Running title: Hydraulic strategies of root xylem

ROOT XYLEM CHARACTERISTICS AND HYDRAULIC STRATEGIES OF SPECIES CO-OCCURRING IN SEMI-ARID AUSTRALIA

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ABSTRACT

Xylem traits such as xylem vessel size can influence the efficiency and safety of water transport and thus plant growth and survival. Root xylem traits are much less frequently examined than those of branches despite such studies being critical to our understanding of plant hydraulics. In this study, we investigated primary lateral and sinker roots of six co-occurring species of semi-arid Australia. Two species are restricted to a floodplain, two were sampled only from the adjacent sand plain, and two species co-occur in both habitats. We assessed root wood density, xylem traits (i.e., vessel diameter, fibre and vessel wall thickness), outer pit aperture diameter and calculated theoretical hydraulic conductivity and vessel implosion resistance. We hypothesized that (1) roots have larger xylem vessel diameters and lower wood density than branches of the same species and that (2) there is an inverse correlation between theoretical sapwood hydraulic conductivity and vessel implosion resistance for roots.

Variation in root wood density was explained by variations in xylem vessel lumen area across the different species (r^2=0.73, p=0.03), as hypothesized. We rejected our second hypothesis, finding instead that the relationship between theoretical hydraulic conductivity and vessel implosion resistance was not maintained in roots of all of our studied species, in contrast to our previous study of branches from the same species. Xylem traits were found to depend upon habitat and eco-hydrological niche, with the groupings including (i) arid-adapted shrubs and trees with shallow lateral roots (*Acacia aneura* and *Psydrax latifolia*), (ii) trees restricted to the floodplain habitat, both evergreen (*Eucalyptus camaldulensis*) and deciduous (*Erythrina vespertilio*) and (iii) evergreen trees co-occurring in both floodplain and adjacent sand plain habitats (*Corymbia opaca* and *Hakea* sp.).

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INTRODUCTION

Xylem traits play an essential role in the efficiency and safety of water transport through the plant, influencing plant growth and survival across a range of environments (Smith & Sperry 2014). Xylem vessels of large diameter (e.g., > 500 μm) can transport water more efficiently and with lower resistance to flow than small xylem vessels (e.g., <20 μm) as a result of the fourth-power relationship between radius and flow through a capillary tube (i.e. Hagen-Poiseuille law; Tyree & Zimmerman 2002). However, the architecture of the pits between large xylem vessels can also increase the risk of xylem cavitation events (e.g. Hargrave *et al.* 1994; Lo Gullo *et al.* 1995; Cai *et al.* 2010; Christman *et al.* 2012). Pits are the structures that interconnect vessels and can influence the efficiency and safety of plant water transport, determining as much as 50% of the xylem hydraulic resistance in species with small pit apertures (Sperry *et al.* 2005; Wheeler *et al.* 2005; Hacke *et al.* 2006). Recent studies also suggest that bubbles known as nanobubbles can move through pit membranes due to their smaller size than that of pit membrane pores (2 to 200 nm) (Schenk *et al.* 2015, Schenk *et al.* 2016). Nanobubbles, different from larger bubbles, are safe from expanding and can dissolve or remain in the pit border (a confined space) until temperatures decrease (at night), thereby increasing gas solubility. Additionally, once formed, nano-bubbles can be stabilized by amphiphilic surfactants present in the xylem sap which can transform surfaces that are hydrophobic into hydrophilic ones (Schenk *et al.* 2015).

Xylem traits of roots are much less frequently studied than those of branches or stems, in part due to difficulty in accessing this plant organ. However, roots are the primary path for water uptake by plants; therefore, knowledge of xylem structure in roots can provide critical information on overall plant water supply (Canadell *et al.* 1996; Martínez-Vilalta *et al.* 2002; McElrone *et al.* 2004; Johnson *et al.* 2014) and are likely to define key hydraulic traits of the entire plant. Previous studies have found that at a species level, root xylem has a larger risk of cavitation (but higher efficiency of water transport) than branch, stem and leaf xylem because of larger vessel diameters in roots (Sperry & Saliendra 1994; Martínez-Vilalta *et al.* 2002; Anfodillo *et al.* 2006).

Our previous work with species from the Ti Tree Basin arid zone, in central Australia (Santini *et al.* 2016) indicates that branch xylem traits can contribute to co-occurrence within close proximity of species in this arid habitat, where water resources are heterogeneously distributed

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122 in space and time (O'Grady *et al.* 2009). For example, species with shallow lateral roots such as

123 *Acacia* spp. that rely on intermittent water resources such as rainwater (Cleverly *et al.* 2016a)

124 had smaller xylem vessels in their stems than that of phreatophytic species with deep tap or

125 sinker roots and thus access to groundwater (e.g. *Eucalyptus* and *Corymbia*). We also found in

126 stem xylem that there was a significant linear regression between lower theoretical hydraulic

127 conductivities (K_s, kg mm^{-1} MPa^{-1} s^{-1}) and higher xylem vessel implosion resistance ($[t/b]^2$, the

128 ratio of vessel double-wall thickness and vessel lumen diameter), concluding that xylem vessels

129 of species with higher theoretical hydraulic conductivities (i.e. *Erythrina vespertilio*, *Corymbia*

130 *opaca* and *Eucalyptus camaldulensis*) were less protected from implosion than xylem vessels of

131 species like *Acacia* spp., *Hakea* sp. and *Psydrax latifolia* which have lower theoretical hydraulic

132 conductivities (Santini *et al.* 2016). Vessel implosion resistance may be an indicative of vessel

133 wall reinforcement, but in roots it might not be indicative of safety as tensile forces in the water

134 column are low (Hacke *et al.* 2001; Pratt *et al.* 2007). We retain the nomenclature of "vessel

135 implosion resistance" merely because it has a history within the literature, whilst recognizing

136 that it may not be a relevant index to assess root xylem vessel safety (Pratt *et al.* 2007).

