Spatio-temporal water dynamics in mature Banksia menziesii trees during drought

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Southwest Australian Banksia woodlands are highly diverse plant communities that are threatened by drought- or temperature-induced mortality due to the region’s changing climate. We examined water relations in dominant Banksia menziesii R. Br. trees using magnetic leaf patch clamp pressure (ZIM-) probes that allow continuous, real-time monitoring of leaf water status. Multiple ZIM-probes across the crown were complemented by traditional ecophysiological measurements. During summer, early stomatal downregulation of transpiration prevented midday balancing pressures from exceeding 2.5 MPa. Diurnal patterns of ZIM-probe and pressure chamber readings agreed reasonably well, however, ZIM-probes recorded short-term dynamics, which are impossible to capture using a pressure chamber. Simultaneous recordings of three ZIM-probes evenly spaced along leaf laminae revealed intrafoliar turgor gradients, which, however, did not develop in a strictly basi- or acropetal fashion and varied with cardinal direction. Drought stress manifested as increasing daily signal amplitude (low leaf water status) and occasionally as rising baseline at night (delayed rehydration). These symptoms occurred more often locally than across the entire crown. Microclimate effects on leaf water status were strongest in crown regions experiencing peak morning radiation (East and North). Extreme spring temperatures preceded the sudden death of B. menziesii trees, suggesting a temperature- or humidity-related tipping point causing rapid hydraulic failure as evidenced by collapsing ZIM-probe readings from an affected tree. In a warmer and drier future, increased frequency of B. menziesii mortality will result in significantly altered community structure and ecosystem function.

Introduction

The Mediterranean biome is characterized by an extraordinarily high level of plant diversity and endemism, which has been attributed to adaptation to its hot, dry summers and cool, wet winters, frequent fires and often nutrient-impoverished soils (Breckle 2002). Plants have evolved a remarkable suite of phenological, physiological and morphological adaptations to cope with these harsh conditions such as sclerophyllous leaves,
lignotubers and epicormic buds for regeneration after fire or cluster roots to enhance nutrient acquisition from the phosphorus-deficient soils (Lambers et al. 2008).

However, despite these diverse adaptations, Mediterranean-type ecosystems are highly vulnerable to climate change (Klausmeyer and Shaw 2009). A plant community that appears particularly vulnerable to climatic drying is Banksia woodland (Sommer and Froend 2011) which is characteristic of the Swan Coastal Plain – an approximately 30 km wide and 400 km long coastal strip that harbors the center of the Southwest Australian global biodiversity hotspot. Banksia menziesii R. Br. and Banksia attenuata R. Br. (Proteaceae) co-dominate the overstorey of this highly diverse woodland (Keighery 2011) that occurs on coarsely textured sandy soils with an extremely low water-holding capacity which causes the soil profile to dry out rapidly to considerab le depths during summer (Groom 2011). These two dominant Banksia species are facultative phreatophytes (Dodd and Bell 1993, Canham et al. 2009) and may access deep soil water resources and groundwater down to a maximum of approximately 10 m through their deep-reaching dimorphic root system (Groom 2004). However, over the past few decades, declining precipitation has resulted in receding groundwater tables and insufficient soil moisture recharge, which has further exacerbated summer drought stress in this region (Timbal et al. 2006, Hughes et al. 2012). In 2010, the southwest of Western Australia experienced its driest and one of the hottest years on record (503.8 mm annual rainfall; Bureau of Meteorology 2011), causing extensive tree mortality in forests and woodlands across the landscape. Monitoring of Perth’s Kings Park urban bushland, found that 60–70% of Banksia spp. populations succumbed to the severe heat and/or drought during that summer (Miller and Easton, unpublished data).

In this study, we investigated water relations of B. menziesii from the leaf to the crown level in response to spring and summer drought in a system with documented significant change in rainfall, temperature and tree cover (Crosti et al. 2007; Australian Bureau of Meteorology). For the first time, we present continuous data on leaf water status across the crown of B. menziesii trees using the novel magnetic leaf patch clamp pressure probe (trade name ZIM-probe™, ZIM Plant Technology GmbH, Hennigsdorf, Germany) for continuous monitoring of leaf water status. On 6 October, four additional leaf patch clamp pressure probes were installed. The tree was equipped with two probes in each cardinal direction on sunlit leaves in the upper and lower crown with the remaining two probes located in the shaded center of the crown and centrally on top of the crown. Because the sclerophyllous leaves are clustered at the end of the branches, the establishment of a radial leaf profile across the crown was not feasible. Canopy access was achieved by using a trailer mounted boom lift. Unexpectedly, this study tree died in late October 2011, following several days of unusually high ambient temperature (see below). On 22 December 2011, we equipped a new 5.5-m tall B. menziesii tree, initially with eight pressure probes, one in the lower and one in the upper crown of each cardinal direction (on average approximately 1.5 m vertical distance from upper to lower crown). In February 2012, we increased the number of probes from one to two in each measuring
location. Moreover, we equipped one upper-crown leaf in each cardinal direction with three leaf patch clamp pressure probes at the base, mid and tip of the blade to test whether within-leaf turgor gradients exist.

The leaf patch clamp pressure probe

The principle of the magnetic leaf patch clamp pressure probe is detailed elsewhere but will briefly be revisited to elaborate on the relationship between probe readings and elastic modulus and hydraulic conductivity (Zimmermann et al. 2008, Westhoff et al. 2009a, Fernández et al. 2011). An intact leaf is clamped between the two planar pads of the probe, each equipped with a toric magnet. One of the pads contains a highly sensitive pressure sensor, the other one a threaded rod for varying the distance between the two magnets. The adjustment of the distance between the two magnets allows the optimum setting of the magnetic clamp pressure, $P_{clamp}$ (which is kept constant during measurements). The small covered leaf patch between the magnets is used as a sensing element for measuring turgor pressure changes in the uncovered leaf surrounding. This implies that the patch is hydrated and in hydraulic (and osmotic) contact with the entire leaf.

