Research paper

Daytime stem swelling and seasonal reversal in the peristaltic depletion of stored water along the stem of *Avicennia marina* (Forssk.) Vierh

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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 is 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 hydraulics in terrestrial trees. We explored the relationship of this pattern to 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 amplitude of stem swelling was strongly positively correlated to daily light sums more often than to measures of water availability in air and soil, especially in winter. 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 to daily temperature, with a clear switch in the direction of peristaltic stem shrinking at the start of the growing season. We 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*.

**Keywords:** bark osmotic turgor, mangrove, phloem swelling, point dendrometer, stem daily cycle, stem diameter variations, tree water relations.

Introduction

Plant communities are key players in Earth’s water cycle. Transpiration is responsible for 60–90% of evapotranspiration in terrestrial ecosystems (Jasechko et al. 2013). Thus, changes in plant communities, their productivity, distribution and abundance, can have significant effects on both local- and global-scale hydrology (Stitch et al. 2008). Further, because water availability itself is a fundamental determinant of plant physiological functioning, global productivity, abundance and distribution, understanding forest response to changing water availability is important (Boisvenue and Running 2006, Allen et al. 2010).

The water status of plants is a fine balance between soil-water supply and atmospheric demand, an exchange that is tightly regulated in order to maintain a favourable 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). 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 and leaf turgor are intimately linked through many not yet entirely understood feedback loops (Zweifel et al. 2001, Schachtman and Goorher 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 water potential tension in the xylem in times of high transpiration demand or low soil-water availability (Zweifel et al. 2001, 2005, De Swaef et al. 2015). Depending on evaporative demand, soil-water availability and the state of internal stores, stored water can contribute significantly to the daily transpiration stream (Oliva Carrasco et al. 2015 and references therein). These elastic storage tissues are usually composed 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).

Stem radius changes (SRC) measured with precision dendrometers provide high-resolution information on these radial stem water dynamics, and are also widely used to study tree seasonal growth, phenology, water deficits and climate–growth relationships, and to parameterize water and sugar transport models (Steppe et al. 2005, Drew and Downes 2009, De Schepper and Steppe 2010, De Swaef et al. 2013). Thus, at a daily resolution, SRC measurements can reflect (i) shrinking and swelling of elastic storage tissues caused by water potential changes and by processes altering osmotic potentials of storage tissues, such as sugar loading in the phloem, (ii) reversible changes in dead conductive xylem elements due to internal changes in water potential, and (iii) irreversible radial stem growth through processes of cell division and enlargement (Zweifel et al. 2001, Mencuccini et al. 2013, De Swaef et al. 2015, Pfautsch et al. 2015a, Steppe et al. 2015a, Chan et al. 2016). On a seasonal scale, 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 cell expansion at the onset of the growing season (Deslauriers et al. 2003, 2014, Zweifel et al. 2006). However, separating water and growth components of stem radius change has not proven a straightforward matter and fully isolating the growth, osmotic and water signal from SRC measurements is still work in progress (Mencuccini et al. 2017). A number of different approaches have been used to this end, from complex multi-parameter models (De Schepper and Steppe 2010, Deslauriers et al. 2011, 2014, Sevanto et al. 2011, Mencuccini et al. 2013) to simpler fractionation techniques (Zweifel et al. 2006, 2016, Mencuccini et al. 2017). Additionally, 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 in 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 (Robert et al. 2011a, 2014, Zweifel et al. 2014, Pfautsch 2016, Mencuccini et al. 2017).

