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ORIGINAL ARTICLE

Xylem traits and water-use efficiency of woody species co-occurring in the Ti Tree Basin arid zone

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Abstract

Key message **Species with low density of intact branches are likely to have higher growth rates than species with high density of intact branches, but at the cost of a lower water-use efficiency and larger sensitivity to xylem embolism.**

Abstract The hydraulic niche separation theory proposes that species co-exist by having a range of traits to allow differential access to resources within heterogeneous environments. Here, we examined variation in branch xylem anatomy and foliar carbon stable isotopes (δ^{13}C) as a measure of water-use efficiency (WUE) in seven co-occurring species, *Acacia aneura*, *Acacia bivenosa*, *Corymbia opaca*, *Eucalyptus camaldulensis*, *Erythrina vespertilio*, *Hakea* sp., and *Psydrax latifolia*, in an arid zone open *Corymbia* savanna on the Ti Tree Basin, Northern Territory, Australia. We test the following hypotheses: (1) Species with large conductive areas exhibit a low density of intact branches, while species with small conductive areas have a significantly higher density of intact branches. (2) Species with smaller conductive areas exhibit more enriched values of δ^{13}C and therefore have larger WUE than those with larger conductive areas and (3) there is an inverse correlation between theoretical sapwood hydraulic conductivity and vessel implosion resistance. The results of this study demonstrated significant variation in density of intact branches, ranging from 0.38 to 0.80 g cm^{-3} and this variation was largely explained by variation in sapwood conductive area. Species with low conductive areas (*P. latifolia*, *Hakea* sp. and *Acacia* species) exhibited large values of WUE ($r^2 = 0.62$, $p < 0.05$). These species are likely to be less vulnerable to cavitation by having small conductive areas and thicker fibre walls. We demonstrated a significant ($r^2 = 0.83$, $p = 0.004$) negative correlation between theoretical sapwood hydraulic conductivity and vessel implosion resistance. These results are discussed in relation to hydraulic niche separation.

Keywords Wood anatomy · Hydraulic niche separation · Arid zone · Water-use efficiency · Carbon isotopes

Introduction

The theory of hydraulic niche separation proposes that different plant species can co-occur because they can each access a given resource, such as water or nutrients, by utilising different strategies to access these resources under a heterogeneous environment (Silvertown et al. 1999; Silvertown 2004). For example, Terradas et al. (2009) demonstrated in a Mediterranean climate that plant species with deep roots can continuously access groundwater resources, while plant species with shallow roots can only access episodic rainwater. In arid environments such as the Ti Tree basin, Northern Territory, water is a limiting resource and variability in xylem hydraulic traits may be crucial for species co-occurrence within the region.

Xylem characteristics such as density of intact branch, vessel size and fibre dimensions can influence water

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transport efficiency and resistance to drought-induced cavitation (Hacke et al. 2001; Chave et al. 2009; Lachenbrunch and McCulloh 2014). Thus, variation of xylem traits forms part of the suite of strategies available to support hydraulic niche separation (e.g. Sterck et al. 2011). Water transport in plants is significantly influenced by xylem vessel size (Enquist et al. 1999; Tyree and Ewers 1991; Chave et al. 2009). Plants that can continuously access water, such as those depending on groundwater, are expected to exhibit large diameter xylem vessels, low density of intact branches and low resistance to water flow with concomitant large rates of transpiration, carbon gain and growth (King et al. 2006). However, large diameter xylem vessels also increase the risk of cavitation (Hacke et al. 2001). Plants occurring in xeric environments often have narrow xylem vessels and a high density of intact branches, traits which increase the resistance to water flow and decrease plant transpiration rates and growth. However, narrow xylem vessels also decrease their vulnerability to drought-induced cavitation (Groom 2004; Froend and Drake 2006). Furthermore, thicker vessel and fibre walls can also improve xylem resistance to cavitation because of their larger resistance to the mechanical stresses associated with the development of large negative xylem water pressure during drought (Hacke et al. 2001; Pratt et al. 2007; Chave et al. 2009).