The turgor pressure, $P_c$, in the leaf patch (given by the osmotic pressure difference between the cellular and apoplastic compartments, is opposed to the magnetic pressure, $P_{clamp}$, applied to the leaf patch. This means that the output patch pressure, $P_p$, sensed by the pressure sensor is low at high turgor pressure and high at low turgor pressure, $P_c$, based on the physics of pressure transfer through the leaf (Zimmermann et al. 2008):

$$P_p = \left(\frac{b}{aP_c + b}\right)^{\frac{1}{2}} \times F_a \times P_{clamp} \quad (1)$$

where $F_a$ is a leaf-specific attenuation factor which takes into account the part of $P_{clamp}$ that is used for compression of air-filled spaces and structural elements such as cuticle and cell walls. $F_a$ can be assumed to be constant down to very low turgor pressures (approximately 50 kPa), even over long measuring periods (Zimmermann et al. 2008, Westhoff et al. 2009a). For the derivation of Eqn 1 a linear dependence of the bulk elastic modulus, $\epsilon$ of the leaf patch on $P_c$ has been assumed (Zimmermann et al. 2008):

$$\epsilon = aP_c + b \quad (2)$$

The magnitude of the coefficients $a$ and $b$ depend on the elasticity of the patch tissue and of the rate constant of turgor pressure changes (depending among other things on the hydraulic conductivity). This can readily be shown if the theoretical considerations of Murphy and Ortega (1995) are taken into account to more accurately describe the changes of the volumetric elastic modulus of the leaf patch on rapid changes of turgor pressure than Eqn 2:

$$\epsilon (P_c) = \epsilon_0 - (\epsilon_\infty - \epsilon_0) e^{kP_c} \quad (3)$$

where $k$ is the rate constant, $\epsilon_0$ the volumetric elastic modulus at $P_c = 0$ and $\epsilon_\infty$ the volumetric elastic modulus at large turgor pressures.

Using Eqn 3 for the integration of the transfer function (Zimmermann et al. 2008) shows that the bracket in Eqn 1 is replaced by:

$$\left[\frac{\epsilon_0}{\epsilon_0 + \epsilon_\infty (e^{kP_c} - 1)}\right]^\frac{1}{2} \quad (4)$$

The exponential term in Eqn 4 can be approximated by a Taylor expansion which can be terminated after 1 + $kP_c$ for relatively small pressures (say approximately 500 kPa or less). Replacing the $e$-function in Eqn 4 by the Taylor expansion leads to Eqn 1 and shows that $b = \epsilon_0$ and $a = k\epsilon_\infty$. Patch pressure measurements, therefore, give in principle access to the hydraulic conductivity and the volumetric elastic moduli at $P_c = 0$ and at high $P_c$ values if two of these parameters can directly be measured. This can only be performed by the cell turgor pressure probe on an intact leaf what is not possible on Banksia leaves because of the high rigidity.

Experimental setup

If not otherwise stated, all probes were attached to the mid-section of leaves, avoiding the main vein. The adaxial side of the leaves of *B. menziesii* is rather smooth, whereas the abaxial side is coarsely structured by prominent veins. Homogenous contact between the leaf and the sensing area of the probe is a mandatory prerequisite for accurate measurements (among other things, this was most likely a major reason why previous attempts to measure turgor pressure by application of an external force failed; see Heathcote et al. 1979, Turner and Sobrado 1983). To ensure optimum contact between sensor and leaf patch, the sensor-holding part of the probe was placed on the adaxial leaf surface. Clamping the sensor-holding part to the abaxial side was much more time-consuming because of the protruding leaf venation, but gave identical results (data not shown; Zimmermann et al. 2008). Real-time probe readings were sent wirelessly by telemetric transmitters (ZIM Plant Technology GmbH) every 5 min to a receiver base station which logged to a GPRS modem linked to an Internet server.

The ZIM-probes were virtually temperature-independent as shown by control experiments where
temperature changes over a range of 20°C only resulted in a change of approximately 1–2 kPa. Compared to the turgor pressure signals (see below) this noise level is sufficiently small to be neglected in the analysis (Zimmermann et al. 2008, Westhoff et al. 2009a).

The magnetic probes measure relative changes of turgor pressure. To allow comparison of diurnal \( P_p \) measurements the \( P_p \) data were offset corrected to \( P_p^* \). For the offset correction the minimum \( P_p \) value of the second day after clamping of the respective probe was used. For further normalization this offset corrected patch pressure \( P_p^* \) was divided by the maximum \( P_p \) reading during the respective day \( [P_p^*/P_p_{max}^*] \). For clarity, \( P_p \) data were smoothed using a 9-point FFT Filter routine in ORIGIN 8.5 (Microcal Software Inc., Northampton, MA).

**Microclimate measurements**

Relative humidity and ambient temperature were recorded in parallel to the ZIM-probe measurements using initially two sensors of each kind in the east and west of the upper canopy (ZIM-temperature probe, ZIM-relative humidity probe, ZIM Plant Technology GmbH, Hennigsdorf, Germany). On January 25, two additional temperature sensors were installed to cover the northern and the southern sections of the canopy.

