

1 **Published in Tree Physiology (2010) 30, 988-1000.**

2
3 **Rates of nocturnal transpiration in two evergreen temperate woodland species with**
4 **differing of water-use strategies**
5
6

7 Melanie Zeppel^{a,c} David Tissue^b Daniel Taylor^a, Catriona Macinnis-Ng^a and Derek Eamus^a

8
9 ^a Plant Functional Biology and Climate Change Cluster, and
10 Department of Environmental Sciences
11 University of Technology
12 Sydney, NSW 2007
13 Australia
14

15 ^b Centre for Plants and the Environment
16 University of Western Sydney
17 Richmond, NSW 2753
18

19 ^c Corresponding author email : Melanie.Zeppel@uts.edu.au

20 Phone: 61-2-9514-8405 Fax: 61-2-6514- 8439
21
22

23 Running head: Different water use strategies and nocturnal sap flow
24

Summary

Nocturnal fluxes may be a significant factor in the annual water budget of forested ecosystems. Here, we assessed sap flow in two co-occurring evergreen species (*Eucalyptus parramattensis* and *Angophora bakeri*) in a temperate woodland for two years in order to quantify the magnitude of seasonal nocturnal sap flow (E_n) under different environmental conditions. The two species showed different diurnal water relations, demonstrated by different diurnal curves of stomatal conductance, sapflow and leaf water potential. The relative influence of several microclimatic variables, including windspeed (U), vapour pressure deficit (D), the product of U and D (UD), and soil moisture content was quantified. D exerted the strongest influence on E_n ($r^2 = 0.59$ to 0.86), soil moisture influenced E_n when D was constant, but U and UD did not generally influence E_n . In both species, cuticular conductance (G_c) was a small proportion of total leaf conductance (G_s) and was not a major pathway for E_n . We found that E_n was primarily a function of water loss from the canopy rather than refilling of stem storage. Mean E_n was 6 to 8 % of 24-h flux across seasons (spring, summer and winter), but was up to 19% of 24-h flux on some days in both species. Despite different day-time strategies in water use of the two species, both species demonstrated low night-time water loss suggesting similar controls on water loss at night. In order to account for the impact of E_n on pre-dawn leaf water potential arising from the influence of disequilibria between root zone and leaf water potential, we also developed a model to more accurately predict soil water potential (ψ_s).

Keywords: Night-time sap flow, stem refilling, stomatal conductance, nocturnal transpiration.

Introduction

Nocturnal sap flow (E_n) occurs across a wide range of species and ecosystems (Novick *et al.* 2009), thereby challenging the paradigms that stomata are closed when radiation is zero and that transpiration does not occur at night (Bucci *et al.* 2004; Phillips *et al.* 2007; Scholz *et al.* 2007). The proportion of E_n to 24-h sap flow (E_n/E_d) is highly variable (1-28%) across diverse ecosystems, including tropical forests (Bucci *et al.* 2004; Dawson *et al.* 2007; Novick *et al.* 2009), boreal forests (Novick *et al.* 2009; Ward *et al.* 2008), and temperate forests (Dawson *et al.* 2007). Interestingly, sap flow at night is often highest (30-60% of 24-h sap flow) in arid desert ecosystems (Snyder *et al.* 2003). Within the same micro-environment, E_n may be variable between species. For example, in co-occurring tree species in a North American deciduous forest, one species exhibited moderate (13%) and two species exhibited low (2-7%) nocturnal fluxes despite similar micrometeorological conditions (Daley and Phillips 2006). In addition, seasonal variation in E_n and E_n/E_d has been observed, particularly in biomes which experience seasonal soil wetting and subsequent drought.

Nocturnal sap flow is associated with two components, mainly stem refilling with water after day-time depletion and transpirational water loss from the canopy (Daley and Phillips 2006; Phillips *et al.* 2009a). Night-time replenishment of water in the stem may contribute a significant proportion of the diurnal sum of 24 hr sap flow, generally 15-25% (Goldstein *et al.* 1998; Phillips *et al.* 2003) but up to 50% (Waring *et al.* 1979); the percentage of night-time water flow used for stem refilling increases with tree size (Phillips *et al.* 2003). To date, there are few studies that simultaneously measure crown and basal sap flow to quantify the proportion of nocturnal sap flow used for stem refilling and that proportion which constitutes transpirational loss of water through the canopy.

Water lost at night through leaf transpiration may be due to the presence of open stomata or to significant flux of water across the leaf cuticle. Although the vast majority of transpirational water loss during the day-time occurs via stomata, a larger proportion of water loss at night could potentially occur across the cuticle. This is especially true when immature leaves are present in the canopy, because immature leaves may lose substantially more water at night than mature leaves in some Eucalypt species (Phillips *et al.* 2009b). Alternatively, immature leaves may have less control of stomatal closure at night than mature leaves, thereby losing more water through transpiration.

Soil water potential (ψ_s) within the root zone is often estimated by measuring pre-dawn leaf water potential (ψ_{pd}) (Eamus, O'Grady *et al.* 2000; Palmer, Fuentes *et al.* 2009) based on the assumption that plant water status has come into equilibrium with that of the soil (Kavanagh *et al.* 2007). However, the occurrence of nocturnal transpiration (Barbour and Buckley 2007; Bucci *et al.* 2005; Dawson *et al.* 2007) may prevent attainment of equilibrium (Kavanagh *et al.* 2007). An accurate estimate of ψ_s is important in studies of hydraulic architecture and ecosystem function. Despite its importance, little research has been conducted on the relationship between nocturnal water loss and pre-dawn water potential (Bucci *et al.* 2004).

In this study, we assessed the magnitude of E_n as a proportion of 24-h sap flow (E_n/E_d) during a two-year period in two different tree genera growing in natural woodland. Our goal was to determine the contribution of nocturnal water loss to total water loss in trees exhibiting different patterns of plant water relations. For example, these two species differ significantly in rates of water use, the diurnal range of leaf water potential and the magnitude of stomatal conductance (Zeppel *et al.* 2008a), and therefore exhibit different day-time water use strategies under common environmental conditions (Zeppel and Eamus 2008). We also test whether environmental variables (*e.g.* soil water content, D , U) and leaf characteristics (immature *versus* mature, G_c , G_s) were important regulators of E_n and E_n/E_d during different seasons (summer, winter, spring). We hypothesised that: (1) D will be the pre-dominant regulator of E_n ; (2) E_n will be greater in the summer than in the winter or spring due to a larger D ; (3) stem refilling will be a larger contributor to E_n than night-time transpiration from the canopy; (4) G_s is a much larger contributor to water loss than G_c at night; and (5) both species will exhibit similar E_n/E_d reflecting very strong environmental control on water loss at night.

Methods and materials

Study site

The study site was located in a remnant Cumberland Plains woodland, near Richmond, west of Sydney, New South Wales, Australia (33° 39'S, 150° 46' E, elevation 32 m). Vegetation at the site consists of open woodland, with an average height of 14 m, dominated by *Angophora bakeri* (E.C.Hall), (narrow-leaved apple) and *Eucalyptus parramattensis* (E.C. Hall) (drooping red gum). These two species account for approximately 80 % of tree basal area at the site, and have very different water-use strategies during the day. For example, pre-dawn and minimum leaf water potentials and diurnal sap flow rates show that *E. parramattensis* experienced much greater water stress and had higher sap flow rates than *A. bakeri* during the day (Fig 1; also see Zeppel *et al.* 2008).

Soils at the site were sandy loams to depths of 60 – 80 cm, underlain by deep sandy clays (Macinnis-Ng *et al.* 2009). Mean tree basal area for the site was $12.3 \pm 3.2 \text{ m}^2 \text{ ha}^{-1}$ with $85.5 \pm 6.5 \text{ stems ha}^{-1}$. Leaf area index of the tree canopy varied from 0.8 to 1.3 throughout the study period, measured using digital photography (MacFarlane *et al.* 2007). The understorey is dominated by shrubs and grasses including *Pultenaea elliptica*, *Cryptandra amara* and *Melaleuca thymifolia*.

Meteorological data

All meteorological data were collected at 15-minute intervals and the mean of the four values within each hour were used in subsequent analyses. Air temperature (°C), wind speed (m s^{-1}), shortwave radiation (W m^{-2}) and rainfall (mm) data were obtained from a meteorological station located approximately 1 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 (4 ha), while total solar radiation was measured above the screen. Vapour pressure deficit (D , kPa) was calculated from wet and dry bulb temperatures.

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, UK) 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 depths at a second plot. Soil moisture storage was estimated over two ranges (0- 60 cm and 0- 110 cm depths) using previously described methods, where soil moisture storage is the product of relative water content for each soil depth and the soil depth (Zeppel *et al.* 2008a).