Water-use efficiency (WUE) can be defined as the ratio of net photosynthesis to transpiration (Farquhar and Richards 1984; Eamus 1991). WUE is correlated with enriched $\delta^{13}C$ (i.e., larger values of $\delta^{13}C$, a measure of the ratio of the stable isotopes of carbon ^{13}C and ^{12}C) in photosynthates of leaves and phloem of twigs and stems (Farquhar 1983; Marshall et al. 2007; Gessler et al. 2009). Plant species with high density of intact branches and small xylem vessels are likely to have a larger WUE (due to their low transpiration rates) and larger resistance to drought-induced cavitation. Alternatively, species with low density of intact branches are likely to have a lower WUE and to exhibit larger growth rates but at the cost of larger vulnerability to xylem embolism (Enquist et al. 1999; Jacobsen et al. 2005).

In this study, we examined variation in branch xylem anatomical traits, i.e., density of intact branches, wood density, conductive area and fibre and vessel wall thickness of seven co-occurring species from the Ti Tree Basin. This basin, with annual average rainfall of approximately 347 mm year^{-1} is classified as an arid basin (Eamus 2003; O' Grady et al. 2009). From our measurements, we determined potential hydraulic conductivity (Zanne et al. 2010), theoretical resistance to vessel implosion (Hacke et al. 2001) and a drought vulnerability index (Carlquist 1977). We also examined foliar carbon stable isotopes ($\delta^{13}C$) as a measure of WUE to examine the relationship between variation of xylem traits and WUE. We tested the following hypotheses: (1) Species with large conductive areas exhibit a low density of intact branches, while species with small conductive areas have a significantly higher density of intact branches. (2) Species with smaller conductive areas exhibit more enriched values of $\delta^{13}C$ and therefore have larger WUE than those with larger conductive areas and (3) there is an inverse correlation between theoretical sapwood hydraulic conductivity and vessel implosion resistance.

Materials and methods

Site description and sample collection

The study site was located 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 site is arid with a mean annual rainfall of 347 mm year^{-1} (Australian Bureau of Meteorology 2014, BoM station 15507), most of which falls in monsoonal summer storms. Mean minimum and maximum annual temperatures are 15 and 31 °C, respectively. The site reaches maximum air temperatures of >40 °C in the summer months (December–February). The Ti Tree Basin extends over 5500 km^2 and contains the ephemeral Hanson and Woodforde rivers, which only flow after intense storms (Harrington et al. 2002).

During July–September 2014, we collected upper canopy terminal branches of seven co-occurring woody species from the Ti Tree Basin: *Acacia aneura*, *Acacia bivenosa*, *Corymbia opaca*, *Eucalyptus camaldulensis*, *Erythrina vespertilio*, *Hakea* sp., and *Psydrax latifolia*. Branches of different species varied in diameter (2–7 mm), but we standardised our collection by sampling the terminal 25 cm of each branch.

Measurement of xylem traits

Branches were collected and stored in sealed vials containing 50 % ethanol. Cross sections of each branch were fixed in formalin-acetic acid-alcohol (FAA) for 7 days and then placed in 70 % ethanol for 2 days; we repeated this washing process twice. Micro-sections of all species with the exception of *E. vespertilio* were prepared with a sledge Leica SM2010R microtome. Micro-sections of *E. vespertilio* were pre-treated with ethanol (8 h), xylene (4 h) and finally embedded in paraffin with a Shandon Histocentre 3 histology-embedding centre (Thermo Fisher Scientific, Australia) before cutting with a Microm HM325 rotatory microtome (Thermo Fisher Scientific, Australia). Micro-sections of all species were double stained with safranin–alcian blue. We photographed the micro-sections with a

Leica DM750 microscope and used the software Image J 1.48v (National Institutes of Health, USA) to measure xylem traits. We determined the minor (a) and major (b) axis diameters of xylem vessels to calculate vessel area (VA) following Eq. (1). We also determined vessel density (VD) (Eq. 2) to calculate conductive area (CA) of each species as in Eq. (3) (Lewis 1992):

$$\mathrm{VA} = \pi ab\, 0.25 \tag{1}$$

$$\mathrm{VD} = \frac{\#\mathrm{vessels}}{\mathrm{mm}^2} \tag{2}$$

$$\mathrm{CA} = \mathrm{VAVD}. \tag{3}$$

We measured fibre wall thickness by measuring the width of 30–50 adjoining fibre walls and dividing the total distance by two (Santini et al. 2012). We also assessed vessel wall thickness by measuring double-vessel walls of 20–30 vessels per species and dividing the total distance by two.