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138 In the present work, we studied six co-occurring species in close proximity within the Ti Tree

139 Basin. These species can be classified into three functional groups with respect to their leaf

140 longevities and root deployment strategies: 1) deciduous trees (*Erythrina vespertilio*), 2)

141 evergreen shrubs and trees (*Acacia aneura*, *Hakea* sp. and *Psydrax latifolia*) and 3) evergreen

142 phreatophytes (*Eucalyptus camaldulensis* and *Corymbia opaca*). There are *ca.* 150 species across

143 Australia in the genus *Hakea*. Although a positive identification could not be made in this case,

144 *Hakea* sp. in this study is likely to be *Hakea arborescens* or *Hakea macrocarpa*. In general,

145 drought-deciduous trees avoid transpiration during the dry season by dropping their leaves for

146 at least one and as many as seven months of the year. Alternatively, evergreen trees retain *ca.*

147 90 % of their full canopy throughout the year (Eamus 1999). *Psydrax latifolia* forms an

148 association with *Acacia aneura*, which has a relatively shallow root system and does not access

149 the groundwater of the site (Cleverly *et al.* 2016a; Cleverly *et al.* 2016b). Phreatophytes like

150 those in the Ti Tree basin do access groundwater by having a taproot and deep sinker roots,

151 thereby allowing retention of their canopy through the dry season (Dawson & Pate 1996;

152 Roberts *et al.* 2016).

153 We measured wood density, xylem traits (i.e., vessel diameter, fibre and vessel wall thickness)

154 and outer pit aperture diameter, and then we calculated theoretical hydraulic conductivity and

155 vessel implosion resistance in a primary sinker root (in the evergreen phreatophytes) or

156 shallow lateral roots (in the remainder of the species). Vessel implosion resistance has only

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been observed in abnormal wood, but it is a measure of vessel wall reinforcement (Baas & Wheeler 2015). We hypothesized that: (1) roots have larger xylem vessel diameters and lower wood density than branches of the same species, placing roots at more risk of cavitation events than stems and (2) there is an inverse correlation between theoretical sapwood hydraulic conductivity and vessel implosion resistance for roots, similar to what we have observed in branches (Santini *et al.* 2016).

MATERIALS AND METHODS

Site description and sample collection

Our study site is located in central Australia in the Ti Tree Basin (22° 7' 48. 56'' S, 133° 24' 57. 67'' E), approximately 180 km north of Alice Springs, Northern Territory, Australia. The Ti Tree Basin is an arid catchment that covers *ca*. 5500 km^2 and contains the ephemeral Woodforde River and Allungra Creek. Mean annual rainfall at the nearest meteorological station is 320 mm year^{-1} (Australian Bureau of Meteorology 2014, BoM station 15643, Territory Grape Farm), and most of this rainfall occurs in monsoonal summer (Dec – Feb) storms (Cleverly *et al.* 2016b). The mean minimum and maximum daily temperature is 15 and 31 °C, respectively, while the maximum air temperature at the site reaches 46 °C in the summer months (December to February) (Harrington *et al.* 2002).

Woody plants were sampled along a 6.5-km transect from the banks of the Woodforde River and its floodplain (*Erythrina vespertilio n=5* and *Eucalyptus camaldulensis n=5*) into the adjacent sand plain (*Acacia aneura n=5*, and *Psydrax latifolia n=5*), and two tree species were sampled from both habitats (*Corymbia opaca n=3* and *Hakea* sp. *n=5*). All of these woody species can be found to co-occur within tens of metres of each other in this semi-arid climate, but they inhabit different eco-hydrological niches (Nolan et al. *In review*). Two trees are groundwater dependent in the Ti Tree Basin: one is restricted to riparian corridors and paleochannels (*Eucalyptus camaldulensis*); and the other (*Corymbia opaca*) is widespread across the basin (O'Grady *et al.* 2009). *Acacia aneura* is also found in both floodplain and sand plain habitats of the Ti Tree Basin (although not on this transect), but these arid-adapted trees are found on siliceous hardpan which provides a near-surface reservoir of soil water storage within their shallow root zone (Cleverly *et al.* 2016a). Plants grow as trees, except for *Psydrax latifolia*, which grows as a shrub (up to 3 m tall).

Root material was collected from three to five roots in each of six woody species (one root per

190 individual plant) of the Ti Tree Basin. Samples were obtained during the austral winter and dry
191 season, June – August 2015 (Figure 1, 2, Table 1). For each species, trees and shrubs were
192 chosen which had roots of a given diameter before sinking below a practical collection depth in
193 the hardpan soil. Roots were sought in a 360° circle around the main trunk and were collected
194 from whichever direction they were found. We collected roots by digging from the main stem to
195 a point where the target primary lateral or sinker root had a diameter of *ca.* 15 mm, at a
196 distance of *ca.* 0.5–6 m from the main trunk (Table 1).

Wood density

198 Root material collected for assessment of wood density was stored in paper bags and kept cool
199 for laboratory analysis. Wood density of roots was measured in 4 cm lengths of sapwood.
200 Sample numbers of individual roots (i.e. one root sample per plant) for assessment of wood
201 density were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n=5), *Hakea* sp. (n=5),
202 *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3) and *Eucalyptus camaldulensis* (n=5). Root
203 sapwood segments were weighed after drying to a constant mass. Wood density was calculated
204 as the ratio of dry weight (5 days at 60 °C) to volume of water displaced by the fully hydrated
205 sapwood segments (i.e., after they were submerged in water for two days; see Santini *et al.*
206 2016).

