

Running head: No elevated CO₂ impact on woodland water-use

Elevated CO₂ did not affect the hydrological balance of a mature native *Eucalyptus* woodland

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1 **ABSTRACT**

2

3 Elevated atmospheric CO₂ concentration (eC_a) might reduce forest water-use, due to
4 decreased transpiration, following partial stomatal closure, thus enhancing water-use
5 efficiency and productivity at low water availability. If evapotranspiration (E_t) is reduced, it
6 may subsequently increase soil water storage (S) or surface runoff (R) and drainage (D_g),
7 although these could be offset or even reversed by changes in vegetation structure, mainly
8 increased leaf area index (L). To understand the effect of eC_a in a water-limited ecosystem,
9 we tested whether two years of eC_a (~40% increase) affected the hydrological partitioning in
10 a mature water-limited *Eucalyptus* woodland exposed to Free-Air CO₂ Enrichment (FACE).
11 This timeframe allowed us to evaluate whether physiological effects of eC_a reduced stand
12 water-use irrespective of L , which was unaffected by eC_a in this timeframe. We hypothesized
13 that eC_a would reduce tree-canopy transpiration (E_{tree}), but excess water from reduced E_{tree}
14 would be lost via increased soil evaporation and understory transpiration (E_{floor}) with no
15 increase in S , R or D_g . We computed E_t , S , R and D_g from measurements of sapflow
16 velocity, L , soil-water content (θ), understory micro-meteorology, throughfall and stemflow.
17 We found that eC_a did not affect E_{tree} , E_{floor} , S or θ at any depth (to 4.5 m) over the
18 experimental period. We closed the water balance for dry seasons with no differences in the
19 partitioning to R and D_g between C_a levels. Soil temperature and θ were the main drivers of
20 E_{floor} while vapour pressure deficit controlled E_{tree} , though eC_a did not significantly affect any
21 of these relationships. Our results suggest that in the short-term, eC_a does not significantly
22 affect ecosystem water-use at this site. We conclude that water-savings under eC_a mediated
23 by either direct effects on plant transpiration or by indirect effects via changes in L or soil
24 moisture availability are unlikely in water-limited mature eucalypt woodlands.

25 **Introduction**

26

27 Rising atmospheric CO₂ concentration (C_a) directly affects several facets of plant physiology,
28 with cascading effects on other biotic and abiotic ecosystem components (Field *et al.*, 1995).
29 At the leaf-level, increases in C_a above the present concentration often enhance
30 photosynthesis, reduce transpiration, due to partially reduced stomatal conductance (g_s), and
31 thus increase water-use efficiency (De Kauwe *et al.*, 2013, Keenan *et al.*, 2013), though the
32 ecosystem-level ramifications of these effects are still debated (Leuzinger & Körner, 2010,
33 Donohue *et al.* 2017). In vegetated areas, evapotranspiration is a major contributor to
34 ecosystem water balance (Zhang *et al.*, 2016). At steady-state conditions, reduced
35 transpiration under elevated C_a (eC_a) may lead to increased soil moisture (Leuzinger &
36 Körner, 2007) and eventually an increase in the amount of precipitation running off the
37 ground surface (R) and into groundwater stores (D_g , Gedney *et al.*, 2006, Zhang *et al.*, 2001).
38 Numerous modelling studies and retrospective analyses have ascribed observed increases in
39 R or soil water storage (S) to rising C_a (Aston, 1984, Betts *et al.*, 2007, Jackson *et al.*, 1998,
40 Macinnis-Ng *et al.*, 2011). Yet, more recent studies highlight that these observations are
41 strongly dependent on the vegetation type and climate (Cheng *et al.*, 2014, Fatichi *et al.*,
42 2016, Huntington, 2008, Leuzinger & Körner, 2010). However, these predictions rely mostly
43 on retrospective analyses encompassing the increase in C_a from pre-industrial to current C_a
44 levels (Betts *et al.*, 2007, Gedney *et al.*, 2006, Ukkola *et al.*, 2016) and these might not
45 necessary apply to further projected increases in C_a for the 21st century.

46 Rising C_a also affects transpiration indirectly (Fatichi *et al.*, 2016), as enhanced total
47 or above-ground productivity would require more water to support more tissue produced in
48 eC_a (Ellsworth *et al.*, 2012, Norby *et al.*, 2005). Satellite observations and model predictions
49 indicate that rising C_a partly underlies the recent global increase in woody biomass and

50 greenness (Zhu *et al.*, 2016), particularly in water-limited regions (Donohue *et al.*, 2009).
51 Increased greenness due to present-day CO₂-fertilization results from greater leaf area per
52 unit of ground area (L , McCarthy *et al.*, 2007, Cheng *et al.*, 2017), which increases
53 transpiration surface area per unit of ground area (Macinnis-Ng *et al.*, 2011). Such an effect
54 may offset or even override potential leaf-level reductions in transpiration. Additionally,
55 increased radiative forcing due to climate change would further offset the potential impacts of
56 reduced stomatal conductance under eC_a (Ukkola *et al.*, 2016, Cheng *et al.* 2014). Indeed,
57 under eC_a , Donohue *et al.* (2017) predict no effective ecosystem-level water-savings in either
58 water-limited sites, where increased L offsets leaf-level water-savings, or in so-called
59 energy-limited sites (cf. Zhang *et al.*, 2001), where little or no change of leaf-level
60 transpiration is expected and where L is already maximised (Yang *et al.*, 2016b).
61 Alternatively, in sub-humid and semi-arid river basins, increased greenness due to eC_a could
62 increase ecosystem-level water-use and reduce streamflow (Trancoso *et al.*, 2017, Ukkola *et*
63 *al.*, 2016).

64 In addition to the impact on transpiration, increased L indirectly alters ecosystem
65 water-use by increasing the evaporative losses due to greater partitioning of incoming
66 precipitation into interception (E_i , Kergoat, 1998), although this might not be the case in
67 forests with vertically-angled leaves where increased L is unlikely to contribute to greater
68 throughfall (Crockford & Richardson, 2000). Additionally, increased foliage shading
69 decreases the amount of radiation reaching the ground surface, thus decreasing understory
70 transpiration and soil evaporation (Crockford & Richardson, 2000, Raz-Yaseef *et al.*, 2010).
71 Ultimately, the contribution of these later components will be strongly determined by the
72 amount and characteristics of the precipitation events and the dynamics of atmospheric
73 evaporative demand.

74 Predictions of the impact of eC_a on forest hydrology are largely derived from leaf-
75 level studies (Field *et al.*, 1995, Gimeno *et al.*, 2016), models (Betts *et al.*, 2007) and
76 retrospective analyses (Yang *et al.*, 2016b), with additional insights from Free-Air CO₂
77 Enrichment (FACE) experiments conducted in forests (Donohue *et al.*, 2017). Some of these
78 FACE studies found partial reductions in g_s (Ellsworth, 1999, Gimeno *et al.*, 2016,
79 Gunderson *et al.*, 2002, Keel *et al.*, 2007) and reduced canopy-transpiration (Cech *et al.*,
80 2003, Wullschleger & Norby, 2001), while others did not find a reduction in either leaf- or
81 canopy-level transpiration (Uddling *et al.*, 2009, Ward *et al.*, 2013). In addition, leaf-level
82 water savings were often offset by increased L (Bobich *et al.*, 2010, Schäfer *et al.*, 2002, Tor-
83 ngern *et al.*, 2015, Warren *et al.*, 2011). All of these studies focused mainly on the effect of
84 eC_a on canopy transpiration (E_{tree}), while other components of evapotranspiration (E_t) were
85 rarely measured (Cheng *et al.*, 2017, but see Schäfer *et al.*, 2002). Furthermore, these studies
86 are primarily restricted to energy-limited or moderately water-limited young trees or forest
87 plantations (Bobich *et al.*, 2010, Ellsworth, 1999, Godbold *et al.*, 2014). In water-limited
88 woodlands, we are less likely to observe a change in R or D_g under eC_a , as potential increases
89 in soil moisture could be lost via ground evaporation and understorey transpiration (Ferretti *et*
90 *al.*, 2003, Nolan *et al.*, 2014, Nowak *et al.*, 2004), although this could be partially offset by
91 reduced g_s under eC_a in the understorey (Morgan *et al.*, 2004). Notwithstanding previous
92 work in tree plantations (Schäfer *et al.*, 2002, Uddling *et al.*, 2009, Wullschleger & Norby,
93 2001), we still lack an experimental test of the effects of eC_a on hydrological partitioning,
94 particularly in mature woodlands experiencing potential water deficits throughout the year.
95 To validate dynamic vegetation models for predicting vegetation-climatic feedbacks, large-
96 scale observations simultaneously addressing the impact of eC_a on all E_t components (not just
97 E_{tree}), R and S are desperately needed (Fisher *et al.*, 2017, Porporato *et al.*, 2004).

98 In our study, a mature and water-limited *Eucalyptus* woodland was exposed to a C_a
99 $150 \mu\text{mol mol}^{-1}$ above ambient, using Free-Air CO_2 Enrichment (the 'EucFACE'
100 experiment). Here, we address the effects of eC_a on precipitation partitioning among
101 components of the hydrological balance (E_t , R , D_g and S) over the first two years of the
102 EucFACE experiment. Duursma *et al.* (2016) demonstrated that L did not respond to eC_a in
103 this woodland, so we hypothesized (i) that partial stomatal closure at the leaf-level (Gimeno
104 *et al.*, 2016) would lead to a decrease in E_{tree} under eC_a ; however, since g_s did not decrease
105 under eC_a in the understorey (Pathare *et al.*, 2017) we expected (ii) that excess water 'saved'
106 by the canopy would be lost via increased soil evaporation together with understorey
107 transpiration (E_{floor}), (iii) thus resulting into no net increase in S , R or D_g . The lack of
108 structural changes induced by eC_a in this timeframe (Duursma *et al.*, 2016), means that we
109 have the advantage that we can carefully examine the partitioning of eC_a effects on water
110 balance components without being confounded by stand structure effects.

