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An analysis of the sensitivity of sap flux to soil and plant variables assessed for an Australian woodland using SPA

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Abstract

Daily and seasonal patterns of tree water use were measured in a temperate, open, evergreen woodland using sap flow sensors. Investigations of soil, leaf, tree and micro-climatological variables were undertaken to validate a soil-plant-atmosphere (SPA) model. A comparison of measured and modelled rates of sap flow showed strong positive correlations across the 115 day study period. Using measured and modelled data we examined (a) the sensitivity of sap flow to abiotic and biotic factors; (b) the depth from which water uptake must occur to achieve observed rates of tree sap flow; (c) the importance of the water content of the upper soil profile as a determinant of sap flow; (d) the occurrence of nocturnal sap flow. We found that sap flow was very sensitive to the leaf area of the stand, whole tree conductance and the critical water potential of the leaves but insensitive to stem capacitance and increases in root biomass. Water uptake must have occurred from depths of up to 3 m and sap flow was independent of the water content of the upper soil profile. Nocturnal sap flow was observed and this was mostly because of incomplete stomatal closure at night.

Introduction

Water loss from soil stores through transpiration of trees is a principle determinant of the water budget of wooded catchments and successful management of water resources requires an understanding of the patterns and regulation of water use by trees. Our understanding of the biological controls of transpiration (rate of water loss per unit leaf area) at leaf- and branch-scales is well developed. For example, the importance of stomatal conductance and its response to soil moisture and vapour pressure deficit (Prior *et al.* 1997; Thomas and Eamus 1999), leaf water potential and xylem embolism (Sperry *et al.* 1993; Prior and Eamus 2000) and leaf-scale cost-benefit analyses, to the adaptive behaviour of Australian tree species have been extensively

documented and reviewed (Eamus and Prior 2001). In contrast, our understanding of the biological controls of tree water use at tree and canopy-scales is less well developed. The importance of canopy conductance (Blanken and Black 2004; Harris *et al.* 2004) and Huber value (McClenahan *et al.* 2004; Macinnis-Ng *et al.* 2004; Choat *et al.* 2007) in regulating tree water use have been examined but the importance of variation in other factors, such as leaf area index (Eamus *et al.* 2000), whole plant hydraulic conductance (Tyree 2003), stem capacitance (Cermak *et al.* 2007) or root biomass has received relatively less attention. A key aim of the work described here is to examine the sensitivity of tree water use to some of these abiotic and biotic factors.

Roots are generally concentrated in the upper soil profile, especially in the upper 0.5 m (Eamus *et al.*, 2002). Typically up to 90 % of the root biomass is observed in this layer (Snowdon *et al.* 2000) and the importance of the water content of this layer as a determinant of tree water use has been established globally (Whitley *et al.* 2007 and *loc. cit.*). However, in Australian woodlands, where deep drainage is generally less than 5% of rainfall (Hatton and Nulsen 1999; Knight *et al.* 2002), we hypothesise that the importance of uptake by roots at depth is likely to be significant, particularly in sandy soils when rainfall events of more than 100 mm will quickly move water to depths lower than 0.5 m. Consequently we examine the depth from which water uptake must occur to achieve the observed rates of tree sap flow and also examine the importance of the water content of the upper soil profile as a determinant of sap flow.

Nocturnal transpiration is a phenomenon that has only recently gained significant attention (Phillips *et al.* 2007), primarily because technology capable of detecting it at the whole-tree scale has only recently become available (Burgess *et al.* 2001). To-date we are aware of only two published accounts of nocturnal transpiration in Australia (Yunusa *et al.* 2000; Dawson *et al.* 2007). However, if, as suggested, open canopies experiencing large vapour pressure deficits are likely to exhibit nocturnal flows (Marks and Lechowicz. 2007), we may expect it to be common in

Australian temperate woodlands. The work described in this paper provides an examination of nocturnal sap flow.

Global climate change will alter the frequency, intensity, timing and distribution of precipitation, in addition to increasing air temperatures and atmospheric carbon dioxide concentrations (IPCC 2007). Such changes will impact on vegetation function (especially carbon and water fluxes) and the outcomes of the interaction of vegetation and climate (for example, species distribution) will also be altered. Consequently we need to understand the mechanisms underlying the links between plant function and climate in order to manage vegetation and water resources efficiently (Porporato *et al.* 2004). This is particularly true in Australia where scarce water resources are becoming increasingly valuable as demand increases and supplies are diminishing at regional scales (Beeton *et al.* 2006). A key tool available to natural resource managers is the application of models of vegetation processes (Eamus *et al.* 2006) but there are relatively few models which can successfully characterise the dynamics of soil-vegetation-atmosphere interactions. One of the most widely and successfully applied models is the soil-plant-atmosphere (SPA) model of Williams *et al.* (1996) which has been tested and validated across a range of diverse ecosystems, including Arctic tundra (Williams *et al.* 2000), Brazilian tropical rainforests (Williams *et al.* 1996; Fisher *et al.* 2006) and temperate Ponderosa pine forests (Williams *et al.* 2001). The SPA model is a mechanistic model which predicts, amongst other parameters, carbon and water fluxes, leaf water relations and changes in soil moisture and is previously untested in Australia. Australian temperate woodlands are distinguished from northern hemisphere woodlands in having a distinctive suite of attributes, including sclerophylly, a dominance by evergreen broadleaved trees, and physiological and morphological adaptations to high solar radiation, low annual rainfall and nutrient poor soils with low soil moisture holding capacity (Eamus *et al.* 2006). Consequently it is not immediately apparent that the SPA model, hitherto applied to northern hemisphere ecosystems, can be successfully applied to Australian ecosystems.

The aims of this paper are:

- First, to validate the SPA model to an Australian woodland, focussing on congruence of measured and modelled sap flow as a test of the model.

Having established that the model performs well, we use the model to answer the question:

- To what extent does variation in a range of abiotic and biotic factors influence sap flow;
- and
- from what depth must water uptake occur to achieve the observed rates of sap flow.

We also use a combination of observed and modelled rates of sap flow to:

- examine the importance of the water content of the upper soil profile as a determinant of sap flow.

Finally we:

- examine measurements of sap flow for patterns and causes of nocturnal sap flow.

Methods and materials

Study site, species composition and plant attributes

The study site was located in a remnant Cumberland Plains woodland, near Richmond, 47 km north-west of Sydney, New South Wales, Australia (33° 39' S, 150° 46' E, elevation 40 m).

Vegetation consists of open woodland, with an average height of 14 m, dominated by *Angophora bakeri* (E.C.Hall), (narrow-leaved apple) and *Eucalyptus sclerophylla* (Blakely) L.A.S. Johnson & Blaxell (Scribbly Gum), which account for approximately 80% of tree basal area at the site. Soils at the site are duplex with a very sandy upper profile to 0.8 m overlying a deep (> 10 m) weakly

pedal orange heavy clay (Bannerman and Hazelton, 1990). Mean tree basal area for the site was $1.45 \pm 0.35 \text{ m}^2 \text{ ha}^{-1}$ with $85.5 \pm 6.5 \text{ stems ha}^{-1}$ and leaf area index was generally about 1.3 throughout the study period, measured using a digital camera (MacFarlane *et al.* 2007). The understorey is dominated by shrubs and grasses including *Pultenaea elliptica*, *Cryptandra amara* and *Melaleuca thymifolia*. The climate is temperate, with mean maximum winter (July) and summer temperatures (Jan) in 2006 of 17.2 and 29.3°C respectively. Mean annual rainfall is 729 mm and is slightly summer dominant (see Figure 1).

Validating SPA

Table 1 describes all the parameters and variables required to run the SPA. The following briefly describes the methods applied to derive input values for plant, soil and climatic variables. The study period spans late winter (late August 2006) to early summer (December 2006) and all measurements were conducted during this period.

Soil physical characteristics

Soil samples (1000 cm^3) were collected at 10 cm intervals to 2 m depth and then transported to the laboratory in zip-lock plastic bags. Sand and clay proportions were determined by weighing dried (60°C for 3 days) samples and wet sieving with a $100 \mu\text{m}$ sieve (Allen, 1989). The portion of the sample remaining in the sieve was dried again and this fraction represents the coarse sand and gravel components. The portion passing through the sieve represents mostly the clay component.

Soil mineral and organic components were determined using the loss on ignition technique in a blast furnace (Allen, 1989). Dried samples of a known mass were combusted at 550°C for five hours. The samples were weighed again and the lost portion was the organic content while the

remaining portion was the mineral content. Bulk density was determined by weighing a known volume of soil, expressed as g cm^{-3} .

Soil hydraulic conductivity was measured four times at two depths in the sand layer (10 and 20 cm) and two depths in the clay layer (70 and 90 cm) using a Guelph Permeameter (Soil Moisture Equipment Corp., CA, USA).

