiration. At night, when transpiration is reduced, an increase of water potential in the xylem allows for the refilling of elastic water stores, resulting in stem expansion. The timing of these processes is important, as it has a strong correlation with the timing of growth, which relies on adequate turgor in cambium tissues (Pfautsch et al. 2015a, Pfautsch 2016, Mencuccini et al. 2017, Coussement et al. 2018).

However, recent studies show a higher diversity of stem water storage patterns than previously thought (Zweifel et al. 2014, Pfautsch et al. 2015a). Work on mangrove water and carbon relations has shown unusual courses of daily stem diameter fluctuations (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b). Instead of the common pattern of daytime shrinking and night time refilling of stem water stores, they observed early morning stem swelling and delayed stem shrinkage. My results in Chapter two confirm these findings for *A. marina*, as peak stem radius was on average at 11:00. This kind of pattern deviates from traditionally studied terrestrial trees which have historically shaped our understanding of SRC, although daytime whole stem swelling has been observed before in commonly studied species e.g. in tomato plants (De Swaef et al. 2013). This unusual daytime stem swelling is thought to be the result of an osmotically driven change in storage tissue water potential, which causes water to flow into the storage tissues at times of high transpiration (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b). The osmotic component of tissue water potential (ψ_{Π}) is always negative, and becomes increasingly more negative at higher solute concentrations. Osmotic changes in storage tissues are in part due to carbon loading and unloading processes in the phloem and these processes are thought to contribute significantly to daily SRC (De Schepper and Steppe 2010, Sevanto et al. 2011, De Swaef et al. 2013, Mencuccini et al. 2013, Chan et al. 2016). Thus, a deeper knowledge of daytime refilling in *A. marina* will not only help us understand how mangroves regulate their water balance, but might also shed light on the contribution of osmotic processes to stem water storage dynamics and alternative mechanisms plants use to ensure turgor and growth (Mencuccini 2017).

In this study I seek to understand the unusual daytime refilling and delayed shrinking of stem elastic water storage tissues in *A. marina*, a pattern which deviates from most terrestrial trees and is hypothetically due to endogenous osmotic adjustment. First, I characterize the diurnal and seasonal relationship of this pattern with other water-related physiological processes such as leaf water potential, stomatal conductance, leaf turgor and sap flow. Second, I analyse the seasonal changes in the timing of peak swelling and shrinking episodes at different stem heights. As I hypothesized that sugar loading plays a role in endogenous osmotic adjustment, I expected daytime swelling to be influenced by proximity to the crown. In order to understand the variation in the direction of this “peristaltic” depletion of internally stored water, I investigate the time lags of peak stem swelling between upper and lower tiers and its relationship to environmental variables. I hypothesise that time lags in swelling episodes along the stem are not driven solely by environmental indicators of water availability (soil water potential, vapour pressure deficit), but also by drivers related to source-sink dynamics.

3.3 Material and Methods

3.3.1 Site description

The study site is located in the Mangawhai estuary, northern New Zealand (36.097°S, 174.573°E), described in full in Chapter 1 and Chapter 2. The study site is located in the upper tidal zone of Tara Creek, furthest from the shore with sandy substrate and short inundation times twice every 24 hours. Annual weather trends during the study period (March 2014 - March 2015) followed patterns typical to subtropical climate. Daily mean relative humidity ranged from 37.68% to 100%, with a mean of 80.8%. Annual temperature extremes ranged from -1.13°C to 29.4°C with a seasonal mean of 16.64°C. Total precipitation sum was 920.9 mm. Soil water potential (SWP) ranged over the whole measuring period from -5 MPa to -1.5 MPa, with a mean of -2.5 MPa. The salinity was measured on three occasions through the year (September 2014, December 2014 and March 2015) in soil pore water at a soil depth of 30cm and ranged from 20‰ to 39‰. Water table depth ranged from -7.0 cm below ground level to +74.4 cm above ground level during tidal inundation (Appendix Figure 1).

3.3.2 Environmental data

A weather station was installed within the canopy in order to explain plant physiological responses to microclimate which is explained in detail in Chapter 1 and Chapter 1. A probe installed within the canopy logged temperature (T, °C) and relative humidity (RH [%]) (Model SHT, Sensirion, Stäfa, Switzerland). Vapour pressure deficit (VPD [kPa]) was calculated from temperature and RH data according to Buck (1981). Solar irradiance (I [W m^{-2}], 380–1120 nm) was measured with a pyranometer (Model PYR, Decagon Devices, WA, USA). Rainfall (PPT [mm]) was measured with a tipping-bucket rain gauge (Model 52202, R. M. Young Company, Traverse City, Michigan, USA). Tidal inundation (WD [cm]), soil-water temperature (SWT [°C]) and soil-water electrical conductivity (EC [µS/cm]) were measured with a CTD sensor (CTD-10, Decagon Devices, WA, USA) fitted into a well dug into the sediment at a depth of 1m in tubing that allowed water flow. Osmotic potential (Ψ_{Π} [MPa]) of the soil and water was calculated using EC and WT following McIntyre (1980). All instruments were logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland). There were two power failures, one from 1 May till 1 June and another from 10 August to 7 September 2014 during which the meteorological data were not collected. Since physiological measurements were not affected by the first power shortage (see *Physiological measurements*) the gap in meteorological measurements of this period was filled in with data from a nearby weather station (<10km).

3.3.3 Physiological measurements

Three mature co-dominant trees of similar height were chosen for continuous physiological measurements (March 2014 - March 2015). Point dendrometers measured stem radius change (SRC) at a µm resolution. These are non-invasive small pistons pressed against the stem connected to a potentiometer, mounted on carbon fibre frames optimized for temperature insensitivity (ZN11-O-WP, Natkon, Oetwil am See, Switzerland). Three dendrometers were installed on each of the three trees, evenly spaced along the stem, the first 70cm above ground level and the third below branching of the crown (190-260 cm). These are referred to as ‘Lower’, ‘Middle’ and ‘Upper’, respectively. Sap flow rate was measured using heat balance gauges (Dynamax, Houston, TX, USA), based on the heat balance method (HB) (Smith and Allen 1996). Two sapflow sensors were installed in each of the three study trees in terminal branches due to limitations set by sensor diameter. All instruments logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland). The power shortage from 10 August to 7 September 2014 led to missing data for these physiological measurements. Turgor pressure probes were also installed with a separate logging system (YARA ZIM Plant Technology, Germany). These are non-invasive probes that measure leaf turgor in real-time (Zimmermann et al. 2008). Briefly, they are a pair of magnets, one of which contains a pressure sensor that applies a constant clamp pressure to the leaf, where the output pressure (*Pp* [kPa]) is inversely proportional to leaf turgor pressure. The procedure for validating and calculating leaf balancing pressure from turgor pressure probes has been described elsewhere (Zimmermann et al. 2008, Bader 2014). Three of these probes were installed in each of the three study trees, dispersed amongst the upper and lower canopy. Water potential (ψ_{leaf} [MPa]) and stomatal conductance (g_s [mmol^{-1}m^{-2}s^{-1}]) measurements were taken during winter (27 May, 4-6 August 2014) and summer (11, 16-17 February 2015) in five trees in the immediate vicinity (< 20 m) of the three main study trees, to avoid study tree denudation. Leaf water potential was measured with a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) following the usual procedure of measuring leaves immediately after excision (Scholander, Hemmingsen et al. 1964). Three to five leaves were measured then averaged per recording and tree. This was done for each tree every hour from pre-dawn until midday (winter) or until dusk (summer). Stomatal conductance was obtained via a steady state porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA) from early morning until evening.

3.3.4 Calculating water induced stem shrinkage and expansion

Stem radius measurements obtained from dendrometers were cleared of jumps and spikes via the procedure described in Chapter 2. Time series of SRC were detrended for growth according to Zweifel (2016) (Chapter 2) resulting in tree water deficit (TWD or ΔW). Thus TWD series show increasingly negative values with increasingly dehydrated stems in reference to full hydration, which is 0. By detrending the dendrometer data for growth, I assume the resulting time series of TWD represents only water related shrinking and swelling of the stem, although the method has its limitations (Zweifel et al. 2016, Mencuccini et al. 2017). The numerical approach to determine duration and amplitude of water related shrinking and swelling of storage tissues was the same as detailed in Chapter 2. Briefly, each circadian cycle of stem oscillations was broken into an amplitude and duration of swelling (stem expansion, $A_{e,}$ T_e) and an amplitude and duration of shrinking (stem contraction, $A_{c,}$ T_c), and their respective the times of day (Table 3.1). A custom function for this analysis was written in *R* software (R Core Team 2017).

3.3.5 Statistical analysis

Wilcoxon Signed-Ranks tests (paired) were used to detect differences in the time of day R_{max} and R_{min} were reached at different trunk tiers (Lower, Middle and Upper) annually and during different seasons (annual, summer and winter). The interquartile range (IQR) is reported for non-normally distributed data instead of the standard deviation.

The time lag in stem swelling peaks between upper and lower tiers (R_{max} *lag*) was calculated as the duration in hours between R_{max} in the upper tier and R_{max} in the lower tier. I applied linear regressions and Kendal tau-b correlations to determine the relationship between $R_{max,}$ *lag* and environmental variables (daily means of temperature, SWP, RH and VPD, daily sums of precipitation, radiation and ET). Additionally, I fitted a linear mixed-effects model (LMM) using the *lme* function in *R* (Pinheiro and Bates 2000). Because of the high collinearity between predictor variables, I used the Kendal correlations to inform the model and eliminated variables that were highly collinear (Zuur et al. 2009). Tree was considered a random factor. Temporal autocorrelation was accounted for by introducing an autoregressive (AR1) parameter φ, using days since the start of the measuring period (Pinheiro and Bates 2000). All analyses were conducted using the free software package *R* (R Core Team, 2017).

Table 3.1 Abreviations used in text and figures for physiological variables studied

Symbol	**Description**	**Units**
ΔW/TWD	Tree Water Deficit, representing water related shrinking and swelling in the stem, calculated according to Zweifel et al. (2016)	mm
Ψleaf Ψpd, ψmd	Plant leaf water potential, at predawn and midday	MPa
gs	Stomatal conductance	mmol $m^{-2}s^{-1}$
Pp	Balancing pressure measured by turgor probes, inversely proportional to leaf turgor	kPa
Ae	Amplitude of the expansion phase in a circadian cycle of ΔW	mm
Ac	Amplitude of the shrinkage phase in a circadian cycle of ΔW	mm
Te	Duration of the expansion phase in a circadian cycle of ΔW	min
Tc	Duration of the shrinkage phase in a circadian cycle of ΔW	min
Rmax	Maximum ΔW reached during a circadian cycle	mm
Rmin	Minimum ΔW reached during a circadian cycle	mm
Rmax lag	Time lag between the upper tier of the tree daily water related stem radius max and the lower tier daily stem radius max.	hours

3.4 Results

3.4.1 Daytime swelling in *A. marina* in relation to other water related physiological processes

Daytime stem water refilling was observed in the diurnal cycles taken both in winter (August 2014) and summer (February 2015, Figure 3.1). Winter radial water dynamics showed slow stem refilling towards dawn, with a rapid increase in stem diameter occurring after sunrise (Figure 3.1i, j). The upper tier reached its maxima first (10:00 h), followed by the lower tier (12:00 h). After an initial rapid decrease stems reached a first minimum at 15:00 h, after which slow refilling started. In summer, stem diameter remained constant during night time, with water related swelling starting just after sunrise, reaching daily maxima first in the lower tiers (10:00 h) and then in the upper tiers (12:00 h) before decreasing steeply again. The initial steep decrease reached a minimum around solar noon (13:30 h) for the lower tiers and after midnight in the upper tiers (Figure 3.1e).

Diurnal courses of ψleaf in summer and winter showed patterns similar to those commonly recorded for terrestrial trees. I observed a gradual ψleaf decline after sunrise, which became steep, reaching its minimum around the same time at which peak stem swelling was reached, at solar noon in winter and 1.5 hours before solar soon in summer (Figure 3.1a, b). Multiple ψ_{pd} measurements across the year showed that midday leaf water potentials (ψ_{md}) were on average lower in summer (ψ_{md} = -4.09 ± 0.16 MPa, n = 3) than in winter (ψ_{md} = -3.78 ± 0.26 MPa, n = 4). However, average predawn water potentials (ψ_{pd}) were higher in summer (ψ_{pd} = -0.59 ± 0.30 MPa, n = 3) than in winter (ψ_{pd} = -1.73 ± 0.18 MPa, n = 4). In both summer and winter ψ_{pd} was less negative than measured soil water potential (winter ψ_{soil} = -2.33 ± 1.4 MPa; summer ψ_{soil} = -3.54 ± 0.25 MPa). The seasonal relationship between ψmd and radial stem water flow amplitudes for all measurement points showed increasingly negative ψ_{leaf} were associated with both larger total daily stem swelling (R^2 = 0.5745, p-value = 0.02, n = 9) and shrinking amplitudes (R^2 = 0.436, p-value = 0.05, n = 9). Thus both larger refilling and depletion of water storage reserves seemed to occur during days with low ψmd (Figure 3.2).

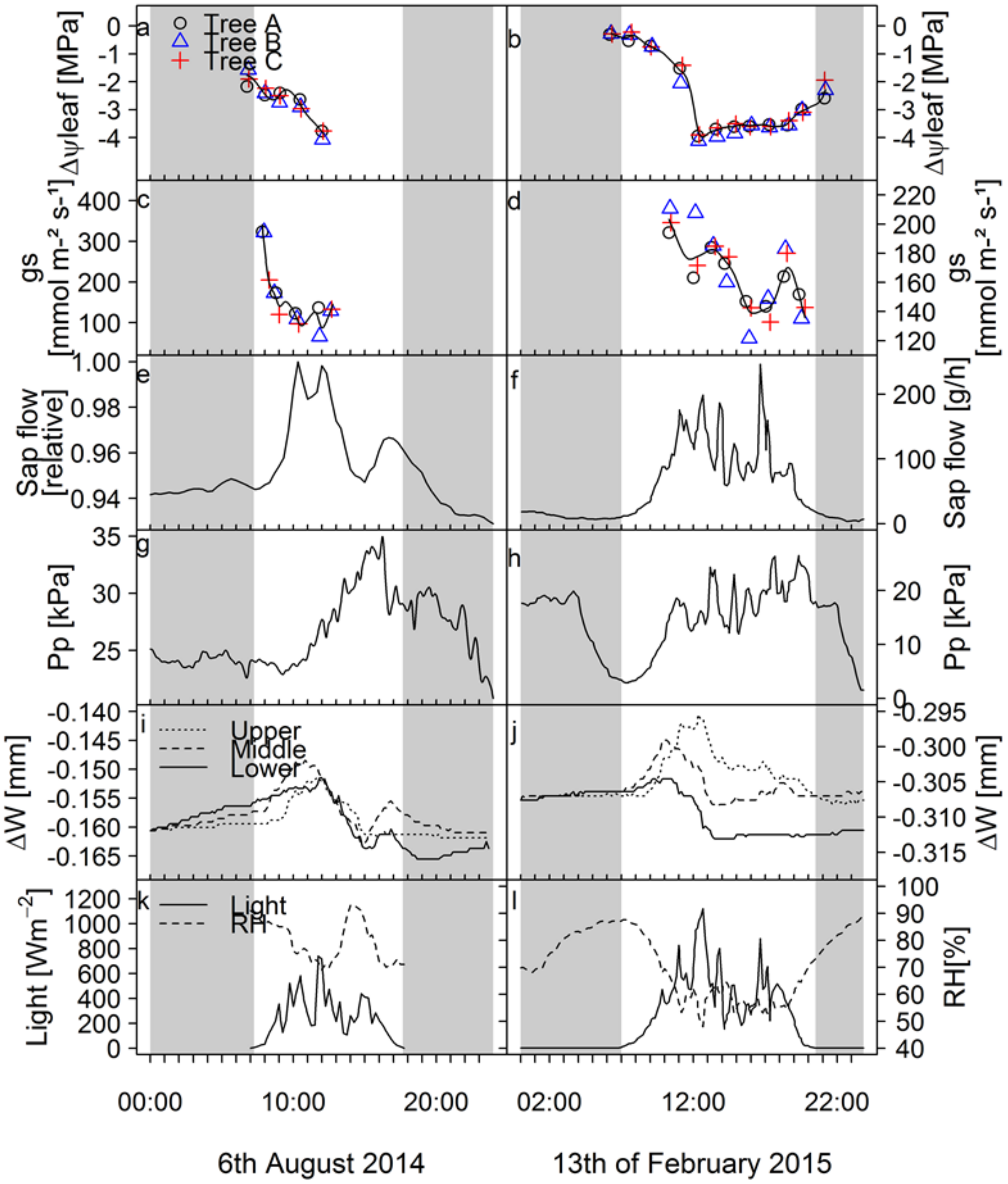


Figure 3.1 Diurnal cycles of physiological parameters of one day in winter (August 6 2014) and summer (February 13 2015) respectively. Leaf water potential (ψleaf, a-b) and stomatal conductance (gs, c-d) are shown for the three tree investigated. Continuous measurements are show for an example tree (Tree 1): sap flow (e-f), balancing pressure (Pp, g-h), water related stem diameter changes (ΔW, i-j; lower, middle and upper trunk correspond to continuous, dashed and dotted lines). Diurnal courses of light and relative humidity are also shown (k-l).

Diel courses of g_s, leaf turgor and sap flow also showed patterns similar to those recorded for terrestrial trees. In summer, g_s showed a decline from morning, but did not reach a minimum until 16:00 h, 3.5 hours after minimum ψ_{leaf} was reached (Figure 3.1d). Overall, P_p and sap flow followed the expected pattern of day-time increase and night time decrease (Figure 3.1e, h). In winter, leaf turgor reached its minima more gradually than in summer, a few hours after minimum stem diameter and ψ_{leaf}. In summer, leaf turgor decreased rapidly after dawn, reaching its first minima just before solar noon as lower stem tiers started to shrink, and second highest minima at 17:00 h, just after g_s started its evening recovery (Figure 3.1e, h).

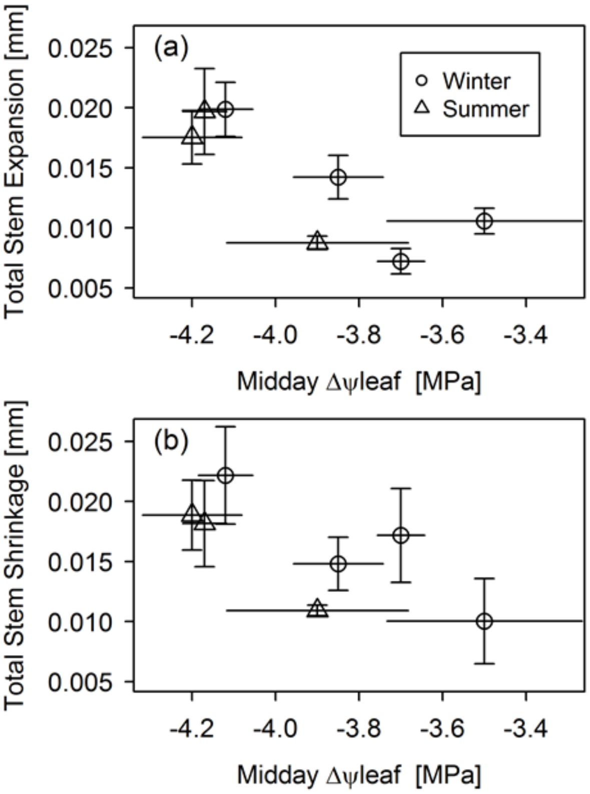


Figure 3.2 Seasonal scale relationship between daily amplitude of water-related stem movements (ΔW) of expansion (a) and shrinkage (b) with leaf water potential at midday (Midday ψ_{leaf}), taken during 7 different days throughout the year in winter and summer (winter: 27 May, 4-6 August 2014; summer: 11, 16-17 February 2015). Bars represent standard error of the mean for the three measurement trees (n=3). There is a significant linear regression between Midday ψ_{leaf} and stem swelling (R^2=0.57, p-value= 0.02) and shrinking amplitudes (R^2 =0.44, p-value = 0.05).

3.4.2 Seasonal variation in the timing of R_{max} and R_{min} at different stem heights and environmental drivers of R_{max} lag

When looking at the timing of stem water storage across all seasons, peak stem swelling occurred most frequently between sunrise and midday (Figure 3.3). Lower areas of the trunk furthest from the crown peaked first (lower tier: median = 10:00 h, IQR = 4 h; middle tier: median = 10:00 h, IQR = 3 h), significantly earlier than those closest to the crown, which peaked later in the day (upper tier: median = 11:00 h, IQR = 3 h; p-value ≤ 0.05). Peak shrinking followed a bimodal distribution, with minima clustered around 18:00 h and just after midnight at 01:00 h (median = 17:00 h, IQR = 16 h) with no significant difference between tiers (Figure 3.3).

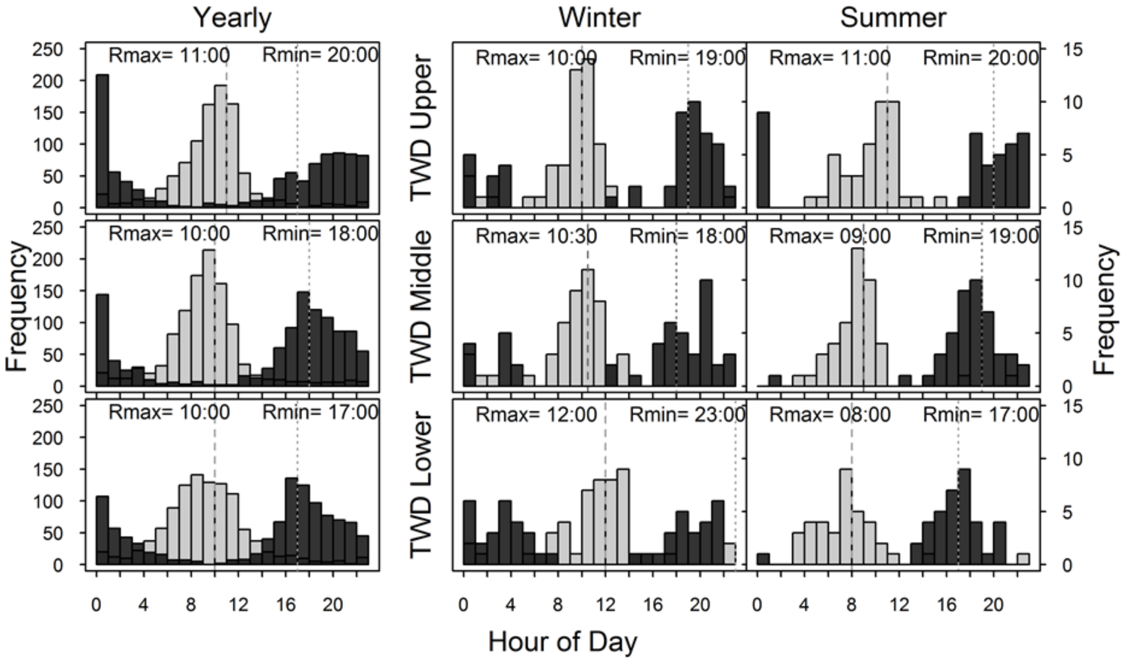


Figure 3.3 Frequency distribution of timing for water related (TWD) stem R_{max} and R_{min} (grey and black bars, respectively) during three periods: yearly (March 2014-March 2015), winter (September 9-23 2014) and summer (January 25 - February 8 2015) and for three heights along the stems (upper, middle lower). The median values of the time of R_{max} and R_{min} water-related stem radial changes are shown by dashed and dotted lines, respectively

However, when comparing summer and winter there was a clear reversal in the timing of peak swelling and shrinking at different stem heights (Figure 3.3

& Figure 3.4). A Wilcoxon Signed-Rank test on differences in peak times between tiers showed that during summer the pattern followed the overall annual trend, where the lower areas of the trunk closest to the soil peaked significantly earlier than the areas closest to the crown (lower tier: median= 8:00 h, IQR = 3.7 h; middle tier: median = 9:00 h, IQR = 2 h; upper tier: median = 11:00 h, IQR = 3 h; p-value ≤ 0.01). In winter however, this pattern reversed and the lower trunks reached their expansion maxima after the upper tiers (lower tier: median = 12:00 h, IQR = 2 h; middle tier: median = 10:30 h, IQR = 2.5 h; upper tier: median = 10:00 h, IQR = 2.2 h; p-value ≤ 0.01) (Figure 3.3 & Figure 3.4). Thus, on an average summer day, upper stems swelled almost until midday, and the coefficient

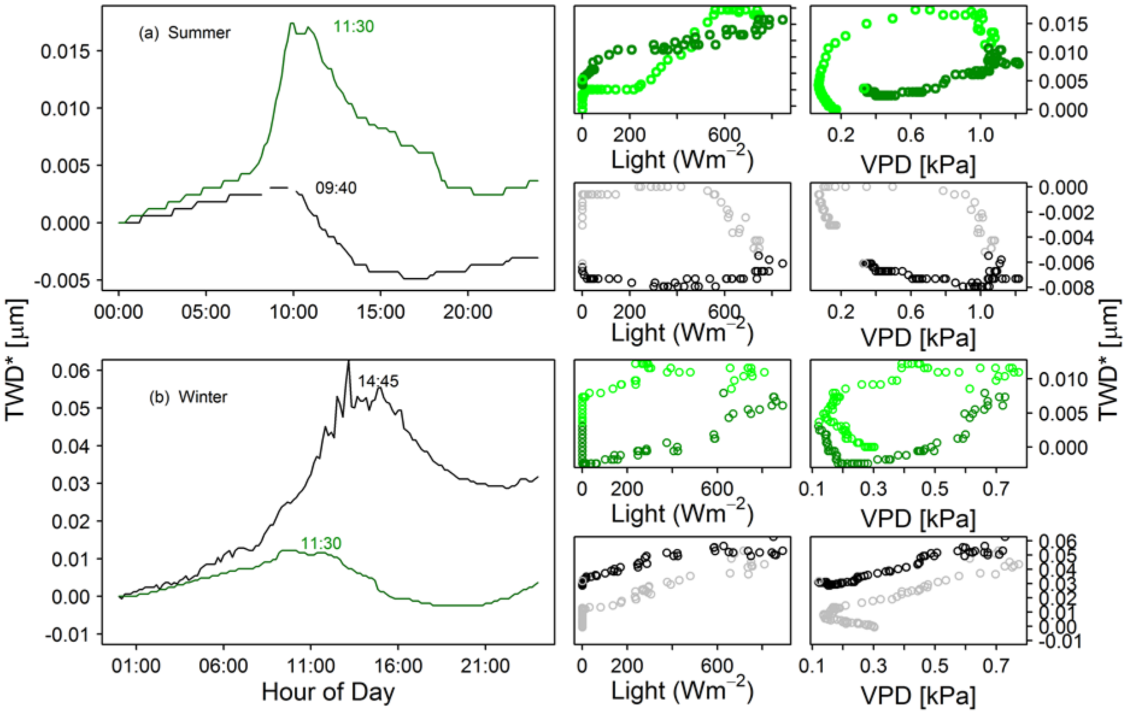


Figure 3.4 Example diel course for Tree A showing the change in timing of upper (green lines and green dots) and lower stem (black lines and black and grey dots) peak TWD* (tree water deficit standardized to start at 0 for comparison) from summer (a) to winter (b). Small right panels show correlation of 10-minute TWD* with light and VPD for upper (green) and lower stem (black), showing the hysteresis between values before midday (light green for upper stem and grey for lower) and after midday (dark green for upper stem and black for lower stem.

of determination in a linear regression between TWD and light was higher in the upper than lower tiers (Upper: $R^2 = 0.69$, p-value ≤ 0.001; Lower: $R^2 = 0.05$, p-value ≤ 0.05), whilst in winter this trend was reversed (Upper: $R^2 = 0.24$, p-value ≤ 0.05 ; Lower: $R^2 = 0.525$, p-value ≤ 0.001) (Figure 3.4).

Timing of peak shrinking in summer followed a similar pattern to peak swelling times, with minima reached significantly earlier by the lower tiers of the trunk, which had started declining first (lower tier: median = 17:00 h, $p \leq 0.05$), followed by the tiers closest to the crown (upper tier: median = 20:00, $p \leq 0.05$). Winter differences in R_{min} time were not significant between tiers, although there was a marked difference in the distribution between the lower and upper tiers, with upper tiers unimodally reaching their minima around 19:00 and lower tiers having a bimodal distribution, reaching their minima either around 20:00 or after midnight (Figure 3.3).

The reversal in the direction of the peristaltic swelling and shrinking along the stem was visible across the whole year when looking at the R_{max} time lag between upper and lower stem tiers (Figure 3.5). From the start of the measuring period until the onset of winter in late May the upper tiers peaked later than the lower tiers (R_{max} *lag* > 0). Between June and November R_{max} *lag* became negative more often, indicating that the upper tiers reached their maxima before the lower tiers. This trend then reversed again towards late November until the end of the measuring period. The start in the shift in R_{max} *lag* from negative to positive values began concurrently to the initiation of stem growth in November (Figure 3.6). Monthly mean R_{max} *lag* was significantly correlated in a linear regression with mean monthly VPD ($R^2 = 0.5$, p-value$\leq$0.01) and temperature ($R^2 = 0.63$, p-value$\leq$0.01) (Figure 3.4). Kendal tau-b correlations and results from a linear mixed effects model (LMM) performed on diurnal-scale values confirmed the significant relationship between temperature, VPD and *R_{max}* *lag* (Table 3.2). The LMM also detected precipitation as a significant driver of *R_{max}* *lag* (Table 3.2). Vapour pressure deficit was excluded from the final model due to the high correlation with both temperature and RH.

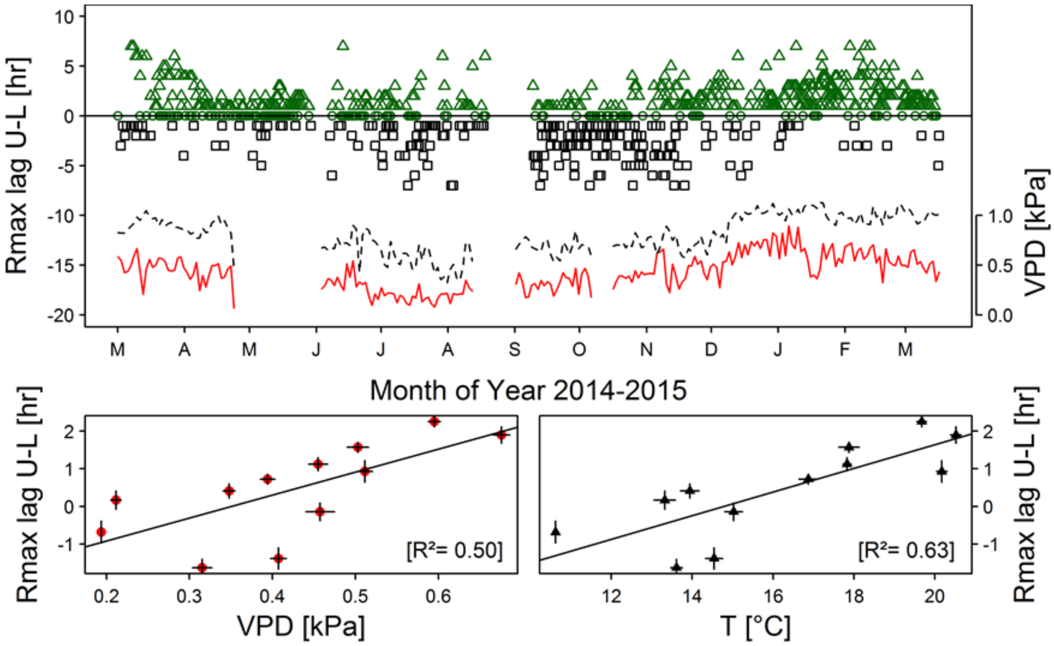


Figure 3.5 (a) Yearly course of the time lag between upper and lower trunk peak water related swelling (*R_{max} lag U-L*) for all three trees investigated. Dashed and solid lines show the daily mean VPD and temperature. Negative time lags (when upper tiers peak before lower) are in black squares, positive time lags (when lower tiers peak before upper) are in green triangles. Correlation between *R_{max} lag* and daily mean VPD (b) and Temperature (c) for monthly mean values with lines representing the standard error of the mean. A highly significant relationship was found in both cases for linear regression ($p \leq 0.001$) and for Kendal correlations and LMM (Table 3.2).

Table 3.2 Relationship between daily mean environmental variables and the daily time lag between upper and lower peak stem swelling (*R_{max} lag*) showing Kendall correlations and description of linear mixed-effects model results (LMM) performed on daily scale data. Variables showing Ø were excluded from LMM due to high collinearity with other variables. Stars indicate level of significance of p-values (*≤0.01, **≤0.001, ***≤0.0001) and n.s. indicates non-significant correlation.

Variable	Kendall correlation ±SE (p-value)	LMM		
		Fixed effect ± SE (p-values)	Random effects SD	Autoregressive parameter φ
T	0.21 (≤0.01)*	0.13±0.035 (≤0.0001)***	0.74	0.46
RH	-0.09 (n.s)	-0.028±0.019 (n.s)		
Light	0.08 (n.s)	1.64.10-5±0.94.10-5(0.08)		
Depth	-0.11 (n.s)	-0.002 ±0.001 (n.s)		
PPT	-0.15 (n.s)	-0.026 ± 0.01 (0.01) *		
SWP	-0.05 (n.s)	-1.28 ± 0.76 (n.s)		
VPD	0.19 (n.s)	Ø'		
ET	0.12 (n.s)	Ø		

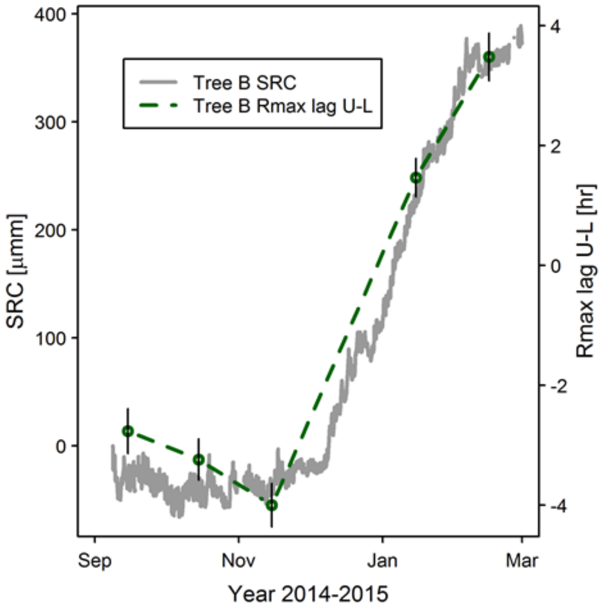


Figure 3.6 Example of stem radius change (SRC) (grey line) during the shift in the time-lag between peak stem radius of upper to lower tree tier (R_{max} lag U-L) (dashed green line) for study Tree B. The beginning of an increase in R_{max} lag U-L (start in the peristaltic direction switch) coincides with the initiation of stem growth at the start of the growing season in November 2014.

3.5 Discussion

3.5.1 Daytime swelling in *A. marina* in relation to other water related physiological processes

Diurnal courses of stem radial water movements are an increasingly well understood component of plant water relations. Isolating water from growth-related components of stem radial change, a far from trivial task, allows for an understanding of the sum of all internal and external conditions affecting tree water relations (Zweifel et al. 2001, Pfautsch et al. 2015a, Steppe et al. 2015a) On a sunny day and with good water supply, observations and plant models of terrestrial species indicate that water flows from elastic tissues (living cells storing water) to xylem conduits. This occurs when xylem water potential is reduced due to transpiration exceeding root water uptake, resulting in early morning stem shrinkage (Zweifel et al. 2000, Zweifel et al. 2001, Sevanto et al. 2002, Deslauriers et al. 2003, Fereres and Goldhamer 2003, Steppe et al. 2006, Cermak et al. 2007, Devine and Harrington 2011, De Schepper et al. 2012, Ehrenberger et al. 2012, Belien et al. 2014, De Swaef et al. 2015)

My findings of diel stem courses deviate from this pattern and confirm previous observations of unusual daytime swelling in the stems of *A. marina* (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b), adding to an increasing body of observations highlighting the diversity of water storage mobilization patterns and strategies (Mencuccini et al. 2017 and references therein). Stem swelling well into the daytime and delayed onset of shrinking occurred despite other diurnal measurements showing patterns commonly observed in terrestrial trees. In *A. marina* leaf water potential dropped and sap flow increased after dawn, with stems still swelling whilst leaf water potentials reached midday lows of down to -4.2 MPa (Figure 3.1). Water flow into storage tissues during this time must be due to a change in inner bark osmotic concentration in the morning, which compensates for the high xylem tensions, a mechanism which was successfully modelled by Vandegehuchte et al. (2014b). Such osmoregulation in storage tissues would allow the plant to maintain turgor necessary for metabolic function, cell division and cell expansion, and hence growth (Krauss and Ball 2012). Although *A. marina* is known to exclude over 90% of salt from the transpiration stream at their roots (Reef and Lovelock 2015), xylem sap still has a high osmotic potential and osmoregulation of xylem and elastic storage tissues during the daytime may help withstand the low water potentials occurring in the xylem at the onset of transpiration (Ball 1988, Flowers and Colmer 2008, Wang et al. 2011, Reef et al. 2012, Lopez-Portillo et al. 2014, Vandegehuchte et al. 2014b). Previous studies have proposed that changes in inner bark osmotic concentration could be related to phloem loading, transport and unloading of sugars (De Schepper and Steppe 2010, De Swaef et al. 2013, Mencuccini et al. 2013, Chan et al. 2016). There is increasing evidence on the role of soluble sugars in osmoregulation, stem radial changes and turgor maintenance (De Swaef et al. 2013, Deslauriers et al. 2014, Pfautsch et al. 2015b), and these could potentially be playing a role in *A. marina* daytime stem swelling. This is especially plausible considering the importance of internal secondary phloem in the wood structure of *A. marina,* and the large phloem/xylem ratios present in this species (Robert 2011, 2014), with the phloem acting as major water store in the stem (Pfautsch et al. 2015b). Additionally, amplitudes of daytime stem swelling were predominantly driven by a positive correlation with cumulative daily solar radiation, as presented in the correlations in Chapter 2, where higher daily light sums were correlated to higher expansion amplitudes. Thus, if phloem loading plays a role in the osmotic signal behind daytime storage tissue expansion, the positive effect of light on swelling amplitudes makes sense, since cumulative daily solar radiation' is often a proxy for photosynthesis (Chan et al. 2016), which in turn is a proxy for phloem loading. It is worth mentioning that the timing of peak swelling for *A. marina* in this study deviates slightly from Vandegehuchte et al. (2014a), who found earlier peaks. The difference compared to my results may be due to differences in measuring heights along the stem, seasonal differences in the study period, microclimatic differences, interspecific competition, or genetic divergence between Australian and New Zealand mangrove populations.

