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

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22  
23 Running head: Different water use strategies and nocturnal sap flow

24

25 **Summary**

26  
27 Nocturnal fluxes may be a significant factor in the annual water budget of forested ecosystems.  
28 Here, we assessed sap flow in two co-occurring evergreen species (*Eucalyptus parramattensis*  
29 and *Angophora bakeri*) in a temperate woodland for two years in order to quantify the magnitude  
30 of seasonal nocturnal sap flow ( $E_n$ ) under different environmental conditions. The two species  
31 showed different diurnal water relations, demonstrated by different diurnal curves of stomatal  
32 conductance, sapflow and leaf water potential. The relative influence of several microclimatic  
33 variables, including windspeed ( $U$ ), vapour pressure deficit ( $D$ ), the product of  $U$  and  $D$  ( $UD$ ),  
34 and soil moisture content was quantified.  $D$  exerted the strongest influence on  $E_n$  ( $r^2 = 0.59$  to  
35  $0.86$ ), soil moisture influenced  $E_n$  when  $D$  was constant, but  $U$  and  $UD$  did not generally  
36 influence  $E_n$ . In both species, cuticular conductance ( $G_c$ ) was a small proportion of total leaf  
37 conductance ( $G_s$ ) and was not a major pathway for  $E_n$ . We found that  $E_n$  was primarily a function  
38 of water loss from the canopy rather than refilling of stem storage. Mean  $E_n$  was 6 to 8 % of 24-h  
39 flux across seasons (spring, summer and winter), but was up to 19% of 24-h flux on some days in  
40 both species. Despite different day-time strategies in water use of the two species, both species  
41 demonstrated low night-time water loss suggesting similar controls on water loss at night. In  
42 order to account for the impact of  $E_n$  on pre-dawn leaf water potential arising from the influence  
43 of disequilibria between root zone and leaf water potential, we also developed a model to more  
44 accurately predict soil water potential ( $\psi_s$ ).

45

46

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

48

49 **Introduction**

50 Nocturnal sap flow ( $E_n$ ) occurs across a wide range of species and ecosystems (Novick *et al.*  
51 2009), thereby challenging the paradigms that stomata are closed when radiation is zero and that  
52 transpiration does not occur at night (Bucci *et al.* 2004; Phillips *et al.* 2007; Scholz *et al.* 2007).  
53 The proportion of  $E_n$  to 24-h sap flow ( $E_n/E_d$ ) is highly variable (1-28%) across diverse  
54 ecosystems, including tropical forests (Bucci *et al.* 2004; Dawson *et al.* 2007; Novick *et al.*  
55 2009), boreal forests (Novick *et al.* 2009; Ward *et al.* 2008), and temperate forests (Dawson *et al.*  
56 2007). Interestingly, sap flow at night is often highest (30-60% of 24-h sap flow) in arid desert  
57 ecosystems (Snyder *et al.* 2003). Within the same micro-environment,  $E_n$  may be variable  
58 between species. For example, in co-occurring tree species in a North American deciduous forest,  
59 one species exhibited moderate (13%) and two species exhibited low (2-7%) nocturnal fluxes  
60 despite similar micrometeorological conditions (Daley and Phillips 2006). In addition, seasonal  
61 variation in  $E_n$  and  $E_n/E_d$  has been observed, particularly in biomes which experience seasonal  
62 soil wetting and subsequent drought.

63  
64 Nocturnal sap flow is associated with two components, mainly stem refilling with water after  
65 day-time depletion and transpirational water loss from the canopy (Daley and Phillips 2006;  
66 Phillips *et al.* 2009a). Night-time replenishment of water in the stem may contribute a significant  
67 proportion of the diurnal sum of 24 hr sap flow, generally 15-25% (Goldstein *et al.* 1998; Phillips  
68 *et al.* 2003) but up to 50% (Waring *et al.* 1979); the percentage of night-time water flow used for  
69 stem refilling increases with tree size (Phillips *et al.* 2003). To date, there are few studies that  
70 simultaneously measure crown and basal sap flow to quantify the proportion of nocturnal sap  
71 flow used for stem refilling and that proportion which constitutes transpirational loss of water  
72 through the canopy.

73  
74 Water lost at night through leaf transpiration may be due to the presence of open stomata or to  
75 significant flux of water across the leaf cuticle. Although the vast majority of transpirational  
76 water loss during the day-time occurs via stomata, a larger proportion of water loss at night could  
77 potentially occur across the cuticle. This is especially true when immature leaves are present in  
78 the canopy, because immature leaves may lose substantially more water at night than mature  
79 leaves in some Eucalypt species (Phillips *et al.* 2009b). Alternatively, immature leaves may have  
80 less control of stomatal closure at night than mature leaves, thereby losing more water through  
81 transpiration.

82

83 Soil water potential ( $\psi_s$ ) within the root zone is often estimated by measuring pre-dawn leaf  
84 water potential ( $\psi_{pd}$ ) (Eamus, O'Grady *et al.* 2000; Palmer, Fuentes *et al.* 2009) based on the  
85 assumption that plant water status has come into equilibrium with that of the soil (Kavanagh *et al.*  
86 2007). However, the occurrence of nocturnal transpiration (Barbour and Buckley 2007; Bucci *et al.*  
87 *al.* 2005; Dawson *et al.* 2007) may prevent attainment of equilibrium (Kavanagh *et al.* 2007).  
88 An accurate estimate of  $\psi_s$  is important in studies of hydraulic architecture and ecosystem  
89 function. Despite its importance, little research has been conducted on the relationship between  
90 nocturnal water loss and pre-dawn water potential (Bucci *et al.* 2004).

