

Xylem as the main origin of stem radius changes in *Eucalyptus*

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Abstract. The state-of-the-art interpretation of stem radius changes (DR_{Total}) for tree water relations is based on knowledge from mostly slow growing tree species. The ratio between diurnal size fluctuations of the rigid xylem (DR_{Xylem}) and the respective fluctuations of the elastic bark (DR_{Bark}) is known to be small (<0.4) and is of importance for the localisation of water storage dynamics in stems. In this study, fast growing *Eucalyptus globulus* Labill. in Tasmania were investigated by point dendrometers in order to investigate tree water relations. Unexpectedly, DR_{Xylem} was found to be the main driver of DR_{Total} with the bark acting as a passive layer on top of the fluctuating xylem under most conditions. Accordingly, the ratio between the diurnal fluctuations of the two tissues was found to be much higher (0.6–1.6) than everything reported before. Based on simulations using a hydraulic plant model, the high tissue-specific elasticity of the *Eucalyptus* xylem was found to explain this atypical response and not osmotically-driven processes or species-specific flow resistances. The wide zone of secondary thickening xylem in various stages of lignification is proposed to be an important component of the high wood elasticity. The tissue acts as additional water storage like the bark and may positively affect the water transport efficiency.

Additional keywords: bark, cambial activity, *Eucalyptus globulus*, *Larix decidua*, lignification, phloem, point dendrometer, stem size fluctuations, Tasmania, hydraulic plant model, tree rings, tree water relations, water tension, wood growth.

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Introduction

Continuous stem radius changes of trees (DR_{Total}) have been measured and described extensively for many woody species around the world (Kozłowski and Winget 1964; Molz and Klepper 1973; Zweifel *et al.* 2001; Deslauriers *et al.* 2003; Daudet *et al.* 2005; Steppe *et al.* 2006; Drew *et al.* 2008; Sevanto *et al.* 2011) and interpretations of DR_{Total} have become an increasingly important source of information to quantify tree physiological responses to climate variability (Steppe *et al.* 2008; Teskey *et al.* 2008; Zweifel *et al.* 2010; Drew *et al.* 2011; Etzold *et al.* 2011; King *et al.* 2013). However, the currently-accepted interpretation of DR_{Total} is derived mainly from measurements of a relatively low number of slow-growing tree species in Europe and North America. This work deals with stem radius changes measured on fast-growing *Eucalyptus globulus* Labill. in Tasmania, Australia and shows the need for an adapted interpretation of such data and the consequent new analysing opportunities.

DR_{Total} consists of two main components: (i) size increments due to growth; and (ii) size fluctuations due to water movement in tissues, inducing swelling or shrinking.

First, stem growth is attributable to the activity in the cambium, a layer of meristematic cells, and enlarging cells, which exist between the bark and the differentiated wood. The cambium builds new cells towards the centre of the stem which mainly differentiate to xylem and it builds cells towards the periphery of the stem which mainly differentiate to phloem (Schweingruber 1996). In both cases, cambial activity leads to increasing DR_{Total} due to growth (Rossi *et al.* 2008; Downes *et al.* 2009; Drew *et al.* 2010). However, in contrast to the wood that is accumulating over time, the cells in the bark undergo different catabolism processes (Gričar *et al.* 2009) which may be size relevant and may lead to decreasing DR_{Total} . Very little is known about the extent of this bark degradation on DR_{Total} and is not further discussed in this work.

Second, the water related processes are driven by modulation in pressure conditions in the tree, mainly induced by transpiration and altered by the hydraulic limitations of the flow and storage system to transport water from the roots to the leaves (Zweifel *et al.* 2007; Ehrenberger *et al.* 2012; Steppe *et al.* 2012) and by osmotic-relevant processes in the bark (De Schepper and Steppe 2010; Sevanto *et al.* 2011; Mencuccini *et al.*

2013). The transpiration-induced negative pressure in the xylem leads to a dehydration of living tissues, which provides the physical force to transport water within the tree (Siau 1984). During the day when transpiration is high the stem loses water from elastic tissues, mainly the bark and the cambium including dividing and enlarging cells as well as phloem. The decrease in DR_{Total} is proportional to this loss of water (Zweifel *et al.* 2000; Drew *et al.* 2011; De Schepper *et al.* 2012; King *et al.* 2013). The origin of decrease in DR_{Total} is mainly related to a decrease in tissue saturation of the bark (DR_{Bark}) and relatively little is attributable to shrinkage of the xylem (DR_{Xylem}), owing to the more limited pressure-induced contraction possible in the rigid sapwood structure (Oertli 1989; Sevanto *et al.* 2005). Methodologically this is quantifiable when comparing measurements of DR_{Total} in parallel to measurements of DR directly on the xylem (DR_{Xylem}) by cutting a window into the bark and setting the sensor onto the wood (Sevanto *et al.* 2011; Mencuccini *et al.* 2013). The difference between the two measurements allows the quantification of DR_{Bark} (Irvine and Grace 1997; Sevanto *et al.* 2002; De Schepper and Steppe 2011; Sevanto *et al.* 2011). It has been found that the water related (i.e. pressure-induced) size changes of the xylem contribute little to the total shrinkage in DR and were usually quantified as <10% (Zweifel *et al.* 2000; Steppe *et al.* 2006) and in one case <40% of DR_{Total} (Sevanto *et al.* 2002).

However, an early experiment with automated point dendrometers at Lewisham, in South-Eastern Tasmania, Australia, in 1995 indicated that fast-growing *Eucalyptus* responded in a different way (Downes *et al.* 1999b). The contribution of DR_{Xylem} to DR_{Total} appeared to be much larger than is presently assumed. The ratio of DR_{Xylem} to DR_{Total} is important in locating the tissues from where water in the stem is released when the stem is shrinking. The lower the ratio, the more water has been withdrawn from the bark. Conversely, larger values indicate that more water is coming from the wood. The functional difference between the two sources of stored water is the additional hydraulic resistance between sapwood and phloem/bark across the cambium (Steppe *et al.* 2012), which is non-existent or very small when the water is withdrawn directly from sapwood. The ratio is therefore of importance in understanding tree water relations since the release of stored water along the water flow and storage system is buffering the water supply system against sudden drops in water potential (Zweifel *et al.* 2007; Steppe *et al.* 2012) with the respective impact on potential embolisms (Johnson *et al.* 2012), stomatal regulation (Zweifel *et al.* 2007), and finally, transpiration.

We compare the ratio of DR_{Xylem} to DR_{Total} from *Eucalyptus* spp. with that of other tree species, highlight the differences and propose anatomical and physiological causes for the observed differences. We further discuss potential physiological consequences for tree water relations of *Eucalyptus* because of these characteristics.

Materials and methods

Site and set up at Lewisham, Tasmania, Australia

The investigation was undertaken in a plantation of 5-year-old *Eucalyptus globulus* Labill. located in south-eastern Tasmania at Lewisham. The trees were planted in mid-1990 at a stand density

of 1428 stems ha^{-1} . The soil consisted of a shallow red-brown loam A horizon and a light brown, medium clay B horizon. Mean soil depth to bedrock was 0.6 m. The annual rainfall at the site is low (on average 515 mm per year, White *et al.* 1998) and below what normally is suitable to plantation establishment. The study trees were periodically irrigated. The management of the site has been described elsewhere (Honeysett *et al.* 1992; White *et al.* 1996).

Point LVDT-based dendrometers (Agricultural Electronics Corporation, Tucson, AZ, USA) and the logging system of the same manufacturer were installed in March 1995 as part of a larger growth and physiology study (Downes *et al.* 1999a; Wimmer *et al.* 2002). The dendrometers were installed on the north-side of the stem of two *E. globulus* trees. These trees were irrigated periodically, so that soil water deficit was kept at a minimum. The study focussed on data measured from August 1995 to December 1995 (corresponding to the spring growth flush). Over this period, irrigation was only applied from 2 November onwards. Three dendrometers were installed on each tree, at ~0.5, 1.5 and 4 m above ground. Dendrometers were mounted on three 4 mm stainless steel threaded rods inserted 40 mm into the wood. Each dendrometer was individually calibrated and a 1 mV change of the reading corresponded to ~3.7 μm in stem radius change. Temperature sensitivity of the dendrometer (including LVDT, frame and anchoring system) was found to be 0.5 $\mu\text{m} \text{ } ^\circ\text{C}^{-1}$ and was corrected accordingly.

On 11 September 1995, one of each of the two dendrometers installed at each height was re-positioned. Dendrometers on tree 1 at 0.5 and 4 m and on tree 2 at 1.5 m were set directly on the xylem. A window of bark (~1 cm^2) was removed and the cambium was scraped from the surface of the wood. The wound was washed cleaned with 100% ethanol. The dendrometer was repositioned against the wood surface, and the area filled with a lanolin paste to avoid drying out the exposed tissue (Fig. 1).