In my diurnal measurements we see a time lag between minimum ψ_{leaf} and minimum leaf turgor and minimum ψ_{leaf} and g_s, where minimum g_s coincides with minimum leaf turgor and minimum daily stem radius, which all occur hours after minimum ψ_{leaf} was reached (Figure 3.1). Usually when transpiration causes water potential to drop, and phloem loading does not increase to compensate for it, leaf turgor drops and stomata close soon after (Hölttä et al. 2005, Nikinmaa et al. 2014). Keeping stomata open would potentially offer the plant the possibility to keep the turgor pressure high by sugar loading in the bark, maintaining overall shoot water potential close to zero and allowing further photosynthesis and sugar loading to occur, a positive feedback maintaining stomatal turgor and aperture (Nikinmaa et al. 2013b). When the plant is not able to compensate for the negative water potentials with sugar loading the stem starts to shrink and the stomata to close, as observed where minimum leaf turgor, g_s and stem turgor occur several hours after minimum water potential. An upregulated turgor pressure could allow for stem growth during this period of the day which otherwise is inhibited (Zweifel et al 2016, Mencuccini et al 2017). This possibility would back the increasing body of literature which points towards a higher diversity of growth timing than previously thought, going beyond the simple theory of growth determination by patterns of daily water potential in the xylem (Mencuccini et al. 2017 and references therein). Given the complexity of separating growth from osmotic and water related changes in SRC, future research should implement a larger set of models and partitioning methods for separating water and carbon components in *A. marina* , comparing results to those given by the method used in this work, which has its limitations (Zweifel et al. 2016, Mencuccini et al. 2017).

Although ΔW was not closely coupled to leaf water potential on an hourly scale, the negative correlation between total daily shrinking amplitudes and midday water potentials (Figure 3.2) reflect relationships recorded in the literature (e.g. Drew et al. 2011, Lechthaler et al. 2017). Thus, higher shrinking amplitudes indicate larger release of stored water from elastic tissues to alleviate more negative water potentials in the leaves. Interestingly however, there was also a negative correlation between leaf ψ_{md} and daily stem swelling amplitude, indicating more negative ψ_{md} were associated with larger stem water refilling amplitudes. This constitutes an unusual finding, which relates to the tight correlation between swelling and shrinking amplitudes found in Chapter 2. It may be the case that more negative water potentials lead to higher levels of osmoregulation responsible for daytime swelling, and thus explain that lower leaf water potentials are accompanied by higher amplitudes of daytime swelling. If carbohydrates are playing a role in osmoregulation of *A. marina*'s storage tissues, then this process could also be explained by osmoregulation causing stomata to stay open past ψ_{md} drop, as seen in our data, thus increasing sugar loading and osmotic turgor in storage tissues at the expense of lowered ψ_{leaf} (Nikinmaa et al. 2013). Further work using a variety of methods to separate water potential from osmotic and growth induced changes in SRC, coupled with osmolyte measurements (organic and inorganic), would help shed light on the relative importance of osmotic controls on stem radius change and their changing relationship to environmental drivers (Pfautsch et al. 2015a, Chan et al. 2016, Pfautsch 2016, Zweifel et al. 2016, Mencuccini et al. 2017, Paljakka et al. 2017). It is also worth noting that my leaf water potential measurements revealed that there was an important imbalance between measured predawn leaf water potentials both in summer and winter, which in summer ranged around -0.2 MPa whilst soil water potential was -3.5 MPa, implying *A. marina* was making use of alternative freshwater sources during this time (see Chapter 6) (Jiang et al. 2017).

3.5.2 Seasonal reversal of timing in peak water related swelling and shrinking episodes at different stem heights

I detected a clear reversal in the timing and direction of the "peristaltic" swelling and shrinking along the stem between summer and winter by studying the time of peak swelling along the stem. This was visible in the yearly trend of R_{max} *lag* monthly mean values which closely followed monthly mean VPD and temperature. In winter R_{max} *lag* was negative with lower tiers peaking later than upper tiers, and as VPD and temperature increased during summer the upper tiers closest to the crown peaked later in the day than the lowest, and thus R_{max} *lag* became positive. To my knowledge, this is the first time such a seasonal reversal in the direction of the "peristaltic" depletion of water along the stem has been recorded.

The direction of the "peristaltic" movement in winter is consistent with water being drawn first from areas closest to evaporation sites, a pattern in accordance with previous observations (Zweifel et al. 2001, Scholz et al. 2008). However, daytime stem swelling was still present and was strongest in the lower tiers, which only started to shrink on average at 12:00, lagging behind upper stem shrinking and ψ_{leaf}. If sugar loading is an important component of the osmotic component of water potential in the stem behind daytime swelling, R_{max} *lag* pattern and seasonal reversal could be explained in terms of the plant's source/sink activity: as shown by Sevanto et al. (2003), according to the Münch theory (Munch 1930) one should expect the loading of sugars to cause stem shrinking near the sources to lag behind xylem shrinking and water potential. At the same time, sugar unloading into active sinks should increase the coupling between whole stem shrinking and xylem tension near the sinks. The patterns observed during winter could be explained by reduced sugar unloading and increased sugar accumulation in the lower stem due to reduced sink activity (Sevanto et al. 2003, Sevanto et al. 2008, Sevanto et al. 2011, Savage et al. 2016) especially since at the study site most of the biomass is located belowground (Tran et al. 2016), which would cause increasingly negative ψ_{Π} of storage tissues (Figure 3.7). Similarly, upper tiers shrinking relatively earlier in the day and following ψ_{leaf} more closely could be due to weaker osmotically driven turgor signal caused by lower photosynthetic rates in winter (data not shown) and thus reduced sugar loading.

On the other hand, the unusual "peristaltic" direction and midday swelling of areas closest to the crown in summer (Figure 3.7) could be the consequence of the upper stem being strongly influenced by phloem sugar loading near the source, which would maintain storage tissue osmotic turgor (and thus lower ψ_{Π}) longer than areas furthest from the source (De Swaef et al. 2013, Mencuccini et al. 2013, Paljakka et al. 2017). Additionally, the earlier onset of shrinking in lower stem sections (around 8:00) could be due to reduced influence of source sugars and increased sugar unloading near sinks after the start of the growing season. This resulted in stem shrinking in lower tiers in summer to be coupled more closely to shoot water potentials, sap flow and atmospheric demand (Figure 3.1). Although the results in this study do not show xylem shrinkage in *A. marina* directly, it seems reasonable to assume that when the contribution of the osmotic signal to whole stem swelling is weak, stems present earlier onset of shrinking, which follows sap flow and leaf and xylem water potentials more closely - thus a parallel can be drawn with the work of Sevanto et al. (Sevanto et al. 2003, Sevanto et al. 2008). Further work is required to investigate the role of the unusual elastic tissue structure of *A. marina* within this framework, especially the multiple layers of

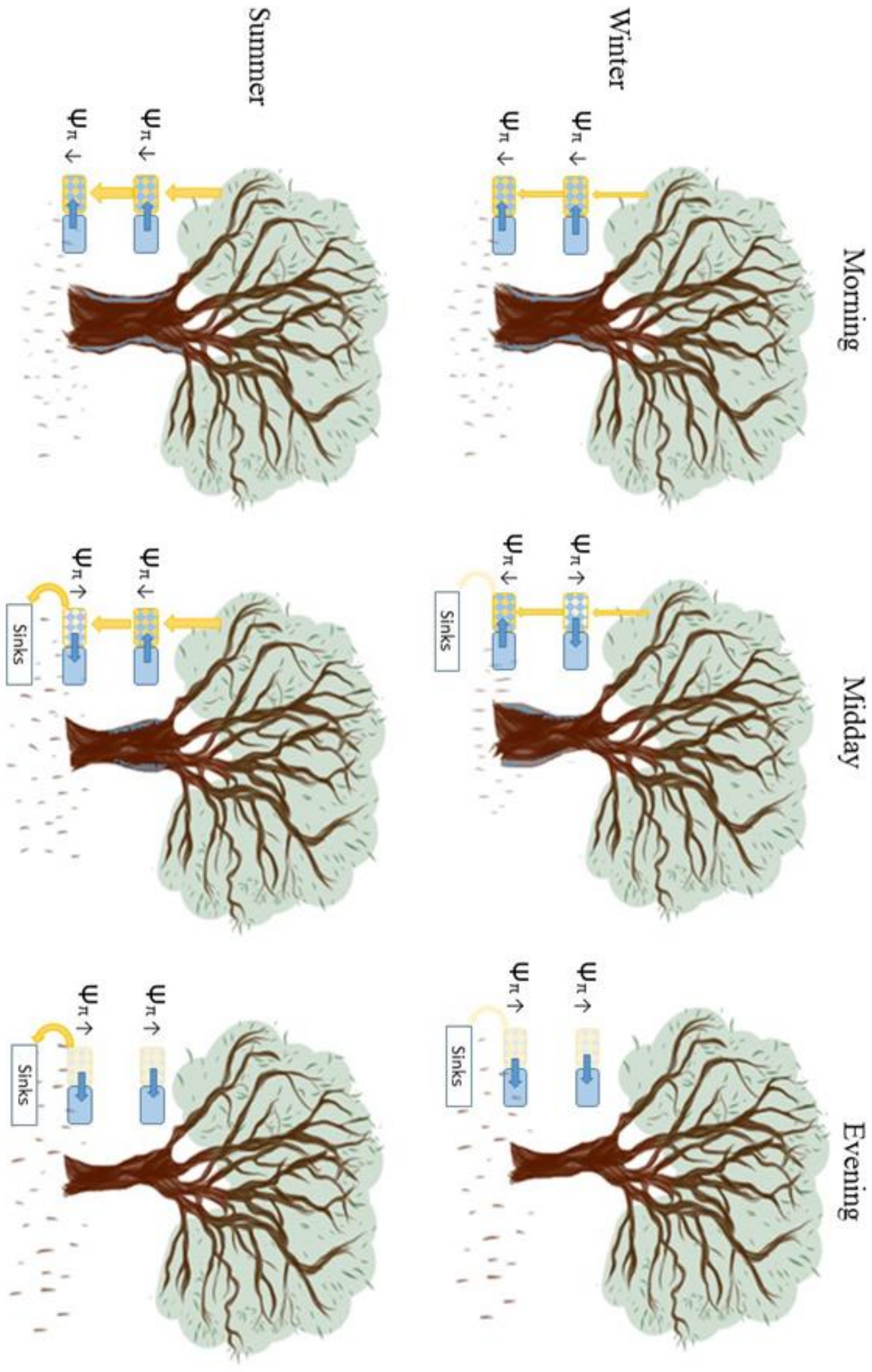


Figure 3.7 Schematic of proposed mechanism driving winter-summer changes in daytime stem swelling and direction of the peristaltic depletion of water from stem storage tissues. Blue boxes represent xylem conduits, whilst diamond boxes represent elastic water storage tissues and the phloem. Blue arrows represent flow of water to and from storage tissues, and presence/absence of blue shading in tree stem represents "swollen"/"depleted" elastic water stores. Yellow arrows indicate hypothesised movement of carbohydrates from sources to sinks, their size proportional to source-sink activity. Sugar loading near sources and unloading near sinks could be partially responsible for the endogenous osmotic regulation, which causes increasingly negative osmotic potential (ψΠ) of storage tissues to draw water from the xylem during the day.

internal secondary phloem (Robert et al. 2011), and confirm the relative contributions of xylem and phloem expansion and contraction to whole stem contraction and expansion, for example by simultaneous measurements on xylem and over-bark stem tissues of *A. marina* (Pfautsch 2015a, Zweifel et al 2014).

The start of a visible shift in R_{max} *lag* at the same time as stem growth start in November (Figure 3.4) and the result of regressions LMMs showing temperature as a major driver of the reversal in R_{max} *lag* provides further backing for the idea of source and sink activity causing delayed shrinking near inactive sinks in winter and in stem areas near the crown in summer. In fact, the timing of R_{max} *lag* becoming positive in late November coincides with the onset of the growing season at the study site, which is known to be between late November and March (Tran et al. 2016, Cusens pers.comm.) and can be seen in the close resemblance between the start of stem growth in raw SRC data and the start of an increasing R_{max} lag U-L (Figure 3.5). The correlation of temperature with photosynthesis, respiration and growth is well documented (Farquhar et al. 1980, Korner 2003), and there is increasing evidence of the relationship between temperature and the osmotic component of SRC (Mencuccini et al. 2013). Chan et al. (2016) found positive correlations of similar magnitude to this study between their modelled estimate of daily osmotic change in elastic tissues to VPD, temperature and light, also linking their results to changes in phloem transport and growth. However, the accumulation of sugars near the source in summertime, a consequence of low water potentials and increased viscosity, could also be responsible for the daytime swelling and delayed onset of shrinking in areas closest to the crown in summer (Savage et al. 2016), which is known to cause interruptions in the downward flow of sugar which can be relieved over-night (Holtta et al. 2006, Knoblauch et al. 2016, Savage et al. 2016).

My data show that time-lags as large as 7h develop between upper and lower stem daytime stem shrinking (Figure 3.5). Changes in the hydraulic conductance of tissues along the stem to the radial water flow through ray parenchyma must play a role in such a notable compartmentation taking place along the stem (Tyree and Zimmermann 2002, Pfautsch et al. 2015a, Pfautsch et al. 2015b). Additionally, one must also consider the role of ions in the xylem, the synthesis and accumulation of other organic compounds and inorganic ions (Popp and Polania 1989, Krauss and Ball 2012, Lopez-Portillo et. al 2014) and the formation of low molecular weight carbohydrates as osmotic adjustors both in the xylem and elastic water storage tissues of *A. marina* (Ball 1988). Further studies should measure the accumulation of soluble sugars, compatible solutes and inorganic osmolites in both the xylem and the phloem to confirm that sugars are indeed the osmolites responsible for the daytime swelling dynamics.

As mentioned previously, the role of the phloem and carbohydrates in producing such notable daytime stem swelling is also plausible when we consider the anatomy of this mangrove species. *Avicennia marina* has successive or multiple cambia, a unique characteristic by which consecutive bands of xylem are interspersed with phloem strands connected by a layer of parenchyma tissue, resulting in very high phloem:xylem ratios (Schmitz et al. 2008, Robert et al. 2011a, Robert et al. 2011b). Santini et al. (2012) found that an increased phloem/xylem ratio in *A. marina* was associated with higher salinity, lower wood density and lower growth rates. It is also thought that increased phloem/xylem ratios may play a role in dealing with water stress through the refilling of embolized xylem vessels, since starch polymerization is postulated to be involved in this process (Nardini et al. 2011, De Baerdemaeker et al. 2017). Thus, it is likely that both the secondary internal phloem and the higher elasticity in storage tissues which this multiple cambia structure confers (Robert 2011a, 2014) explains that phloem loading and unloading processes have such a strong effect on daily stem radial measurements.

3.6 Conclusion

My results show for the first time year-round presence of daytime stem swelling in *Avicennia marina* due to endogenous osmotic adjustment of elastic water storage tissues. With daytime swelling magnitude being driven mostly by light sum and often uncoupled from water availability in air and soil, it seems likely that synthesis of sugars and phloem loading play a part in endogenous osmotic adjustment of storage tissues. My results also show a previously undescribed reversal in the timing and direction of the “peristaltic” depletion of water stores along the stem. This reversal occurred at the onset of the growing season, and I propose changes in phloem loading/unloading due to source-sink activity as the cause. Further research is required to identify the exact mechanisms behind endogenous osmotic adjustment completed with osmolyte measurements, as well as advantages such a trait may confer to growth and survival. Lastly, additional studies are needed to investigate the mechanism and prevalence of changes in the direction of peristaltic water store depletion and replenishment along the stem amongst other species especially those which are underrepresented in tree water hydraulics literature.

3.7 References

Ball, M. C. 1988. Ecophysiology of mangroves. Trees **2**:129-142.

Belien, E., S. Rossi, H. Morin, and A. Deslauriers. 2014. High-resolution analysis of stem radius variations in black spruce Picea mariana (Mill.) BSP subjected to rain exclusion for three summers. Trees-Structure and Function **28**:1257-1265.

Buck, A. L. 1981. New equations for computing vapor-pressure and enhancement factor. Pages 1527-1532 Journal of Applied Meteorology.

Buckley, T. N. 2005. The control of stomata by water balance. New Phytol **168**:275-292.

Cermak, J., J. Kucera, W. L. Bauerle, N. Phillips, and T. M. Hinckley. 2007. Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiology **27**:181-198.

Chan, T., T. Holtta, F. Berninger, H. Makinen, P. Nojd, M. Mencuccini, and E. Nikinmaa. 2016. Separating water-potential induced swelling and shrinking from measured radial stem variations reveals a cambial growth and osmotic concentration signal. Plant Cell Environ **39**:233-244.

Coussement, J. R., T. De Swaef, P. Lootens, I. Roldán-Ruiz, and K. Steppe. 2018. Introducing turgor-driven growth dynamics into functional–structural plant models. Annals of Botany:mcx144-mcx144.

De Baerdemaeker, N. J. F., R. L. Salomon, L. De Roo, and K. Steppe. 2017. Sugars from woody tissue photosynthesis reduce xylem vulnerability to cavitation. New Phytologist **216**:720-727.

De Schepper, V. and K. Steppe. 2010. Development and verification of a water and sugar transport model using measured stem diameter variations. J Exp Bot **61**:2083-2099.

De Schepper, V., D. van Dusschoten, P. Copini, S. Jahnke, and K. Steppe. 2012. MRI links stem water content to stem diameter variations in transpiring trees. J Exp Bot **63**:2645-2653.

De Swaef, T., V. De Schepper, M. W. Vandegehuchte, and K. Steppe. 2015. Stem diameter variations as a versatile research tool in ecophysiology. Tree Physiol **35**:1047-1061.

De Swaef, T., S. M. Driever, L. Van Meulebroek, L. Vanhaecke, L. F. Marcelis, and K. Steppe. 2013. Understanding the effect of carbon status on stem diameter variations. Ann Bot **111**:31-46.

Deslauriers, A., M. Beaulieu, L. Balducci, A. Giovannelli, M. J. Gagnon, and S. Rossi. 2014. Impact of warming and drought on carbon balance related to wood formation in black spruce. Ann Bot **114**:335-345.

Deslauriers, A., H. Morin, C. Urbinati, and M. Carrer. 2003. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees **17**:477-484.

Devine, W. D. and C. A. Harrington. 2011. Factors affecting diurnal stem contraction in young Douglas-fir. Agricultural and Forest Meteorology **151**:414-419.

Dodd. 2013. Abscisic acid and stomatal closure: a hydraulic conductance conundrum? New Phytologist **197**.

Drew, D. M. and G. M. Downes. 2009. The use of precision dendrometers in research on daily stem size and wood property variation: a review. Dendrochronologia **27**:159-172.

Drew, D. M., A. E. Richards, G. M. Downes, G. D. Cook, and P. Baker. 2011. The development of seasonal tree water deficit in Callitris intratropica. Tree Physiol **31**:953-964.

Ehrenberger, W., S. Rüger, R. Fitzke, P. Vollenweider, M. Günthardt-Goerg, T. Kuster, U. Zimmermann, and M. Arend. 2012. Concomitant dendrometer and leaf patch pressure probe measurements reveal the effect of microclimate and soil moisture on diurnal stem water and leaf turgor variations in young oak trees. Functional Plant Biology **39**:297-305.

Farquhar, G. D., S. von Caemmerer, and J. A. Berry. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 plants. Planta **149**:78-90.

Fereres, E. and D. A. Goldhamer. 2003. Suitability of stem diameter variations and water potential as indicators for irrigation scheduling of almond trees. Journal of Horticultural Science & Biotechnology **78**:139-144.

Flowers, T. J. and T. D. Colmer. 2008. Salinity tolerance in halophytes*. New Phytologist **179**:945-963.

Herzog, K. M., R. Häsler, and R. Thum. 1995. Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees **10**:94-101.

Holtta, T., T. Vesala, S. Sevanto, M. Peramaki, and E. Nikinmaa. 2006. Modeling xylem and phloem water flows in trees according to cohesion theory and Munch hypothesis. Trees-Structure and Function **20**:67-78.

Hsiao, T. C. and E. Acevedo. 1974. Plant responses to water deficits, water use efficiency and drought resistance. Agricultural Meteorology **14**: 59--84.

Knoblauch, M., J. Knoblauch, D. L. Mullendore, J. A. Savage, B. A. Babst, S. D. Beecher, A. C. Dodgen, K. H. Jensen, and N. M. Holbrook. 2016. Testing the Munch hypothesis of long distance phloem transport in plants. eLife **5**.

Korner, C. 2003. Carbon limitation in trees. Journal of Ecology **91** 4–17.

Krauss, K. W. and M. C. Ball. 2012. On the halophytic nature of mangroves. Trees **27**:7-11.

Lambers, H., F. S. Chapin III, and T. L. Pons. 2008. Plant Physical Ecology. Second edition. Springer Science+Business Media, New York.

Lopez-Portillo, J., F. W. Ewers, R. Mendez-Alonzo, C. L. Paredes Lopez, G. Angeles, A. L. Alarcon Jimenez, A. L. Lara-Dominguez, and M. D. Torres Barrera. 2014. Dynamic control of osmolality and ionic composition of the xylem sap in two mangrove species. Am J Bot **101**:1013-1022.

McIntyre, D. S. 1980. Basic relationships for salinity evaluation from measurements on soil solution. Autralian Journal of Soil Research **18**:199-206.

Mencuccini, M., T. Holtta, S. Sevanto, and E. Nikinmaa. 2013. Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytol **198**:1143-1154.

Mencuccini, M., Y. Salmon, P. Mitchell, T. Holtta, B. Choat, P. Meir, A. O'Grady, D. Tissue, R. Zweifel, S. Sevanto, and S. Pfautsch. 2017. An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant Cell Environ **40**:290-303.

Merilo, E., I. Joesaar, M. Brosche, and H. Kollist. 2014. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. New Phytol **202**:499-508.

Nardini, A., M. A. Lo Gullo, and S. Salleo. 2011. Refilling embolized xylem conduits: is it a matter of phloem unloading? Plant Sci **180**:604-611.

Nikinmaa, E., T. Holtta, P. Hari, P. Kolari, A. Makela, S. Sevanto, and T. Vesala. 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. Plant Cell and Environment **36**:655-669.

Nikinmaa, E., R. Sievanen, and T. Holtta. 2014. Dynamics of leaf gas exchange, xylem and phloem transport, water potential and carbohydrate concentration in a realistic 3-D model tree crown. Ann Bot **114**:653-666.

Oliva Carrasco, L., S. J. Bucci, D. Di Francescantonio, O. A. Lezcano, P. I. Campanello, F. G. Scholz, S. Rodriguez, N. Madanes, P. M. Cristiano, G. Y. Hao, N. M. Holbrook, and G. Goldstein. 2015. Water storage dynamics in the main stem of subtropical tree species differing in wood density, growth rate and life history traits. Tree Physiol **35**:354-365.

Paljakka, T., T. Jyske, A. Lintunen, H. Aaltonen, E. Nikinmaa, and T. Holtta. 2017. Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies L. Karst). Plant Cell and Environment **40**:2160-2173.

Pantin, F., F. Monnet, D. Jannaud, J. M. Costa, J. Renaud, B. Muller, T. Simonneau, and B. Genty. 2013. The dual effect of abscisic acid on stomata. New Phytol **197**:65-72.

Pfautsch, S. 2016. Hydraulic anatomy and function of trees—basics and critical developments. Current Forestry Reports **2**:236-248.

Pfautsch, S., T. Hölttä, and M. Mencuccini. 2015a. Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology **35**:706-722.

Pfautsch, S., J. Renard, M. G. Tjoelker, and A. Salih. 2015b. Phloem as Capacitor: Radial Transfer of Water into Xylem of Tree Stems Occurs via Symplastic Transport in Ray Parenchyma. Plant Physiology **167**:963-971.

Pinheiro, J. C. and D. M. Bates. 2000. Mixed Effects Model in S and S-Plus. Springer New York.

Reef, R. and C. E. Lovelock. 2015. Regulation of water balance in mangroves. Ann Bot **115**:385-395.

Reef, R., N. Schmitz, B. A. Rogers, M. C. Ball, and C. E. Lovelock. 2012. Differential responses of the mangrove Avicennia marina to salinity and abscisic acid. Functional Plant Biology **39**:1038-1046.

Robert, E. M. R., N. Schmitz, I. Boeren, T. Driessens, K. Herremans, J. De Mey, E. Van de Casteele, H. Beeckman, and N. Koedam. 2011a. Successive Cambia: A Developmental Oddity or an Adaptive Structure? PLOS ONE **6**:e16558.

Robert, E. M. R., N. Schmitz, J. A. Okello, I. Boeren, H. Beeckman, and N. Koedam. 2011b. Mangrove growth rings: fact or fiction? Trees **25**:49-58.

Sallo, F. D., L. Sanches, V. R. D. Dias, R. D. Palacios, and J. D. Nogueira. 2017. Stem water storage dynamics of Vochysia divergens in a seasonally flooded environment. Agricultural and Forest Meteorology **232**:566-575.

Santini, N. S., N. Schmitz, and C. E. Lovelock. 2012. Variation in wood density and anatomy in a widespread mangrove species. Trees **26**:1555-1563.

Savage, J. A., M. J. Clearwater, D. F. Haines, T. Klein, M. Mencuccini, S. Sevanto, R. Turgeon, and C. Zhang. 2016. Allocation, stress tolerance and carbon transport in plants: how does phloem physiology affect plant ecology? Plant Cell Environ **39**:709-725.

Schachtman, D. P. and J. Q. Goodger. 2008. Chemical root to shoot signaling under drought. Trends Plant Sci **13**:281-287.

Schmitz, N., E. M. Robert, A. Verheyden, J. G. Kairo, H. Beeckman, and N. Koedam. 2008. A patchy growth via successive and simultaneous cambia: key to success of the most widespread mangrove species Avicennia marina? Ann Bot **101**:49-58.

Scholz, F. C., S. J. Bucci, G. Goldstein, F. C. Meinzer, A. C. Franco, and F. Miralles-Wilhelm. 2008. Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water. Tree Physiology **28**:469-480.

Sevanto, S., T. Holtta, and N. M. Holbrook. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant Cell Environ **34**:690-703.

Sevanto, S., E. Nikinmaa, A. Riikonen, M. Daley, J. C. Pettijohn, T. N. Mikkelsen, N. Phillips, and N. M. Holbrook. 2008. Linking xylem diameter variations with sap flow measurements. Plant and Soil **305**:77-90.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2002. Time lags for xylem and stem diameter variations in a Scots pine tree. Plant Cell and Environment **25**:1071-1077.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2003. Sugar transport together with environmental conditions controls time lags between xylem and stem diameter changes. Plant Cell and Environment **26**:1257-1265.

Smith, D. M. and S. J. Allen. 1996. Measurement of sap flow in plant stems. J Exp Bot **47**:1833-1844.

Steppe, K., D. De Pauw, R. Lemeur, and P. Vanrollegheim. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257–273.

Steppe, K., F. Sterck, and A. Deslauriers. 2015a. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends Plant Sci **20**:335-343.

Steppe, K., M. W. Vandegehuchte, R. Tognetti, and M. Mencuccini. 2015b. Sap flow as a key trait in the understanding of plant hydraulic functioning. Tree Physiol **35**:341-345.

Tilman, D. 1982. Resource Competition and Community Structure. Princeton University Press.

Tran, P., I. Gritcan, J. Cusens, A. C. Alfaro, and S. Leuzinger. 2016. Biomass and nutrient composition of temperate mangroves (Avicennia marina var. australasica) in New Zealand. New Zealand Journal of Marine and Freshwater Research:1-16.

Tyree, M. and M. H. Zimmermann. 2002. Xylem Structure and the Ascent of Sap. Second edition. Spriner-Verlag, Berlin.

Urrutia-Jalabert, R., S. Rossi, A. Deslauriers, Y. Malhi, and A. Lara. 2015. Environmental correlates of stem radius change in the endangered Fitzroya cupressoides forests of southern Chile. Agricultural and Forest Meteorology **200**:209-221.

Vandegehuchte, M. W., A. Guyot, M. Hubau, S. R. E. De Groote, N. J. F. De Baerdemaeker, M. Hayes, N. Welti, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014a. Long-term versus daily stem diameter variation in co-occurring mangrove species: Environmental versus ecophysiological drivers. Agricultural and Forest Meteorology **192-193**:51-58.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, T. De Swaef, D. A. Lockington, and K. Steppe. 2014b. Modelling reveals endogenous osmotic adaptation of storage tissue water potential as an important driver determining different stem diameter variation patterns in the mangrove species *Avicennia marina* and *Rhizophora stylosa*. Annals of Botany **114**:667-676.

Wang, W., Z. Yan, S. You, Y. Zhang, L. Chen, and G. Lin. 2011. Mangroves: obligate or facultative halophytes? A review. Trees **25**:953-963.

Zimmermann, D., R. Reuss, M. Westhoff, P. Gessner, W. Bauer, E. Bamberg, F. W. Bentrup, and U. Zimmermann. 2008. A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. Journal of Experimental Botany **59**:3157-3167.

Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer New York.

Zweifel, R., D. M. Drew, F. Schweingruber, and G. M. Downes. 2014. Xylem as the main origin of stem radius changes in *Eucalyptus*. Functional Plant Biology **41**:520-534.

Zweifel, R., M. Haeni, N. Buchmann, and W. Eugster. 2016. Are trees able to grow in periods of stem shrinkage? New Phytol **211**:839-849.

Zweifel, R., H. Item, and R. Hasler 2001. Link between diurnal stem radius changes and tree water relations. Tree Physiology **21**: 869–877.

Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees **15**:50-57.

Zweifel, R., K. Steppe, and F. J. Sterck. 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. J Exp Bot **58**:2113-2131.

Zweifel, R., L. Zimmermann, and D. M. Newbery. 2005. Modeling tree water deficit from microclimate: an approach to quantifying drought stress. Tree Physiology **25**:147-156.

Chapter 4 - Disentangling the net: Separating components of *Avicennia marina* (Forssk.) Vierh stem radial water dynamics by concomitant xylem and over-bark measurements

“Although you hide in the ebb and flow
Of the pale tide when the moon has set,
The people of coming days will know
About the casting out of my net,
And how you have leaped times out of mind
Over the little silver cords“
W. B. Yeats – Extract from “The Fish”

“If you're doing an experiment, you should report everything that you think might make it invalid -
not only what you think is right about it”
Richard Feynman

4.1 Abstract

Daytime stem shrinking is a well recorded phenomenon, as trees dip into internal water stores to meet imbalances between water supply and demand. Uncertainty surrounds the mechanisms behind the unusual pattern of daytime stem swelling, presented by species like the mangrove *Avicennia marina*, and which is thought to originate in the osmotic adjustment of storage tissues.

I performed on-xylem radius change (XRC) and whole stem (SRC) measurements with point dendrometers to ascertain if the swelling of the stem is due to an increase in the thickness of the phloem and inner-bark (BRC). I measured leaf water potential (ψ_{leaf}), sap-flow, leaf turgor and microclimate to understand the coupling between BRC and transpiration-driven changes in XRC.

Our results present direct evidence of the type of mechanism responsible for daytime stem swelling. Inner-bark thickness increase concurrent with XRC decrease, sap-flow increase and leaf turgor loss, reveals a phloem-generated turgor signal behind daytime stem swelling of tree stems. On-xylem measurements were highly heterogeneous due to the variability in the three dimensional fish-net wood structure of the stem of *A. marina*. As daytime decreases in XRC were compensated by BRC and correlated with conditions of high water demand, I suggest a potential role of daytime stem swelling in the hydraulic safety of *A. marina*.

4.2 Introduction

Stem radius changes (SRC) caused by the expansion or contraction of stem tissues are predominantly due to changes in water potentials within the plant (Herzog et al. 1995, Zweifel et al. 2000, Zweifel et al. 2005, Steppe et al. 2006, López-Portillo et al. 2014, De Swaef et al. 2015). Low water potentials in the air, due to high solar radiation, VPD and wind, induce transpiration. The water loss from the crown by transpiration lowers the crown water potential and induces a water potential gradient within the plant which forces water to flow from the point of highest water potential (soil/roots) to the point of lowest water potential in the leaves (axial water transport). Within this hydraulic system there is not only axial water transport but also radial water movement between xylem and inner-bark which moves from high to low water potential (Zweifel et al. 2000, Holtta et al. 2006, Steppe et al. 2006, Sevanto et al. 2011, De Swaef et al. 2015, Pfautsch et al. 2015a). During the day, transpiration causes xylem water potential to drop, and water is drawn radially from the elastic water storage tissues into the axial sap flow stream. During the night, this process is reversed and water storage tissues are refilled (Whitehead & Jarvis 1981). This depletion and refilling of water stores results in the diurnal cycles of stem swelling and shrinking, which are thought to reflect the changes predominantly of the elastic tissues of the cambium and bark, including the outer parenchyma and especially the phloem, referred to here as inner-bark (Sevanto et al. 2002, Holtta et al. 2006, Drew and Downes 2009, De Schepper and Steppe 2010, Sevanto et al. 2011, Mencuccini et al. 2013, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Savage et al. 2016) and excluding the dead outermost layer of the bark. However, this view is based on the study of comparatively few North-American and European slow-growing tree species and recent research shows that stem radius patterns are more diverse and that inner-bark may not be the only tissue types responsible for stem radius changes (Pfautsch 2015a, Pfautsch 2016 and references therein). For example, non-lignified xylem and sapwood tissues are known to also change their thickness in response to diurnal changes in tree water status, despite their generally lower elasticity (Sevanto et al. 2002, Sevanto et al. 2011, Woodruff and Meinzer 2011, Zweifel et al. 2014, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Pfautsch 2016, Mencuccini et al. 2017).

Measurements on-xylem and on the inner-bark allow us not only to discover the relative water storage role of each tissue, but also give information on the drivers of tissue water potentials and theirs relationship to stem radius changes. There are two main drivers of tissue water potentials in a tree: (i) the physical and (ii) the biological ones. When drivers are mostly physical, flow and storage dynamics of trees are explicable with a hydraulic system where the water potential of a certain tissue can be calculated according to Ohm’s law. Several model approaches proved this concept to be valuable in simulating stem radius variations to a very high degree (Zweifel et al. 2000, Zweifel et al. 2005, Steppe et al. 2006). However, tree water relations do not always follow the patterns of a purely physical system, but are additionally altered by biological drivers, such as osmoregulation or stomatal regulation (Sevanto et al., 2002, 2003, 2011, Zweifel et al 2014, De Schepper & Steppe 2010, Pfautsch 2015). Sugar loading and unloading processes in the phloem allow plants to actively change the osmotic potentials and thus the water potential of storage tissues, consequently affecting the water flow and storage dynamics from the xylem to the phloem (Sevanto et al. 2002, 2003, De Schepper and Steppe 2010, Sevanto et al. 2011, De Schepper et al. 2013, Pfautsch et al. 2015b, Savage et al. 2016). As a purely physical approach has proven relatively successful in explaining and modelling stem radius change, it is not until recently that the role in stem radius change of other mechanisms such as osmoregulation, the Mūnch flow hypothesis, and carbohydrate dynamics have been explored (Sevanto et al. 2002, 2003, Holtta et al. 2006, De Schepper and Steppe 2010, Sevanto et al. 2011, Mencuccini et al. 2013, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Savage et al. 2016, Mencuccini et al. 2017, Paljakka et al. 2017). However, part of this success rested on SRC measurements near the base of trees where osmoregulation processes may not be as important as nearer to the tree crown (Zweifel, personal comm., Paljakka et. al 2017).