91  
92 In this study, we assessed the magnitude of  $E_n$  as a proportion of 24-h sap flow ( $E_n/E_d$ ) during a  
93 two-year period in two different tree genera growing in natural woodland. Our goal was to  
94 determine the contribution of nocturnal water loss to total water loss in trees exhibiting different  
95 patterns of plant water relations. For example, these two species differ significantly in rates of  
96 water use, the diurnal range of leaf water potential and the magnitude of stomatal conductance  
97 (Zeppel *et al.* 2008a), and therefore exhibit different day-time water use strategies under common  
98 environmental conditions (Zeppel and Eamus 2008). We also test whether environmental  
99 variables (*e.g.* soil water content,  $D$ ,  $U$ ) and leaf characteristics (immature *versus* mature,  $G_c$ ,  $G_s$ )  
100 were important regulators of  $E_n$  and  $E_n/E_d$  during different seasons (summer, winter, spring). We  
101 hypothesised that: (1)  $D$  will be the pre-dominant regulator of  $E_n$ ; (2)  $E_n$  will be greater in the  
102 summer than in the winter or spring due to a larger  $D$ ; (3) stem refilling will be a larger  
103 contributor to  $E_n$  than night-time transpiration from the canopy; (4)  $G_s$  is a much larger  
104 contributor to water loss than  $G_c$  at night; and (5) both species will exhibit similar  $E_n/E_d$  reflecting  
105 very strong environmental control on water loss at night.

106

107

108 **Methods and materials**

109

110 *Study site*

111

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

121

122 Soils at the site were sandy loams to depths of 60 – 80 cm, underlain by deep sandy clays  
123 (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  $85.5 \pm$   
124  $6.5 \text{ stems ha}^{-1}$ . Leaf area index of the tree canopy varied from 0.8 to 1.3 throughout the study  
125 period, measured using digital photography (MacFarlane *et al.* 2007). The understory is  
126 dominated by shrubs and grasses including *Pultenaea elliptica*, *Cryptandra amara* and  
127 *Melaleuca thymifolia*.

128

129 *Meteorological data*

130 All meteorological data were collected at 15-minute intervals and the mean of the four values  
131 within each hour were used in subsequent analyses. Air temperature (°C), wind speed ( $\text{m s}^{-1}$ ),  
132 shortwave radiation ( $\text{W m}^{-2}$ ) and rainfall (mm) data were obtained from a meteorological station  
133 located approximately 1 km west of the study site. Aspirated wet and dry bulb temperatures were  
134 obtained from a screened weather station (Environdata Pty Ltd, Australia) located approximately  
135 500 m from the study site in a cleared field (4 ha), while total solar radiation was measured above  
136 the screen. Vapour pressure deficit ( $D$ , kPa) was calculated from wet and dry bulb temperatures.

137

138 *Soil moisture content*

139 Volumetric soil moisture content was measured with an array of frequency domain reflectometry  
140 sensors (Theta Probe, ML2-X, Delta-T devices, Cambridge, UK) in two plots. Theta probes were  
141 buried horizontally at 10, 20, 40 and 60 cm depths in one plot and 10, 40 and 70 cm depths at a  
142 second plot. Soil moisture storage was estimated over two ranges (0- 60 cm and 0- 110 cm  
143 depths) using previously described methods, where soil moisture storage is the product of relative  
144 water content for each soil depth and the soil depth (Zeppel *et al.* 2008a).

145

#### 146 *Sapwood area, diameter at breast height, tree height*

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

155

#### 156 *Sap flow*

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

167

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

173 microclimatic variables on  $E_n$  during different seasonal periods: spring (26 days in October –  
174 November 2006), summer (16 days January to February 2007) and winter (32 days in May and  
175 June 2007). The summer period included a 2-day rain event (total 32 mm), and the winter a large  
176 3-day rain event (total 168 mm), thereby generating substantial differences in soil moisture  
177 during the experimental period.

178  
179 We measured sap flow in 3 trees of each species for one month at (1) the junction between the  
180 lowest branch and the bole of the tree or the ‘base of live crown’; and (2) 30 cm above the  
181 ground. As the tree height was limited to 10 to 12 m, the base of live crown was 2 m above  
182 ground; therefore, the bottom probe set was installed at 50 cm rather than 1.3 m. The difference  
183 between the volume of sap flow recorded at the top of the stem and the volume of sap flow  
184 recorded at the base of the stem represented stem recharge (Goldstein *et al.* 1998; Phillips *et al.*  
185 2009a). We calculated the time required for crown sap flow and basal sap flow to become equal.  
186 This transition occurred when sap flow ceased to be transpiration out of the canopy (*i.e.*  
187 dehydration of the stem) and became refilling of the stem (*i.e.* rehydration) (Fig 2). This diurnal  
188 pattern was typical of clear sunny days and occurred for 78% (21 of 27) of the days sampled. *E.*  
189 *parramattensis* showed similar diurnal patterns (Fig. 2b,c) with rehydration of the stem  
190 completed between 17:00 and 19:00; thereafter and until sunrise, crown flow exceeded base flow  
191 indicating sap flow was transpirational water loss and not stem recharge.

192  
193 We calculated the sum  $E_n$  from 2300 to 0500 and divided this by the 24-h sum of sap flow for  
194 that day, to estimate the proportional contribution of total daily sap flow ( $E_n/E_d$ ) (Daley and  
195 Phillips 2006). The beginning of the ‘night time’ period differs for different studies, with some  
196 using the time when radiation becomes less than  $5 \text{ W m}^{-2}$  or zero (Daley and Phillips 2006;  
197 Phillips *et al.* 2009b) whereas others use midnight (Benyon 1999). To provide a conservative  
198 estimate of when stem refilling ceases, we used 2300 as the start of ‘night’. Whole-tree water use  
199 was scaled to stand water use using a method described previously (Zeppel *et al.* 2008b) by  
200 multiplying mean sap flux density by the sapwood area of the stand for each species.

201  
202 *Leaf stomatal conductance*

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

207 Devices, Cambridge, UK) which was calibrated every two or three hours, before each sampling  
208 period. Measurements of leaf conductance taken using a Delta-T porometer have greater than 90  
209 % accuracy within a reading range of 5 to 800  $\text{mmol m}^{-2} \text{s}^{-1}$  and when ambient conditions of  
210 humidity are between 10 and 90 % (Delta-T Instruction Manual, 2004). Measurements were  
211 taken at approximately 9:00, 11:00, 13:00 16:00; 19:00, 22:00, 01:00, 04:00, 07:00, and ended at  
212 9:00 the following morning.