Assessment of xylem traits

208 Preparation of tissues and measurements of xylem traits were performed following Santini *et al.*
209 (2016). Root samples were cut into segments of two centimetres in length for analysis of cross
210 sections. Each root segment was fixed in formalin-acetic acid-alcohol (FAA) for ~10 days and
211 then placed in 70% ethanol for 2 days. After this washing process, samples were dehydrated for
212 one day each in increasing concentrations of ethanol: 70%, 96% and 100%. From the ~15 mm-
213 diameter samples, we prepared 10–20 μm-thick cross sections using a sledge Leica SM2010R
214 microtome. Number of samples (each representing one individual tree or shrub) for measuring
215 xylem traits were as follows: *Erythrina vespertilio n=5, Acacia aneura n=3*, *Hakea* sp. *n=4,*
216 *Psydrax latifolia n=5, Corymbia opaca n=3 and Eucalyptus camaldulensis n=5*. Next, *Erythrina*
217 *vespertilio, Acacia aneura*, *Hakea* sp. and *Psydrax latifolia* were double-stained with safranin and
218 alcian blue (1:2). *Corymbia opaca* and *Eucalyptus camaldulensis* were assessed with no-staining
219 due to the presence of resins that complicated the assessment of wood xylem traits after
220 staining. Sections were photographed for measurements using a Leica DM750 microscope.
221 Vessel diameters (minor and major axes), fibre wall thickness and vessel wall thickness were
222 measured in ImageJ v1.48 (National Institutes of Health, USA). The cross-sectional vessel lumen

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area (VA) was determined as an ellipse (i.e. as a product of π and the semi-minor and semi-major radii), which was then multiplied by vessel density (VD, number of vessels per unit cross-sectional area) to determine conductive lumen fraction (F, named CA in Santini *et al.* 2016). We determined fibre wall thickness in 30 - 50 adjoining fibres (Santini *et al.* 2013). Additionally, xylem vessel wall thickness was measured at three positions (i.e. pie-shaped sectors of 20 - 50 vessels) in the cross section (Santini *et al.* 2016).

Scanning Electron Microscopy (SEM) preparation and imaging

We measured the diameter of pit apertures in three individual roots per species (one sample per individual plant) through the analysis of SEM imagery. Samples of root tissue (which corresponded to similar positions in which we assessed the xylem traits) were hand-sectioned longitudinally to a thickness of 2 - 4 mm. The sections were treated with a series of ethanol and hexamethydisilizane as detailed in Johnson *et al.* (2014). After treatment, samples were placed into the stage of an EVO LS15 SEM (Zeiss, Germany) and were visualized in variable pressure mode. We used an accelerated voltage of 15 kV and took images with magnifications of 250X, 500X and 2kX for all our samples. Diameters of pit apertures were measured as recommended by Scholz *et al.* (2013) by assessing the widest part of the opening of a minimum of 50 pits per species with the software Image J 1.48v (National Institutes of Health, USA).

Theoretical hydraulic conductivity and resistance to vessel implosion

Theoretical hydraulic conductivity (K_s) was determined as a function of (i) conductive lumen fraction (F, equal to VA·VD) and (ii) the ratio of vessel size to vessel density (S, VA/VD; Zanne *et al.* 2010) (Equation 1).

$$K_S \propto F^{1.5} S^{0.5} \hspace{4cm} \text{(Equation 1)}$$

Vessel implosion resistance was estimated as the ratio of the double-wall thickness (t, the total vessel wall thickness between adjacent cells) to the mean vessel diameter (Hacke *et al.* 2001; Zanne *et al.* 2010). Mean hydraulic diameter was defined as $DH=(\Sigma D^4/N)^{1/4}$ and represents the mean diameter that all of the vessels in a root would have in order to correspond to the same overall conductivity for the numbers of conduits (Tyree & Zimmerman 2002).

Data analyses

We used linear regression analyses to test relationships between univariate pairs: wood density and vessel lumen area, log (vessel density) and vessel lumen area, outer pit aperture and log (vessel diameter), theoretical hydraulic conductivity (K_s) and log (xylem vessel implosion

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resistance), and vessel wall thickness and vessel diameter. Significant differences among species in fibre wall thickness, vessel wall thickness, vessel diameter, outer pit aperture, the conductive lumen fraction and wood density were assessed with a one-way ANOVA. Statistical analyses were performed with the software Prism version 7.0c (GraphPad Software, La Jolla, CA, USA).

We also analyzed data obtained from the same population of trees or shrubs used in this study (Santini *et al.* 2016). Santini *et al.* (2016) sampled three replicate branches in three individual trees/shrubs per species (n= 3 x 3 per species); in contrast, we sampled one root from each of three to five individuals per species (n = 3 to 5 per species). We tested differences in means ($\pm$ standard errors) between roots and branches in wood density, xylem traits (i.e. vessel diameter, fibre and vessel wall thickness and conductive lumen fraction) and theoretical hydraulic conductivity and vessel implosion resistance by using a t-test with the Holm-Sidak approach. This method assumes that means are sampled from populations with the same standard deviations.

<div align="center">RESULTS</div>

In the root samples, wood density values ranged from 0.21 ± 0.009 g cm^{-3} in *Erythrina vespertilio* to 0.82 ± 0.023 g cm^{-3} in *Hakea* sp. (Table 2). Vessel diameter values were different across species and varied from 24.4 ± 0.29 μm in *Psydrax latifolia* to 188 ± 6.15 μm in *Erythrina vespertilio*. Fibre walls were thinnest in *Acacia aneura* (3.9 ± 0.10 μm) and thickest in *Corymbia opaca*, *Hakea* sp. and *Eucalyptus camaldulensis* (5.7 ± 0.15 μm, 5.4 ± 0.12 μm, 5.3 ± 0.08 μm, respectively). Vessel walls were thinnest in *Psydrax latifolia* (4.12 ± 0.05 μm) and thickest in *Corymbia opaca* (11.5 ± 0.29 μm). Outer pit apertures were smallest in *Psydrax latifolia* (3.73 ± 0.05 μm) and largest in *Erythrina vespertilio* (7.13 ± 0.11 μm) (Table 2). Conductive lumen fraction ranged from 0.05 ± 0.003 in *Erythrina vespertilio* to 0.37 ± 0.008 in *Psydrax latifolia* (Table 2).