There was considerably less rain during the measurement period from August to December 1995 compared with the average from 1990 to 2000 (300–350 mm) and it was ~1°C cooler than on average (11.1–12.1°C). Soil moisture content was monitored regularly throughout the site using a neutron moisture probe (CPN 503 Hydroprobe, Concord, CA, USA). Soil water deficit was quantified as the amount of water required to return the site to field capacity.

Site and set up at Sandy Bay, Hobart, Tasmania

To provide further insight into these findings, an additional experimental set up with point dendrometers were installed in a patch of natural forest next to the CSIRO laboratories in Sandy Bay, Hobart, Tasmania between November and December 2010. Data used in this study were measured between 5 and 11 November 2010. Two point dendrometers of the type ZB06 (Natkon.ch, Hombrechtikon, Switzerland) were installed on the stem of a ~8-year-old *E. globulus* tree at 1.4 and 1.8 m above the ground. Stem size at breast height (~1.3 m above the ground) was 0.5 m in diameter. Bark thickness, including the cambium, was on average 15 mm. The carbon fibre frame of the dendrometers was fixed to the stem by three stainless steel rods (4 mm in diameter) anchored ~4–5 cm in the wood. The sensor head of the upper dendrometer was placed on the bark surface from which dead and

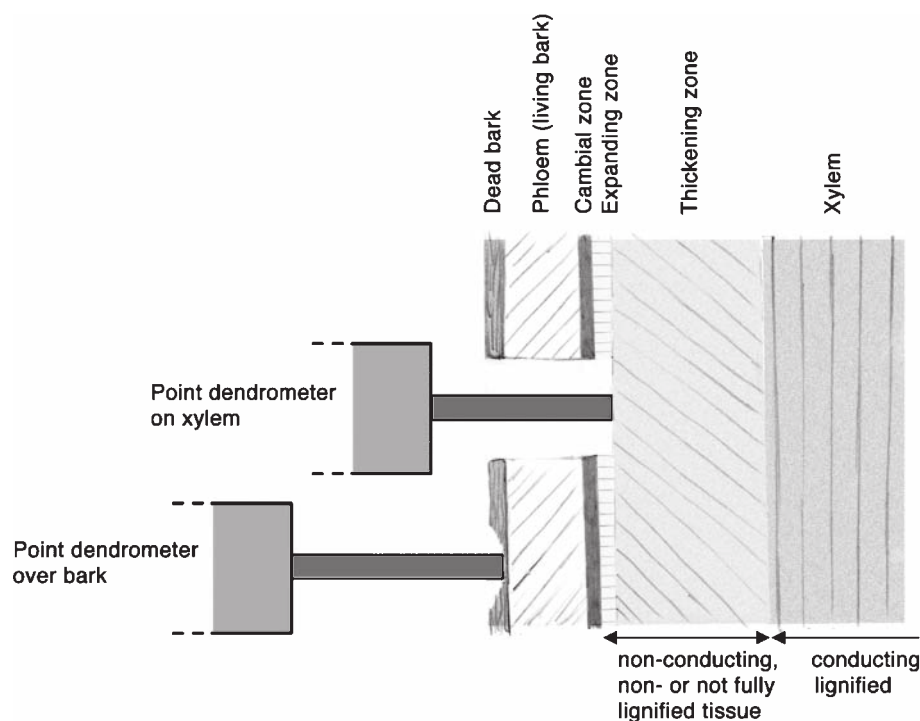


Fig. 1. Set-up of dendrometers over bark and on the xylem of stems of *Eucalyptus globulus*. To place the sensor on the xylem, a hole was cut into the bark to remove the dead bark, the phloem, the cambial zone, and the soft tissue in the expanding zone. The partly lignified thickening zone was at least partly retained. Loose parts of the dead bark were removed to place the sensor over bark.

flaking material had been removed. A window of $\sim 1 \text{ cm}^2$ was cut out of the bark to reach the wood and the sensing head of the lower dendrometer was placed directly against the xylem (Fig. 1). Temperature sensitivity of the ZB06 (including electronic part, frame and anchoring system) was measured to be $0.27 \mu\text{m } ^\circ\text{C}^{-1}$ and not further corrected (data not shown). Sap flow was measured with a Dynagage SGB25 (Dynamax, Houston, TX, USA) on a north-exposed branch above the dendrometers (branch diameter $\sim 3 \text{ cm}$). Measurements were executed every 10 s, and averaged and stored every 2 min with a wireless data acquisition system (DecentLab GmbH, Dübendorf, Switzerland).

Microscopy

Wood cores 2 mm in diameter were extracted at 1.3 m height from stems of *E. globulus* trees previously monitored using dendrometers at the Sandy Bay site on January 10, 2013 (summer) using a Trephor puncher (University of Padua, Italy), and immediately placed in FAA-solution (formalin-acetic-alcohol-water). For comparison, 4 mm diameter cores taken from slower-growing *Larix deciduas* Mill. located at Gampel, Switzerland on June 29, 2012 (summer) were also analysed. Cores from both species were stained in order to colour lignified xylem (red) and living cells (blue).

After sectioning the cores with a sliding microtome and washing them with water, the thin sections ($\sim 20 \mu\text{m}$ thick) were covered with Nawashin solution (10 parts of chromic acid, four parts of formaldehyde and one part of acetic acid) for 10 min in order to fix the cell contents. Then the samples were

simultaneously stained with Astrablue (0.5 g Astrablue-powder, 100 mL distilled water, and 2 mL acetic acid) and Safranin (0.8 g Safranin-powder, 100 mL distilled water) for 3 min and afterwards washed with water. The samples were then covered with picric aniline blue solution (one part saturated aniline blue and four parts saturated picric acid resolved in 95% ethanol) and heated for a few seconds to $\sim 80^\circ\text{C}$. Afterwards, they were washed with water and 3 times with dehydrated ethanol (75, 96 and 100%) and finally xylol. The samples were embedded in Canada balsam, analysed under the microscope with polarised light, and photographed with a digital camera (Canon EOS 5; Canon, Tokyo, Japan), connected to the microscope.

Hydraulic plant model

The hydraulic plant model HPMZ07 based on the water flow and storage concepts of Zweifel *et al.* (2002) and Steppe *et al.* (2006, 2008) was applied to compare the measured stem radius changes of xylem and bark to simulate water tensions inside the respective tissues (Zweifel *et al.* 2007, 2012). The model was run on the modelling platform PhytoSim V1.2 (PhytoIT, Ghent, Belgium) using total crown transpiration and soil water potential as input. Transpiration was estimated as potential evapotranspiration from microclimate including net radiation, vapour pressure deficit, wind speed, air temperature according to Zweifel *et al.* (2002, 2007) and assuming no stomatal regulation. The model was parameterised for individual *Eucalyptus* trees from Lewisham to fit best the measured stem radius changes (DR_{Total}). The parameterisation procedure

includes a sensitivity and an identifiability analysis in order to find those model parameters that are mathematically reasonable for a best-fit parameter optimisation according to De Pauw *et al.* (2008b, 2008a). The procedure led to the following parameters and values: the hydraulic flow resistance between xylem and bark ($R_C = 0.03 \text{ MPa s mg}^{-1}$), the storage capacity of the stem ($C_{\text{Stem}} = 1200 \text{ g MPa}^{-1}$), an allometric parameter to calculate stem size changes (d_S) from stem diameter ($b = 9.3414 \text{ m}^{-1}$) and a dimensionless conversion factor for soil water potentials into root water potentials ($k_{\text{Soil}} = 8.4$). Further parameters were pre-set by using data subsamples and known or estimated physiological limits (e.g. minimum leaf water potential = -2 MPa) to constrain the model output: the hydraulic stem resistance in the xylem ($R_X = 0.002 \text{ MPa s mg}^{-1}$), the storage capacity of the crown ($C_{\text{Crown}} = 18.9 \text{ g MPa}^{-1}$), the maximum bark size ($a = 18.2 \text{ mm}$), tree height ($l = 12 \text{ m}$). More details about the model can be found in Appendix A. The model was used to calculate water flow and storage conditions inside the tree including water potentials in the crown, the stem wood and the corresponding bark tissue.

Results

Little variation in stem radius changes due to sensor position

Stem radius fluctuations (DR) measured with point dendrometers relied on measurement of an area of $4\text{--}5 \text{ mm}^2$ of stem surface. It is therefore relevant to know to what extent the position of

the sensor affects the results, particularly when comparing DR measurements over bark (DR_{Total}) with those directly positioned on the xylem (DR_{Xylem}). Daily patterns and magnitudes of variation between the six measurements, made at three different heights on two *E. globulus* trees at Lewisham, were very homogenous (Fig. 2). DR fluctuations were closely in phase for all the measurement positions on both trees (Fig. 2a). Relatively little variation occurred between the measurement positions in terms of absolute increments over longer periods, which was more attributable to slightly different growth rates than to the water related changes in DR that is the main focus of this paper.