Sapwood area, diameter at breast height, tree height

Sapwood cross-sectional area was measured by taking two 5 mm diameter cores from the trunk of the tree at approximately 1.3 m height. Sapwood area was measured on each tree instrumented with a heat ratio method (HRM) sap flow system (Burgess *et al.* 2001). Distinct colour changes were observed between bark, sapwood and heartwood in both species. Diameter at breast height (DBH cm) was measured at 1.3 m from the ground using a diameter tape and tree height was recorded using an inclinometer within 2 plots of 50 m x 50 m. Regressions between sapwood area and DBH were used to develop allometric relationships to estimate total sapwood area of the stand.

Sap flow

Sap velocity was measured using the HRM technique as described by Burgess *et al.*, (2001). Two probe sets (4 sensors) were inserted at 90° to each other in each tree at *ca.* 1.3 m. For each species, 6 trees were chosen to represent the size distribution at the site and were instrumented with four sensors per tree (2 probe sets per tree). The sensors were stratified with depth to account for variation in sap flow across the radial profile of each tree (Ford *et al.* 2004). Sap flow was measured continuously at hourly intervals from June 2006 to November 2006, and then at half-hourly intervals from November 2006 to March 2008. Sap flow data were corrected for the effects of wounding, radial variability in flow, sapwood area and moisture content of wood using algorithms described by Burgess *et al.*, (2001). Wound widths, wood and water contents are described in (Zeppel *et al.* 2008a).

The zero-set of each sensor (four per tree) was determined using two methods: (1) examining the flow rates at night when *D* and *U* were *ca.* zero during the 18-month period; and (2) at the end of the study, cutting into the sapwood beneath the sensors in 4 trees to determine the zero-flow rates. There were no significant differences ($P < 0.05$) between the two different methods used to estimate zero-flow rates. We examined the comparative influence of soil moisture and

microclimatic variables on E_n during different seasonal periods: spring (26 days in October – November 2006), summer (16 days January to February 2007) and winter (32 days in May and June 2007). The summer period included a 2-day rain event (total 32 mm), and the winter a large 3-day rain event (total 168 mm), thereby generating substantial differences in soil moisture during the experimental period.

We measured sap flow in 3 trees of each species for one month at (1) the junction between the lowest branch and the bole of the tree or the ‘base of live crown’; and (2) 30 cm above the ground. As the tree height was limited to 10 to 12 m, the base of live crown was 2 m above ground; therefore, the bottom probe set was installed at 50 cm rather than 1.3 m. The difference between the volume of sap flow recorded at the top of the stem and the volume of sap flow recorded at the base of the stem represented stem recharge (Goldstein *et al.* 1998; Phillips *et al.* 2009a). We calculated the time required for crown sap flow and basal sap flow to become equal. This transition occurred when sap flow ceased to be transpiration out of the canopy (*i.e.* dehydration of the stem) and became refilling of the stem (*i.e.* rehydration) (Fig 2). This diurnal pattern was typical of clear sunny days and occurred for 78% (21 of 27) of the days sampled. *E. parramattensis* showed similar diurnal patterns (Fig. 2b,c) with rehydration of the stem completed between 17:00 and 19:00; thereafter and until sunrise, crown flow exceeded base flow indicating sap flow was transpirational water loss and not stem recharge.

We calculated the sum E_n from 2300 to 0500 and divided this by the 24-h sum of sap flow for that day, to estimate the proportional contribution of total daily sap flow (E_n/E_d) (Daley and Phillips 2006). The beginning of the ‘night time’ period differs for different studies, with some using the time when radiation becomes less than 5 W m^{-2} or zero (Daley and Phillips 2006; Phillips *et al.* 2009b) whereas others use midnight (Benyon 1999). To provide a conservative estimate of when stem refilling ceases, we used 2300 as the start of ‘night’. Whole-tree water use was scaled to stand water use using a method described previously (Zeppel *et al.* 2008b) by multiplying mean sap flux density by the sapwood area of the stand for each species.

Leaf stomatal conductance

A 24-h campaign (March 2009) was conducted to directly evaluate G_L . Aluminium A-frame ladders were installed beneath 3 trees of each species which contained immature, flushing leaves. In each tree, two or three mature, fully-expanded, healthy upper crown leaves, and two or three bright green immature leaves were used to measure leaf conductance using a porometer (Delta-T

Devices, Cambridge, UK) which was calibrated every two or three hours, before each sampling period. Measurements of leaf conductance taken using a Delta-T porometer have greater than 90 % accuracy within a reading range of 5 to 800 mmol m⁻² s⁻¹ and when ambient conditions of humidity are between 10 and 90 % (Delta-T Instruction Manual, 2004). Measurements were taken at approximately 9:00, 11:00, 13:00 16:00; 19:00, 22:00, 01:00, 04:00, 07:00, and ended at 9:00 the following morning.

Comparing cuticular conductance of immature and mature leaves

We measured G_c in 12 excised immature (recently flushed) leaves and 12 mature (fully expanded) leaves of each species. Leaves were excised in the morning, wrapped in aluminium foil and sealed in plastic bags, and immediately brought back to the laboratory. Leaves were weighed, and then placed on an 'airing rack' with both top and lower surfaces exposed to air. Changes in leaf fresh mass, air and leaf temperature and atmospheric relative humidity were measured repeatedly over a 2.5 hour period. Leaf temperature was measured using a thermocouple and air temperature was measured using wet and dry bulbs to estimate Leaf-to-Air-Vapour-Pressure-Deficit during the measurement period. Initial measurements were taken at intervals of 3 minutes until 21 minutes elapsed, and then leaves were weighed every 30 minutes until 2.5 hours elapsed. G_c was calculated from the rate of water loss from 90 to 150 min when stomates were closed; leaf mass regressed against time indicated stomatal closure occurred after 50 minutes. Water loss was converted from g s⁻¹ to mmol m⁻² s⁻¹ following (Pearcy *et al.* 1989).

Leaf water potential

Leaf water potential (Ψ_l) was measured on three bagged and three unbagged leaves of both species, on each of six trees that were instrumented for sap flow. Measurements were taken on two or three consecutive days in winter 2006, and summer 2006/7, 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 0630 and 1800 h. Bagged leaves were wrapped in aluminium foil to exclude sunlight and contained within plastic bags to prevent transpiration. In the first field campaign (winter 2006), we used bagged leaves 2 hours after sunrise as a surrogate for pre-dawn leaf water potential because the mean ψ_l of bagged leaves and pre-dawn water potential was not significantly different ($P < 0.05$).

No-flow xylem water potential

When the plant and soil do not reach equilibrium overnight, it is possible to estimate bulk soil ψ by extrapolating the trajectory of the relationship between E vs ψ_{pd} to when $E = 0$, to determine the ‘no-flow rate’ (Sperry *et al.* 2002). If an estimate of bulk soil water potential is required, the no-flow rate is more accurate than ψ_{pd} when E_n is occurring. We examine relationships between sap flow and leaf water potential across seasons, and provide a simple correction model for estimating ψ_s when ψ_l does not reach equilibrium with soil.

To assess the disequilibrium between soil matric potential and pre-dawn leaf water potential, we measured leaf water potential throughout the morning and plotted it against the corresponding sap flow for two or three consecutive days in December 2006 and May, June, July and August 2007. Where a strong relationship was found between leaf water potential and sap velocity, this relationship was extrapolated to determine the leaf water potential when sap velocity was zero (*i.e.*, no-flow xylem water potential) (Bucci *et al.* 2004, O’Grady *et al.* 2005). Data were pooled into two groups (summer 2006 and winter 2007) and a general linear model (SPSS version 14, SPSS Inc. Chicago, IL) was used to determine whether pre-dawn leaf water potentials were significantly different between the seasons. For both species, there was a significant interaction between season and the dependant variable, so the seasons were treated as separate samples. The relationship between pre-dawn leaf water potential and no-flow xylem water potential was examined using regression analysis.

Statistical analyses of drivers of E_n

The responses of E_n to D , U , UD and soil moisture storage at shallow (60 cm) and deep soil layers (110 cm) were assessed using a multiple linear regression (MLR). In order to examine the effect of soil moisture on E_n , analyses were conducted during periods of contrasting soil moisture content in summer (14 January to 16 February) and winter (1 June to 31 July). Soil moisture content did not vary significantly in spring, so these data were excluded from the analyses. We used linear regression analysis (SPSS v12.0 for Windows) to explore the unique contribution of each predictor to explain the variance in E_n . The unique relationship of each predictor was assessed in terms of a partial slope and partial r^2 value. The use of multiple regression allowed us to look at the unique relationship between two variables while holding potentially confounding effects of other variables constant (Hair *et al.* 2006). All statistical results were significant based on a P -value < 0.05 .