213

#### 214 *Comparing cuticular conductance of immature and mature leaves*

215 We measured  $G_c$  in 12 excised immature (recently flushed) leaves and 12 mature (fully  
216 expanded) leaves of each species. Leaves were excised in the morning, wrapped in aluminium  
217 foil and sealed in plastic bags, and immediately brought back to the laboratory. Leaves were  
218 weighed, and then placed on an 'airing rack' with both top and lower surfaces exposed to air.  
219 Changes in leaf fresh mass, air and leaf temperature and atmospheric relative humidity were  
220 measured repeatedly over a 2.5 hour period. Leaf temperature was measured using a  
221 thermocouple and air temperature was measured using wet and dry bulbs to estimate Leaf-to-Air-  
222 Vapour-Pressure-Deficit during the measurement period. Initial measurements were taken at  
223 intervals of 3 minutes until 21 minutes elapsed, and then leaves were weighed every 30 minutes  
224 until 2.5 hours elapsed.  $G_c$  was calculated from the rate of water loss from 90 to 150 min when  
225 stomates were closed; leaf mass regressed against time indicated stomatal closure occurred after  
226 50 minutes. Water loss was converted from  $\text{g s}^{-1}$  to  $\text{mmol m}^{-2} \text{s}^{-1}$  following (Percy *et al.* 1989).

227

#### 228 *Leaf water potential*

229 Leaf water potential ( $\Psi_1$ ) was measured on three bagged and three unbagged leaves of both  
230 species, on each of six trees that were instrumented for sap flow. Measurements were taken on  
231 two or three consecutive days in winter 2006, and summer 2006/7, using a Scholander-type  
232 pressure bomb (Plant Water Status Console, Soil Moisture Equipment Corporation, USA). Fully  
233 expanded, sunlit, mature leaves were sampled in the outer canopy between 2 – 8 m height  
234 between 0630 and 1800 h. Bagged leaves were wrapped in aluminium foil to exclude sunlight  
235 and contained within plastic bags to prevent transpiration. In the first field campaign (winter  
236 2006), we used bagged leaves 2 hours after sunrise as a surrogate for pre-dawn leaf water  
237 potential because the mean  $\psi_1$  of bagged leaves and pre-dawn water potential was not  
238 significantly different ( $P < 0.05$ ).

239

#### 240 *No-flow xylem water potential*



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

247  
248 To assess the disequilibrium between soil matric potential and pre-dawn leaf water potential, we  
249 measured leaf water potential throughout the morning and plotted it against the corresponding sap  
250 flow for two or three consecutive days in December 2006 and May, June, July and August 2007.  
251 Where a strong relationship was found between leaf water potential and sap velocity, this  
252 relationship was extrapolated to determine the leaf water potential when sap velocity was zero  
253 (*i.e.*, no-flow xylem water potential) (Bucci *et al.* 2004, O’Grady *et al.* 2005). Data were pooled  
254 into two groups (summer 2006 and winter 2007) and a general linear model (SPSS version 14,  
255 SPSS Inc. Chicago, IL) was used to determine whether pre-dawn leaf water potentials were  
256 significantly different between the seasons. For both species, there was a significant interaction  
257 between season and the dependant variable, so the seasons were treated as separate samples. The  
258 relationship between pre-dawn leaf water potential and no-flow xylem water potential was  
259 examined using regression analysis.

260

### 261 *Statistical analyses of drivers of $E_n$*

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

273

274 **Results**

275 *Soil moisture and micrometeorological conditions*

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

280  
281 In both spring and summer,  $D$  was high before rain events, reaching maximum values of 5.0 kPa  
282 and 5.5 kPa, respectively. After rain events,  $D$  was reduced in spring, ranging from 1.0 to 3.1  
283 kPa. In summer,  $D$  remained relatively high after rain events, reaching a peak of 4.0 kPa four  
284 days after the rain event. In contrast,  $D$  was low both before and after rain events in winter, and  
285 did not exceed 1.2 kPa. As expected, radiation was high in spring and summer (maximum 900  
286 and 1000  $\text{W m}^{-2}$ , respectively) and low in winter (maximum 600  $\text{W m}^{-2}$ ). Highly variable soil  
287 moisture, atmospheric demand, and energy levels during the study period provided an excellent  
288 platform for the analysis of environmental controls on  $E_n$ .

289

290 *Differing water use strategies for the two species during the day*

291 A comparison of diurnal curves of  $\psi_1$  and transpiration across seasons demonstrated that  $E$ .  
292 *parramattensis* was a more profligate user of water and experienced greater water stress than  $A$ .  
293 *bakeri*. Diurnal  $\psi_1$  in all seasons showed that  $E$ . *parramattensis* generally experienced greater  
294 water stress than  $A$ . *bakeri* (Fig. 1a). During the hottest sampling period on a clear day,  $A$ . *bakeri*  
295 reached a minimum of -1.9 MPa whereas  $E$ . *parramattensis* reached a minimum of -3.0 MPa.

296

297 Diurnal curves of sap velocity for  $E$ . *parramattensis* show higher rates of water use than for  $A$ .  
298 *bakeri* in the afternoon in both spring and winter.  $E$ . *parramattensis* had higher maximum rates of  
299 sap flow than  $A$ . *bakeri* in summer and winter, although on hot, dry summer days,  $E$ .

300 *parramattensis* had higher morning and lower afternoon sap flow than  $A$ . *bakeri* (Fig 1b). These  
301 diurnal curves suggest  $E$ . *parramattensis* is a greater consumer of water compared with  $A$ . *bakeri*.

302 Mean hourly sap flow rates (mean of 24 h) of  $E$ . *parramattensis* and  $A$ . *bakeri* were similar in  
303 spring ( $62.3 \pm 2.0$  and  $61.9 \pm 2.2$   $\text{mm hr}^{-1}$  respectively,  $n = 624$ ) whereas values of  $E$ .