Potential hydraulic conductivity, resistance to vessel implosion and a vulnerability index

We calculated the potential hydraulic conductivity (K_s) as in Eq. (4):

$$K_s \propto F^{1.5} S^{0.5}, \tag{4}$$

where F is the vessel fraction and S is the ratio of vessel size to vessel number, calculated as in Eq. (5) and (6) (Zanne et al. 2010):

$$\mathrm{F} = \mathrm{VAVD} \tag{5}$$

$$\mathrm{S} = \frac{\mathrm{VA}}{\mathrm{VD}}. \tag{6}$$

Vessel implosion resistance was calculated as:

$$(t/b)^2. \tag{7}$$

where t is the double-wall thickness and b is the hydraulic mean vessel diameter, both expressed in µm (Hacke et al. 2001).

Finally, we determined a drought vulnerability index (VI) by dividing vessel diameter (D_h) by vessel density (Carlquist 1977) Eq. (8) and (9):

$$\mathrm{VI} = \frac{D_\mathrm{h}}{\mathrm{VD}} \tag{8}$$

where D_h is,

$$D_\mathrm{h} = \sqrt{\frac{2a^2b^2}{a^2 + b^2}} \tag{9}$$

The vulnerability index is a measure of the redundancy of the number of vessels present per unit sapwood area. A low value is taken to indicate a large resistance to drought (Carlquist 1977).

Density of intact branches and wood density

Material to measure density of intact branches and wood density was stored in paper bags and was maintained cool in an insulated container until laboratory analysis. Density of intact branches was measured from ~5 cm length branch segments. Branches were placed in water for 2 days to obtain full branch hydration. We measured the mass of the displaced hydrated branch. The same branches were oven dried for 5 days at 60 °C to attain constant weight. Density of intact branches was calculated by dividing dry mass by hydrated branch volume. Wood density was measured following the same methods as for measuring density of intact branches but after carefully debarking our intact branches with a razor blade.

Analysis of stable carbon isotopes δ^{13}C

Samples stored in paper bags were completely dried in an oven at 60 °C for 5 days. Dry leaf samples were ground with a Retsch MM300 grinding mill (Verder Group, Netherlands). The ground material was placed in 3.5 mm × 5 mm tin capsules for analysis of δ^{13}C. Analyses were performed with a Picarro G2121-i Analyser (Picarro, Santa Clara, CA, USA) for isotopic $\mathrm{CO_2}$. Values of δ^{13}C were quantified as in Eq. (10), where R corresponds to the isotopic value $^{13}\mathrm{CO_2}/^{12}\mathrm{CO_2}$ of the sample (R_a) or the standard (R_b). We used atropine and acetanilide as laboratory standard references. Results were normalised with the international standards sucrose (IAEA-CH-6, $\delta^{13}\mathrm{C_{VPDB}} = -10.45$), cellulose (IAEA-CH-3, $\delta^{13}\mathrm{C_{VPDB}} = -24.72$) and graphite (USGS24, $\delta^{13}\mathrm{C_{VPDB}} = -16.05$).

$$\delta^{13}C = \left(\frac{R_\mathrm{a}}{R_\mathrm{b}} - 1\right) \times 1000. \tag{10}$$

Data analyses

We used linear regression analyses to test the relationships between density of intact branches and conductive area, vessel area and fibre wall thickness, vessel density and fibre wall thickness, potential hydraulic conductivity and log (vessel implosion resistance) and density of intact branches and wood density. One-way ANOVA tests were used to compare values of density of intact branches, conductive area, fibre wall thickness, vessel wall thickness, δ^{13}C, theoretical hydraulic conductivity, vessel implosion resistance and vulnerability index between species. We used a paired *t* test to compare mean and standard errors of density of intact branches and wood density within species.