Soil moisture content

Volumetric soil moisture content was measured with an array of frequency domain reflectometry sensors (Theta Probe, ML2-X, Delta-T devices, Cambridge) in two plots. Theta probes were buried horizontally at 10, 20, 40 and 60 cm depths in one plot and 10, 40 and 70 cm in a second plot. Soil moisture at each depth was calculated by multiplying soil depth (mm) by the soil moisture fraction of the soil. The water contained in each layer was summed to estimate total soil moisture content to 70 cm. Soil relative water content was estimated by dividing actual total soil moisture content by maximum soil moisture content ever observed over the entire study period.

Root biomass and root density

Root biomass was measured in soil cores of known volume (typically $500 - 1000 \text{ cm}^3$) taken at 10, 40, 50, 70, 100 and 150 cm depths at each of forty locations at the site. Each sample was sealed in plastic bags and transported to the laboratory where they were spread in a thin layer and roots collected for 10 min per sample. Roots were then oven dried, weighed and root density calculated as grams of root material per volume of soil (g m^{-3}). Rooting depth was greater than 1.5 m depth throughout the site but it was not possible to investigate below this depth.

Sapwood area, diameter at breast height, tree height

Sapwood cross-sectional area was measured by taking two 5 mm diameter cores from the trunk at approximately 1.3 m height. Sapwood area was measured on each tree that was instrumented with an HRM sap flow system (see below). Distinct colour changes were observed between bark, sapwood and heartwood in both species. Diameter at breast height (DBH) was measured at 1.3 m from the ground using a diameter tape, while tree height was recorded using an inclinometer. A census of the DBH of each tree within 2 plots of 50 m x 50 m was taken. Regressions between sapwood area and DBH were estimated to provide allometric relationships. The allometric relationship for each species was used in conjunction with the census data to provide an estimate of the sapwood area per hectare of the stand.

Leaf area index

Leaf area index (LAI) was estimated as described by Macfarlane *et al.* (2007). Two randomly located 50 × 50 m plots were marked at the site, and 25 upward looking canopy images per plot were taken with the camera located at a height of 1.5 m. All canopy photographs were collected as FINE JPEG format with a resolution of 3,871,488 pixels, using a Nikon Coolpix 995 digital camera. The lens was set to F2 (which provided a viewing angle of approximately 35°); exposure was set to automatic and aperture-priority modes. The aperture was set to a maximum possible F-stop of 9.6. All other camera settings follow those described in Macfarlane *et al.* (2007). Within each plot, canopy images were collected at 10 m intervals, along 5 linear transects spaced 10 m apart (giving a total of 25 images per plot). Each image was taken with the camera lens pointed directly upwards (zenith angle of 0°) with the aid of a bubble level fixed to the camera. All images were then analysed for the total number of pixels in canopy large gaps, as well as total canopy gaps, using the Adobe Photoshop® CS2 program. Leaf area index was then calculated from these

variables using equations detailed in Macfarlane *et al.* (2007). The leaf area index of the site was taken as the average LAI of all 50 images.

Leaf water potential

Leaf water potential (Ψ_1) was measured on three bagged and three unbagged leaves of six trees of each of the two species that were instrumented for sap flow (see below). Bagged leaves were wrapped in aluminium foil and covered with a plastic bag the day before measurement. The water potential of bagged leaves is close to the soil water potential in the rooting zone when measured pre-dawn (O'Grady *et al.* 2006) or is equal to xylem water potential, which approximates to soil moisture water content when measured during the day.

Measurements were taken at approximately 90 minute intervals on two or three consecutive days in late winter 2006, and summer 2006 using a Scholander-type pressure bomb (Plant Water Status Console, Soil Moisture Equipment Corporation, USA). Fully expanded, sunlit, mature leaves were sampled in the outer canopy between 2 – 8 m height between pre-dawn and 1800 h.

Whole plant hydraulic conductance

Whole plant hydraulic conductance (k_h) was calculated using the method of O'Grady *et al.* 2006. This method calculates conductance from the inverse of the slope of the relationship between sapflow (E) and the gradient of water potential between the root surface and leaf ($\Psi_1 - \Psi_s$). Thus, $k_h = E / (\Psi_1 - \Psi_s)$. Soil water potential was estimated by measuring Ψ_1 of leaves which had been sealed in aluminium foil and plastic bags the previous evening and leaf water potential was measured in unbagged leaves. Sapflow was measured with heat ratio sensors as described below. K_h is called G_{plant} in SPA.

Stomatal conductance and photosynthesis

Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) and instantaneous assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) were measured for trees of both dominant species using a portable photosynthesis system (LCpro+, ADC BioScientific, Hoddesdon UK) throughout the day on the same days as leaf water potential was measured. Two or three expanded, sunlit, mature leaves were sampled in the outer canopy between 2 m and 8 m height from three replicate trees of the two dominant species.

Maximum carboxylation rate and maximum electron transport rate

Maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) were determined by measuring assimilation rate over a range of internal leaf CO_2 concentrations with the LCpro+ portable photosynthesis system. Internal leaf CO_2 concentration (C_i) was manipulated using a CO_2 dosing cylinder and a scrubber attached to the leaf chamber as part of the leaf gas exchange system. CO_2 concentrations in the leaf chamber were approximately 300, 200, 100, 400, 600, 800, 1000, 1600 and 2000 ppm. This equated to an internal partial pressure (C_i) ranging from approximately 8 to 150 Pa. Leaf chamber CO_2 concentration was allowed to stabilise for at least 5 minutes at each concentration before assimilation rate was recorded. Light was maintained at 1000 (± 50) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ using the light attachment on the leaf chamber. Air temperature in the chamber was maintained at 20 °C (± 0.5 °C). The rate of assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$, on a leaf area basis) was plotted against the C_i (Pa) from which V_{cmax} and J_{max} were calculated (Manter and Kerrigan 2004).

Foliar Nitrogen

Leaf samples were collected from a variety of heights in the canopy via a hydraulic platform. On return to the laboratory, they were oven-dried (70°C for 3 d), ground with a ball grinder and nitrogen content was measured with the total carbon and nitrogen analyser (TruSpec CN 2000, Leco Corporation, Michigan). Foliar nitrogen was expressed as g of nitrogen per m² leaf.

Sap flow

Sap velocity was measured using the heat ratio technique (Burgess *et al.* 2001). For each species, six trees were chosen to cover the range in size present at the site for each species. These trees were instrumented with four sensors per tree (two probesets per tree) oriented at 90° to each other. Sensors were stratified with depth to account for variation in sap flow across the radial profile of each tree (Medhurst *et al.* 2002). Sap flow was measured at hourly intervals continually from June 2006 to November 2006, and then at half hourly intervals from November 2006 to December 2006. Sap flow data were corrected for the effects of wounding, radial variability in flow, sapwood area and volumetric fractions of water and wood as reported previously using algorithms described by Burgess *et al.* (2001). Accurate estimation of the zero-set of each sensor is important (Regalado and Ritter 2007) and in this study the zero-set of each sensor was determined by examining the flow rates at night time when VPD and wind speed were at or close to zero, during winter and spring.

Wound width and wood and water fractions

Volume fractions of wood and water in the sapwood were determined gravimetrically on 5 mm diameter cores taken from 10 trees of each species on two occasions. For *A. bakeri*, mean wood

fraction was 0.27 ± 0.02 and mean water fraction was 0.62 ± 0.03 . In *E. sclerophylla* the mean wood fraction was 0.34 ± 0.02 and mean water fraction was 0.52 ± 0.02 . The width of the wound around the holes used to insert the probes was measured twice in six trees of each species using a binocular microscope (O'Grady *et al.* 1999). A wound width of 2.55 mm for *A. bakeri* and 2.67 mm for *E. sclerophylla* was used to correct velocity estimates. Sap velocity was calculated for each sensor after allowing five days for development of the wound resulting from inserting sensors (Olbrich 1991).

Stand water use

Sap flow was scaled to stand water use for each species by obtaining the product of mean sap flow of each species (mm h^{-1}) and the total sapwood area of that species within the stand ($\text{m}^2 \text{ha}^{-1}$). Sap flow of the two dominant species was summed to estimate transpiration of the whole stand, then divided by total ground area to stand water use ($\text{m}^3 \text{m}^{-2} \text{ground area h}^{-1}$).

Meteorological data

All meteorological data were collected at half-hourly intervals throughout the sample period. Air temperature ($^{\circ}\text{C}$), wind speed (m s^{-1}), solar radiation (W m^{-2}) and rainfall (mm) were obtained from a meteorological station located approximately 2 km west of the study site. Aspirated wet and dry bulb temperatures were obtained from a screened weather station (Envirodata Pty Ltd, Australia) located approximately 500 m from the study site in a cleared field (approximately 4 ha). Vapour pressure deficit (D, kPa) was calculated from wet and dry bulb temperature data.

Photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$) was estimated by multiplying total shortwave radiation by 2.3.

Data analysis

Regression analysis and calculation of root mean square error (RMSE) was used to compare and evaluate measured and modelled rates of stand water use (SPSS v. 14.0, 2005).

Results

Meteorological conditions

Throughout the 115 day study period, maximum daily solar radiation levels increased from an average of about 700 W m^{-2} in spring (September) to almost 1000 W m^{-2} in summer (December), with occasional declines in daily maxima to $150 - 400 \text{ W m}^{-2}$ on cloudy days (Fig. 1). Mean daily maximum vapour pressure deficits (D) were lower in spring (typically $1 - 3 \text{ kPa}$) than summer (typically $2 - 6 \text{ kPa}$). There were several small rainfall events distributed throughout the study period, although during one week in early September over 50 mm was received (Fig. 1).

Vapour pressure deficit, temperature and solar radiation data are also presented in more detail for the periods of intensive field study (Fig. 2). Day 2 of the study (August 29th) was the first day of intensive field study of leaf water potential and photosynthesis. This day was characterised by a stable D throughout the day (around 1.3 kPa), a relatively small fluctuation in daytime air temperature (maximum temperature of $20 \text{ }^{\circ}\text{C}$) and large fluctuation in solar radiation throughout the day as cloud cover varied. The second day of intensive field study (1st September) was characterised by a large amplitude fluctuation in D throughout the day, increasing to a maximum of around 2.3 kPa . Solar radiation showed a smooth uniform increase throughout the day because of the absence of cloud. Diurnal variation in air temperature was larger than that on August 29th and peaked in the mid-afternoon at around $26 \text{ }^{\circ}\text{C}$.

The second intensive field study occurred in summer (December 13th and 14th). These days were hotter, with a larger diurnal range than those of late winter and spring. Similarly, the maxima and range in D and solar radiation were larger in summer than late winter and spring (Fig. 2). Day 108 (Summer, Dec 13th) had a maximum D of approximately 1.3 kPa, maximum radiation of about 900 W m⁻² and a maximum temperature of approximately 27°C (Fig. 2); these values were smaller than those experienced on the following day (day 109 of the study), when maximum D was almost 5 kPa, maximum radiation was about 1100 W m⁻² and maximum temperature was over 35°C.

Leaf water potential

Diel leaf water potentials in winter mostly ranged between -0.25 MPa and -3.13 MPa for *E. sclerophylla* and between -0.18 MPa and -2.44 MPa for *A. bakeri* (Table 2). There was a trend for *E. sclerophylla* to exhibit lower water potentials than *A. bakeri* in winter. During summer, diel leaf water potentials predominantly ranged between -0.66 MPa and -2.23 MPa for *E. sclerophylla* and between -0.78 MPa and -3.6 MPa for *A. bakeri*.

Modelled and measured hourly sap flow

Modelled and measured hourly sap flow values were similar in magnitude and daily patterns during both winter and summer sampling periods. A representative consecutive 16 day period for each of spring, and summer are shown in Fig. 3. From September to October 2006, the maximum hourly measured and modelled sap flow for each day increased, with peaks around 0.15 to 0.2 mm h⁻¹ respectively. One notable difference between measured and modelled values is that the SPA model shows sap flow to be decreasing during the evening on most nights to zero and then increasing at sunrise. In contrast observed rates of sap flow rarely reached zero at night. Generally measured sap flow decreased to a minimum around 0200 and 0400 h, after which sap flow started to increase.

In summer, maximum rates of modelled and measured sap flow ranged from 0.125 to 0.2 mm h⁻¹ (Fig. 3). Nocturnal sap flow was observed at all times of year, especially when soils were moist and nocturnal D was large (Fig. 3) but the SPA model did not replicate this behaviour.

There was a strong positive linear relationship between modelled and measured hourly values of sap flow (Fig. 4) and the slopes were close to 1 (0.91 in spring and 1.04 in summer; R² = 0.80 and 0.73 respectively) and the intercepts were not significantly different from zero.

Daily sap flow and cumulative totals

Daily values of measured and modelled sap flow showed similar trends throughout the study period (spring, day 5 to 27 of the study period and summer, days 87 to 115; Fig.5). In spring, daily sap flow was typically 1 – 1.5 mm d⁻¹, with a minimum of 0.4 mm d⁻¹ observed on a cool and cloudy day 13. There was a trend of increasing rates of sap flux during the spring as temperatures and D increased. Throughout the spring period, measured rates of sap flux tended to be about 5 - 10% lower than modelled values, whilst in summer, measured values tended to be about 5% larger than modelled values (Figs. 5, 6). In contrast to the upward trend observed in spring, both measured and modelled rates of sap flow tended to decline over the summer period from an average of about 1.6 mm d⁻¹ to an average of less than 1 mm d⁻¹. There was a strong positive correlation (slope 0.55) between measured and modelled daily sums of sap flux across the entire study period (Fig. 6).

Modelled and measured cumulative water fluxes over the study period were very similar across the 115 day study period, particularly for the first 80 days (Fig. 7). Only in the last 25 days was there a

slight (4%) divergence between measured and modelled sap flow, consistent with the slightly larger slope for the regression of daily summer rates of sap flow (Fig. 7).

Both measured and modelled sap flow were substantially larger (by almost 40 mm) than precipitation (Fig. 7). The sum of soil evaporation (estimated to be 40 mm by the SPA model), interception (estimated to be 11 mm) and sap flow was approximately 200 mm, or 80 mm larger than rainfall, indicating a significant contribution to tree water use of water stored at depths of up to 3 m.

Daily sap flow and soil moisture interactions

There was no effect of soil moisture content of the upper 80 cm of soil on the rate of water use of the stand (Fig. 8a, b). Daily stand water use ranged from 0.4 mm d⁻¹ to almost 1.8 mm d⁻¹, and soil moisture content varied from about 8% to about 32%. The gradual decline in total soil moisture content for the upper 80 cm of the profile from day 13 to day 50 was associated with a trend of increasing stand water use as daily average temperature, D and light flux density increased from early spring through to summer. Similarly the trend of a slight decline in stand water use in the last 25 days of the study was not associated with a declining soil moisture content of the upper 80 cm. The local minima in stand water use observed on days 10/11, 50, 70 and 84 reflect the influence of cloudy rainy days on transpiration.

A comparison of the modelled changes in soil moisture content for a range of depths in the sand (10 cm and 20 cm) and clay layers (120 – 200 cm) across the entire study period is shown in Figure 9. Whilst changes in the upper 10 and 20 cm of soil are large and rapid, in response to individual rainfall events, the deeper clay layers show no change in water content throughout the

initial 40 days. The upper clay layers (one layer at 120 cm is represented in Fig. 9) shows the largest change in soil content, falling from 30% to 17% over the 115 day period.

Sensitivity analysis

Daily sap flow (mean, minimum and maximum) during the 115 day study period was most sensitive to changes in LAI, critical leaf water potential (LWP) and whole plant hydraulic conductance (Fig. 10 a, b, c). Thus, for these three variables, a decrease or increase in their respective value of 50% resulted in a large decrease or increase in modelled sap flow, typically of 30 – 60% (for the \pm 50% change). For all three variables the response of sap flow to changes in the value of the variable was close to linear but deviated from linearity at the largest percentage decline in value. Proportional changes in LAI and the critical leaf water potential were approximately equivalent in their effects on mean sap flow. For instance, decreasing LAI by 50% and increasing LWP by 50% resulted in similar changes in mean sap flow. In contrast, changes in maximum rates of sap flow were larger for LWP than LAI. For instance, reducing LWP by 50% (Fig. 10b) caused a larger increase in maximum rate of sap flow than increasing LAI to 150% (Fig. 10a). Similarly, increasing LWP by 50% reduced minimum sap flow to close to zero but the same was not seen when LAI was reduced to 50%.

In contrast to the large sensitivity of sap flow to changes in LAI, critical leaf water potential and whole plant conductance, there was only a small change in rates of sap flow when stem capacitance, fine root radius or root biomass were altered by up to 500% from the value used in the simulations (Fig. 10 d, e & f respectively). Similarly, changing soil resistance from 10% to 500% of the value used in the simulation altered mean sap flow by approximately 5%, whilst changing soil porosity or optimal leaf temperature had minimal impact on rates of sap flow (data not shown).

Discussion

In order to achieve the aims of this study we first had to successfully parameterise the SPA model for the woodland site. Success in model parameterisation is defined here as a good correlation between observed and modelled rates of sap flow. Therefore we discuss this first.