Root wood density was negatively correlated with root xylem vessel lumen area (i.e. wood density was smallest in roots with large xylem vessels; r^2=0.73, p=0.03, Figure 3). Roots of *Hakea* sp. fell outside of this relationship with a much higher root wood density for its root vessel lumen area than in the other five species. We also found strong linear regressions between log (root vessel density) *versus* root vessel lumen area (r^2=0.72, p=0.03, Figure 4) and outer pit aperture in root xylem *versus* log (root vessel diameter) (r^2=0.92, p=0.002, Figure 5). Outer pit apertures and vessel diameters were smallest in *Psydrax latifolia* (3.73 ± 0.05 μm and 24.4 ± 0.29 μm) and largest in *Erythrina vespertilio* (7.13 ± 0.11 μm and 188 ± 6.15 μm) (Table

287 2, Figure 5). The two species which were collected from only the sand plain (*Psydrax latifolia*

288 and *Acacia aneura*) had the highest vessel density and lowest vessel lumen area, vessel

289 diameter and outer pit aperture (Figures 3 - 5). By contrast, the two riparian trees (*Eucalyptus*

290 *camaldulensis* and *Erythrina vespertilio*) had root xylem with the largest vessel lumen area,

291 vessel diameter and theoretical hydraulic conductivity, and the smallest theoretical vessel

292 implosion resistance, among the six-woody species (Figures 3, 5 and 6).

293 In root xylem, we found a strong effect of habitat (i.e., obligate riparian *versus* widespread or

294 arid-adapted species) on the relationship between theoretical hydraulic conductivity and log

295 xylem vessel implosion resistance (Figure 6). Theoretical hydraulic conductivity was smallest,

296 and theoretical implosion resistance largest, in the group of species which are not restricted to

297 riparian corridors (*Acacia aneura*, *Psydrax latifolia*, *Corymbia opaca* and *Hakea* sp.). The largest

298 theoretical hydraulic conductivity was found in *Eucalyptus camaldulensis* and was lowest in

299 *Psydrax latifolia*, whereas vessel implosion resistance followed a gradient across species: *Acacia*

300 *aneura > Psydrax latifolia > Hakea* sp. > *Corymbia opaca > Eucalyptus camaldulensis > Erythrina*

301 *vespertilio* (Table 3, Figure 6).

302 We also found a significant regression between vessel diameter and vessel wall thickness for

303 root xylem in the four species which are not restricted to riparian habitats (r^2=0.99, p=0.004,

304 Figure 7). The small number of points in the regression reduces statistical power, but the

305 regression was not significant when *Eucalyptus camaldulensis* and *Erythrina vespertilio* were

306 included in the analysis (r^2=0.02, p=0.70). It was furthermore exceptional that these four-

307 species fit so tightly to a single regression line, suggesting that those species which are not

308 restricted to riparian habitats have tight co-constraints of root vessel diameter and wall

309 thickness. Clear species pair-groupings by habitat and eco-hydrological niche were found in

310 vessel diameter and wall thickness: arid-adapted *Acacia aneura* and *Psydrax latifolia*, obligate

311 riparian *Eucalyptus camaldulensis* and *Erythrina vespertilio*, and *Corymbia opaca* and *Hakea* sp.

312 which occurred in both habitats (Figure 7).

313 Wood density was significantly larger in branches than in roots (Table 2). Our t-test

314 comparisons of fibre wall thickness between roots and branches indicated that *Hakea* sp.,

315 *Corymbia opaca* and *Eucalyptus camaldulensis* exhibited thicker fibre walls in roots than in

316 branches. Fibre wall thickness in *Acacia aneura* was similar for roots and branches, whereas

317 *Psydrax latifolia* had thinner fibre walls in roots than in branches. Vessel wall thickness was

318 larger in roots than in branches of *Erythrina vespertilio, Acacia aneura, Hakea sp., Corymbia*

319 *opaca* and *Eucalyptus camaldulensis,* but was similar in roots and branches of *Psydrax latifolia.*

320 Vessel diameter was larger in roots than in branches for *Erythrina vespertilio*, *Hakea sp., Psydrax*

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321 *latifolia, Corymbia opaca,* and *Eucalyptus camaldulensis* but was similar in roots and branches of

322 *Acacia aneura.* Conductive lumen fraction in *Erythrina vespertilio* and *Corymbia opaca* was

323 smaller in roots than in branches, whereas it was larger in *Psydrax latifolia* roots compared to

324 branches. Conductive lumen fraction was similar in roots and branches of *Acacia aneura*, *Hakea*

325 sp. and *Eucalyptus camaldulensis* (Table 2). Theoretical hydraulic conductivity was consistently

326 larger in roots than in branches; but differences were not significant for all species (Table 3).

327 Vessel implosion resistance was larger in roots than in branches of *Acacia aneura*, *Hakea* sp. and

328 *Corymbia opaca* but was lower in roots of *Psydrax latifolia* than in branches. Vessel implosion

329 resistance was similar in roots and branches of *Erythrina vespertilio* and *Eucalyptus*

330 *camaldulensis* (Table 3). Finally, we also observed tyloses or vascular occlusion by parenchyma

331 cells in some of the vessels of *Eucalyptus camaldulensis* and *Corymbia opaca*.