In a purely physically driven system, changes in bark water potentials are closely linked to changes in xylem water potentials, and thus their radial changes should run in parallel with a certain delay, depending on the hydraulic coupling (resistance) and elastic properties of both tissues. Consequently, if xylem and bark tissue water potentials, and the radial change linked to them, develop in opposite directions there must be biological processes involved (Sevanto et al. 2002, 2003, Sevanto et al. 2011, Mencuccini et al. 2013, Pfautsch et al. 2015b, Chan et al. 2016, Mencuccini et al. 2017). In this manner, the difference between stem radius measurements on xylem and over-bark can help an estimation of the biological and the purely transpiration-driven contributions to the hydraulic system, the phloem generated turgor signal, as well as inform us on the relative contributions of inner-bark and xylem to SRC, and the hydraulic coupling and elastic properties of both tissues (Sevanto et al. 2002, 2003, Sevanto et al. 2011, Mencuccini et al. 2013, Zweifel 2014, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Chan et al. 2016, Mencuccini 2017).

Avicennia marina (Forssk.) Vierh. presents an unusual SRC diurnal cycle, characterized by early morning stem swelling and delayed shrinking. This pattern is thought to be due to endogenous osmotic adjustment which causes water to be drawn radially into elastic water storage tissues even after the onset of transpiration and leaf water potential drop (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b). Carbohydrates, together with phloem loading and unloading processes, are hypothesized to play a role in the endogenous adjustment which explains daytime stem swelling, causing the changes in the osmotic water potential of storage tissues (Vandegehuchte 2014b, Donnellan Barraclough et al. 2018). *Avicennia marina* has unusually high phloem to xylem ratios, which could explain why the phloem generated turgor signal has such a strong effect on the SRC patterns (Donnellan Barraclough et al. 2018). These high phloem to xylem ratios are a characteristic of a stem structure possessed by *A. marina* called successive reticulate cambia (Robert et al. 2011b). In mangroves, *Avicennia* is the only genus known to possess successive cambia, a wood structure found also in lianas, shrubs and trees belonging to over 34 different families (Robert et al. 2011a, Robert et al. 2014). This structural trait is characterized by multiple layers of vascular cambia, which either can develop successively or simultaneously, producing several layers of xylem and internal secondary phloem (Schmitz et al. 2008, Robert et al. 2009, Robert et al. 2011a, Robert et al. 2011b, Robert et al. 2014). Two models have been proposed for the three-dimensional structure of these consecutive bands of cambia in *A. marina*, (1) the fishnet stocking model, where multiple layers of cambia sheets are discontinued along and around the stem and (2) the broken cylinders model, where continuous cambia sheets are broken up longitudinally or radially after formation (Robert 2011). Robert found about

85% of trees and shrubs with concentric internal secondary phloem grow in dry or saline habitats (Robert et al. 2011a). Therefore, this vascular structure is thought to confer advantages for water transport, storage and safety, where the phloem has a role in supplying stored water and carbohydrates, and potentially helps in embolism repair (Schmitz et al. 2007b, Schmitz et al. 2008b, Salleo et al. 2009). This highly complex “net-like” wood structure is also known to result in high heterogeneity or *patchiness* in many processes measured by point dendrometers, both in the case if radial water movements and stem growth measurements (Robert 2011b, 2014).

To our knowledge, xylem radius change (XRC) has never been measured simultaneously with SRC in trees presenting daytime stem swelling. Concomitant measurements of XRC and SRC in *A. marina* could help investigate whether daytime stem swelling originates in the inner-bark and is decoupled from transpiration induced xylem water potential drop. Thus, by calculating inner bark thickness changes (BRC) from the two measurements (SRC minus XRC) it may be possible to reveal the osmotically driven turgor signal driving the unusual whole stem SRC. Additionally, our capacity to measure xylem radius changes with point dendrometers on species with multiple reticulate cambia, like *A. marina*, remains unclear. Research shows that the net of wood-patches of xylem and phloem which make up the stem are highly heterogeneous and can change within a few millimetres, affecting dendrometer measurements.

This chapter measures on-xylem concomitantly to SRC in the stem of *A. marina* at different stem heights and times of year together with other water-related physiological processes over several years. The first main hypothesis addressed in this work is that daytime stem radius swelling in *A. marina* originates in swelling of the inner bark, caused by osmotic adjustment. To address this, I calculate BRC from XRC and SRC measurements. If BRC is affected by osmoregulation processes, I expect that inner-bark will swell whilst xylem shrinks, going against transpiration-induced changes in water potential, sap flow and leaf turgor (BRC will be opposed to XRC – Type I pattern). As I hypothesize that daytime swelling is functionally important in *A. marina*, I characterize the changes of SRC, BRC and XRC in *A. marina* in response to different environmental conditions and their relationship with other physiological processes such as leaf water potential, leaf turgor and sap flow. On the one hand, I expect that BRC will be more pronounced in conditions of high water demand, which will cause increased osmoregulation of storage tissues. On the other hand, I expect that since XRC is driven predominantly by physically-induced water potential changes, it will follow the dynamics of leaf water potential, leaf turgor, sap flow and indicators of water supply and demand more closely than BRC and SRC. The second main hypothesis I address is that on-xylem measurements are heterogeneous in space and time, and XRC shrinkage concomitant to SRC swelling (Type I pattern) is not always possible to detect. To address this, I evaluate the nature of the heterogeneity in SRC and XRC measurements caused by the high variability in *A. marina*’s reticulate wood anatomy by performing concomitant measurements of SRC and XRC at different stem locations throughout the year and classifying resulting patterns.

4.3 Materials and methods

4.3.1 Study site

The study site is located in the Mangawhai estuary, northern New Zealand (36.097°S, 174.573°E). The estuary consists of two main waterways and approximately 80 ha of mangrove forest. The study site is located in the upper tidal zone of Tara Creek, furthest from the shore with sandy substrate and short inundation times twice every 24 hours. The stand is monospecific and homogeneous, composed of A. marina individuals 3-4 m in height. Within the stand, I picked an area at least 50 m from the channel to avoid edge effects. Annual weather trends during the study period (February 2015 – December 2015, August 2017) followed patterns typical to temperate climate of the north island with the exception of the month of November 2015, which had no precipitation. Daily mean relative humidity ranged from 38% to 100%, with a mean of 80.8%. Annual temperature extremes ranged from -1.5○C to 29.4○C with a seasonal mean of 15.4○C. Total precipitation sum was 1062.9 mm. Soil water potential (SWP) ranged over the whole measuring period from -5 MPa to -1.4 MPa, with a mean of -2.5 MPa. The salinity was measured on two occasions during the experimental period (March 2015 and December 2015) in soil pore water at a soil depth of 30cm and ranged from 20‰ to 39‰. Water table depth ranged from -12.8 cm below ground level to +64.4 cm above ground level during tidal inundation (Appendix Figure 1).

4.3.2 Environmental data

A weather station was installed within the canopy in order to explain plant physiological responses to microclimate. Briefly, a probe installed within the canopy logged temperature and relative humidity (RH) (Model SHT, Sensirion, Stäfa, Switzerland). Vapour Pressure Deficit (VPD) was calculated from this data according to Buck (1981). Solar irradiance (I [W m^{-2}]) was measured with a pyranometer (Model PYR, Decagon Devices, WA, USA). Rainfall was measured with a tipping-bucket rain gauge (Model 52202, R. M. Young Company, Traverse City, Michigan, USA). Tidal inundation (WD [cm]), soil-water temperature (SWT [°C]) and soil-water electrical conductivity (EC [µS/cm]) were measured with a CTD sensor (CTD-10, Decagon Devices, WA, USA) fitted into a well dug into the sediment at a depth of 1 m in tubing that allowed water flow. Osmotic potential ($\Psi\prod$ [MPa]) of the soil and water was calculated using soil-water electrical conductivity and temperature (McIntyre 1980). All instruments logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland).

4.3.3 Physiological measurements

Physiological measurements of tissue radius change were done by placing point dendrometer pairs on over-bark to measure total stem radius change (SRC) and directly on-xylem to measure xylem radial change (XRC). Because of uncertainty surrounding the capacity to measure xylem radius changes in reticulate wood structure, I refer to sensor head measurements positioned on-xylem measurements as XRC for clarity and brevity, although this does not mean they in fact measure only xylem radius changes.

Dendrometers are non-invasive small pistons pressed against the stem connected to a potentiometer, all mounted on carbon fibre frames optimized for insensitivity to temperature changes (ZN11-O-WP, Natkon, Oetwil am See, Switzerland). Measurements were carried out intermittently at different times, along the stem of different tree individuals over a period of two years, from February 2015 until August 2017 (Table 4.1).

The sensor-heads of the first top dendrometers at each stem tier (Table 4.1) were placed directly on the stem to measure SRC. The sensor-heads of the second dendrometer of each pair were placed directly on the xylem tissue by cutting a small window of ~1 cm^2 out of the bark to measure XRC. To avoid the drying out of the xylem when exposed to the air, I applied a thin layer of non-conductive non-absorbent silicon grease. Despite this, xylem measurements are liable to become unreliable over time due to tissue drying

Table 4.1 Details of different on-xylem measurement campaigns carried out over a two year period from February 2015-July 2017 with automatic point dendrometers on four different trees. Position and height refer to the location of the dendrometer pair (two dendrometers per tier) in reference to ground level. Tree ID indicates name of different tree individuals used in measurements

Measuring period (date/ number of days)	**Position**	**Height (cm)**	**Tree ID**
February 2015 (30 days)	Upper	270	Tree A
July-August 2015 (60 days)	Upper	190-260	Tree B
	Middle	150	
	Lower	90	
November-December 2015 (45 days)	Upper	190-260	Tree B
	Middle	150	
	Lower	90	
December-January 2015 (15 days)	Upper	190-260	Tree B
	Middle	150	
	Lower	90	
August 2017 (15 days)	Upper	270	Tree C
	Lower	80	
	Upper	270	Tree D
	Lower	80	

or repair (Zweifel 2014) and measurement were restarted with fresh windows cut into the bark at slightly displaced locations at the same height on five different occasions: February 17, July 25, November 3 and December 3 of 2015, and August 13 2017 (Table 4.1).

Sap flow rate was measured using heat balance gauges (Dynamax, Houston, TX, USA), based on the heat balance method (HB) (Smith and Allen 1996). Two sapflow sensors were installed in each of the study trees in upper branches of ~20 mm diameter. All instruments logged at 10 minute intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland). Turgor pressure probes were installed with a separate login system (YARA ZIM Plant Technology, Germany). These are non-invasive probes that measure leaf turgor in real time by measuring the balancing pressure of a magnet clamp (Zimmerman 2004). The balancing pressure (Pp) is inversely proportional to leaf turgor. I report the inverse of Pp, which is directly correlated to leaf turgor (1/Pp). Three of these probes were installed in each of the study trees, dispersed amongst the upper and lower canopy.

Leaf water potential (ψ_{leaf}) was measured on February 18 during pre-dawn and midday on two neighbouring trees (<10 m). Leaf water potential was measured with a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) following the usual procedure of measuring leaves immediately after excision (Scholander, Hemmingsen et al. 1964). For each time point, three to five leaves were measured then averaged per time and tree.

4.3.4 Calculation of physiological parameters: Elements of radial change in over-bark and xylem.

Both SRC and XRC measurements obtained from dendrometers were cleared of jumps and spikes with a custom written function in R (as per Chapter 2). I calculated the thickness changes of the inner-bark as the difference between total SRC measurements and XRC measurements, resulting in the signal of bark radius change (BRC) (Sevanto et al. 2011, Zweifel et al. 2014).

$$BRC(t) = SRC(t) - XRC(t)$$

I then used a numerical approach to determine duration and amplitude of shrinking and swelling of both total SRC, BRC and XRC, implementing an approach similar to the one developed by Deslauriers et al. (2011) by building a custom function (Donnellan Barraclough et al. 2018). Briefly, I broke down stem and xylem circadian cycles into shrinking periods between a radius maximum (R_{max}) and the following minimum (R_{min}) when stored water is depleted, and expansion periods between R_{min} and the next occurring R_{max} when water stores are being replenished. This allowed us to extract contraction amplitude (A_c), expansion amplitude (A_e), contraction duration (T_c) and expansion duration (T_e) in addition to the times of the day R_{max} and R_{min} occurred.

4.3.5 Statistical analysis and other calculations

Diel relationships between environmental and physiological variables were tested through multiple correlations performed on data at a 10-minute resolution for a two week period (16-30 December 2015). This period was chosen as it presented two different precipitation regimes (presence/absence) in close temporal proximity and stem radius measurements and leaf turgor measurements were available concurrently with no interruptions. Analyses were conducted using the free software package R (R Core Team, 2017)

4.4 Results

4.4.1 Xylem shrinkage concomitant to inner-bark swelling: diel cycle patterns in relation to other plant ecophysiological metrics

Measurements detected xylem shrinkage concomitant to inner-bark daytime stem swelling (Type I pattern) during several measurement periods. This Type I XRC pattern had clearly opposed timings to SRC maxima and minima (Type I: Figure 4.1, Figure 4.6), which persisted for several weeks. Examples are the upper stem position in February 2015 of Tree A and the lower stem position of Tree B during July to December 2015. During this time, XRC showed shrinking during early morning, reaching its minima before midday, a pattern which was clearly different from the daytime swelling of SRC and BRC measurements (Figure 4.1, a). Calculated BRC showed a morning thickness increase which was superior to

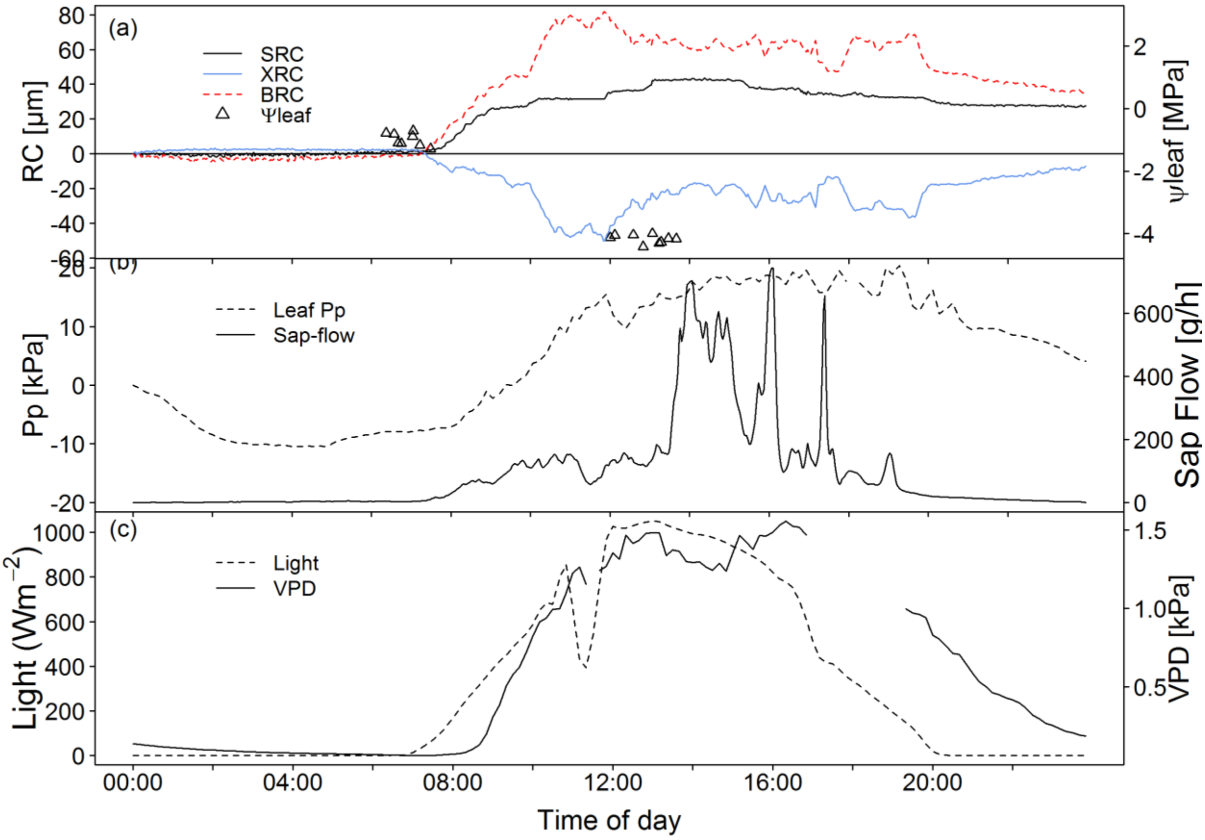


Figure 4.1 Example of a diurnal cycle measured on February 18 2015 of (a) leaf water potential (ψleaf), over-bark SRCob, xylem XRC and calculated inner-bark BRC for the upper dendrometer pair of Tree A and (b) leaf turgor balancing pressure (Pp) and stem sap flow for Tree A.

total SRC, thus compensating for XRC shrinkage and contributing to whole stem increase at midday (Figure 4.1). Xylem shrinking occurred concurrently with the morning $\Delta\psi_{leaf}$ drop and stem sap flow increase (Figure 4.1, a, b). Xylem radial changes also closely mirrored leaf turgor, with balancing pressure (Pp) morning increase and evening drop coinciding with xylem morning shrinking and evening recovery (Figure 4.1). Xylem radius seemed to undergo more fluctuations than total SRC, making XRC look more similar to leaf Pp in the amount of fluctuations and peaks during sun-lit hours.

Over all four measurement periods of 2015 a total of 82 days showed a distinct Type I pattern in both measurement trees (Table 4.4). On average XRC R_{max} was reached most frequently in the two hours before and after midnight (XRC R_{max}: median = 16.5 h, IQR = 16 h) and xylem R_{min} around midday (XRC R_{min}: median = 12 h, IQR = 5.25 h) (Figure 4.1, lower stem). Total SRC measurements showed a reversed pattern to xylem, with R_{max} occurring most frequently during midday (SRC R_{max}: median = 11 h, IQR=5) and R_{min} in the first hours after midnight (SRC R_{min}: median= 7 h, IQR=20).

4.4.2 Responsiveness of Type 1 XRC to diel and seasonal environmental changes

Analysis of Type I XRC measurements showed that XRC patterns responded to changing environmental conditions and held a correlation with the responses of other ecophysiological measurements at both diel and 10-minute scales. When comparing a summer (February 2015) and a winter (July 2015) period, seasonal changes were seen to affect XRC, SRC and BRC patterns, which accompanied changes in leaf turgor and sap flow (Figure 4.2). For the summer period analysed, sunny days showed large amplitudes for XRC, BRC and SRC (Table 4.2) which coincided with high sap flow rates (Figure 4.2). Thus, BRC seemed to increase and compensate large decreases in xylem radius (Figure 4.2). During the same period, summer days with increased cloudiness resulted in lowered sap flow rates, and reduced amplitudes XRC, BRC and SRC. The winter period analysed presented much lower amplitudes of radius change (Table 4.2). Circadian cycles of swelling and shrinking disappeared almost completely and whole-stem steadily increased during a rainy period where humidity stayed above 90% for several days

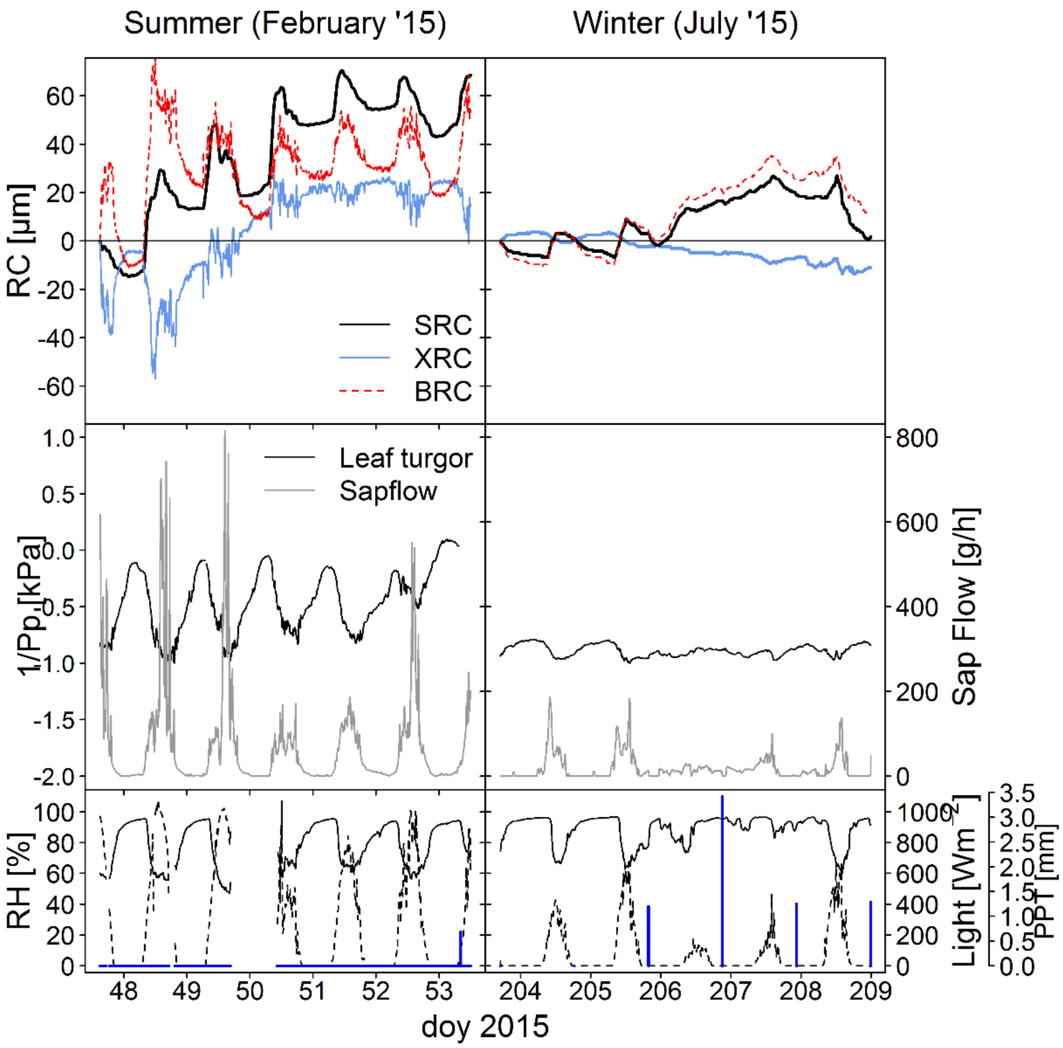


Figure 4.2 Diurnal ecophysiological patterns for an example A.marina tree during a week in summer (February 2015) and a week in winter (July 2015). Upper panel shows stem radial variations measured on over-bark tissue (SRC), black, xylem radial changes (XRC, blue) and calculated inner b, redark (BRC). Mid panel shows leaf turgor balancing pressure and stem sap flow, and lower panel shows daily courses of relative humidity (RH), light and precipitation.

(Figure 4.2). The effects of precipitation and high humidity also resulted in lowered sap flow rates and the lack of strong diurnal leaf turgor fluctuations. Both stem radial changes, leaf turgor and sap flow began to fluctuate again once humidity dropped below 80%.

Table 4.2 Diurnal cycle summary characteristics, amplitude of expansion (Ae) and contraction (Ac), day to day change (Ad) and times of radius maxima (Rmax time) and minima (Rmin time) of whole stem SRC, on-xylem XRC and inner-bark BRC for seven days measured in summer (February 2015) and seven days in winter (July 2015).

	Summer			Winter		
	XRC	BRC	SRC	XRC	BRC	SRC
Ae (µm)	7.5±30	41.2 ± 21.3	31.02 ± 12	5.1 ±0.3	19.9 ±11.2	16.8±8.6
Ac (µm)	4.2±30	36.8±12.2	20.0 ± 6	10.6±6.9	11±2.3	13±8.15
Ad (µm)	1.1	5.5 ± 20	8.05 ± 9	-2.1 ±6.5	10.6±14	7.9±6.9
Rmax time	5:30	12:15	10:00	8:00	12:00	11:00
Rmin time	11:00	03:00	00:00	18:00	02:00	02:30

The analysis of two weeks in December 2015 (16 -29 December) at the 10-minute time-scale showed that XRC was also clearly sensitive to environmental drivers of transpiration. During the two analysed weeks XRC response to environmental drivers depended on the presence of precipitation (Figure 4.3). During a week of high precipitation, both XRC and leaf turgor lost the strong diurnal signal, whilst SRC and BRC still showed more notable diurnal shrinking and swelling. At this time, XRC lost any significant correlation with environmental variables (Table 4.3). However, when precipitation was absent, both XRC and leaf turgor were strongly coupled, showing strong fluctuations which closely mirrored RH, and which were opposite to the cycles of SRC and BRC shrinking and swelling (Figure 4.3). During this time, XRC and leaf turgor were highly positively correlated with

RH and negatively with VPD, light and temperature (Table 4.3), whilst SRC and BRC had the inverse relationship to these variables (Table 4.3).

Table 4.3 Correlation coefficients for correlation of on-xylem radius changes (XRC), total stem radius change (SRC), inner-bark radius change (BRC) and leaf turgor (1/Pp) to environmental variables at a 10 minute time scale.

	16-22 December (PPT present)				23- 30 December 2015 (PPT absent)			
	XRC	SRC	BRC	Turgor	XRC	SRC	BRC	Turgor
Humidity	0.16	-0.43*	-0.35	0.54*	0.62*	-0.53*	-0.69*	0.74*
Light	-0.1	0.52*	-0.08	-0.60*	-0.47*	0.52*	0.56*	-0.8*
Temperature	-0.18	0.55*	0.4*	-0.55*	-0.58*	0.35	0.55*	-0.77*
VPD	-0.2	0.54*	0.4*	-0.55*	-0.65*	0.5*	0.7*	-0.74*
PPT	0.04	-0.03	-0.03	0.04	0.06	-0.15	-0.14	-0.11
SWP	0.18	-0.19	0.05	0.18	-0.05	0.06	0.0	-0.09
depth	-0.28	-0.2	0.18	0.2	0.07	-0.04	0.07	-0.00

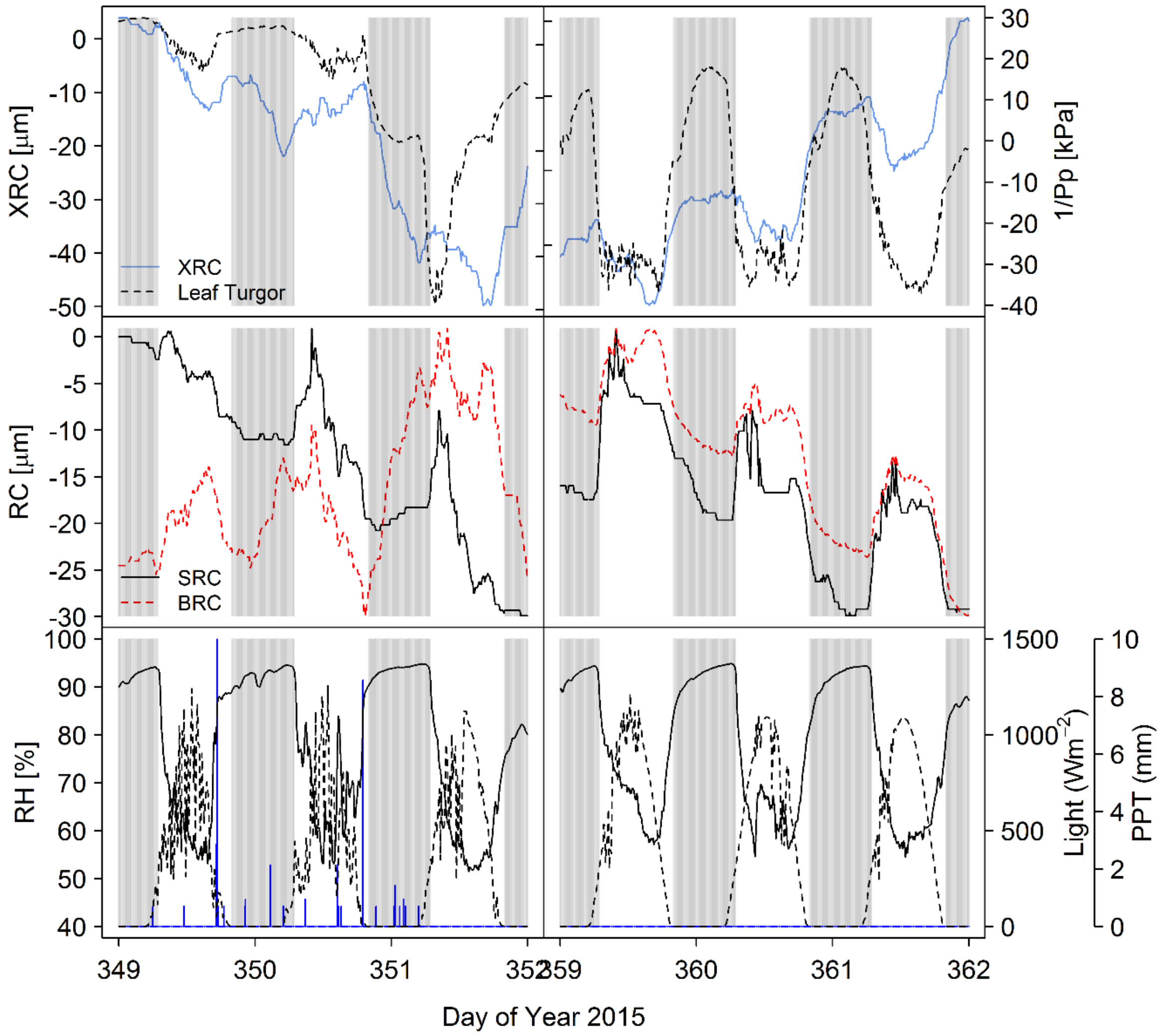


Figure 4.3 Three example days within each of the analysed 10 day periods of December 2015 showing precipitation presence (left panels) and absence (right panels) for on-xylem radius change (XRC), leaf turgor (1/Pp), stem radius change (SRC) and inner-bark radius change (BRC). Bottom row shows diurnal courses of humidity (RH, black line), precipitation (PPT, blue bars) and light (dotted line). Grey areas indicate night-time periods.

4.4.3 Characterizing the patchiness and heterogeneity in on-xylem and SRC measurements

Although XRC measurements showed a clear pattern which was distinctive from SRC measurements, this pattern was not always consistent or present at all measurement points and periods (Table 4.4). The heterogeneity between XRC measurement points occurred within individual stems at the same time period and at different time periods (Table 4.4 & Table 4.5).

Xylem radius change measurements showed four distinct patterns, based on their shape and timing relative to SRC, allowing for a classification into Type I – IV which is detailed in Table 4.4. Firstly, Type I XRC pattern had clearly opposed timings to SRC maxima and minima (Type I: Figure 4.4, lower stem), which persisted for several weeks. Thus, opposed timing resulted in SRC maxima coinciding with XRC minima around midday. Examples are the upper stem position in February 2015 and the lower stem position during July to December 2015 (Figure 4.1, 4.6 and Supplementary Figure 4.1). Secondly, there was Type II XRC pattern, which also had opposed timing to concomitant SRC, with XRC showing evidence of day-time shrinking and night-time refilling, but after 2-3 days the XRC pattern became very similar or almost identical to their corresponding pair SRC (Figure 4.6 & Supplementary Figure 4.1, Period I vs II). Thirdly, there was Type III XRC, a pattern which differed from SRC

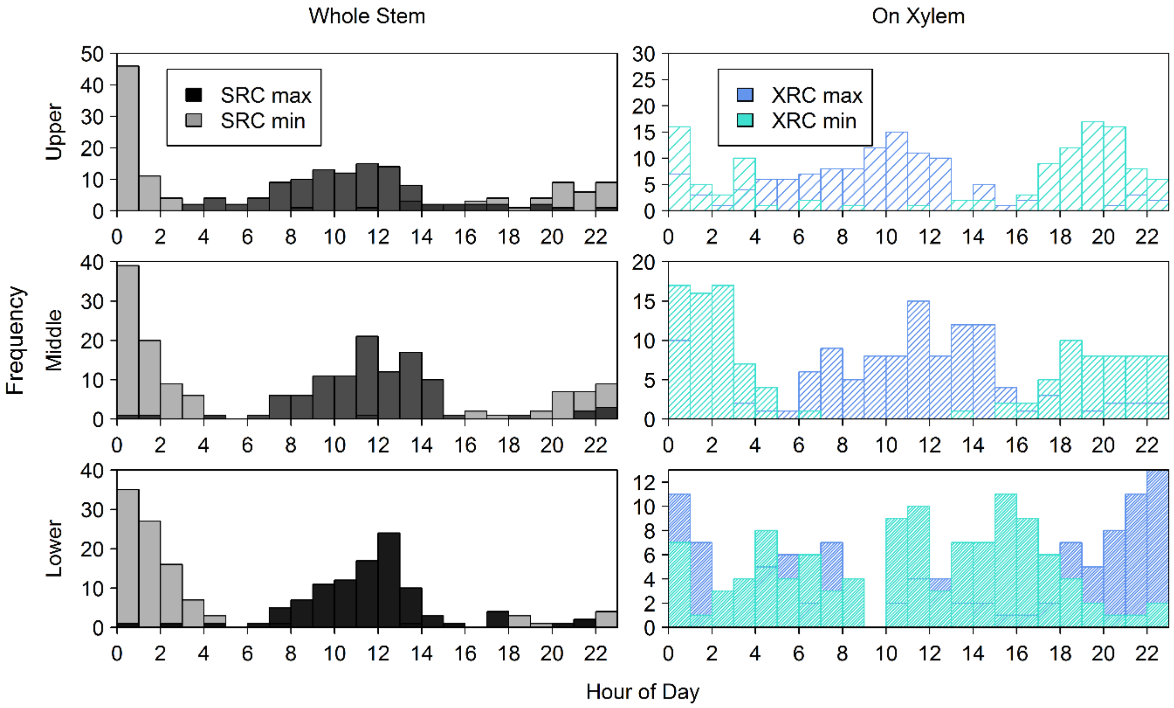


Figure 4.4 Frequency distribution of timing of maximum and minimum radius for whole stem (black, SRC max and SRC min) and for on xylem measurements (blue, XRC max and XRC min) for Tree B from June until December 2015

measurements but where XRC did not have opposed peak timings to SRC, thus making them clearly different from Type I patterns. Examples of Type III are measurements taken in August 2017 (Figure 4.5 & Figure 4.6 Period I). Lastly, there was Type IV, a pattern indistinguishable from SRC measurements (Table 4.4, Figure 4.6 & Supplementary Figure 4.1, upper stem).

Table 4.4 Results obtained in the different dendrometer measurement campaigns on four different trees at different times between 2015-2017. Details give the position on the tree stem (upper, middle or lower stem) and the type of xylem radius change (XRC) pattern according to its similarity to stem radius change (SRC) as described in text, briefly: (Type I) Distinctive pattern and timing opposed to SRC (Type II) Distinctive pattern and timing but disappears after a few days (Type III) Distinctive pattern but timing not opposed to SRC (Type IV) Identical to SRC. The position shows the location within the stem (upper, middle or lower) and the individual tree ID (A-D). Figure shows a references to figures in the text showing measurement examples.