A comparison of measured and modelled sap flow and the site water balance

There was close correlation between measured and modelled rates of sap flow at both hourly and daily time-steps (Figs. 4, 5, 6) across the entire study period and slopes of the regressions were close to one with a zero offset, in all cases. That the model described the data successfully is further revealed in the cumulative rates of sap flow (Fig. 7). After 115 days of modelling, the final modelled value of water use was only 4% larger than that of the measured value. Such congruence of modelled and measured rates of sap flow across periods of relatively high rates of rainfall (mid-September received about 50 mm of rainfall in 6 days) and extended periods of minimal rainfall (approximately 10 mm of rain fell in the 45 days preceding 30th October) demonstrates that the SPA model, with appropriate adjustments, can successfully be applied to Australian conditions.

Peak hourly rates of sap flow were typically 0.125 to 0.2 mm h⁻¹, whilst daily rates of sap flow were typically 0.8 to 2.0 mm d⁻¹ across the entire study period. Both diurnal and seasonal patterns for modelled and measured sap flow were highly comparable and comparable to those observed in Amazonian forest trees (Fisher *et al.* 2006) and an open woodland of temperate Australia (Zeppel *et al.* 2006), but slightly larger than rates observed in trees of an open savanna of monsoonal Australia (Hutley *et al.* 2000). Over the study period total precipitation received at the site was almost 120 mm. Soil evaporation was modelled to be about 45 mm and interception losses were estimated to be about 10 mm. In addition, understory water use is significant at this site and

estimated to be in the range 20 – 40 mm. Total water transpired by the trees was about 150 mm. Consequently the sum of these evaporative fluxes is 225 to 245 mm, or about double the rainfall received. Clearly this woodland site is using deep (> 1 m) stores of water, presumably in the clay layer since the sandy upper profile has a total storage at this site of approximately 70 mm of water and much of this will be accessed by the understorey. The importance of deep stores of water and the uncoupling of sap flow from the soil moisture content of the upper profile is discussed later.

Sensitivity analysis of model outputs – insight to tree function

An analysis of the sensitivity of model outputs to variation in input values can be used for two purposes. The first is to inform us about plant function: how does plant function vary when attributes such as LAI or critical leaf water potential or root biomass change? The second is to answer the question: given limited resources (time and money), where should we allocate those resources to ensure that we optimise the performance of the model? We now discuss these two issues.

The sensitivity analysis indicated that leaf area index, critical leaf water potential, whole plant hydraulic conductance and soil-root properties are the factors which most strongly influence sap flow. Previous sensitivity analyses of SPA have focused on impacts on gross primary productivity and latent energy outputs (Williams *et al.* 1996, William *et al.* 2001), but we focus on sap flow because we are most interested in catchment water balances. Williams *et al.* (2001) reported that GPP and LE were most sensitive to LAI and rooting depth, whilst whole plant conductivity, decreases but not increases in root biomass, root resistivity and critical LWP were of secondary but significant importance as determinants. We also found that if the value of root biomass used as an input to the model was decreased to 50% of the observed biomass a small decline in sap flow occurred but there were no changes in sap flow if root biomass was increased. Clearly, once sufficient rooting biomass (and depth) is present to supply the water demands of the canopy (itself

determined by LAI and micro-meteorological conditions), then additional root biomass does not result in increased water use as other factors (LAI and micro-meteorology) become limiting. The issue of root depth is discussed separately later.

Maximum LAI in this vegetation is low in comparison to other temperate woodlands and increasing LAI as a modelled input increased rates of water use. Clearly the LAI of this site is not in ecohydrological equilibrium, that is, it has not reached Eagleson's optimality (Eagleson 1982, Eagleson and Tellers 1982, Hatton *et al.* 1997). This is likely to be because of the impact of the long-term drought that has affected the eastern seaboard of Australia for the past 5 years (Eamus *et al.* 2006) which is likely to have reduced the LAI from a larger, long-term average and because of a fire that affected the site approximately seven years ago. Whilst increasing the LAI may increase sap flow, the model showed that this is associated with a very rapid depletion of soil moisture and a greater risk of drought stress, similar to that observed at a ponderosa pine plantation (Williams *et al.* 2001). However, in agreement with the conclusion from an earlier study (Eamus *et al.* 2000), it is likely that changes in LAI over the medium and longer term (seasons and years) are mechanisms by which trees can regulate water use in response to seasonal and inter-annual variation in water availability. It is also clear that obtaining accurate assessments of changes in LAI are required for successful application of the SPA model.

The critical leaf water potential used in the model (-2.8 MPa) was the mean minimum leaf water potential across the study period. This was not as low as the lowest measured field value of -3.8 MPa, which was observed infrequently and only in one species. Using a critical leaf water potential of -3.8 MPa in the model produced unrealistically high sap flow rates for the canopy as a whole and the more representative value of -2.8 MPa (the mean minimum of the two species) was found to produce peak and average sap flow rates that were good representations of measured values. It is likely that adjustment of critical water potentials through osmoregulation (Fordyce *et*

al. 1997) or changes in volumetric elastic modulus of cell walls (Eamus and Narayan 1990) allows some adjustment to tree water uptake in response to changes in water availability experienced by Australian trees.

The values for G_{plant} measured in this study varied by an order of magnitude across individual trees (approximately 0.8 to 9 $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) but the mean value used in the model (3.5 $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) whilst less than 20% of the value used by Williams *et al.* (2001; 20 $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), is comparable to values applied by Fisher *et al.* (2007) and Schwarz *et al.* (2004). When G_{plant} was varied between 10 and 200% of the value used in the model, peak and average sap flow increased by 300 and 500% respectively, highlighting the importance of getting good estimates of G_{plant} for successful application of the SPA model.

From what depth did water uptake occur?

The majority of applications of the SPA, to-date, have been to sites with relatively uniform soil profiles and relatively shallow rooting depths (generally less than 2.0 m) (Williams *et al.* 1996; Williams *et al.* 1998; Williams *et al.* 2001; Schwarz *et al.* 2004) although recent applications to Amazonian rainforest simulate root depths to 10 m (Fisher *et al.* 2006; 2007). In contrast, the current site has a duplex soil profile with very different properties between the upper sandy soil (to 80 cm) and the lower deep (> 10 m) clay layer. The upper profile has a low water storage capacity and high saturated conductivity; the clay layer has a high storage capacity and low saturated conductivity. Significant root density was observed to 1.5 m and roots were clearly growing to greater depths but we were unable to access greater depths. In order to satisfactorily describe the observed rates and patterns of sap flow, root depths to 3.0 m was required as an input value to the SPA model for sufficient stored water to be available to account, for example for the sustained rate of sap flow during a 27 d period without rain (day 18 to day 45). Similar adjustments to root depth

to account for observed rates of sap flow during dry periods have been required in previous applications (Fisher *et al.* 2006). The presence of tree roots to such depths has been extensively documented in open woodlands such as these (Canadell *et al.* 1996). The cause of the early cessation of modelled sap flow when a root depth of 2 m was used in the model was because when leaf water potential and soil water potential both reached the critical leaf water potential, uptake of water ceased.

Daily stand water use is uncoupled from soil moisture content in the upper profile

The importance of soil moisture content of the upper soil profile in regulating transpiration has been established in many studies world-wide (Oren and Pataki 2001; Lagergren and Lindroth 2002; Warren *et al.* 2005; Ford *et al.* 2007; Kume *et al.* 2007). This is generally correlated with the fact that the majority of the biomass of roots of trees is found in the upper 50 cm of the soil profile (Jackson *et al.* 1996; Eamus *et al.* 2002). Similarly, rapid responses of transpiration to short-pulses of rain highlight the importance of this part of the soil profile (Ogle and Reynolds 2004; Burgess 2006; Zeppel *et al.* 2007). However, in the present study, stand water use was decoupled from the soil moisture content of the upper profile, despite most of the roots being located there.

Uncoupling of sap flow and soil moisture content of the upper 80 cm is observed in Fig. 9, where the large decline in soil moisture content between days 18 and 45 are associated with a trend of increasing sap flow and a constant or slight increase in soil moisture between days 80 and 115 is associated with a trend of decreasing sap flow. We conclude that the presence of the large water resource present in the shallow clay from 80 cm to 3 m depth provides a large buffering capacity for tree water use. In support of this conclusion is the observation that the modelled water content of the clay at 120 and 160 cm depth exhibits a decline from day 8 of the study and even at a depth of 2 m, the clay show a decline in water content after day 50. A similar uncoupling of tree water use from soil drying in the upper profile was observed by Cleverly *et al.* (2006) who observed that

drought had minimal impact on tree water use at sites where groundwater was kept within three meters of the soil surface.