Mangrove forests are a diverse biome of halophytic salt-tolerant plants that occupy the intertidal zones of tropical and subtropical latitudes around the world. Avicennia marina (Forssk.) Vierh., the grey mangrove, is one of the most common species, with the widest latitudinal range (Quisthoudt et al. 2012). Studying water dynamics of mangroves in New Zealand is especially appealing due to the homogeneity and monospecificity of the stands as this causes a reduction in the confounding factors that often affect water and carbon relations studies, e.g., interspecific competition (Leuzinger and Hattenschwiler 2013). Partly due to the difficult accessibility and adverse environmental conditions, we still have little knowledge of the water relations of A. marina (Reef and Lovelock 2015), despite their recognized importance to humans, wildlife, coastal processes and global carbon cycles (Kristensen et al. 2008, Alongi 2014). Some recent studies on mangrove water and carbon relations showed unusual courses of daily stem diameter fluctuations (Vandegehuchte et al. 2014a, 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. This kind of pattern deviates from traditionally studied terrestrial trees that 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, 2014b) (Fig. 1). This osmotic component of tissue water potential ($\psi_t$) is always negative, and becomes increasingly so 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).

Studying SRC in A. marina is also of special interest due to the unusual wood anatomy of this mangrove species, 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, 2011b, 2014). Internal secondary phloem in A. marina is thought to be an adaptive trait in order to cope with physiological drought, by contributing to internal water storage and security (Robert et al. 2011a, Santini et al. 2012) (Robert et al. 2011a, 2014). Additionally, radial change measurements in A. marina have been shown to be especially challenging due to its anatomical peculiarities...
The high and variable elasticity of storage tissues and the patchy activation of growth within the network of multiple growth cambium have been seen to cause high heterogeneity and patchiness of point SRC measurements (Robert et al. 2014). Thus, a deeper knowledge of daytime refilling in A. marina will not only help us understand how mangroves regulate their water balance, but also increase our understanding of our capacity to measure and understand a greater diversity of different radial water storage strategies used by a more diverse array of tree species with the tools and techniques available.

In this study, we seek to understand the unusual daytime refilling and delayed shrinking of stem elastic water storage tissues in A. marina, a pattern that deviates from most terrestrial trees and is due to endogenous osmotic adjustment. First, we characterize the diurnal and seasonal relationship of this pattern to other water-related physiological processes such as leaf water potential, stomatal conductance, leaf turgor and sap flow. Second, we analyse the seasonal changes in the timing of peak swelling and shrinking episodes at different stem heights. As we hypothesized that sugar loading plays a role in endogenous osmotic adjustment, we 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, we investigate the time lags of peak stem swelling between upper and lower tiers. Lastly, we analyse the seasonal shift in environmental drivers of water-related swelling and shrinking amplitude and duration along the stem. We hypothesize that swelling episodes are not driven solely by environmental indicators of water availability (soil-water potential, vapour pressure deficit), but also by endogenous drivers (osmoregulation).

Materials and methods

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 h. The stand is monospecific and homogeneous, composed of A. 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. Annual weather trends during the study period (March 2014–March 2015) followed patterns typical to subtropical climate. Daily mean relative humidity (RH) 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 from over the whole measuring period −0.7 MPa to −0.01 MPa with a mean of −0.25 MPa. Water table depth ranged from −7.0 cm below ground level to +74.4 cm above ground level during tidal inundation.

Environmental data

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 (°C) and 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, Pullman, WA, USA). Rainfall (mm) was measured with a tipping-bucket rain gauge (Model 52202, R. M. Young Company, Traverse City, MI, USA). Evapotranspiration (ET, mm) was measured with an ETgage Model E #54 (ETgage Company, Loveland, CO, USA) fitted with a cover that simulates the convention reference standard of alfalfa leaves. Pyranometer, rain-gauge and ETgage were installed on a scaffolding platform so they rested just above canopy maximum height. Tidal inundation (WD, cm), soil-water temperature (°C) and soil-water electrical conductivity (EC, μS/cm) were measured with a CTD sensor (CTD-10, Decagon Devices) fitted into a well dug into the sediment at a depth of 1 m below ground level.