DISCUSSION

334 In this study, wood density of roots was significantly smaller than that of branches of the same

335 species (cf. Table 2, O' Grady *et al.* 2009; Santini *et al.* 2016). Our linear regression analysis

336 indicated that variation in root wood density was explained by variations in xylem vessel lumen

337 area across the different species (r^2=0.73, p=0.03, Figure 3). Thus, our first hypothesis, that

338 roots within a given species have larger xylem vessel diameters (and consequently larger vessel

339 lumen areas) and lower wood density than branches was supported. Numerous studies have

340 found that xylem vessel size is larger in roots than in branches (and that wood density is lower

341 in roots than in branches of the same species), and these studies also suggest that roots exhibit

342 larger hydraulic conductivities and are at more risk of cavitation events (Pate *et al.* 1998;

343 Martínez-Vilalta *et al.* 2002). The larger vessel size observed in roots compared to branches has

344 been suggested to be a continuation of the hydraulic tapering observed in aboveground xylem

345 with increasing tree height (Petit *et al.* 2008; Petit *et al.* 2010; Anfodillo *et al.* 2013). Whereas

346 differences in vessel diameter among the tallest trees (10 – 15 m; *Corymbia opaca*, *Eucalyptus*

347 *camaldulensis* and *Erythrina vespertilio*) were not determined by tree height, which was similar

348 across these species, tapering cannot be discarded as a possible explanation to smaller vessel

349 diameter in the shorter trees and shrubs.

350 We can confirm that for our studied species, theoretical hydraulic conductivity (K_s) was larger

351 in roots than in branches, but that differences were not significant for all species. Lower wood

352 densities in roots compared to branches can allow for shrinkage adjustments at high negative

353 water potentials and reduce mechanical strength of this plant organ (Jacobsen *et al.* 2005;

354 Santini *et al.* 2013). Roots are contained in a soil matrix that provides mechanical protection,

355 indicating that safety demands may be decoupled from mechanical demands in plant roots

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356 compared to branches. The arrangement of xylem traits and the total fraction of tissues (i.e.

357 fibre wall fraction but also fibre lumen fraction, fraction of parenchyma) also influence wood

358 density (e.g. Zieminska *et al.* 2013, Lachenbrunch and McCulloh 2014), but this was not

359 considered in the present study.

360 Our previous work (Santini *et al.* 2016) found a strong negative relationship between K_s and

361 vessel implosion resistance in stems of seven species, including all of the species from this

362 study: i.e. *Erythrina vespertilio*, *Acacia aneura*, *Hakea sp.*, *Psydrax latifolia*, *Corymbia opaca and*

363 *Eucalyptus camaldulensis*. A meaningful relationship between K_s and vessel implosion resistance

364 was not found in the current study. Instead, the arid-adapted species clustered with the species

365 sampled in both habitats and exhibited the lowest K_s and highest vessel implosion resistance,

366 whereas K_s and vessel implosion resistance was decoupled in the obligate riparian species (one

367 deciduous, one evergreen). Thus, we rejected our second hypothesis, finding instead that the

368 relationship between K_s and vessel implosion resistance was not maintained in roots of all of

369 our studied species as it was for branches. Vessel implosion resistance is a vessel wall

370 reinforcement metric, but in roots it might not be a relevant indicator of safety as in roots

371 tensile forces in the water column are minimal and low wood densities in roots compared to

372 branches may allow for adjustments at high negative water potentials (Jacobsen *et al.* 2005;

373 Pratt *et al.* 2007). In the present root study, we found the sinker roots of *Eucalyptus*

374 *camaldulensis* to have an extraordinarily high K_s, which is characteristic of this species (Roberts

375 *et al.* 2016). The high K_s in *Eucalyptus camaldulensis* roots was the result of larger vessel lumen

376 area and vessel density than in *Erythrina vespertilio* and *Corymbia opaca*, which have similarly

377 large branch K_s to that of *Eucalyptus camaldulensis* branches (Santini *et al.* 2016). Tyloses (or

378 vascular occlusion by parenchyma cells) are expected to reduce K_s, thus the large values of K_s in

379 *Eucalyptus camaldulensis* and in *Corymbia opaca* suggests that flow restriction in root vessels

380 with tyloses is compensated by large K_s in root vessels without tyloses. Tylose formation has

381 been observed to occur in response to wounding and pathogen attack as to compartmentalize

382 infection (Saitoh *et al.* 1993; Sun *et al.* 2008). Tylose development was observed infrequently in

383 roots, and therefore had only minimal impact on our understanding of K_s for *Eucalyptus*

384 *camaldulensis* and *Corymbia opaca*, particularly in light of the fact that the difference in K_s

385 between these two species was more than ten-fold.

386

387 We found a strong relationship between log (vessel density) and vessel lumen area in roots

388 (r^2=0.72, p=0.03, Figure 4). Thus, *Erythrina vespertilio*, *Corymbia opaca* and *Eucalyptus*

389 *camaldulensis* had the largest xylem vessels but also had low density of xylem vessels per

390 sapwood area. This relationship between vessel density and vessel lumen area has been widely

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observed across plant species (Preston *et al.* 2006). In addition, there was a strong relationship between outer pit aperture and log (vessel diameter) (r^2=0.92, p=0.002, Figure 5), which is consistent with previous findings that pit anatomy scales with lumen diameter (Lazzarin *et al.* 2016). Li *et al.* (2016) found that samples stored in ethanol (such as samples from the current study) exhibited shrinkage of pit membranes. We cannot confirm shrinking in our study, nonetheless our results are comparable with our previous study (Santini *et al.* 2016). Further measurements are required to assess relationships among pit membrane pores, nanobubble size, outer pit aperture size, vessel size and vulnerability to cavitation events. However, cavitation events can occur when the pressure difference between adjacent air and water filled vessels becomes large enough to pull the air-water interface through the nanopores of the pit membranes (Hacke *et al.* 2001; Martínez-Vilalta *et al.* 2002; McElrone *et al.* 2004; Schenk *et al.* 2015).