Measuring period	Position (Tree ID)	Pattern type	Figure
February 2015	Upper (T-A)	I	Fig. 1
July 2015	Upper (T-B)	II	S Fig. 1
	Middle (T-B)	II	
	Lower (T-B)	I	
November 2015	Upper (T-B)	IV	S Fig. 1
	Middle (T-B)	IV	
	Lower (T-B)	I	
December 2015	Upper (T-B)	III	Fig. 6
	Middle (T-B)	III	
	Lower (T-B)	I	
August 2017	Upper (T-C)	III	Fig 6
	Lower (T-C)	III	
	Upper (T-D)	III	
	Lower (T-D)	III	

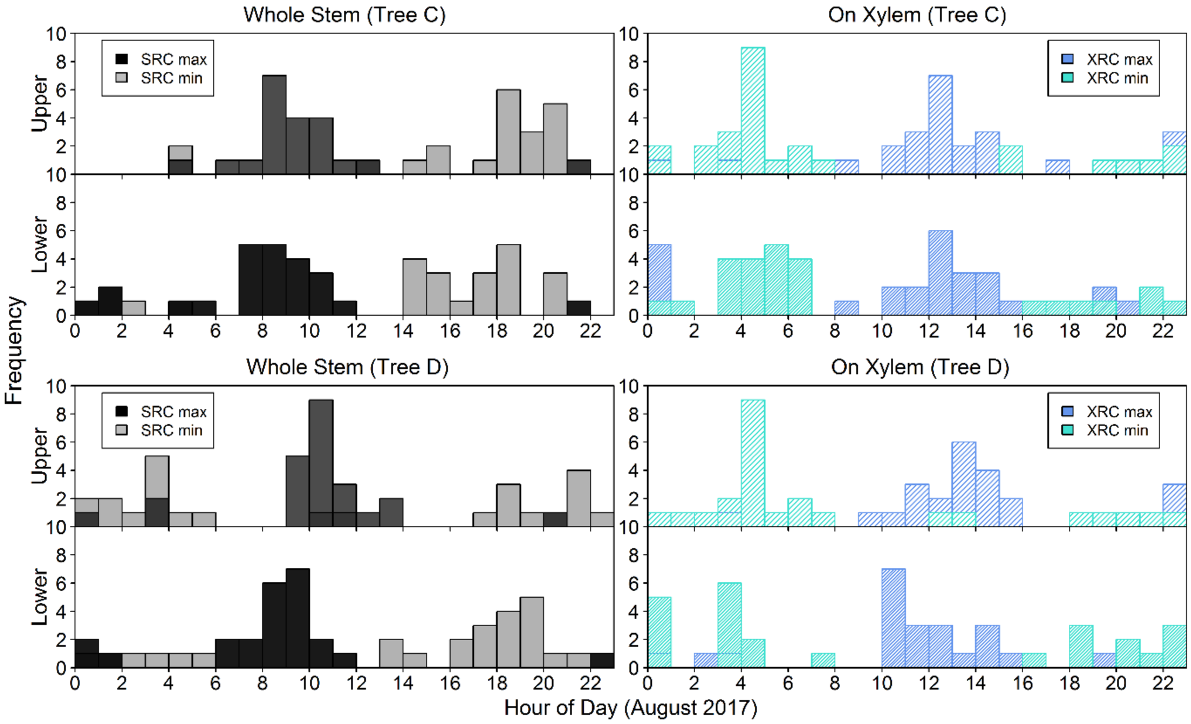


Figure 4.5 Frequency distribution of timing of maximum and minimum radius for whole stem (black, SRC max and SRC min) and for on xylem measurements (blue, XRC max and XRC min) for in upper and lower positions for Tree C and Tree D for August 2017

Briefly, during the first measurement period of February 2015, the dendrometer pair showed XRC Type I & II pattern with timing clearly opposed to SRC measurements (Table 4.4 & Table 4.5), as did the lower position the measured tree during the measurement periods from July to December 2015 (Figure 4.4, Table 4.4 & Table 4.5). The upper and middle positions during July to December 2015 showed a Type II or IV pattern, with XRC maxima and minima timings which were mostly indistinguishable from SRC at the start of

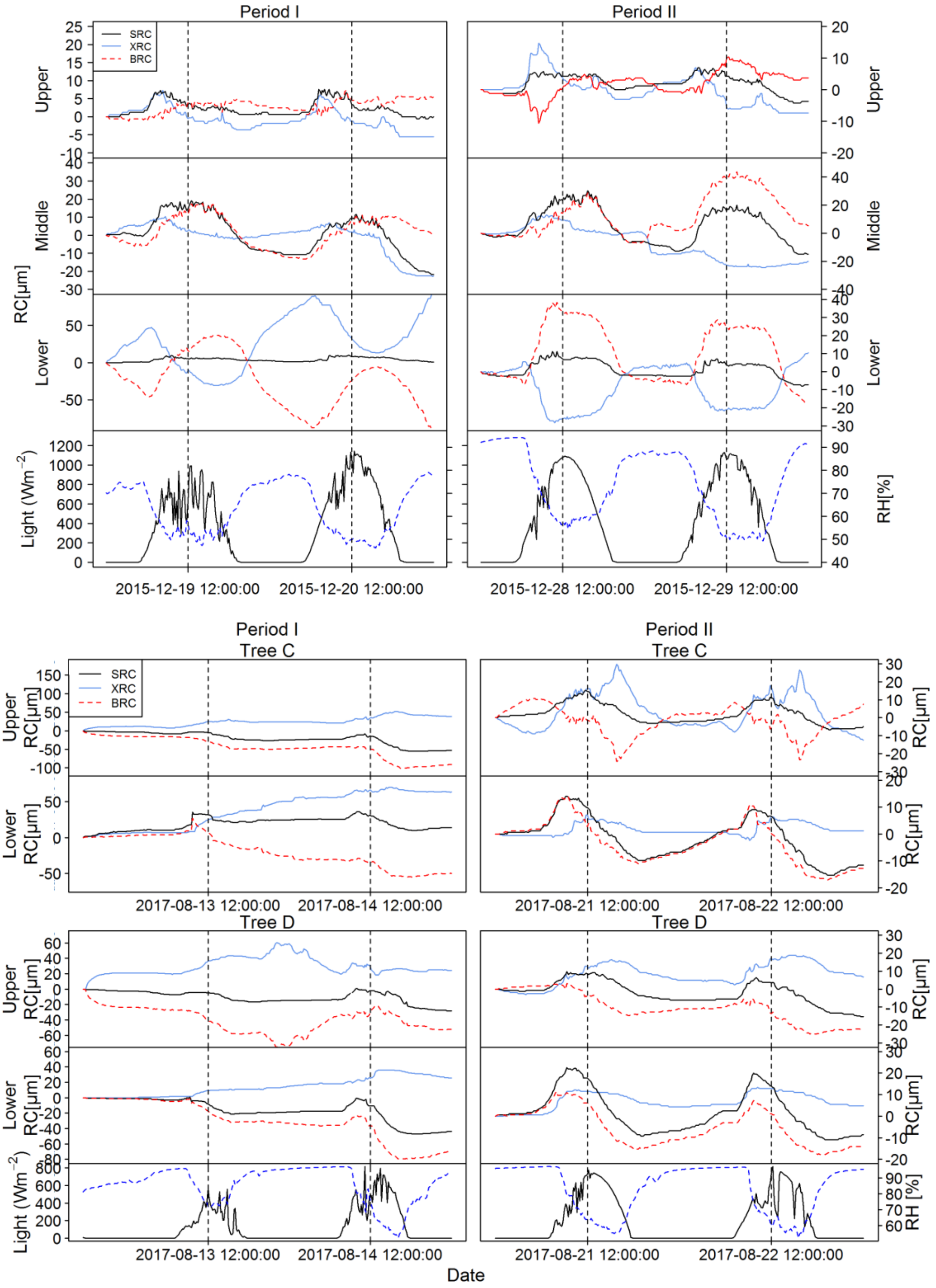


Figure 4.6 Example of radius change measurement heterogeneity for first two days (Period I) and last two days (Period II) of December 2015 (upper panel) and August 2017(lower panel) showing whole stem (SRC, black line), on-xylem (XRC, blue line) and calculated-inner bark (BRC, red dotted line) radius changes (RC) for Upper, Middle and Lower stem positions for Tree B of December 2015 and for Upper and Lower positions in Tree C and D of August 2017. Also the diurnal cycles of light (black line) and relative humidity (RH, blue dotted line)

measurement or after a few days (Figure 4.4, Table 4.4 & Table 4.5). During the measurement campaign of August 2017, XRC patterns were predominantly of Type III. There was a clear difference between timing of XRC and SRC maxima and minima, which lagged behind each other but were not opposed (SRC maxima did not coincide with XRC minima) and this was consistent between trees and stem tiers. On average, SRC_{min} occurred in the late hours of the evening (Figure 4.5, Figure 4.6 & Table 4.5), whilst XRC_{min} was reached in the early hours of the morning (Figure 4.5, Table 4.5). On average SRC_{max} was reached on average three hours before XRC_{max} (Figure 4.5, Table 4.5).

Table 4.5 Timing of peak and minimum radius change for whole-stem measurements (SRCmax and SRCmin) and on-xylem measurements (XRCmax and XRCmin)

Measuring period	Position (Tree ID)	SRCmax	XRCmax	SRCmin	XRCmin
July 2015	Upper (T-B)	12:30±3	11:00±7	01:00±2	18:00±16
	Middle (T-B)	13:00± 3	11:00±5	02:00±19	20:00± 17
	Lower (T-B)	13:00± 2	12:00±12	2:00±2	15:00±12
November 2015	Upper (T-B)	11:30± 3	11:00±4	00:00±20	23:00±22
	Middle (T-B)	12:00± 2	14:00±2	01:30±15	2:00±2
	Lower (T-B)	12:00± 2	19:00±9	2:00±2	11:00±6
December 2015	Upper (T-B)	9:00±3	8:00±4	00:00±18	20:00±3
	Middle (T-B)	12:00± 4	10:00±4	03:00±14	20:00±18
	Lower (T-B)	11:00± 3	5:00±19	3:00±2	13:00± 3.5
August 2017	Upper (T-C)	10:00 ±2	13:00±3	19:00 ±4.6	5:00 ±7.5
	Lower (T-C)	9:00 ±2	13:00±4	16:30 ±4	6:00 ±9.5
	Upper (T-D)	11:00 ±2	14:00±3	19:30 ±6	5:00 ±8
	Lower (T-D)	9:00 ±2	12:00±3.5	18:00 ±6	5:00 ±15

4.5 Discussion

4.5.1 Results reveal phloem-generated turgor signal behind daytime stem swelling

Simultaneous measurements of whole stem and xylem radius change are an important tool in understanding the origins and mechanisms that drive stem elastic water storage (Sevanto et al. 2011, Mencuccini et al. 2013, De Swaef et al. 2015, Pfautsch et al. 2015a, Chan et al. 2016). Not only do they provide insight into the contributions of inner bark and xylem tissues to whole stem radial change, but they can also provide information on relative source-sink strength, sugar loading and the contributions of osmotic potential induced changes to the total water potential of storage tissues (Mencuccini 2013, Chan 2016, Pfautsch 2015a).

My study shows that simultaneous xylem and whole stem radius measurements have the capacity to reveal the mechanisms behind daytime stem swelling patterns present in *A. marina* (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b, Donnellan Barraclough et al. 2018). Sensors recorded that the initiation of morning transpiration and leaf water potential drop was accompanied by the shrinking of xylem tissues, which was concurrent to whole stem radius increase. These results reveal an increase in inner-bark thickness which must be due to a turgor signal originating in the phloem. Phloem-generated turgor signals have been successfully modelled in the past through an osmotic component which decouples elastic storage tissue water potential from water pressure potential decrease in the xylem (Mencuccini 2013, Chan 2016, Pfautsch 2015a, Mencuccini 2017).

The daytime shrinking of the xylem concurrent to whole stem swelling recorded in this work (Type I pattern) is highly novel and is, to the author's knowledge, the first time such a pattern has been recorded in tree stems. My work also provides backing for the theory that osmoregulation in the phloem explains morning swelling in the stem of *A. marina* (Vandegehuchte 2014a, Vandegehuchte 2014b, Donnellan Barraclough et al. 2018). It is thought that loading and unloading of carbohydrates plays an important role in turgor changes of the phloem, and contributes a significant osmotic component to storage tissue water potential and thus SRC (Sevanto et al. 2002, 2003, De Schepper and Steppe 2010, Sevanto et al. 2011, De Schepper et al. 2013, Pfautsch et al. 2015b, Chan et al. 2016, Savage et al. 2016, Paljakka et al. 2017). The relationship found in this study of BRC with temperature and light in early summer back this notion, as it coincides with other work showing that the phloem generated turgor signal was highly correlated with environmental variables related to carbohydrate production (Mecuccini 2013, Chan 2016). In *A. marina* it appears that the phloem-generated turgor signal has a major effect on total stem radius, and BRC seemed to compensate for large decreases in xylem radius, allowing for a refilling of xylem tissues overnight. This explains unusual SRC patterns observed in this species, where night-time refilling of elastic water stores is often absent, an absence which could be due to xylem refilling at the cost of elastic water stores (Figure 4.6, Donnellan Barraclough et al. 2018). These results back the idea of the phloem as a major capacitator in tree stems (Pfautsch et al. 2015b). In fact, internal secondary phloem is thought to have an important role in water storage in *A. marina*, a theory confirmed by MRI studies on young *A. marina* saplings (Robert 2014).

Additional to carbohydrates, it is important to consider that other osmotic active substances may be playing a role in the osmotic adjustment of *A. marina* storage tissues, such as inorganic ions and other compatible solutes. A study by

López-Portillo et al. (2014) postulated that parenchyma cells “download” inorganic ions (mainly Na^+ and Cl^-) to the transpiration stream, in order to avoid cavitation due to extreme water potential gradients in the xylem and facilitate water transport from storage tissues, a mechanism described in *Arabidopsis* studies (Sunarpi et al. 2005). The study of López-Portillo et al. (2014) however, did not measure stem radius change and thus it seems unlikely that parenchyma tissues transport inorganic ions to the xylem at the same time as these elastic tissues are swelling. Therefore, the potential role in hydraulic security and embolism refilling in *A. marina* of daytime stem swelling, secondary internal phloem and osmolites (including both inorganic ions and non-structural carbohydrates dynamics) should also be a matter for further investigation, as strategies to survive the low water potential pressures experienced by this halophyte.

4.5.2 Reliability of Type I on-xylem patterns due to confirmation via other water-relations measurements and environmental correlations.

Xylem measurements showing morning tissue size decrease (Type I measurements) held a strong relationship to diel courses of sap-flow, leaf turgor and leaf water potential. Xylem shrinkage was recorded in the morning at the onset of transpiration, concurrent to leaf turgor and leaf water potential decrease. The shrinking of xylem tissues at the onset of transpiration is a pattern commonly recorded in the literature, as xylem water potential drops and water flows through the stem to supply transpiring leaves, explicable with a physical model analogue to the Ohm’s law (Scholander et al. 1965, Sevanto et al. 2002, Angeles et al. 2004, Holtta et al. 2006, Steppe et al. 2006, Pfautsch et al. 2015a, Pfautsch 2016). Although changes in thickness of the xylem are generally recorded to be lower than the characteristic contraction of the inner bark during daytime, this is species and stem position dependent (Zweifel et al. 2014, Pfautsch et al. 2015a). Thus, the volume changes in the xylem under a certain negative pressure are dependent on the elastic modulus and xylem hydraulic conductivity (Steppe et al. 2006, Baert et al. 2015, Pfautsch et al. 2015a). My results showed that xylem radial change was only slightly lower than whole stem and inner bark radius change in magnitude, suggesting a relatively high elasticity in the xylem of this species which could be partly explained with non-fully lignified xylem, as found for eucalyptus trees (Zweifel et al. 2014).

Xylem radial movements were also strongly correlated with environmental indicators of water supply and demand, such as precipitation, humidity and VPD. Thus, xylem fluctuations were larger in conditions of higher atmospheric demand, and became irregular during cloudy conditions or times of high precipitation. Xylem dynamics were very closely following the dynamics of leaf turgor, implying a strong hydraulic coupling between these tissues. Such a close hydraulic coupling between xylem and leaves is important to ensure leaf water supply for maintaining turgor, photosynthesis sugar loading and to prevent cavitation and is supported by several studies comparing pure physical soil-plant-atmosphere tree models with field measurements (Zweifel et al. 2000, Zweifel et al. 2005, Steppe et al. 2006).

4.5.3 The patchiness of measurements

Despite the detection of Type I XRC pattern over 82 days in different trees and stem locations, this study also confirms that the "patchiness" described for dendrometer measurements (Robert 2011a, Robert 2014) extends to on-xylem measurements. This heterogeneity is inherent to trees with a fish-net reticulate cambia like *A. marina* and highlights the difficulty of using this method on species with multiple reticulate cambia (Schmitz et al. 2008, Robert et al. 2011, Robert et. al. 2014). As dendrometers were changed and checked at each new measurement period, sensor failure cannot explain the heterogeneity in these results.

The backing of xylem patterns by other ecophysiological measures also rules out that there is no physiologically valid signal amongst the heterogeneity (Figure 4.1 & 4.2). Multiple reticulate cambia results in a complex network of non-cylindrical wood patches composed of xylem and internal secondary phloem. Robert et al. (2011) compared the wood structure of *A. marina* to a complex three-dimensional fishnet, where the thickness and pairing between patches of xylem and internal phloem changes within a few millimetres along the height of the tree. Research has shown that *A. marina's* hydraulic architecture causes heterogeneity in radial water movements measured with dendrometers, a variability which depends on the section of stem measured and which can change depending on the environmental conditions (Robert et al. 2014, Donnellan Barraclough et. al 2018). In a similar way Van de Wal et al. (2014) found heterogeneous sap-flow rates which varied significantly throughout the sapwood of *A. marina*. Thus, I propose that the variation in water transport processes measured over different wood patches must be due both to the high variation of the three dimensional organization itself and to the variation in the properties of xylem patches and the phloem patches which compose it, mainly their thickness, elasticity, age and hydraulic conductivity.

This study shows an array of different on-xylem patterns which have been classified depending on the similarity of maxima and minima timing to SRC measurements. I propose that the hydraulic architecture is responsible for heterogeneity in XRC measurements in one or more of the following ways. Firstly, where exactly the dendrometer piston lies on the measured tissue is not trivial. Robert et al (2011) describe the high complexity of the “fishnet” structure, where phloem bridges form to connect bands of outer-bark phloem to internal secondary phloem. Thus, even when peeling away the bark phloem, there is no guarantee that the sensor head rests on a band of xylem or in fact rests over one of the “phloem bridges”. If this were the case, then the signal would be that of an elastic tissue similar to the inner bark, which result in Type IV patterns. Secondly, under the assumption that a sensor head lies on a xylem strand, if this xylem section is thick or inelastic enough to mask thickness changes occurring in the phloem tissues beneath (assuming these occur), then it would show very different radius changes to a thin or elastic xylem strand. This is evident in some of my XRC Type I patterns, which showed opposed diel courses to SRC, which can only be possible if XRC is not affected by thickness changes of phloem strands beneath it or if there is no secondary phloem beneath (assuming these behave as the outer phloem). Thirdly, if xylem elasticity is high in a measured region, the xylem vessels may either show a different response compared to an inelastic vessel, or the sensor head may be measuring thickness changes of tissues beneath it. In these cases, BRC calculations are unreliable as a way of understanding the dynamics of the phloem, since there is no guarantee that XRC measurements carry only a xylem signal (Type III-IV patterns). In this manner the elasticity and thickness of the xylem, i.e. its capacity to deform under a certain pressure, may have a determining effect on the pattern which is detected by XRC measurements and should be measured in future studies.

Three-dimensional studies on reticulate wood structure of *A. marina* have also shown that the degree of branching and xylem to phloem ratios increase with tree height (Robert 2011). Although I could not find a systematic variation with height in my study (Figure 4.4 & 4.5), there is some anecdotal evidence since Type I XRC pattern (where xylem shrunk concomitantly to whole stem increase) was most often found in the lower stem. Future work should combine short-term XRC measurements with detailed histological analysis along the tree stem in order to understand the effect of tissue structure and properties on detected patterns. Additionally, if carbohydrate dynamics are indeed involved in osmoregulation, it seems reasonable to expect changes in the time-lags between xylem and phloem radius change as one nears the crown or across the seasons which should be studied in detail (Mencuccini et al. 2013, Chan et al. 2016, Donnellan Barraclough et al. 2018). Lastly, the similarity to SRC of some of the XRC patterns recorded here might point towards a water storage and depletion of the layers of secondary internal phloem. Despite the difficulties in studying internal secondary phloem, additional work, such as those using MRI techniques, are needed in order to understand whether the external bark phloem and the internal secondary phloem behave in a similar way.

Finally, the results of this Chapter could provide an additional explanation for the heterogeneity of whole SRC measurements (Robert 2014), as these contain two distinct signals, XRC and BRC, which can sometimes be opposed in direction. Thus, this begs the question on how reliable are whole stem SRC measurements, considering they can contain different contributions of opposing XRC and BRC depending on the structure of the wood under the dendrometer measuring location. However, SRC still is the total sum of forces affecting radial water movement, and correlations to environmental conditions found in Chapter 2 show it is still a physiologically significant measure.

4.6 Conclusion

This work presents the first direct evidence of the exact mechanisms behind daytime stem swelling, by measuring thickness increases in the elastic tissues of the inner bark concurrent to daytime xylem shrinkage. Such a pattern provides support for the theories of osmotic adjustment in storage tissues of *A. marina* having a role in daytime stem swelling (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b). It is reasonable to assume that the inner bark measurements presented here are due to a phloem generated turgor signal caused by phloem loading and unloading processes, and considerable evidence points towards the importance of sugar as osmolites in the phloem of *A. marina* (Donnellan Barraclough et al 2018). Lastly, my work also shows that daytime inner bark swelling in *A. marina* was related to conditions of atmospheric water demand, and thus reticulate cambia and daytime stem swelling likely have a role in guaranteeing hydraulic safety. My results constitute an important advancement in revealing the role of the phloem within stem water relations. Further work should address the possible role of phloem-generated turgor signals in maintaining hydraulic safety in other species, especially those with internal secondary phloem and patterns of daytime stem swelling.

4.7 References

Angeles, G., B. Bond, J. S. Boyer, T. Brodribb, J. Renée Brooks, M. J. Burns, J. Cavender-Bares, M. Clearwater, H. Cochard, J. Comstock, S. D. Davis, J.-S. Domec, L. Donovan, F. Ewers, B. Gartner, U. Hacke, T. Hinckley, N. M. Holbrook, H. G. Jones, K. Kavanagh, B. Law, J. López-Portillo, C. Lovisolo, T. Martin, J. Martínez-Vilalta, S. Mayr, F. C. Meinzer, P. Melcher, M. Mencuccini, S. Mulkey, A. Nardini, H. S. Neufeld, J. Passioura, W. T. Pockman, R. B. Pratt, S. Rambal, H. Richter, L. Sack, S. Salleo, A. Schubert, P. Schulte, J. P. Sparks, J. Sperry, R. Teskey, and M. Tyree. 2004. The cohesion-tension theory. New Phytologist **163**:451-452.

Baert, A., V. De Schepper, and K. Steppe. 2015. Variable hydraulic resistances and their impact on plant drought response modelling. Tree Physiology **35**:439-449.

Chan, T., T. Holtta, F. Berninger, H. Makinen, P. Nojd, M. Mencuccini, and E. Nikinmaa. 2016. Separating water-potential induced swelling and shrinking from measured radial stem variations reveals a cambial growth and osmotic concentration signal. Plant Cell and Environment **39**:233-244.

De Schepper, V., T. De Swaef, I. Bauweraerts, and K. Steppe. 2013. Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany **64**:4839-4850.

De Schepper, V. and K. Steppe. 2010. Development and verification of a water and sugar transport model using measured stem diameter variations. Journal of Experimental Botany **61**:2083-2099.

De Swaef, T., V. De Schepper, M. W. Vandegehuchte, and K. Steppe. 2015. Stem diameter variations as a versatile research tool in ecophysiology. Tree Physiology **35**:1047-1061.

Deslauriers, A., S. Rossi, A. Turcotte, H. Morin, and C. Krause. 2011. A three-step procedure in SAS to analyze the time series from automatic dendrometers. Dendrochronologia **29**:151-161.

Donnellan Barraclough, A., R. Zweifel, J. Cusens, and S. Leuzinger. 2018. Daytime stem swelling and seasonal reversal in the peristaltic depletion of stored water along the stem of Avicennia marina (Forssk.) Vierh. Tree Physiol.

Drew, D. M. and G. M. Downes. 2009. The use of precision dendrometers in research on daily stem size and wood property variation: a review. Dendrochronologia **27**:159-172.

Herzog, K. M., R. Häsler, and R. Thum. 1995. Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees **10**:94-101.

Holtta, T., T. Vesala, S. Sevanto, M. Peramaki, and E. Nikinmaa. 2006. Modeling xylem and phloem water flows in trees according to cohesion theory and Munch hypothesis. Trees-Structure and Function **20**:67-78.

López-Portillo, J., F. W. Ewers, R. Méndez-Alonzo, C. L. Paredes López, G. Angeles, A. L. Alarcón Jiménez, A. L. Lara-Domínguez, and M. d. C. Torres Barrera. 2014. Dynamic control of osmolality and ionic composition of the xylem sap in two mangrove species. American Journal of Botany.

McIntyre, D. S. 1980. Basic relationships for salinity evaluation from measurements on soil solution. Autralian Journal of Soil Research **18**:199-206.

Mencuccini, M., T. Holtta, S. Sevanto, and E. Nikinmaa. 2013. Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytologist **198**:1143-1154.

Mencuccini, M., Y. Salmon, P. Mitchell, T. Hölttä, B. Choat, P. Meir, A. O'Grady, D. Tissue, R. Zweifel, S. Sevanto, and S. Pfautsch. 2017. An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant, Cell & Environment **40**:290-303.

Paljakka, T., T. Jyske, A. Lintunen, H. Aaltonen, E. Nikinmaa, and T. Holtta. 2017. Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies L. Karst). Plant Cell and Environment **40**:2160-2173.

Pfautsch, S. 2016. Hydraulic Anatomy and Function of Trees—Basics and Critical Developments. Current Forestry Reports **2**:236-248.

Pfautsch, S., T. Hölttä, and M. Mencuccini. 2015a. Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology **35**:706-722.

Pfautsch, S., J. Renard, M. G. Tjoelker, and A. Salih. 2015b. Phloem as Capacitor: Radial Transfer of Water into Xylem of Tree Stems Occurs via Symplastic Transport in Ray Parenchyma. Plant Physiology **167**:963-971.

Robert, E. M. R., A. H. Jambia, N. Schmitz, D. J. R. De Ryck, J. De Mey, J. G. Kairo, F. Dahdouh-Guebas, H. Beeckman, and N. Koedam. 2014. How to catch the patch? A dendrometer study of the radial increment through successive cambia in the mangrove Avicennia marina. Annals of Botany **113**:741-752.

Robert, E. M. R., N. Koedam, H. Beeckman, and N. Schmitz. 2009. A safe hydraulic architecture as wood anatomical explanation for the difference in distribution of the mangroves Avicennia and Rhizophora. Functional Ecology **23**:649-657.

Robert, E. M. R., N. Schmitz, I. Boeren, T. Driessens, K. Herremans, J. De Mey, E. Van de Casteele, H. Beeckman, and N. Koedam. 2011a. Successive Cambia: A Developmental Oddity or an Adaptive Structure? Plos One **6**.

Robert, E. M. R., N. Schmitz, J. A. Okello, I. Boeren, H. Beeckman, and N. Koedam. 2011c. Mangrove growth rings: fact or fiction? Trees **25**:49-58.

Savage, J. A., M. J. Clearwater, D. F. Haines, T. Klein, M. Mencuccini, S. Sevanto, R. Turgeon, and C. Zhang. 2016. Allocation, stress tolerance and carbon transport in plants: how does phloem physiology affect plant ecology? Plant Cell and Environment **39**:709-725.

Schmitz, N., E. M. R. Robert, A. Verheyden, J. G. Kairo, H. Beeckman, and N. Koedam. 2008. A patchy growth via successive and simultaneous cambia: Key to success of the most widespread mangrove species Avicennia marina? Annals of Botany **101**:49-58.

Scholander, P. F., E. D. Bradstreet, E. A. Hemmingsen, and H. T. Hammel. 1965. Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science **148**:339-346.

Sevanto, S., T. Holtta, and N. M. Holbrook. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant Cell and Environment **34**:690-703.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2002. Time lags for xylem and stem diameter variations in a Scots pine tree. Plant Cell and Environment **25**:1071-1077.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2003. Sugar transport together with environmental conditions controls time lags between xylem and stem diameter changes. Plant Cell and Environment **26**:1257-1265.

Steppe, K., D. J. W. De Pauw, R. Lemeur, and P. A. Vanrolleghem. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257-273.

Sunarpi, T. Horie, J. Motoda, M. Kubo, H. Yang, K. Yoda, R. Horie, W. Y. Chan, H. Y. Leung, K. Hattori, M. Konomi, M. Osumi, M. Yamagami, J. I. Schroeder, and N. Uozumi. 2005. Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na+ unloading from xylem vessels to xylem parenchyma cells. Plant Journal **44**:928-938.

Van de Wal, B. A. E., A. Guyot, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014. Influence of temporospatial variation in sap flux density on estimates of whole-tree water use in Avicennia marina. Trees **29**:215-222.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, S. R. E. De Groote, N. J. F. De Baerdemaeker, M. Hayes, N. Welti, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014a. Long-term versus daily stem diameter variation in co-occurring mangrove species: environmental versus ecophysiological drivers. Agricultural and Forest Meteorology **192**:51-58.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, S. R. E. De Groote, N. J. F. H. De Baerdemaeker, M., N. Welti, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014b. Long-term versus daily stem diameter variation in co-occurring mangrove species: environmental versus ecophysiological drivers. Agricultural and Forest Meteorology **192**:51-58.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, T. De Swaef, D. A. Lockington, and K. Steppe. 2014c. Modelling reveals endogenous osmotic adaptation of storage tissue water potential as an important driver determining different stem diameter variation patterns in the mangrove species Avicennia marina and Rhizophora stylosa. Annals of Botany.

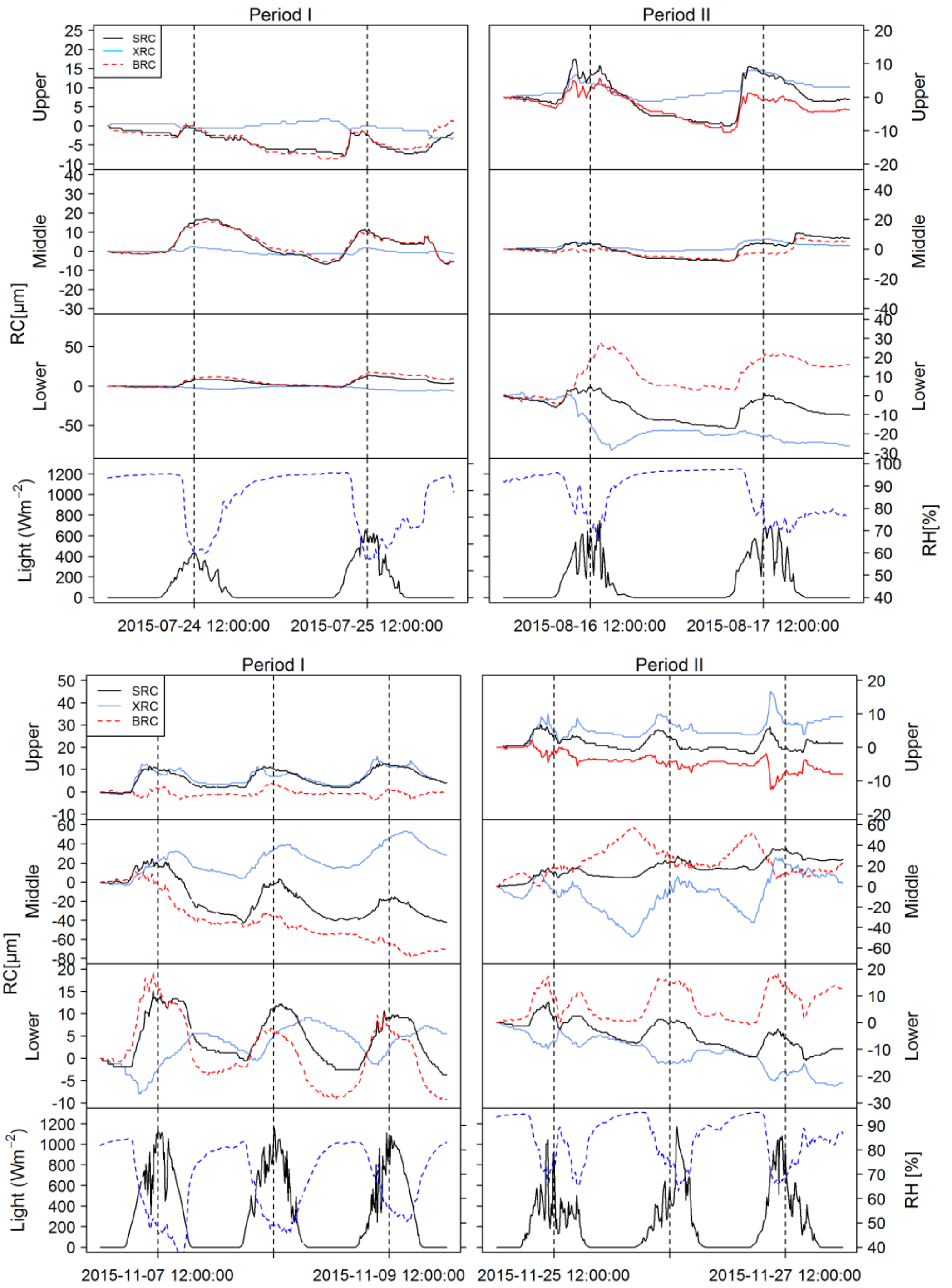
Woodruff, D. R. and F. C. Meinzer. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant, Cell & Environment **34**:1920-1930.

Zweifel, R., D. M. Drew, F. Schweingruber, and G. M. Downes. 2014. Xylem as the main origin of stem radius changes in Eucalyptus. Functional Plant Biology **41**:520-534.

Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees **15**:50-57.

Zweifel, R., L. Zimmermann, and D. M. Newbery. 2005. Modeling tree water deficit from microclimate: an approach to quantifying drought stress. Tree Physiology **25**:147-156.

4.8 Supplementary Information



Supplementary Figure 4.1 Example of measurement heterogeneity for Tree B first three days (Period I) and last three days (Period II) of July-August 2015 (upper panel) and November 2015 (lower panel) measurement period showing whole stem (SRC, black line), on-xylem (XRC, blue line) and calculated-inner bark (BRC, red dashed line) radius changes (RC) for Upper, Middle and Lower tiers. Also shown are the diurnal cycles of light (black line) and relative humidity (RH, blue dashed line)

Chapter 5 - No leaf is an island: using turgor pressure probes to study the relationship of leaf turgor to microclimate and water relations in *Avicennia marina* (Forssk.) Vierh.

> *"My roots go down to the depths of the world, through earth dry with brick, and damp earth, through veins of lead and silver. I am all fibre. All tremors shake me, and the weight of the earth is pressed to my ribs. Up here my eyes are green leaves, unseeing."*
>
> *Virginia Woolf – The Waves*

5.1 Abstract

Leaves are the central hub of water and carbon exchange in plants. Despite the inevitable loss of water to transpiration, leaves must maintain a level of hydration and turgor which supports carbon dioxide uptake through stomata. In this chapter, I investigate short and long term dynamics of leaf turgor in *Avicennia marina* with high temporal resolution leaf patch pressure probes. My work shows that patch pressure measured by pressure probes provides a useful measure of leaf water status which is highly correlated with leaf water potential. Leaf patch pressure measured at a 10-minute resolution correlated with environmental drivers of transpiration and stomatal regulation, such as light, temperature and vapour pressure deficit. Precipitation was a strong driver of leaf turgor in *A. marina* both in the long and the short term. Small precipitation events during the day increased leaf turgor pressure, and the presence of precipitation at a seasonal scale increased the amplitude of leaf turgor fluctuations. These results support recent work showing mangrove reliance on fresh water inputs due both to increased soil water availability and potential atmospheric water uptake through leaves. My results also seem show that the internal controls behind day time stem swelling in *A. marina* temporarily override the needs of transpiring leaves, and that this causes delayed recovery of leaf turgor in the evenings. In particular, the lower stem seemed to respond more sensitively to water demands of leaf transpiration than the upper stem, and the time-lag between lower stem water mobilization and leaf turgor recovery followed VPD closely throughout the year.

5.2 Introduction

As early as 1896, H.H. Dixon proposed that the force pulling water up through trees is generated when water evaporates from plant leaves. This idea later became the basis of the Cohesion-Tension Theory (CTT). The CTT states that the high level of cohesion between molecules, together with adhesion to a plant's conduit walls, assures formation of continuous water columns within plants' vascular system (Tyree 1997, Angeles et al. 2004). These continuous water columns are subjected to negative pressures, or tensions, generated by the evaporation of water from leaf surfaces through tiny pores called stomata. In 1948, Van den Honert quantitatively described water flux through plants as a catenary process, establishing parallels with Ohm's law, applied in electrical systems. A plant's roots, xylem and leaves, together with soil and leaf boundary layer, become resistances to water flow, and in our "plant circuit" capacitances are represented by the ability of soil and plant tissues to store water. Water potential gradients within a tree caused by differences in the water potential between the soil and the atmosphere drive the stream of water up the xylem, facilitating long distance transport of water and solutes throughout the plant (Tyree 1997, Meinzer et al. 2001, Tyree and Zimmermann 2002, Steppe et al. 2006, Zweifel et al. 2007, Pfautsch 2016). Water which is stored in the stems, especially in the softer elastic tissues, is able to counterbalance temporal water deficits caused by low soil or atmospheric water potentials in a flux known to cause measurable stem radius changes (SRC) (Herzog et al. 1995, Zweifel et al. 2000, Deslauriers et al. 2003, Daudet et al. 2005, Steppe et al. 2006, Cermak et al. 2007, Sevanto et al. 2011, Pfautsch et al. 2015). In this “plant circuit” framework, leaves constitute the final stop-over before the atmosphere, where water is lost to transpiration.