Table 1. Abbreviations used in text and figures for physiological variables studied.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔW/TWD</td>
<td>Tree water deficit, representing water-related shrinking and swelling in the stem, calculated according to Zweifel et al. (2016)</td>
<td>mm</td>
</tr>
<tr>
<td>(\Psi_{\text{leaf}})</td>
<td>Plant leaf water potential, at predawn and midday</td>
<td>MPa</td>
</tr>
<tr>
<td>(\Psi_{\text{pd}})</td>
<td>Stomatal conductance</td>
<td>mmol m⁻² s⁻¹</td>
</tr>
<tr>
<td>(\Psi_{\text{mid}})</td>
<td>Balancing pressure measured by turgor probes, inversely proportional to leaf turgor</td>
<td>kPa</td>
</tr>
<tr>
<td>(A_e)</td>
<td>Amplitude of the expansion phase in a circadian cycle of (\Delta W)</td>
<td>mm</td>
</tr>
<tr>
<td>(A_c)</td>
<td>Amplitude of the shrinkage phase in a circadian cycle of (\Delta W)</td>
<td>mm</td>
</tr>
<tr>
<td>(T_e)</td>
<td>Duration of the expansion phase in a circadian cycle of (\Delta W)</td>
<td>min</td>
</tr>
<tr>
<td>(T_c)</td>
<td>Duration of the shrinkage phase in a circadian cycle of (\Delta W)</td>
<td>min</td>
</tr>
<tr>
<td>(R_{\text{max}})</td>
<td>Maximum (\Delta W) reached during a circadian cycle</td>
<td>mm</td>
</tr>
<tr>
<td>(R_{\text{min}})</td>
<td>Minimum (\Delta W) reached during a circadian cycle</td>
<td>mm</td>
</tr>
<tr>
<td>(R_{\text{max lag}})</td>
<td>Time lag between the upper tier of the tree daily water-related stem radius max and the lower tier daily stem radius max.</td>
<td>h</td>
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Downloaded from https://academic.oup.com/treephys/article-abstract/38/7/965/4942248 by Lib4RI - Library of Eawag, Empa, PSI, WSL user on 19 October 2018
in tubing that allowed water flow. Osmotic potential ($\Psi_{w}$, MPa) of the soil and water was calculated using electrical conductivity and water temperature 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 (<10 km).

**Physiological measurements**

Three mature co-dominant trees of similar height were chosen for continuous physiological measurements. 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 90 cm above ground level and the third below branching of the crown (190–260 cm). These are referred to as ‘lower’, ‘middle’ and ‘upper’. Sap flow rate was measured using heat balance gauges (Dynamax, Houston, TX, USA), based on the heat balance method (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). 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, Dübendorf, Switzerland). Three dendrometers were installed in each of the three trees, evenly spaced along the stem, from early morning until evening.

Calculating water-induced stem shrinkage and expansion

Stem radius measurements obtained from dendrometers were cleared of jumps and spikes. Since SRC includes both growth of new cells and water-related swelling and shrinking, the dendrometer data were detrended for growth according to Zweifel et al. (2016). Briefly, this method assumes no growth during periods of stem shrinkage. The resulting values (ΔW or TWD) 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, we assume that the resulting time series of TWD (also called 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). We 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 et al. (2003). We broke down stem circadian cycles of TWD (Figure 1) 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. To do this, we fitted a smoothing spline to the processed data and, by calculating the first derivative [$d_{i}$] at any point in the cycle, we defined the length and the amplitude of times of expansion (positive gradient, [$d_{i}$] > 0) and contraction (negative gradient, [$d_{i}$] < 0). Thus, we extracted 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. This is different from using the standard 24 h day to find daily maxima and minima, and allowed us to pinpoint key characteristics of each circadian cycle, independent of their length or any variability of occurrence of maxima and minima. A custom function for this analysis was written in R software (R Development Core Team 2017).

**Statistical analysis**

Wilcoxon Signed-Ranks paired tests 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.
Daytime stem swelling and seasonal reversal in peristaltic stem water depletion

Figure 1. Parameters of water-related stem radius changes at 10 min intervals detrended for growth signal. Each cycle is defined as the time between a cycle maximum ($R_{\text{max}}$) and the subsequent maximum. Cycles are separated into a contraction phase (in black), which goes from a $R_{\text{max}}$ to the next $R_{\text{min}}$, and expansion phase (in red), which goes from $R_{\text{min}}$ to the next $R_{\text{max}}$. Each cycle is characterized by a contraction amplitude ($A_c$), expansion amplitude ($A_e$), contraction duration ($T_c$), expansion duration ($T_e$) and total cycle duration ($T_{\text{cycle}}$). Total daily change ($A_d$) is the difference between a cycle maximum and the subsequent maximum (data are not shown in Results section).