111

112 **Materials and Methods**

113

114 *Study site and experimental design*

115 EucFACE is located on an ancient alluvial floodplain, 3.6 km from the Hawkesbury River, in
116 western Sydney (NSW, Australia, 33°37'S, 150°44'E, 23 m a.s.l.), in a 270 ha patch of native
117 Cumberland Plain woodland (Fig. S1). The site is flat (maximum slope: 0.004°), with the
118 lowest land-surface elevations found near ring 5 (Fig. S1). The site is characterized by a
119 humid temperate-subtropical transitional climate with a mean annual temperature of 17°C,
120 with January being the hottest (mean daily maximum 30°C) and July the coldest (mean daily
121 minimum 3.6°C), and a mean annual precipitation (P) of $730 \pm 30 \text{ mm y}^{-1}$, with February
122 being the wettest month ($123 \pm 16 \text{ mm month}^{-1}$) and July the driest ($29 \pm 5 \text{ mm month}^{-1}$),

123 mean \pm se, 1992-2014, Bureau of Meteorology, station 067105, 5 km away). Satellite-
124 estimated actual E_t is 739 ± 34 mm y^{-1} (1981-2012, Zhang *et al.*, 2016), which means that the
125 site is water-limited.

126 The upper soil (up to 30-50 cm) is a loamy sand ($> 75\%$ sand), slightly-acidic
127 (pH = 4.5) and with low-organic C ($< 1\%$) and overall low phosphorus (Ellsworth *et al.*,
128 2017). At 30-70 cm depth, there is a layer of higher clay content (15-35% clay), below which
129 the soil is a sandy loam or sandy clay loam. Between 300-350 and 450 cm depth, the soil is
130 clay ($> 40\%$ clay). Groundwater is present at ~ 12 m below the surface (Fig. S2).

131 Tree density ranges from 600-1000 trees ha^{-1} (basal area, BA = 27.6 ± 2.7 $m^2 ha^{-1}$,
132 $n = 6$ plots), L is ~ 2 $m^2 m^{-2}$ (Duursma *et al.*, 2016), the canopy height is 18-23 m tall and
133 mean tree diameter at 1.3 m (DBH) is 18.8 ± 0.6 cm. The main canopy forming tree is
134 *Eucalyptus tereticornis* Sm with an understory mainly composed of grasses, with low
135 densities of forbs and occasional shrubs (Pathare *et al.*, 2017).

136 At EucFACE, there are six 25-m diameter plots (hereafter "rings"). Each ring
137 comprises a cylindrical frame of 28 m-high vertical pipes extending above the canopy
138 (treetops ranging 18-23 m). Vegetation within rings 1, 4 and 5 (Fig. S1) was exposed to a C_a
139 $150 \mu mol mol^{-1}$ above ambient, whereas the other three rings received ambient C_a (see
140 Gimeno *et al.*, 2016 for further details). In contrast to previous FACE experiments, here C_a
141 was ramped-up gradually to minimize potential transient effects. Here, the C_a was increased
142 at a rate of $\sim 30 \mu mol mol^{-1}$ per month over a ~ 6 month period until C_a reached
143 $150 \mu mol mol^{-1}$ above ambient in the e C_a rings on the 5 February 2013 (see Drake *et al.*,
144 2016 for a detailed description of the ramp-up).

145

146 *Meteorological and soil moisture measurements*

147 On top of a central tower (23.5 m) in each ring, an array of sensors measured air temperature
148 and relative humidity (HUMICAP ® HMP 155, Vaisala, Vantaa, Finland), net radiation (R_n ,
149 CNR2 Kipp & Zonen, Delf, The Netherlands), photosynthetically active radiation (PAR, LI-
150 190, LI-COR, Inc., Lincoln, NE, USA) and wind speed (Wincap Ultrasonic WMT700
151 Vaisala, only on the three eC_a rings, Fig. S1). These variables were measured every second
152 and one- (wind) or ten-minute (all other variables) averages were recorded on data loggers
153 (CR3000, Campbell Scientific Australia, Townsville, Australia). The average of the six (three
154 for wind) rings was used to characterize the meteorological conditions on site. Daily Penman
155 potential evapotranspiration (E_p) was calculated as (Donohue *et al.*, 2010):

156 (Eq. 1)

$$E_p = \frac{\Delta}{\Delta + \gamma} R_n + \frac{\gamma}{\Delta + \gamma} \frac{6430(1 + 0.536u)}{\lambda}$$

157 where γ is the psychrometric constant (65.3 Pa K⁻¹), daily R_n integral is in mm day⁻¹, D is
158 mean daily water vapour pressure deficit (in Pa), u is mean daily wind speed (in m s⁻¹), λ is
159 the latent heat of vaporisation of water (2.45 MJ kg⁻¹) and $\frac{d e_s}{dT}$ is the rate of change of saturated
160 water vapour with temperature (Pa K⁻¹).

161 Soil volumetric water content (θ_v) was monitored in each ring at eight locations with
162 frequency-domain reflectometers installed at 30 cm depth (TDRs, CS650 Soil Water Content
163 Reflectometer, Campbell Scientific). Soil temperature (T_{soil}) was measured at two locations in
164 each ring with temperature probes at 5 cm depth (TH3-s, UMS GmbH, Frankfurt, Germany).
165 θ_v and T_{soil} were measured every second and 15-minute averages were logged on CR3000s.

166

167 *Canopy leaf area measurements*

168 A detailed description of the methods for L (in m² m⁻²) quantification is found in Duursma *et al.*
169 *al.* (2016). Briefly, L was estimated from diffuse canopy transmittance (τ_d) calculated from

170 the ratio of above- and below-canopy PAR measured in each ring with one and three sensors
171 (LI-190), at 23.5 and 1.5 m height, respectively. For these calculations we used only PAR
172 measurements under highly diffuse conditions (diffuse fraction [F_{diff}] > 0.98). We measured
173 F_{diff} with a BF5 Sunshine sensor (Delta-T Instruments, Cambridge, UK) installed on a tower
174 extending 5 m above the canopy at a nearby site (within 2 km). We then calibrated L
175 estimates from ρ_a against cumulative litter production over 4-months (Duursma *et al.*, 2016).

176

177 *The water balance components*

178 Mass balance provides a framework for assessing the impact of eC_a on the partitioning of
179 precipitation (P). The mass balance for water can be expressed as:

180 (Eq. 2)

$$\Delta S = P - R - D_g - E_t + \Delta S$$

181 where R is surface runoff, D_g is drainage, S is the change in root-zone soil water storage and
182 E_t is evapotranspiration. All variables measured in mm day^{-1} . In this study, we assessed the
183 effect of eC_a on E_t and S , while we did not expect a significant effect on R or D_g . Therefore,
184 S , E_t and all of its components were quantified at the ring-level (except for stem-flow, see
185 below) and R and D_g at the site-level. Eq. 2 was implemented for 30 months starting on June
186 2012, including the pre-treatment and ramp-up periods

187 1. *Precipitation*

188 We defined a P event as a continuous series of hours with $P > 0 \text{ mm h}^{-1}$ interrupted for one
189 hour or less with $P = 0 \text{ mm h}^{-1}$. Site P was the average of three automated tipping bucket rain
190 gauges (TB4, Hydrological Services Pty Ltd, Liverpool, NSW, Australia) located at the top
191 of the central tower in rings 1, 4 and 3 (Fig. S1). P from each bucket was logged every 15
192 minutes onto CR3000 data loggers.

193

194 2. *Surface runoff*

195 Surface runoff (R) was calculated as the excess of P minus E_p when the upper soil was
196 saturated and precipitation intensity either exceeded soil infiltration capacity (17 mm h^{-1} for
197 sandy soils, Campbell & Norman, 1998) or cumulative precipitation (P_{cum}) for each event
198 minus E_p exceeded maximum soil storage capacity. In our site, with a sandy soil with
199 $\theta_v = 3\%$ at permanent wilting point and $\theta_v = 30\%$ at saturation and an effective depth of
200 400 mm, the maximum soil storage capacity was calculated as: $400 \times (30-3)/100 = 108 \text{ mm}$.
201 This approach should be valid for our flat study site with a stratified (or duplex) soil texture
202 with a defined shallow layer of relatively impermeable clay at $\sim 400 \text{ mm}$.

203 3. *Drainage and soil water*

204 The D_g from Eq. 2 represents the amount of water that drained below the assumed root-zone
205 and was not accessible for transpiration. To determine whether the vegetation in our study
206 site was accessing groundwater, we monitored changes of the water table level on-site and
207 analysed the isotopic composition of xylem water and potential water sources
208 (Supplementary methods). Neither the dynamics of the water table depth (seasonal or intra-
209 day), nor the isotopic composition of the tree xylem water suggested that the vegetation at our
210 site used groundwater (Fig. S2 and S3).

211 We assumed that D_g would be water lost from the deep soil layer and calculated D_g as
212 the inverse of the change in soil water storage from 3 to 4.5 m depth. This approach was
213 justified for our site where the soil has a marked multilayered texture: the upper sandy soil
214 (from 0 to 0.3-0.5 m) is where the majority of roots are located (Piñeiro et al., unpublished
215 data). Below this depth (up to 3 m), the soil is a sandy clay loam (up to 3 m depth) where
216 only a few live roots are present and below 3 m, a clay horizon starts and continues beyond
217 4.5 m depth. Our observations for root distribution across the depth profile are consistent with
218 the results of Macinnis-Ng *et al.* (2010) from a nearby (within 7 km) site where $\sim 90\%$ of the

219 tree roots were found in the upper soil (0.7 m) with only occasional roots present in the
 220 deeper (up to 1.5 m) clay horizon. We assumed that changes in soil moisture below 3 m are
 221 unlikely affected by direct vegetation water uptake or hydraulic lift. Thus, we calculated D_g
 222 as water lost below 3 m for a given time interval (t_1-t_2) according to:

223 (Eq. 3)

$$\theta_{z_1, z_2} - \theta_{z_1, z_2} = - \int_{z_1}^{z_2} \theta_{z_1, z_2} - \theta_{z_1, z_1} - \theta_{z_1-1} \theta_{z_1-1}$$

224 where θ_{z_1, z_2} is the soil water content at depth z_i and z_{\max} is 4.5 m (Duursma *et al.*, 2011). There
 225 were no differences in deep soil water storage between ambient and eC_a rings over time
 226 ($t = -0.94, p = 0.35$), so D_g was calculated at the site level from averaged deep soil water
 227 storage from all rings.