We used δ^{13}C of leaves to calculate water-use efficiency (WUE) following Eq. (11) and Eq. (12) (Marshall et al. 2007):

$$\mathrm{WUE}_i = \frac{c_\mathrm{a}\,(b - \Delta)}{1.6\,(b - a)}, \quad (11)$$

where c_a is the atmospheric concentration of CO_2, which is ~390 ppm, a corresponds to discrimination of $^{13}CO_2$ due to slower motion from the atmosphere through the leaf stomata (~−4.4 ‰) and b is the discrimination against $^{13}CO_2$ molecules from the enzyme ribulose biphosphate carboxylase/oxygenase within the leaf (~−27 ‰). The Δ value was calculated as:

$$\Delta = (\delta^{13}\mathrm{C}_\mathrm{atm} - \delta^{13}\mathrm{C}_\mathrm{plant}) / \left(1 + \frac{\delta^{13}\mathrm{C}_\mathrm{plant}}{1000}\right), \quad (12)$$

where $\delta^{13}\mathrm{C}_\mathrm{atm}$ is −8.1 ‰ (Carbon Dioxide Information Analysis Center 2014) and δ^{13}C plant are the values obtained from leaves as indicated in Eq. (10).

We determined the relationship between WUE and conductive area with a Spearman correlation analysis. All analyses were performed using Prism version 6.0a (GraphPad Software, La Jolla, CA, USA) and the R software package, functions *glm* and *lm* (R Development Core Team 2008).

Results

Xylem traits

The density of intact branches was significantly lower in *E. vespertilio* than in five of the six remaining species (Table 1) whilst the density of intact branches of *C. opaca* was significantly lower than four of the remaining species (Table 1).

Fibre wall thickness was significantly larger in *P. latifolia* than the remaining six species (*E. vespertilio*, *C. opaca, E. camaldulensis*, *Hakea* sp., *A. aneura* and *A. bivenosa)* (Table 1). Vessel wall thickness was highest in *A. bivenosa*, *A. aneura* and *P. latifolia* and lowest in *E. vespertilio* (Table 1). Conductive areas per mm^2 of cross section of branch varied from 0.098 ± 0.008 mm^2 mm^{-2} in *A. aneura* to 0.258 ± 0.053 mm^2 mm^{-2} in *E. camaldulensis* (Table 1).

Density of intact branches was largely explained by xylem conductive area ($r^2 = 0.83$; $p = 0.004$, Fig. 1). Thus species in which vessel size and vessel density combine as larger conductive areas also exhibited a lower density of intact branches. There was a significant negative relationship between vessel area and fibre wall thickness ($r^2 = 0.80$; $p = 0.006$, Fig. 2). We found a positive relationship between vessel density and fibre wall thickness ($r^2 = 0.61$; $p = 0.037$, Fig. S1). However, when *P. latifolia* was excluded from the analysis, the regression was not significant ($r^2 = 0.05$; $p = 0.6$, Fig. 3).

Values of potential hydraulic conductivity (K_s) were significantly larger ($p < 0.05$) in *E. vespertilio*, *C. opaca* and *E. camaldulensis* compared to *P. latifolia*, *Hakea* sp., *A. aneura* and *A. bivenosa* (Table 3).

The theoretical resistance to vessel implosion $(t/b)^2$ ranged from 0.0096 ± 0.00006 in *E. vespertilio* to 0.14 ± 0.00092 in *P. latifolia* (Table 3). There was a significant negative log linear relationship between potential hydraulic conductivity and resistance to vessel implosion ($r^2 = 0.83$; $p = 0.004$, Fig. 4) across the seven species.

The Vulnerability index (VI) was significantly larger in *A. aneura* and *A. bivenosa* compared to most other species (Table 3).

Our paired *t* test analysis to compare density of intact branches and wood density showed that in five of our seven

Table 1 Xylem characteristics, density of intact branches and wood density of different species of branches collected in the Ti Tree Basin, Northern Territory