**Dry leaf replica experiments**

Eqn 1 was derived under the assumption that the probe is applied to samples containing incompressible water. Furthermore, it is assumed that the elastic coefficients of the leaves are temperature-independent and that the cellular osmotic pressure is constant over a wide turgor pressure range (Zimmermann et al. 2008). The assumption of constant cellular osmotic pressure holds more or less over a large turgor pressure range because of the relatively high volumetric elastic modulus of the cell walls. Close to the plasmolytic point a significant increase of the cellular osmotic pressure is, however, expected (by 10–20%). At turgor pressure \( P_c \)=0, Eqn 1 loses validity. Porous samples such as dried plant material, but also filters will show a marked response to ambient temperature changes because of the air trapped within the pores of the sample material. Air has a very low specific heat capacity. For distinction of these effects from real turgor pressure effects measurements were performed on dry *Banksia* leaves and on different filter and paper types (e.g. 0.2 \( \mu m \) Millipore).

**Transpiration rate and stomatal conductance**

On 7 February 2012, diurnal measurements of transpiration rate (E) and stomatal conductance (g_s) to water vapor were performed at roughly 2 hourly intervals using a portable photosynthesis system (LI-6400XT, LI-COR Biosciences, Lincoln, NE). All measurements of \( g_s \) were conducted at ambient temperature, ambient vapor pressure deficit (VPD), ambient light intensity [photosynthetic photon flux density (PPFD)] and 385 ppm leaf chamber CO\(_2\) concentration. Pre-dawn recordings were taken before 06:00 h (Australian Western Standard Time, AWST) with the leaf chamber illumination turned off. For subsequent measurements the LED light unit was set to track ambient light levels. Individual recordings were taken as soon as \( g_s \) had reached steady-state which commonly occurred in less than 3 min.

**Pressure chamber measurements**

The diurnal measurement campaign also included measurements of balancing pressure (\( P_b \)) using a Scholander-type pressure bomb (PMS Instruments Co., Model 1000, Albany, OR). Sample leaves were harvested in close vicinity to leaves equipped with a ZIM-probe, using a sharp razor blade. Samples were immediately sealed in a zip-lock bag containing a moist tissue and placed into the pressure chamber within 2 min after excision. During measurements the chamber pressure was increased at a rate of approximately 0.3 MPa min\(^{-1}\).

**Sap flow measurements**

Sap flow was recorded from 31 January 2012 until the end of February 2012. We used one heat ratio method (HRM) sap flow sensor (Burgess et al. 2001; ICT International, Armidale, NSW, Australia) installed at 1.3 m height on the south-facing side of the trunk. The HRM sensor was protected against environmental effects using silver foil bubble wrap insulation. Readings were logged every 10 min. The sap velocity data were corrected for the zero offset at night by using the values from the night with the lowest VPD with no observed rainfall.

**Statistical analysis**

All statistical analyses were performed with the free software \( R \), version 2.13.2 (R Development Core Team, 2011; www.r-project.org). We used generalized additive mixed models (GAMM, package mgcv) to investigate the diurnal patterns of leaf physiological parameters and the response of normalized \( P_p \) to VPD. Model selection was based on the Akaike Information Criterion (AIC) considering differences between AIC values (\( \Delta AIC \)) ≥10 statistically significant (Burnham and Anderson 2002).

For the analysis of the diurnal leaf physiological recordings we applied GAMMs comprising the entire
day. First we formulated a GAMM with one common smoother term (i.e. smooth curve from a thin plate regression spline) for all cardinal directions or canopy positions (upper or lower), respectively. This model comprised ‘cardinal direction’ and ‘vertical canopy position’ as nominal explanatory variables. To test for differences between cardinal directions, this general model was compared to nested models using individual smoother terms for each cardinal direction or any grouping combination of cardinal directions. To test for differences related to vertical canopy position, the general model was compared to a model with two individual smoothers for upper and lower canopy.

For the analysis of the VPD response we binned VPD in 0.1 kPa intervals and derived the 95th percentiles of the \( P_p \) values or normalized sap flow within these intervals for the hot and rainless period from 6 to 13 February 2012. To test for differences in \( P_p \), VPD response between cardinal directions and canopy height we compared several models with a smoother term for VPD and ‘cardinal direction’ and ‘vertical canopy position’ (upper or lower canopy) as nominal explanatory variables. Testing for differences between cardinal directions and between upper and lower canopy was based on model comparisons as described above. A mixed model approach was required to include variance structures (varIdent, varExp or a combination thereof, package \textit{nlme}) in the models to deal with variance heterogeneity.

The response of normalized sap flow rates to VPD was modeled by generalized nonlinear least squares regression using a Mitscherlich-type function:

\[
SF = SF_{\text{max}} \left(1 - e^{-\alpha (\text{VPD} - \text{VPD}_{\text{c}})/SF_{\text{max}}} \right)
\]  

(5)

where \( SF \) is actual sap flow rate, \( SF_{\text{max}} \) is maximum sap flow, \( \alpha \) is the initial slope, VPD is vapor pressure deficit and \( \text{VPD}_{\text{c}} \) is the VPD at which sap flow reaches zero. In this model we applied an exponential variance structure (varExp) to model variance heterogeneity.

For all models, the underlying assumptions were assessed using quantile–quantile plots and histograms to test for normally distributed normalized residuals and plots of these residuals vs fitted values and each explanatory variable to check for homogeneity of variance.

\section*{Results}

\subsection*{Diurnal course of stomatal conductance, patch pressure, balancing pressure and sap flow}

On a cloudless and sunny summer day in the beginning of February 2012, we performed diurnal measurements of gas-exchange and balancing pressure (\( P_b \)) to complement the continuous \( P_p \) and sap flow recordings.