228 Soil volumetric water content (θ_v) across the soil profile (25-450 cm) was monitored
 229 every 15-20 days at two locations in each ring with a neutron probe (NMM, 503DR
 230 Hydroprobe ®, Instroteck, NC, USA). We measured θ_v in 25 cm intervals from 25 to 150 cm
 231 depth and in 50 cm intervals from 150 to 450 cm depth (see Supplementary Methods).

232 4. *Change in soil water storage*

233 We calculated the change in soil water storage (ΔS) for a soil column to 3 m depth. Since we
 234 expected to observe an effect of eC_a on ΔS , we calculated ΔS for each ring over a given time
 235 (t) interval (t_1-t_2) as:

236 (Eq. 4)

$$\Delta \theta_{z_1- z_2} = \int_{z_1}^{z_2} \theta_{z_1, z_2} - \theta_{z_1, z_1} - \theta_{z_1-1} \theta_{z_1-1}$$

237 where θ_{z_1} is the soil water content at depth z_i and z_{\max} is 3 m (Duursma *et al.*, 2011). Here, θ_{z_1}
 238 is the mean of two measurements at the same depth from two locations within each ring.

239 5. *Evapotranspiration*

240 Total ecosystem evapotranspiration (E_t) consists of:

241 (Eq. 5)

$$E_t = E_i + E_{\text{tree}} + E_{\text{floor}}$$

242 where E_i is the canopy interception loss, E_{tree} is overstorey canopy transpiration and E_{floor} is
243 soil evaporation and understorey transpiration.

244 *a. Interception*

245 Canopy interception loss (E_i) was calculated as:

246 (Eq. 6)

$$E_i = P - (T_f + S_f)$$

247 where T_f is throughfall and S_f is stemflow. Throughfall (T_f) was measured under the canopy
248 with one custom-built fixed trough in each ring. Each trough consisted of an 8×0.25 m
249 gutter set with a maximum inclination of 1° . In each ring, T_f was calculated taking into
250 account the projected area of each gutter. The troughs drained into a large volume tipping
251 bucket flow gauge (TB1L, Hydrological Services Pty Ltd). Total T_f was logged every 15-
252 minutes onto CR3000 data loggers. One additional trough was located in an open space (Fig.
253 S1) to act as a control and we found that this trough underestimated P by $4.4 \pm 0.6\%$
254 ($R^2 = 0.98$, $p < 0.001$). We assumed that the troughs in the rings underestimated T_f to a
255 similar extent to the control through and corrected accordingly to calculate E_i .

256 Stem flow (S_f) was measured in ten trees across EucFACE, adjacent to the study
257 rings, (DBH: 24.6 ± 2.4 cm, range: 14-38 cm). Stem flow collectors were custom-built and
258 consisted of a collar, constructed from a half-split 25 mm diameter vinyl pipe glued with
259 silicone around the trunk. Collectors channelled S_f into automated tipping buckets (TB4) and
260 total S_f was recorded every 15 minutes onto a CR3000. We used this dataset to model the
261 amount of S_f in each ring. We fitted a linear mixed model to S_f volume collected in each
262 precipitation event as a function of tree basal area, P event size and duration (Crockford &
263 Richardson, 2000), including event as a random factor (Table S1). The obtained coefficients

264 for each fixed factor (tree basal area, P_{cum} and duration) were used to predict S_f of each tree
265 inside each ring, during each precipitation event.

266 *b. Overstorey canopy transpiration*

267 Whole tree overstorey canopy transpiration (E_{tree}) was computed from measurements of tree
268 sapflow velocity (v_s , Oren *et al.*, 1998). We measured v_s every ten minutes using the heat
269 pulse compensation technique (Marshall, 1958) with equipment manufactured by Edwards
270 Industries (Havelock North, New Zealand) and connected to a CR3000 data loggers. Each
271 sensor consisted of two temperature probes, constructed of sealed Teflon @ 1.8 mm diameter
272 tubing, with each probe containing a negative temperature coefficient thermistor. The
273 temperature sensors were placed 10 mm (downstream) and 5 mm (upstream) from a 1.8 mm
274 diameter stainless steel heater probe. The holes for the temperature and heater probe were
275 drilled with a drill guide to ensure accurate positioning of the probes and parallel alignment.
276 Distances between implanted probes and bark depth were recorded at the time of installation.
277 Measured heat pulse velocity was corrected for the appropriate wound size. The wound width
278 was measured from cut sections of sapwood where dummy probes were inserted for 30 days
279 from five trees adjacent to the study plots. Wound size (2.7 ± 0.2 mm) was measured under
280 the light microscope ($\times 40$ magnification). v_s was calculated from heat pulse velocity given
281 the fractions of wood and liquid in the sapwood (Swanson & Whitfield, 1981).

282 In winter 2012, four dominant or co-dominant trees with positive growth rates for the
283 previous year were selected for monitoring of v_s , in each ring. Two sets of thermocouples and
284 heater element were installed in each tree at 1.3 m height on two randomly selected azimuths.
285 At each tree location, the two thermocouples were positioned at the specific depth below the
286 cambium at which v_s was maximised along the sapwood profile (i.e. both probes in each tree
287 were set at the same depth to get an estimate of within tree radial v_s variability). This depth
288 was determined empirically for each tree as follows: within each tree, one probe was set at

289 10 mm below the cambium (fixed) and the second probe (mobile) at 25 mm below the
290 cambium. Then, on a cloudless spring day in September 2012, the mobile probe was
291 incrementally pulled out from 25 to 0 mm below the cambium at 5 mm intervals every
292 90 minutes (Wullschleger & Norby, 2001). In sensors that were not manipulated, v_s remained
293 relatively constant. With these observations, we determined the depth below the cambium at
294 which v_s was maximal by comparing v_s between the fixed and the mobile probe.

295 Sapwood area (A_s) and fractions of wood and liquid within the sapwood matrix were
296 calculated from cores extracted from 35 trees adjacent to the study rings. Cores were
297 collected from trees reflecting the size distribution of trees within the rings (DBH:
298 31.3 ± 2.2 cm). Wood cores were 5 mm in diameter and were extracted using a standard
299 Pressler increment borer (Haglöf Västernorrland, Sweden). Sapwood depth was measured
300 with a digital calliper after staining wood cores with methyl-orange that provided visual
301 differentiation of the sapwood from the heartwood (Pfausch *et al.*, 2012). We calculated the
302 correlation coefficients between basal area and A_s measured in these trees to predict A_s per
303 unit of ground area from basal area inside each ring.

304 Mean tree v_s was calculated from the average of the two sets of thermocouples and
305 heater element installed on each tree. We calculated mean hourly v_s for each ring (\bar{v}_s) from
306 the four (three in rings 2 and 6) measured trees. Hourly E_{tree} for each ring was calculated as:
307 (Eq. 7)

$$\bar{v}_{\text{tree}} = \bar{v}_s \bar{A}_s$$

308 where A_s is the sapwood area per unit of ground area of each ring. Daily and seasonal water-
309 use per unit ground area for each ring were calculated by integrating E_{tree} over time.

310 *c. Soil evaporation and understorey transpiration*

311 Soil evaporation together with understorey transpiration (E_{floor}) was estimated from the
312 change in soil moisture over 5 cm depth measured at two locations in each ring with two

313 theta probes (ThetaProbe ML2x, Delta-T). Changes in soil moisture at this depth are likely to
 314 reflect E_{floor} because: (i) most understorey vegetation roots are found between 0 and 5 cm
 315 depth (Piñeiro et al. unpublished data); and (ii) changes in soil moisture at this depth are
 316 likely to capture water losses due to soil evaporation, given the soil's sandy texture
 317 (Campbell & Norman, 1998). Hourly E_{floor} was calculated as:

318 (Eq. 8)

$$E_{\text{floor}, t_2 - t_1} = \frac{z}{\rho_w} \frac{\Delta \theta_v}{\Delta t}$$

319 where z is the depth of the theta probes (5 cm) and $\frac{\Delta \theta_v}{\Delta t}$ is the difference in θ_v between
 320 consecutive hourly averages ($t_1 - t_2$). A decrease in θ_v from 0 to 5 cm results from
 321 evapotranspiration, but also from water infiltration (Schreiner-McGraw *et al.*, 2016); thus, to
 322 avoid overestimation of E_{floor} , we only calculated E_{floor} for days with $P = 0 \text{ mm d}^{-1}$ and
 323 preceded by a day with $P < 2 \text{ mm d}^{-1}$ (454 of 730 days passed these criteria). We validated
 324 our approach with E_{floor} measurements made at one location adjacent to ring 1 (Fig. S1). An
 325 automated long-term clear chamber (LI-8100-104C, LI-COR,) coupled to an IRGA (LI-
 326 8100A, LI-COR) measured E_{floor} every 30 min on a permanently installed PVC collar. The
 327 automated chamber was deployed from winter 2013 to spring 2014 and rendered 222 days of
 328 measurements without errors (Fig. S4). We found that E_{floor} measured with the clear chamber
 329 followed an exponential correlation with site E_p (Fig. S4). Also, daily E_{floor} estimated from
 330 θ was significantly correlated with daily E_{floor} measured with the clear chamber ($p < 0.05$,
 331 Fig. S4). For those dates with $P > 0 \text{ mm d}^{-1}$ and/or preceded by a day with $P > 2 \text{ mm d}^{-1}$ (276
 332 days), E_{floor} was estimated from site E_p , from the exponential correlation of E_{floor} and E_p from
 333 the clear chamber measurements (Fig. S4).