The gatekeepers that wait at the leaves, marking the end of water's pathway through the plant to the atmosphere, are the stomata, where the bulk of CO_2 uptake and water loss takes place. Stomatal aperture can actively regulate transpirational water loss and thus plant water uptake, flux and storage. Stomata must minimize water loss whilst maximizing carbon uptake for photosynthesis and growth (Buckley and Mott 2013). Stomatal response to environmental signals occurs through changing stomatal aperture, by changes in turgor of the cells that make up the stomatal pore, the guard and epithelial cells. These changes in aperture modify stomatal conductance (mmol m^{-2} s^{-1}) to CO_2 and water vapour for a given boundary layer conductance (Buckley 2005). Stomatal guard and epithelial cells are known to respond predominantly to light, CO_2, temperature, water availability, soil dryness and wind speed. Though the metabolic mechanisms by which stomatal guard cells change turgor are relatively well described (Sirichandra et al. 2009) the complex response to multiple environmental and internal factors is not fully understood (Merilo et al. 2014). The level of stomatal control in a species is part of determining water loss and risk of cavitation, nutrient uptake via promotion of mass flow, regulation of leaf turgor and temperature, resistance to heat stress, and carbon assimilation (Haworth et al. 2011).

As the last port before the atmosphere, understanding leaf hydration and turgor is essential to understanding tree water relations and carbon uptake. Although stomatal aperture and leaf turgor are intimately linked through the effect of transpirational water loss and soil water availability, many of the feedback loops controlling this relationship are not fully understood and the relationship is not always clear (Zweifel et al. 2007, Schachtman and Goodger 2008, Dodd 2013, Pantin et al. 2013, Merilo et al. 2014). Overall leaf turgor is a reflection of the hydration status of the cells that make up the leaf, which in angiosperm trees is usually a palisade mesophyll and spongy mesophyll, tissues that are made up of photosynthetically active palisade cells. The spongy mesophyll allows for large air spaces, especially in the vicinity of stomata, where gas exchange takes place. The vascular tissue of each leaf is a continuation of the vascular tissue of the stem, and these vascular bundles extend in the form of veins dividing amongst the mesophyll (Chen et al. 1973, Clearwater and Meinzer 2001). Within these bundles, there is intimate contact between the xylem and the phloem, with the latter collecting sugars from photosynthetic tissue in a process known as phloem loading ready to distribute it to the rest of the plant (Turgeon 1996, Oparka and Turgeon 1999, Rennie and Turgeon 2009, Turgeon and Wolf 2009, De Schepper et al. 2013, Savage et al. 2016). Thus, maintaining leaf turgor and flow of water between xylem and phloem is essential for maintaining the aforementioned functions of gas exchange, photosynthesis and sugar export. In fact, recognizing cell turgor loss is thought to be one of the classical indicators of plant water stress, as it has such clear impacts on cellular structural integrity, metabolism and whole-plant performance (Breshears et al. 2008, McDowell et al. 2008, Bartlett et al. 2012). One of these derived traits is for example the leaf water potential at turgor loss, or bulk turgor loss point, which has been used to assess physiological drought tolerance for decades (Bartlett 2012 and references therein).

A novel method for non-invasively measuring leaf turgor has been introduced in recent years (Zimmermann et al. 2007, Zimmermann et al. 2008). This instrument is a leaf-patch clamp pressure probe (known by the commercial name of ZIM probe) which detects a pressure signal from the leaf (P_p) that is inversely correlated with leaf turgor pressure. This method has been tested on oak and olive trees, maize, and wheat, amongst others, and has been shown to detect plant responses to changing microclimate, low water availability, and to be of potential use, for example, in irrigation scheduling (Zimmermann et al. 2008, Westhoff et al. 2009, Fernandez et al. 2011, Ehrenberger et al. 2012a, Ehrenberger et al. 2012b, Rodriguez-Dominguez et al. 2012, Bader et al. 2014, Aissaoui et al. 2016, Martinez-Gimeno et al. 2017). Recent work also investigated the relationship between measured P_p and stem radius change, showing that there are insights to be gained on the hydraulic relationship between stem and leaves from the use of this technology (Ehrenberger et al. 2012a). Despite the mounting evidence of their reliability, turgor pressure probes are not widely used; in some cases neglected and in others dismissed, some of the mistrust surrounding these probes may be understood in the context of the controversy around the theories of Zimmermann on the CTT (Meinzer et al. 2001, Zimmermann et al. 2004).

Living in salty waters, there are still many unknowns as to how *Avicennia marina* (Forssk.) Vierh experiences changes in salinity and water availability, in what is already an extreme environment. At the leaf level, *A. marina* has specialized anatomical and physiological features as a result of adaptations to its halophytic home. Many of its leaf hydraulic and photosynthetic traits are related to its salt management strategy (Jiang et al. 2017). Although *A. marina* excludes most salt at the root level, it can also excrete salt at the leaf level through specialized salt glands, in a process facilitated by abundant trichomes (hairs) on leaf abaxial surfaces (Reef and Lovelock 2015). Accumulation of salt in leaves can lead to succulence or accumulation in senescent leaves and subsequent abscission (Zheng et al. 1999). Leaf salt accumulation in cell vacuoles is facilitated by compatible solutes in the cytoplasm (e.g. Parida et al. 2004).

Additionally, more recent research points towards the existence of specialized extracellular structures in *A. marina* leaves, which may have a role in foliar water retention (Nguyen et al. 2017b). Despite extensive knowledge on *A. marina* leaf traits, comparatively little is known about high resolution *A. marina* leaf hydration and turgor dynamics in the field, and how they are influenced by microclimate. Some of this work suggests that freshwater inputs and atmospherically derived water are essential for achieving full leaf hydration (Uddin 2014, Lovelock et al. 2017, Nguyen et al. 2017a). Understanding leaf hydration is also important as recent research has shown that leaf water storage in *A. marina* saplings can be an essential water store in times of low supply or high salinity (Lechthaler et al. 2016, Nguyen et al. 2017a, Nguyen et al. 2017b). Real-time leaf turgor changes have never been studied in *A. marina,* and such work might help reveal the effect on leaf hydration of changing environmental conditions and, more specifically, freshwater inputs. Additionally, as turgor pressure probes have also proven useful in helping understand the balance and dynamics of water depletion and storage in trees (Ehrenberger et al. 2012a), they might help elucidate the consequence of daytime stem swelling for diel leaf hydration dynamics in *A. marina* and the nature of the hydraulic connection between the elastic water stores in the stem and leaves (Chapter 3 of this thesis).

In this study, I wish to complement my previous work on *A. marina* water relations with information on leaf turgor dynamics as measured by leaf-patch clamp pressure probes. My questions are:

1. Are turgor pressure probes a reliable tool for measuring leaf water dynamics in *A. marina*? How do measured changes in turgor pressure relate to other

water-related processes within the tree, such as leaf water potential, stomatal conductance, sap flow and stem radius change?

2. What are the main environmental drivers of leaf turgor changes at hourly and day-to-day resolutions? More specifically, do fresh water inputs through precipitation have a measureable effect on leaf turgor in *A. marina*?
3. What do the time-lags between stem water storage mobilization and leaf turgor fluctuations tell us about the effect of diurnal stem swelling on leaf turgor dynamics in *A. marina*? Are these time-lags influenced by environmental conditions as well as osmoregulation?

5.3 Material and Methods

5.3.1 Site and microclimate monitoring

The study took place over a two year period from August 2014 until December 2016 at the research site in the Mangawhai estuary, 100 km north of Auckland, New Zealand (36.097°S, 174.573°E). The site is described in full in Chapter 2 of this thesis. Briefly, it is located at the upper tidal zone of an estuary with approximately 80 ha of mangrove forest. The site has a sandy substrate and short inundation times, twice every 24 hours. The stand is monospecific and homogeneous, composed of *Avicennia marina* individuals 3-4 m in height. During the measurement period, the weather followed patterns usual to the temperate climate of the north of New Zealand's North-Island (Appendix Figure 1). The month of November 2015 was characterized by zero precipitation, an event which had not been recorded in the area for at least 10 years (see Chapter 2 for more details). During the measurement period, pore water salinity was measured four times at a depth of 30 cm, and ranged from 26‰ to 39‰, with an average of 31‰.

A weather station was installed within the canopy to measure microclimate during the two year study period, described in full in Chapter 2 of this thesis. Briefly, loggers recorded temperature and relative humidity (RH) (Model SHT, Sensirion, Stäfa, Switzerland) which allowed calculation of Vapour Pressure Deficit (VPD) according to Buck (1981). Solar irradiance (I [W m^{-2}], 380–1120 nm) was measured with a pyranometer (Model PYR, Decagon Devices, WA, USA). Rainfall was measured with a tipping-bucket rain gauge (Model 52202, R. M. Young Company, Traverse City, Michigan, USA). Tidal inundation (WD [cm]), soil-water temperature (SWT [°C]) and soil-water electrical conductivity (EC [µS/cm]) were measured with a CTD sensor (CTD-10, Decagon Devices, WA, USA). Osmotic potential (Ψ_{Π} [MPa]) of the soil and water was calculated using soil-water electrical conductivity and temperature following McIntyre (1980). All instruments logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland). Any gaps in meteorological measurements during this period were filled in with data from a nearby weather station (<10km).

5.3.2 Ecophysiological data

Continuous physiological measurements were performed on three mature co-dominant trees from August 2014 until July 2015 and reduced to two trees from July 2015 until the end of the experiment (tree details in Chapter 2). Physiological measurements and instruments are described in detail in Chapter 1-3 of this thesis. Briefly, point dendrometers measured stem radius change (SRC) at a µm resolution. Three dendrometers were installed in each of the study trees, evenly spaced along the stem, the first 90cm above ground level and the third below branching of the crown (190-260 cm). Sap flow rate was measured using heat balance gauges (Dynamax, Houston, TX, USA), based on the heat balance method (HB) (Smith and Allen 1996). All instruments logged at 10 min intervals via a wireless logging system (DecentLab GmbH, Dübendorf, Switzerland). Water potential and stomatal conductance measurements were taken during winter (August 4-6 2014) and summer (February 11, 16-17 2015) in 5 trees in the immediate vicinity (<20 m) of the three main study trees. Leaf water potential (ψleaf [MPa]) was measured with a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) following the usual procedure of measuring leaves immediately after excision (Scholander et al. 1965). Three to five leaves were measured then averaged per recording and tree. This was done for each tree approximately every hour from pre-dawn until midday (winter) or until dusk (summer). Leaf stomatal conductance (g_s [mmol/ m^2 s]) was obtained via a steady state porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA) from early morning until evening.

5.3.3 Turgor pressure probes

Turgor pressure probes were installed (YARA ZIM Plant Technology, Germany), three of them in each of the study trees, dispersed amongst the upper and lower canopy. Turgor pressure probes (YARA ZIM Plant Technology GmbH) are minimally invasive and continuously measure leaf patch pressure, which is correlated with leaf turgor. Probes are a pair of magnets with a diameter of 10 mm clamped to a leaf, one of which contains a pressure sensor. The magnets apply a constant clamp pressure (P_{clamp}), so that the pressure sensor is able to detect relative changes in the pressure under the measured leaf patch. The output pressure signal is called patch pressure (P_p [kPa]) and it is inversely correlated with leaf turgor pressure, e.g. when the leaf dehydrates during stomatal opening, leaf turgor decreases and P_p increases, and vice versa. The mathematical procedure for validating and calculating leaf balancing patch pressure from pressure probes output has been described elsewhere (Zimmermann et al. 2008, Bader et al. 2014). The probe signals were logged every 10 minutes, and transmitted via ZIM wireless transmitters to a controller which sent the data to an internet server. Three probes were installed, dispersed within the canopy of each of the three study trees. Over the two year study (2014-2015) measuring periods had a maximum duration of three months at a time, after which the probes were removed for cleaning and maintenance, due to the harshly oxidising conditions in the mangrove forest, and then put out again. Thus, probes were deployed during (1) August 2014, (2) September and October 2014, (3) November and December 2014, (4) February and March 2015, (5) April and May 2015, (7) June to September 2015 (8) October 2015 to December 2015. For the last period, wireless ZIM loggers failed during November 2015 and no turgor probe measurements were collected until loggers were charged and signals restarted.

5.3.4 Calculations

Previous research shows that P_p is a relative measure, and that P_p change (and thus P_p amplitude) can be affected by P_{clamp} applied during a particular installation (Bramley 2013). Thus, before analysis, the P_p time-series for each measuring period (time periods stated above) was standardized using an approach similar to Bader et al. (2014). Patch pressure values were standardized (P_p*) (*Equation 1*) by considering the first measured P_p at the start of the measurement

($P_{p\ (t0)}$) as P_p =0, and then dividing P_p values either by the daily or monthly maximum P_p value ($P_{p\ \max}$), for hourly and inter-diel analysis, respectively:

$$Equation\ 1:\ Pp^{*}(t) = \frac{Pp(t) - Pp(t_0)}{Pp_{max}}$$

Because under certain conditions (extreme drought, cavitation or irreversible leaf turgor loss) patch pressure is not always inversely proportional to leaf turgor, I follow the convention of reporting P_p* (Zimmerman 2008). However for clarity of graph visualization some figures show the inverse of P_p* (1/P_p*) in order to show a trend which is directly proportional to leaf turgor.

The time of daily minimum and maximum P_p* was extracted for each 24 h period (from midnight to following midnight). Times of stem radius change (SRC) minima and maxima were extracted via the stem cycle method as described in Chapter 2. A time lag (Time-lag P_p – $\mathrm{SRC_{max}}$) in hours was calculated between the time of day of peak SRC (maximum stem radius) and peak P_p* (minimum leaf turgor) in order to obtain the time taken from the start of water mobilization in the stem to the beginning of leaf turgor recovery.

Due to the large number of fluctuations in a diel P_p curve, I choose to analyse the area under the curve as a proxy of the amplitude of P_p diel courses. The area under a 24 hour diel course (from midnight to midnight) of a P_p curve (AUC) was calculated using the natural spline interpolation method via the *auc* function of the *MESS* package of the software R (Ekstrom 2017). Each day’s AUC was calculated by setting midnight P_p to P_p=0. For statistical analysis comparing daily AUC values on a monthly scale (see below), AUC was normalized every 30 days as in *Equation 1* by replacing Pp (t) by daily AUC and diving it by maximum monthly AUC. All calculations and statistical procedures were done in R Core Team (2017).

5.3.5 Statistics

Hourly scale (also called intra-diel or within day) drivers of P_p* were analysed through multiple correlations performed on data at a 10 minute resolution for a period of 10 days in summer (28 February–9 March 2015) and 10 days in winter (5–10 June 2015). These periods were chosen as they presented high quality data for all sensors. Individual correlations were performed for each tree and each day, and the mean correlation of all three trees and of all 10 days is reported.

All measurement periods across 2014 to 2015 were included in an analysis of *Pp* at a yearly scale. To ensure the validity of different approaches, linear mixed effects models (LME) were performed on daily AUC calculated as both (1) normalized AUC for all measuring periods and (2) for each individual measuring period for non-normalized AUC (results shown in Supplementary information). Time-series autocorrelation was accounted for with an ARMA correlation structure with an autoregressive parameter φ established by the day of the year. The model was weighted by normalization time periods (every 30 days) to account for group heteroscedasticity caused by possible installation differences between sampling periods. The turgor pressure probe used for each measurement was set as random factor. The model with the optimum residual diagnostic plots and lowest Akaike information criterion (AIC) was chosen.

5.4 Results

5.4.1 Are turgor pressure probes reliable in *A. marina*? Dynamics of P_p in relation to the other water-related physiological processes.

Diurnal cycles of turgor pressure probe measurements throughout the year showed that P_p readings were highly correlated with other measures of plant water status (Figure 5.1). Even at very short time scales, leaf water potential fluctuations (Ψ_{leaf}) were closely mirrored by P_p measurements (Figure 5.1b and Figure 5.2). This coupling was not as strong in SRC measurements.

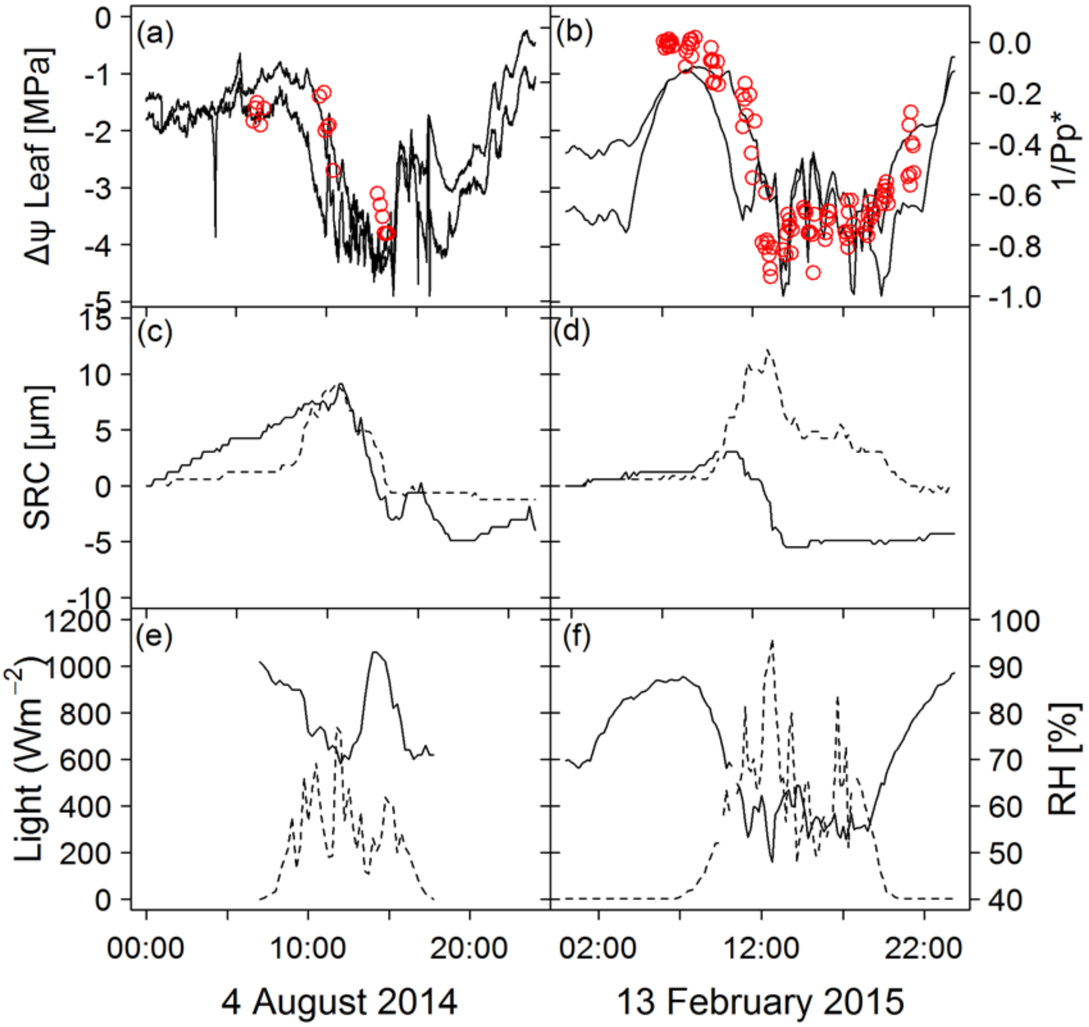


Figure 5.1 Summer and winter diurnal cycles of leaf water potential measurements [Δψ] (red dots) for all five measured trees (n=5) together with example Tree A leaf turgor (black lines) expressed as the inverse of patch pressure (a-b) together with SRC for upper (dashed line) and lower (black line) tree ties (c-d) and diurnal course of relative humidity [RH] (black line) and light (dashed line) (e-f)

When plotting P_p * against all other water related physiological measurements for an average summer day (Figure 5.2), a significant linear regression was strongest with Ψ_{leaf} (Ψ_{leaf}: $R^2 = 0.71$, p-value ≤ 0.01), with low P_p* (high leaf turgor) associated with less negative Ψ_{leaf}. Stomatal conductance also showed high g_s values associated with low P_p* (and thus high turgor) although the relationship was not as strong ($g_{s:}$ $R^2 = 0.2$, p-value ≤ 0.05). The relationship between P_p* and sap flow was not linear and there was a clear hysteresis in play (Figure 5.2b). A hysteresis effect was also apparent in the relationship between P_p* and SRC, with a marked difference in the relationship shape and strength between stem tiers, with a significant linear negative correlation between P_p* and the lower tier (SRC_{lower}: $R^2 = 0.55$, p-value ≤ 0.01) with reduced SRC associated to high P_p*, but no significant linear relationship with upper stem tier (SRC_{upper}: $R^2 = 0.15$, p-value ≥ 0.05).

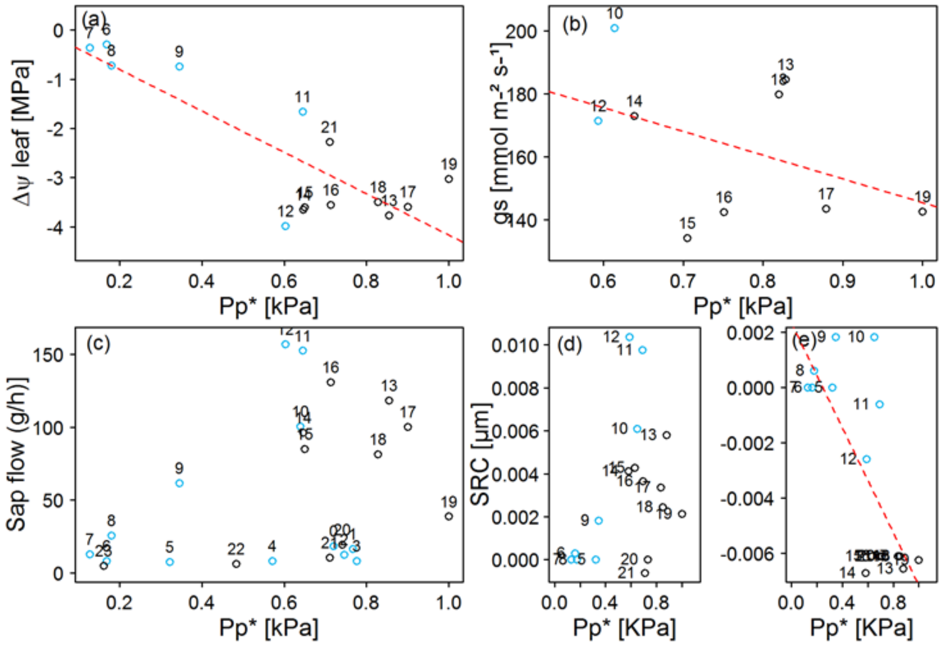


Figure 5.2 Relationships between leaf balancing pressure (P_p*) and water related physiological measurements: water potential (a), stomatal conductance (b), branch sap flow (c) and tree water deficit for the upper (d) and lower (e) stem, for an example day in summer 13th February 2015. Time of day is indicated with a label number and circle colour, with morning values up to and including midday (blue) to afternoon values (black). Red lines indicate significant linear regressions. Points represent the mean of all measured trees (Pp, SRC: n=3; ψleaf and gs: n=5)

The correlation between P_p and Ψ_{leaf} was maintained at an inter-daily scale (Figure 5.3), where the amplitude of P_p* (scaled at a monthly level) followed the difference between predawn Ψ_{leaf} and midday Ψ_{leaf}, although this relationship was non-significant ($R^2 = 0.3$, p-value ≥ 0.05).

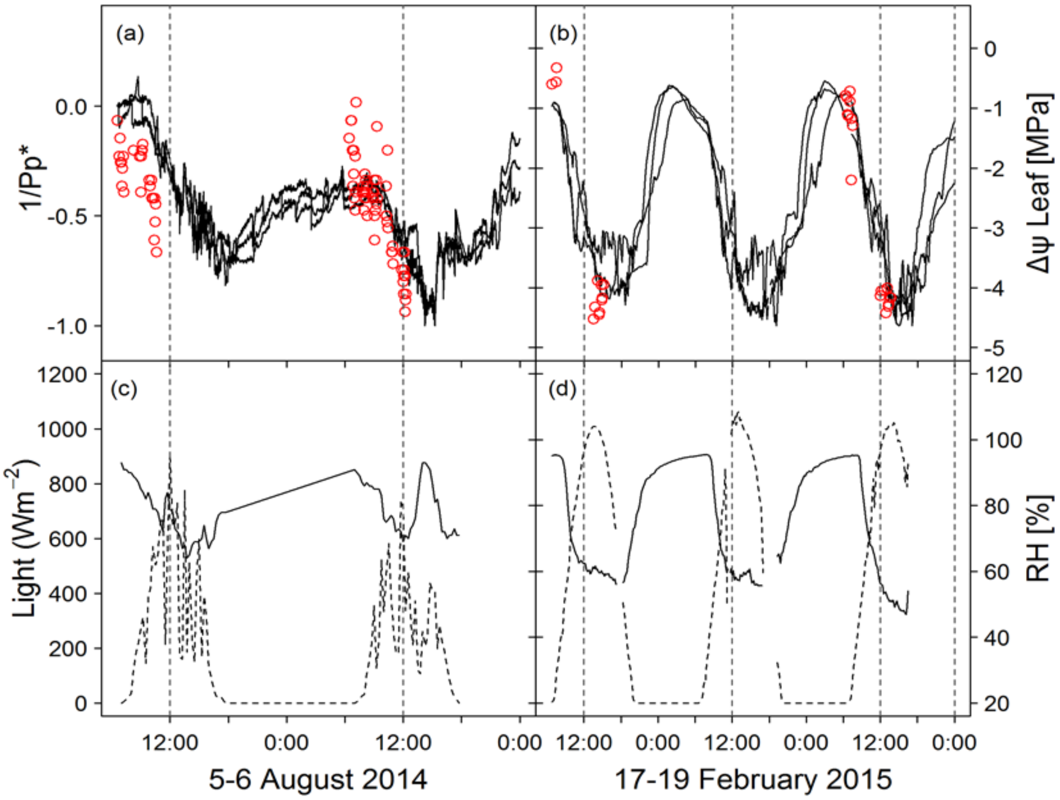


Figure 5.3 Leaf turgor expressed as the standardized inverse of patch pressure (1/P_p*) standardized over two (a) or three (b) days for each Tree A-C, together with leaf water potential (Δψ) (red circles) measured from pre-dawn till midday on five trees (n=5) for two consecutive days in winter (a) and summer (b) together with light and relative humidity (RH) (c-d).

5.4.2 Environmental drivers of P_p at hourly and to monthly scales and effect on leaf turgor of fresh-water inputs through precipitation events

a. *Environmental correlates of hourly intra-diel P_p*

At a daily 10 minute resolution during an average sunny summer day, P_p* was significantly correlated with T ($R^2 = 0.82$, p-value ≤ 0.01), RH ($R^2 = 0.85$, p-value ≤ 0.01) and VPD ($R^2 = 0.84$, p-value ≤ 0.01) (Figure 5.2 a, c ,d). There was also a strong correlation with light ($R^2 = 0.45$, p-value ≤ 0.01) during the morning hours which became less strong in the evening, due to a lag between dropping light levels and lowering P_p* (Figure 5.4). There was no apparent correlation with soil water potential and tidal water depth, although there was a sharp drop in soil water potential which coincided with a drop in P_p*. There was a clear hysteresis effect in the correlations from morning to afternoon which was especially notable in T, Light, RH and VPD, revealing delayed recovery of P_p* values (Figure 5.4, blue versus black dots). The

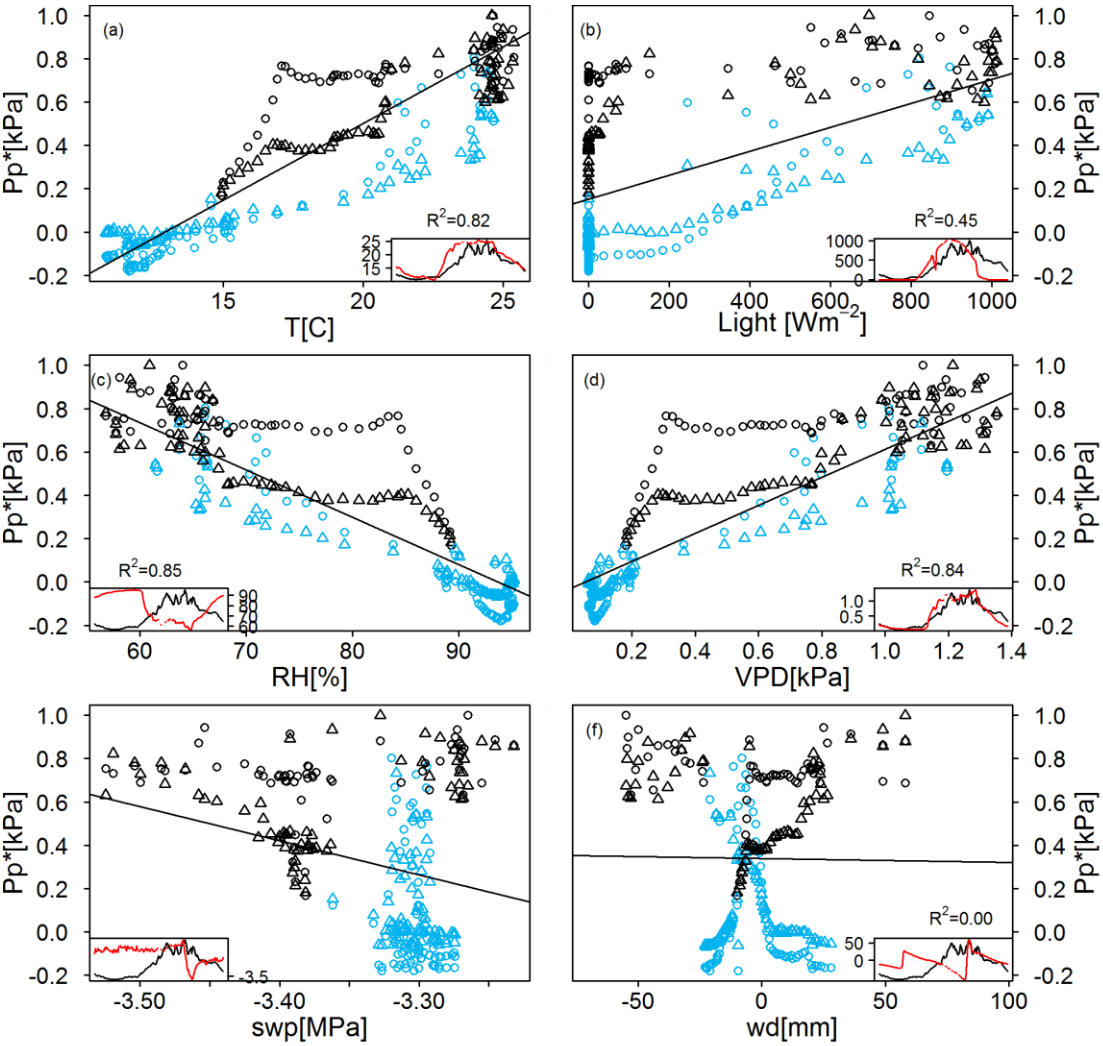


Figure 5.4 Daily scale correlations for an example sunny day in summer (13th March 2015) for tree A (open circles) and tree B (open triangles) for temperature (a), light (b), relative humidity (c) vapour pressure deficit (d), soil water potential (e) and water depth (f). The bottom of each panel shows the diurnal course of tree A and the corresponding environmental variable of each panel. Note the hysteresis effect from morning values up to and including midday (blue) to afternoon values (black).

strong correlation at an intra-diel scale of P_p* with T, RH, VPD and light was maintained in the analysis performed for 10 days in summer and 10 days in winter (Table 5.1). Overall, summer P_p* correlations with T, RH, VPD and light were higher than winter correlations. Additionally, summer 10-minute P_p* seemed most strongly correlated with RH, whilst winter 10-minute P_p* was more to temperature (Table 5.1).

Table 5.1 Mean of daily correlation coefficients between intra-diel P_p* dynamics and environmental conditions at a 10 minute scale. Correlations were calculated individually for 10 days in summer (28 February - 9 March 2015) and 10 days in winter (5 - 15 June 2015) for all three measurement trees (n=3). Star indicates significant correlation at 0.01 significance level.

	RH	T	Light	VPD	Depth	SWP	PPT
P_p* summer	-0.84*	0.84*	0.64*	0.82*	-0.13	0.25	0.03
P_p* winter	-0.65*	0.7*	0.48*	0.65*	-0.35	0.16	0.07

b. *Environmental drivers of diel to monthly P_p* AUC*

Results of the final model using normalized P_p* data pooled from all measuring periods of 2014-2015 showed that primary drivers of P_p* AUC were light and precipitation, which were both positively related to P_p* (Table 5.2). This model was chosen for optimal residual distribution and is presented in Table 5.2. Some models showed that, in certain individual measuring periods, soil water potential and temperature were significant drivers of P_p* AUC, whilst VPD and RH were positive correlates in most cases (see Supplementary Table 5.1). Results from all LME models investigated using individual measuring periods of non-normalized data are shown in the Supplemetary Information.

Table 5.2 Linear Mixed Effects model for diel to monthly environmental drivers of P_p* area under curve (AUC) over all measurement periods (May 2014, September and October 2014, November 2014, February and March 2015, April and May 2015, July and September 2015, October 2015 and December 2015). Asterix in the p-value column indicate statistical significance.

Variable	Fixed effects β1 (estimate ± SE)	p-value	Random effects b1 (SD σb1)	Autoregressive parameter φ
RH	0.0± 0.1*10-6	0.94	SD 1.5 10-5 Residual 0.18	0.41
T	-0.1*10-6 ±0.1*10-6	0.30		
Depth	0.0±0.1*10-6	0.90		
VPD	-0.2*10-5 ± 0.4*10-5	0.54		
SWP	0.0± 0.1*10-6	0.89		
Light	0.1 * 10-5± 0.1 *10-7	0.0000**		
PPT	0.32 *10-4 ±0.9*10-5	0.001*		

c. *Effect on Pp of fresh-water inputs at an hourly and monthly scales*

Precipitation had a clear effect on both hourly and diel P_p trends and P_p AUC (Table 5.2), significant in LME results, but not significant in Kendal tau correlations (Table 5.1). However, the hourly scale effects could be seen directly when plotting P_p* curves during different precipitation regimes (Figure 5.5). At an intra-diel scale high precipitation levels (PPT > 10mm) resulted in an almost complete loss of a pronounced P_p* curve, thus reducing AUC in the short term. Smaller precipitation events (10mm > PPT > 1) occurring during the day resulted in temporarily lowered P_p* values (and thus increased leaf turgor) (Figure 5.5). Precipitation events which occurred at night did not seem to markedly affect P_p trends (Figure 5.5).

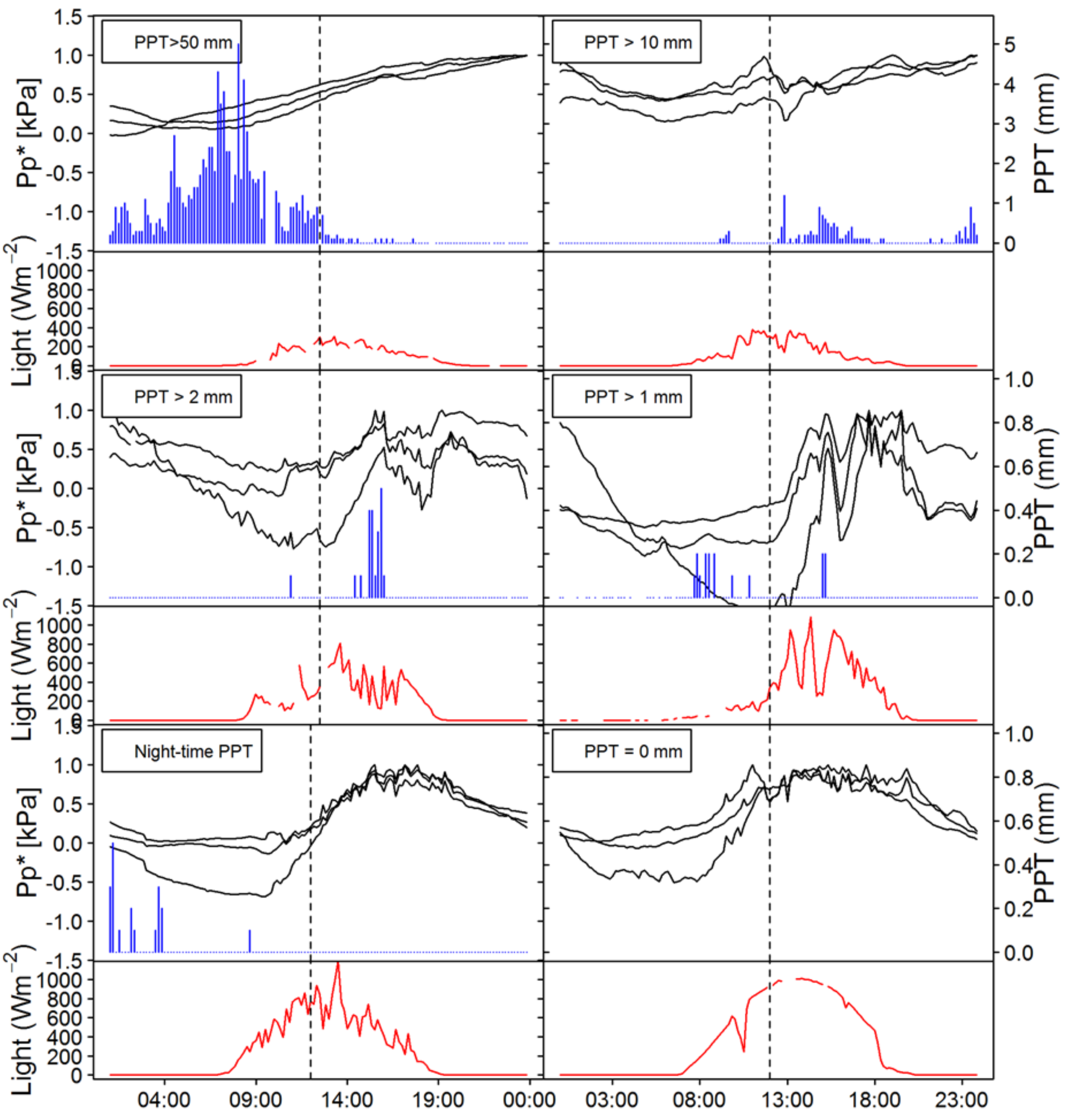


Figure 5.5 Effect on diurnal P_p* trends of different precipitation (PPT) regimes for all three measurement trees (Tree A-C, n=3): left to right top to bottom (1) PP > 50mm (2) 50 mm > PPT > 10 mm (3) 10 mm > PPT > 2 mm (4) 2 mm > PPT > 1 mm (5) Night time PPT, and (6) PPT = 0 mm. Higher precipitation levels result in the loss of a pronounced P_p* curve.