Results

Soil moisture and micrometeorological conditions

Soil moisture storage in shallow soil at 60 cm (Θ_s) during spring was consistently low (*ca.* 50 mm), increasing in summer (*ca.* 90 mm) after rain events > 20 mm, and then declining to pre-rain levels 10 days after the rain events. During winter, Θ_s was high (up to 250 mm) and remained elevated after numerous rain events in mid-June, including 130 mm over a three-day period.

In both spring and summer, D was high before rain events, reaching maximum values of 5.0 kPa and 5.5 kPa, respectively. After rain events, D was reduced in spring, ranging from 1.0 to 3.1 kPa. In summer, D remained relatively high after rain events, reaching a peak of 4.0 kPa four days after the rain event. In contrast, D was low both before and after rain events in winter, and did not exceed 1.2 kPa. As expected, radiation was high in spring and summer (maximum 900 and 1000 W m^{-2} , respectively) and low in winter (maximum 600 W m^{-2}). Highly variable soil moisture, atmospheric demand, and energy levels during the study period provided an excellent platform for the analysis of environmental controls on E_n .

Differing water use strategies for the two species during the day

A comparison of diurnal curves of ψ_l and transpiration across seasons demonstrated that *E. parramattensis* was a more profligate user of water and experienced greater water stress than *A. bakeri*. Diurnal ψ_l in all seasons showed that *E. parramattensis* generally experienced greater water stress than *A. bakeri* (Fig. 1a). During the hottest sampling period on a clear day, *A. bakeri* reached a minimum of -1.9 MPa whereas *E. parramattensis* reached a minimum of -3.0 MPa.

Diurnal curves of sap velocity for *E. parramattensis* show higher rates of water use than for *A. bakeri* in the afternoon in both spring and winter. *E. parramattensis* had higher maximum rates of sap flow than *A. bakeri* in summer and winter, although on hot, dry summer days, *E. parramattensis* had higher morning and lower afternoon sap flow than *A. bakeri* (Fig 1b). These diurnal curves suggest *E. parramattensis* is a greater consumer of water compared with *A. bakeri*. Mean hourly sap flow rates (mean of 24 h) of *E. parramattensis* and *A. bakeri* were similar in spring (62.3 ± 2.0 and 61.9 ± 2.2 mm hr^{-1} respectively, $n = 624$) whereas values of *E. parramattensis* were higher than *A. bakeri* in summer (70.4 ± 2.3 and 56.4 ± 1.8 mm hr^{-1} respectively, $n = 672$), and in winter (58.7 ± 1.6 and 31.5 ± 0.9 mm hr^{-1} respectively, $n = 2144$). Mean rates of E_n between 23:00 and 05:00 for *A. bakeri* were higher in summer and spring (1.4

mm hr⁻¹ and 1.6 mm hr⁻¹ respectively) than in winter (0.6 mm hr⁻¹). Similarly, E_n for *E. parramattensis* was higher in summer and spring (1.8 mm hr⁻¹ for both seasons) than in winter (1.2 mm hr⁻¹). Generally, *E. parramattensis* had higher rates of both day-time and night time sap flow than *A. bakeri* (Fig. 1a) meaning that E_n/E_d for both species was similar on most days.

Environmental regulators of E_n

UD is the product of windspeed (U) and D , and therefore it is not appropriate to use partial correlations for auto-correlated variables such as U and UD . Therefore a comparison of Pearson's correlation coefficients was conducted to determine which independent variables had the strongest influence on E_n . In both species, hourly E_n was more strongly correlated with D (coefficients of 0.70 to 0.83) than UD (0.41 to 0.43) or U alone (< 0.17) across all seasons and for all seasons pooled (Table 1). E_n was significantly correlated with D (Fig. 4) and UD ($p < 0.05$) for each species and each season (excluding winter for *E. parramattensis*), and seasons pooled (Table 1). In contrast, E_n was not significantly correlated with U when all seasons were pooled ($p > 0.05$). E_n was more strongly correlated with shallow Θ (Θ_s) than deep Θ (Θ_d) for both species in each season and for all seasons pooled (Table 1). Therefore, further statistical analyses were conducted on hourly E_n , D and Θ_s rather than Θ_d .

Hourly E_n , D and Θ_s

Partial correlations showed that as D increased, E_n increased and the correlation was significant when Θ_s was held constant (Table 2). In contrast, when D was held constant, Θ_s had no significant influence on E_n in spring and winter; however, in summer and across all seasons pooled, Θ_s had a significant influence on E_n for both species. In all seasons, and across seasons, the influence of D on E_n was greater in *A. bakeri* than *E. parramattensis*.

Night-time stem refilling and transpiration from the canopy

Stem refilling contributed 24-31% of E_n in *E. parramattensis* and 22-50% of E_n for *A. bakeri* before rain events. Following rain events, stem refilling increased to 67% for the smallest *A. bakeri* (DBH = 15.7 cm) and to $> 85\%$ of E_n for the smallest *E. parramattensis* (DBH = 9.8 cm); however, larger trees (DBH 17.5 and 20 cm) were not responsive to rain events. During nights with extremely low D , stem refilling was *ca.* 95% of E_n . However, for most nights the proportion

of stem refilling was < 31% for *E. parramattensis* and < 50% for *A. bakeri*. Consequently, night-time transpiration was the predominant contributor to E_n during the study period.

Contribution of g_s and g_c to water loss at night

Water loss occurred every night and was largely due to open stomata rather than to transpiration across the cuticle. Night-time leaf conductance for both species ranged from 12 to 20 mmol m⁻² s⁻¹, with only 5-10% of E_n attributable to loss across the cuticle. There were differences in g_c depending upon leaf age such that g_c of immature leaves (1.11 mmol m⁻² s⁻¹ for both species) was much higher than for mature leaves (0.76 and 0.22 mmol m⁻² s⁻¹) for *E. parramattensis* and *A. bakeri*, respectively. In general, g_s was higher in immature leaves than in mature leaves of *A. bakeri* at dawn, but otherwise similar; differences in g_s at night between immature and mature leaves of *E. parramattensis* were minimal (Fig 3). Leaf conductance was comparable between immature and mature leaves during daylight hours (data not shown).

Determination of E_n/E_d

Mean E_n/E_d was 6-8 % across seasons (spring, summer and winter), but was up to 19% on some days in both species (Fig 5). In spring, mean E_n/E_d was 8% for both species, with a maximum of 18% and 19% for *E. parramattensis* and *A. bakeri*, respectively. In summer, mean E_n/E_d was 7% for both species, with a maximum of 10% and 13% for *E. parramattensis* and *A. bakeri*, respectively, occurring on 22 January, a night with high D . In winter, mean E_n/E_d was 6% for both species; maximum values of 14 and 19% occurred for the *E. parramattensis* and *A. bakeri*, respectively, on June 7 when D was high.

Correction model for predicting soil water potential when E_n occurs

In summer, there were strong negative linear relationships between ψ_1 and morning sap flow (r^2 from 0.97 to 0.53). We found that the calculated no-flow xylem water potentials ranged between -0.21 and -1.39 MPa and the measured pre-dawn ψ_1 ranged between -0.4 and -1.5 MP. Consequently, the measured pre-dawn ψ_1 were more negative than the water potential calculated from the no-flow xylem water potentials. However, there was a strong relationship between these two parameters (Fig. 6). For both species, there was a larger off-set between pre-dawn leaf water potential and no-flow xylem water potential in summer than winter, as indicated by the larger y-intercept calculated with the regression analysis (Fig. 6).

Discussion

D was the predominant regulator of E_n

The predominant regulator of E_n was D (with a strong positive correlation), followed by UD , Θ_s , Θ_d and U . E_n increased under conditions of high D , in agreement with observations from a large number of other species (Barbour and Buckley 2007; Barbour *et al.* 2005; Christman *et al.* 2009; Dawson *et al.* 2007; Fisher *et al.* 2007; Rawson and Clarke 1988). However, E_n was not strongly correlated with U , similar to observations in shrubs and trees of California (Fisher *et al.* 2007). Additionally, we found a weaker dependence of E_n on UD than D for both species, in all seasons, contrasting to observations by Phillips *et al.* (2009) who reported that E_n was more strongly correlated with UD than D . One possible reason for this is that the field site used by Phillips *et al.* (2009) was a small stand of trees surrounded by low lying vegetation and as such was likely to experience a larger wind speed than our site which was a much larger woodland. E_n was higher in summer than winter or spring, due to the high values of D experienced during this season.