334

335 *Statistical analyses*

336 We tested for significant differences ($p < 0.05$) between C_a levels over time on E_{tree} , E_{floor} and
337 soil water storage by fitting general additive mixed models (GAMMs). For this purpose, we
338 considered the ring as our experimental unit and assessed for random ring-to-ring variability
339 within each C_a level (Wood, 2006). We used the *mgcv* package in R version 3.2.2 (R
340 Development Core Team, 2014). In all fitted GAMMs, we used a cubic regression spline. For
341 the smoothed term in the model, we used up to 5-20 degrees of freedom, which resulted in
342 biologically realistic smoothed dynamics. Additionally, to quantify soil water dynamics under
343 ambient and eC_a , irrespective of horizontal and vertical heterogeneity in soil texture, we
344 estimated the numerical derivative of soil water storage (dS/dt) and its confidence interval
345 (Duursma *et al.*, 2016) as estimated from the GAMM fitted to dS/dt for ambient and eC_a , for
346 the whole vertical profile (0-4.5 m) and for specific depths.

347 To assess the effect of eC_a on the relationships between climatic (D , T_{soil} and PAR)
348 and other environmental drivers (θ_v and L) and transpiration components (E_i , E_{tree} , E_{floor}), we
349 used either GAMMs or linear mixed models (LMM). We used a LMM to assess the effects of
350 eC_a on the relationships of E_i with L ; precipitation event duration and size; with ring and
351 precipitation event as random factors. We used GAMMs to test for the effect of eC_a on the
352 climatic forcing of E_{tree} and E_{floor} , with ring and date as random factors. For E_{tree} , we included
353 D , u and PAR as predictors and L as a covariate (Duursma *et al.*, 2014). To further assess the
354 eC_a effect on the climatic forcing of E_{tree} we performed an additional GAMM with day-length
355 normalised D (D_z , Tor-ngren *et al.*, 2015). For E_{floor} (computed only from θ measured inside
356 the rings), we included understorey D and PAR, T_{soil} and θ_v (Raz-Yaseef *et al.*, 2012).

357 We estimated our ability to close the water balance by calculating P minus
358 $(R + D_g + S + E_t)$ from Eq. 2. We computed the overall water balance for the study site, with
359 site P , R , D_g and average ($n = 6$ rings) S and E_t . We calculated the terms of the water

360 balance for each season (summer, DJF; autumn, MAM; winter, JJA; and spring, SON). We
361 considered that we had closed the water balance when the sum of the water balance
362 components (Eq. 2) was at least 75% of precipitation for that period, i.e.
363 $|(P - R + D_g + S + E_t)/P| < 0.25$ (Schreiner-McGraw *et al.*, 2016).

364

365 **Results**

366

367 *Meteorological parameters and precipitation*

368 All the data and analyses presented in this manuscript are published here for the first time,
369 except for the raw climatic data (temperature, precipitation and air relative humidity, Gimeno
370 *et al.*, 2016) and the leaf area index (L , Duursma *et al.*, 2016). Prior to the start of the eC_a
371 treatment, the EucFACE site experienced a very wet summer and early autumn (P_{cum}
372 December 2011-April 2012: 647 mm, or 2/3rds of long-term annual average), followed by an
373 average autumn and winter. During the eC_a ramp-up, the site experienced a wet and warm
374 spring and summer, and a heat wave when we recorded the highest D on site for the study
375 period (7.9 kPa, Fig. 1). In late January 2013, the site received $P_{cum} = 191$ mm in 7 days (Fig.
376 2) that led to temporary standing water on site for 48 h. The first year of full eC_a treatment
377 (commencing on February 2013) was characterized by a warm and dry winter, followed by
378 an unusually hot and dry early spring that boosted daily D and E_p close to typical midsummer
379 values (Fig. 1). This was followed by a rainy spring ($P_{cum} = 218$ mm in November 2013, Fig.
380 2) that preceded a drier than usual summer ($P_{cum} = 87$ mm in February 2014) and finally an
381 average autumn and winter in 2014 (Fig. 1, Table 1).

382

383 *Surface runoff, drainage and groundwater depth*

384 Over the study period, P_{cum} exceeded maximum soil water storage capacity (108 mm) during
385 one precipitation event (27 January 2013; Fig. 2) with an excess of 39 mm over 24 h. During
386 this period E_p was 8 mm, so R was estimated as 31 mm (Table 1). Furthermore, the site
387 experienced one event (15 November 2013) that should have exceeded soil infiltration
388 capacity, but the soil was not saturated and standing water was absorbed before any R was
389 generated.

390 From July 2013 to October 2014, mean groundwater depth was 12.8 m and ranged
391 from 12.64 to 12.96 m. The variability in the groundwater depth did not show any daily or
392 seasonal patterns (Fig. S2). The isotopic signature of xylem water under dry and wet
393 conditions did not match the signature of groundwater (Fig. S3). These analyses, together
394 with the absence of live roots below 1 m depth observed during the augering of 15 holes of
395 4.5 m depth each, suggests that the EucFACE deep-rooted vegetation (trees) did not access
396 groundwater. Henceforth, we argue that observed changes in the groundwater depth were
397 likely associated with regional groundwater δ surface water interactions governed by the
398 water-level in the Hawkesbury River. Our approach to calculate D_g (Eq. 3) should be valid
399 for the temporal (2 years) and spatial (~1 ha of instrumented study area) scale of this study.

400 The contribution of D_g to the water balance varied from 20% (spring 2012) to 2% in
401 summer 2013 (Table 1), although it should be noted that seasonal D_g and P could be
402 temporally uncoupled. Coupling depends on the P dynamics and the level of antecedent
403 saturation in the soil column. At our site, D_g might be lagged with respect to seasonal P when
404 the upper soil column (0-3 m, sand and sandy clay loam) was saturated with water, but not
405 the deep soil (below 3 m, clay). This would have led to an initial increase in deep soil water
406 storage that would have later drained below the root-zone. This was the case in autumn 2013
407 when D_g according to our calculation was negative (i.e. apparent reduction in the amount of

408 water below the rooting zone). With the exception of this latter season, our approach showed
409 that over the study period, there was an excess of water draining beyond the root zone.

410

411 *Effect of elevated CO₂ on soil water storage (S)*

412 Initial measurements of S (June 2012) were the maximum over the study period. S_{\max} varied
413 among rings due to differences in soil texture (ring 6 with the highest clay content had the
414 highest S_{\max} : 828 mm) and micro-topography (S_{\max} in ring 5 with the lowest elevation was
415 758 mm, while in ring 1: 657 mm). We found that S decreased continuously in all rings
416 during 2012, until early 2013, when large precipitation events increased S close to S_{\max} (Figs.
417 2 and 3a). In 2013, S also decreased until another series of rain events in November, when S
418 increased, but did not reach S_{\max} (Fig. 3a). During the first half of 2014, S decreased until a
419 series of precipitation events in the middle of the year (August) interrupted this trend (Fig.
420 3a), yet S did not reach S_{\max} . The complete overlapping of the 95% CI of the fitted GAMM to
421 S over time between C_a levels indicated that there were no significant differences in S
422 between C_a levels (Fig. 3a). Similarly, we found that S integrated over 0.25 or 0.5 m depth
423 intervals did not differ between C_a (Fig. S5).

424 We found that S decreased significantly for most of the study period, ($dS/dt < 0$,
425 $p < 0.05$), particularly during the autumn-winter (Fig. 3b). During wet periods, S increased,
426 but dS/dt was significantly positive only in summer 2013 and spring 2014. The 95% CI of the
427 fitted GAMM for ambient and eC_a overlapped over the entire period, indicating that there
428 were no significant differences in dS/dt (Fig. 3b). We found similar results for dS/dt
429 calculated for specific depths up to 2 m (Fig. S6). Below 2 m depth, in 2012 (pre-treatment
430 and ramp-up), dS/dt in eC_a was positive or zero when our measurements commenced and
431 then it declined progressively to stable negative values, non-significantly different from those
432 in ambient C_a . This result was strongly driven by the contrasting trends observed in two of

433 the study rings that happened to be randomly assigned to different C_a levels and it is not
434 likely to have been generated by the eC_a treatment *per se*. Instead, this resulted from the
435 preceding heavy rainfall that most likely led to lateral water redistribution towards ring 5,
436 which has the lowest elevation and which happened to be randomly assigned to the eC_a
437 treatment. Additionally, this could have also resulted from a greater rate of soil water decline
438 observed in one of the ambient C_a rings (ring 6), which was not mimicked by the other two
439 ambient rings (2 and 3), but yet affected the overall mean of the ambient C_a treatment. We
440 suggest that vertical heterogeneity in the soil texture structure was responsible for differences
441 among ambient C_a rings.

442

443 *Stem flow, throughflow and interception under elevated CO_2*

444 We found that S_f , measured adjacent to the EucFACE rings, was significantly correlated
445 ($p < 0.001$) to tree basal area, and the quantity and duration of P events (Table S1). The
446 estimated contribution of S_f within each ring was less than 2% of precipitation.

447 E_i did not differ between C_a levels (Table 2). The best model for E_i included L ,
448 quantity and duration of the P event, but it did not include C_a , or its interactions (Table 2).
449 Since L did not respond to eC_a , indirect eC_a effects on E_i were discarded. Across the seasons,
450 the contribution of E_i to E_t was not negligible (Fig. 4) and ranged from 5% (in winter 2013)
451 to 24% (summer 2013). The main driver of E_i was P_{cum} (Table 2) so differences in the
452 relative contribution of E_i to E_t between years and within season were due to differences in P
453 (Fig. 4 and Table S2).

454

455 *Canopy transpiration and understorey evapotranspiration under elevated CO_2*

456 A leaf flushing event occurred shortly after the start of the implementation of the full eC_a
457 treatment, thus the potential direct effect of eC_a on leaf-level transpiration would have been

458 realized since the beginning of the experimental treatment. Over the study period, neither
459 E_{tree} , nor E_{floor} differed between C_a levels, as evidenced by the consistent overlap of the 95%
460 GAMM confidence intervals (Fig. 5, 6, S7 and S8). E_{tree} constituted the largest proportion of
461 seasonal E_t , followed by E_{floor} , in all seasons (Fig. 4). The mean contributions of E_{tree} and
462 E_{floor} to E_t were 63% and 20%, respectively, and ranged from 72% (winter 2013) to 58%
463 (summer 2013) for E_{tree} and from 23% (spring 2013) to 15% (winter 2014) for E_{floor} . Daily
464 E_{tree} showed the typical three phase response to D_z variation, initially rising with increasing
465 D_z until it reached a plateau and then decreasing, with no significant differences between C_a
466 levels (Fig. 6). The GAMM also showed that E_{tree} was strongly driven by total daily PAR,
467 again with no significant differences between C_a levels (Fig. S7). E_{floor} was strongly
468 controlled by θ_v followed by T_{soil} and understorey D with no significant differences between
469 C_a levels (Fig. S8).