Following repositioning of three of the six dendrometers on the xylem, readings on xylem were almost identical in the three measurement locations (Fig. 2b). This was despite one sensor being mounted on tree 1 (T1) and two sensors on tree 2 (T2) and the location of the measurement positions varying between 0.5 and 4 m above ground.

How long are measurements on xylem reliable?

Xylem measurements on the two trees at Lewisham (DR_{Xylem}) were found to run in parallel for $\sim 3\text{--}4$ months (Fig. 3), clearly distinguishable from the measurements over bark (DR_{Total}). Diurnal fluctuations in DR_{Xylem} remained within a range of $\sim 100 \mu\text{m}$ and no growth occurred at these positions (Figs 2, 3). In contrast, DR_{Total} showed marked growth, particularly after 2

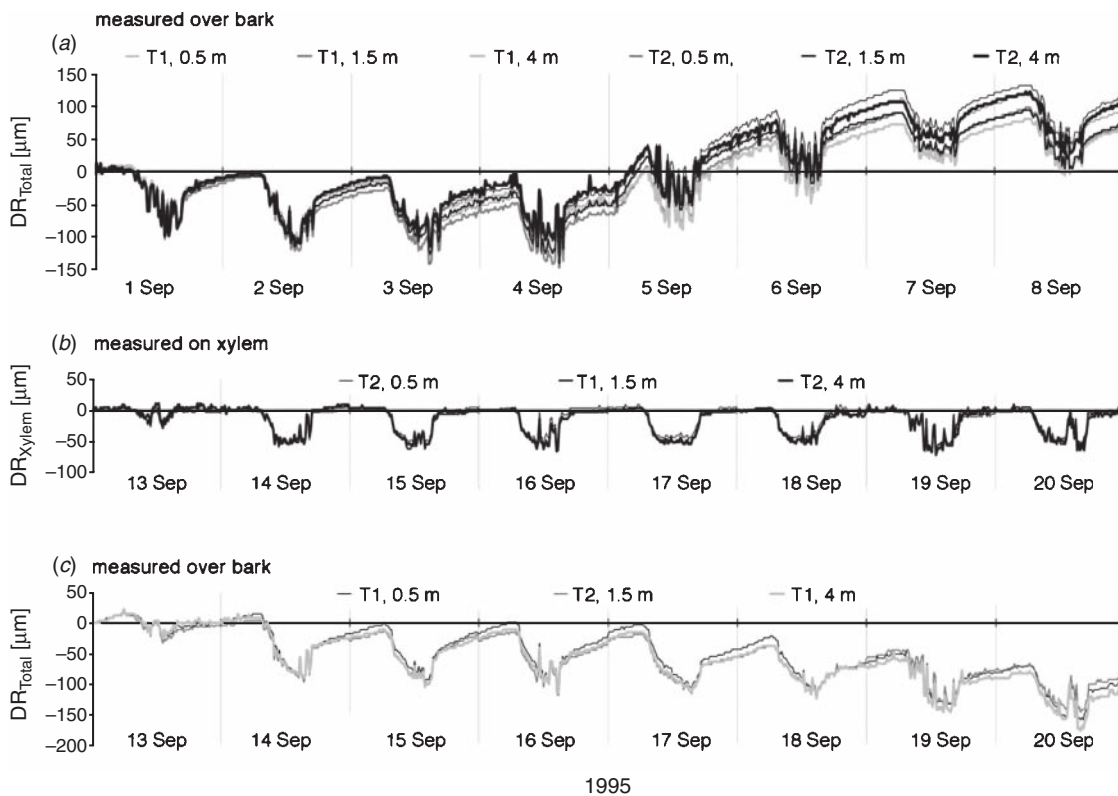


Fig. 2. Stem radius changes (DR) of two *Eucalyptus globulus* (T1 and T2) at Lewisham, Tasmania. (a) Six sensors were mounted at three different heights above ground and total radial fluctuation over bark (DR_{Total}) was measured. After an initial phase, (b) three of the sensors were set on the xylem on 11 September 1995 and (c) three sensors remained untouched. Temporal resolution of data is 15 min.

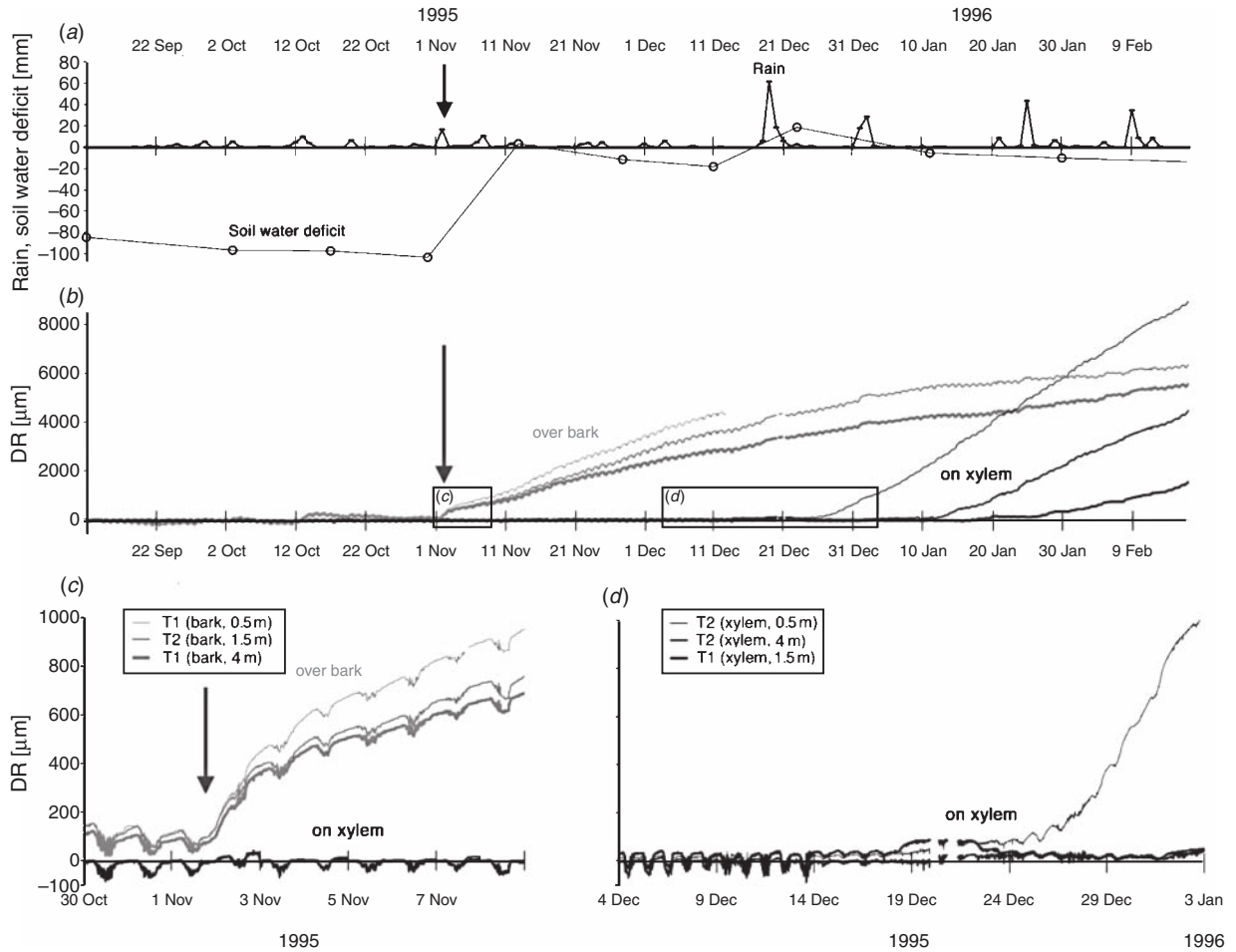


Fig. 3. Stem radius changes (DR) and the respective soil water conditions at Lewisham in the period between September 1995 and January 1996. (a) Precipitation and soil water deficit. (b) DR over bark (DR_{Total}) increased after starting the irrigation on 2 November 1995 indicated by an arrow, whereas DR on xylem (DR_{Xylem}) remained unaffected until mid-December 1995 to January '96 when the three readings suddenly began to increase. (c, d) are enlarged views of the two time windows of interest.