304 *parramattensis* were higher than  $A$ . *bakeri* in summer ( $70.4 \pm 2.3$  and  $56.4 \pm 1.8$   $\text{mm hr}^{-1}$

305 respectively,  $n = 672$ ), and in winter ( $58.7 \pm 1.6$  and  $31.5 \pm 0.9$   $\text{mm hr}^{-1}$  respectively,  $n = 2144$ ).

306 Mean rates of  $E_n$  between 23:00 and 05:00 for  $A$ . *bakeri* were higher in summer and spring (1.4

307 mm hr<sup>-1</sup> and 1.6 mm hr<sup>-1</sup> respectively) than in winter (0.6 mm hr<sup>-1</sup>). Similarly,  $E_n$  for *E.*  
308 *parramattensis* was higher in summer and spring (1.8 mm hr<sup>-1</sup> for both seasons) than in winter  
309 (1.2 mm hr<sup>-1</sup>). Generally, *E. parramattensis* had higher rates of both day-time and night time sap  
310 flow than *A. bakeri* (Fig. 1a) meaning that  $E_n/E_d$  for both species was similar on most days.

311  
312 *Environmental regulators of  $E_n$*   
313  $UD$  is the product of windspeed ( $U$ ) and  $D$ , and therefore it is not appropriate to use partial  
314 correlations for auto-correlated variables such as  $U$  and  $UD$ . Therefore a comparison of Pearson's  
315 correlation coefficients was conducted to determine which independent variables had the  
316 strongest influence on  $E_n$ . In both species, hourly  $E_n$  was more strongly correlated with  $D$   
317 (coefficients of 0.70 to 0.83) than  $UD$  (0.41 to 0.43) or  $U$  alone ( $< 0.17$ ) across all seasons and for  
318 all seasons pooled (Table 1).  $E_n$  was significantly correlated with  $D$  (Fig. 4) and  $UD$  ( $p < 0.05$ ) for  
319 each species and each season (excluding winter for *E. parramattensis*), and seasons pooled  
320 (Table 1). In contrast,  $E_n$  was not significantly correlated with  $U$  when all seasons were pooled  
321 ( $p > 0.05$ ).  $E_n$  was more strongly correlated with shallow  $\Theta$  ( $\Theta_s$ ) than deep  $\Theta$  ( $\Theta_d$ ) for both species  
322 in each season and for all seasons pooled (Table 1). Therefore, further statistical analyses were  
323 conducted on hourly  $E_n$ ,  $D$  and  $\Theta_s$  rather than  $\Theta_d$ .

324  
325 *Hourly  $E_n$ ,  $D$  and  $\Theta_s$*   
326 Partial correlations showed that as  $D$  increased,  $E_n$  increased and the correlation was significant  
327 when  $\Theta_s$  was held constant (Table 2). In contrast, when  $D$  was held constant,  $\Theta_s$  had no  
328 significant influence on  $E_n$  in spring and winter; however, in summer and across all seasons  
329 pooled,  $\Theta_s$  had a significant influence on  $E_n$  for both species. In all seasons, and across seasons,  
330 the influence of  $D$  on  $E_n$  was greater in *A. bakeri* than *E. parramattensis*.

331  
332 *Night-time stem refilling and transpiration from the canopy*  
333 Stem refilling contributed 24-31% of  $E_n$  in *E. parramattensis* and 22-50% of  $E_n$  for *A. bakeri*  
334 before rain events. Following rain events, stem refilling increased to 67% for the smallest *A.*  
335 *bakeri* (DBH = 15.7 cm) and to  $> 85\%$  of  $E_n$  for the smallest *E. parramattensis* (DBH = 9.8 cm);  
336 however, larger trees (DBH 17.5 and 20 cm) were not responsive to rain events. During nights  
337 with extremely low  $D$ , stem refilling was *ca.* 95% of  $E_n$ . However, for most nights the proportion

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

340

#### 341 *Contribution of $g_s$ and $g_c$ to water loss at night*

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

351

#### 352 *Determination of $E_n/E_d$*

353 Mean  $E_n/E_d$  was 6-8 % across seasons (spring, summer and winter), but was up to 19% on some  
354 days in both species (Fig 5). In spring, mean  $E_n/E_d$  was 8% for both species, with a maximum of  
355 18% and 19% for *E. parramattensis* and *A. bakeri*, respectively. In summer, mean  $E_n/E_d$  was 7%  
356 for both species, with a maximum of 10% and 13% for *E. parramattensis* and *A. bakeri*,  
357 respectively, occurring on 22 January, a night with high  $D$ . In winter, mean  $E_n/E_d$  was 6% for  
358 both species; maximum values of 14 and 19% occurred for the *E. parramattensis* and *A. bakeri*,  
359 respectively, on June 7 when  $D$  was high.

360

#### 361 *Correction model for predicting soil water potential when $E_n$ occurs*

362 In summer, there were strong negative linear relationships between  $\psi_1$  and morning sap flow ( $r^2$   
363 from 0.97 to 0.53). We found that the calculated no-flow xylem water potentials ranged between  
364 -0.21 and -1.39 MPa and the measured pre-dawn  $\psi_1$  ranged between -0.4 and -1.5 MP.  
365 Consequently, the measured pre-dawn  $\psi_1$  were more negative than the water potential calculated  
366 from the no-flow xylem water potentials. However, there was a strong relationship between these  
367 two parameters (Fig. 6). For both species, there was a larger off-set between pre-dawn leaf water  
368 potential and no-flow xylem water potential in summer than winter, as indicated by the larger y-  
369 intercept calculated with the regression analysis (Fig. 6).

370

## 371 **Discussion**

372 *D* was the predominant regulator of  $E_n$

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

384

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

395

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

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

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

412

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

414 Water loss at night was primarily through open stomata rather than across the cuticle, with only  
415 5-10% of water loss occurring across the cuticle at night. Our results are similar to those for a  
416 number of studies that found cuticular water loss accounted for 8 – 30 % of total water loss  
417 (Caird et al. 2007; Howard and Donovan 2007). The results from this study demonstrate that  
418 stomatal conductance is much higher than cuticular conductance at night, thereby generating  
419 transpirational water loss from the canopy.