Species	Fibre wall thickness (μm)	Vessel wall thickness (μm)	Conductive area (mm^2 mm^{-2})	Density of intact branches (g cm^{-3})	*n*	Wood density (g cm^{-3})	*n*	Paired *t* test *p* (*t*; *df*)
E. vespertilio	2.80 ± 0.11^{A}	1.98 ± 0.09^{A}	0.249 ± 0.020^{AB}	0.38 ± 0.03^{A}	9	0.40 ± 0.016	6	0.83 (0.22; 5)
C. opaca	3.36 ± 0.16^{AC}	2.88 ± 0.17^{B}	0.258 ± 0.032^{AB}	0.42 ± 0.08^{AB}	9	0.68 ± 0.022	6	0.02 (3.06; 5)
E. camaldulensis	3.31 ± 0.12^{AC}	2.61 ± 0.09^{B}	0.258 ± 0.053^{B}	0.51 ± 0.01^{BC}	9	0.65 ± 0.03	6	0.04 (2.6; 5)
P. latifolia	6.79 ± 0.42^{B}	3.78 ± 0.13^{C}	0.133 ± 0.013^{AC}	0.56 ± 0.02^{CD}	9	0.73 ± 0.017	6	0.0005 (7.9; 5)
Hakea sp.	4.26 ± 0.15^{C}	2.71 ± 0.13^{B}	0.123 ± 0.023^{AC}	0.65 ± 0.01^{DE}	9	0.719 ± 0.03	6	0.04 (2.7; 5)
A. aneura	4.01 ± 0.18^{C}	4.27 ± 0.19^{C}	0.098 ± 0.008^{C}	0.78 ± 0.01^{EF}	9	0.95 ± 0.07	6	0.03 (2.9; 5)
A. bivenosa	3.52 ± 0.20^{AC}	4.42 ± 0.17^{C}	0.099 ± 0.007^{C}	0.80 ± 0.03^{E}	9	0.88 ± 0.09	6	0.28 (1.2; 5)

Values are means and standard errors, *n* indicates sample size. Different letters among columns indicate means were 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 density of intact branches and wood density within species

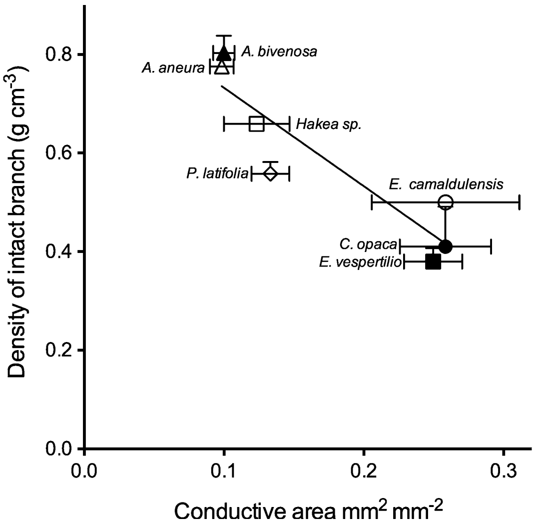

Fig. 1 Relationship between density of intact branch and conductive area ($r^2 = 0.83$; $p = 0.004$) for seven co-occurring species from the Ti Tree basin, Northern Territory. The regression was: density of intact branch = −1.99 Conductive area +0.93

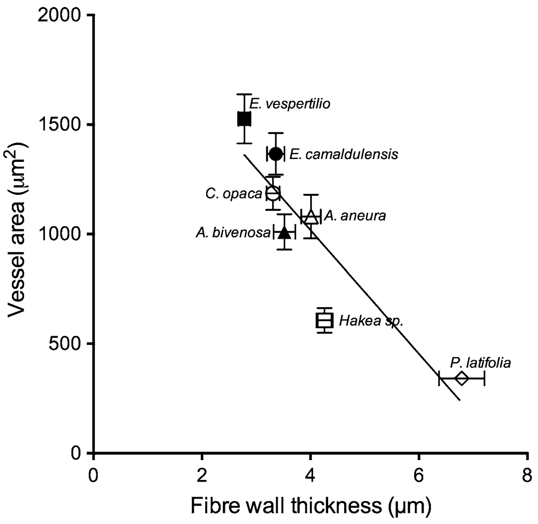

Fig. 2 Relationship between vessel area and fibre wall thickness ($r^2 = 0.80$; $p = 0.006$) for seven co-occurring species from the Ti Tree basin, Northern Territory. The regression was: Vessel area = −282 fibre wall thickness +2145

species: *C. opaca*, *E. camaldulensis*, *P. latifolia*, *Hakea* sp. and *A. aneura*, wood density was significantly higher ($p < 0.05$) than the density of intact branches. However, in *E. vespertilio* and in *A. bivenosa* differences between density of intact branch and wood density were not significant (Table 1). Density of intact branches and wood density exhibited a large positive correlation coefficient ($r^2 = 0.93$, $p = 0.0003$, Fig. 5).