The time lag in stem swelling peaks between upper and lower tiers ($R_{\text{max lag}}$) was calculated as the duration in hours between $R_{\text{max}}$ in the upper tier and $R_{\text{max}}$ in the lower tier. We applied linear regressions and Kendal tau-b correlations to determine the relationship between $R_{\text{max lag}}$ and environmental variables (daily means of temperature, SWP, RH and VPD, daily sums of precipitation, radiation and ET). Additionally, we 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, we 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 $\phi$, using days since the start of the measuring period (Pinheiro and Bates 2000).

To study seasonal differences in the response of water-related shrinking and swelling to environmental variables we chose 2 weeks in summer (25 January–8 February 2015) and 2 weeks in winter (9–23 September 2014). Periods with similar precipitation sums were selected in order to minimize the dominating effect of precipitation on cycle length (Deslauriers et al. 2007). Bootstrapped Kendal tau-b correlations were calculated for expansion and shrinking properties ($A_c$, $A_e$, $T_c$, $T_e$) at each stem tier and the environmental variables occurring during each circadian cycle. 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. 2007) we performed partial correlations using duration as a partial correlate to confirm these did not change (results not shown). Additionally, to investigate shrinking and swelling amplitudes further, we fitted LMMs or generalized additive mixed models with Poisson distribution when required to $A_c$ and $A_e$ for each stem tier following the same procedure as described above for $R_{\text{max lag}}$. All analyses were conducted using the free software package R (R Development Core Team 2014).

Results

**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 in summer (February 2015, Figure 2). Winter radial water dynamics showed slow stem refilling towards dawn, with a rapid increase in stem diameter occurring after sunrise (Figure 2i and 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 2e).

Diurnal courses of $\psi_{\text{leaf}}$ in summer and winter showed patterns similar to those commonly recorded for terrestrial trees. We observed a gradual $\psi_{\text{leaf}}$ 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 h before solar noon in summer (Figure 2a and b). Multiple $\psi_{\text{pd}}$ measurements across the year showed that midday leaf water potentials ($\psi_{\text{md}}$) were on average lower in summer ($\psi_{\text{md}} = -4.09 \pm 0.16 \text{ MPa}$, $n = 3$) than in winter ($\psi_{\text{md}} = -3.78 \pm 0.26$, $\text{MPa}$, $n = 4$). However, average predawn water potentials ($\psi_{\text{pd}}$) were higher in summer ($\psi_{\text{pd}} = -0.59 \pm 0.30$, $\text{MPa}$, $n = 3$) than in winter ($\psi_{\text{pd}} = -1.85 \pm 0.18$, $\text{MPa}$, $n = 4$), suggesting that in winter, trees did not reach water saturation overnight. The seasonal relationship between $\psi_{\text{md}}$ and radial stem water flow amplitudes showed increasingly negative $\psi_{\text{md}}$ were associated to both larger total daily stem swelling ($R^2 = 0.5745$, $P$-value = 0.02) and shrinking amplitudes ($R^2 = 0.436$, $P$-value = 0.05). Thus both larger refilling and depletion of water storage reserves seemed to occur during days with low $\psi_{\text{md}}$ (Figure 3).
Diurnal 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 typical decline from morning, but did not reach a minimum until 16:00 h, 3.5 h after minimum \( \psi_{\text{leaf}} \) was reached (Figure 2d). Overall, \( P_p \) and sap flow followed the expected pattern of daytime increase and night time decrease (Figure 2e–h). In winter, leaf turgor reached its minima more gradually than in summer, a few hours after minimum stem diameter and \( \psi_{\text{leaf}} \). 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 2e–h).

**Seasonal variation in the timing of \( R_{\text{max}} \) and \( R_{\text{min}} \) at different stem heights and environmental drivers of \( R_{\text{max}} \) lag**

When looking at the timing of stem water storage across all seasons, peak stem swelling occurred most frequently between sunrise and midday (Figure 4). 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 4).