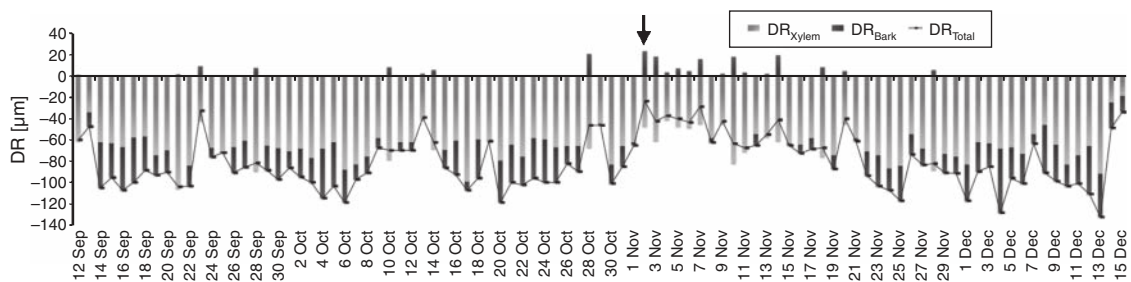


Fig. 4. Daily radial stem shrinkage measured over bark (DR_{Total}) and resolved into the fractions xylem (DR_{Xylem}) and bark (DR_{Bark}). DR_{Total} and DR_{Xylem} were measured, DR_{Bark} is the difference between the two measurements (*Eucalyptus globulus*, Lewisham, Tasmania). Positive values of DR_{Bark} indicate an expansion in bark size in parallel to a shrinkage of DR_{Total}. Irrigation of the trees started on 2 November 1995, indicated by an arrow.

November when irrigation was initiated and soil water deficit was reduced (Fig. 3). After mid-December, however, the DR_{Xylem} readings began to show net increment as well (Fig. 3), indicating that cambial initials had regenerated, and the variation was not entirely due to water potential fluctuations in the xylem. The

callus growth produced by wounded cambium, especially from the sides, developed progressively over time until it started to touch the dendrometer sensor. The differences between the dendrometers reflect the differences in time when the callus started touching the sensor. To analyse the dynamics and

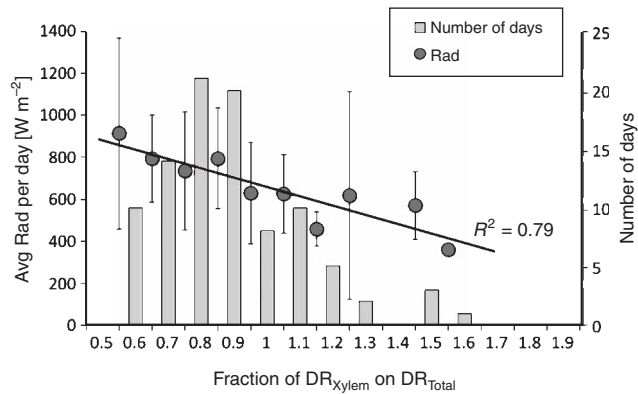


Fig. 5. Distribution of specific daily ratios between shrinkage of xylem (DR_{Xylem}) and total shrinkage of stem measured over bark (DR_{Total}) (grey bars), and the relationship to average daytime light conditions (Rad). Error bars indicate s.d. of Rad per group of days. The linear regression between DR_{Xylem}/DR_{Total} and Rad explains 79% of the relationship. A ratio of $DR_{Xylem}/DR_{Total} < 1$ means that bark and xylem were shrinking. A ratio = 1 means that only the xylem was shrinking, and a ratio > 1 means that the xylem was shrinking whereas the bark was expanding. (*Eucalyptus globulus*, Lewisham, Tasmania, 1995).

origin of DR, we focus on data generated between 12 September and 15 December 1995.

Quantifying the fraction of xylem size fluctuations on total stem radius changes

Daily radial xylem shrinkage (DR_{Xylem}) at Lewisham was between -18 and $-105 \mu\text{m}$ (Fig. 4). This was unexpectedly large in comparison to the range of DR_{Total} , which was in about the same range (-23 to $-132 \mu\text{m}$). The difference between these two measurements was calculated as the resulting fluctuation of the bark (DR_{Bark}) which ranged between -60 and $+23 \mu\text{m}$ (Fig. 4). On some occasions, 100% of shrinkage in DR_{Total} was attributable to xylem shrinkage whereas the bark size remained unchanged. Also, there were days when the bark was expanding whereas the xylem was shrinking at the same time (Figs 4, 5). The intensity of solar radiation was strongly, negatively correlated with DR_{Xylem}/DR_{Total} ($R^2 = 0.79$). Bark shrinkage occurred under sunny,

relatively dry conditions when DR_{Xylem}/DR_{Total} was < 1 . Under moderately sunny conditions, stem shrinkage was more attributable to the xylem and under conditions of low light, shrinking xylem occurred at the same time as expanding bark (Fig. 5, days with a ratio of $DR_{Xylem}/DR_{Total} > 1$). Coupled to this result was the finding that the contribution of the xylem to DR_{Total} was largest for days with relatively small DR_{Total} , corresponding to the low light conditions (data not shown). The bark showed almost no diurnal fluctuation after rain or irrigation, whereas the xylem was more uniform in terms of diurnal fluctuations.

Sandy Bay

To further verify the reliability of the Lewisham findings from 1995, we conducted measurements on trees of the same species (*E. globulus*) growing in a stand of trees on the CSIRO campus at Sandy Bay (Hobart) in 2010 (Fig. 6). A different point dendrometer model and a different data-logging technique were used to exclude potential technical artefacts. Furthermore, the combination of sap flow data with measured DR_{Total} and DR_{Xylem} showed very clearly that: (i) the amplitude of the fluctuation of the xylem was in the same range as at Lewisham; (ii) the size changes in the bark and in the xylem had sometimes opposing directions; and (iii) the xylem tended to respond quicker and was more sensitive to changes in sap flow than the bark (Fig. 6).

Inner-daily patterns of bark and xylem size fluctuations

The responses of xylem (DR_{Xylem}) and bark (DR_{Bark}) at both sites showed distinctly different patterns over a day (Fig. 7). Consequently, the total change in stem radius (DR_{Total}) exhibited a unique pattern. DR_{Xylem} responded much faster to changes in ambient weather than DR_{Bark} and, thus, varying light conditions were more closely related to DR_{Xylem} than to DR_{Bark} (Fig. 8). By comparison, responses in the bark were more buffered (Fig. 7, e.g. 13 September) or in some cases almost completely decoupled from water relations in the xylem (Fig. 7, e.g. 28 October). In general, shrinkage and subsequent expansion began earlier in the xylem than in the bark and its duration was much shorter than in the bark. During periods of irreversible net expansion (Fig. 7, e.g. 28 September, 8–9 November), the total stem radius increase over one day was up to $300 \mu\text{m}$.

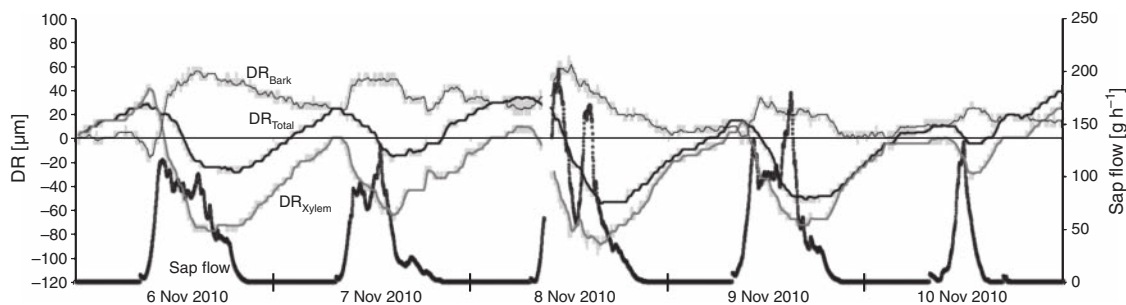


Fig. 6. Diurnal courses of stem radius changes and sap flow of *Eucalyptus globulus* at Hobart, Tasmania. Stem radius changes were measured over bark (DR_{Total}) and on the xylem (DR_{Xylem}). Bark size fluctuations (DR_{Bark}) were calculated as the difference between DR_{Total} and DR_{Xylem} . Light grey lines show the measured data, darker grey and black lines show a moving average. Sap flow was measured on one of the two main branches of the tree. Temporal resolution of data: 1 min.