This study advances our understanding of root anatomy and hydraulic strategies of Australian semi-arid species and confirms that roots exhibit larger xylem vessel diameters and lower wood density than branches of the same species. In terms of functional grouping, *Erythrina vespertilio,* the deciduous species exhibited high K_s (and thus a relatively efficient water transport system) and low xylem vessel implosion resistance relative to *Corymbia opaca* and the evergreen species. Thus, theoretically, *Erythrina vespertilio* can maintain relatively high transpiration rates and in turn metabolic carbon uptake during the wet season (Enquist *et al.* 1999). At the same time, *Erythrina vespertilio* can survive the dry season by reducing its canopy area to zero. *Erythrina vespertilio* can also store large amounts of water and sugars in its parenchyma tissue in order to support root and shoot respiration during the dry season when the canopy is absent and carbon capture does not occur (Carlquist 2001). The phreatophytic riparian species *Eucalyptus camaldulensis* exhibited the largest values of K_s and the lowest values of xylem vessel implosion resistance. By having deep sinker roots and sustained access to the shallow groundwater reservoir at *ca.* 3 m depth all year, *Eucalyptus camaldulensis* is at low risk of cavitation during the dry season. *Corymbia opaca*, the second phreatophyte, exhibited much smaller K_s values than *Eucalyptus camaldulensis*. In turn, *Corymbia opaca* roots exhibited similar K_s and vessel implosion resistance values to the evergreen species with lateral shallow roots. Relatively low K_s in *Corymbia opaca* can be explained as this species deploys deeper sinker roots than *Eucalyptus camaldulensis* (up to 10 m depth) and because groundwater use in *Corymbia opaca* is limited by high temperature and vapour pressure deficit (Cleverly *et al.* 2016b). Alternatively, low K_s in the evergreen species: *Acacia aneura*, *Hakea* sp. and *Psydrax latifolia* that deploy lateral shallow roots and rely on intermittent water resources such as rainwater can be explained as a strategy to survive or tolerate drought.

CONCLUSIONS

Deep sinker roots of *Eucalyptus camaldulensis* exhibited a highly efficient water transport system with the largest theoretical hydraulic conductivity (K_s) of all species as a result of its riparian habit and continuous access to shallow groundwater at *ca.* 3 m depth. Despite also exhibiting deep sinker roots (to 8 m) and groundwater use, *Corymbia opaca* had root xylem traits similar to those of the other evergreen trees found in both floodplain and sand plain habitats: *Hakea* sp. Root xylem anatomy in the six-woody species of this study tended to cluster by habitat or eco-hydrological niche. At one extreme are species with shallow lateral roots which occupy the extraordinarily dry hardpans of the Ti Tree Basin and which access intermittent water resources (i.e. rainwater) (i.e. arid-adapted *Acacia aneura* and *Psydrax latifolia*). Roots of these arid-adapted species have the smallest vessel diameter, vessel lumen area (and thus the largest vessel density) and outer pit aperture. At the other extreme are the species which are restricted to riparian corridors, paleochannels and topographic depressions in the floodplain habitat (*Eucalyptus camaldulensis* and *Erythrina vespertilio*), which exhibited the largest root K_s, vessel diameter and vessel lumen area, with the smallest root wood density and theoretical implosion resistance, indicating low overall investment in root xylem. Vessel implosion resistance as a concept can and has been used as an indicator of vessel wall reinforcement. Whilst we do not suggest that vessels do implode within roots (because tensile forces in the water column are low) the metric can be better viewed as a measure of investment in cell walls. The very low density of xylem vessels in roots of *Erythrina vespertilio* was due to a large fraction of parenchyma that is likely to store large amounts of water. Thus, *Erythrina vespertilio* may be able to support root and shoot respiration during the dry season when the canopy is absent and carbon capture does not occur (Carlquist 2001). Xylem traits were found to depend upon habitat and eco-hydrological niche, with the groupings including (i) arid-adapted shrubs and trees with shallow roots (*Acacia aneura* and *Psydrax latifolia*), (ii) trees restricted to the floodplain habitat, both evergreen (*Eucalyptus camaldulensis*) and deciduous (*Erythrina vespertilio*) and (iii) evergreen trees co-occurring in both floodplain and adjacent sand plain habitats (*Corymbia opaca* and *Hakea* sp.).

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Figures

Figure 1. (A – D) Photographs of roots of four of our studied species, *Erythrina vespertilio*, *Acacia aneura* and *Psydrax latifolia* exhibit shallow lateral roots while *Corymbia opaca* exhibits deep sinker roots.

Figure 2. (A – F) Photographs of root wood micro-sections of the studied species from the Ti Tree Basin, Northern Territory. Photographs illustrate xylem vessels (V), vessel walls (VW) and fibre walls (FW). **(A – D)** Species were double-stained with safranin and alcian blue (1:2). **(F - E)** *Corymbia opaca* and *Eucalyptus camaldulensis* were not stained due to the presence of resins. Tyloses (Ty) were observed in some of the vessels.

Figure 3. Relationship between root wood density and root vessel lumen area, the regression was: $Y = -14.78 X + 0.67$ ($r^2=0.73$, $p=0.03$). Sample numbers of individual primary roots (one root per individual plant) for assessment of wood density were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 5), *Hakea* sp. (n=5), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus camaldulensis* (n=5). Sample numbers of individual primary roots (one root per individual plant) for assessment of root vessel lumen area were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 3), *Hakea* sp. (n=4), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus camaldulensis* (n=5). Symbols represent mean values $\pm$ standard errors for different species: *Erythrina vespertilio* (crossed-circle), *Acacia aneura* (triangle), *Hakea* sp. (square), *Psydrax latifolia* (diamond), *Corymbia opaca* (upside-down triangle), *Eucalyptus camaldulensis* (circle). Different coloured symbols indicate functional groups (Yellow: deciduous, green: evergreen and blue: evergreen phreatophytes).