However, when comparing summer and winter there was a clear reversal in the timing of peak swelling and shrinking at different stem heights (Figure 4). A Wilcoxon signed-ranks 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). 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 ≤ 0.05), followed by the tiers closest to the crown (upper tier: median = 20:00, P ≤ 0.05). Winter differences in \( R_{\text{min}} \) 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 h and lower tiers having a bimodal distribution, reaching their minima either around 20:00 h or after midnight (Figure 4).

The reversal in the direction of the peristaltic swelling and shrinking along the stem was visible across the whole year when looking at the \( R_{\text{max}} \) time lag between upper and lower stem tiers (Figure 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 (\( R_{\text{max}} \) lag > 0). Between June and November \( R_{\text{max}} \) 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 \( R_{\text{max}} \) lag from negative to positive values began concurrently to the initiation of stem growth in November (Figure 6). Monthly mean \( R_{\text{max}} \) lag was significantly correlated in a linear regression to mean monthly VPD (\( R^2 = 0.5, P\text{-value} \leq 0.01 \)) and temperature (\( R^2 = 0.63, P\text{-value} \leq 0.01 \)) (Figure 5). Kendal tau-b correlations and results from a LMM performed on diurnal-scale values confirmed the significant relationship between temperature, VPD and \( R_{\text{max}} \) lag (Table 2). The LMM also detected precipitation as a significant driver of \( R_{\text{max}} \) lag (Table 2). Vapour pressure...
deficit was excluded from the final model due to the high correlation to both temperature and RH.

Changing seasonal drivers of water-related swelling and shrinking at different stem tiers

Correlation analysis and linear mixed effects modelling carried out for a period of 2 weeks in summer and 2 weeks in winter revealed cumulative daily solar radiation as the predominant driver of stem swelling, consistent in its effect during both summer and winter periods by either affecting swelling amplitude or duration (see Table S1 available as Supplementary Data at Tree Physiology Online and Figure 7). Physical indicators of water availability (RH, VPD and SWP) did not significantly affect $A_e$ during winter, but did during the summer period in upper tiers only (see Table S1 available as Supplementary Data at Tree Physiology Online and Figure 7). Additionally there was a positive effect of precipitation on $T_e$ expansion duration. On the other hand, periods of stem shrinking in both winter and summer were predominantly correlated with indicators of atmospheric water demand such as RH, VPD and ET. Thus, in winter, RH, VPD and ET affected both shrinking duration and amplitude in upper and lower tiers (see Table S1 available as Supplementary Data at Tree Physiology Online and Figure 7). Light sum was also positively correlated with $A_e$ and $T_e$ (see Table S1 available as Supplementary Data at Tree Physiology Online and Figure 7). In summer the variables maintained their effect, although they predominantly affected the upper tiers closest to the crown (Figure 7). Water depth was found to have no significant effect on stem shrinking or swelling in both analysis, and was excluded.

Figure 5. (a) Yearly course of the time lag between upper and lower trunk peak water-related swelling ($R_{\text{max}}$ 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 $R_{\text{max}}$ 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 2).

Figure 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 ($R_{\text{max}}$ lag U–L) (dashed green line) for study Tree B. The beginning of an increase in $R_{\text{max}}$ 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.
Discussion

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 ample 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 (Steppe et al. 2015a and references therein). Our findings deviate from this pattern and confirm previous observations of unusual daytime swelling in the stems of A. marina (Vandegehuchte et al. 2014a, 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 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