470

471 *The overall water balance*

472 We quantified the water balance components for our site and according to our criteria,
473 $|(P - R - D_g - S - E_t)/P| < 0.25$, we achieved good closure in summer and spring 2013 and in
474 winter 2014. Also, we were able to account for 44% of P in winter 2013 (Table 3). We were
475 unable to close the water balance in spring 2012, autumn 2013 and summer 2014; three
476 seasons preceded by large precipitation events (Fig. 2), when soil water storage increased by
477 more than 100 mm (Table 1).

478

479 **Discussion**

480

481 Our study constitutes the most comprehensive quantification of the hydrological balance of a
482 tree-dominated FACE experiment (Leuzinger & Körner, 2010, Schäfer *et al.*, 2002). Previous

483 forest FACE studies found reduced tree water-use (Warren *et al.*, 2011) but neglected the eC_a
484 effect on understorey evapotranspiration (except from Schäfer *et al.*, 2002) or on the
485 partitioning to S , D_g and R . Furthermore, none of these were conducted in water-limited
486 sites and while some climatic land-surface models predict an increase in S and eventually in
487 R and D_g (Betts *et al.*, 2007, Gedney *et al.*, 2006); this prediction does not appear to hold for
488 water-limited regions (Ukkola *et al.*, 2016). Furthermore, all these predictions are based on
489 retrospective analyses and thus are limited to present-day C_a and cannot account for projected
490 C_a increases for most of the 21st century. For water-limited regions, process-based theoretical
491 models predict that increased L offsets reduced leaf-level transpiration under eC_a (Donohue *et*
492 *al.*, 2017, Macinnis-Ng *et al.*, 2011). Here, for our water-limited woodland, we had
493 hypothesised that reduced E_{tree} under eC_a , would not be offset by increased L (Duursma *et al.*,
494 2016) and since the site is water-limited, any excess water resulting from reduced E_{tree} would
495 be quickly lost via E_{floor} , meaning no net increase in S . We found no changes in E_{tree} , E_{floor}
496 or S , thus eC_a did not reduce stand water-use in this mature water-limited woodland.

497

498 *Canopy transpiration under ambient and elevated CO_2*

499 Contrary to our expectations, we did not find a reduction in E_{tree} under eC_a , neither in daily
500 mean nor maximum v_s . Some previous forest FACE studies had found non-significant
501 reductions in E_{tree} under eC_a (Bobich *et al.*, 2010, Ward *et al.*, 2013); but in these studies,
502 reductions in leaf-level transpiration were offset by increased L , which we did not observe
503 (Duursma *et al.*, 2016). In our case, g_s of the canopy-dominant tree (*E. tereticornis*)
504 temporarily decreased by 20%, but this reduction was transient and became non-significant
505 when water availability became most limiting and D peaked (Gimeno *et al.*, 2016).
506 Furthermore, any given decrease in g_s is usually translated into a weaker transpiration
507 response because there are additional sources of variability affecting the upscaling from leaf-

508 to canopy-level processes such as micrometeorology, canopy patchiness and/or vertical
509 variations in leaf anatomy, even in well-coupled canopies, such as ours (Jarvis &
510 McNaughton, 1986). Hence it is not surprising that the partial reduction observed in g_s from
511 discrete campaigns restricted to the upper part of dominant trees did not scale to the canopy
512 level. Additionally, our observations from this native woodland are inherently affected by the
513 natural variability. For example, the mean coefficient of variation for mean daily v_s within
514 rings (i.e. among trees) was 38%, whereas within C_a levels (i.e. among rings) it was 24%,
515 despite selection of the most representative and comparable trees that contributed up to 50%
516 of the total basal area within each ring. Given that maximum measured reduction in g_s was
517 20% (Gimeno *et al.*, 2016); we cannot discard that potential transient reductions in canopy
518 transpiration could have been obscured by the natural variability among trees for this two-
519 year study (Paschalis *et al.*, 2017).

520 Besides the expected direct effect of eC_a on E_{tree} , we also expected indirect effects,
521 beyond changes in L that did not occur (Duursma *et al.*, 2016). Elevated C_a could have also
522 indirectly affected E_{tree} by modifying climatic forcing of transpiration; for example, the slope
523 of the relationship between D and transpiration can decrease under eC_a (Duursma *et al.*, 2014,
524 Wullschleger & Norby, 2001). In our study, there were no significant differences between C_a
525 levels in the response of E_{tree} to D , including a much larger D (up to 7.9 kPa) than
526 experienced previously at another forested FACE experiment (Tor-ngern *et al.*, 2015). This
527 result is consistent with the lack of a significant effect of eC_a on the combined sensitivity of
528 stomata to C_a and D in eucalypts (Gimeno *et al.*, 2016, Kelly *et al.*, 2016). Taken together,
529 these results suggest that eC_a is unlikely to alleviate increasing atmospheric drought stress in
530 a climate change scenario with warmer temperatures and higher D (Nelson *et al.*, 2004), at
531 least in this mature eucalypt woodland.

532

533 *Understorey evapotranspiration and canopy interception under elevated CO₂*

534 We had hypothesised that excess water resulting from reduced E_{tree} under eC_a would be lost
535 via enhanced E_{floor} , but here, E_{tree} did not decrease under eC_a and there was no increase in
536 E_{floor} . Consistently, soil water storage (S) did not differ between ambient and eC_a plots at any
537 depth and neither did S over the study period, which vertically extends similar results for
538 the upper soil (0-30 cm) on the same site (Drake *et al.*, 2016, Pathare *et al.*, 2017). Previous
539 studies had reported that soil moisture was not conserved in eC_a in water-limited regions
540 (Nowak *et al.*, 2004) and without increased water availability; it is not surprising that there
541 was no change in E_{floor} . We did not find an indirect effect on E_{floor} either, as the response of
542 E_{floor} to T_{soil} and θ_v was unaffected by eC_a . With our approach, we cannot separate the
543 contributions of understorey transpiration and soil evaporation to E_{floor} , which in this type of
544 woodland are both likely to constitute an important fraction of ecosystem water-use (Ferretti
545 *et al.*, 2003, Nolan *et al.*, 2014). In our study, the evaporative component could not have been
546 affected by eC_a because soil water did not increase (Drake *et al.*, 2016) and the amount of
547 incident radiation in the understorey did not decrease, as L was unaffected (Duursma *et al.*,
548 2016). In contrast, we could have still expected that transpiration by the understorey
549 responded to eC_a , in addition to indirect environmental effects (via changes in soil moisture
550 or incident radiation). Nevertheless, three years of measurements on the dominant
551 understorey grasses on-site revealed that under eC_a neither g_s decreased, nor did herbaceous
552 biomass increase (Collins *et al.*, 2018, Pathare *et al.*, 2017).

553 We quantified the contribution of E_i to E_t and whether this component changed under
554 eC_a . In agreement with previous studies (Crockford & Richardson, 2000, Gash, 1979, Soubie
555 *et al.*, 2016), quantity and duration of the P events were the main drivers of E_i , T_f and S_f .
556 More abundant and longer events resulted into larger E_i , but contrary to expectations, we
557 found a negative effect of L on E_i . The prediction that E_i increases with L is based on

558 observations from densely packed canopies with horizontally angled leaves (Kergoat, 1998).
559 However, in most *Eucalypt* woodlands, including ours, leaves are angled vertically or near-
560 vertically; hence, P falling on the vegetation mostly contributes to T_f instead of E_i (Crockford
561 & Richardson, 2000). Nevertheless, given that neither L (Duursma *et al.*, 2016) nor tree radial
562 growth rate increased under eC_a (Ellsworth *et al.*, 2017), we are unlikely to observe any
563 change in the partitioning of P into T_f , S_f and E_i under eC_a , in mature *Eucalypt* woodlands.
564 Even under a scenario where L and/or growth responded to eC_a , we would still predict that E_i
565 would not change because the contribution of S_f is negligible (and thus so would be potential
566 increases in S_f due to radial growth increments) and greater L would not increase E_i with
567 these leaf angles. This later result is relevant for improving our ability to estimate ecosystem
568 water-use and to predict vegetation-atmospheric coupling under future atmospheric
569 conditions. Currently, most process-based models assume that E_i increases with eC_a (De
570 Kauwe *et al.*, 2013, Zhang *et al.*, 2016), but our results suggest otherwise for this type of
571 woodland, over the study period.

572

573 *Lack of effective water-savings under elevated CO₂*

574 Over the study period, potential evapotranspiration (E_p) exceeded precipitation (P) in all
575 seasons and total E_t was always less than E_p , which indicates that our site was water-limited
576 during the study period, at this time-step. We found that despite being water-limited, seasonal
577 E_t was often less than P , which allowed some P to be partitioned to S , R and D_g . Here, we
578 followed a conservative approach to calculate D_g from deep soil water dynamics assuming an
579 effective rooting depth of 3 m. With this approach, we are likely overestimating the decrease
580 (or underestimating the increase) in S and thus we are underestimating the amount
581 partitioned to D_g . For those seasons where we calculated a decrease in S , our estimate of E_t
582 was not similar to S plus P , minus R and D_g . Our estimates of E_{tree} and E_{floor} are comparable

583 to observations from similar (Nolan *et al.*, 2014) and nearby forests (Bourne *et al.*, 2015) and
584 the proportion of seasonal E_i is within the range of other studies (Kergoat, 1998, Soubie *et*
585 *al.*, 2016). Hence, it is not reasonable to believe that we largely underestimated E_t (by nearly
586 100%). A more plausible explanation is that we overestimated the effective rooting depth
587 (Macinnis-Ng *et al.*, 2010), but without a detailed survey of root distribution we are unable to
588 provide a more realistic S . This latter explanation would agree with the estimates from a
589 recent modelling study (Yang *et al.*, 2016a), which estimated an effective rooting depth of
590 1.2 m in the region. Here, we opted for a more conservative approach and established an
591 effective rooting depth based our own observations and characterization of the soil texture
592 profile. Nevertheless, despite the uncertainty regarding effective rooting depth, we found that
593 eC_a had no effect on the temporal dynamics of S or S at any depth during the entire study
594 period, further supporting our argument that eC_a did not increased soil water-storage at any
595 depth in this woodland.