Across the tree crown, leaf transpiration (\( E \)) was more tightly linked to light intensity than to VPD (Fig. 1). Transpiration rates showed pronounced differences between cardinal directions but the diurnal courses seen in the upper and lower canopy did not warrant separate fits (\( \Delta \text{AIC} = 24 \) in favor of the GAMM with individual smoothers for cardinal direction; \( \Delta \text{AIC} = 0.7 \) for height-varying canopy models). In east- and north-facing leaves \( E \) increased linearly with increasing light intensity and reached peak values of around 6 mmol m\(^{-2}\) s\(^{-1}\) before noon coinciding with maximum light intensity. Peak values of \( E \) in the West and South reached maxima at similar times but were about a third lower compared to East and North and showed a more gradual decline (apart from the upper south-facing foliage).

The diurnal recordings of \( g_s \) also showed similar patterns between upper and lower canopy foliage (\( \Delta \text{AIC} = 21 \) in favor of the GAMM with one common smoother for vertical canopy position). When testing for differences in \( g_s \) across cardinal directions in the \textit{B. menziesii} canopy, the lowest AIC was associated with the model where ‘N + E’ and ‘S + W’ groups were formed (\( \Delta \text{AIC} = 106.8 \)). Overall, pre-dawn \( g_s \) was low and did not exceed values of approximately 60 mmol m\(^{-2}\) s\(^{-1}\), irrespective of vertical crown position and cardinal direction (Fig. 1). Maximum \( g_s \) occurred in the morning between 08:00 and 10:00 h coinciding with VPDs of 1.5–2 kPa (Fig. 1). Peak values of \( g_s \) ranged between 175–250 mmol m\(^{-2}\) s\(^{-1}\), except for west-facing leaves in the lower crown whose \( g_s \) only reached about 100 mmol m\(^{-2}\) s\(^{-1}\). On average, maximum stomatal conductance across the canopy was 184 ± 10 mmol m\(^{-2}\) s\(^{-1}\) (mean ± se, \( n = 25 \) leaves). For the remainder of the day, \( g_s \) continuously declined in all leaves and returned to pre-dawn levels between 17:00 and 18:00 h.

The normalized \( P_p \) curves derived from ZIM-probe recordings on upper and lower crown leaves showed the typical diurnal pattern, minimum values during the night (highest turgor), increasing after sunrise (decreasing turgor pressure due to transpirational water loss), peaking between 00:00 and 14:00 h when VPD reached very high maximum values between 5 and 6 kPa (lowest turgor) and decreasing in the afternoon (turgor recovery phase). Except for west-facing foliage, the \( P_p \) curves roughly matched the pattern derived from spot measurements of \( P_b \) using a Scholander-type pressure chamber. At all times, \( P_b \) values remained below 2.5 MPa regardless of vertical crown position or cardinal direction. Differences in \( P_b \) values between upper and lower crown largely reflected differences in \( g_s \) and in the west- and south-facing leaves also the more markedly differing light intensities between upper and lower crown. However, when
comparing the entire daily courses, these differences were statistically not significant (vertical crown position: ΔAIC = 16.8; cardinal direction: ΔAIC = 11 in both cases in favor of the ‘one common smoother’ GAMM). While the continuous ZIM-probe measurements allowed tracing the dynamics of leaf water status in response to small changes in microclimate during the day, this variation was certainly not detectable in $P_b$. Plotting the normalized $P_p$ vs the pooled and averaged $P_b$ data yielded a strong positive linear correlation ($r^2 = 0.61$, $P < 0.0001$; Fig. 2). Normalized sap flow increased steeply after sunrise and leveled off into a plateau with near maximal values at around 10:00 h (Fig. 3). The plateau extended to 18:00 h and subsequently normalized sap flow declined rapidly to values around 0.45 until sunset. Afterwards, the return to nighttime values occurred much more slowly and was not yet completed at midnight (Fig. 3).

$P_p$ and sap flow response to VPD

During mid-summer, the VPD response of normalized $P_p$ differed significantly between cardinal directions ($ΔAIC = 176.9$, in favor of the GAMM with individual smoother terms for each cardinal direction; Fig. 4). Pooling cardinal directions into ‘N + E’ and/or ‘S + W’ groups did not improve the model. In the east- and north-facing leaves, normalized $P_p$ increased steeply to values around 1 with rising VPD up to VPD values between 2 and 3 kPa and then either increased more slowly, remained constant or declined to values around 0.6 (Fig. 4A–D). West- and south-facing leaves showed a more uniform response, their $P_p$ increased in a more linear fashion.
with increasing VPD (Fig. 4E–H). In the south and west, upper and lower crown leaves showed similar responses to VPD ($\Delta$AIC < 1). In contrast, in the east and north the VPD response differed markedly between upper and lower crown due to the decrease of $P_p$ at higher VPD seen in an east-facing leaf from the lower crown and a north-facing leaf in the upper crown (east: $\Delta$AIC = 27.4, north: $\Delta$AIC = 46.1; Fig. 4B, C). Normalized sap flow showed a typical saturation response to VPD with fairly steady plateau values up to very high VPDs of approximately 8 kPa ($R^2 = 0.97, P < 0.0001$). At 2.5 kPa VPD normalized sap flow reached 95% of its maximum (Fig. 4I).