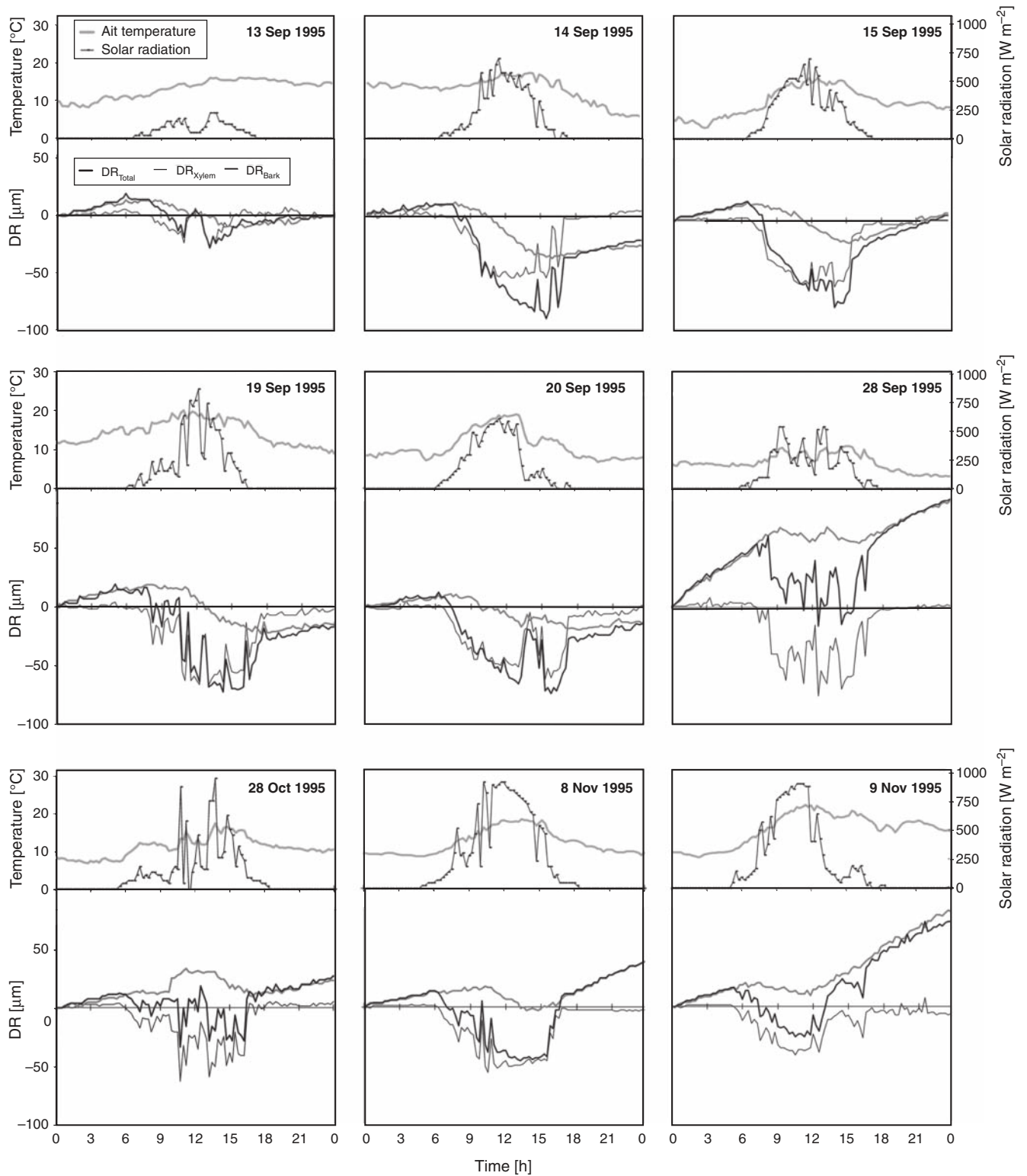


Fig. 7. Selection of days with intra-day courses of air temperature, solar radiation, and stem radius changes of *Eucalyptus globulus* at Lewisham, Tasmania, in 1995. Stem radius changes were measured over bark (DR_{Total}) and on the xylem (DR_{xylem}). Bark size fluctuations (DR_{Bark}) were calculated as the difference between DR_{Total} and DR_{xylem} . Temporal resolution of data: 15 min.

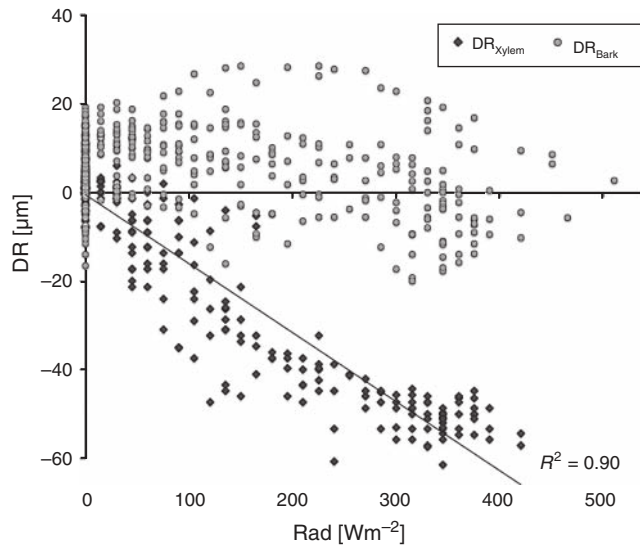


Fig. 8. Relationship between light conditions (Rad) and size fluctuations of xylem and bark (DR). Data at a 15 min resolution include morning records (1–13h) from Lewisham between 12 and 22 September 1995.

Water tensions in xylem and bark

The application of the hydraulic tree model (HPMZ07) showed that changes in xylem water potentials explained the radial size fluctuations in the xylem very well (Fig. 9). The same was true for water potential changes in the bark and the respective radial bark size fluctuations. The two tissues followed the respective courses of water potentials very closely. The appropriate response delay of the bark to water potential changes in the xylem in the HPMZ07 model was achieved by adjusting the hydraulic resistance between xylem and bark (R_C). R_C was found to be 15 times higher than the hydraulic resistance in axial direction (R_X) causing a strong decoupling of the bark from the xylem.

The very close relationship between tissue water tensions and the respective tissue size fluctuations did not hold for days with little light (Fig. 9). On such days, the bark tissue was increasing in size despite the increasing (modelled) water tensions. As an example, bark size considerably increased around noon on 28 October 1995 (Fig. 9), whereas the (modelled) bark water potential was decreasing at the same time. After a relatively short period of about 3 h, the bark size fluctuation returned to a ‘normal’ pattern in line with the modelled water tensions.

Wood anatomy

Wood samples of *E. globulus* (Tasmania) showed a remarkable difference in size of the cambial, enlarging and particularly the thickening zones in comparison to a typical example of a slow growing species (*L. decidua*, Valais, Switzerland). The thickening zone of *E. globulus* with non- or only partially lignified immature xylem reached 200–350 μm in depth, whereas the same zone of *L. decidua* was \sim 20–40 μm only (Fig. 10; Table 1). Both tree species were sampled within their growth period. Indeed, in the case of *E. globulus*, the samples were taken at the end of a relatively hot, dry period with little growth in beginning of January 2013. The samples of *L. decidua* were taken at the end of June 2012, a time when wood growth has

produced most of its annual increment; however, growth continued for some weeks. The limited measured anatomical observations are discussed in the context of a collection of xylem thickening zone sizes deduced from literature (Table 1).

Discussion

The fast growing *Eucalyptus globulus* showed unexpectedly large stem diameter fluctuations attributable to size changes in the xylem (DR_{Xylem} , Figs 2–4). This is in contrast to tree species in which bark size changes (DR_{Bark}) are known to be the main driver for total stem radius changes (DR_{Total}) (Zweifel *et al.* 2000; Sevanto *et al.* 2002; Steppe *et al.* 2006). We found this to be true for a dataset collected from an irrigation experiment in 1995 (White *et al.* 1998; Downes *et al.* 1999a) (Figs 1–5, 7–10) as well as in data collected in 2010 (Fig. 6). DR_{Xylem} exhibited a faster response to weather conditions than DR_{Total} including DR_{Bark} (Fig. 8) and was sometimes completely decoupled from the dynamics measurable in the bark. In the following we discuss stem radius fluctuations in *E. globulus*, the difference of this species compared with the more extensively studied European/American tree species and the origin and functionality of xylem size changes in *E. globulus*.