Figure 4. Relationship between log (root vessel density) and root vessel lumen area, the regression was: $Log (Y) = -67.69 X + 2.17$ ($r^2=0.72$, $p=0.03$). Sample numbers of individual primary roots (one root per individual plant) for assessment of root vessel density and root vessel lumen area were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 3), *Hakea* sp. (n=4), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus camaldulensis* (n=5). Symbols represent mean values $\pm$ standard errors for different species: *Erythrina vespertilio* (crossed-circle), *Acacia aneura* (triangle), *Hakea* sp. (square), *Psydrax latifolia* (diamond), *Corymbia opaca* (upside-down triangle), *Eucalyptus camaldulensis* (circle). Different coloured symbols indicate functional groups (Yellow: deciduous, green: evergreen and blue: evergreen phreatophytes).

Figure 5. Relationship between root outer pit aperture and the logarithm (Log) of root vessel diameter, the regression was: $Y = 1.56 X - 0.93$ ($r^2=0.92$, $p=0.002$). Sample numbers of individual primary roots (one root per individual plant) for assessment of pit apertures were n = 3 per species. Sample numbers of individual primary roots (one root per individual plant) for assessment of root vessel diameter were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 3), *Hakea* sp. (n=4), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus camaldulensis* (n=5). Symbols represent mean values $\pm$ standard errors for different species: *Erythrina vespertilio* (crossed-circle), *Acacia aneura* (triangle), *Hakea* sp. (square), *Psydrax latifolia* (diamond), *Corymbia opaca* (upside-down triangle), *Eucalyptus camaldulensis* (circle). Different coloured symbols indicate functional groups (Yellow: deciduous, green: evergreen and blue: evergreen phreatophytes).

Figure 6. Relationship between root theoretical hydraulic conductivity (K_s) and log root xylem vessel implosion resistance $(t/b)^2$. Sample numbers of individual primary roots (one root per

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656 individual plant) for assessment of root K_s and log root $(t/b)^2$ were as follows: Acacia *aneura* (n
657 = 3), *Hakea* sp. (n=4), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus*
658 *camaldulensis* (n=5) and *Erythrina vespertilio* (n=5). Symbols represent mean values $\pm$
659 standard errors for different species: *Erythrina vespertilio* (crossed-circle), *Acacia aneura*
660 (triangle), *Hakea* sp. (square), *Psydrax latifolia* (diamond), *Corymbia opaca* (upside-down
661 triangle), *Eucalyptus camaldulensis* (circle). Different coloured symbols indicate functional
662 groups (Yellow: deciduous, green: evergreen and blue: evergreen phreatophytes).

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664 **Figure 7.** Relationship between root vessel diameter and root vessel wall thickness, the
665 regression was: Y = 0.13 X + 1.18 (r^2=0.99, p=0.004). Sample numbers of individual primary
666 roots (one root per individual plant) for assessment of root vessel diameter and root vessel wall
667 thickness were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 3), *Hakea* sp. (n=4),
668 *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3). *Erythrina vespertilio* (n=5) and *Eucalyptus*
669 *camaldulensis* (n=5) were not included in the regression. Symbols represent mean values $\pm$
670 standard errors for different species: *Erythrina vespertilio* (crossed-circle), *Acacia aneura*
671 (triangle), *Hakea* sp. (square), *Psydrax latifolia* (diamond), *Corymbia opaca* (upside-down
672 triangle), *Eucalyptus camaldulensis* (circle). Different coloured symbols indicate functional
673 groups (Yellow: deciduous, green: evergreen and blue: evergreen phreatophytes).

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Tables

Table 1. Characteristics of the studied species and sampling areas at the Ti Tree Basin, Northern Territory. Xylem traits were vessel diameter, fibre and vessel wall thickness.

Functional group	Species	Growth form	Area utilized for assessment of xylem traits (mm^2)*	Number of assessed xylem vessels (range)	Type of collected primary roots	Distance of collected primary lateral/sinker root from the main stem, range (m)	Tree height (m)
Deciduous	*Erythrina vespertilio*	Tree	~ 8	7 - 12	Lateral / Shallow	~ 2 - 6	10 – 15
Evergreen	*Acacia aneura*	Tree	~ 0.3	37 - 55	Lateral / Shallow	~ 0.5 - 1	5 – 10
	Hakea sp.	Tree	~ 1.3	39 - 41	Lateral / Shallow	~ 1 - 2	5 – 10
	Psydrax latifolia	Shrub	~ 0.08	60 - 85	Lateral / Shallow	~ 0.5 - 2	2 – 3
Evergreen phreatophytes	*Corymbia opaca*	Tree	~ 1.3	16 - 24	Sinker / Deep	~ 1 - 6	10 – 15
	Eucalyptus camaldulensis	Tree	~ 1.3	15 - 35	Sinker / Deep	~ 2 - 3	10 – 15

* Areas are approximate values per image.

Table 2. Xylem characteristics and wood density of tap roots of our collected species from the Ti Tree Basin, Northern Territory. NA indicates not assessed. Data from branches were provided by Santini *et al.* (2016). Values are means and standard errors. Values with the same letter within a column were not significantly different (p > 0.05) as tested with a one-way Analysis of Variance. A paired t-test was used to compare mean and standard errors of xylem characteristics and wood density between roots and branches, (*) indicates p < 0.05 and ns indicates non-significant differences. Sample number of individual tap roots for assessment of xylem traits (i.e. vessel diameter, fibre and vessel wall thickness) were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 3), *Hakea* sp. (n=4), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus camaldulensis* (n=5). Samples number of individual tap roots for assessment of pit apertures were n = 3 per species. Sample number of individual tap roots for assessment of wood density were as follows: *Erythrina vespertilio* (n=5), *Acacia aneura* (n = 5), *Hakea* sp. (n=5), *Psydrax latifolia* (n=5), *Corymbia opaca* (n=3), *Eucalyptus camaldulensis* (n=5).