**Pp leaf gradients**

*Banksia menziesii* has highly sclerophyllous, undulate leaves with oblong blades that are 2–3 cm wide and 15–20 cm long. In adaptation to the seasonally dry climate and/or nutrient-limitation a thick cuticle has evolved and the stomata are aggregated and sunken in crypts on the abaxial leaf surface (see Fig. S2). Simultaneous patch pressure ($P_p$) recordings at the base, mid and apex of individual leaves gave evidence for the temporary development of $P_p$ gradients within the leaf during day- and/or night-time (Fig. 5). The initial clamp pressures of the three ZIM-probes on a north-facing leaf were coincidentally almost identical (Fig. 5B), whereas the initial clamp pressures of the probes on east-, west- and south-facing leaves differed markedly from each other due to the inhomogeneous properties of the leaves (Fig. 5A, C, D). All ZIM-probe measurements in Fig. 5 were normalized even though the initial clamp pressures of the three ZIM-probes in Fig. 5B did not necessary

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**Fig. 2.** Relationship between normalized $P_p$ values and corresponding balancing pressure values $P_b$ derived from pressure chamber measurements performed on 7 February 2012. Symbols represent mean values averaged over all cardinal directions and the upper and lower canopy.

**Fig. 3.** Diurnal course of normalized sap flow in *Banksia menziesii* on 7 February 2012. AWST denotes Australian Western Standard Time.

**Fig. 4.** The response of normalized patch pressure ($P_p$) in upper and lower leaves from all cardinal directions (A–H) and normalized sap flow (I) to vapor pressure deficit (VPD) during a rainless period in peak summer 2012 (6–13 February).
require normalization. The daily courses clearly showed that water loss (i.e. increase in \(P_p\)) was sometimes larger at the base (Fig. 5A), sometimes at the mid (Fig. 5B) or sometimes at the leaf apex (Fig. 5C, D). Evaluation of measurements performed over 6 weeks confirmed this finding. Similar fluctuations in water loss from base to apex were found when the probe located in the mid of the leaf was omitted or clamped on the opposite side of the mid vein thus excluding the possibility of interference of the mid probe with base-to-apex water transport.

**Diurnal \(P_p\) measurements**

Typical diurnal \(P_p\) measurements showed a positive relationship with air temperature and a negative relationship with relative humidity (RH; Fig. 6). Consequently, minimal \(P_p\) values (highest turgor) occurred during nights and maxima (lowest turgor) around noon (Fig. 6). Data shown are derived from a probe clamped on an east-facing leaf in the lower crown at the end of summer in March 2012. Probes clamped on leaves in other crown locations yielded qualitatively similar results. Data from the last week of March 2012 were selected because the diurnal temperature variations were very similar from day to day. Maximum temperature values at noon ranged between 31 and 35°C and minimum temperature at night varied between 14 and 17°C. In contrast, RH values showed a less consistent pattern. The maximum nighttime RH values increased from around 60% on 22 March reaching values of nearly 100% on 26 March. The changes in temperature and RH were only partly reflected in the \(P_p\) curves. Despite similar daily peak temperatures the corresponding \(P_p\) peak values increased continuously and reached maximum plateau values on 26 March. In the middle of the night of 26 March, at constant air
temperature of 15°C and RH > 90%, $P_p$ started to rise dramatically from typical night time levels around 5–17 kPa at dawn (see arrow in Fig. 6). Such signal increases at night occur when the absolute turgor pressure drops below a certain threshold (<50 kPa; Ehrenberger et al. 2012) resulting from substantial drought stress. In this state, the intercellular air spaces increasingly affect the magnitude of $P_p$ (state II according to the nomenclature of Ehrenberger et al. 2012). Interestingly, on the following days the increase of $P_p$ disappeared during the night indicating sufficient water supply to the leaves for rehydration. On 8 April, a similar increase in nighttime $P_p$ values at constant air temperature was observed (data not shown). Throughout the entire measuring period, state II occurred from time to time (see Table 1), but its frequency increased appreciably in February and April. Temporary drought stress was very often limited to individual branches. For example, during April probes clamped on east- and west-facing leaves in the lower canopy recorded state II more frequently than probes at other canopy locations (Table 1). On a few days, nearly all probes recorded strong drought stress signals indicating that the entire tree suffered from water shortage (e.g. on 1 February, 10 February, 22 February, 26 March, 28 April). Overall, state II was observed 177 times between January and April. Interestingly, the occurrence of state II coincided with high RH during the night (80–100%).

Commonly, drought effects are also manifested by an increase in the length of the turgor pressure recovery phase during the afternoon. However, this was not observed in *B. menziesii* leaves. Estimates of relaxation times derived from the roughly exponential $P_p$ decline during the afternoon showed rapid $P_p$ recovery within roughly 60 min with little day-to-day variation throughout the measuring period.

**Collapsing $P_p$ readings due to tree desiccation**

Towards the end of October 2011, peak air temperature sharply increased from 28 to 36°C over the course of a few days inducing high VPD > 4 kPa (Fig. 7). At that time probe readings started to crash in the upper north-facing leaves, closely followed by the signal collapse in the upper east-facing leaves. This process continued with the lower east- and north-facing leaves and the signal collapse occurred somewhat delayed in the west- and south-facing foliage.