Table 2. Relationship between daily mean environmental variables and the daily time lag between upper and lower peak stem swelling ($R_{\max \text{ lag}}$) showing Kendall correlations and description of LMM results performed on daily scale data. Variables showing Ø were excluded from LMM due to high collinearity with other variables. Asterisks indicate level of significance of $P$-values (* ≤0.01, ** ≤0.0001) and n.s. indicates non-significant correlation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kendall correlation ± SE (P-value)</th>
<th>LMM Fixed effect ± SE (P-values)</th>
<th>Random effects SD</th>
<th>Autoregressive parameter $\phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>0.21 (≤0.01)*</td>
<td>0.13 ± 0.035 (≤0.0001)**</td>
<td>0.74</td>
<td>0.46</td>
</tr>
<tr>
<td>Humidity</td>
<td>−0.09 (n.s.)</td>
<td>−0.028 ± 0.019 (n.s.)</td>
<td></td>
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<tr>
<td>Light</td>
<td>0.08 (n.s.)</td>
<td>1.64·10^{-5} ± 0.94·10^{-5} (0.08)</td>
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<tr>
<td>Water depth</td>
<td>−0.11 (n.s.)</td>
<td>−0.002 ± 0.001 (n.s.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precipitation</td>
<td>−0.15 (n.s.)</td>
<td>−0.026 ± 0.01 (0.01)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil water potential</td>
<td>−0.05 (n.s.)</td>
<td>−1.28 ± 0.76 (n.s.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vapour pressure deficit</td>
<td>0.19 (n.s.)</td>
<td>Ø</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evapotranspiration</td>
<td>0.12 (n.s.)</td>
<td>Ø</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 7. Kendall tau-b correlation coefficients between environmental variables and different $\Delta W$ cycle properties (expansion time, shrinkage time, expansion amplitude, shrinking amplitude) calculated for 2 weeks in the winter of August 2015 (left column of each panel) and 2 weeks in the summer of February 2016 (right column of each panel) for upper and lower tiers of the tree (hatched and grey bars, respectively). Crosses and asterisks indicate significant correlations for upper and lower tiers of the tree at 95% confidence after 1000 bootstrapped correlations.
flow increased after dawn, with stems still swelling whilst leaf water potentials reached midday lows of down to $-4.2$ MPa (Figure 2). 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 Vandeghuchte et al. (2014b). Such osmoregulation in storage tissues would allow the plant to maintain turgor necessary for metabolic function, cell division and cell expansion (hence growth), enabling the plant to manage the low tissue water potentials typical of halophytes (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), 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, Lopez-Portillo et al. 2014). 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 et al. 2011a, 2011b, 2014), with the phloem acting as major water store in the stem (Pfautsch et al. 2015b).

In our diurnal measurements, we see a time lag between minimum $\psi_{\text{leaf}}$ and minimum leaf turgor and minimum $\psi_{\text{leaf}}$ and $g_s$, where minimum $g_s$ coincides with minimum leaf turgor and minimum daily stem radius, which all occur hours after minimum $\psi_{\text{leaf}}$ was reached (Figure 2). 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. 2006, 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. 2013). 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 we 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 that 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 (Mencuccini et al. 2017 and references therein). Given the complexity of separating growth from osmotic and water-related changes in SRC, we suggest that future research implements a larger set of models and partitioning methods for separating water and carbon elements in A. marina, comparing results to those given by the method used in this work, which has its limitations (Zweifel et al. 2016).

Although $\Delta W$ 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) reflects seasonal relationships recorded in the literature (e.g., Drew et al. 2011). 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 $\psi_{\text{md}}$ and daily stem swelling amplitude, indicating more negative $\psi_{\text{md}}$ were associated with larger stem water refilling amplitudes. This constitutes an unusual finding, which could perhaps be due to either a tight correlation between swelling and shrinking amplitudes or due to stomata staying open past $\psi_{\text{md}}$ drop, thus increasing sugar loading and osmotic turgor in storage tissues at the expense of lowered $\psi_{\text{leaf}}$.