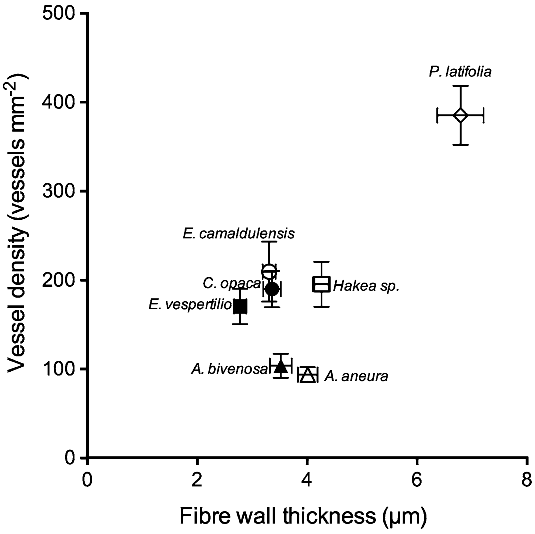

Fig. 3 Relationship between vessel density and fibre wall thickness ($r^2 = 0.05$; $p = 0.67$) for six co-occurring species from the Ti tree basin, Northern Territory

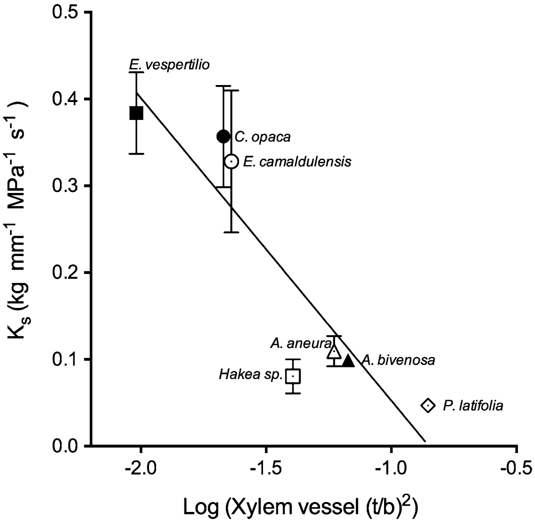

Fig. 4 Relationship between theoretical hydraulic conductivity (K_s) and log xylem vessel implosion resistance $(t/b)^2$, the regression was $K_s = -0.3484$ xylem vessel $(t/b)^2 - 0.2958$ ($r^2 = 0.83$, $p = 0.004$)

Stable carbon isotopes δ^{13}C

Values of δ^{13}C from leaves were significantly more depleted in *E. camaldulensis* than five of the remaining six species whilst there were no significant differences amongst the remaining six species (Table 2). There was a significant negative correlation between water-use efficiency and conductive area ($r^2 = 0.81$, $p < 0.05$, Fig. 6).

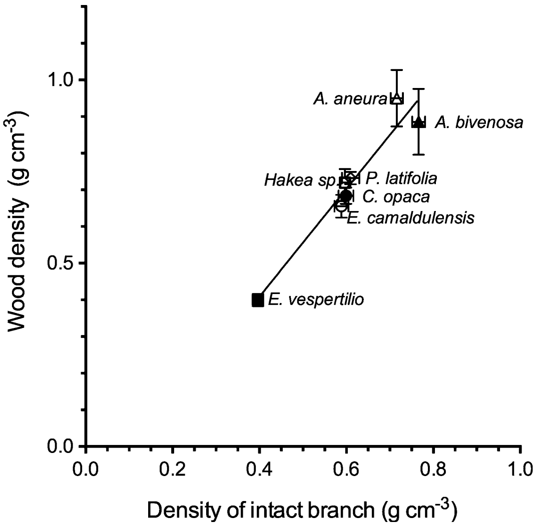

Fig. 5 Relationship between wood density and density of intact branch ($r^2 = 0.93$; $p = 0.0003$) for seven co-occurring species from the Ti Tree basin, Northern Territory. The regression was: Wood density = 1.47 Density of intact branch −0.18