420

421  *$E_n/E_d$  was similar in all seasons and in both species*

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

430

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

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

445  
446 Despite experiencing similar microclimatic and soil conditions, the two species exhibited  
447 different strategies for minimising water loss while maximising carbon gain. *A. bakeri*  
448 experienced  $\psi_L$  close to zero in all seasons, low summer rates of water use, similar sap flow  
449 across the day and  $E_n$  was strongly correlated with  $D$ . In contrast, *E. parramattensis* experienced  
450 comparatively lower water potentials, higher rates of water use during summer, and higher  
451 morning and lower afternoon rates of water use, with  $E_n$  less correlated with  $D$  than in *A. bakeri*.  
452 In summary, *A. bakeri* exhibited attributes of a xeric vegetation type (*i.e.* water saver) and *E.*  
453 *parramattensis* exhibited comparatively more mesic attributes (*i.e.* greater consumer of water).

454  
455 Although different day-time water use strategies were employed, both species constrained  
456 nocturnal water fluxes to conservatively low seasonal values of 6 to 8% for  $E_n/E_d$  for each season.  
457 Interestingly, co-occurring woodland (Hatton *et al.* 1998; Zeppel and Eamus 2008) and semi-arid  
458 (O'Grady *et al.* 2009) species have also used different strategies to achieve similar rates of water  
459 loss. At a similar Eucalypt woodland, two sympatric species which had different hydraulic  
460 architecture (*i.e.* different relationships between leaf area, sapwood area and tree size), showed  
461 functional convergence between tree size and water use (Zeppel and Eamus 2008). Eucalypt  
462 woodlands along a Western Australian aridity gradient progressively reduced leaf area as sites  
463 became more arid, whereas sapwood area was largely unaffected by aridity (Pekin *et al.* 2009). In  
464 addition, low rates of  $E_n/E_d$  have been reported in three other Eucalypt dominated woodlands  
465 (Benyon 1999; Mitchell *et al.* 2008; Phillips *et al.* 2009b) suggesting a strong level of  
466 environmental control on nocturnal fluxes.

467

468 *Seasonality in the disequilibrium between  $\psi_{pd}$  and  $\psi_L$  and  $E_n/E_d$*

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

483

484

485



486 **Conclusion**

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

501

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508

509

510 **Figures and Tables.**

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

	D (kPa)	UxD (kW m <sup>-2</sup> )	Windspeed (m s <sup>-1</sup> )	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*

514

515 Table 2. Partial correlation co-efficients for hourly  $E_n$ , D and shallow soil storage (\* = significant  
 516 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*

517

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,  $\text{cm hr}^{-1}$ ) for each  
537 species in (a) spring, (b) summer, and (c) winter.

538

539 Fig 5. The proportion of summed stand water use ( $\text{mm day}^{-1}$ ) 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

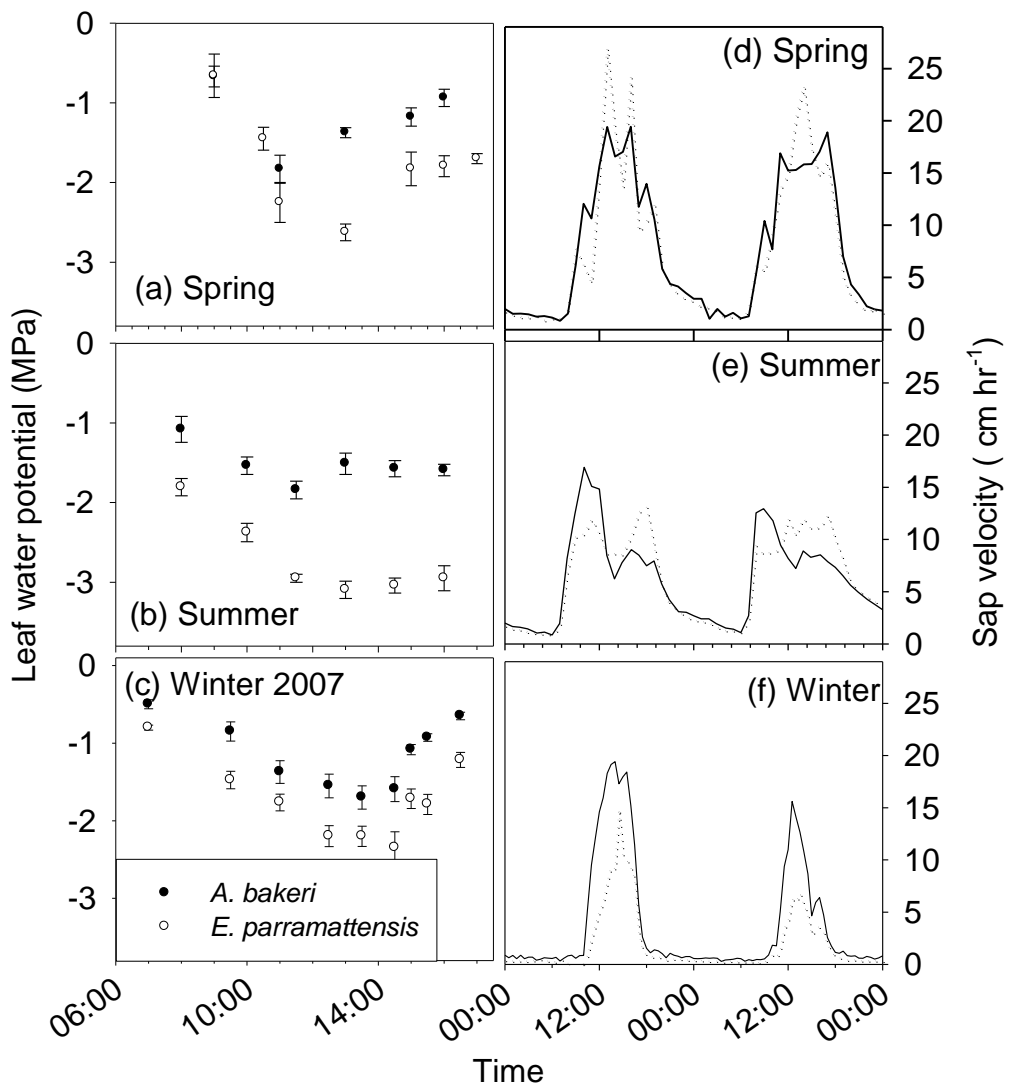


Fig. 1.