E_n increased as soil moisture increased when D was held constant. Our findings support the observation of lower E_n or lower g_s at night associated with decreased plant water availability in a number of species (Donovan *et al.* 2003; Ludwig *et al.* 2006; Muchow *et al.* 1980). In addition, Dawson *et al.* (2007) reported that the magnitude of E_n was negatively correlated with the number of days since rain, and subsequently soil moisture. Here, we demonstrate that in a mature forest both D and soil moisture influence E_n , and that D is a much stronger influence on E_n than soil moisture. Increasing night-time temperatures in conjunction with reduced rainfall, as predicted for many regions under climate change projections (Dunlop and Brown 2008), may lead to higher D assuming specific humidity remains constant. Consequently, this may generate higher E_n and greater water loss at night under these climate change projections.

Night-time transpiration from the canopy is a greater contributor to E_n than stem refilling

Our results challenge the paradigm that nocturnal sap flow is predominantly the result of refilling of stems at night (Caird *et al.* 2007). In this study, instrumented trees of both species for a similar range of tree sizes (DBH of 11.0 to 17.5 cm for *A. bakeri* and 9.8 to 20.0 cm for *E. parramattensis* respectively) showed that stem refilling was not the main contributor to nocturnal sap flow. Generally, stem refilling was smaller in *E. parramattensis* (<31% of sap flow at night)

and *A. bakeri* (<50% of sap flow at night) than water loss from night-time transpiration from the canopy, although stem refilling was a significant proportion of stem flow when D was low or in the smallest trees following rain. Therefore, stem refilling primarily occurred during the day or in the early evening, and water loss sometimes occurred during the night which has been observed previously. For example, daytime rehydration and water loss from the canopy was reported in several *Eucalyptus* species growing in Australia (Benyon 1999; Phillips et al. 2009), and in *Betula papyrifera* in a temperate forest in North America (Daley and Phillips 2006). In this study, we demonstrated that sap flow at night was more than stem refilling and that nocturnal sap flow represented the transpirational loss of water from the tree canopy. To date, no sufficient explanation has been offered for night-time transpirational water loss.

Water loss through stomata is the principle path for water loss, not cuticular water loss

Water loss at night was primarily through open stomata rather than across the cuticle, with only 5-10% of water loss occurring across the cuticle at night. Our results are similar to those for a number of studies that found cuticular water loss accounted for 8 – 30 % of total water loss (Caird et al. 2007; Howard and Donovan 2007). The results from this study demonstrate that stomatal conductance is much higher than cuticular conductance at night, thereby generating transpirational water loss from the canopy.

E_n/E_d was similar in all seasons and in both species

Mean values of E_n/E_d were 8% in spring, 7% in summer and 6% in winter, and remarkably, were similar for both species during each season. Similarly, eight species of *Eucalyptus* growing in a common garden exhibited E_n/E_d of 5-7% over the entire year (Phillips et al. 2009b), 2 – 8% in a Eucalypt woodland (Mitchell et al. 2008) and 5% in a *E. grandis* plantation (Benyon 1999). During winter and spring, our maximum E_n/E_d was approximately 15 to 20%, whereas in summer, when both day and night sap flow was greater than in winter and spring, maximum E_n/E_d was 10 to 13%. These values are comparable to maximum E_n/E_d of 15% in a Eucalypt woodland in Western Australia (Dawson et al. 2007).

The remarkably consistent and low values of E_n/E_d across seasons in our study were comparable to other *Eucalyptus* species at three other woodlands (Benyon 1999; Mitchell et al. 2008; Phillips et al. 2009b); higher sap flow during both day and night for *E. parramattensis* compared with *A. bakeri* generated similar E_n/E_d for both species. In contrast, E_n/E_d in 11 of 17 desert grasses and

shrubs frequently exceeded 30% and reached maximum values of 60% (Snyder *et al.* 2003). Similarly, tropical and temperate forests found E_n/E_d to range extensively in response to variable environmental conditions (see reviews by Novick *et al.* 2009 and Dawson *et al.* 2007), whereas we observed low E_n/E_d across all seasons. In plants, consistently low E_n/E_d may reflect low water loss at night due to tight stomatal control (low E_n) or conversely, high water use during the day (high E_d). In our eucalypts, low E_n/E_d primarily reflected low E_n rather than high E_d . In desert ecosystems, large E_n/E_d ratios are frequently observed (Snyder *et al.* 2003), apparently largely due to low E_d rather than high E_n . Ecosystems that experience regular seasonal drought often exhibit highly variable E_n/E_d across seasons, reflecting the large range of E_d observed in these systems.

Despite experiencing similar microclimatic and soil conditions, the two species exhibited different strategies for minimising water loss while maximising carbon gain. *A. bakeri* experienced ψ_L close to zero in all seasons, low summer rates of water use, similar sap flow across the day and E_n was strongly correlated with D . In contrast, *E. parramattensis* experienced comparatively lower water potentials, higher rates of water use during summer, and higher morning and lower afternoon rates of water use, with E_n less correlated with D than in *A. bakeri*. In summary, *A. bakeri* exhibited attributes of a xeric vegetation type (*i.e.* water saver) and *E. parramattensis* exhibited comparatively more mesic attributes (*i.e.* greater consumer of water).

Although different day-time water use strategies were employed, both species constrained nocturnal water fluxes to conservatively low seasonal values of 6 to 8% for E_n/E_d for each season. Interestingly, co-occurring woodland (Hatton *et al.* 1998; Zeppel and Eamus 2008) and semi-arid (O'Grady *et al.* 2009) species have also used different strategies to achieve similar rates of water loss. At a similar Eucalypt woodland, two sympatric species which had different hydraulic architecture (*i.e.* different relationships between leaf area, sapwood area and tree size), showed functional convergence between tree size and water use (Zeppel and Eamus 2008). Eucalypt woodlands along a Western Australian aridity gradient progressively reduced leaf area as sites became more arid, whereas sapwood area was largely unaffected by aridity (Pekin *et al.* 2009). In addition, low rates of E_n/E_d have been reported in three other Eucalypt dominated woodlands (Benyon 1999; Mitchell *et al.* 2008; Phillips *et al.* 2009b) suggesting a strong level of environmental control on nocturnal fluxes.

Seasonality in the disequilibrium between ψ_{pd} and ψ_L and E_n/E_d

Pre-dawn leaf water potential has frequently been used as a surrogate approximation for soil moisture availability (O'Grady *et al.* 1999; Palmer *et al.* 2008) based on the assumption that ψ_L has equilibrated overnight. However, it is clear that this assumption is not valid when E_n occurs. Furthermore, estimation of whole plant hydraulic conductance from the relationship between transpiration and the gradient in water potential between the roots and leaves also uses pre-dawn water potential as an estimate of root water potential. Sensitivity analysis of a soil-plant-atmosphere exchange model showed that the output of the model was especially sensitive to estimations of whole plant hydraulic conductance (Zeppel *et al.* 2008a). To our knowledge, this is the first time the correction factor, originally described by Kavanagh *et al.* (2007), has been utilised to generate a more accurate prediction of soil water potential and whole plant hydraulic conductance. In the present study E_n/E_d was relatively small, indicating that the disequilibrium between soil and leaf water potentials was also relatively small (approximately 0.2 MPa). However, at sites where E_n/E_d is large, we recommend application of this methodology to more accurately estimate root surface water potential and whole plant hydraulic conductance.

Conclusion

We found that E_n was relatively small (6-8%) across all seasons in two co-occurring evergreen species (*Eucalyptus parramattensis* and *Angophora bakeri*) in a temperate woodland, suggesting that water loss at night was a minor component of the total water budget of this ecosystem. In determining the relative influence of microclimatic variables, including windspeed (U), vapour pressure deficit (D), the product of U and D (UD), and soil moisture content, on E_n we found that D exerted the strongest influence on E_n , soil moisture influenced E_n when D was constant, but U and UD did not generally influence E_n . In both species, cuticular conductance (G_c) was a small proportion of total leaf conductance (G_s) and was not a major contributor to E_n . We found that E_n was primarily a function of transpirational water loss from the canopy rather than refilling of stem storage. Despite different day-time water use strategies, both species demonstrated low night-time water loss suggesting similar controls on water loss at night. Finally, in order to account for the impact of E_n on pre-dawn leaf water potential arising from the influence of disequilibria between root zone and leaf water potential, we developed a model to more accurately predict soil water potential (ψ_s) when E_n is relatively high.

Acknowledgements

We are grateful to WSN at Castlereagh for allowing site access and field assistance from Rhys Whitley, Isa Yunusa, Tony Palmer, and Andy Leigh. We thank Nathan Phillips and Tom Buckley for providing useful comments on earlier versions of the manuscript. This project was supported by ARC Linkage Project 0669063, ARC Discovery Project 0877722, and a UTS Early Career Research Grant awarded to MZ and CM.