Measurements of sap flow reveal nocturnal flows

Modelled hourly rates of sap flow consistently showed zero sap flow at some point in every night, but the measured data rarely showed zero flow at night. The HRM method for measuring sap flow is particularly sensitive to detecting low flows (Dawson *et al.* 2007) and we are confident that such night time flow (not necessarily nocturnal transpiration as transpiration requires water loss from leaves) are actual flows rather than an artefact of the technique. Furthermore, the SPA is able to predict nocturnal flows (Williams *et al.* 2001) and therefore the absence of modelled nocturnal sap flow is not a result of the structure of the model. Nocturnal sap flow is receiving increasing attention (Marks and Lechowicz 2007; Dawson *et al.* 2007) as sensors become able to detect low flow rates. Such night-time flows may occur because of recharge of depleted water stores in the stem and canopy or because of stomata remaining slightly open. Nocturnal uptake of water may occur to enhance nutrient uptake or to support other metabolic activities (Marks and Lechowicz 2007). Marks and Lechowicz (2007) report that nocturnal sap flow occurs often in species adapted to high light environments, as is observed in the present study site where LAI is low and solar radiation levels are high.

There are two potential causes of nocturnal flow. First, it may result from a water potential gradient between the root surface and the stem and canopy of trees. Thus, water flow is occurring to recharge the storage volume of the tree. Second, it may result from incomplete closure of stomata at night. In the former, nocturnal flow should be independent of night-time vapour pressure deficits (D) and should decline throughout the night as the gradient of water potential between soil and canopy is lost. In the second, a strong dependence on D should be observed

(Yunusa *et al.* 2000). In the present study, patterns of nocturnal sap flow before and after rain events support some stem and canopy refilling (that is, nocturnal flows are driven by water potential gradients) but there appears to be much more sap flow arising from incomplete stomatal closure. Thus, nocturnal sap flow was low after an extended period (20+ days) without rain, and increased after rain, when the gradient in water potential between soil and canopy increased. However, during long rain events (of several days duration), nocturnal sap flow remained low because the night-time values of D were low and this dependence on D can be explained by partially open stomata. Variation in night time D explained much of the variation in sap flow amongst different nights. Because of the interaction of soil moisture content and D , in the days immediately preceding rainfall, there was generally insufficient soil moisture for nocturnal flows to occur despite a large D , and stomata remained more effectively closed at night and so rates of nocturnal sap flow did not respond to large overnight values of D . However, once the soil was recharged through rainfall, nocturnal sap flow was strongly affected by overnight D and D explained up to 70% of the variation in nocturnal sap flow. Thus, if refilling of storage sites in the canopy was the only reason for nocturnal sap flow, sap flow should have increased during the rainfall event (not after) and should not have been so closely associated with nocturnal D when soil moisture was plentiful. Proportionally, sap flow due to stomatal opening may be eight times higher than sap flow due to stem and canopy refilling.

Conclusions

Using the validated SPA model we were able to demonstrate that (a) variation in some abiotic and biotic factors strongly influenced sap flow; (b) water uptake was occurring from depths of up to 3 m, despite the majority of the root biomass occurring in the top 0.5 m of the soil; (c) sap flow was found to be independent of the water content of the top 0.8 m of soil and it was concluded that the deep clay layer was able to sustain observed rates of sap flow even as the upper soil moisture

content declined from almost 30% to less than 10%; nocturnal sap flow was frequently observed and because of the relationship between D and water potential gradients between soil and canopy it was concluded that incomplete stomatal closure at night was the major cause of nocturnal sap flow.

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Figures and tables

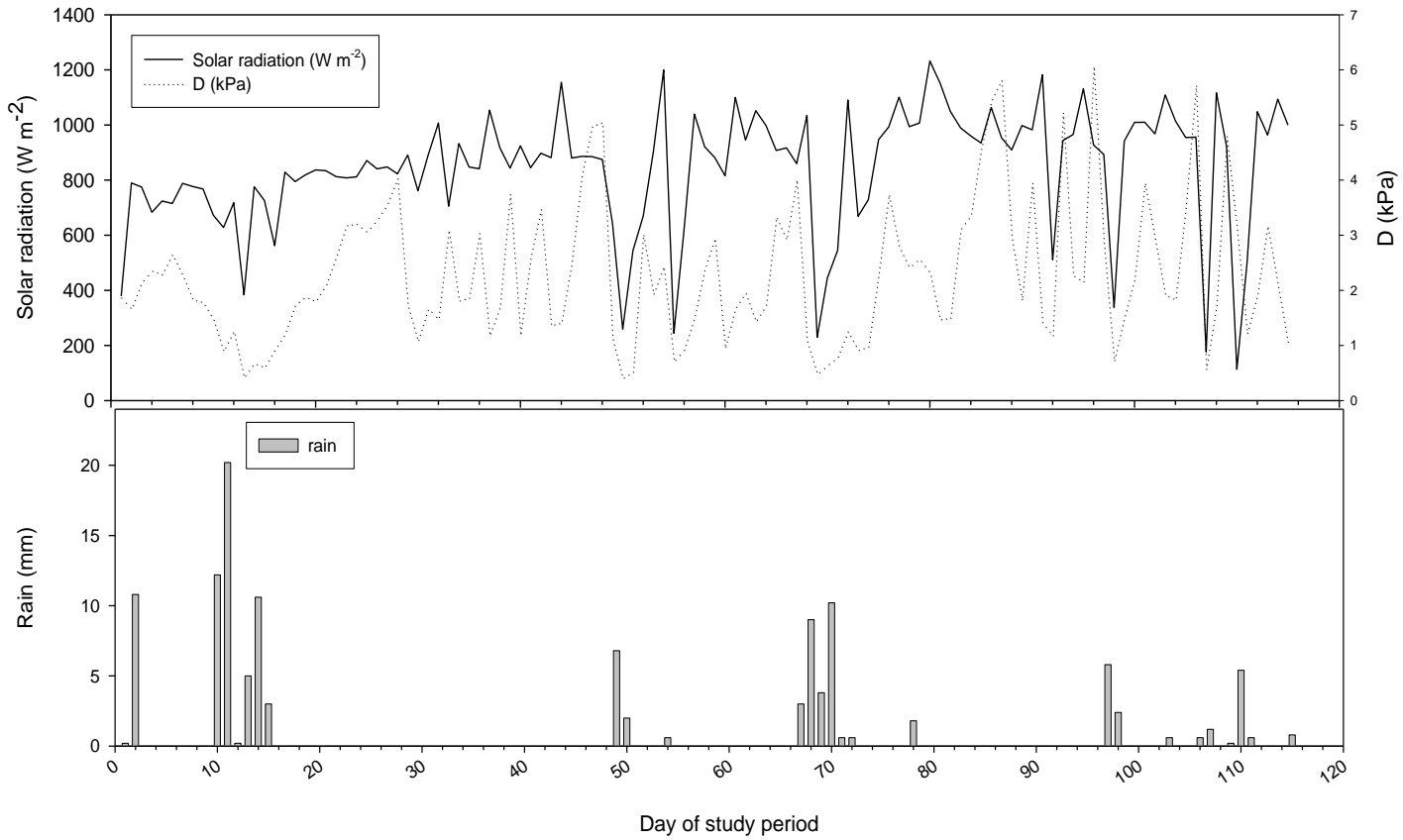


Fig. 1. Solar radiation (shown as daily maximum, W m^{-2}), vapour pressure deficit (D, daily maximum, kPa) and total daily rainfall (mm) measured at the site during the study period.