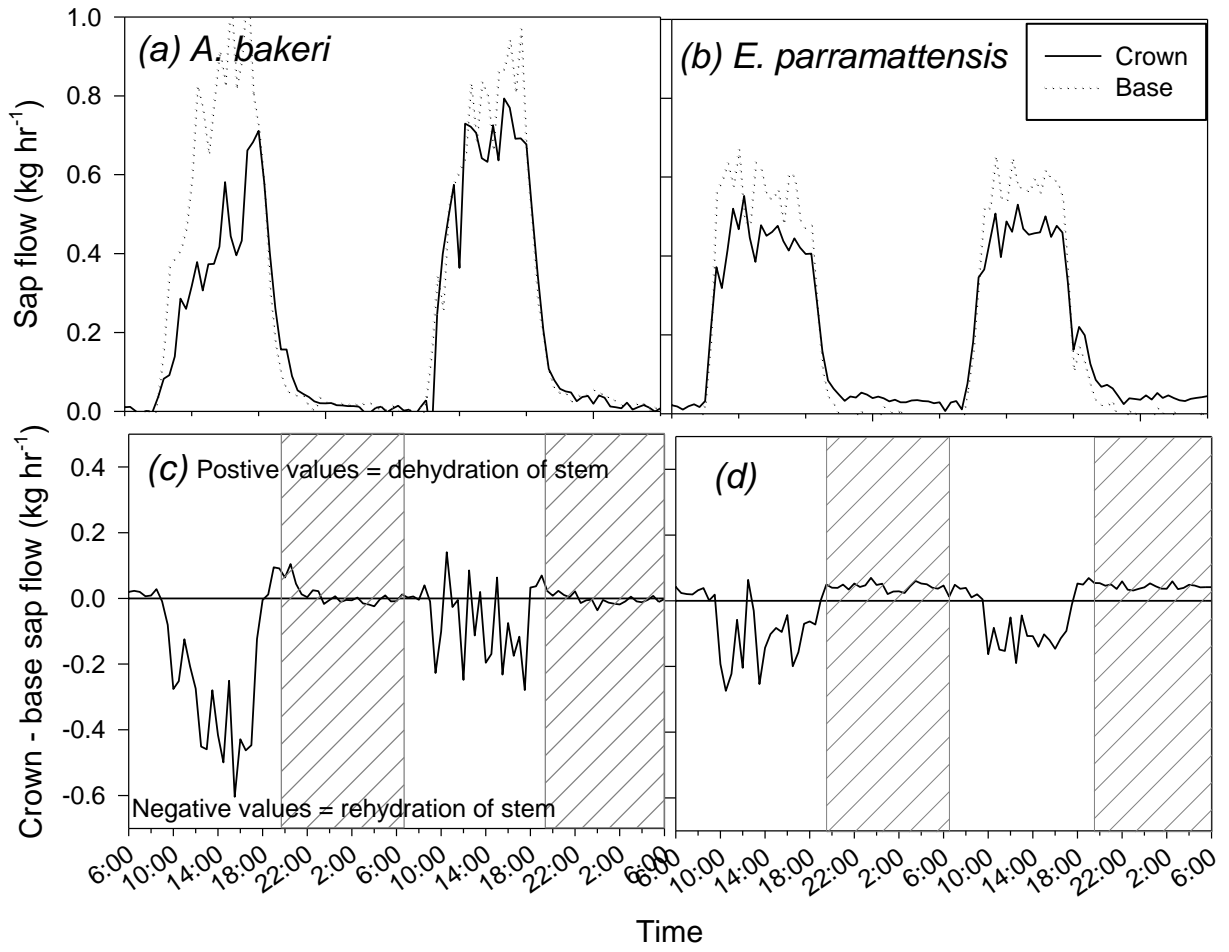


Fig. 2.