Figures and Tables.

Table 1. Pearson's correlation co-efficients between micro-meteorological variables and soil moisture storage at 60 cm and 110 cm, for each species during spring, summer, winter and all seasons pooled (* = significant at $p < 0.05$).

	D (kPa)	UxD (kW m ⁻²)	Windspeed (m s ⁻¹)	Shallow soil storage (to 60 cm) (mm)	Deep soil storage (to 110 cm) (mm)
Spring – <i>A. bakeri</i>	0.87*	0.60*	0.28*	-0.39*	-.30
Spring – <i>E. parramattensis</i>	0.75*	0.34*	n/s	-0.34*	-0.26
Summer – <i>A. bakeri</i>	0.82*	0.45*	-0.26*	-0.43*	-0.32
Summer – <i>E. parramattensis</i>	0.68*	0.340	-0.19*	-0.12*	n/s
Winter – <i>A. bakeri</i>	0.56*	0.41*	0.24*	-0.45*	-0.45*
Winter – <i>E. parramattensis</i>	0.28*	n/s	n/s	n/s	n/s
All seasons pooled – <i>A. bakeri</i>	0.83*	0.41*	n/s	-0.24*	-0.16*
All seasons pooled – <i>E. parramattensis</i>	0.70*	0.43*	n/s	-0.13*	-0.08*

Table 2. Partial correlation co-efficients for hourly E_n , D and shallow soil storage (* = significant at $p < 0.05$).

Hourly E_n	Model summary R^2	D(kPa) R^2	Shallow soil storage (60 cm) (mm) R^2
Spring – <i>A. bakeri</i>	0.76*	0.72*	0.00
Spring – <i>E. parramattensis</i>	0.56*	0.51*	0.00
Summer – <i>A. bakeri</i>	0.73*	0.67*	0.10*
Summer – <i>E. parramattensis</i>	0.47*	0.46*	0.02*
Winter – <i>A. bakeri</i>	0.60*	0.57*	0.01
Winter – <i>E. parramattensis</i>	0.56*	0.51*	0.00
All seasons pooled – <i>A. bakeri</i>	0.67*	0.66*	0.02*
All seasons pooled – <i>E. parramattensis</i>	0.45*	0.45*	0.01*

518 **Figure captions.**

519 Fig. 1. A comparison of different water use strategies used by each species, leaf water potential
520 during cloud-free days on (a) Spring, (b) Summer, (c) winter. Mean of two or three leaves
521 measured are shown (with s.e. bars). The mean sap flow of six trees of each species during (d)
522 spring, (e) summer and (f) winter. Solid lines are *E. parramattensis*, dotted lines are *A. bakeri*.

523

524 Fig. 2. Diurnal time series of crown and basal sap flow for each species (a and b) and the difference
525 between crown and basal sap flow for the same species (c and d). Positive values indicate time
526 periods when water transpired was preferentially withdrawn from stem water storage, and negative
527 values indicate time periods when water from the soil was refilling stem storage. Note that refilling
528 of stem storage ceased by 18:00 to 19:00 for both species. Net radiation reached zero at 19:30. Data
529 were collected for 60 days and on three trees of each species, time series for one day is shown to
530 provide clarity, and represents typical branch to basal sap flow patterns on clear sunny days.

531

532 Fig. 3. Leaf conductance of immature (flushing) and mature leaves of each species between 18:00
533 and 09:00. Double stars represent significant differences ($p < 0.05$) between phenological type
534 within each species, for each time period.

535

536 Fig. 4. The relationship between D (kPa) and nocturnal sap flow (sap flux density, cm hr^{-1}) for each
537 species in (a) spring, (b) summer, and (c) winter.

538

539 Fig 5. The proportion of summed stand water use (mm day^{-1}) for day (grey bars, 05:30 to 22:30)
540 and night (black bars, 23:00 to 05:00) for each species in (a) spring, (b) summer and (c) winter.

541

542 Fig 6. The relationship between predawn leaf water potential and no-flow xylem water potential for
543 a) *A. bakeri* and b) *E. parramattensis* in summer 2006 (closed circles and bold line) and winter
544 2007 (open circles and plain line), including 95% confidence intervals for each regression. Seasons
545 were separated because a general linear model showed there was a significant interaction between
546 the effect of season and predawn leaf water potential ($p < 0.01$). All regression analyses were
547 $p < 0.01$. No-flow xylem water potential was calculated by extrapolating the relationship between
548 sap flow and leaf water potential to zero flow.

549

550

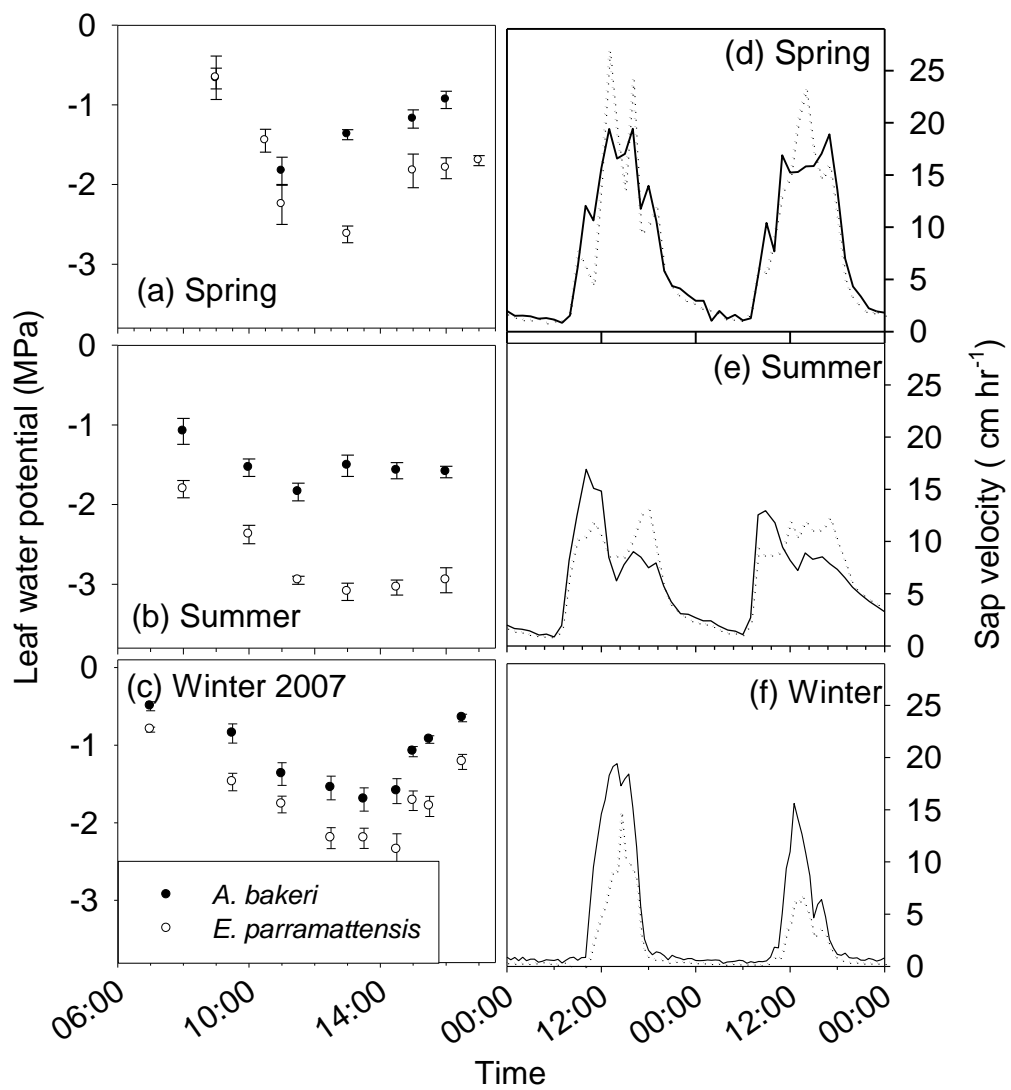


Fig. 1.