596 In addition to our estimate of rooting depth, there may be other sources of uncertainty,
597 such as our coarse approach to estimate R from measurements of P and soil properties. We
598 established that R would only occur at times when the soil was fully saturated and
599 precipitation exceeded the infiltration capacity, yet since EucFACE occurs on an alluvial
600 floodplain, soil saturation may not occur homogeneously across the site. Indeed, some
601 unquantified R might have been generated in areas where the soil saturated faster than the
602 overall site mean due to spatial heterogeneity in soil texture. Furthermore, we did not account
603 for the contribution of possible deep lateral flow that can occur in multilayered strongly
604 contrasting textured soils (Cox & Pitman, 2002), such as at our site. These uncertainties could
605 explain our inability to fully close the overall water balance for our study site for those
606 seasons preceded by very rainy season, when we would have been more likely to
607 underestimate D_g , R and lateral flow.

608 We examined the impact of eC_a on the hydrological balance of a native, mature
609 woodland at the stand level for 30 months and during periods of water-limitation. Elevated C_a
610 did not alter S , E_{tree} , E_{floor} , E_i or E_t during this time. Furthermore, eC_a did not indirectly affect
611 E_t through changes in growth, phenology or L . In addition, we did not find significant effects
612 of eC_a on the climatic forcing of transpiration, such that under a future climate change
613 scenario (i.e. altered precipitation patterns and warmer global surface temperatures), more
614 severe water-stress due to an increase in evaporative demand would not be alleviated under
615 eC_a in this type of woodland. Based on this study, in water-limited catchments dominated by
616 mature woodlands we should not expect changes in the amounts of precipitation partitioned
617 to R and D_g in response to future increases in C_a .

618

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620

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638

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851 **Tables and Figures**

852

853 **Table 1.** Total seasonal potential evapotranspiration (E_p), precipitation (P), surface runoff (R), drainage (D_g) change in soil water storage (S)
854 and total evapotranspiration (E_t). E_t is the sum of canopy interception (E_i), canopy transpiration (E_{tree}) and soil evaporation and understorey
855 transpiration (E_{floor}). E_p , P , R and D_g were measured or calculated at the site level. Depicted values of S , E_t , E_i , E_{tree} and E_{floor} are the mean ($\pm se$)
856 of the three rings for each atmospheric CO₂ level (ambient, a, and elevated, e). There were no significant differences ($p > 0.01$) between CO₂
857 levels for any of the hydrological components. All values in mm per season.

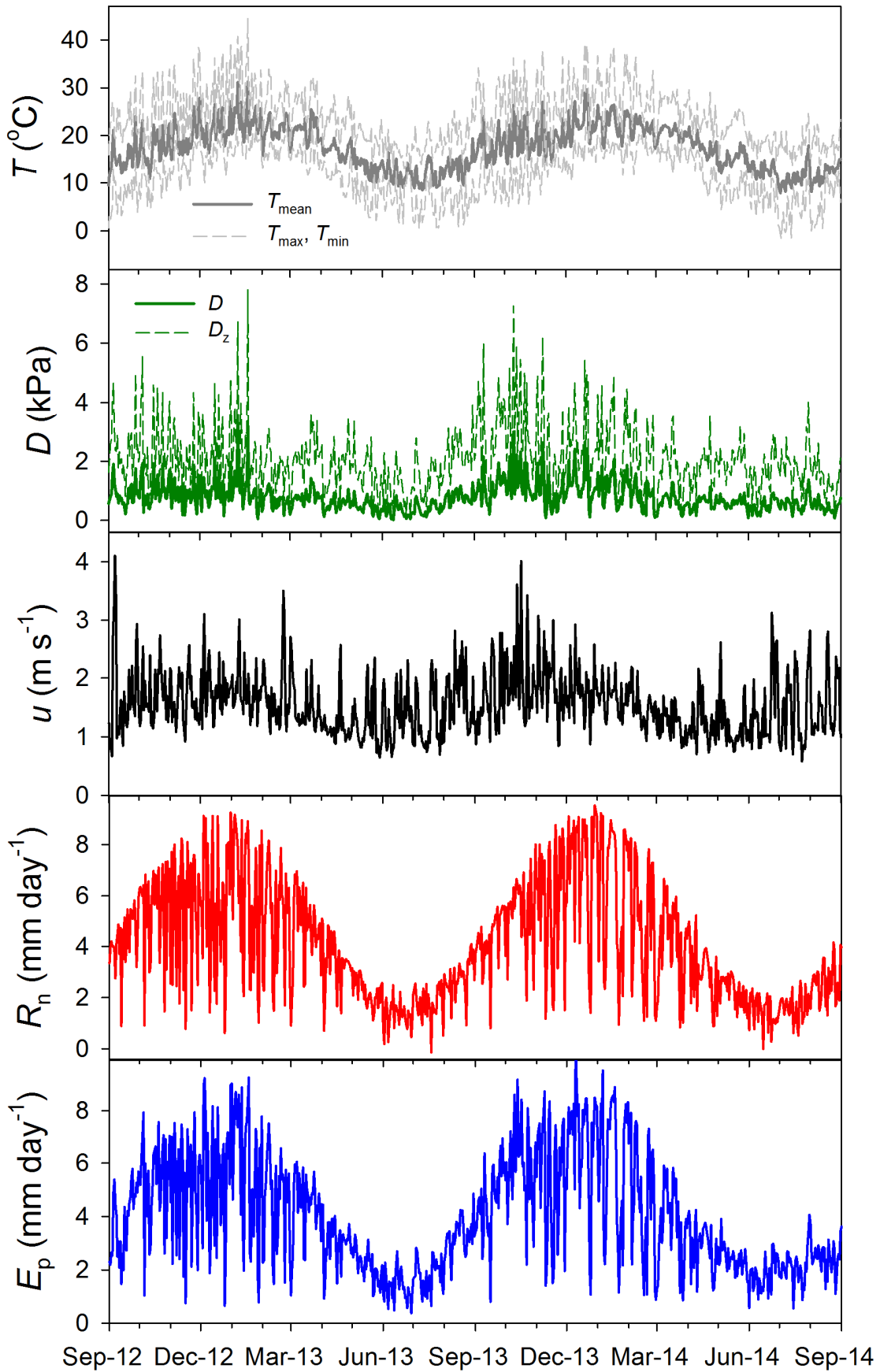
Season	CO ₂	E_p	P	R	ΔS	E_t	E_i	E_{tree}	E_{floor}
Spring-2012	a	429	92	0	-106±10	97±5	10±2	64±3	23±1
	e				-109±23	108±20	17±9	68±11	23±1
Summer-2013	a	486	377	31	133±17	190±22	47±9	109±15	35±2
	e				119±26	206±32	50±10	122±21	33±2
Autum-2013	a	296	155	0	-113±13	153±9	20±1	99±12	34±4
	e				-89±26	151±21	24±7	100±18	28±3
Winter-2013	a	197	84	0	-45±17	84±9	3±1	61±8	19±1
	e				-48±18	81±10	5±2	57±11	19±1
Spring-2013	a	488	250	0	114±40	120±13	21±8	75±6	24±1
	e				110±40	126±16	32±8	69±12	24±1
Summer-2014	a	536	151	0	-149±30	159±10	16±4	106±8	36±1
	e				-139±23	172±23	23±6	111±21	38±4
Autum-2014	a	275	170	0	-26±12	132±3	30±7	76±9	27±2
	e				-34±4	142±22	28±5	89±17	15±1
Winter-2014	a	195	150	0	25±3	80±6	18±1	50±6	13±0.2
	e				14±4	87±11	20±6	54±12	13±0.5

858 **Table 2.** Results (estimated coefficients \pm se, t and p -value) of the best linear mixed model fit
 859 to canopy interception (E_i , log-transformed). Selected fixed factors were: event precipitation
 860 (P_{cum} in mm, log-transformed), event duration (in h) and leaf area index (L , in $m^2 m^{-2}$). The
 861 CO_2 treatment did not significantly affect E_i or the any of the effect sizes.