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

We 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 peak of swelling along the stem. This was visible in the yearly trend of $R_{\text{max}}$ lag monthly mean values that closely followed monthly mean VPD and temperature. In winter, $R_{\text{max}}$ 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 $R_{\text{max}}$ lag became positive. To our 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 h, lagging behind upper stem shrinking and $\psi_{\text{leaf}}$. If sugar loading is an important component of the osmotic component of water potential in the stem behind daytime swelling, $R_{\text{max}}$ 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 (Münch 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, 2008, 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 \( \psi_U \) of storage tissues. Similarly, upper tiers shrinking relatively earlier in the day and following \( \psi_{leaf} \) 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 8) 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 \( \psi_U \)) longer than areas furthest from the source (De Swaef et al. 2013, Mencuccini et al. 2013). Additionally, the earlier onset of shrinking in lower stem sections (around 8:00 h) 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 2). 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. (2003, 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 internal secondary phloem (Robert et al. 2011a, 2011b), 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 (Zweifel et al. 2014, Pfautsch 2015).

The start of a visible shift in \( R_{max} \) lag at the same time as stem growth start in November (Figure 6) 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, J. Cusens personal communication) 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 6). 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,

![Figure 8](https://academic.oup.com/treephys/article-abstract/38/7/965/4942248)

**Figure 8.** 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 hypothesized 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 (\( \psi_U \)) of storage tissues to draw water from the xylem during the day.
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 (Hölttä et al. 2006, Savage 2016).

Our data show that time-lags as large as 7 h develop between upper and lower stem daytime stem shrinking (Figure 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 2015a, 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 Polanía 1989, Krauss and Ball 2012) 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 and osmolites in both the xylem and the phloem to confirm that sugars are indeed the osmolites responsible for the daytime swelling dynamics.

It is worth mentioning that the timing of peak swelling for A. marina in our study deviates slightly from VanDegehuette et al. (2014a), who found earlier peaks. The difference compared with our 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.

Changing seasonal correlates of water-related swelling and shrinking episodes at different stem heights

There were clear differences between environmental correlates of A. marina stem swelling and shrinking between seasons and stem tiers. Amplitudes of daytime stem swelling were predominantly driven by a positive correlation to cumulative daily solar radiation, especially during winter periods, even after controlling for cycle length (Deslauriers et al. 2007). This is an unusual finding, as studies usually report radiation as having a negative effect on water-related stem expansion amplitudes, e.g., King et al. (2013) and Urrutia-Jalabert et al. (2015). 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. Physical drivers of SRC in air and soil were much less strongly correlated than in other studies (Zweifel et al. 2005, Devine and Harrington 2011). Relative humidity and VPD still played an important role in summer stem refilling, becoming more highly correlated to stem swelling than cumulative solar radiation. This is a more usual finding, as RH helps maintain turgor by reducing the negative pressure in the plant, especially during summer months when water is scarce (e.g., Deslauriers et al. 2003, Kocher et al. 2012). However, especially in the case of lower tiers, these variables were not significantly related to stem swelling in either summer or winter, and thus further work is required to understand what drives stem swelling amplitudes in lower stem tiers. This was also an interesting correlation between soil-water potential and expansion amplitude in winter, which indicates higher expansion amplitudes occurring at more negative soil-water potentials, which could point towards accumulation of ions during this time, contributing to expansion or a higher osmoregulatory response occurring during times of low water potentials. Despite the fact that water-related swelling was not always correlated to the most common transpiration and water storage drivers, stem shrinking drivers were similar to those recorded in previous studies (Deslauriers et al. 2003, Devine and Harrington 2011, King et al. 2013, Urrutia-Jalabert et al. 2015), with atmospheric variables such as VPD, RH and ET strongly correlated to shrinking amplitudes. Further work using a variety of methods to separate water potential from osmotic and growth induced changes in SRC, coupled with osmolite measurements, would help shed light on the relative importance of water vs 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).

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, 2011b). Santini et al. (2012) found that an increased phloem:xylem ratios 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). Thus, it is likely that both the secondary internal phloem and the higher elasticity in storage tissues which this multiple cambia structure confers (Robert et al. 2011a, 2014) explains that phloem loading and unloading processes have such a strong effect on daily stem radial measurements.

Conclusions

Our results show for the first time year-round presence of daytime stem swelling in A. 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. Additionally, we found 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 we 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 osmolite 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 that are underrepresented in tree water hydraulics literature.

**Supplementary Data**

Supplementary Data for this article are available at Tree Physiology Online.

**Conflict of interest**

None declared.

**References**