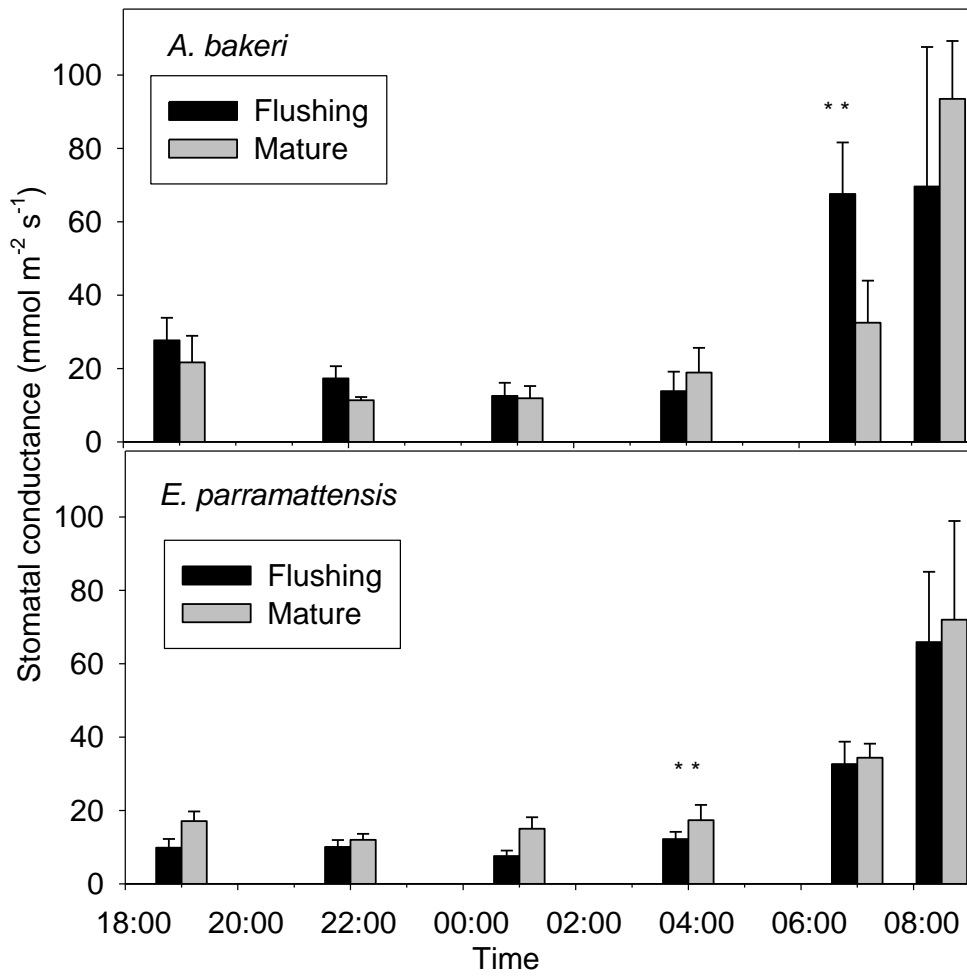


Fig. 3.

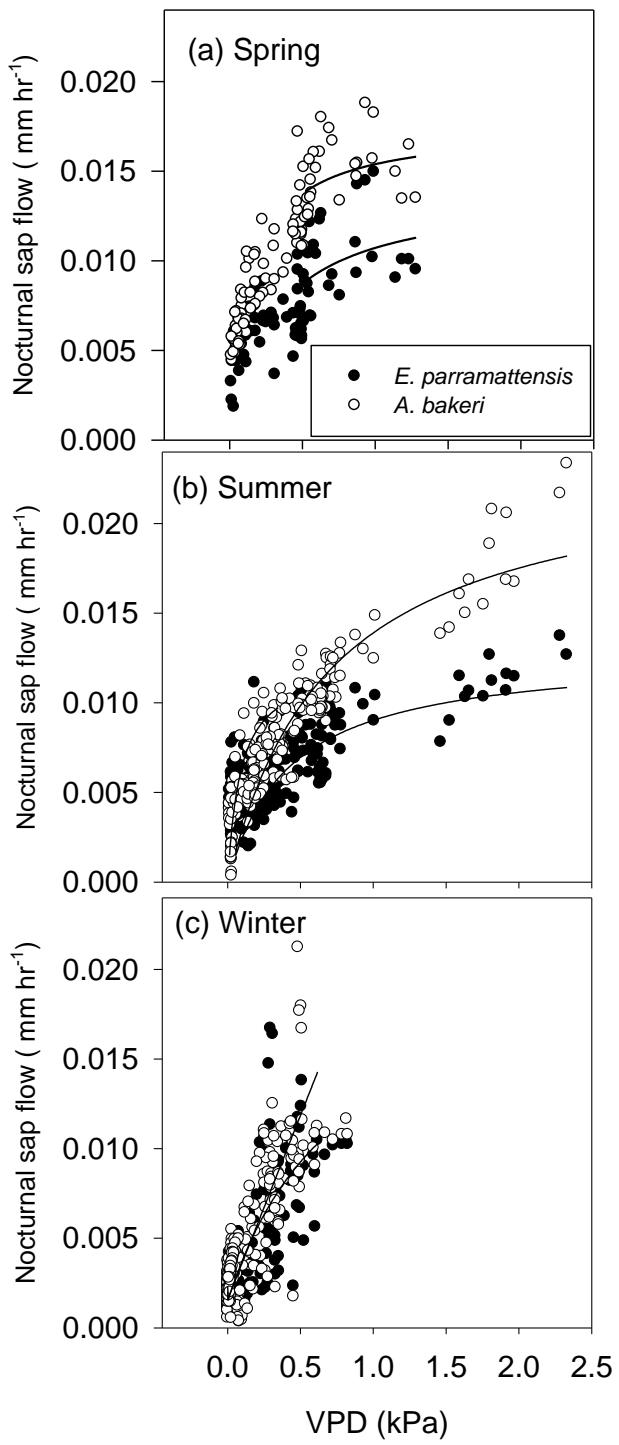


Fig. 4.