Investigating the long-term effect of precipitation on leaf turgor trends and P_p AUC during different measurement periods, precipitation had the long-term effect of increasing P_p AUC (Table 5.2 and Supplementary Table 5.1). This was especially notable in the December measurement period, which took place at the end of a month-long drought occurring in November, during which tree stem radius shrank steadily (data not shown, see Chapter 2). The effect of this drought on P_p is illustrated in Figure 5.6, where two responses of P_p to precipitation were present: (1) a longer term effect of precipitation lack, and (2) a shorter term response to precipitation after the drought. The first effect was a marked difference between the long-term P_p AUC and peak P_p timing from before (October 2015) and after (December 2015) the month-long drought in November. Prolonged precipitation absence and decreasing soil water potential caused an overall reduction in P_p AUC mean and variation before (AUC: mean = 3064.2 a.u, SD = 2142 a.u) and immediately after the drought (AUC: mean = 1824 a.u, SD = 876.9 a.u) (Supplementary Table 5.1). There was also a shift toward later peak P_p timing mean and smaller variation after precipitation absence (Peak P_p time: mean = 14:30 h, SD = 2.5 h) than before the absence (Peak P_p time: mean = 10:30 h, SD = 5 h) (Figure 5.6 a, b). The longer term effect of precipitation absence could also be seen in the close similarity of progressively reduced P_p AUC and soil water potential in December (Figure 5.6a). Peak P_p timing before and after the drought also closely followed VPD daily mean (Figure 5.6b) as did P_p AUC (Supplementary Table 5.1). In the second effect, which could be seen once precipitation restarted in December, precipitation events caused a sudden increase in P_p AUC mean and variance (Figure 5.6a, b). This effect could also be seen directly in December P_p diurnal curves, where the precipitation caused a big increase in P_p fluctuations.

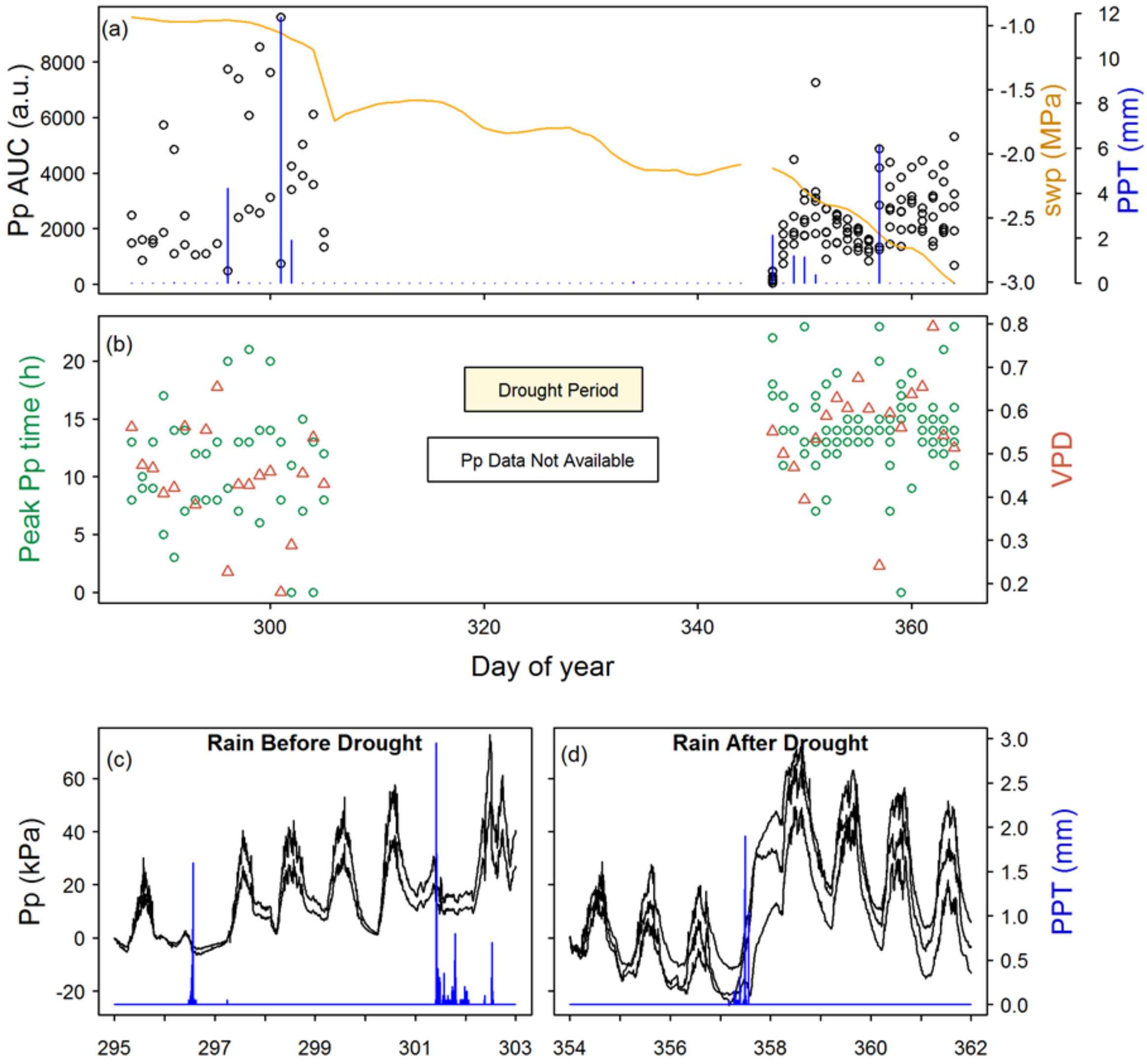


Figure 5.6 Changes in P_p before and after the month-long drought of November 2015. Top panel (a) shows area under curve of P_p diurnal cycle (AUC, arbitrary units) for three probes per tree, together with soil water potential (swp, orange line) and precipitation (PPT, blue bars). Note that there are no P_p data for the month of November, but environmental data and SRC data were still recorded. Mid panel (b) shows changes in time of day of P_p peak (green circles), note the increase in spread before and after drought and similarity to mean daily VPD (red triangles). Bottom panel left (c) shows diurnal P_p cycles for three example probes in Tree A and Tree D before the drought and right panel (d) for a week after the drought, note the different effect of PPT on P_p curves.

5.4.3 Peak timing and lags between SRC and P_p as an indicator of environmental and physiological change

a. *Yearly trends and environmental correlates of Pp and SRC timing and lags*

A seasonal shift towards later $P_{p\ \max}$ in summer and a return to earlier $P_{p\ \max}$ in winter was observed during the late winter 2014 to early winter 2015 (Table 5.3). Thus, minimum leaf turgor was reached later in summer than in winter months. This shift was reflected in a larger P_p-SRC$_{max}$ time-lag between P_p and SRC peaks during summer, changing from a lag of under two hours at the end of winter to a lag of up to 7 hours in summer (Table 5.3, Figure 5.7). The monthly median P_p-SRC$_{max}$ time-lag between leaf $P_{p\ max}$ and lower stem SRC (Figure 5.7a) closely followed monthly mean VPD ($R^2 = 0.67$, p-value < 0.01) and temperature ($R^2 = 0.74$, p-value < 0.001). This trend was also present in the time-lag to upper stem tiers, if not as strong (Figure 5.7b) and the correlation to VPD and temperature was not significant. The relationship of P_p-SRC$_{max}$ time-lag to temperature and VPD continued into the late winter of 2015 after the experimental trees were changed (data not shown). Full histograms with changes in timing distributions, including SRC minima and P_p minima timing are shown Supplementary Information. Time-lags using these peaks did not show significant correlations to studied environmental variables (results not shown).

Table 5.3 Time of peak $P_{p\ max}$ and SRC averaged for the three trees during the study period from September 2014 to June 2015 and the time-lag from leaf $P_{p\ max}$ to SRC$_{max}$ (P_p-SRC$_{max}$).

Year	2014				2015					
Month	09	10	11	12	01	02	03	04	05	06
$P_{p\ max}$	13:30	14:00	14:00	14:00	NA	15:00	14:00	NA	12:00	11:30
SRC$_{max}$ U	10:00	11:00	12:00	12:00	NA	11:00	10:00	NA	11:00	11:00
SRC$_{max}$ L	13:00	12:00	10:00	10:00	NA	8:00	7:00	NA	10:00	09:30
P_p-SRC$_{max}$ U	4.9h	3.2h	2.1h	2.1h	NA	4h	3h	NA	1h	0.6h
P_p-SRC$_{max}$ L	1.8h	1.3h	2.8h	2.3h	NA	7h	7h	NA	2.4h	2h

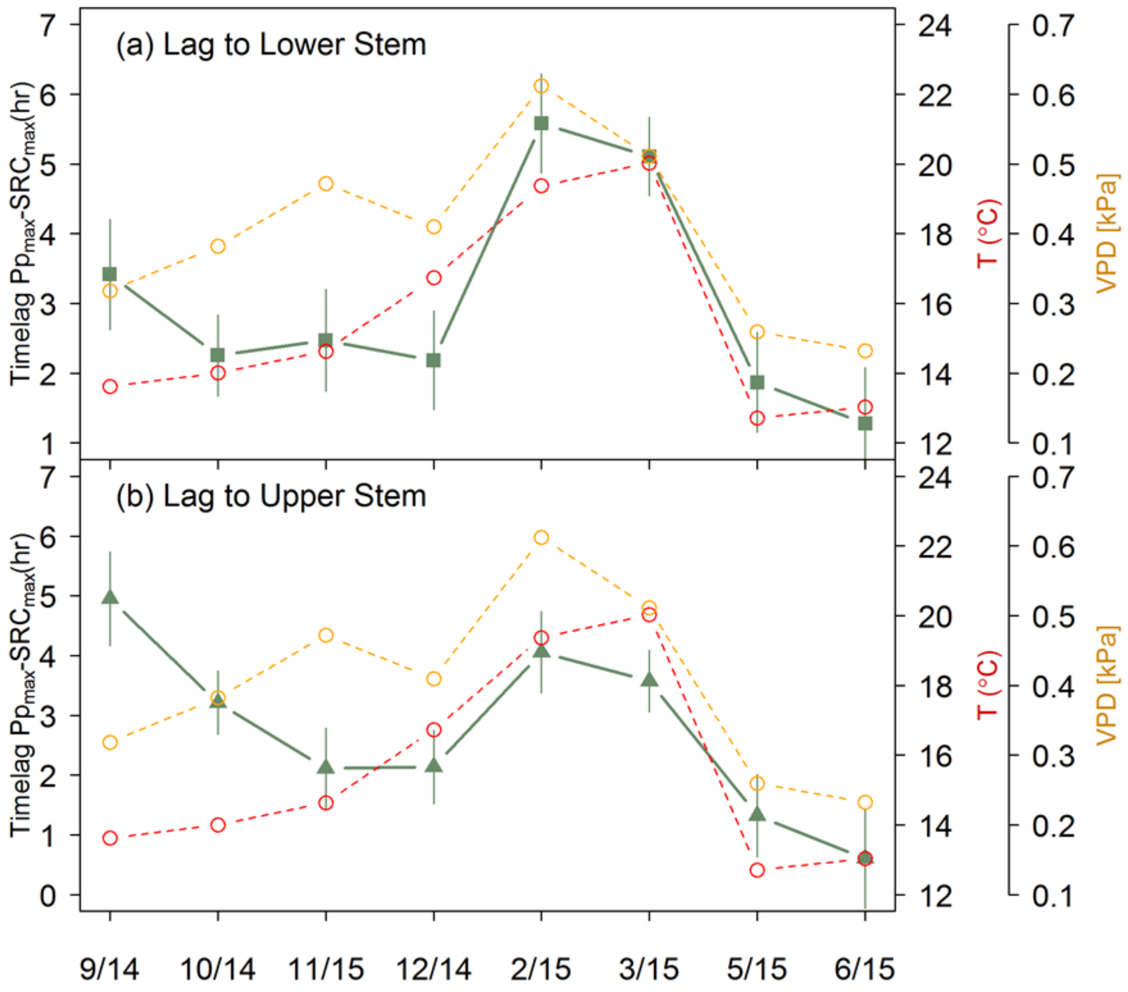


Figure 5.7 Time-lag in hours (P_p-SRC$_{max}$) between daily P_p peak (minimum leaf turgor) and SRC peak (maximum stem radius) of (a) lower (green squares) and (b) upper (green triagles) stem. Monthly mean values of temperature (red lines) and VPD (orange lines). Vertical lines for timelags indicate standard error of the monthly mean for three measured trees (n=3).

b. *Effect of precipitation absence on P_p and SRC time-lags*

The P_p-SRC$_{max}$ time-lag between peak P_p and peak SRC shifted from having a smaller mean lag with a larger variance before November 2015 (Time-lag P_p - SRC$_{max}$: mean = 2 h, sd = 6 h), to having a larger mean lag and smaller variance after November 2015 (Time-lag P_p - SRC$_{max}$: mean = 4 h, sd = 5 h) (Figure 5.8a). This shift could be seen in the change in P_p peak timing from a more variable and almost binomial spread ($P_{p\ max}$ time: mean = 11:00, sd = 4.8 h) (Figure 5.8 b, c) to a later and less variable P_p peak timing ($P_{p\ max}$ time: mean = 15:00, sd = 3.5 h).

Peak SRC timing seemed to shift from a later and more variable peak timing to an earlier and less variable time after November 2015 (Figure 5.8 b,c).

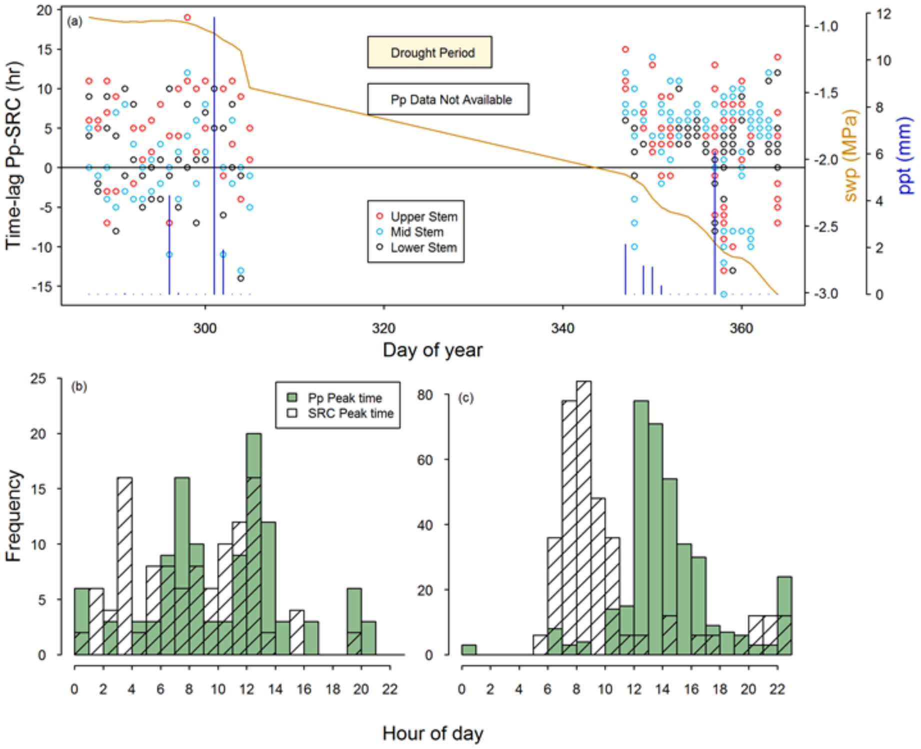


Figure 5.8 Changes in the daily time-lag in hours between time of peak P_p and peak SRC for (a) upper, middle and lower stem for Tree A and Tree D (n=2), for before and after the month-long drought of November 2015, together with soil water potential (swp, ochre line) and precipitation (ppt, blue bars). Distribution of peak $P_{p\ max}$ time (green) and peak SRC time (white dashed) for all sensors on Tree A and Tree D (n=2) for (b) before and (c) after the month of November 2015.

5.5 Discussion

My results show that leaf turgor pressure probes provide a useful measure of leaf water status in *A. marina*. The reliability of P_p was evidenced by the relationship of leaf balancing pressure with other measures of *A. marina* water relations and environmental indicators of water availability and demand. Obtaining high-resolution leaf turgor information for *A. marina* has the potential to deepen our understanding of the role that leaf water dynamics play within the whole of mangrove water relations (Reef and Lovelock 2015). Thus, my results also show the capacity of leaf turgor dynamics to provide insights on the hydraulic connection between stem and leaves, as *A. marina* showed delayed leaf turgor recovery in the evenings caused by the late supply of water stored in the stems. These results are encouraging as, in comparison with extensive knowledge of stem water storage dynamics and stem sap flow, and despite increasing interest in the movement of water through leaves (Sack et al. 2003, Nardini et al. 2005, Sack et al. 2005, Zwieniecki et al. 2007, Jiang et al. 2017, Nguyen et al. 2017a), our knowledge of high resolution temporal dynamics of leaf turgor and leaf hydrology in field experiments is still scarce.

5.5.1 Short time-scale dynamics of P_p in relation to the other water-related physiological processes.

The strong correlation in this study between ψ_{leaf} and measured P_p (Figure 5.1–Figure 5.3) coincides with the work of Zimmermann et al. (2008) or Ehrenberger et al. (2012b) which, in addition to using the Scholander pressure chamber, backed their P_p theory with individual cell turgor pressure probe measurements (Zimmermann et al. 2008, Westhoff et al. 2009, Bramley et al. 2013). Thus, the turgor pressure probe is a promising tool for monitoring changes in leaf turgor continuously and non-destructively, although these changes are relative and need calibration with pressure chamber measurements (Zimmermann et al. 2013). In the stems, mid-morning sap-flow increase was accompanied by leaf turgor loss, but early evening leaf turgor recovery lagged behind sap-flow decrease. Thus, the hourly relationship between sap-flow and leaf turgor was not linear, and there was a strong hysteresis in play. These results coincide with work showing a direct relationship between stem sap-flow and P_p, where it was also shown that increasing hysteresis was a sign of water stress (Ehrenberger et al. 2012a, Ehrenberger et al. 2012b, Rodriguez-Dominguez et al. 2012). At an intra-diel resolution P_p and sap-flow responded in a similar fashion to environmental conditions, for example, both sap-flow and P_p presented decreased rates and decreased AUC, respectively, during times of low evapotranspiration pressure (see also Chapter 4). The nature of the relationship between P_p and stem water storage movements seems more complex due to the existence of daytime stem swelling in *A. marina*, and the results of this work differ from those shown in previous studies (Ehrenberger et al. 2012a). My measurements evidenced that rapid morning decrease in leaf turgor was not matched by a decrease of stem radius. The fact that there was a higher direct correlation of hourly P_p with lower stem SRC than to upper stem SRC in summer can be explained by lower stem tier SRC being more closely coupled to leaf water potential in summer (see Chapter 3). This is not the case for upper stem tiers at this time of year, which present a more pronounced daytime stem swelling (see Chapter 3). In this case, the differences in the relationship between upper and lower stem to leaf turgor at the hourly scale provide backing for the idea that in summer the lower stem functions as a capacitor whilst the upper stem is more influenced by osmoregulation (see Chapter 2 and 3).

5.5.2 Leaf turgor dynamics response to microclimate

High resolution measurements across summer and winter evidenced the sensitivity o*f A. marina* leaves to common drivers of transpiration and stomatal control, such as light, T, VPD and RH across summer and winter (Table 5.1). Thus, at 10-minute resolution, leaf turgor decreased with higher evapotranspiration pressure whilst it increased with increased humidity. However, at larger scales (diel to monthly) the strongest drivers were precipitation and light, backing the increasing amount of studies which show the importance of freshwater inputs for maintaining leaf hydration in mangroves (Lovelock et al. 2017, Nguyen et al. 2017a, Nguyen et al. 2017b).

Diel measurements showed a palpable hysteresis present in the relationship between P_p and environmental drivers of transpiration, differentiating morning from afternoon trends. The strong positive correlation present in the morning as P_p increased simultaneous to transpiration, was weakened when P_p then failed to recover at the same rate in the evening after T, VPD and light levels dropped. Such close correlations between P_p and T, VPD, RH and light have been observed before (Bramley et al. 2013, Zimmermann et al. 2013), and some studies hypothesize that the presence of a hysteresis is an indication of water stress and cavitation in the leaf (Bramley et al. 2013). Thus, an increase in the area of the hysteresis (difference between morning increase and afternoon recovery) would imply an increase of water stress in the leaf. One of the mechanisms proposed is that slow recovery and tissue rehydration is a sign of cavitation increasing resistance along the flow pathway (Zimmerman et al. 2013 and references therein).

Leaf turgor in *A. marina* seems to respond strongly to freshwater inputs. In the short term small precipitation events resulted in instantaneous improvements of leaf turgor and rainy days resulted in reduced amplitudes of leaf turgor fluctuations. In the longer term, LME analysis showed that precipitation had a positive effect on the magnitude of leaf turgor fluctuations, implying an enhancement of water storage and mobilization in the leaves in days after rain (Figure 5.6). The alleviation of transpiration pressure in the leaves and the availability of fresh water to the roots provided by precipitation must cause relaxation of negative tensions in the leaves, allowing for increased leaf turgor and increased fluctuations. Fast responses of P_p to fresh-water inputs have been observed before in irrigation studies (Fernandez et al. 2011, Rodriguez-Dominguez et al. 2012, Bramley et al. 2013, Martinez-Gimeno et al. 2017), although usually just through leaf turgor improvements (lowered P_p) but not increased amplitude of the fluctuations, as seen here (but see Martínez‑Gimeno et al. 2017).

The double effect of rain on P_p observed in this study could be explained with Ehrenberger's 2012 theory of two stage correlation of P_p to leaf turgor depending on leaf hydration. Thus, after a long period of precipitation absence leaves could have reached turgor loss point, causing decrease of P_p fluctuations, and the loss of a direct correlation between absolute P_p values and leaf turgor. This loss of a direct correlation between P_p values and leaf turgor is thought to be caused by water replacement for air in the parenchyma tissue of leaf cells (Ehrenberger et al. 2012a, Martínez‑Gimeno et al. 2017). When precipitation returned in December, it had the effect of increasing P_p AUC by allowing leaves to fully rehydrate and increase leaf water storage and mobilization. Since leaf water has been shown to be an important water store in *A. marina* and other mangrove species (Lechthaler et al. 2016, Nguyen et al. 2017a), an increase in water storage after fresh-water inputs would explain the positive effect of precipitation on the magnitude of P_p fluctuations in this study. Additionally, my results also imply a strong regulation of stomatal aperture in *A. marina* in situations of water stress, although stomatal conductance was not measured during this time. The low amplitude of leaf turgor fluctuations after prolonged rainless period could be due to tight stomatal regulation and downregulation of transpiration, especially since soil water potential during this time was low (Figure 5.6). After precipitation, increased water availability and a potential increase of stomatal aperture could explain the fast response and larger fluctuations in P_p. This coincides with the work of Reef and Lovelock (2015) which showed an increase of stomatal conductance in the presence of fresh water. Lowered leaf turgor during times of water shortage could be key for *A. marina*, since it has been hypothesized that turgor loss plays a role in signalling water stress and inducing the cessation of growth and stomatal conductance, although the link is complex (Bunce 1997, Kolb and Sperry 1999, Buckley 2005, Addington et al. 2006, Ache et al. 2010, Aasamaa and Sober 2011, Merilo et al. 2014). Further research should address long term effects on P_p of fresh-water inputs shortage across several seasons, and if inverted P_p curves caused by leaf turgor loss and cavitation appear in *A. marina* (Ehrenberger et al. 2012b).

It is worth noting, that short-term improvements on leaf turgor occurred even in response to precipitation events as small as 1 mm. Although these results could be due to the instantaneous alleviation of transpiration tension due to increased water vapour around the leaf they could also be the result of leaf water uptake. Leaf water uptake can occur in humid or foggy weather conditions and is thought to be an important source of water in tall trees (Zimmermann et al. 2007, Berry et al. 2013, Uddin 2014). It is also thought to be an important source of water in arid ecosystems where small precipitation events do little to change soil water moisture but help maintain leaf hydration (Breshears et al. 2008). It seems plausible that foliar water uptake could occur as a way of alleviating very low water potentials in *A. marina* leaves, and recent research has shown that alternative sweet water sources are necessary in order to explain leaf hydration and different water sources in *A. marina* (Reef et al. 2015, Santini et al. 2015, Lovelock et al. 2017, Nguyen et al. 2017a). However, direct evidence of leaf foliar uptake in *A. marina* is still missing and should be a matter for further research.

5.5.3 Relationship between P_p and SRC peaks and timing as a valuable source of information

In my results, both the hysteresis and timing of P_p in *A. marina* seem to point to a slow recovery of leaf turgor in the evening, especially in summer (Figure 5.1). This could be partially explained both in the context of *A. marina's* exceptionally low leaf water potential values and through potential imbalances between water supply and demand (Tardieu and Simonneau 1998, Kolb and Sperry 1999, Clearwater and Meinzer 2001, Meinzer et al. 2001, Sperry et al. 2002, Nardini et al. 2005). These imbalances are especially plausible in the context of daytime stem swelling, as the late onset of stem water storage mobilization could mean a delayed supply of stored water to leaves. Thus delayed P_p recovery in the evening could be caused by timescale differences between the short-term variation in water loss rates and the corresponding response in stomatal aperture and water storage mobilization in the stem, causing leaves to undergo imbalances in supply and demand (Zwieniecki et al. 2007, Melcher et al. 2012). Delayed recovery and eventual return to predawn P_p values has been observed in other species under water stress (known as *Phase II* of water stress set in) (Ehrenberger 2012a). However, despite late initiation of leaf turgor increase (i.e. $P_{p\,max}$ timing) our results showed that leaf turgor and leaf water potential were able to recover overnight, if slowly (Figure 5.1 and Figure 5.3). Although few studies exist on leaf turgor courses of night-time leaf rehydration, the process is well studied through the night time recovery of Ψ_{leaf} to predawn levels, which reflect soil water potential. Thus, trees in a positive water balance are able to return their leaves to predawn Ψ_{leaf} levels, a process which can rely heavily on internally stored water if water availability in soil is low (Bucci et al. 2005, Scholz et al. 2007).

My results show that the time-lag between the start of water storage mobilization (peak SRC) and the initiation of leaf turgor recovery after reaching peak P_p changed throughout the year (Figure 5.7). Previous work has shown that the time-lags between stem water storage mobilization and P_p dynamics can be an interesting source of information of plant responses to both changing microclimate and physiology (Ehrenberger 2012a). The increasing P_p-SRC_{max} time-lag linked to higher temperature and VPD shows that in conditions of high atmospheric water demand a larger amount of time is needed from the start of water reserve mobilization in the stem to the initiation of turgor recovery in the leaves. Additionally, the increased P_p-SRC_{max} time-lag after an extended period of no rain (Figure 5.8) also seems to indicate that such time-lag increases could be an indication of water stress in the tree. In fact, increased time-lags between peak SRC and peak P_p was also reported in a drought experiment (Ehrenberger et al. 2012a). If sap-flow alone cannot meet the needs of transpiring leaves, delayed supply of stored water due to daytime stem swelling and increased water stress may cause leaves to rely increasingly on leaf water storage. Indeed, the work of Lechthaler et al (2016) on other mangrove species showed an increased reliance on leaf water storage in conditions of high salinity as transport of water to leaves did always not meet demand.

In the case of *A. marina* the seasonal shift in the time-lag between leaf P_p and SRC peak was again different in the lower and upper stem, mostly due to a shift in summer towards earlier SRC peak in the lower stem. The potentially different hydraulic linkage of lower and upper stem and sensitivity to the water demand of leaves is seen both in the intra-diurnal correlations and the seasonal time-lag, and raises the question of what are the internal controls overriding the transpiration requirements of leaves to different parts of the stem. As pointed out by Ehrenberger (2012a), further work using high-resolution stem SRC and leaf P_p measurements is needed to determine the nature of the hydraulic connection between water demand of leaves and water storage in stems. As mentioned previously, a potential explanation might be found in the postulated osmoregulation of water storage tissues behind daytime stem swelling (Vandegehuchte et al. 2014). This osmoregulation, especially if it is reliant on phloem sugar loading, could influence the upper vs. lower stem differently depending on the time of year, causing different sensitivities to the water demand of transpiring leaves (Chapter 2 and Chapter 3). Thus, in summer, lower stem tiers may be less influenced by osmoregulation, and respond more readily to the water demands of leaves. On the other hand, in summer, the relative strength of the osmotic signal in the upper tiers would make stem water storage mobilization less sensitive to the water demand of leaves, hence the lack of correlation of upper stem P_p-SRC_{max} time-lag to measures of evaporative water demand. In the same way, these results confirm the work of Chapter 3, where late recovery of leaf turgor may be “the price to pay” for maintaining stomata open in order increase sugar production, sugar loading, and hence maintain the osmotic turgor signal of the phloem (Nikinmaa et al. 2013). Thus, P_p-SRC time-lags not only contain information on changes in water supply and demand, but potentially also of the strength of the osmotic signal in different parts of the tree.

On the other hand, time-lag differences could be caused by changes along the stem of the capacity for withdrawing water from living cells in the inner-bark. These differences could in turn could be due to changes in the hydraulic conductance of tissues to the radial water flow through symplastic pathways caused by structural resistances and changes in aquaporin expression (Tyree and Zimmermann 2002, Steppe et al. 2012, Baert et al. 2015, Pfautsch et al. 2015). In this study only time-lags between peak P_p and peak SRC were analysed, reflecting the time taken between the start of water storage mobilization into the sap flow stream and the start of leaf turgor recovery. The time-lag between initiation of leaf turgor loss and stem shrinkage could also reveal important information on the hydraulic connection between stem and leaves and the strength of the osmotic signal in storage tissues. Although I did not find significant relationships between peak leaf turgor (min P_p) and $\mathrm{SRC}_{\mathrm{max}}$ in this study, additional work should also investigate other time-lags.

Despite the fact that turgor pressure probes provide an exciting new tool for non-destructive high temporal resolution measurements of relative changes in leaf turgor, a note on caution is needed. Firstly, the relationship between leaf turgor and other measures of leaf hydration, such as leaf water content per area, is not always straight forward. Thus, further work should complement turgor pressure measurements with other measures of leaf hydration and pressure-volume curves in *A. marina* leaves (Jiang et al. 2017, Nguyen et al. 2017a, Nguyen et al. 2017b). Secondly, the conditions of calibration and relationship between measured leaf patch pressure and leaf turgor make assumptions about osmotic turgor which might not always be met in *A. marina*. This is in fact also the case for measurements of leaf water potential performed with Scholander pressure bomb.

These measurements do not include the osmotic, but only the hydrostatic component of total leaf water potential. Thus, caution is needed when interpreting these values, as the osmotic component to water potential of leaves in *A. marina* may be significant. Future studies of P_p fluctuations and leaf water potential should be coupled with measurements of osmotic changes in the leaves, which could be essential to understand the importance of osmotic adjustment in halophytic species, especially salt secreting species like *A. marina*.

5.6 Conclusion

My work shows that leaf patch pressure probe measurements provide a valuable information source on high temporal resolution *A. marina* leaf water relations and their response to the environment, opening up many new avenues of potential field research in *A. marina*. Leaf turgor dynamics evidence that the internal controls behind day time stem swelling override the needs of transpiring leaves and that this causes delayed recovery of leaf turgor in the evenings. My results also support the work of Chapter 2 and 3 showing the lower stem seems to respond more sensitively to transpiration and leaf water needs than the upper stem. Lastly, the results of this work show the importance of precipitation in helping relieve leaf tensions both in the long and the short term, supporting the increasing amount of work that shows a reliance of mangroves on fresh water inputs both through relieving soil water availability and facilitating atmospheric water uptake.

5.7 References

Aasamaa, K. and A. Sober. 2011. Stomatal sensitivities to changes in leaf water potential, air humidity, CO2 concentration and light intensity, and the effect of abscisic acid on the sensitivities in six temperate deciduous tree species. Environmental and Experimental Botany **71**:72-78.

Ache, P., H. T. Bauer, H. Kollist, K. A. S. Al-Rasheid, S. Lautner, W. Hartung, and R. Hedrich. 2010. Stomatal action directly feeds back on leaf turgor: new insights into the regulation of the plant water status from non-invasive pressure probe measurements. The Plant Journal **62**:1072-1082.

Addington, R. N., L. A. Donovan, R. J. Mitchell, J. M. Vose, S. D. Pecot, S. B. Jack, U. G. Hacke, J. S. Sperry, and R. Oren. 2006. Adjustments in hydraulic architecture of *Pinus palustris* maintain similar stomatal conductance in xeric and mesic habitats. Plant, Cell & Environment **29**:535-545.

Aissaoui, F., H. Chehab, B. Bader, A. Ben Salem, N. M'Barki, S. Laamari, B. Chihaoui, Z. Mahjoub, and D. Boujnah. 2016. Early water stress detection on olive trees (*Olea europaea* L. cvs 'chemlali' and 'Chetoui') using the leaf patch clamp pressure probe. Computers and Electronics in Agriculture **131**:20-28.

Angeles, G., B. Bond, J. S. Boyer, T. Brodribb, J. Renée Brooks, M. J. Burns, J. Cavender-Bares, M. Clearwater, H. Cochard, J. Comstock, S. D. Davis, J.-S. Domec, L. Donovan, F. Ewers, B. Gartner, U. Hacke, T. Hinckley, N. M. Holbrook, H. G. Jones, K. Kavanagh, B. Law, J. López-Portillo, C. Lovisolo, T. Martin, J. Martínez-Vilalta, S. Mayr, F. C. Meinzer, P. Melcher, M. Mencuccini, S. Mulkey, A. Nardini, H. S. Neufeld, J. Passioura, W. T. Pockman, R. B. Pratt, S. Rambal, H. Richter, L. Sack, S. Salleo, A. Schubert, P. Schulte, J. P. Sparks, J. Sperry, R. Teskey, and M. Tyree. 2004. The cohesion-tension theory. New Phytologist **163**:451-452.

Bader, M. K. F., W. Ehrenberger, R. Bitter, J. Stevens, B. P. Miller, J. Chopard, S. Rüger, G. E. S. J. Hardy, P. Poot, K. W. Dixon, U. Zimmermann, and E. J. Veneklaas. 2014. Spatio-temporal water dynamics in mature *Banksia menziesii* trees during drought. Physiologia Plantarum:n/a-n/a.

Baert, A., V. De Schepper, and K. Steppe. 2015. Variable hydraulic resistances and their impact on plant drought response modelling. Tree Physiology **35**:439-449.

Bartlett, M. K., C. Scoffoni, and L. Sack. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters **15**:393-405.

Berry, Z. C., N. M. Hughes, and W. K. Smith. 2013. Cloud immersion: an important water source for spruce and fir saplings in the southern Appalachian Mountains. Oecologia **174**:319-326.

Bramley, H., W. Ehrenberger, U. Zimmermann, J. A. Palta, S. Ruger, and K. H. M. Siddique. 2013. Non-invasive pressure probes magnetically clamped to leaves to monitor the water status of wheat. Plant and Soil **369**:257-268.

Breshears, D. D., N. G. McDowell, K. L. Goddard, K. E. Dayem, S. N. Martens, C. W. Meyer, and K. M. Brown. 2008. Foliar absorption of intercepted rainfall improves woody plant water status most during drought. Ecology **89**:41-47.

Bucci, S. J., G. Goldstein, F. C. Meinzer, A. C. Franco, P. Campanello, and F. G. Scholz. 2005. Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in neotropical savanna trees. Trees-Structure and Function **19**:296-304.

Buck, A. L. 1981. New equations for computing vapor-pressure and enhancement factor. Pages 1527-1532 Journal of Applied Meteorology.

Buckley, T. N. 2005. The control of stomata by water balance. New Phytologist **168**:275-292.

Buckley, T. N. and K. A. Mott. 2013. Modelling stomatal conductance in response to environmental factors. Plant Cell and Environment **36**:1691-1699.