East-facing leaves in the upper crown initially responded with an increase in $P_p$ peak values over 3 days (October 26–28), but then $P_p$ peaks declined in a nearly linear fashion until 2 November when the signal collapsed (see straight line in Fig. 7A). Subsequently, only noisy traces were recorded. Nighttime $P_p$ values also declined over the last 4 days before the signal collapse (Fig. 7A). In west- and south-facing leaves irrespective of crown location, the pronounced temperature rise caused a continuous increase in $P_p$ peak values until 2 November when the $P_p$ signal suddenly broke down (see solid line in Fig. 7B). Surprisingly, minimal nighttime $P_p$ values remained largely unaffected by increasing temperature, but during the last two nights prior to the signal collapse, $P_p$ only reached minima

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**Table 1.** Frequency of $P_p$ night increases (state II) from 22 December 2011 to 30 April 2012 measured on leaves of a approximately 5 m tall *Banksia menziesii* tree in each cardinal direction in the upper and lower crown (Note: each row in the table reflects a different branch in the crown).

<table>
<thead>
<tr>
<th></th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
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<tr>
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<td>0</td>
<td>5</td>
<td>4</td>
<td>14</td>
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<td>−</td>
<td>6</td>
<td>1</td>
<td>3</td>
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<tr>
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<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Upper leaf 2</td>
<td>−</td>
<td>−</td>
<td>2</td>
<td>0</td>
<td>3</td>
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<tr>
<td>North</td>
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<tr>
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<td>0</td>
<td>4</td>
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<td>3</td>
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<tr>
<td>Lower leaf 2</td>
<td>−</td>
<td>−</td>
<td>2</td>
<td>5</td>
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<td>3</td>
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shortly before dawn indicating severely delayed leaf rehydration (Fig. 7B). The signal collapse in the evening of 2 November was followed by a “flatline” trace. The signal collapse indicated the death of the tree. Consistent with the probe readings complete leaf desiccation occurred within a few days. Interestingly, when monitoring desiccated leaves continued post mortem, the signal returned a few days after the death of the tree showing pronounced, diurnal “Pp changes” (Fig. 7A, B; start at the dotted line). Such changes in Pp may occur if the leaf pore spaces, including xylem vessels, become progressively filled with air during the desiccation process. The trapped air in the leaf patch under the probe expands and shrinks according to changes in air temperature and thus exerts a corresponding pressure on the sensor of the probe. Model experiments using porous filter papers instead of desiccated B. menziesii leaves confirmed this explanation (Fig. 8). When the probes were removed from the dry pale leaves the patch previously covered by the probe magnets was also dry but less pale, presumably due to protection from radiation. Also, the spot was not impressed, i.e. the edge of the patch ended homogenously with the surrounding leaf area.

The temperature-dependency of the ZIM-probes was checked in a control experiment after the removal of the probes from the desiccated leaves. The ‘empty’ probes (i.e. the two magnets clamped together without sample in between) were left hanging free at their previous measurement sites in the tree crown where they were exposed to large temperature variations. Temperature changes over a range of 20°C only resulted in a signal change of approximately 1 kPa (inset Fig. 7B).

Discussion

Diurnal course of stomatal conductance, patch pressure, balancing pressure and sap flow

Diurnal measurements revealed a distinct hysteresis pattern between gs and E, which seems to reflect microclimatic drivers. E strongly correlated with light and thus temperature with peak values starting from noon. In contrast, gs peaked around mid-morning and progressively declined once VPD exceeded 2 kPa (lacking recovery with decreasing VPD) indicating high sensitivity to evapotranspirative demand. This pattern was consistent across the B. menziesii crown (Fig. 1) and matches the Physiol. Plant. 2014
within the desiccated leaf. "Pp" changes measured on other types reflect the expansion and shrinkage of air-filled spaces (vessels etc.) to the maximum values of around 150 mmol m$^{-2}$ s$^{-1}$.

The "Pp" data reflect the expansion and shrinkage of air-filled spaces (vessels etc.) within the desiccated leaf. "Pp" changes measured on other types of filter and typewriting paper were much smaller indicating that the air-filled pore structure plays an important role. Gray rectangles indicate nighttime hours.

\[ \begin{align*}
\text{Relative humidity (RH)} &\text{ on a } 0.2 \text{ mm Millipore filter} \\
\text{recorded at the same} \\
\text{crown location at the beginning of December, 2011. The "Pp" data} \\
\text{reflect the expansion and shrinkage of air-filled spaces (vessels etc.)} \\
\text{within the desiccated leaf. "Pp" changes measured on other types} \\
\text{of filter and typewriting paper were much smaller indicating that the} \\
\text{air-filled pore structure plays an important role. Gray rectangles indicate} \\
\text{nighttime hours.}
\end{align*} \]

Fig. 8. ZIM-probe readings together with air temperature (T) and relative humidity (RH) on a 0.2 mm Millipore filter recorded at the same crown location at the beginning of December, 2011. The "Pp" data reflect the expansion and shrinkage of air-filled spaces (vessels etc.) within the desiccated leaf. "Pp" changes measured on other types of filter and typewriting paper were much smaller indicating that the air-filled pore structure plays an important role. Gray rectangles indicate nighttime hours.

‘apparent’ feed-forward stomata response described by Franks et al. (1997) who concluded that it was a rare phenomenon. However, *Eucalyptus globulus* growing in a plantation forest in Western Australia also showed this type of feed-forward control of gs during peak summer (Macfarlane et al. 2004) and recently Ocheltree et al. (2013) reported that 8 out of 19 studied grass species exhibited this phenomenon, which correlated strongly with root conductance. These findings suggest that this type of stomatal response to VPD may be more common than previously thought and deserves closer attention.