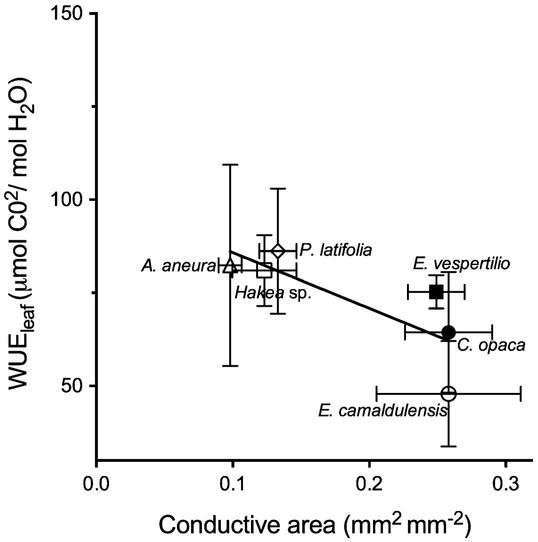

Fig. 6 Correlation between water-use efficiency (WUE, obtained with $\delta^{13}C$ from leaves) and conductive area ($r^2 = 0.81$, $p < 0.05$)

Table 2 Values of $\delta^{13}C$ for leaves ($n = 9$ leaves per species) of six species collected in the Ti Tree Basin arid zone

Species	$\delta^{13}C$ in leaves
E. vespertilio	-27.45 ± 0.07^{A}
C. opaca	-28.43 ± 0.47^{AB}
E. camaldulensis	-29.89 ± 0.41^{B}
P. latifolia	-26.50 ± 0.49^{A}
Hakea sp.	-26.84 ± 0.80^{A}
A. aneura	-26.96 ± 0.27^{A}

Different letters among columns indicate means were significantly different $p < 0.05$ as tested with a one-way analysis of variance

Values are means and standard errors

Discussion

Density of intact branches was highly variable across the seven co-occurring species from the arid zone Ti Tree Basin. Density of intact branches values ranged from 0.38 ± 0.007 g cm^{-3} in *E. vespertilio* to 0.80 ± 0.03 g cm^{-3} in *A. bivenosa*. These values agree with previous results of O'Grady et al. (2009) who observed a similar range for their 12 species study in arid Australia. This large variation in values reflects the variability in a number of anatomical and functional traits. Anatomical characteristics determining density, including conductive areas and fibre content, influence the hydraulic and mechanical properties of woody plants (Santini et al. 2012; Lachenbrunch and McCulloh 2014). Our linear regression analysis indicated that variation in density of intact branches was largely explained by differences in conductive areas across species. *Acacia bivenosa* and *A. aneura* had the highest density and also exhibited the smallest conductive areas, while *E. vespertilio*, *E. camaldulensis* and *C. opaca* exhibited lower density and larger conductive areas. The negative relationship between density of intact branch and conductive area is in agreement with our first hypothesis and with previous studies (Chave et al. 2009; Lachenbrunch and McCulloh 2014 and references therein). This relationship may reflect that species with larger conductive areas (*E. camaldulensis*, *C. opaca* and *E. vespertilio*) may also exhibit larger growth rates compared to those species with smaller conductive areas (*A. aneura*, *A. bivenosa*, *P. latifolia* and *Hakea* sp.). Larger growth rates in species with larger conductive areas are likely to be due to larger lumen areas enabling larger transpiration rates and concomitant larger rates of carbon gain (Enquist et al. 1999). O' Grady et al. (2009) found that *E. camaldulensis*, which has large conductive areas, exhibited significantly larger rates of water use and larger specific leaf areas (the ratio of leaf area to leaf dry weight) than *A. aneura*, that has small conductive areas. In addition, we found that *E. vespertilio*, *C. opaca* and *E. camaldulensis* exhibited larger theoretical hydraulic conductivities compared to *Acacia* species, *Hakea* sp. and *Psydrax latifolia*, further suggesting that the three former species will exhibit larger transpiration rates and hence larger growth rates than the three latter species. Given that growth and photosynthetic rate correlate with specific leaf area and in many cases determine plant productivity, these findings and our results of potential hydraulic conductivity (Table 3; Martínez-Cabrera and Estrada-Ruiz 2014) support our second hypothesis, that species with larger