Water tensions as main driver for stem radius fluctuations

Deformation of tissues and the effect of this phenomenon on stem radius is mainly induced by the dynamic water potentials within the plant (Steppe *et al.* 2006; Zweifel *et al.* 2007; Sevanto *et al.* 2011; Mencuccini *et al.* 2013). Such water potentials in the hydraulic system of a tree are mainly induced by the evaporative demand of the air surrounding the foliage and the respective transpiration of the leaves (Dixon and Joly 1895; Zimmermann 1983; Milburn 1996). The resulting gradients in water potentials between different tree parts drive the water movement up the stem. At any location up the stem, the xylem water potential gradients not only affect the movement of water up the tree, but also determine the water movement in the radial direction between xylem and bark (Zweifel *et al.* 2001; Sevanto *et al.* 2011; Steppe *et al.* 2012). With decreasing water potentials during the day, water is withdrawn from bark tissue into the xylem and the reverse occurs with increasing water potentials during the night. These two processes are coupled to observable shrinkage or expansion of elastic tissues in the radial direction (Figs 2, 9). In previous research, most of this radial size change has been attributed to size changes of the bark, while size changes in the wood were small (1–40%, Zweifel *et al.* 2000; Sevanto *et al.* 2002; Steppe *et al.* 2006). However, in *E. globulus*, it is evident that bark contributes only a small proportion to DR_{Total} (Figs 2, 3). Rather, the main part of the variation is caused by changes in DR_{Xylem} (Fig. 4). The ratio of contributions of xylem and bark to DR_{Total} varies with weather conditions (Fig. 5) and is assumed to be linked to the level of drought stress and the respective evaporative demand of the air. Furthermore, the bark appears to function as a ‘hydraulically passive’ layer on top of the more environmentally sensitive xylem (Figs 7, 8). An increased radial hydraulic resistance decouples the size fluctuations of the bark from the xylem, indicated by increased time lags of size changes in the bark on water potential changes in the xylem (Fig. 9). This effect has recently been shown by Sevanto *et al.* (2011), who

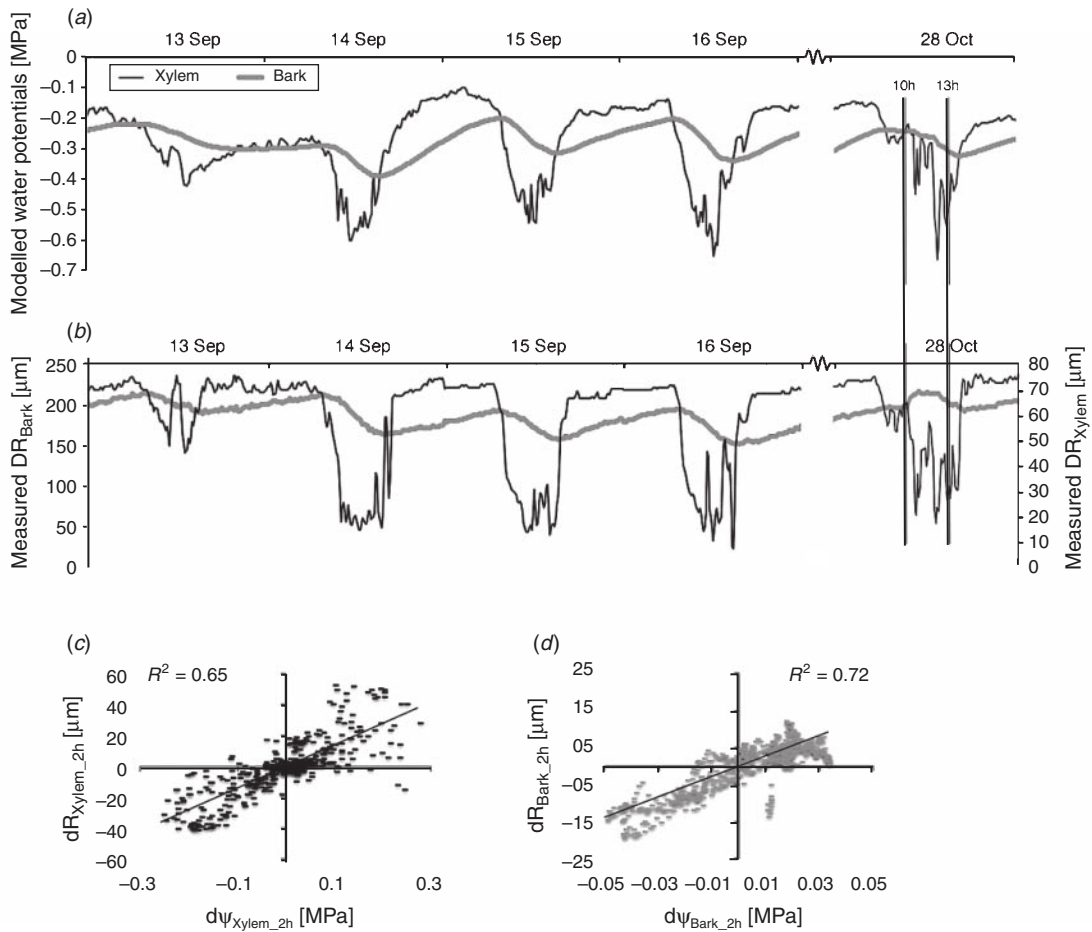


Fig. 9. Modelled water potentials in comparison to measured radial tissue size changes. (a) Water potentials in xylem and bark were simulated with a hydraulic plant model (HPMZ07). (b) Radial tissue size changes were measured with point dendrometers (*Eucalyptus globulus*, Lewisham, Tasmania, 1995) mounted on the stem surface (DR_{Total}) and on the xylem (DR_{Xylem}). Bark size fluctuations (DR_{Bark}) were calculated as the difference between DR_{Total} and DR_{Xylem} . (c) Changes of xylem water potentials over time intervals of 2 h ($d\Psi_{\text{Xylem}_2\text{h}}$) were strongly correlated with changes of DR_{Xylem} over the same interval ($dR_{\text{Xylem}_2\text{h}}$). (d) The same was true for the bark ($d\Psi_{\text{Bark}_2\text{h}}$ and $dR_{\text{Bark}_2\text{h}}$). Not caught by the model and not included in the correlations is the bark size swelling over noon on 28 October 1995.

experimentally decoupled the bark from the xylem by inserting aluminium foil between the two tissues.

Explanations deduced from a hydraulic plant model

We tested the relationship between size fluctuations in xylem and bark and the respective tissue water potentials based on pure hydraulic controls with the hydraulic plant model HPMZ07 (Zweifel *et al.* 2007; Appendix A). We found that the modelled water potentials in the respective tissues explained the measured size fluctuations of xylem and bark to a very high degree including the observed response delay between xylem and bark (Fig. 9). As expected, the degree of delayed response of the bark tissue could be related to the radial hydraulic resistance (R_C) between the two tissues (Sevanto *et al.* 2011; Steppe *et al.* 2012) and also explained the smoother pattern of diurnal variation in DR_{Bark} compared with DR_{Xylem} (Fig. 7). The ratio between the radial hydraulic resistance (R_C) and the axial hydraulic resistance (R_X) was found to be 15, which is slightly below the range of

values found in other modelling studies (Steppe *et al.* (2008): $R_C/R_X = 30$, Zweifel *et al.* (2007): $R_C/R_X = 200$, Sevanto *et al.* (2011): $R_C/R_X = 330$) but not exceptionally different. Also the absolute value of $R_C = 0.03 \text{ (MPa} \cdot \text{mg}^{-1}\text{)}$ found in this model study for *E. globulus* did not differ appreciably from most other studies: *Pinus contorta*: $R_C = 0.025$ (Hunt *et al.* 1991), *Malus pumila* $R_C = 0.02$ (Hunt *et al.* 1991), but *Picea sitchensis* $R_C = 0.009$ (Milne and Young 1985). We conclude, therefore, that the atypical behaviour of *E. globulus* cannot be fundamentally explained by differences in average R_C , or R_C/R_X , but rather, the explanation lies with differences in the elasticity of parts of the xylem.

Origin of xylem size fluctuations in *Eucalyptus*

According to our DR_{Xylem} measurements and the conclusions from the hydraulic plant model, the xylem of the investigated *Eucalyptus* species seems to be more elastic than the one of species investigated with dendrometers so far (Irvine and Grace

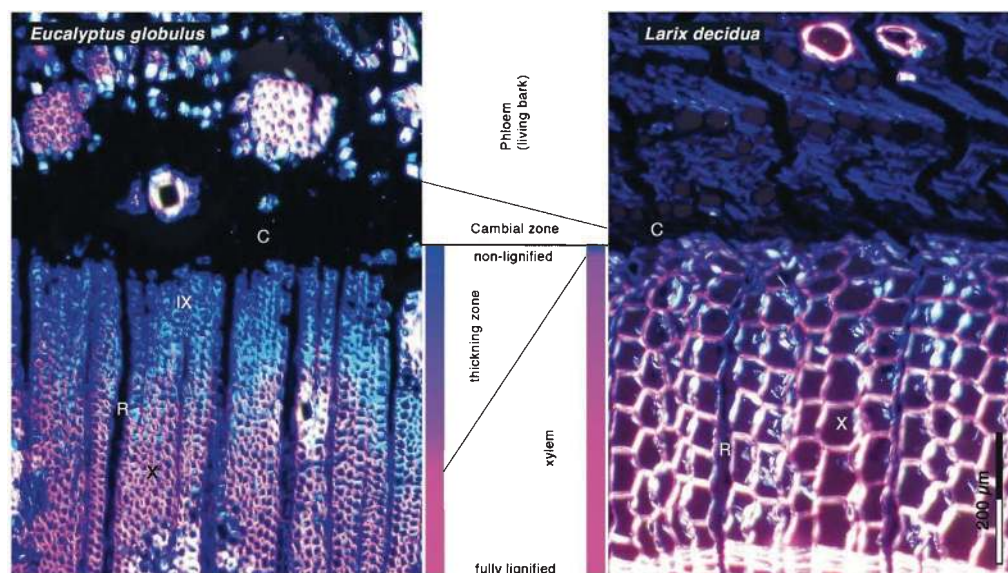


Fig. 10. Magnified and stained stem slices of *Eucalyptus globulus* and *Larix decidua* under polarised light. Red coloured cell walls indicate lignified structures, the blue coloured and black areas indicate living cells. Tissues are specifiable into bark (mainly phloem cells), cambial zone, thickening zone (immature xylem, non- or partially-lignified), and fully lignified xylem (sapwood). Abbreviations: X, xylem; IX, immature xylem; R, ray parenchyma; C, cambium.