Certain pairs of aspects (N + E vs W + S) produced similar gs which may reflect the fact that these aspects share similar light exposure times and levels (Fig. 1). Overall, strong stomatal control over transpiration prevented excessive loss of water and thus limited the drop of leaf turgor across the crown. This finding confirms the lack of groundwater access at our location as *B. menziesii* has been reported to rarely show stomatal closure at sites where the roots tap into groundwater (Dodd and Bell 1993). The mean maximum gs of 184 mmol m$^{-2}$ s$^{-1}$ across the *Banksia* crown is close to the maximum values of around 150 mmol m$^{-2}$ s$^{-1}$ reported for deep-rooting *Banksia* woodland species during summer but is nearly 50% lower than the value quoted for *B. menziesii* under optimal conditions (Veneklaas and Poot 2003, Drake et al. 2013). As a consequence of the strong stomatal regulation, Pp values peaked around 2 MPa matching peak shoot water potentials for this species quoted by Dodd and Bell (1993). This value also coincides with the figure reported to cause a 50% loss of stem hydraulic conductivity measured in *B. menziesii* at a similar site (Canham et al. 2009), suggesting that fine-tuned stomatal regulation reduces the danger of complete hydraulic failure. This was confirmed by the normalized sap flow values, which were already leveling into a plateau by the time stomatal conductance started to decrease on the day of the diurnal measurement campaign (Fig. 3). However, *B. menziesii* has rather large stomata, resulting in a relatively slow stomatal response to changes in VPD and hence prolonged exposure times to steep leaf-to-air VPD gradients making this species more susceptible to leaf hydraulic failure than co-occurring *Banksia* species (Drake et al. 2013). A comparative study of four *Banksia* species (including *B. menziesii*) along a pronounced soil moisture gradient found no evidence of hydraulic adjustment in stem- and leaf-specific conductivities or Huber values in response to soil water availability (Canham et al. 2009). Leaf-specific conductivities of *B. menziesii* ranged from $0.9 \times 10^{-4}$ to $1.0 \times 10^{-3}$ kg m$^{-1}$ MPa$^{-1}$ s$^{-1}$. Water relations of *B. menziesii* are very similar to the co-dominant *B. attenuata* (Canham et al. 2009) for which Carter et al. (2006) found higher osmotic potential than in co-occurring woodland species and interestingly no osmotic adjustment between summer and winter (always higher than $-3$ MPa). However, the bulk elastic modulus was 28% higher during summer (less elastic cells) compared to winter suggesting that turgor maintenance relies on changes in cell wall elasticity rather than adjustments in osmotic potential. Our findings and those from other studies indicate that *B. menziesii* operates with a narrow safety margin before catastrophic xylem dysfunction.

The minor variations seen in the normalized Pp traces of the ZIM probes indicate stomatal oscillations in response to small, transient changes in microclimate, which have been reported previously (Rüger et al. 2010, Zimmermann et al. 2010). Picking up such minor changes demonstrates the high sensitivity of the magnetic leaf turgor probe and highlights its suitability for detailed studies of stomatal responses and functioning.

**Pp and sap flow response to VPD**

The response of normalized Pp to VPD illustrates the within-crown variability in terms of leaf water balance (Fig. 4). East- and north-facing leaves are more exposed to solar radiation during the morning and midday period when peak transpiration occurs, and hence are more prone to drought stress than leaves from the other cardinal directions. A leveling off or decline in signal in these leaves could be observed that coincided with the VPD range over which we assessed downregulation of stomatal conductance during our diurnal measurements (2–3
kPa). However, we have no straightforward explanation for the lack of this stomatal feedback on $P_p$ signals in west- and south-facing leaves. Remarkably, in most of the west- and south-facing leaves $P_p$ values were not at their lowest values at night when VPD was lowest (Fig. 4), which may point to water reallocation within the crown at night from the less sun-exposed west and south to the east and north where insolation is higher and water therefore in greater demand during the day (Westhoff et al. 2009b). We found that sap flow reached 95% of its maximum rate at 2.5 kPa VPD, which again agrees very well with the VPD values marking the onset of stomatal closure observed during the diurnal measurements.

**$P_p$ leaf gradients**

At the leaf-level, we suspected turgor gradients along the sclerophyllous lamina due to its large dimensions and structural heterogeneity. The installation of three ZIM-probes evenly spaced along the length of the leaf blade confirmed the presence of leaf internal turgor pressure gradients in this species (Fig. 5). However, we did not find strictly acro- or basipetal gradients. The occurrence of the highest $P_p$ (i.e. lowest turgor) was not tied to a particular section of the leaf, which may indicate patchy stomatal conductance (Mott and Buckley 2000) or could result from leaf undulations that may cause different local transpiration rates for similar stomatal conductances due to differences in light exposure. Fast-developing turgor pressure gradients within transpiring leaves have recently been reported for the large-leaved species *Tetrastigma voinierianum* and *Musa acuminata* (Zimmermann et al. 2008, 2010). Our findings confirm the results of Turner et al. (1984) who measured differences in water potential within large *Helianthus annuus* leaves using in situ psychrometers. Interestingly, their study also showed that pressure chamber measurements only reflected the water potential of cells near the midrib and did not yield an average for entire *H. annuus* leaves.