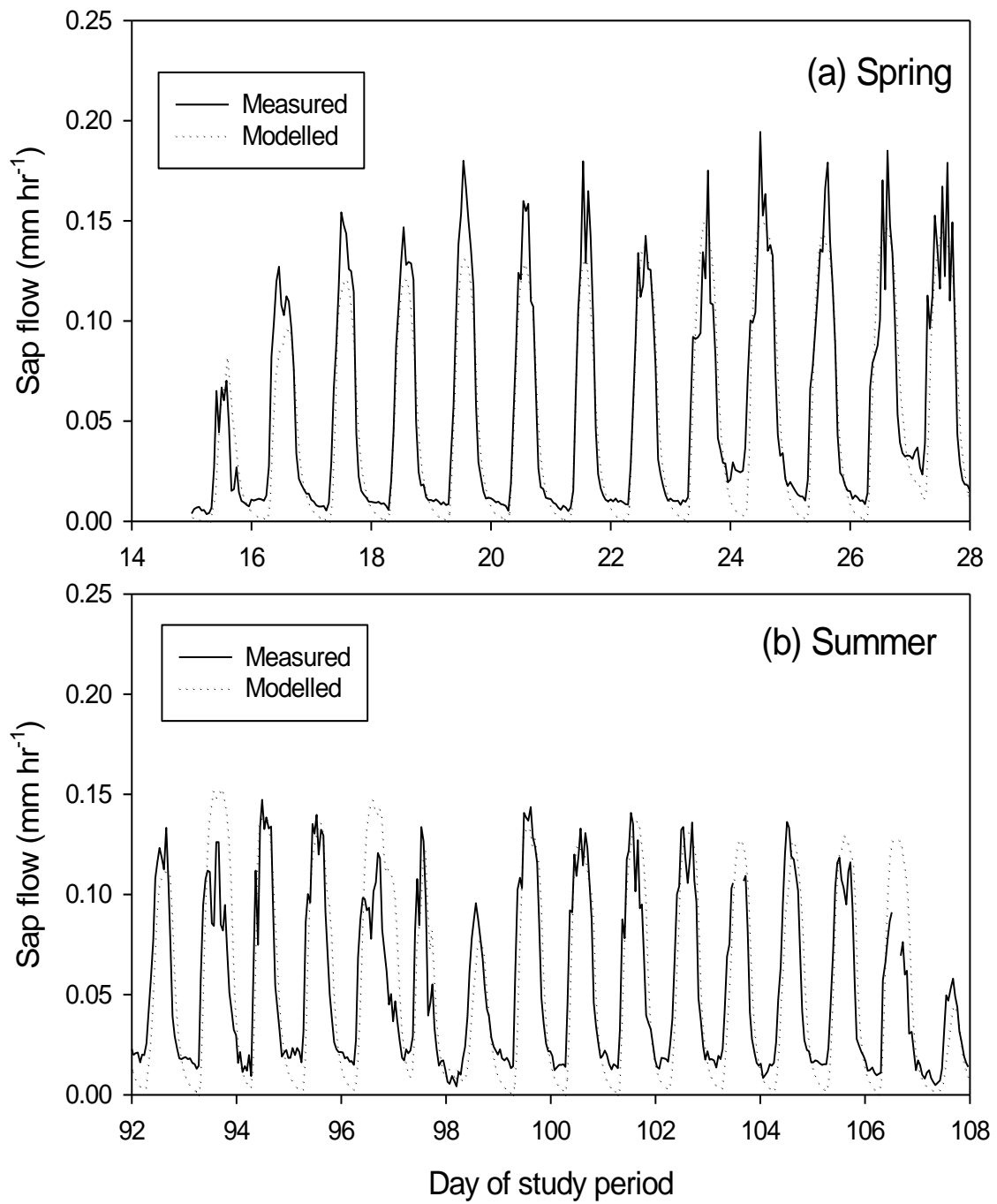


Fig. 2. A comparison of measured and modelled hourly sap flux of the stand. Data shown represent (a) Spring 2006 and (b) Summer 2006/7.

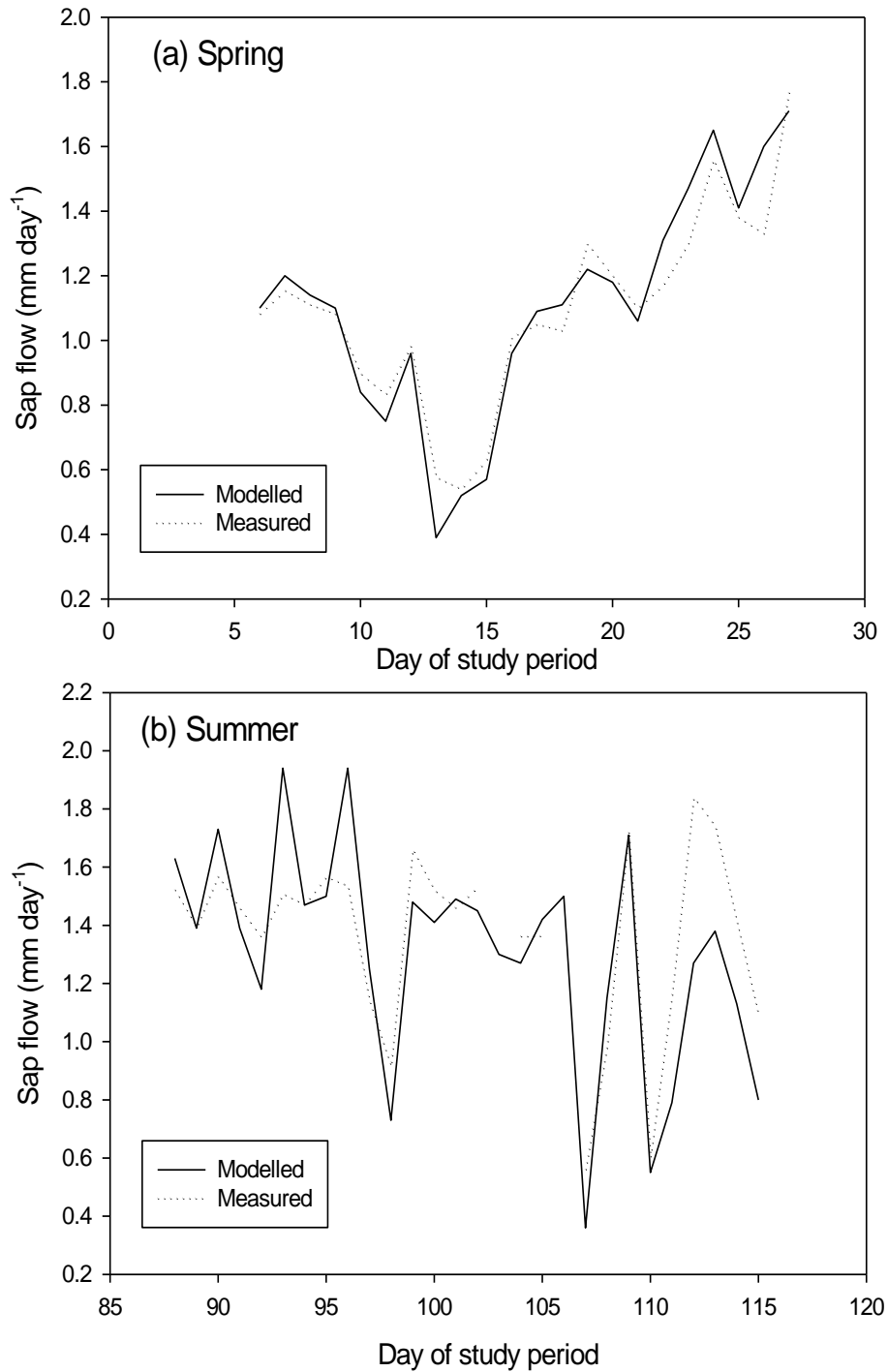


Fig. 3. Examples of modelled and measured daily estimates of sap flow during (a) spring 2006 and (b) summer 2006/7.

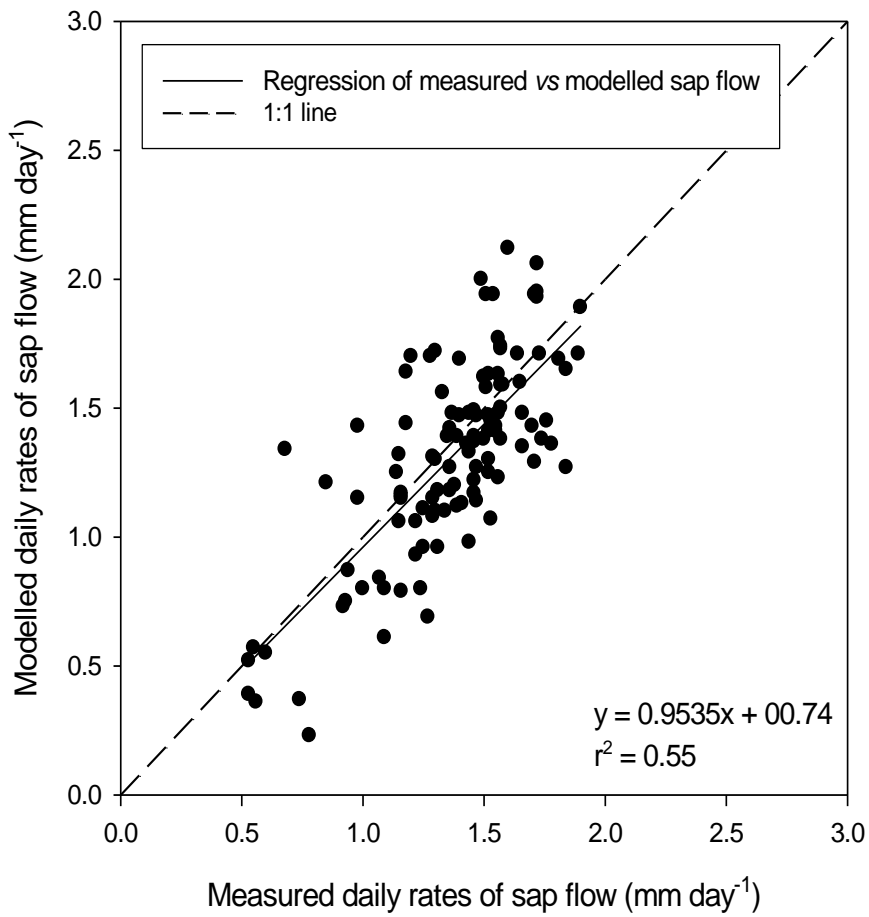


Fig. 4. A regression of daily values of measured and modelled sap flow (mm day^{-1}) during for the entire study period.