Table 1. Size of the thickening zone of a selection of tree species
ND, data not available

Species	Number of cells in thickening zone	Size of thickening zone (μm)	Source
<i>Eucalyptus globulus</i>	25–35	200–350	Fig. 10
<i>Eucalyptus globulus</i>	~100	~500–1500	Ridoutt and Sands (1994); Ridoutt et al. (1995)
<i>Eucalyptus nitens</i>	>35	>400	D. M. Drew and G. M. Downes, unpubl. data
<i>Eucalyptus grandis</i> \times <i>urophylla</i>	20–25	~300	Drew and Pammenter (2007)
<i>Eucalyptus grandis</i> \times <i>camuldulensis</i>	20–40	360	Drew and Pammenter (2007)
<i>Quercus robur</i>	10–13	250–300	Schweingruber et al. (2008), fig. 3.45
<i>Fraxinus excelsior</i>	0–4	0–50	Schweingruber et al. (2013), fig. 5.5
<i>Larix decidua</i>	1–2	20–40	Fig. 10
<i>Larix decidua</i>	0–5	ND	Rossi et al. (2007), fig. 2
<i>Pinus cembra</i>	2–3	5–10	Schweingruber et al. (2008), fig. 3.62
<i>Pinus cembra</i>	0–6	NA	Rossi et al. (2007), fig. 2
<i>Abies alba</i>	0–2	0–15	Schweingruber et al. (2008), fig. 4.72
<i>Picea abies</i>	0–2	0–15	Schweingruber et al. (2008), fig. 4.74
<i>Picea abies</i>	3–5	25–35	Schweingruber et al. (2008), fig. 7.26
<i>Picea abies</i>	0–8	ND	Rossi et al. (2007), fig. 2
<i>Picea abies</i>	0–17	ND	Gričar et al. (2007), fig. 4
<i>Picea abies</i>	5	50	Gričar et al. (2007), fig. 5
<i>Alnus glutinosa</i>	1–2	10–15	Schweingruber et al. (2008), fig. 6.27
<i>Juniperus</i> sp.	1–2	1–2	Schweingruber et al. (2008), fig. 6.26
<i>Malus sylvestris</i>	1–4	10–30	Schweingruber et al. (2008), fig. 6.25

1997; Zweifel et al. 2001; Sevanto et al. 2002; Steppe et al. 2006). Therefore, it can be expected to undergo more distinct size fluctuations under changing stem water potentials. Based on the results that are available in the literature, and from data in Table 1 and Fig. 10, we conclude that the relatively large xylem elasticity is to a large extent a species-specific function of the large thickening zone of secondary thickening cells in *Eucalyptus*

spp. in general, and in *E. globulus* in particular (Ridoutt and Sands 1993; Ridoutt and Sands 1994; Drew and Pammenter 2007). The thickening zone with immature, non- or partially-lignified xylem was found to be much larger in the *Eucalyptus* spp. in general (0.2–1.5 mm) than in the slower growing European species (0–0.3 mm) (Table 1). These juvenile xylem elements are not functional in terms of transporting water and are not yet fully

lignified. They are therefore more elastic under varying water potentials across the developing zone than fully differentiated and lignified xylem cells. Consequently, while they remain hydraulically connected to the conducting xylem, they would tend to shrink to a greater extent under decreasing xylem water potentials than fully lignified tissues. The lignification process of *Eucalyptus* may be more prolonged, or the rate of cell production is higher and thus the duration of subsequent differentiation takes longer than in slower-growing European tree species on which detailed, dendrometer-based stem size variation has mostly been studied. The lignification of new built xylem cells in the slow-growing species seems to be rapid enough so that fewer cells exist in the immature (and thus elastic) state compared with the case of *Eucalyptus*. Besides the species-specific differences in thickening zone sizes (Table 1, Fig. 10), there is good evidence that the number of cells in the thickening zone varies with season (Gričar *et al.* 2007; Rossi *et al.* 2007; Gričar and Čufar 2008; Gričar *et al.* 2009). The number of cambial cells (Gričar *et al.* 2007) and most likely also the number of immature xylem cells are highest during the most intensive growth phase (Rossi *et al.* 2006). That means that the fraction of DR_{Xylem} on DR_{Total} may additionally vary due to thickening size changes over the seasons independent of the species. Furthermore, in all likelihood, the limited data available for *Eucalyptus* sp. (see Table 1) does potentially underestimate the potential widths of the zones in most cases, as samples were not always taken during periods of peak growth.

Biological drivers for stem radius fluctuations

Besides water potentials, radial hydraulic resistance, and tissue elasticity there are also biological processes reported to affect bark size changes (De Schepper and Steppe 2010; Sevanto *et al.* 2011; Steppe *et al.* 2012; Mencuccini *et al.* 2013). These biological processes are related to the hydraulic resistance in radial direction between xylem and bark (R_C), sugar content changes in the active phloem, and growth. There are strong indications that neither R_C (Steppe *et al.* 2012) nor sugar content are stable over time (De Schepper and Steppe 2010; Sevanto *et al.* 2011). R_C may be altered by aquaporins (Steppe *et al.* 2012), and osmotic potentials are affected by sugar loading, unloading and, phloem transport processes, which themselves depend on biotic and abiotic variables (De Schepper and Steppe 2010, 2011). Further, the turgor-driven growth processes (cell division and cell elongation), occurring under moderate xylem water tensions only (Lockhart 1965; Zweifel *et al.* 2006), alter the shrinkage of DR_{Bark} and DR_{Xylem} and consequently influence the shrinking ratio between the two tissues (De Schepper and Steppe 2010).

In our study the presence of such biological drivers is concluded from DR measurements on days with increasing bark size in parallel to decreasing xylem size (Figs 6, 7, 9) and a consequent ratio of DR_{Xylem} to $DR_{\text{Total}} > 1$ (Figs 4, 5). Such behaviour is not explicable with passive hydraulic traits as R_C or the tissue elasticity and thus cannot be simulated with a purely hydraulic plant model (Fig. 9, e.g. 28 October). The biological processes (or at least some of them) are assumed to occur every day (De Schepper and Steppe 2010; Mencuccini and Holttá 2010) but become only visible on days with a low evaporative demand and humid soil water conditions (Figs 4, 5)

and, thus, on days with moderate water potentials in the stem. The shrinkage of DR_{Bark} is further assumed to be more strongly altered by growth processes due to the buffering effect of the additional hydraulic resistance R_C on low water potentials (Zweifel *et al.* 2007) and a potentially higher cell enlargement rate under lowered water potentials on the bark side of the cambium. It is therefore possible that increasing DR_{Bark} is at least partially induced by enlarging bark cells.

We conclude that all of these biological processes induce relatively small size-relevant forces and are therefore masked on days with increased transpiration rates and thus low xylem water potentials. They become visible on days with relatively low transpiration rates only (Figs 5, 7).

Potential implications on tree water relations

The release of tree-internally stored water smooth peaks in xylem water potentials, reduces the likelihood of embolism (Steppe *et al.* 2012) and therefore indirectly affects stomatal control of transpiration via the modulated water potentials inside the tree (Zweifel *et al.* 2007). Water stored on the sapwood side of the cambium is more easily accessible than on the bark side, as the hydraulic resistance across the cambium (R_C) is known to be considerable; ~10–350 times larger than the hydraulic resistance of the xylem (see above). We suggest that xylem water potentials are more efficiently buffered in *Eucalyptus* compared with other tree species with no such water storage on the sapwood side of the cambium. Our results suggest that the buffering system of *Eucalyptus* is two-stage since the bark water storage is not responding to moderately low water potentials but becomes activated with increasing evaporative demand of the air (Figs 5, 7) and consequently increasing water potential gradients.