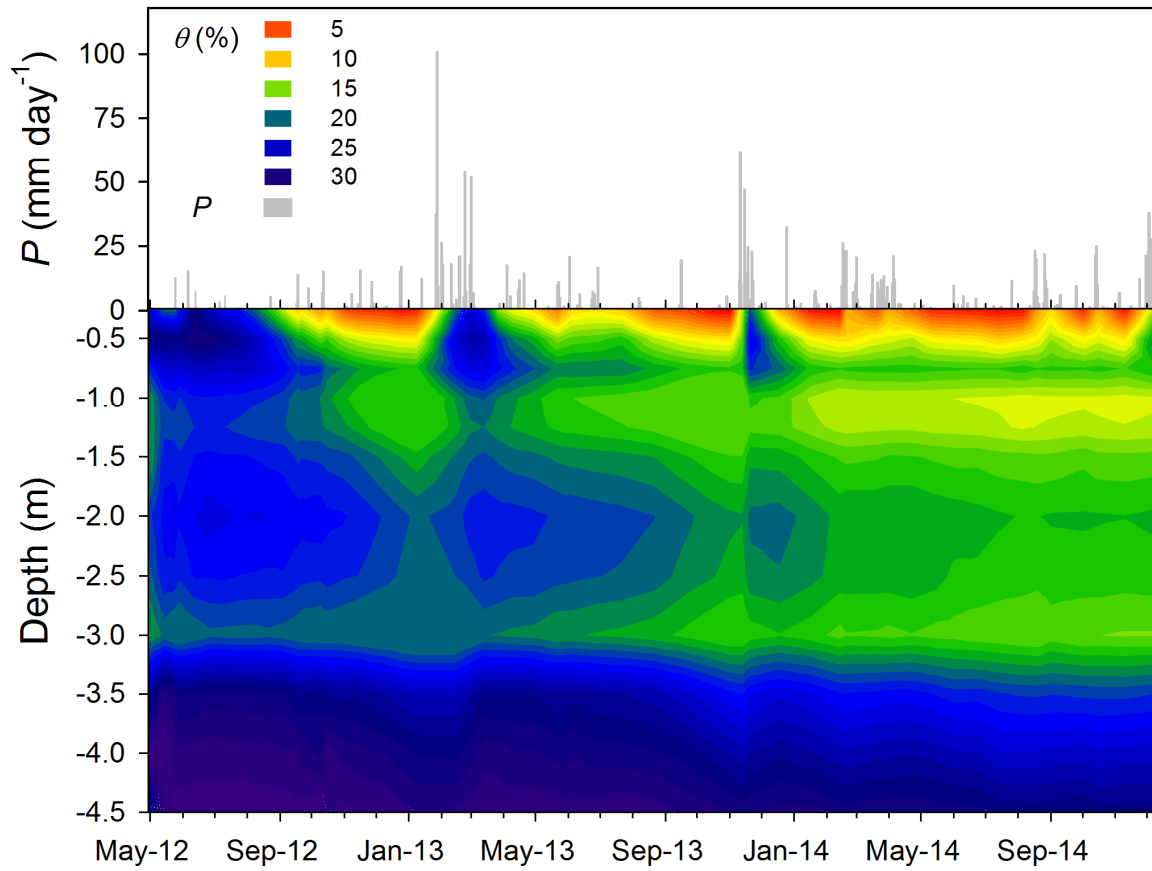
Fixed	Estimate	t	p
Log(P_{cum})	0.322 ± 0.02	15.2	<0.001
Duration	0.03 ± 0.006	5.5	<0.001
L	-0.18 ± 0.09	-2.1	0.038

862 **Table 3.** Seasonal values of the ratio of precipitation (P) to potential evapotranspiration (E_p), P , surface runoff (R), drainage (D_g) change in soil
863 water storage (S) and total evapotranspiration (E_t). For terms measured at the ring level (S and E_t), depicted values are the mean (se) of the six
864 rings. The error denotes the disequilibrium from the closure of the water balanced calculated as: $P \pm R \pm D_g \pm S \pm E_t = 0$. All values, except for
865 P/E_p and $|\text{Error}/P|$ (both unitless), are in mm per season.

Year	2012			2013			2014		
Season	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	
P/E_p	0.21	0.78	0.53	0.42	0.51	0.28	0.62	0.77	
P	92	377	155	84	250	151	170	150	
R	0	31	0	0	0	0	0	0	
D_g	19	6	-4	11	20	18	4	11	
S	-107 (11)	126 (14)	-101 (14)	-47 (11)	112 (25)	-144 (17)	-30 (6)	19 (3)	
E_t	103 (9)	198 (18)	152 (10)	82 (6)	123 (9)	165 (12)	137 (10)	84 (6)	
Error	78	16	108	37	-5	111	58	36	
$ \text{Error}/P $	0.84	0.04	0.69	0.44	0.02	0.74	0.34	0.24	



867 **Fig. 1** Meteorological variables during the study period from top to bottom: maximum (T_{\max})
868 and minimum (T_{\min}) daily temperatures (dashed grey lines), mean daily temperature (T_{mean}),
869 mean daily water pressure deficit (D , continuous green line) and day-length normalized D
870 ($D_z = D n_d/24$, where n_d is the number of daylight hours dashed green line), mean daily wind
871 speed (u), total net radiation (R_n) and total Penman potential evapotranspiration (E_p).
872

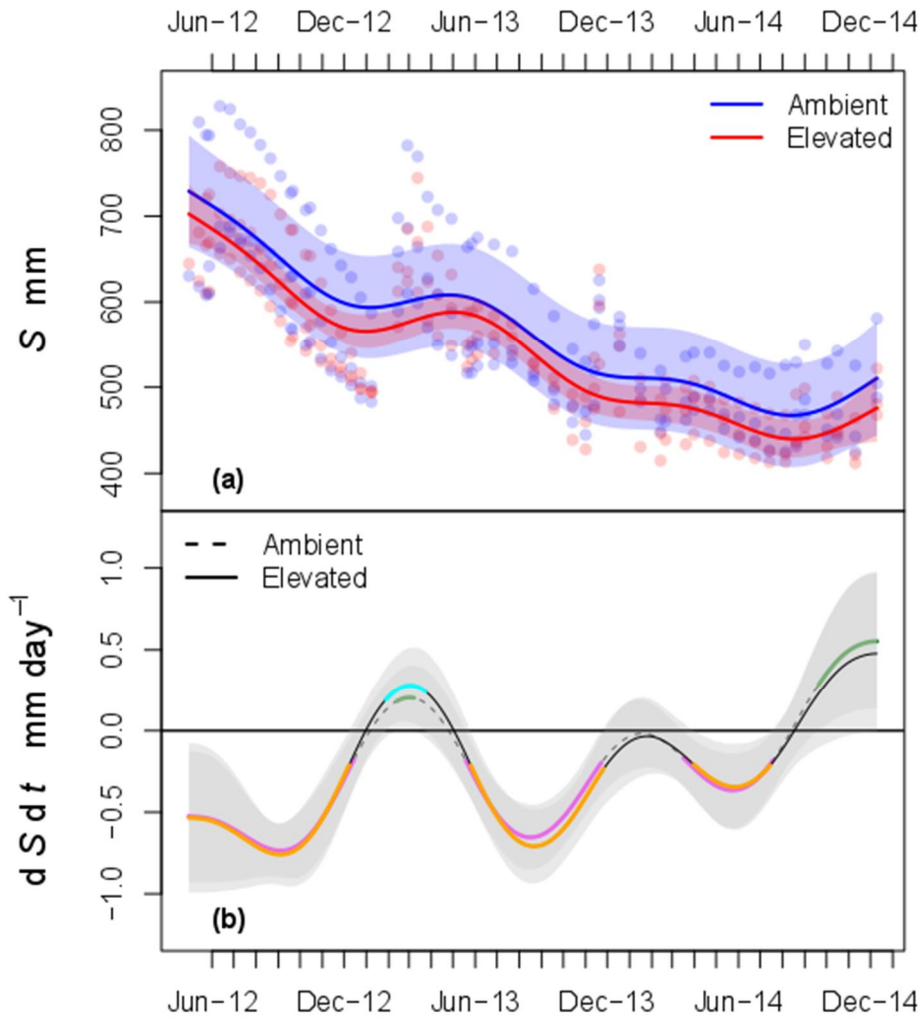


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875 **Fig. 2** Daily precipitation (P , grey bars) and temporal evolution of the vertical profile of soil
 876 volumetric water content (θ) inferred from mean ($n = 6$ rings) periodical measurements.

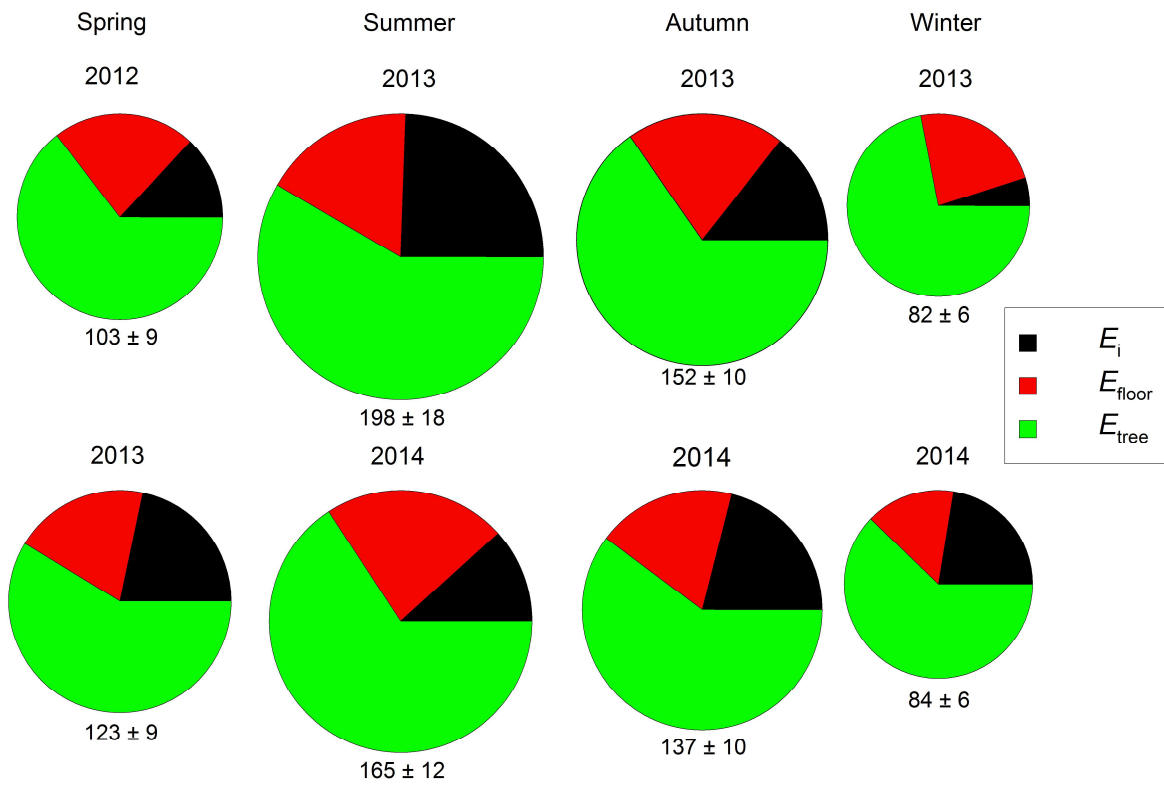
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879 **Fig. 3 (a)** Soil water storage (S) over a soil column of 3 m depth, each point is the mean of
 880 two locations within each ring, lines and polygons represent the fitted generalized additive
 881 models with their 95% confidence intervals (CI) for each CO_2 treatment level. **(b)** Mean
 882 estimated daily changes in S (dS/dt , lines) and 95% CIs (polygons). Rates of change not
 883 significantly different from 0 are indicated with dashed (ambient) or continuous (elevated)
 884 black lines. Significantly negative changes are indicated with pink (ambient) and orange
 885 (elevated) lines and positive changes with cyan (elevated) and green (ambient) lines.