**Diurnal $P_p$ measurements**

The high-resolution ZIM-probe recordings revealed pronounced fluctuations of $P_p$ values at noon and early afternoon which are most likely the result of passing clouds intercepting sunlight and wind gusts transiently removing the boundary layer of the leaves and thus stimulating transpiration. The observed increase in the daily signal amplitude in response to very similar daily temperature patterns (Fig. 6) suggests increasing loss of leaf turgor and has previously been observed in a number of crop species (e.g. grapevine, citrus, olive, banana, canola, wheat; Rüger et al. 2010, Bramley et al. 2013) following the termination of irrigation and is thus a clear indication for increasing water shortage. In the cited studies drought stress also caused an upward shift in the baseline signal, i.e. an increase in the minimum values at night, indicating incomplete leaf rehydration. However, *B. menziesii* rarely showed this phenomenon, implying complete turgor recovery overnight. The tight stomatal regulation observed in *B. menziesii* may help prevent excessive leaf dehydration and thus facilitate turgor recovery. Given the frequently high relative humidity and concomitant dew formation on leaf surfaces at night, foliar moisture uptake supported by hydrogels (mucilage) is very likely to occur as has previously been shown for a number of tree species from various habitats (Zimmermann et al. 2007). In a cleverly designed experiment using severed forked branches, Yates and Hutley (1995) have conclusively demonstrated the occurrence of horizontal water transport in tree crowns associated with foliar water uptake. Water absorbed by wetted leaves from one arm of a fork was transferred through the branch xylem and progressively improved the water status in the leaves of the second arm, which was kept dry.

**Collapsing $P_p$ readings due to tree desiccation**

Coincidentally, we obtained data from a *B. menziesii* tree that succumbed to sudden death in early spring (Fig. 7). The monitored tree had been growing on a crest where groundwater levels are well below 30 m throughout the year (BP Miller and S Easton, unpublished data), which is far beyond the reach of *B. menziesii* roots (Groom 2004). However, given the ample precipitation during October, especially during the last week prior to the tree’s death (54.8 mm rainfall; see Fig. S1), it seems rather unlikely that the studied tree was limited by low soil water availability when maximum daily spring temperatures unexpectedly rose above 35°C. Therefore, it is more plausible that the sudden increase in temperature and VPD have caused xylem embolisms which in turn have eventually reduced hydraulic conductance to the extent of complete hydraulic failure. This implies higher xylem vulnerability to embolism due to rapid temperature changes in spring compared to summer when such temperature patterns occur more frequently. In the east-facing leaf in the upper canopy of this tree, the decline in $P_p$ peak values during the days prior to its death suggests gradual desiccation (Fig. 7A). However, with progressive water loss and more air entering the leaf, the system became increasingly compressible rendering Eqn 1 invalid, i.e. under these conditions, the declining peak signal must not be mistaken as improving leaf turgor. In contrast, the south-facing leaf from the
upper crown, seemed better supplied with water: despite increased drought stress at daytime (increasing $P_p$ peak values), complete turgor recovery was achieved at night and only became impaired during the last two nights before the tree’s death (Fig. 7B). Here, the abrupt signal collapse is probably indicative of sudden hydraulic failure. When all leaf water had been replaced by air in the desiccating leaves, the $P_p$ signal picked up again now only reflecting the temperature-driven expansion and retraction of air in the pore spaces (Figs 7 and 8). As drought stress signals were first observed in the northern and eastern section of the crown, north- and east-facing foliage should be given particular attention for monitoring and quantifying drought stress in seasonally dry ecosystems in the Southern hemisphere. Sudden, non-fire related Banksia deaths without any prior symptoms of deterioration in plant health have been noticed since the 1960s but have not yet been linked to a particular cause (Crosti et al. 2007). Groom et al. (2000) also reported sudden temperature-related deaths of $B. \text{menziesii}$ but those were associated with unusually high summer temperatures at a site that had been subjected to ground water abstraction. In their case the Banksia trees were already facing summer drought as well as depleted soil water resources due to drawdown but apparently they had been able cope with these harsh conditions until temperatures surpassed a certain threshold for some days. In the light of these and our findings, it appears that temperature and/or VPD seem to play a critical role in pushing $B. \text{menziesii}$ beyond their tipping point. With the future prospect of more frequent and longer lasting heat waves, increasing mortality rates in Banksia trees are likely to occur and suggest that, in the long run, $B. \text{menziesii}$ will give way to more resilient species shifting not only community structure but – given their dominance in Banksia woodlands – the whole ecosystem into a new state.

The mechanisms driving water transport in plants and the links with carbon metabolism and drought are a matter of ongoing debate (Meinzer et al. 1999a, McDowell 2011). Many trees have been shown to perform multidirectional hydraulic redistribution of soil water resources via their root system, thus enhancing plant water balance and nutrient availability in the upper soil horizon. Our data indicate that horizontal water redistribution may also happen in the canopy making moisture available from crown locations less prone to heat/drought damage to meet higher transpiration demands and mitigate the risk of hydraulic disruption in more exposed crown areas (Yates and Hutley 1995). Future investigations will focus on sectorial watering and shading approaches to elucidate within-crown water (re)allocation in this species.

From a conservation perspective, our data imply that the ZIM-probe is a sensitive tool for detecting incipient drought stress in highly sclerophyllous species and is therefore an ideal instrument for evaluating adaptive management strategies to sustain woodland health and natural water resources.

**Author contributions**

M. K.-F. B. and U. Z. conceived and designed the experiments. M. K.-F. B., J. C., J. S. and B. P. M. performed the experiments. M. K.-F. B. and W. E. analyzed the data. M. K.-F. B. and U. Z. wrote the manuscript; E. J. V. and G. H. won the overarching grant; other authors provided editorial advice.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig S1.** Daily minimal/maximal temperature and precipitation for winter/spring 2011 and summer/autumn 2012 from the Perth ‘Metro’ weather station, approximately 5 km from the study site.

**Fig S2.** Cross section of *Banksia menziesii* leaf embedded in glycomethacrylate and stained with toluidine blue indicating hair filled stomatal crypt on abaxial surface, thickened cuticle on adaxial surface and sclerenchyma cells that account for the rigid structure of the leaf.

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