We further speculate that such a water flow and storage system might increase the water transport efficiency and might even be a functional factor for the ability of this species to grow so fast. Having the cambium better buffered against low water potentials might lead to an increased overall growth rate (Lockhart 1965; Steppe *et al.* 2006). Furthermore, an optimised water supply system with e.g. height-dependent buffering capacities could help to compensate for height-induced limitations of the hydraulic system, additionally to e.g. the recently proposed tapering effect of the conduits in *Eucalyptus regnans* where increasing conduit tapering towards the tree periphery is suggested to progressively reduce hydraulic constraints caused by tree height (Petit *et al.* 2010, 2011).

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Appendix A

HPMZ07 Model

The hydraulic plant model HPMZ07 (Fig. A1) is based on the functional relationships between tree water relations and stem radius fluctuations described by Zweifel *et al.* (2001). A functional relationship between the tree water relations-driven turgor pressure in the cambium and its direct link to cambial activity and growth (RCGro-Models) was added by Steppe *et al.* (2006, 2008). The RCGro2 model was then combined with the stomatal regulation approach by Zweifel *et al.* (2002, 2007) leading to the currently used HPMZ07 model.

Model parameterisation procedure

The model consists of 16 parameters of which a small number can be mathematically set by a parameter optimisation procedure. The maximum number of freely adjustable parameters in an identifiable model depends on the model and data structure and on the number of independently measured variables the model output is fit to (De Pauw *et al.* 2008b, 2008a). In this case, the model output was fitted to time series of stem radius data (DR_{Total}). The integrated sensitivity and identifiability tools in the software PhytoSim (De Pauw *et al.* 2008a), in which the model HPMZ07 was operated, led us to four parameters that fulfilled the mathematical needs for an identifiable model. Identifiable parameters drive most of the variability in the model output and are largely independent of each other. Three parameters affecting the stomatal sensitivity to light, crown water potential, and stem water status were pre-set in order to keep the stomata fully open during daylight. The rest of the parameters needed to be pre-set by measurements (e.g. a = maximum bark thickness) or by model runs of subsamples. A list of parameters and their origins is given below.

Optimised parameters (red symbols in Fig. A1)

b (m^{-1}), allometric parameter to calculate the dynamic bark thickness (d_S) in relation to the stem diameter (D_{outer}) and the maximum bark thickness (a). $b = 9.3414$.

C_{Stem} ($g\ MPa^{-1}$), water storage capacitance of the bark. $C_{\text{Stem}} = 1200$.

R_C ($MPa \cdot s \cdot mg^{-1}$), hydraulic resistance between xylem and bark. $R_C = 0.03$.

k_{soil} (dimensionless), transformation parameter to calculate root water potentials from measured soil water potentials. $k_{\text{soil}} = 8.4$.

Measured or estimated parameters (orange symbols in Fig. A1)

a (m^{-1}), maximum bark thickness d_S . $a = 0.018$.

$D_{\text{outer_initial}}$ (m), measured stem diameter. $D_{\text{outer_initial}} = 0.15$.

l (m), measured tree height. $l = 12$.

Pre-set parameters obtained from model runs of data subsamples (orange symbols in Fig. A1)

Φ ($MPa^{-1} s^{-1}$), growth parameter for radial wood growth. $\Phi = 2.45 \times 10^{-9}$.

C_{Crown} ($g\ MPa^{-1}$), water storage capacitance of the crown. $C_{\text{Crown}} = 19$.

R_X ($MPa\ s\ mg^{-1}$), hydraulic resistance of the xylem. $R_X = 0.002$.

ϵ_0 (m^{-1}), proportionality parameter for the bulk elasticity of the stem. $\epsilon_0 = 115$.

Γ (MPa), bark turgor threshold for cambial activity and thus growth. $\Gamma = 0.9$.

$\Phi_{\text{Rad_threshold}}$ ($W\ m^{-2}$), threshold for stomatal responsiveness to light. $\Phi_{\text{Rad_threshold}} = 2.6$.

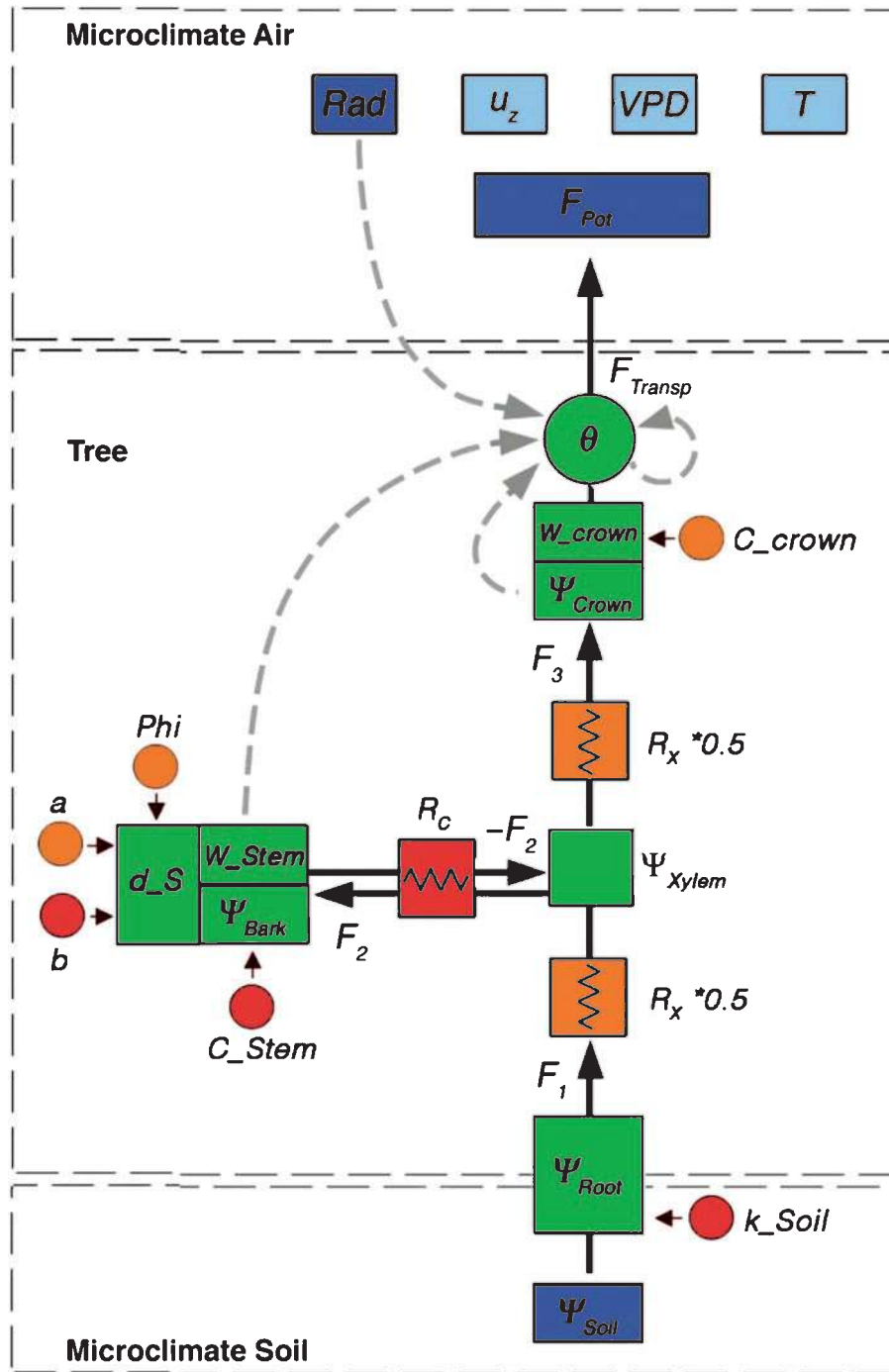


Fig. A1. The model HPMZ07 with some key parameters. The model is based on hydraulic flow and storage principles with the components: water storage in the stem (W_{Stem}) and crown (W_{Crown}) with the respective storage capacities in stem (C_{Stem}) and crown (C_{Crown}), flow path with corresponding water fluxes (F_1 to F_3), flow resistances (R_C = hydraulic resistance between xylem and bark, and R_x = hydraulic resistance of the xylem), and the corresponding water potentials (Ψ) in soil, stem, bark, and crown. Variables are calculated (green), parameters are optimised (red) or pre-set (orange), and input factors were measured (blue). Light-blue elements are input factors for the calculation of the potential transpiration (F_{Pot}), and dark-blue elements are input parameters for the tree water relations model. Abbreviations: Φ , growth parameter; d_S , dynamic stem size; a, maximum d_S ; b, allometric parameter to calculate d_S from stem diameter; k_{Soil} , transformation parameter to calculate root water potentials from measured soil water potentials.