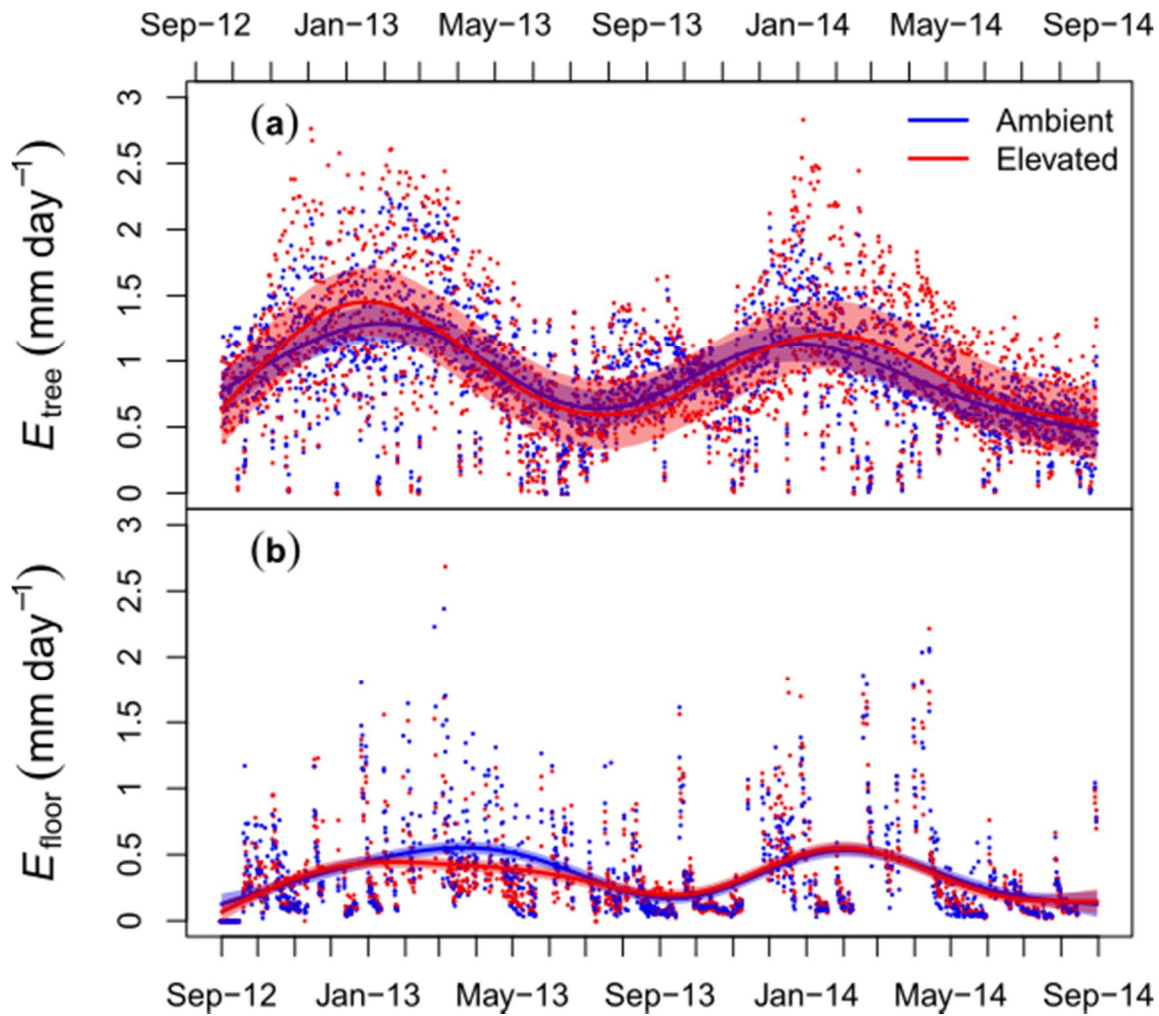
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888 **Fig. 4** Partitioning of total evapotranspiration among: canopy interception (E_i), canopy
 889 transpiration (E_{tree}) and understory transpiration together with floor evaporation (E_{floor}). The
 890 size of each pie-chart is proportional to the corresponding seasonal evapotranspiration (in mm
 891 per season) indicated (site mean \pm se, $n = 6$ rings).

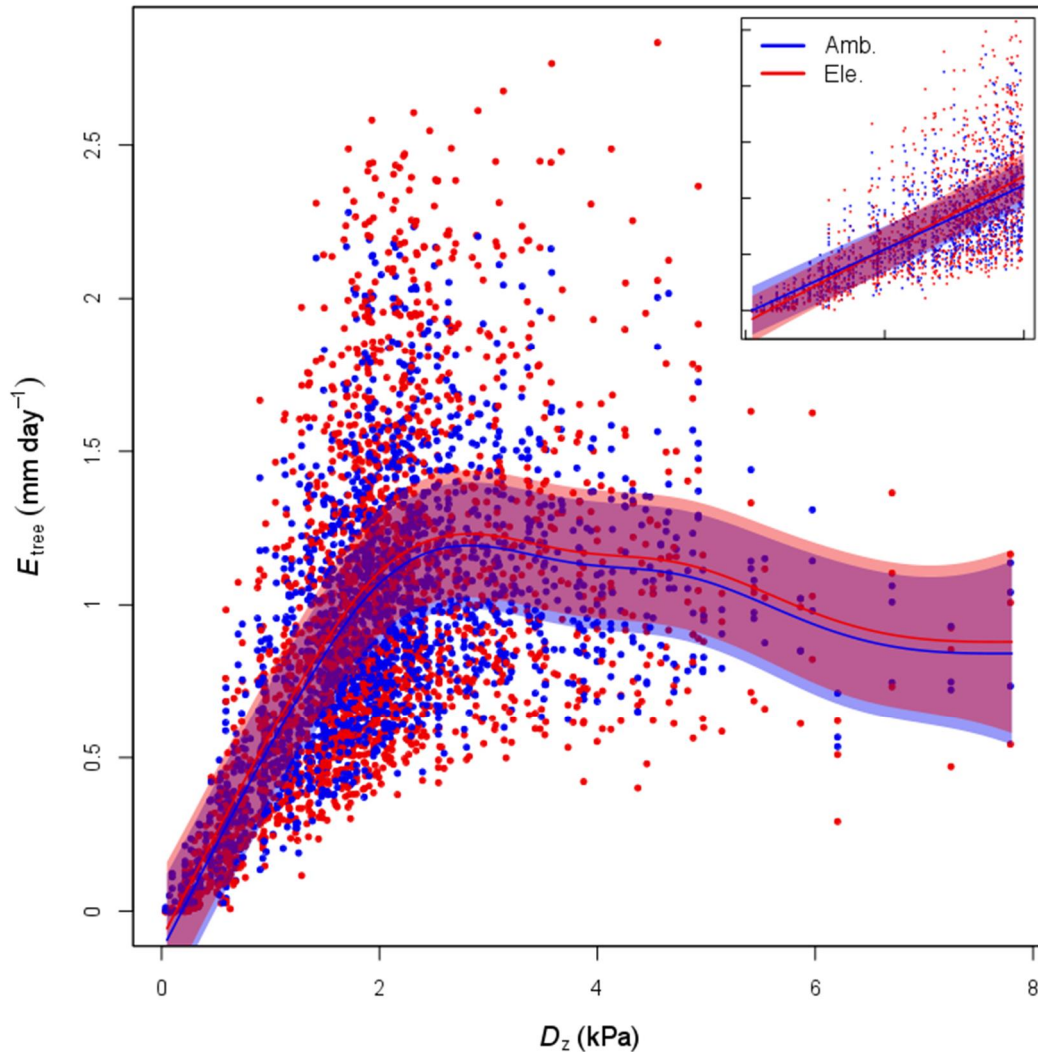
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894 **Fig. 5** (a) Estimated daily canopy tree transpiration (E_{tree}) and (b) understorey
 895 evapotranspiration (E_{floor}) for each ring under different C_a concentrations levels: ambient
 896 (blue) and elevated (red). Lines and polygons represent the fit of the GAMM for each C_a
 897 level with their 95% confidence intervals. E_{tree} was estimated from mean ($n = 3-4$ trees per
 898 ring) sapflow velocities and E_{floor} was estimated from mean ($n = 2$ locations per ring) changes
 899 in shallow (5 cm) soil volumetric water content.

900



901

902 **Fig. 6** Estimated daily canopy tree transpiration (E_{tree}) under different CO_2 concentrations:
 903 ambient (blue) and elevated (red) plotted against day-length normalised mean daily vapour
 904 pressure deficit (D_z). Lines and polygons represent the estimated fit of the GAMM for each
 905 CO_2 level with their 95% confidence intervals (CI). E_{tree} was estimated from mean
 906 ($n=3-4$ trees per ring) sapflow velocities. Figure inset represent the fits and CIs of a linear
 907 mixed model restricted to $D_z < 2$ kPa, where daily E_{tree} increased linearly with D_z ($t = 20.6$,
 908 $p < 0.01$) and depicted lines have slopes (\pm se) of 0.57 ± 0.03 and 0.67 ± 0.01 $\text{mm day}^{-1} \text{kPa}^{-1}$,
 909 for ambient and elevated CO_2 levels, respectively.