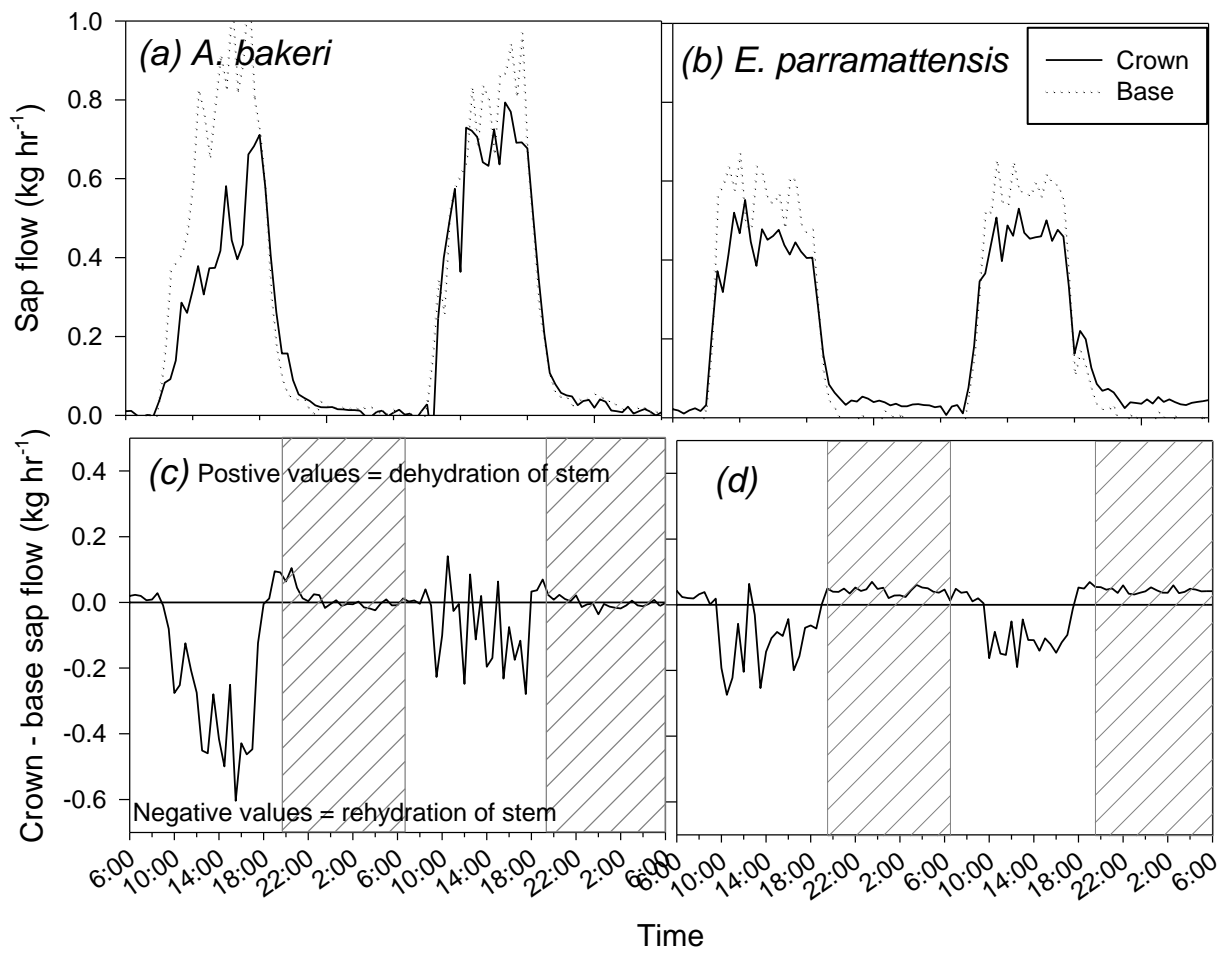


Fig. 2.

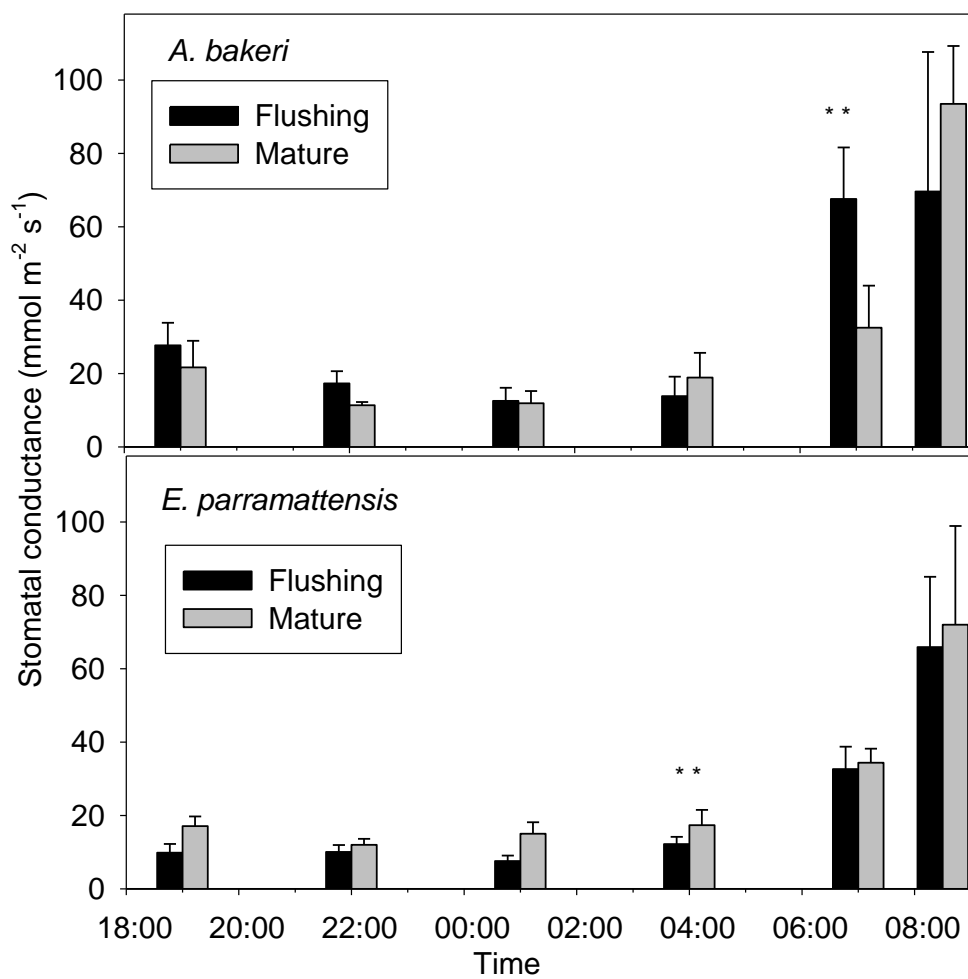


Fig. 3.

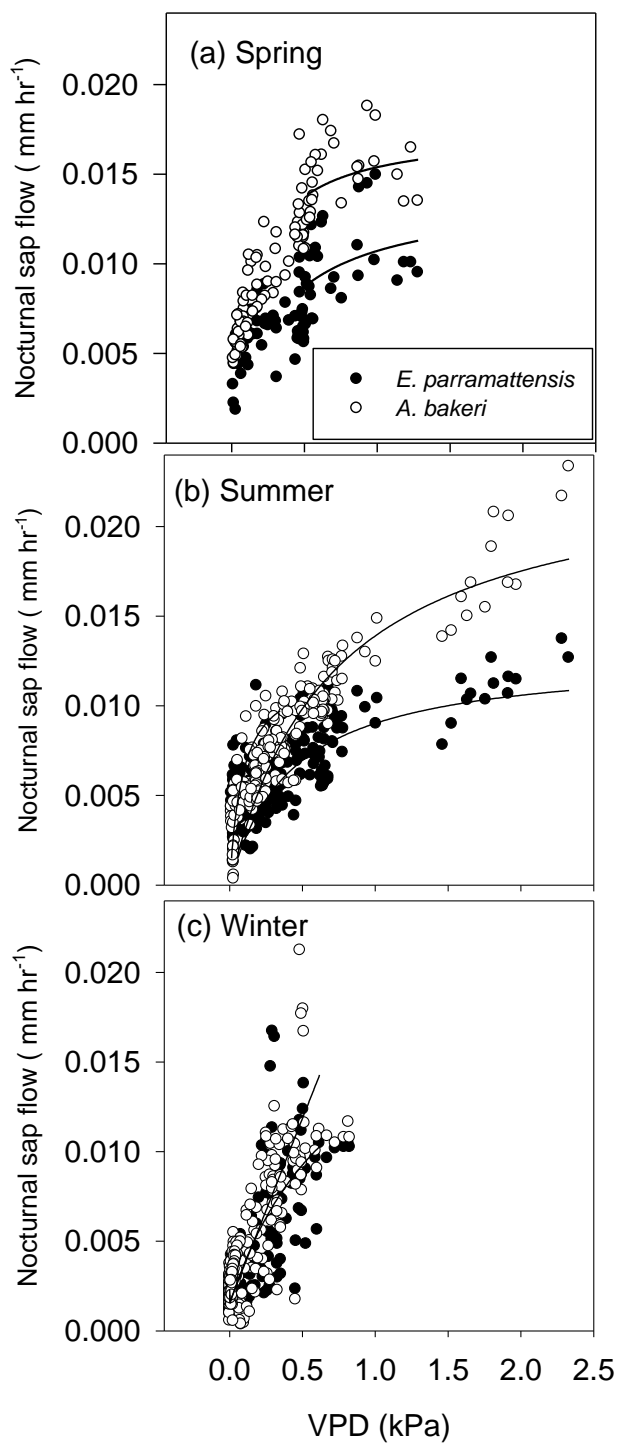


Fig. 4.