Species	Fibre wall thickness (μm)	Vessel wall thickness (μm)	Vessel diameter (μm)	Outer pit aperture (μm)	Conductive lumen fraction (mm² mm⁻²)	Wood density (g cm⁻³)
Erythrina vespertilio Roots	NA	6.97 ± 0.21^{AD}	188 ± 6.15^{A}	7.13 ± 0.11^{A}	0.05 ± 0.003^{A}	0.21 ± 0.009^{A}
Branches	2.80 ± 0.11	1.98 ± 0.09	40.44 ± 1.634	NA	0.249 ± 0.020	0.40 ± 0.016
Paired *t* test (t;df)	NA	$(21.59; 72)^*$	$(29.75; 12)^*$		$(4.97; 72)^*$	$(3.73; 72)^*$
Acacia aneura Roots	3.9 ± 0.10^{C}	6.07 ± 0.19^{D}	34.8 ± 1.08^{E}	4.62 ± 0.05^{D}	0.17 ± 0.014^{D}	0.61 ± 0.021^{D}
Branches	4.01 ± 0.18	4.27 ± 0.19	35.14 ± 1.61	NA	0.098 ± 0.008	0.95 ± 0.07
Paired *t* test (t;df)	ns	$(7.78; 72)^*$	ns		ns	$(6.69; 72)^*$
Hakea sp. Roots	5.4 ± 0.12^{A}	9.48 ± 0.15^{E}	60.3 ± 1.91^{F}	6.16 ± 0.15^{B}	0.12 ± 0.009^{DE}	0.82 ± 0.023^{E}
Branches	4.26 ± 0.15	2.71 ± 0.13	27.01 ± 2.18	NA	0.123 ± 0.023	0.712 ± 0.03
Paired *t* test (t;df)	$(3.47; 60)^*$	$(29.29; 72)^*$	$(10.14; 12)^*$		ns	$(2.12; 72)^*$
Psydrax latifolia Roots	4.5 ± 0.07^{B}	4.12 ± 0.05^{C}	24.4 ± 0.29^{D}	3.73 ± 0.05^{C}	0.37 ± 0.008^{C}	0.58 ± 0.01^{CD}
Branches	6.79 ± 0.42	3.78 ± 0.13	20.19 ± 0.58	NA	0.133 ± 0.013	0.73 ± 0.017
Paired *t* test (t;df)	$(6.99; 60)^*$	ns	$(5.13; 12)^*$		$(5.92; 72)^*$	$(2.95; 72)^*$
Corymbia opaca Roots	5.7 ± 0.15^{A}	11.5 ± 0.29^{B}	79.4 ± 3.12^{C}	5.88 ± 0.16^{B}	0.08 ± 0.006^{AE}	0.49 ± 0.002^{BC}
Branches	3.36 ± 0.16	2.88 ± 0.17	39.41 ± 1.57	NA	0.258 ± 0.032	0.68 ± 0.022
Paired *t* test (t;df)	$(7.14; 60)^*$	$(37.30; 72)^*$	$(12.87; 12)^*$		$(4.45; 72)^*$	$(3.73; 72)^*$
Eucalyptus camaldulensis Roots	5.3 ± 0.08^{A}	7.25 ± 0.12^{A}	131 ± 3.42^{B}	6.49 ± 0.14^{B}	0.25 ± 0.011^{B}	0.44 ± 0.03^{B}
Branches	3.31 ± 0.12	2.61 ± 0.09	36.67 ± 1.38	NA	0.258 ± 0.053	0.65 ± 0.03
Paired *t* test (t;df)	$(6.07; 60)^*$	$(20; 72)^*$	$(30.41; 12)^*$		ns	$(4.13; 72)^*$

Table 3. Calculated means and standard errors of theoretical hydraulic conductivity (K_s) and vessel implosion resistance $(t/b)^2$, where t is the double-wall thickness (in μm) and b is the hydraulic mean vessel diameter (in μm). Different letters among columns indicate means were significantly different p < 0.05 as tested with a one- way Analysis of Variance.

Species	K_s (kg mm^{-1} MPa^{-1} s^{-1})	n	$(t/b)^2$	n
Erythrina vespertilio **Roots**	1.86 ± 0.1984	5	0.0068 ± 0.00037	5
Branches	0.383 ± 0.047	9	0.0096 ± 0.00006	3
Paired *t* test (t;df)	(6.48; 72)*		ns	
Acacia aneura **Roots**	0.4192 ± 0.1166	3	0.1179 ± 0.01017	3
Branches	0.109 ± 0.017	9	0.059 ± 0.00009	3
Paired *t* test (t;df)	ns		(10.86; 72)*	
Hakea **sp. Roots**	0.8140 ± 0.1511	4	0.0995 ± 0.0040	4
Branches	0.08 ± 0.019	9	0.0404 ± 0.00154	3
Paired *t* test (t;df)	(3.22; 72)*		(10.90; 72)*	
Psydrax latifolia **Roots**	0.2103 ± 0.0090	5	0.1094 ± 0.012	5
Branches	0.047 ± 0.006	9	0.14 ± 0.00092	3
Paired *t* test (t;df)	ns		(5.64; 72)*	
Corymbia opaca **Roots**	0.6654 ± 0.14	3	0.08536 ± 0.0079	3
Branches	0.356 ± 0.058	9	0.0213 ± 0.00047	3
Paired *t* test (t;df)	ns		(11.81; 72)*	
Eucalyptus camaldulensis **Roots**	6.837 ± 0.677	5	0.01617 ± 0.0013	5
Branches	0.328 ± 0.082	9	0.0237 ± 0.00001	3
Paired *t* test (t;df)	(28.57; 72)*		ns	

Figure 1

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A *Erythrina vespertilio*

B *Acacia aneura*

C *Psydrax latifolia*

D *Corymbia opaca*

Figure 2 Click here to download Figure Figure 2_VF.tiff ⬇

Figure 2. A *Erythrina vespertilio*, B *Acacia aneura*, C *Hakea sp.*, D *Psydrax latifolia*, E *Corymbia opaca*, F *Eucalyptus camaldulensis*

Figure 3

Figure 4

Figure 4

Figure 5

Figure 6

Figure 6

Figure 7