Bunce, J. A. 1997. Does transpiration control stomatal responses to water vapour pressure deficit? Plant, Cell & Environment **20**:131-135.

Cermak, J., J. Kucera, W. L. Bauerle, N. Phillips, and T. M. Hinckley. 2007. Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiology **27**:181-198.

Chen, T. M., P. Dittrich, W. H. Campbell, and C. C. Black. 1973. Resolution of a mature nutsedge leaf into upper, and lower epidemis, mesophyll cells, and bundle sheath strands and their enzyme compliments. Plant Physiology **51**:6-6.

Clearwater, M. J. and F. C. Meinzer. 2001. Relationships between hydraulic architecture and leaf photosynthetic capacity in nitrogen-fertilized *Eucalyptus grandis* trees. Tree Physiology **21**:683-690.

Daudet, F. A., T. Ameglio, H. Cochard, O. Archilla, and A. Lacointe. 2005. Experimental analysis of the role of water and carbon in tree stem diameter variations. Journal of Experimental Botany **56**:135-144.

De Schepper, V., T. De Swaef, I. Bauweraerts, and K. Steppe. 2013. Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany **64**:4839-4850.

Deslauriers, A., H. Morin, C. Urbinati, and M. Carrer. 2003. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees **17**:477-484.

Dodd. 2013. Abscisic acid and stomatal closure: a hydraulic conductance conundrum? New Phytologist **197**.

Ehrenberger, W., S. Rüger, R. Fitzke, P. Vollenweider, M. Günthardt-Goerg, T. Kuster, U. Zimmermann, and M. Arend. 2012a. Concomitant dendrometer and leaf patch pressure probe measurements reveal the effect of microclimate and soil moisture on diurnal stem water and leaf turgor variations in young oak trees. Functional Plant Biology **39**:297-305.

Ehrenberger, W., S. Ruger, C. M. Rodriguez-Dominguez, A. Diaz-Espejo, J. E. Fernandez, J. Moreno, D. Zimmermann, V. L. Sukhorukov, and U. Zimmermann. 2012b. Leaf patch clamp pressure probe measurements on olive leaves in a nearly turgorless state. Plant Biology **14**:666-674.

Fernandez, J. E., C. M. Rodriguez-Dominguez, A. Perez-Martin, U. Zimmermann, S. Ruger, M. J. Martin-Palomo, J. M. Torres-Ruiz, M. V. Cuevas, C. Sann, W. Ehrenberger, and A. Diaz-Espejo. 2011. Online-monitoring of tree water stress in a hedgerow olive orchard using the leaf patch clamp pressure probe. Agricultural Water Management **100**:25-35.

Haworth, M., C. Elliott-Kingston, and J. C. McElwain. 2011. Stomatal control as a driver of plant evolution. Journal of Experimental Botany **62**:2419-2423.

Herzog, K. M., R. Häsler, and R. Thum. 1995. Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees **10**:94-101.

Jiang, G. F., U. M. Goodale, Y. Y. Liu, G. Y. Hao, and K. F. Cao. 2017. Salt management strategy defines the stem and leaf hydraulic characteristics of six mangrove tree species. Tree Physiology **37**:389-401.

Kolb, K. J. and J. S. Sperry. 1999. Differences in drought adaptation between subspecies of sagebrush (Artemisia tridentata). Ecology **80**:2373-2384.

Lechthaler, S., E. M. R. Robert, N. Tonne, A. Prusova, E. Gerkema, H. Van As, N. Koedam, and C. W. Windt. 2016. Rhizophoraceae Mangrove Saplings Use Hypocotyl and Leaf Water Storage Capacity to Cope with Soil Water Salinity Changes. Frontiers in Plant Science **7**.

Lovelock, C. E., R. Reef, and M. C. Ball. 2017. Isotopic signatures of stem water reveal differences in water sources accessed by mangrove tree species. Hydrobiologia **803**:133-145.

Martinez-Gimeno, M. A., M. Castiella, S. Ruger, D. S. Intrigliolo, and C. Ballester. 2017. Evaluating the usefulness of continuous leaf turgor pressure measurements for the assessment of Persimmon tree water status. Irrigation Science **35**:159-167.

McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D. G. Williams, and E. A. Yepez. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist **178**:719-739.

McIntyre, D. S. 1980. Basic relationships for salinity evaluation from measurements on soil solution. Autralian Journal of Soil Research **18**:199-206.

Meinzer, F. C., M. J. Clearwater, and G. Goldstein. 2001. Water transport in trees: current perspectives, new insights and some controversies. Environmental and Experimental Botany **45**:239-262.

Melcher, P. J., M. H. N., M. J. Burns, M. A. Zwieniecki, A. R. Cobb, T. J. Brodribb, B. Choat, and L. Sack. 2012. Measurements of stem xylem hydraulic conductivity in the laboratory and field. Methods in Ecology and Evolution **3**:685-694.

Merilo, E., I. Joesaar, M. Brosche, and H. Kollist. 2014. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. New Phytol **202**:499-508.

Nardini, A., E. Gortan, and S. Salleo. 2005. Hydraulic efficiency of the leaf venation system in sun- and shade-adapted species. Functional Plant Biology **32**:953-961.

Nguyen, H. T., P. Meir, L. Sack, J. Evans, R. S. Oliveira, and M. C. Ball. 2017a. Leaf water storage increases with salinity and aridity in the mangrove *Avicennia marina*: integration of leaf structure, osmotic adjustment and access to multiple water sources. Plant Cell and Environment **40**:1576-1591.

Nguyen, H. T., P. Meir, J. Wolfe, M. Mencuccini, and M. C. Ball. 2017b. Plumbing the depths: extracellular water storage in specialized leaf structures and its functional expression in a three-domain pressure-volume relationship. Plant Cell and Environment **40**:1021-1038.

Nikinmaa, E., T. Holtta, P. Hari, P. Kolari, A. Makela, S. Sevanto, and T. Vesala. 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. Plant Cell and Environment **36**:655-669.

Oparka, K. J. and R. Turgeon. 1999. Sieve elements and companion cells - Traffic control centers of the phloem. Plant Cell **11**:739-750.

Pantin, F., F. Monnet, D. Jannaud, J. M. Costa, J. Renaud, B. Muller, T. Simonneau, and B. Genty. 2013. The dual effect of abscisic acid on stomata. New Phytol **197**:65-72.

Parida, A. K., A. B. Das, Y. Sanada, and P. Mohanty. 2004. Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*. Aquatic Botany **80**:77-87.

Pfautsch, S. 2016. Hydraulic anatomy and function of trees—basics and critical developments. Current Forestry Reports **2**:236-248.

Pfautsch, S., T. Hölttä, and M. Mencuccini. 2015. Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology **35**:706-722.

Reef, R. and C. E. Lovelock. 2015. Regulation of water balance in mangroves. Annals of Botany **115**:385-395.

Reef, R., H. L. Markham, N. S. Santini, and C. E. Lovelock. 2015. The response of the mangrove *Avicennia marina* to heterogeneous salinity measured using a split-root approach. Plant and Soil **393**:297-305.

Rennie, E. A. and R. Turgeon. 2009. A comprehensive picture of phloem loading strategies. Proceedings of the National Academy of Sciences of the United States of America **106**:14162-14167.

Rodriguez-Dominguez, C. M., W. Ehrenberger, C. Sann, S. Ruger, V. Sukhorukov, M. J. Martin-Palomo, A. Diaz-Espejo, M. V. Cuevas, J. M. Torres-Ruiz, A. Perez-Martin, U. Zimmermann, and J. E. Fernandez. 2012. Concomitant measurements of stem sap flow and leaf turgor pressure in olive trees using the leaf patch clamp pressure probe. Agricultural Water Management **114**:50-58.

Sack, L., P. D. Cowan, N. Jaikumar, and N. M. Holbrook. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. Plant Cell and Environment **26**:1343-1356.

Sack, L., M. T. Tyree, and N. M. Holbrook. 2005. Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. New Phytologist **167**:403-413.

Santini, N. S., R. Reef, D. A. Lockington, and C. E. Lovelock. 2015. The use of fresh and saline water sources by the mangrove *Avicennia marina*. Hydrobiologia **745**:59-68.

Savage, J. A., M. J. Clearwater, D. F. Haines, T. Klein, M. Mencuccini, S. Sevanto, R. Turgeon, and C. Zhang. 2016. Allocation, stress tolerance and carbon transport in plants: how does phloem physiology affect plant ecology? Plant Cell and Environment **39**:709-725.

Schachtman, D. P. and J. Q. Goodger. 2008. Chemical root to shoot signaling under drought. Trends Plant Sci **13**:281-287.

Scholander, P. F., E. D. Bradstreet, E. A. Hemmingsen, and H. T. Hammel. 1965. Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science **148**:339-346.

Scholz, F. G., S. J. Bucci, G. Goldstein, F. C. Meinzer, A. C. Franco, and F. Miralles-Wilhelm. 2007. Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. Plant Cell and Environment **30**:236-248.

Sevanto, S., T. Holtta, and N. M. Holbrook. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant Cell and Environment **34**:690-703.

Sirichandra, C., A. Wasilewska, F. Vlad, C. Valon, and J. Leung. 2009. The guard cell as a single-cell model towards understanding drought tolerance and abscisic acid action. Journal of Experimental Botany **60**:1439-1463.

Smith, D. M. and S. J. Allen. 1996. Measurement of sap flow in plant stems. J Exp Bot **47**:1833-1844.

Sperry, J. S., U. G. Hacke, R. Oren, and J. P. Comstock. 2002. Water deficits and hydraulic limits to leaf water supply. Plant Cell and Environment **25**:251-263.

Steppe, K., H. Cochard, A. Lacointe, and T. Ameglio. 2012. Could rapid diameter changes be facilitated by a variable hydraulic conductance? Plant Cell and Environment **35**:150-157.

Steppe, K., D. J. W. De Pauw, R. Lemeur, and P. A. Vanrolleghem. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257-273.

Tardieu, F. and T. Simonneau. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. Journal of Experimental Botany **49**:419-432.

Turgeon, R. 1996. Phloem loading and plasmodesmata. Trends in Plant Science **1**:418-423.

Turgeon, R. and S. Wolf. 2009. Phloem Transport: Cellular Pathways and Molecular Trafficking. Pages 207-221 Annual Review of Plant Biology. Annual Reviews, Palo Alto.

Tyree, M. and M. H. Zimmermann. 2002. Xylem Structure and the Ascent of Sap. Second edition. Spriner-Verlag, Berlin.

Tyree, M. T. 1997. The Cohesion-Tension theory of sap ascent: current controversies. Journal of Experimental Botany **48**:1753-1765.

Uddin, S. 2014. Canopy water uptake: An important survival mechanism of mangroves. Ghent University and Vrije Universiteit Brussel, Belgium.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, T. De Swaef, D. A. Lockington, and K. Steppe. 2014. Modelling reveals endogenous osmotic adaptation of storage tissue water potential as an important driver determining different stem diameter variation patterns in the mangrove species *Avicennia marina* and *Rhizophora stylosa*. Annals of Botany.

Vandenhonert, T. H. 1948. Water transport in plants as a catenary process. **3**:146-153.

Westhoff, M., R. Reuss, D. Zimmermann, Y. Netzer, A. Gessner, P. Gessner, G. Zimmermann, L. H. Wegner, E. Bamberg, A. Schwartz, and U. Zimmermann. 2009. A non-invasive probe for online-monitoring of turgor pressure changes under field conditions. Plant Biology **11**:701-712.

Zheng, W. J., W. Q. Wang, and P. Lin. 1999. Dynamics of element contents during the development of hypocotyles and leaves of certain mangrove species. Journal of Experimental Marine Biology and Ecology **233**:247-257.

Zimmermann, D., R. Reuss, M. Westhoff, P. Geßner, W. Bauer, E. Bamberg, F.-W. Bentrup, and U. Zimmermann. 2008. A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. Journal of Experimental Botany **59**:3157-3167.

Zimmermann, D., M. Westhoff, G. Zimmermann, P. Geßner, A. Gessner, L. H. Wegner, M. Rokitta, P. Ache, H. Schneider, J. A. Vásquez, W. Kruck, S. Shirley, P. Jakob, R. Hedrich, F. W. Bentrup, E. Bamberg, and U. Zimmermann. 2007. Foliar water supply of tall trees: evidence for mucilage-facilitated moisture uptake from the atmosphere and the impact on pressure bomb measurements. Protoplasma **232**:11-34.

Zimmermann, U., R. Bitter, P. E. R. Marchiori, S. Ruger, W. Ehrenberger, V. L. Sukhorukov, A. Schuttler, and R. V. Ribeiro. 2013. A non-invasive plant-based probe for continuous monitoring of water stress in real time: a new tool for irrigation scheduling and deeper insight into drought and salinity stress physiology. Theoretical and Experimental Plant Physiology **25**:2-11.

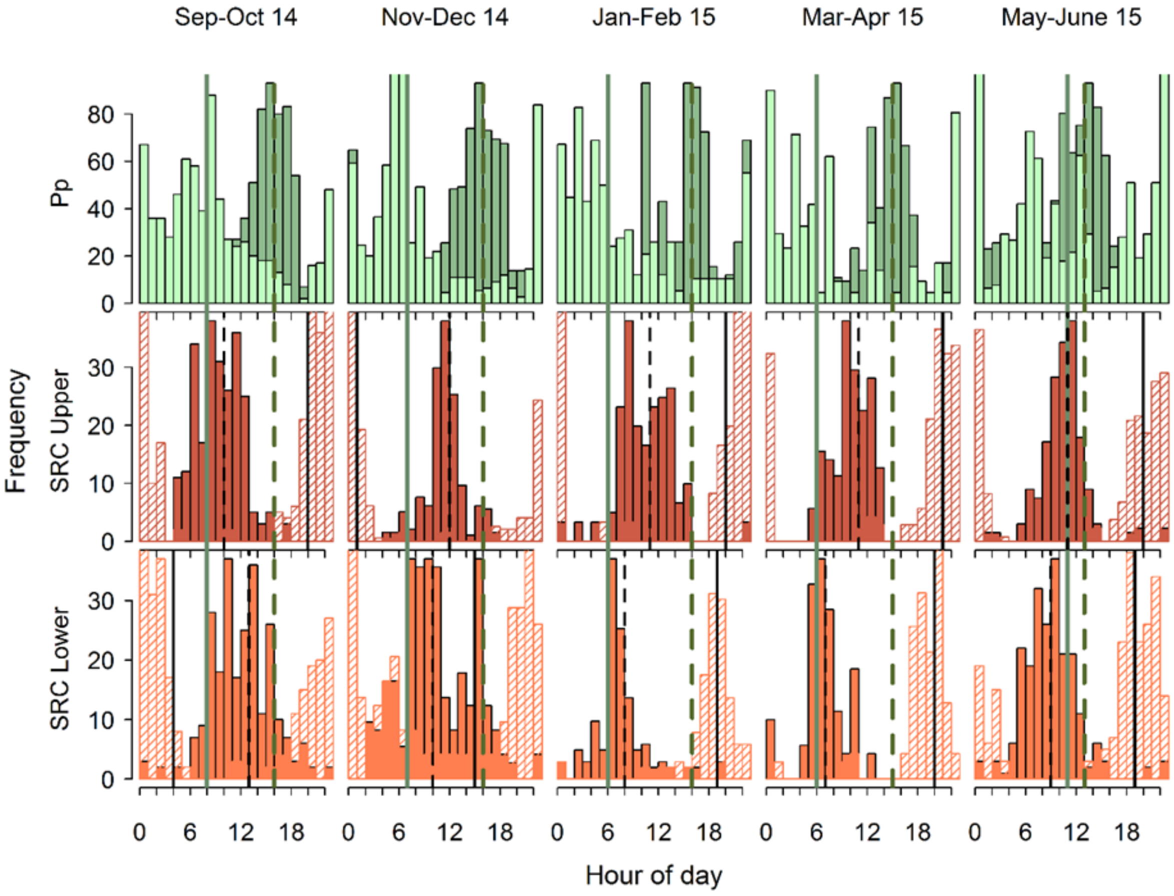
Zimmermann, U., H. Schneider, L. H. Wegner, and A. Haase. 2004. Water ascent in tall trees: does evolution of land plants rely on a highly metastable state? New Phytologist **162**:575-615.

Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees **15**:50-57.

Zweifel, R., K. Steppe, and F. J. Sterck. 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. J Exp Bot **58**:2113-2131.

Zwieniecki, M. A., T. J. Brodribb, and N. M. Holbrook. 2007. Hydraulic design of leaves: insights from rehydration kinetics. Plant Cell and Environment **30**:910-921.

5.8 Supplementary Information



Supplementary Figure 5.1 Timing Pp maxima (dark green bars) and minima (light green) (and thus minimum and maximum turgor, respectively) and SRC maxima (dark salmon and dark coral) and minima (dashed salmon and dashed coral) for upper (SRC upper) and lower stem (SRC lower) for the continuous measuring period from September 2014 until June 2015 for all measurement trees (n=3). Continuous and dashed lines indicate mean time of minima and maxima respectively, for Pp (green) and SRC (black).

Supplementary Table 5.1 Results of LME models on each individual measurement period in 2014 (1) September (2) October (3) November to December, and 2015 (4) February to March (5) May (6) June to August and (7) November to December. Those models where residual variance is smaller than random effects intercept variance (b_1) are not suitable predictive models and results were discarded from main results section.

	Variable	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
Sep-14	**RH**	2.34± 0.65	0.00*	SD: 10.85 Residual: 11.51	**0.49**
	T	-3.1± 0.67	0.00*		
	Depth	-0.02± 0.02	0.02		
	VPD	164 ±43.14	0.00*		
	SWP	-0.26± 0.07	0.00*		
	Light	-0.005± 0.02	0.8		
	PPT	0.62 ±0.26	0.02*		
	Variable	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
Oct-14	**RH**	0.7	0.28	SD: 1.518713 Residual: 16.4199	**0.8**
	T	-2.94	0.00*		
	Depth	0.03	0.05*		
	VPD	62.48	0.1		
	SWP	0.17	0.2		
	Light	0.05	0.00*		
	PPT	0.68	0.13		
	Variable	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
Nov - Dec 14	**RH**	1.1	0.00*	SD: 16 Residual: 20	**0.46**
	T	-1.44	0		
	Depth	0	0.61		
	VPD	53.56	0		
	SWP	0.021	0.15		
	Light	0.07	0.46		
	PPT	0.11	0.001*		
	Variable	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
Feb - Mar 15	**RH**	0.59	0.09	SD: 15 Residual: 10	**0.48**
	T	-0.49	0.16		
	Depth	-0.01	0.01		
	VPD	34.24	0.02		
	SWP	0.003	0.47		
	Light	0.002	0.7		

	Variable	Fixed effects β1 (estimate ± SE)	p-value	Random effects b1 (SD σb1)	Autoregressive parameter ϕ
	PPT	0.66	0		
May-15	**Variable**	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
	RH	-0.02	0.96	SD: 20 Residual: 10	**0.43**
	T	-1.05	0.38		
	Depth	0.06	0.12		
	VPD	-18	0.6		
	SWP	-0.01	0.66		
	Light	0.009	0.88		
	PPT	-0.003	0.9		
Jun - Aug 15	**Variable**	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
	RH	0.01	0.1	SD: 1228 Residual: 1920	**0.51**
	T	0	0.56		
	Depth	0	0.41		
	VPD	0.065	0.11		
	SWP	0	0.64		
	Light	0.01	0.05*		
	PPT	0.102	0.05*		
Oct - Dec 15	**Variable**	**Fixed effects β1 (estimate ± SE)**	**p-value**	**Random effects b1 (SD σb1)**	**Autoregressive parameter ϕ**
	RH	-0.001	0.15	SD: 230.4 Residual: 424.8	**0.37**
	T	0	0.84		
	Depth	0	0.23		
	VPD	-0.067	0.05*		
	SWP	0	0.19		
	Light	0.001	0.0*		
	PPT	0.085	0.5		

Chapter 6 - In sight of land: Final Discussion and Comments

“non canimus surdis;
respondent omnia silvae”

we sing not to the deaf;
the trees echo every word
Virgil, Eclogues 10:8

6.1 Introduction

> *"To me, an experiment is a kind of conversation with plants: I have a question for them, but since we don't speak the same language, I can't ask them directly and they can't answer verbally. But plants can be eloquent in their physical responses and behaviours.*
>
> *Plants answer questions by the way they live, by their responses to change; you just need to learn how to ask"*
>
> *Robin Wall Kimmerer – Braiding Sweetgrass*

The curiosity which drives the study of tree ecophysiology is ancient, and our knowledge has grown hand in hand with the tools and skills to capture it. Our understanding of tree responses to their environment is becoming greater with every passing year, to the point where we are able to simulate tree function and incorporate individual scale tree responses into landscape scale models. There are still, however, many unknowns, and even the mechanism by which a plant's sugar transport system is thought to work, the Münch flow hypothesis, has only been indirectly measured (Knoblauch et al. 2016, Knoblauch and Peters 2017). Gaining a deeper understanding of the water related aspect of tree ecophysiology is vital if we are to understand how they will respond to a changing climate. Mangrove forests are ecosystems which harbour high species diversity, are thought to be one of the largest blue carbon sinks (McLeod et al. 2011, Atwood et al. 2017) and provide vital services to human communities in the tropics (Malik et al. 2015). Thus, the scientific community has both the need and the responsibility to increase our understanding of environmental changes on mangrove function and growth, in order to help inform models which predict landscape scale changes and thus increase our capacity to implement measures to guarantee their protection (Record et al. 2013, Daru and le Roux 2016).

In the following text I discuss the main findings of my thesis on the water relations of *Avicennia marina*. Firstly I review the each individual chapter, then I synthesize and discuss them in conjunction and finally I lay out my study limitations with suggestions for further research

6.2 Core chapter findings

Deciding on the scope and focus of each chapter was one of the most challenging parts of writing this thesis. In some cases, I decided for a broad approach, a traditional "setting of the scene", as many of the methods employed in this thesis had not been used before in *A. marina* (Chapter 4 & 5). In other cases, the questions were more specific as there was more previous research available on the topic (Chapter 3). Nonetheless, each of the chapters successfully addressed the questions laid out in Chapter 1. The core finds of each chapter are presented below chapter by chapter, and then synthesised in conjunction in Section 6.3 *General Synthesis*.

Chapter two - Testing the Waters: Analysing the heterogeneous stem radial change dynamics of the mangrove Avicennia marina (Forssk.) Vierh looking for indicators of environmental influence on radial water storage

Chapter two sets the scene for this thesis, by taking a broad look at the study of stem radius change in *A. marina,* using point dendrometers over one and a half years. As the wood structure of *A. marina* has been reported to cause heterogeneous results both in growth and water dynamics dendrometer studies, the aim of this chapter was firstly to establish if dendrometer measurements are purely stochastic, as proposed by Robert et al. (2014). My results show that long term trends of SRC and water induced stem radius changes (ΔW) are indeed more heterogeneous than those seen in other studies. However, detailed analysis of water-related stem cycle components, derived in a method similar to Deslauriers et al. (2011), still showed discernible seasonal trends and strong correlations with environmental variables. I propose that some of the heterogeneity in the SRC measurements is due to changes in wood structure and properties along the stem, as well as changes in the strength of osmoregulation with stem height, which causes different sensitivities to environmental variables along the stem. In some cases the heterogeneity is caused by different amplitudes in ΔW, but not the direction of the response, e.g. all points tend to have relative increases in stem radius during high humidity. In these cases I propose that the standardization by measurement point maxima can help discern relative trends and strengthen regressions with environmental variables, as seen in my results. Thus, although caution is needed when upscaling dendrometer measurements to the tree level, it is still perfectly possible to observe relative changes and responses to changing environmental conditions. Another element of my analysis, which allowed to discern the origin of the heterogeneity in measurement points, was the segregation of the data by stem tiers. Thus, I found that the upper tiers were more affected by light and atmospheric demand of water, whilst the lower tiers were more sensitive to soil water potential and precipitation levels.

Another keystone finding in this chapter is that the most important driver of expansion in *A. marina* at short time-scales of 30 days is light. These results provide backing for the theory that sugars play a role in the osmoregulation responsible for daytime stem swelling in *A. marina*, as proposed by Vandegehuchte et al. (2014b). This chapter also evidenced the importance of freshwater inputs through precipitation, confirming that *A. marina* makes use of freshwater when and where available for increasing its water stores. It also showed the importance of atmospheric water demand, in driving storage tissue contraction and there was no confirmation of maximum daily shrinkage as an indicator of seasonal water stress during this study (Goldhamer and Fereres 2001, Deslauriers et al. 2003, Fereres and Goldhamer 2003, Belien et al. 2014, Robert et al. 2014). The effect of soil salinity on stem water storage was hard to discern since the correlation changed sign throughout the analysed period. However, it often had a negative correlation with stem expansion and contraction amplitude, which coincides with the work of Lechthaler et al. (2016) suggesting larger reliance of mangroves on stored water during times of high salinity.

The results of this work are promising as they show that measuring stem radius change in *A. marina* with point dendrometers is a reliable way of gaining understanding of this mangrove species' water relations. Point dendrometers are widely used, as they provide an easy method for studying water and growth dynamics of tree stems. Hence, their correct employment in the study of *A. marina* will undoubtedly allow us to further our understanding of water storage dynamics and their role in the water relations of this enigmatic species.

Chapter three - Catching the swell: Daytime stem expansion and seasonal reversal in the peristaltic wave of stored water use along the stem of Avicennia marina (Forssk.) Vierh

Chapter three is a thorough investigation of daytime stem swelling, analysing both diel courses and seasonal changes along the stem. My results show how the stem of *A. marina* swells well into the day, even after the start of transpiration, decrease in leaf turgor and a drop in leaf water potential to values as low as -4.5 MPa. Water flow into storage tissues during this time is thus decoupled from transpiration induced water potential drop, and must be due to an osmotic adjustment of inner bark elastic tissues (Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b). I propose that the changes in inner bark osmotic concentration originate in the phloem and are related to phloem loading, transport and unloading of sugars, which are known osmolytes (De Schepper and Steppe 2010, De Swaef et al. 2013, Mencuccini et al. 2013, Chan et al. 2016, Paljakka et al. 2017). My measurements of stomatal conductance and leaf turgor showed that minima were reached late in the day, implying that stomata stay open to facilitate sugar loading and thus maintain turgor of storage tissues (Nikinmaa et al. 2013). These results are exciting, as upregulated turgor pressure at midday could allow stem growth during daytime, a process which otherwise is inhibited (Zweifel et al. 2016, Mencuccini et al. 2017, Coussement et al. 2018).

The theory of the phloem as an essential actor in daytime swelling is backed by the seasonal reversal in the direction along the stem of the peristaltic movement of water storage depletion. During winter, the lower areas of the stem swelled for longer than upper areas, which started shrinking first. In summer however, this trend was reversed, and the lower stem started shrinking first whilst the upper stem lagged behind. This switch in the direction of swelling coincided with the start of the growing season and was correlated with temperature, and thus must be related to changes in the strength of osmoregulation along the stem caused by changing carbohydrates supply and demand. Thus, my results back the work of Sevanto et al. (2002, 2003 and 2011), which showed that phloem loading and unloading processes coupled to relative source sink strength, have a strong effect on the turgor signal in the phloem, and thus the coupling and time-lags between phloem and xylem water potential. The results of Chapter 2 showing that light is the strongest correlate of stem swelling strongly back the conclusions of Chapter 3, as light sum is a proxy of sugar production, and hence source strength and osmolyte production.

My findings are compelling, not only do they show the wealth of different water storage and growth strategies used by plants, but also provide backing for current theories on the importance of the phloem and soluble carbon in elastic water store dynamics (Sevanto et al. 2003, Holtta et al. 2006, De Schepper and Steppe 2010, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Paljakka et al. 2017). The special wood structure of *A. marina*, which has multiple layers of internal phloem, and the role of the phloem turgor signal in water storage dynamics, no doubt has a role to play in guaranteeing hydraulic safety and growth, and might be the key to the success of this halophyte (Schmitz et al. 2008).

Chapter four – Disentangling the net: Separating components of Avicennia marina (Forssk.) Vierh stem radial water dynamics by concomitant xylem and over-bark measurements

The aim of Chapter four was to help resolve the mechanisms responsible for daytime stem swelling in *A. marina* by performing simultaneous measurements of over-bark and xylem radius change. My results showed than daytime stem swelling is due to an increase in inner bark thickness which overcompensates for the shrinking of the xylem at the onset of transpiration. This uncoupling between the inner bark and the transpiration-driven changes in the leaf and xylem reveals an osmotic phloem generated turgor signal (Chan et al. 2016) reinforcing the theory that phloem physiology has an essential role in day time stem swelling of *A. marina* (Chapter 3). Inner-bark increase compensated for sharp xylem shrinking, and thus osmoregulation may have a role in guaranteeing water safety during times of high transpiration, especially considering that the salinity of sap in *A. marina* is higher than in other species (Krauss and Ball 2013, López-Portillo et al. 2014). My measurements also showed xylem radius changes behaved much more as a physically driven component of the plant hydraulic system. Thus, xylem was closely influenced by atmospheric water demand and coupled to leaf turgor changes, more so than whole stem radius measurements. Therefore, the results of this chapter provide additional support for osmotic adjustment of inner bark tissues (Vandegehuchte et al. 2014b), with a potential role in helping maintain hydraulic safety, and the turgor necessary for metabolic function, cell division, cell expansion and hence growth.

Despite the results obtained by on-xylem measurements, this chapter also reveals that point dendrometer measurements of xylem tissue are heterogeneous, and this variability is likely due to the wood structure of *A. marina*, composed of multiple reticulate cambia. Thus, on-xylem measurements should be used with caution as they may be unpredictable, especially if one disposes of a limited amount of time to perform measurements. With care and time however, xylem measurements in *A. marina* offer an exciting avenue for further research. Amongst other possibilities, they could help understand the mechanisms behind changes in daytime stem swelling dynamics, such as the peristaltic switch, and reveal if source-sink strength and phloem loading and unloading processes are indeed responsible (Sevanto et al. 2002, 2003).

Chapter five - No leaf is an island: using turgor pressure probes to study the relationship of leaf turgor to microclimate and water relations in Avicennia marina (Forssk.) Vierh.

Chapter five is the first ever high-resolution leaf turgor study in *A. marina* using the leaf patch pressure clamp technology (Zimmermann et al. 2008, Zimmermann et al. 2013). Diel leaf turgor dynamics in *A. marina* revealed a delayed recovery of leaf turgor in the evening, which was most pronounced in summer. Thus, conditions of water shortage increased the hysteresis between morning and afternoon leaf turgor values. The delayed supply to thirsty leaves of stored water due to daytime stem swelling no doubt contributes to this late recovery of leaf turgor in the evening. The study of time lags between leaf turgor and stem radius change dynamics revealed the influence of atmospheric water demand on the time it took for leaf turgor to recover from the start of water mobilization. The findings of this chapter also confirm the different behaviours of the upper and lower stem and their changing sensitivity to the water demand of leaves. My work also confirms the increasing amount of evidence of the importance of fresh water inputs for mangrove trees (Robert et al. 2014, Reef and Lovelock 2015, Reef et al. 2015, Santini et al. 2015, Lovelock et al. 2017, Nguyen et al. 2017a, Nguyen et al. 2017b). Instant alleviation of leaf turgor through small precipitation events are also an additional line of evidence of leaf water uptake in *A. marina*

The results of this chapter open a promising horizon for the study of leaf turgor and its part in the water relations of *A. marina*. The majority of literature using the turgor pressure probe relates to irrigation and horticultural sciences (Martinez-Gimeno et al. 2017), and wider usage in ecophysiology studies of natural ecosystems could greatly improve our understanding of forest responses to water availability. Considering the sensitivity of leaf turgor to water shortages seen in other work, the use of turgor pressure probes might be key in understanding tree responses to drought. Advancing this avenue of research is becoming critical, since large tree die-off events due to climate change related drought are already happening across the globe (Allen et al. 2010). Since our understanding of these events is often limited by our capacity to measure tree water stress at a high enough resolution, any technical developments in this field, such as the use of leaf turgor pressure probes, should be pursued further .

6.3 General synthesis and comments

My work on *A. marina* contains several important findings which are not only relevant for furthering our understanding of the physiology of mangroves and their response to a changing environment. My results also provide general insights on physiological processes within a plant's hydraulic system, and the mechanisms essential for maintaining a favourable balance of water pools and fluxes in trees. As research begins to encompass a broader array of plant species from a greater diversity of environments, we are starting to attain an understanding of the multiple strategies and mechanisms present in plants to guarantee an adequate state of hydration and growth (Mencuccini et al. 2017). Understanding the hydraulic dynamics of leaves and stems is essential for understanding how trees will respond to changing environmental conditions, both through potential changes in growth or death through hydraulic failure (Allen et al. 2010, Hartmann et al. 2013, Trenberth et al. 2013, Sperry and Love 2015, Pivovaroff et al. 2016).

My findings show that daytime stem expansion is an important feature of *A. marina* radial water storage dynamics. My work establishes that this process is caused by the swelling of the inner bark, which must be due to an osmotic adjustment of storage tissues as in the short term it is uncoupled from transpiration, leaf turgor, leaf water potential, and xylem shrinkage. Since the swelling of the stem is strongly affected by light and the relative proximity to sources and sinks, soluble sugars must certainly play a role in this osmoregulation. Stem swelling was often not strongly correlated with expected variables like relative humidity or VPD, and such a strong positive influence of light sum on stem swelling is remarkable, and easily explained if sugars are involved in osmoregulation. Thus my work provides backing for and furthers the work of Vandegehuchte et al. (2014 a, b) which modelled and explained daytime stem swelling in mangroves as a consequence of storage tissue osmotic adjustment. My work showed that the inner bark swelling compensated for decreases in xylem radius in times of high water demand. Delayed shrinking of water stores would mean more water is available in storage tissues at the end of a day to potentially push water back into the transpiration stream, possibly playing a role in embolism refilling in xylem and leaves. The role of phloem-generated turgor signal in guaranteeing hydraulic safety is especially plausible considering that the wood structure of *A. marina*, comprised of multiple layers of internal secondary phloem, is associated with water stressed environments in other species (Schmitz et al. 2008, Robert et al. 2011).

My work fits well into our developing understanding of the effect of osmotic adjustment and soluble sugar dynamics in the phloem on stem radius changes, as well as the important links between water relations and non-structural carbon (NSC), and their potential role in osmoregulation, hydraulic safety, embolism refilling, turgor maintenance and eventually, growth. (Sevanto et al.

2003, Daudet et al. 2005, De Schepper and Steppe 2010, Woodruff and Meinzer 2011, De Schepper et al. 2013, Mencuccini et al. 2013, De Swaef et al. 2015, Pfautsch et al. 2015a, Chan et al. 2016, De Baerdemaeker et al. 2017, Mencuccini et al. 2017). The implications of phloem generated turgor signal in enabling growth in *A. marina* during the daytime should be the object of future study (Mencuccini et al. 2017, Coussement et al. 2018). Further research of stem radius changes on other species with multiple reticulate cambia will help understand if daytime swelling is a characteristic shared by other species with internal secondary phloem.

Despite the potential adaptive function of daytime stem swelling, my work also shows for the first time that diurnal stem swelling seems to have consequences for leaf hydration, causing delayed leaf turgor recovery in the evenings. This finding confirms the work of other authors which have also suggested that mangrove leaves must use internal water stores in order to manage imbalances between supply and demand (Lechthaler et al. 2016, Nguyen et al. 2017a). Although over the last decade, many studies have pointed towards the importance of foliar water uptake in relieving leaf water potential of tall trees, it is only recently that there is evidence it occurs in mangroves (Breshears et al. 2008, Goldsmith 2013, Gotsch et al. 2014, Uddin 2014, Reef and Lovelock 2015, Lovelock et al. 2017, Nguyen et al. 2017a). My results back this notion, since there was evidence that small precipitation events relieved leaf turgor, and further work should address the long term reliance of leaves on atmospherically derived water.

The work of Mencuccini et al. (2013) and Sevanto et al. (2002, 2003 and 2011) linking carbon and water radial dynamics along the stem were essential for understanding the peristaltic switch which occurs along the stem at the start of the growing season. On the one hand these patterns can easily be explained through proximity to the source of sugars of the upper stem, and thus proximity to sites of sugar loading and strength of the phloem generated osmotic signal. On the other hand, the influence of osmoregulation on the lower stem can be explained by changes in sink strength (and thus soluble sugar concentrations) throughout the year. The peristaltic switch has never been measured before in other trees to my knowledge, and it would be exciting to ascertain if it occurs in other trees, even those without internal secondary phloem. Another finding in this thesis is the apparent difference between lower and upper stem which was found throughout all chapters. The difference between tiers was not just present in terms of the peristaltic switch but also in their different relationships to environmental conditions and the water demand of leaves. These differences are undoubtedly due to the changing influence of osmoregulation along the stem and changes in tissues structure. Thus, the lower stem seemed more sensitive to rainwater availability, soil water potential and also to the transpiration requirements of leaves. Conversely, the upper stem seemed more sensitive to light and atmospheric water demand. Although the semi-autonomous nature of tree branches is a matter researched in the field of bud and twig development (Hoch 2005), high spatial definition dynamics of tree stems are rarely reported for dendrometer studies (but see Zweifel and Häsler 2001, Sevanto et al. 2002, Mencuccini et al. 2013), and should be a matter for further study.