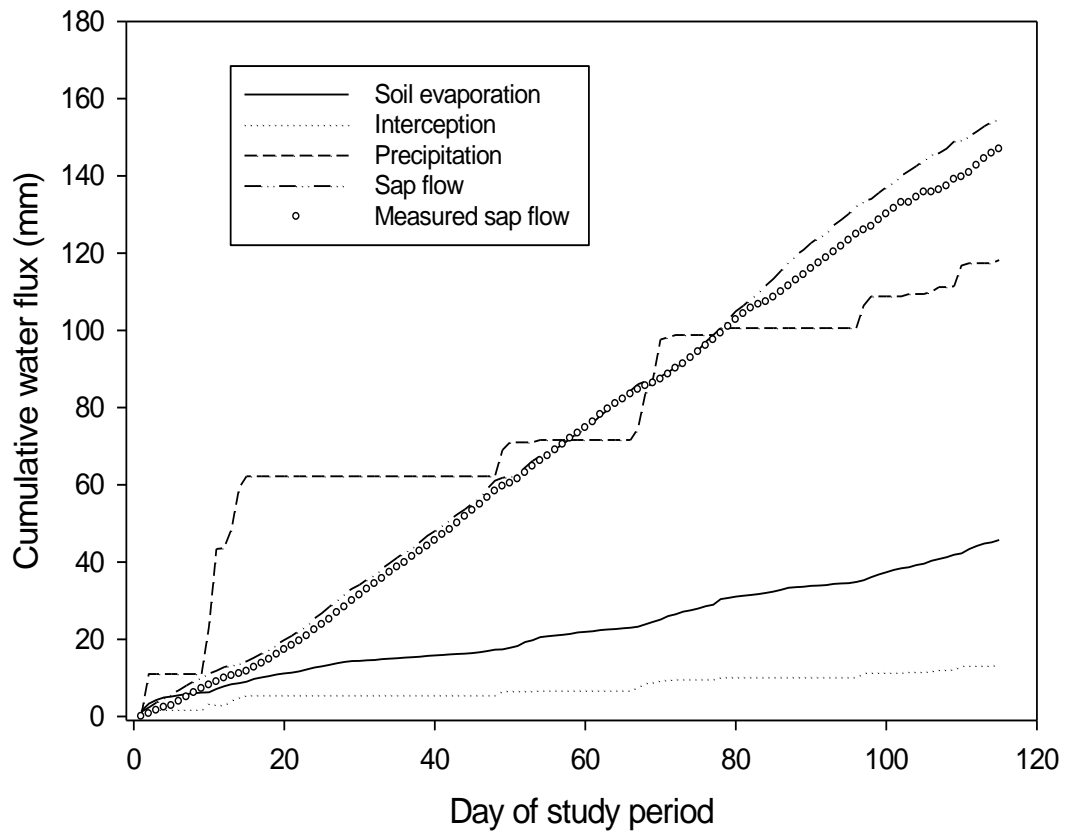


Fig. 5. Cumulative water fluxes, including modelled soil evaporation, interception, precipitation, transpiration. Measured sap flow is shown by open circles.

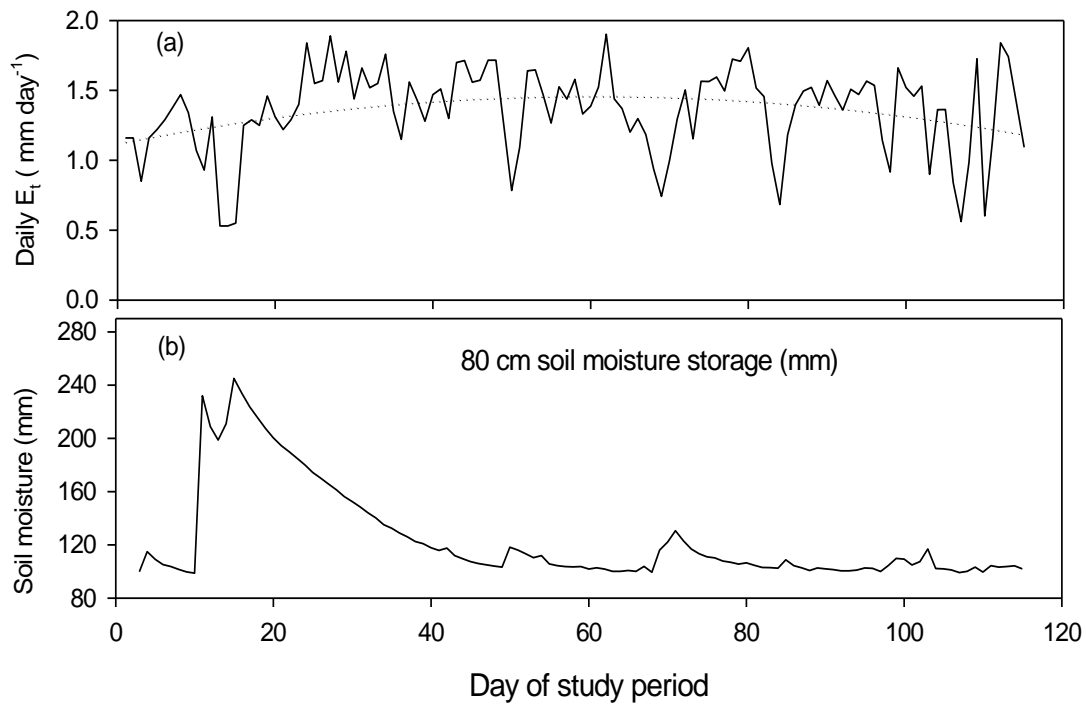


Fig. 6. Changes in daily stand E_t (a), (solid line) with a second order quadratic regression (dotted line) fitted to show long-term seasonal trends; and (b) measured total soil moisture content for the upper 80 cm of soil. Soil moisture shows a large increase in early September following a large rain event on days 10 and 17. The subsequent decline in soil moisture content between days 18 and 50 is not reflected in a decline in stand water use over the same period (a). The small decline in stand water use in the last 25 days of the study was similarly not associated with a decline in soil moisture store for the top 80 cm.

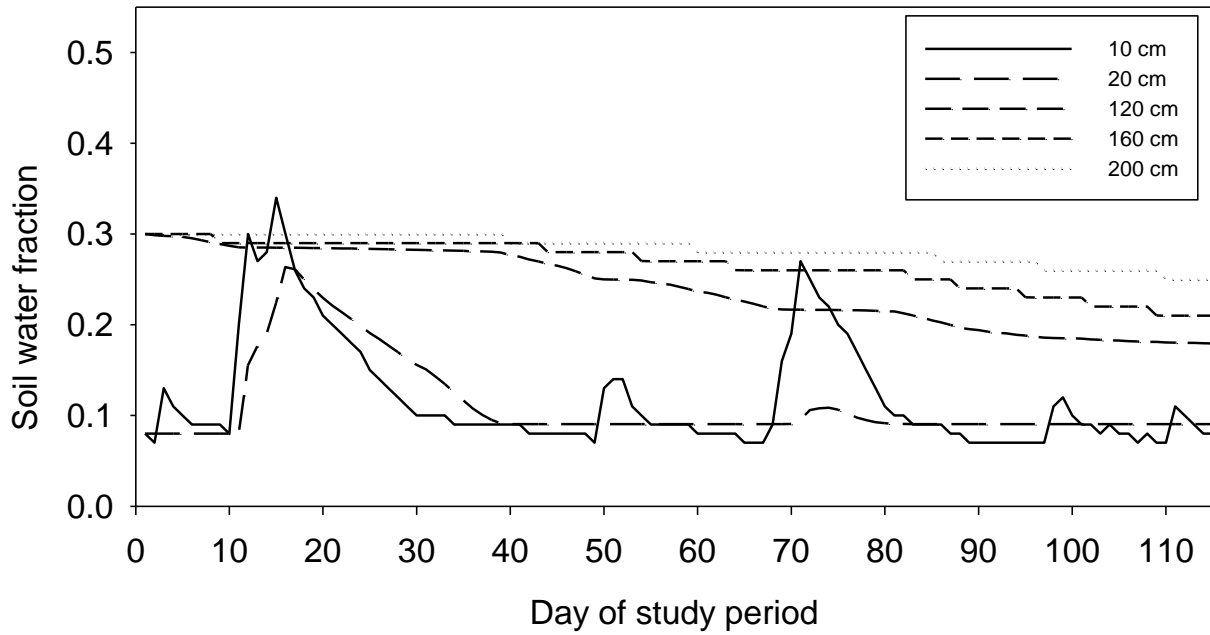


Fig. 7. A comparison of the modelled changes in soil moisture content for a range of depths in the sand (10 cm and 20 cm) and clay layers (120 – 200 cm).