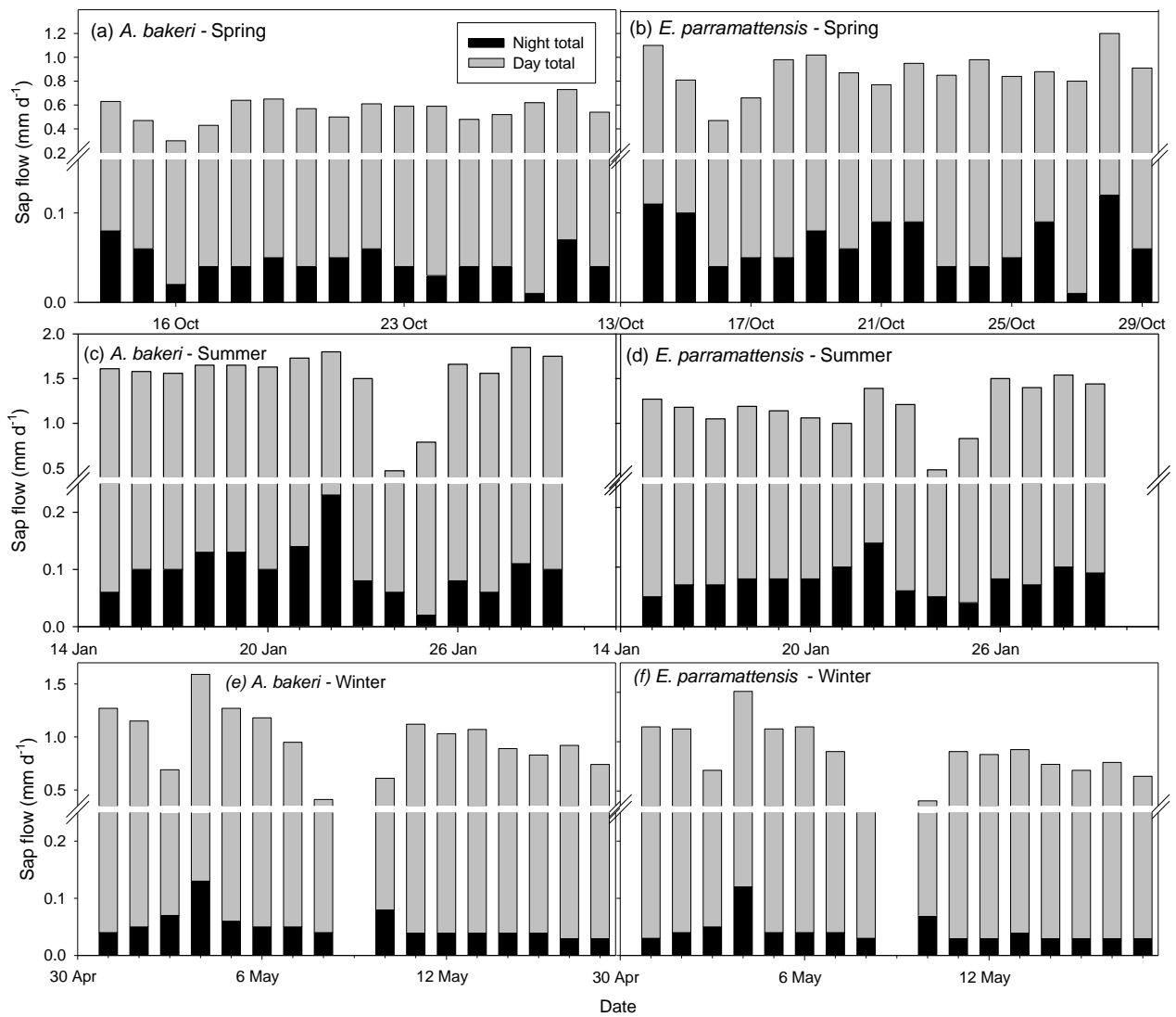


Fig 5a-f. The proportion of summed stand water use (mm d⁻¹) for day (grey bars, 05:30 to 22:30) and night (black bars, 23:00 to 05:00) for each species in spring.

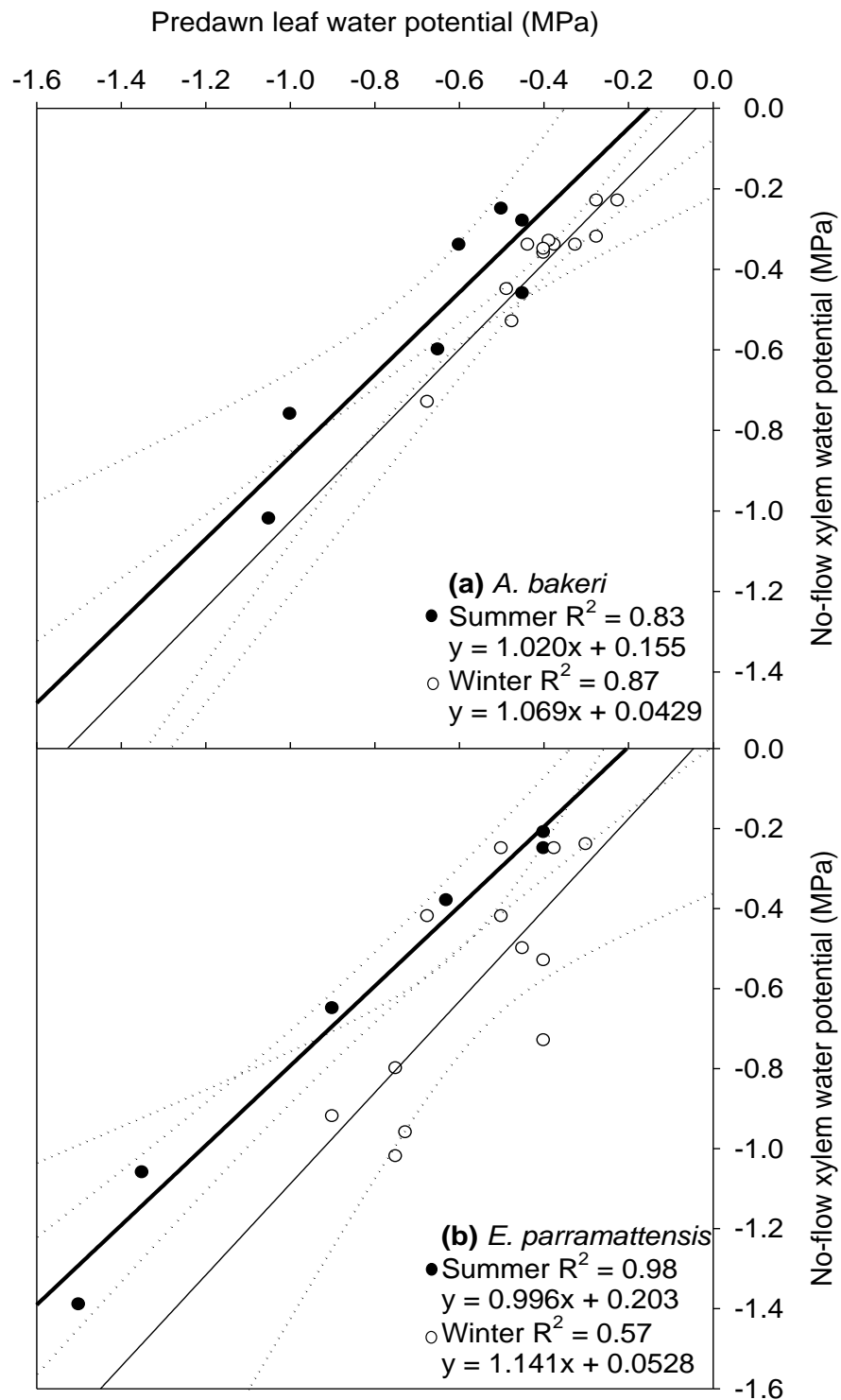


Fig 6.