The insights obtained through the study of *A. marina* osmotic regulation of storage tissues and changing role of phloem dynamics have the capacity to reveal much more than the physiology of this mangrove species. Studying the phloem is hard, due to the nature of the tissues and the processes which it harbours (Turgeon 1996, Oparka and Turgeon 1999, Rennie and Turgeon 2009, Turgeon and Wolf 2009, Jensen et al. 2011, De Schepper et al. 2013, Savage et al. 2013, Pfautsch et al. 2015a, Pfautsch et al. 2015b, Knoblauch et al. 2016). Thus, insights into the phloem generated turgor signal in *A. marina* and the role of multiple internal secondary phloem might reveal important insights into the role of the phloem and carbohydrate dynamics in general, for turgor maintenance, water storage dynamics, water safety, embolism refilling and growth.

A note on water sources and salinity

My work shows the importance of freshwater inputs for *A. marina*, which was evidenced both in the response of stem radius and leaf turgor to precipitation events. Precipitation allowed for replenishment of water stores both through increase leaf turgor and stem radius. In the same manner, precipitation lack sometimes resulted in dramatic reductions in both leaf and radial stem water storage. Thus, my work complements recent research showing the importance of fresh water availability and its impact on leaf and stem water storage (Ball 1988b, a, Robert et al. 2014, Reef and Lovelock 2015, Reef et al. 2015, Santini et al. 2015, Jiang et al. 2017, Lovelock et al. 2017). These results are vital for an understanding of how mangroves will be affected by changes in the earth's climate (Record et al. 2013, Krauss et al. 2014). This is especially true in regions which are accustomed to high levels of precipitation, like New Zealand, where mangroves may be especially vulnerable to increases in drought duration and frequency.

It is evidently interesting that throughout the analysis made in this thesis, soil salinity was not often strongly correlated to *A. marina* measured ecophysiology. In some cases soil water potential was negatively correlated with stem cycle amplitudes, implying larger reliance on water stores and higher water storage in times of high salinity, which coincides with the recent work by Lechthaler et al. (2016). However this relationship was not always present, and sometimes the correlations became positive (Chapter 2). An explanation for the lack of strong or consistent soil water potential effects found in this study may lie in the predawn water potential imbalance detected in my results (Chapter 3). The predawn water potentials of *A. marina* ranged from -0.1 to -1.2 MPa, with a mean of -0.5 MPa. This is surprising, considering that soil water potential measured with the CTD was considerably below this during most of the measurement period, on average -2.5 MPa, with an average site salinity of 30‰. The extreme case of water potential imbalance was seen on February 13th, when predawn water potential was of -0.25 MPa whilst soil water potential was of -3.8 MPa (Chapter 3). Theory states predawn leaf and xylem water potential reflects soil water potential, if trees are able to reach full rehydration overnight, or tree water potential is somewhat more negative than that of soil if night time transpiration occurs (e.g. Bucci et al. 2005, Jiang et al. 2017). Although water potential imbalances where leaves have more negative water potentials than that of soil are commonly observed in halophytes due to presence of osmolites (Scholander 1968, Wang et al. 2011, Jiang et al. 2017), my study trees show the opposite. Thus, as my results show that leaf water potential was much less negative than that of soil, I must conclude that trees were accessing alternative water sources, either in the form of groundwater, precipitation or atmospheric water. Such an imbalance has been observed before in a field study measuring *A. marina* leaf water potential, and was also attributed to the use of fresh water (Jiang et al. 2017). The mangroves in this study are within 100 m of the estuary stream which carries fresh water from the mainland. However, although the salinity of pore water is lower than in other sites in the estuary, it has never been measured to be below 23‰. Thus, the possibility of my study mangroves accessing fresh water could explain the low correlations with measured soil water potential encountered throughout the study. In this sense it is worth mentioning that in mangrove systems there is a correlation between *A. marina* water-related physiology and measured soil salinity. Mangroves are known to affect the salinity of their soil, as the tight filtration of water at the root level leaves salt behind (Scholander 1968, Passioura et al. 1992, Marchand et al. 2004, Krauss et al. 2015). This adds an additional level of complexity to salt measurements in the field, as the direction of the causality, e.g. the effects of salinity on transpiration vs the effects of transpiration on salinity, may not be clear cut.

Additionally, it is important to keep in mind that the method for measuring soil water potential, the CTD gage, might not be optimal or reflect the experienced water potential of the trees. Firstly, the approximation for converting conductivity to osmotic water potential based on McIntyre’s (1980) work is theoretical and might not always be accurate, depending on the temperature and solute ranges. Secondly, the water measured in the CTD chamber might not be a true reflection of the soil pore water tree roots are exposed to. Thirdly, the CTD also requires high maintenance, as it has a tendency to be filled with sediment and that leads to erroneous readings.

Given the large amount of work showing the importance of salinity for mangrove development, functioning, water relations and growth (Ball 1988b, a, Ball and Pidsley 1995, Wang et al. 2011, Reef et al. 2012, Krauss and Ball 2013, Nguyen et al. 2015), it is undoubtedly important to understand the effects of natural salinity changes and gradients, and find ways to reliably measure them in the field. However, it is important to keep in mind that many of the aforementioned studies look at the effect of salinity differences either in manipulation experiments with different salinity treatments on saplings or, more rarely, in natural spatial salinity gradients. Studies including high resolution temporal changes in salinity of a study location are rare. Those studies that, to my knowledge, have measured continuous changes in salinity at a site often report no measurable effects of temporal variation in salinity (Robert et al. 2014, Vandegehuchte et al. 2014a, Vandegehuchte et al. 2014b). Additionally, many of those experiments reporting effects of salinity are done on seedlings and saplings and some of their results may not be transferable to adult trees, due for example, to important morphological changes in wood structure with age. An example is that recent studies on seedlings showed that salinity was essential for growth and development of *A. marina* seedlings, as low salinity impaired wood structure formation (Nguyen et al. 2015). However, a field study by Santini et al. (2015) performed on adult *A. marina*, found that it used fresh water sources for growth, and that the use of freshwater increased with its availability, and this had determinant effect on growth rates. Thus, caution is needed when making generalizations on the importance of salt for growth (and water relations) from results of experiments done using seedlings. However, it is clear that salinity must have an effect on the water relations of *A. marina*, on stomatal regulation, transpiration and water storage (Ball 1988b, Munns 2002, Naidoo 2006, Krauss et al. 2008, Wang et al. 2011, Krauss and Ball 2013, López-Portillo et al. 2014, Nguyen et al. 2015, Reef and Lovelock 2015, Reef et al. 2015, Santini et al. 2015, Lechthaler et al. 2016, Nguyen et al. 2017a). Hence, finding reliable and accurate ways of measuring the salinity and soil water potential experienced by mangrove roots in the field, may be an important step towards furthering our understanding of mangrove ecosystems' responses to soil water potential and salinity.

Additional remarks

It is worth remarking that a conclusion of this study is also that a wealth of information is held in the times of day and time lags, additional to amplitudes of stem radius change or leaf turgor. Many studies look at changing amplitudes, which are undoubtedly a valuable source of information especially for upscaling plant water use (Zweifel et al. 2000, Deslauriers et al. 2003, Steppe et al. 2006, Cermak et al. 2007, Drew and Downes 2009, Fernandez and Cuevas 2010, Drew et al. 2011, Turcotte et al. 2011, Belien et al. 2014, De Swaef et al. 2015, Steppe et al. 2015, Sallo et al. 2017). However, the study of timing and time-lags contributes an important aspect to the understanding the coordination of leaf turgor, leaf conductance, transpiration and radial water storage. Thus, integrating the work of past dendrometer studies, like those of Vadegehuchte et al. (2014a) and Robert et al. (2014), my work shows that, despite the patchiness, dendrometers measurements are still an important source of information of radial water storage dynamics in *A. marina*, especially when combined with other ecophysiological measurements.

Another conclusion to be drawn from this work is the importance of integration periods when conducting ecophysiological research. Much of the published work in ecophysiology of mangroves covers only a few weeks or months and the definitions of long-term and short-term are somewhat poorly defined (Vadegehuchte et al. 2014a). Perhaps because of the rarity and high value of insights provided by research spanning more than a year, the word long-term is perhaps overused. Thus, my work reinforces the importance of spanning long periods of time of at least a year and also analysing different periods separately. This approach is essential in order to truly understand the dynamics of the forest and its changing drivers, as short time windows of a single season introduce confounding factors and might not be representative, and thus lead to conclusions with limited extrapolation capacity.

6.4 Study limitations and suggestions for further research

> *"In studying a philosopher, the right attitude is neither reverence nor contempt, but first a kind of hypothetical sympathy, until it is possible to know what it feels like to believe his theories, and only then a revival of the critical attitude, which should resemble the state of mind of a person abandoning opinions which he has hitherto held"*
>
> Bertrand Russel – The History of Western Philosophy

Bertrand Russel gives us useful advice in the understanding of any system of thought, be it philosophy or empirical science (Russel 1945). The conclusions presented in this work suffer from the centuries-long ailment of any empirical science – they represent a story pieced together from the scraps offered by those things which can be measured and interpreted within the framework of our current knowledge. It is warrant that some of the interpretations made in my work are incomplete, but hopefully my work sets the scene for further research to build and improve upon.

The lines of evidence which are lacking and should guide further research are fairly obvious to the reader I’m sure. Mainly, measurements of osmotica and osmotic potential would contribute immensely to completing the picture. Many of the explanations I offer draw on osmotica and phloem physiology to explain trends of daytime stem swelling, such as the peristaltic switch (Chapter 3) or the different environmental drivers of upper and lower stem dynamics (Chapter 2). Although these ideas are backed by modelling studies (Vandegehuchte et al. 2014b) they remain unproven, until measurements of osmotica are made. These measurements should include carbohydrates but also salts, together with evaluations of relative source-sink strength. It is important to remember that inorganic ions and other compatible solutes in the xylem and phloem must also play a role in *A. marina,* and should be taken into account in future work (Sunarpi et al. 2005, López-Portillo et al. 2014). Direct measurements of osmotica and osmotic potential of storage tissues, although rare, are now beginning to appear in the literature (De Swaef et al. 2013, De Swaef et al. 2015, Paljakka et al. 2017, von Arx et al. 2017). Measurements of NSC are much more common and would be an important contribution to this work also, especially for elucidating the role of multiple phloem layers in daytime stem swelling and its consequences for potential cavitation refilling. In fact, extensive tissue sampling was originally conducted in this study with the idea of NSC analysis, as a complement to Chapter 4. However, analysis was not possible since traditional protocols for NSC analysis did not work for *A. marina*, due to the high levels of polyphenols in tissue samples, and a new analysis method is currently being optimized as part of the Ph.D. work of another student (Ravi, pers. comm.).

A second line of evidence would be, in addition to a detailed analysis of different carbohydrates, a metabolomics profiling of carbohydrate and osmotica-related metabolism. The environmental correlations of expansion and contraction show that, depending on the integration period, the relationships can change. Considering that osmoregulation is likely a biologically regulated process, making the assumption that the nature of relationships between radial change and environmental conditions are constant is undoubtedly inaccurate. Changes in the regulation of osmotic processes, providing physiological adjustment to changing environmental conditions, might be an important feature of *A. marina* water relations.

A third line of evidence which would contribute greatly to my work is a histological study of *A. marina* tissues. Such work should be conducted both with a functional approach, such as using dyes to see the passage of water between tissues (Pfautsch et al. 2015a, Pfautsch et al. 2015b, Pfautsch 2016), and a more anatomical approach, for microscopy and measurement of tissue properties. Thus, our understanding of the effect of *A. marina* wood structure on stem radial change could increase greatly if stem radius measurements were complemented with a study of tissue structure and properties such as capacitance, elasticity and conductance.

A note on statistical methods and analysis

Experimental design and statistical analyses in ecophysiology are challenging, and can be quite different to what is taught in traditional ecology classes. Firstly, large replication becomes challenging when expensive equipment is employed, with individual sensors costing up to NZD 10,000. Despite this, time series measurements afford an increased replication in time, which can in some ways make up for (or mask) the lack of spatial and individual replication. Nonetheless, the constraints of low replication can impose limits to the scope of our questions and our capacity to extrapolate results of our work. Secondly, working with time-series analysis where lots of collinear environmental variables are involved is challenging. I remember being both amused and exasperated when, after trudging my way through Zuur's chapter (Zuur 2014) on complex mixed effects model with time series, he concluded in the end that Pearson correlations were the most reliable way to test model conclusions. Zuur remarks that in these cases it is very hard to pinpoint "the" driving variable, and this is where manipulative experiments come in.

Despite being challenging, much can be gained by combining field studies with manipulation experiments in mangrove systems. Thus, theories of the role of sugars and osmotica could be tested with girdling experiments, defoliation and shading experiments, and the importance of freshwater inputs through rain exclusion experiments.

6.5 Final Conclusion

> *"Because we can't speak the same language, our work as scientists is to piece the story together as best we can. (...) We measure and record and analyze in ways that might seem lifeless but to us are the conduits to understanding the incrutable lives of species not our own. Doing science with awe and humility is a powerful act of reciprocity with the non-human world"*
>
> *Robin Wall Kimmerer – Braiding Sweetgrass*

Trees have ensured their survival by evolving an incredible array of dynamic responses to their ever changing homes. Some trees however, are hard pressed to keep up with the speed of our changing climate. Amongst the forests, mangroves are certainly vulnerable to the changes in the Earth's climate are already underway. Straddling the boundaries of a sea that is rising and relying on rainfall which is less predictable each year, it is important to understand the response of mangrove forests to environmental conditions. Additionally mangrove research also allows us to complete the glossary of plant hydraulics, by showing less well-studied mechanisms and processes by which plants ensure their water supply. The work presented in my thesis deepens our understanding both of *A. marina's* ecophysiology and response to environmental conditions, and our knowledge of general physiological processes operating in the plant world.

6.7 References

Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.-H. Lim, G. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management **259**:660-684.

Atwood, T. B., R. M. Connolly, H. Almahasheer, P. E. Carnell, C. M. Duarte, C. J. E. Lewis, X. Irigoien, J. J. Kelleway, P. S. Lavery, P. I. Macreadie, O. Serrano, C. J. Sanders, I. Santos, A. D. L. Steven, and C. E. Lovelock. 2017. Global patterns in mangrove soil carbon stocks and losses. Nature Climate Change **7**:523-+.

Ball, M. C. 1988a. Ecophysiology of mangroves. Trees **2**:129-142.

Ball, M. C. 1988b. Salinity tolerance in the mangroves Aegiceras corniculatum and Avicennia marina. I. Water use in relation to growth, carbon partitioning, and salt balance. Functional Plant Biology **15**:447-464.

Ball, M. C. and S. M. Pidsley. 1995. Growth responses to salinity in relation to distribution of two mangrove species, *Sonneratia alba* and *S.* alba, in northern Australia. Functional Ecology **9**:77-85.

Belien, E., S. Rossi, H. Morin, and A. Deslauriers. 2014. High-resolution analysis of stem radius variations in black spruce Picea mariana (Mill.) BSP subjected to rain exclusion for three summers. Trees-Structure and Function **28**:1257-1265.

Breshears, D. D., N. G. McDowell, K. L. Goddard, K. E. Dayem, S. N. Martens, C. W. Meyer, and K. M. Brown. 2008. Foliar absorption of intercepted rainfall improves woody plant water status most during drought. Ecology **89**:41-47.

Bucci, S. J., G. Goldstein, F. C. Meinzer, A. C. Franco, P. Campanello, and F. G. Scholz. 2005. Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in neotropical savanna trees. Trees-Structure and Function **19**:296-304.

Cermak, J., J. Kucera, W. L. Bauerle, N. Phillips, and T. M. Hinckley. 2007. Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiology **27**:181-198.

Chan, T., T. Holtta, F. Berninger, H. Makinen, P. Nojd, M. Mencuccini, and E. Nikinmaa. 2016. Separating water-potential induced swelling and shrinking from measured radial stem variations reveals a cambial growth and osmotic concentration signal. Plant Cell Environ **39**:233-244.

Coussement, J. R., T. De Swaef, P. Lootens, I. Roldán-Ruiz, and K. Steppe. 2018. Introducing turgor-driven growth dynamics into functional–structural plant models. Annals of Botany:mcx144-mcx144.

Daru, B. H. and P. C. le Roux. 2016. Marine protected areas are insufficient to conserve global marine plant diversity. Global Ecology and Biogeography **25**:324-334.

Daudet, F. A., T. Ameglio, H. Cochard, O. Archilla, and A. Lacointe. 2005. Experimental analysis of the role of water and carbon in tree stem diameter variations. Journal of Experimental Botany **56**:135-144.

De Baerdemaeker, N. J. F., R. L. Salomon, L. De Roo, and K. Steppe. 2017. Sugars from woody tissue photosynthesis reduce xylem vulnerability to cavitation. New Phytologist **216**:720-727.

De Schepper, V., T. De Swaef, I. Bauweraerts, and K. Steppe. 2013. Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany **64**:4839-4850.

De Schepper, V. and K. Steppe. 2010a Development and verification of a water and sugar transport model using measured stem diameter variations. Journal of Experimental Botany **61**:2083-2099.

De Swaef, T., V. De Schepper, M. W. Vandegehuchte, and K. Steppe. 2015. Stem diameter variations as a versatile research tool in ecophysiology. Tree Physiology **35**:1047-1061.

De Swaef, T., S. M. Driever, L. Van Meulebroek, L. Vanhaecke, L. F. Marcelis, and K. Steppe. 2013. Understanding the effect of carbon status on stem diameter variations. Ann Bot **111**:31-46.

Deslauriers, A., H. Morin, C. Urbinati, and M. Carrer. 2003. Daily weather response of balsam fir (Abies balsamea (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees **17**:477-484.

Deslauriers, A., S. Rossi, A. Turcotte, H. Morin, and C. Krause. 2011. A three-step procedure in SAS to analyze the time series from automatic dendrometers. Dendrochronologia **29**:151-161.

Drew, D. M. and G. M. Downes. 2009. The use of precision dendrometers in research on daily stem size and wood property variation: a review. Dendrochronologia **27**:159-172.

Drew, D. M., A. E. Richards, G. M. Downes, G. D. Cook, and P. Baker. 2011. The development of seasonal tree water deficit in Callitris intratropica. Tree Physiology **31**:953-964.

Fereres, E. and D. A. Goldhamer. 2003. Suitability of stem diameter variations and water potential as indicators for irrigation scheduling of almond trees. Journal of Horticultural Science & Biotechnology **78**:139-144.

Fernandez, J. E. and M. V. Cuevas. 2010. Irrigation scheduling from stem diameter variations: A review. Agricultural and Forest Meteorology **150**:135-151.

Goldhamer, D. A. and E. Fereres. 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrigation Science **20**:115-125.

Goldsmith, G. R. 2013. Changing directions: the atmosphere-plant-soil continuum. New Phytologist **199**:4-6.

Gotsch, S. G., H. Asbjornsen, F. Holwerda, G. R. Goldsmith, A. E. Weintraub, and T. E. Dawson. 2014. Foggy days and dry nights determine crown-level water balance in a seasonal tropical montane cloud forest. Plant Cell and Environment **37**:261-272.

Hartmann, H., W. Ziegler, O. Kolle, and S. Trumbore. 2013. Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. New Phytologist **200**:340-349.

Hoch, G. 2005. Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. Plant Cell and Environment **28**:651-659.

Holtta, T., T. Vesala, S. Sevanto, M. Peramaki, and E. Nikinmaa. 2006. Modeling xylem and phloem water flows in trees according to cohesion theory and Munch hypothesis. Trees-Structure and Function **20**:67-78.

Jensen, K. H., J. Lee, T. Bohr, H. Bruus, N. M. Holbrook, and M. A. Zwieniecki. 2011. Optimality of the Munch mechanism for translocation of sugars in plants. Journal of the Royal Society Interface **8**:1155-1165.

Jiang, G. F., U. M. Goodale, Y. Y. Liu, G. Y. Hao, and K. F. Cao. 2017. Salt management strategy defines the stem and leaf hydraulic characteristics of six mangrove tree species. Tree Physiology **37**:389-401.

Knoblauch, M., J. Knoblauch, D. L. Mullendore, J. A. Savage, B. A. Babst, S. D. Beecher, A. C. Dodgen, K. H. Jensen, and N. M. Holbrook. 2016. Testing the Munch hypothesis of long distance phloem transport in plants. eLife **5**.

Knoblauch, M. and W. S. Peters. 2017. What actually is the Munch hypothesis? A short history of assimilate transport by mass flow. Journal of Integrative Plant Biology **59**:292-310.

Krauss, K. W. and M. C. Ball. 2013. On the halophytic nature of mangroves. Trees **27**:7-11.

Krauss, K. W., J. G. Barr, V. Engel, J. D. Fuentes, and H. Q. Wang. 2015. Approximations of stand water use versus evapotranspiration from three mangrove forests in southwest Florida, USA. Agricultural and Forest Meteorology **213**:291-303.

Krauss, K. W., C. E. Lovelock, K. L. McKee, L. López-Hoffman, S. M. L. Ewe, and W. P. Sousa. 2008. Environmental drivers in mangrove establishment and early development: a review. Aquatic Botany **89**:105-127.

Krauss, K. W., K. L. McKee, C. E. Lovelock, D. R. Cahoon, N. Saintilan, R. Reef, and L. Z. Chen. 2014. How mangrove forests adjust to rising sea level. New Phytologist **202**:19-34.

Lechthaler, S., E. M. R. Robert, N. Tonne, A. Prusova, E. Gerkema, H. Van As, N. Koedam, and C. W. Windt. 2016. Rhizophoraceae Mangrove Saplings Use Hypocotyl and Leaf Water Storage Capacity to Cope with Soil Water Salinity Changes. Frontiers in Plant Science **7**.

López-Portillo, J., F. W. Ewers, R. Méndez-Alonzo, C. L. Paredes López, G. Angeles, A. L. Alarcón Jiménez, A. L. Lara-Domínguez, and M. d. C. Torres Barrera. 2014. Dynamic control of osmolality and ionic composition of the xylem sap in two mangrove species. American Journal of Botany.

Lovelock, C. E., R. Reef, and M. C. Ball. 2017. Isotopic signatures of stem water reveal differences in water sources accessed by mangrove tree species. Hydrobiologia **803**:133-145.

Malik, A., R. Fensholt, and O. Mertz. 2015. Mangrove exploitation effects on biodiversity and ecosystem services. Biodiversity and Conservation **24**:3543-3557.

Marchand, C., F. Baltzer, E. Lallier-Verges, and P. Alberic. 2004. Pore-water chemistry in mangrove sediments: relationship with species composition and developmental stages (French Guiana). Marine Geology **208**:361-381.

Martinez-Gimeno, M. A., M. Castiella, S. Ruger, D. S. Intrigliolo, and C. Ballester. 2017. Evaluating the usefulness of continuous leaf turgor pressure measurements for the assessment of Persimmon tree water status. Irrigation Science **35**:159-167.

McLeod, E., G. L. Chmura, S. Bouillon, R. Salm, M. Björk, C. M. Duarte, C. E. Lovelock, W. H. Schlesinger, and B. R. Silliman. 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2. Frontiers in Ecology and the Environment **9**:552-560.

Mencuccini, M., T. Holtta, S. Sevanto, and E. Nikinmaa. 2013. Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytologist **198**:1143-1154.

Mencuccini, M., Y. Salmon, P. Mitchell, T. Holtta, B. Choat, P. Meir, A. O'Grady, D. Tissue, R. Zweifel, S. Sevanto, and S. Pfautsch. 2017. An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant Cell Environ **40**:290-303.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant, Cell & Environment **25**:239-250.

Naidoo, G. 2006. Factors contributing to dwarfing in the mangrove *Avicennia marina*. Annals of Botany **97**:1095-1101.

Nguyen, H. T., P. Meir, L. Sack, J. Evans, R. S. Oliveira, and M. C. Ball. 2017a. Leaf water storage increases with salinity and aridity in the mangrove *Avicennia marina*: integration of leaf structure, osmotic adjustment and access to multiple water sources. Plant Cell and Environment **40**:1576-1591.

Nguyen, H. T., P. Meir, J. Wolfe, M. Mencuccini, and M. C. Ball. 2017b. Plumbing the depths: extracellular water storage in specialized leaf structures and its functional expression in a three-domain pressure-volume relationship. Plant Cell and Environment **40**:1021-1038.

Nguyen, H. T., D. E. Stanton, N. Schmitz, G. D. Farquhar, and M. C. Ball. 2015. Growth responses of the mangrove *Avicennia marina* to salinity: development and function of shoot hydraulic systems require saline conditions. Ann Bot **115**:397-407.

Nikinmaa, E., T. Holtta, P. Hari, P. Kolari, A. Makela, S. Sevanto, and T. Vesala. 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. Plant Cell and Environment **36**:655-669.

Oparka, K. J. and R. Turgeon. 1999. Sieve elements and companion cells - Traffic control centers of the phloem. Plant Cell **11**:739-750.

Paljakka, T., T. Jyske, A. Lintunen, H. Aaltonen, E. Nikinmaa, and T. Holtta. 2017. Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst). Plant Cell and Environment **40**:2160-2173.

Passioura, J. B., M. C. Ball, and J. H. Knight. 1992. Mangrpves may salinize soil and in doing so limit their transpiration rate. Functional Ecology **6**:476-481.

Pfautsch, S. 2016. Hydraulic anatomy and function of trees—basics and critical developments. Current Forestry Reports **2**:236-248.

Pfautsch, S., T. Hölttä, and M. Mencuccini. 2015a. Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology **35**:706-722.

Pfautsch, S., J. Renard, M. G. Tjoelker, and A. Salih. 2015b. Phloem as capacitor: cadial transfer of water into xylem of tree Stems occurs via symplastic transport in ray parenchyma. Plant Physiology **167**:963-971.

Pivovaroff, A. L., S. C. Pasquini, M. E. De Guzman, K. P. Alstad, J. S. Stemke, L. S. Santiago, and K. Field. 2016. Multiple strategies for drought survival among woody plant species. Functional Ecology **30**:517-526.

Record, S., N. D. Charney, R. M. Zakaria, and A. M. Ellison. 2013. Projecting global mangrove species and community distributions under climate change. Ecosphere **4**.

Reef, R. and C. E. Lovelock. 2015. Regulation of water balance in mangroves. Annals of Botany **115**:385-395.

Reef, R., H. L. Markham, N. S. Santini, and C. E. Lovelock. 2015. The response of the mangrove *Avicennia marina* to heterogeneous salinity measured using a split-root approach. Plant and Soil **393**:297-305.

Reef, R., N. Schmitz, B. A. Rogers, M. C. Ball, and C. E. Lovelock. 2012. Differential responses of the mangrove Avicennia marina to salinity and abscisic acid. Functional Plant Biology **39**:1038-1046.

Rennie, E. A. and R. Turgeon. 2009. A comprehensive picture of phloem loading strategies. Proceedings of the National Academy of Sciences of the United States of America **106**:14162-14167.

Robert, E. M. R., A. H. Jambia, N. Schmitz, D. J. R. De Ryck, J. De Mey, J. G. Kairo, F. Dahdouh-Guebas, H. Beeckman, and N. Koedam. 2014. How to catch the patch? A dendrometer study of the radial increment through successive cambia in the mangrove *Avicennia marina*. Annals of Botany **113**:741-752.

Robert, E. M. R., N. Schmitz, I. Boeren, T. Driessens, K. Herremans, J. De Mey, E. Van de Casteele, H. Beeckman, and N. Koedam. 2011. Successive Cambia: A Developmental Oddity or an Adaptive Structure? Plos One **6**.

Sallo, F. D., L. Sanches, V. R. D. Dias, R. D. Palacios, and J. D. Nogueira. 2017. Stem water storage dynamics of Vochysia divergens in a seasonally flooded environment. Agricultural and Forest Meteorology **232**:566-575.

Santini, N. S., R. Reef, D. A. Lockington, and C. E. Lovelock. 2015. The use of fresh and saline water sources by the mangrove Avicennia marina. Hydrobiologia **745**:59-68.

Savage, J. A., M. A. Zwieniecki, and N. M. Holbrook. 2013. Phloem Transport Velocity Varies over Time and among Vascular Bundles during Early Cucumber Seedling Development. Plant Physiology **163**:1409-1418.

Schmitz, N., E. M. Robert, A. Verheyden, J. G. Kairo, H. Beeckman, and N. Koedam. 2008. A patchy growth via successive and simultaneous cambia: key to success of the most widespread mangrove species Avicennia marina? Ann Bot **101**:49-58.

Scholander, P. F. 1968. How mangroves desalinate seawater. Physiologia Plantarum **21**:251-261.

Sevanto, S., T. Holtta, and N. M. Holbrook. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant Cell and Environment **34**:690-703.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2002. Time lags for xylem and stem diameter variations in a Scots pine tree. Plant Cell and Environment **25**:1071-1077.

Sevanto, S., T. Vesala, M. Peramaki, and E. Nikinmaa. 2003. Sugar transport together with environmental conditions controls time lags between xylem and stem diameter changes. Plant Cell and Environment **26**:1257-1265.

Sperry, J. S. and D. M. Love. 2015. What plant hydraulics can tell us about responses to climate-change droughts. New Phytol **207**:14-27.

Steppe, K., D. De Pauw, R. Lemeur, and P. A. Vanrolleghem. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology **26**:257-273.

Steppe, K., F. Sterck, and A. Deslauriers. 2015. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends Plant Sci **20**:335-343.

Sunarpi, T. Horie, J. Motoda, M. Kubo, H. Yang, K. Yoda, R. Horie, W. Y. Chan, H. Y. Leung, K. Hattori, M. Konomi, M. Osumi, M. Yamagami, J. I. Schroeder, and N. Uozumi. 2005. Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na+ unloading from xylem vessels to xylem parenchyma cells. Plant Journal **44**:928-938.

Trenberth, K. E., A. Dai, G. van der Schrier, P. D. Jones, J. Barichivich, K. R. Briffa, and J. Sheffield. 2013. Global warming and changes in drought. Nature Climate Change **4**:17-22.

Turcotte, A., S. Rossi, A. Deslauriers, C. Krause, and H. Morin. 2011. Dynamics of depletion and replenishment of water storage in stem and roots of black spruce measured by dendrometers. Front Plant Sci **2**:21.

Turgeon, R. 1996. Phloem loading and plasmodesmata. Trends in Plant Science **1**:418-423.

Turgeon, R. and S. Wolf. 2009. Phloem Transport: Cellular Pathways and Molecular Trafficking. Pages 207-221 Annual Review of Plant Biology. Annual Reviews, Palo Alto.

Uddin, S. 2014. Canopy water uptake: An important survival mechanism of mangroves. Ghent University and Vrije Universiteit Brussel, Belgium.

Vandegehuchte, M. W., A. Guyot, M. Hubau, S. R. E. De Groote, N. J. F. De Baerdemaeker, M. Hayes, N. Welti, C. E. Lovelock, D. A. Lockington, and K. Steppe. 2014a. Long-term versus daily stem diameter variation in co-occurring mangrove species: Environmental versus ecophysiological drivers. Agricultural and Forest Meteorology **192-193**:51-58.

Vandegehuchte, M. W., A. Guyot, M. Hubeau, T. De Swaef, D. A. Lockington, and K. Steppe. 2014b. Modelling reveals endogenous osmotic adaptation of storage tissue water potential as an important driver determining different stem diameter variation patterns in the mangrove species Avicennia marina and Rhizophora stylosa. Annals of Botany.

von Arx, G., A. Arzac, P. Fonti, D. Frank, R. Zweifel, A. Rigling, L. Galiano, A. Gessler, and J. M. Olano. 2017. Responses of sapwood ray parenchyma and non-structural carbohydrates of Pinus sylvestris to drought and long-term irrigation. Functional Ecology **31**:1371-1382.

Wang, W., Z. Yan, S. You, Y. Zhang, L. Chen, and G. Lin. 2011. Mangroves: obligate or facultative halophytes? A review. Trees **25**:953-963.

Woodruff, D. R. and F. C. Meinzer. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant, Cell & Environment **34**:1920-1930.

Zimmermann, D., R. Reuss, M. Westhoff, P. Geßner, W. Bauer, E. Bamberg, F.-W. Bentrup, and U. Zimmermann. 2008. A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. Journal of Experimental Botany **59**:3157-3167.

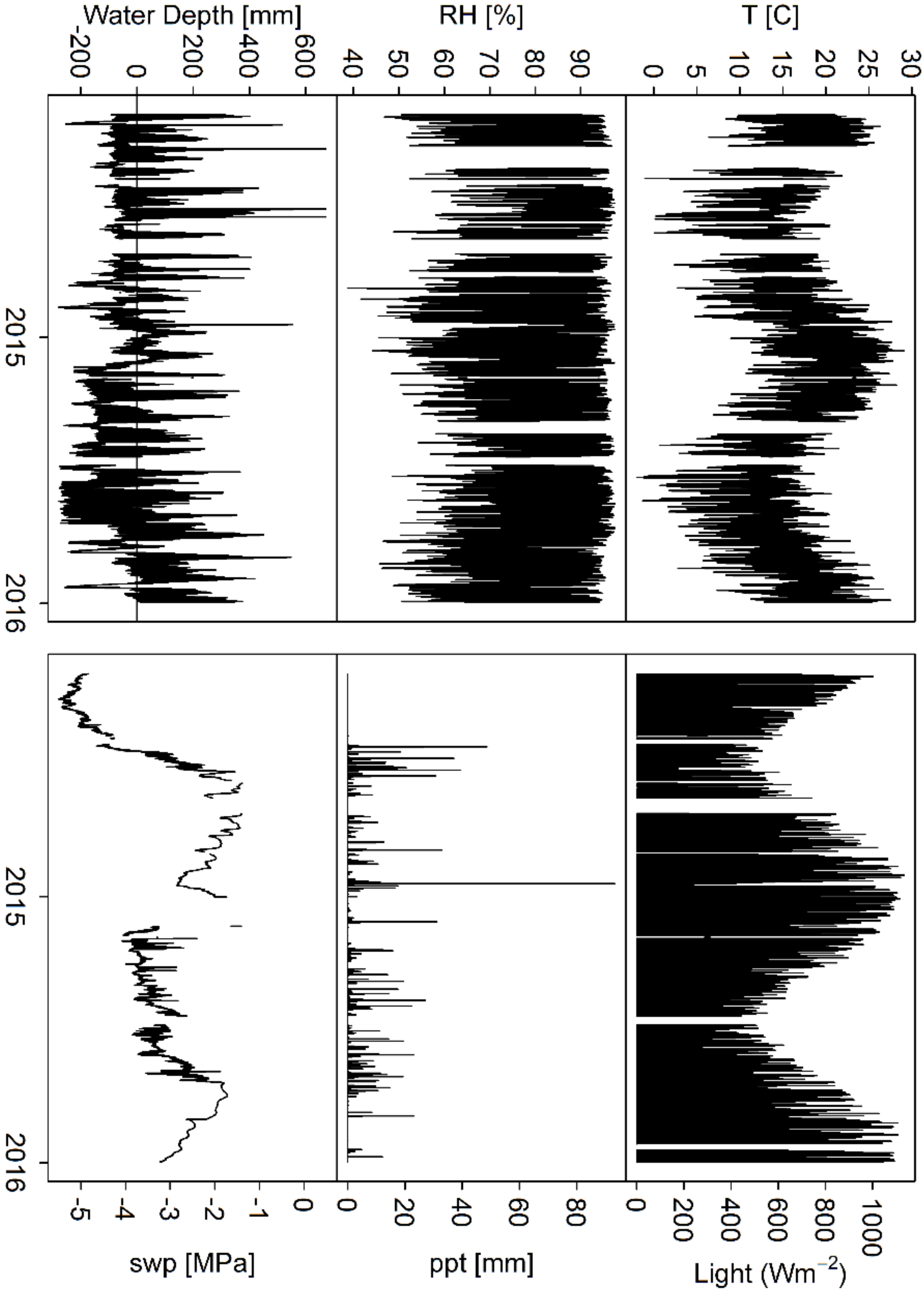
Zimmermann, U., R. Bitter, P. E. R. Marchiori, S. Ruger, W. Ehrenberger, V. L. Sukhorukov, A. Schuttler, and R. V. Ribeiro. 2013. A non-invasive plant-based probe for continuous monitoring of water stress in real time: a new tool for irrigation scheduling and deeper insight into drought and salinity stress physiology. Theoretical and Experimental Plant Physiology **25**:2-11.

Zweifel, R., M. Haeni, N. Buchmann, and W. Eugster. 2016. Are trees able to grow in periods of stem shrinkage? New Phytol **211**:839-849.

Zweifel, R. and R. Häsler. 2001. Dynamics of water storage in mature subalpine *Picea abies*: temporal and spatial patterns of change in stem radius. Tree Physiology **21**, 561–569.

Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees **15**:50-57.

APPENDIX



Appendix Figure 1 Courses of main environmental variables during the whole study period of this thesis from March 2014 until December 2015. Left to right top to bottom are temperature (T), light, relative humidity (RH), precipitation (ppt), water depth, and soil water potential (swp).