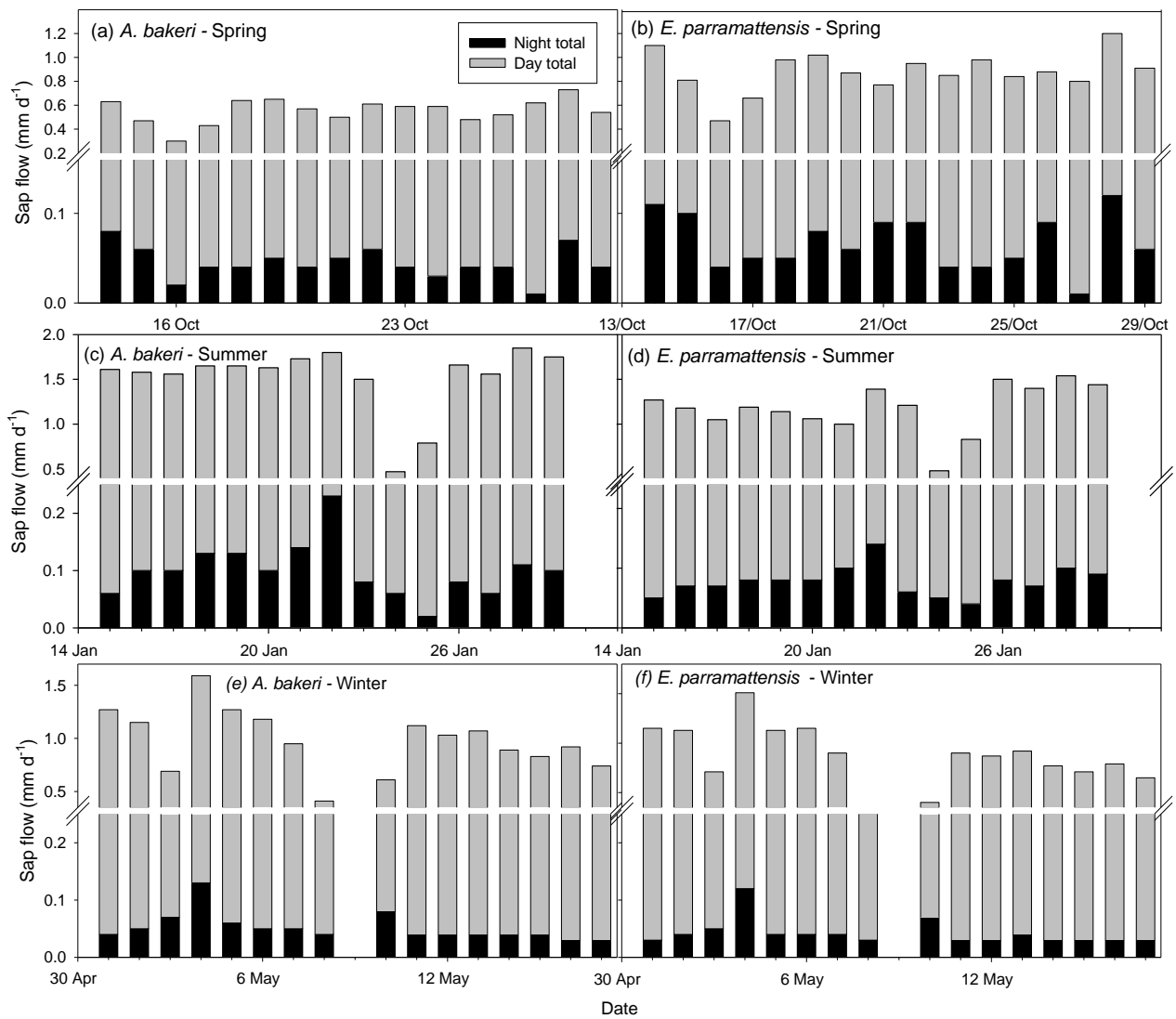


Fig 5a-f. The proportion of summed stand water use (mm d<sup>-1</sup>) for day (grey bars, 05:30 to 22:30) and night (black bars, 23:00 to 05:00) for each species in spring.



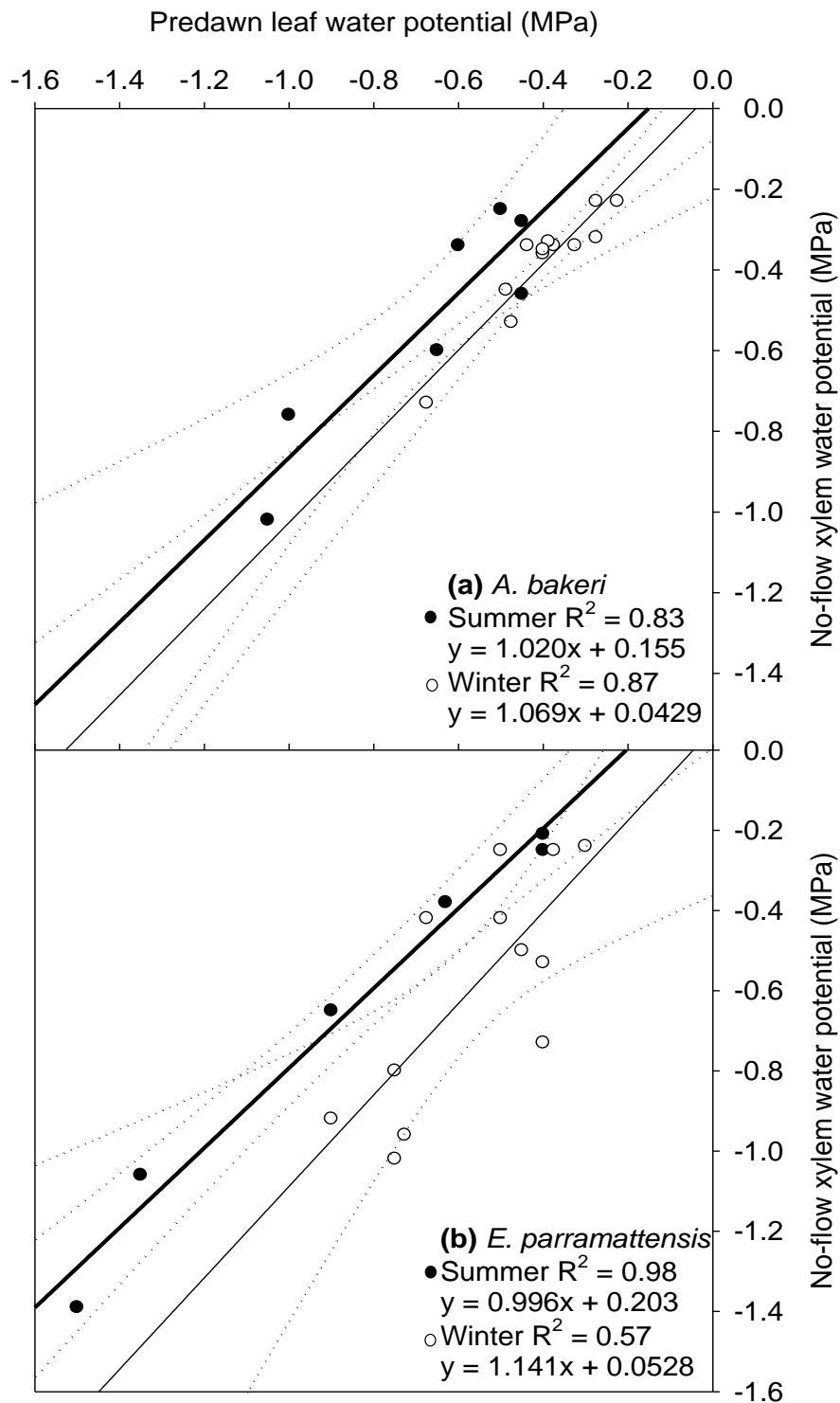


Fig 6.

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