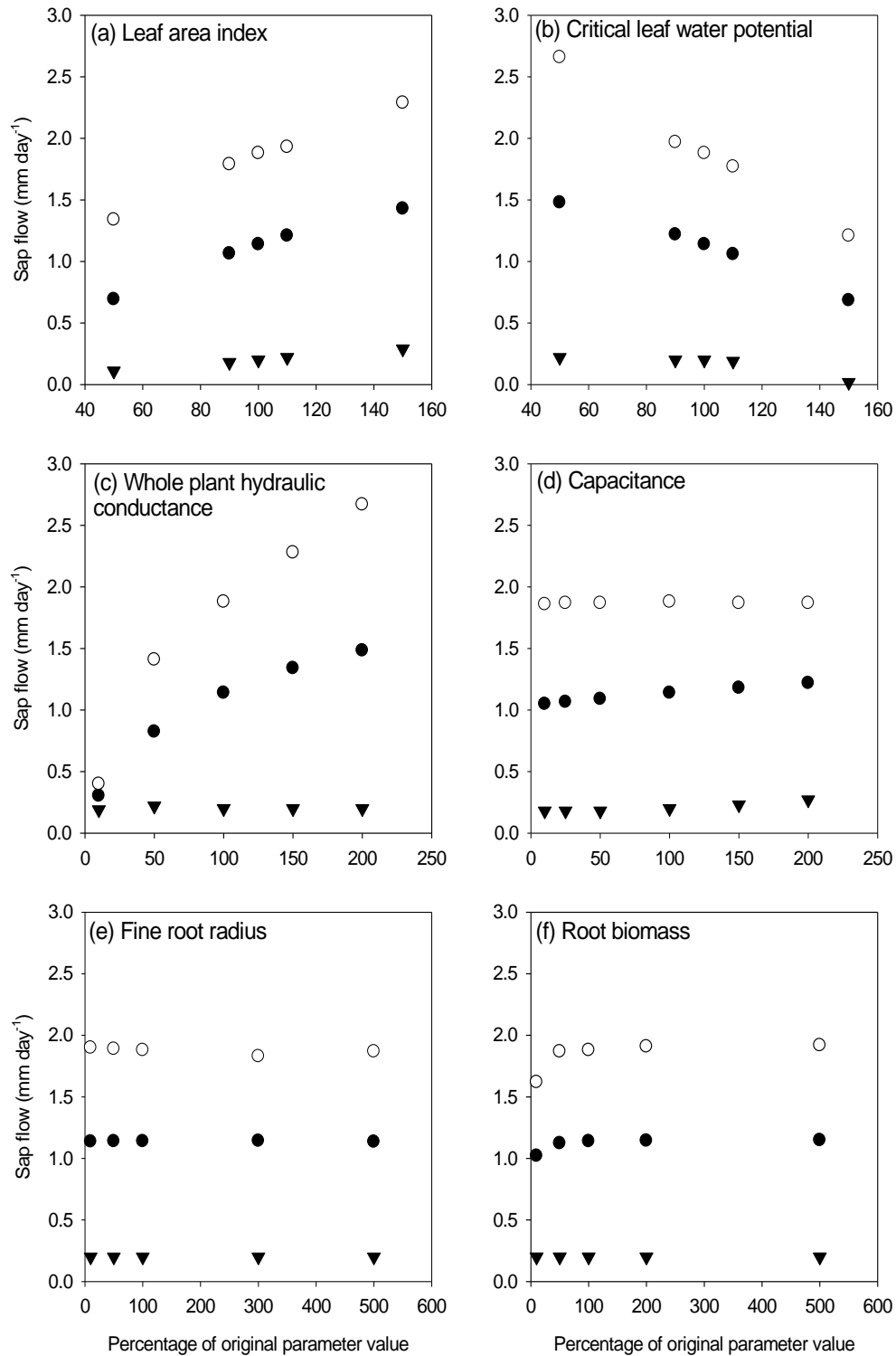


Fig. 8. Sensitivity analysis of the mean, minimum and maximum daily sap flow during the study period (115 days) to changes in the values of six key parameters, (a) LAI, (b) critical leaf water potential, (c) whole plant hydraulic conductance, G_{plant} , (d) capacitance, (e) fine root radius, and (f) root biomass. Axes show the percentage of the baseline parameter (from table 2), and mean (closed circle), minimum (closed triangle) and maximum (open circle) sap flow. Note the different scales on x axes.

Table 1. Values used in SPA for this study.

SPA model input values indicating the name, symbol, units, value used and whether the data were measured or estimated for the study site.

<i>Parameter/Variable</i>	<i>Symbol</i>	<i>Units</i>	<i>Value</i>	<i>Source</i>
Ambient atmospheric CO ₂ concentration	C _a	mmol mol ⁻¹	374	Measured, this study.
Canopy layer capacitance	C _n		5000	Williams <i>et al.</i> , 1996
Canopy hydraulic conductivity	G _p	mmol m ⁻¹ s ⁻¹ MPa ⁻¹	3.5	Measured, this study.
Layer height of soil	H	m	0.1 to 0.8 m depth, then 0.2 to 3.2 m	Site estimate
Leaf Area Index	L	m ⁻² m ²	1.3 to 1.9	Measured, this study.
Proportion of LAI in top layer	L _{top}		0.125	Estimated using Weibull cumulative distribution function
Fine root length per m ² ground area; total/layer	l _R /l _r		1930/3450	Measured, this study
Saturated hydraulic conductivity of clay	L _{clay}	mm h ⁻¹	0.66	Measured, this study.
Saturated hydraulic conductivity of sand	L _{sand}	mm h ⁻¹	124.2	Measured, this study.
Areal concentration of leaf N	N	g m ⁻² ground area	2.7	Measured, this study.
Proportion of total canopy N in top layer	N _{top}		0.125	Measured, this study.
Fine root radius	r _r	M	0.0001	Measured, this study.
Air temperature	T _a	°C	Variable	Measured, this study.
Leaf temperature	T _i	°C	Variable	Measured, this study.
RuBP carboxylation capacity	V _{cmax}	μmol g ⁻¹ s ⁻¹	73.6	A/Ci curve, this study
Maximum electron transport rate	J _{max}	μmol g ⁻¹ s ⁻¹	129.8	A/Ci curve, this study
δA/δg _s threshold for stomatal opening	τ	%	1.0007	Williams <i>et al.</i> , 1996
Minimum sustainable leaf water potential	Ψ _{lmin}	MPa	-2.8	Measured, this study.
Soil water potential	Ψ _s	MPa	-0.5	Pre-dawn leaf water potential, this study
% soil clay content in top 10 cm		%	9.79	Measured, this study.

% soil sand content in top 10 cm	%	85.62	Measured, this study.
Draincheck – field capacity as fraction of total porosity	fraction	0.5	Measured, this study.
Latitude	°	33	Measured, this study.
Dimension of leaves	m ²	0.08	Estimated
Root resistivity	MPa s g mmol ⁻¹	100	Estimated
Root mass	g	3450	Measured, this study.
Rooting depth	m	3.2	Estimated.

Table 2: Variables measured at the study site.

Predawn and minimum leaf water potentials, whole plant hydraulic conductance (G_{plant}), leaf area index (LAI), maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) on the days of intensive sampling. Values shown are mean and standard error of the mean.

	29 th Aug	1 st Sept	13 th Dec	14 th Dec
Predawn leaf water potential (MPa)				
<i>A. bakeri</i>	-0.96±0.15	-0.18±0.03	-0.78±0.13	-0.62±0.04
<i>E. sclerophylla</i>	-1.77±0.07	-0.25±0.06	-1.58±0.13	-0.66±0.11
Minimum leaf water potential (MPa)				
<i>A. bakeri</i>	-1.88±0.24	-2.44±0.34	-2.18±0.13	-1.81±0.18
<i>E. sclerophylla</i>	-2.56±0.09	-3.13±0.12	-3.60±0.21	-2.23±0.13
G_{plant} (mmol s ⁻¹ m ⁻² MPa ⁻¹)				
<i>A. bakeri</i>	5.04±2.10			
<i>E. sclerophylla</i>	2.22±0.50			
LAI	1.1	1.1	1.5	1.5
V_{cmax} (μmol g ⁻¹ s ⁻¹)			73.6±11.5	
J_{max} (μmol g ⁻¹ s ⁻¹)			129.8±19.5	