References

Barbour MM, Buckley TN (2007) The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance. *Plant Cell and Environment* **30**, 711-721.

- Barbour MM, Cernusak LA, Whitehead D, Griffin KL, Turnbull MH, Tissue DT, Farquhar GD (2005) Nocturnal stomatal conductance and implications for modelling delta O-18 of leaf-respired CO2 in temperate tree species. *Functional Plant Biology* **32**, 1107-1121.
- Benyon RG (1999) Nighttime water use in an irrigated *Eucalyptus grandis* plantation. *Tree Physiology* **19**, 853-859.
- Bucci S, Scholz F, Goldstein, Meinzer F, Hinojosa J, Hoffman W, Franco A (2004) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiology* **24**, 1119-1127.
- Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Campanello P, Scholz FNG (2005) Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in Neotropical savanna trees. *Trees - Structure and Function* **19**, 296-304.
- Caird MA, Richards JH, Donovan LA (2007) Nighttime stomatal conductance and transpiration in C-3 and C-4 plants. *Plant Physiology* **143**, 4-10.
- Cavender-Bares J, Sack L, Savage J (2005) Atmospheric and soil drought reduce nocturnal conductance in live oaks. In '90th Annual Meeting of the Ecological-Society-of-America/9th International Congress of Ecology'. Montreal, CANADA pp. 611-620
- Christman MA, James JJ, Drenovsky RE, Richards JH (2009) Environmental stress and genetics influence night-time leaf conductance in the C-4 grass *Distichlis spicata*. *Functional Plant Biology* **36**, 50-55.
- Daley MJ, Phillips NG (2006) Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiology* **26**, 411-419.
- Dawson T, Burgess S, Tu K, Oliveira R, Stantiago L, Fisher J, Simonin K, Ambrose A (2007) Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology* **27**, 561-576.
- Donovan LA, Richards JH, Linton MJ (2003) Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials. *Ecology* **84**, 463-470.
- Dunlop M, Brown PR (2008) 'Implications of climate change for Australia's National Reserve System: A preliminary assessment.' Report to the Department of Climate Change, February 2008. Department of Climate Change, Canberra, Australia.
- Fisher JB, Baldocchi DD, Misson L, Dawson TE, Goldstein AH (2007) What the towers don't see at night: nocturnal sap flow in trees and shrubs at two Ameriflux sites in California. *Tree Physiology* **27**, 597-610.
- Ford CR, McGuire MA, Mitchell RJ, Teskey RO (2004) Assessing variation in the radial profile of sap flux density in *Pinus* species and its effect on daily water use. *Tree Physiology* **24**, 241-249.
- Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A (1998) Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell & Environment* **21**, 397-406.

- Hair JF, Black WC, Babin BJ, Anderson RE, Tatham RL (2006) 'Multivariate data analysis.'
- Hatton T, Reece P, Taylor P, McEwan K (1998) Does leaf water efficiency vary among eucalypts in water-limited environments? *Tree Physiology* **18**, 529-536.
- Howard AR, Donovan LA (2007) Helianthus nighttime conductance and transpiration respond to soil water but not nutrient availability. *Plant Physiology* **143**, 145-155.
- Jiang GM, Zhu GJ (2001) Different patterns of gas exchange and photochemical efficiency in three desert shrub species under two natural temperatures and irradiances in Mu Us Sandy Area of China. *Photosynthetica* **39**, 257-262.
- Kavanagh K, Pangle R, Schotzko A (2007) Nocturnal transpiration causing disequilibrium between soil and stem predawn water potential in mixed conifer forests of Idaho. *Tree Physiology* **27**, 621-630.
- Ludwig F, Jewitt RA, Donovan LA (2006) Nutrient and water addition effects on day- and night-time conductance and transpiration in a C-3 desert annual. *Oecologia* **148**, 219-225.
- MacFarlane C, Hoffman M, Eamus D, Kerp N, Higginson S, McMurtrie R, Adams M (2007) Estimation of leaf area index in eucalypt forest using digital photography. *Agricultural & Forest Meteorology* **143**, 176-188.
- Macinnis-Ng C, Fuentes S, *et al.* (2009) Root biomass distribution and soil properties of an open woodland on a duplex soil. *Plant and Soil* **In Review**.
- Mitchell P, Veneklaas E, Lambers H, Burgess S (2008) Using multiple trait associations to define hydraulic functional types in plant communities of south-western Australia. *Oecologia* **158**, 385-397.
- Muchow RC, Ludlow MM, Fisher MJ, Myers RJK (1980) Stomatal behaviour of Kenaf and Sorghum in a semi-arid tropical environment. 1. During the night. *Australian Journal of Plant Physiology* **7**, 609-619.
- Norby RJ, Luo YQ (2004) Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytologist* **162**, 281-293.
- Novick KA, Oren R, Stoy PC, Siqueira MBS, Katul GG (2009) Nocturnal evapotranspiration in eddy-covariance records from three co-located ecosystems in the Southeastern U.S.: Implications for annual fluxes. *Agricultural and Forest Meteorology* **149**, 1491-1504.
- O'Grady AP, Cook PG, Eamus D, Duguid A, Wischusen JDH, Fass T, Worldege D (2009) Convergence of tree water use within an arid-zone woodland. *Oecologia* **160**, 643-655.
- O'Grady AP, Eamus D, Hutley LB (1999) Transpiration increases during the dry season: Patterns of tree water use in eucalypt open-forests of northern Australia. *Tree Physiology* **19**, 591-597.
- Palmer AR, Fuentes S, Taylor D, Macinnis-Ng C, Zeppel M, Yunusa I, February E, Eamus D (2008) The use of pre-dawn leaf water potential and MODIS LAI to explore seasonal trends in the

phenology of Australian and southern African woodlands and savannas. *Australian Journal of Botany* **56**, 557-563.

Pearcy RW, Ehleringer J, Mooney HA, Rundel PW (1989) 'Plant Physiological Ecology.' (London, UK)

Pekin BK, Boer MM, Macfarlane C, Grierson PF (2009) Impacts of increased fire frequency and aridity on eucalypt forest structure, biomass and composition in southwest Australia. *Forest Ecology and Management* **258**, 2136-2142.

Phillips N, Barbour M, Dawson T (2007) Casting light on nocturnal stomatal and canopy conductance. *Tree Physiology* **27**, 550.

Phillips N, Scholz F, Bucci S, Goldstein G, Meinzer F (2009a) Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: comment on Burgess and Dawson (2008). *Plant and Soil* **315**, 315-324.

Phillips NG, Lewis JD, Logan B, Tissue DT (2009b) Nocturnal gas exchange is conservative but sensitive to crown foliar dynamics across diverse Eucalyptus species. *Tree Physiology* **In review**.

Phillips NG, Ryan MG, Bond BJ, McDowell N, Hinckley TM, Cermak J (2003) Reliance on stored water increases with tree size in three species in the Pacific Northwest. *Tree Physiology* **23**, 237-245.

Rawson HM, Clarke JM (1988) Nocturnal Transpiration in Wheat. *Functional Plant Biology* **15**, 397-406.

Scholz FG, Bucci SJ, Goldstein G, Meinzer FC (2007) Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiology*.

Snyder KA, Richards JH, Donovan LA (2003) Night-time conductance in C3 and C4 species: do plants lose water at night? *J. Exp. Bot.* **54**, 861-865.

Sperry JS, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. *Plant, Cell & Environment* **25**, 251-263.

Ward EJ, Oren R, Sigurdsson BD, Jarvis PG, Linder S (2008) Fertilization effects on mean stomatal conductance are mediated through changes in the hydraulic attributes of mature Norway spruce trees. *Tree Physiology* **28**, 579-596.

Waring RH, Whitehead D, Jarvis PG (1979) The contribution of stored water to transpiration in Scots pine. *Plant, Cell & Environment* **2**, 309-317.

Zeppel M, Eamus D (2008) Coordination of leaf area, sapwood area and canopy conductance leads to species convergence of tree water use in a remnant evergreen woodland. *Australian Journal of Botany* **56**, 97-108.

Zeppel MJB, Macinnis-Ng C, Palmer A, Taylor D, Whitley R, Fuentes S, Yunusa I, Williams M, Eamus D (2008a) An analysis of the sensitivity of sap flux to soil and plant variables assessed for an Australian woodland using a soil-plant-atmosphere model. *Functional Plant Biology* **35**, 509-520.

Zeppel MJB, Macinnis-Ng CMO, Yunusa IAM, Whitley RJ, Eamus D (2008b) Long term trends of stand transpiration in a remnant forest during wet and dry years. *Journal of Hydrology* **349**, 200-213.