Table 3 Calculated means and standard errors of theoretical hydraulic conductivity (K_s), Vulnerability Index (VI) 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)

Species	K_s (kg mm^{-1} MPa^{-1} s^{-1})	VI	*n*	$(t/b)^2$	*n*
E. vespertilio	0.383 ± 0.047^{A}	0.26 ± 0.03^{AC}	9	0.0096 ± 0.00006^{A}	3
C. opaca	0.356 ± 0.058^{A}	0.21 ± 0.02^{A}	9	0.0213 ± 0.00047^{B}	3
E. camaldulensis	0.328 ± 0.082^{A}	0.20 ± 0.02^{A}	9	0.0237 ± 0.00001^{B}	3
P. latifolia	0.047 ± 0.006^{B}	0.05 ± 0.004^{B}	9	0.14 ± 0.00092^{C}	3
Hakea sp.	0.08 ± 0.019^{B}	0.14 ± 0.01^{AB}	9	0.0404 ± 0.00154^{D}	3
A. aneura	0.109 ± 0.017^{B}	0.40 ± 0.04^{C}	9	0.059 ± 0.00009^{E}	3
A. bivenosa	0.099 ± 0.004^{B}	0.37 ± 0.06^{C}	9	0.067 ± 0.00011^{F}	3

Different letters among columns indicate means were significantly different $p < 0.05$ as tested with a one-way Analysis of Variance

conductive areas and lower density of intact branch utilise more water and gain more carbon than species with lower density of intact branch (Kriedemann 1986; Enquist et al. 2007; Cornelissen et al. 2003).

We determined a significant negative relationship between intrinsic water-use efficiency and conductive area. High WUE is associated with slow growth species that exhibit low transpiration rates (Ball 1988; Hasselquist et al. 2010; Craven et al. 2013; Table S1). Our results indicated that *Acacia* species, *Hakea* sp. and *P. latifolia,* characterised by small conductive areas, small potential hydraulic conductivities and larger resistance to vessel implosion exhibited a large WUE. These species are also characterised by their low stature in the field and extreme tolerance of very low soil and foliar water potentials (<−6 MPa). Furthermore, *Acacia* species have shallow roots, which can laterally extend 13 m and can only access shallow soil water in our study site (Dunkerley 2002; Rolf Faux field observation). In contrast, *E. camaldulensis* is known to access the shallow (<3 m) groundwater at our riparian site in the Ti Tree (O' Grady et al. 2009; Rolf Faux field observation). High WUE and high density of intact branches for *Acacia* spp., *Hakea* sp. and *P. latifolia* are likely to contribute significantly to their tolerance of aridity in the Ti Tree Basin, where a thick fibre matrix, small conductive areas and large vessel implosion resistance confer a larger resistance to xylem embolism. Our results support our third hypothesis that there is an inverse correlation between theoretical sapwood hydraulic conductivity and vessel implosion resistance. The pre-dawn and mid-day foliar water potential of *Acacia* spp. is much lower than that of several co-occurring tree species (especially *E. camaldulensis*; O' Grady et al. 2009), indicative of an effective drought tolerant strategy for such species. Additionally, a low specific leaf area in *Acacia* spp. may help them to avoid excessive water loss (O'Grady et al. 2006; O' Grady et al. 2009). In contrast, *E. camaldulensis*, *C. opaca* and *E. vespertilio* exhibited low WUE (Table 2) and these species access groundwater (O' Grady et al. 2009). In these phreatophytic species, where water is not a limiting resource, larger conductive areas and transpiration rates do not compromise their hydraulic safety (O' Grady et al. 2009) and support the large growth form of these species.

Wood density was correlated with density of intact branches ($r^2 = 0.93$, $p = 0.0003$). However, wood density was significantly higher than the density of intact branches in most species. These results agree with Santini et al. (2012) that found that bark in small branches is less dense that sapwood and do not contribute to mechanical support. Our results indicate that in small branches (∼2–7 mm diameter) density of intact branch can be used as a proxy for wood density. In species from the Ti Tree Basin bark may be important in protection from high temperatures; inner bark has also been demonstrated to play a role in reducing water loss and in isolating tree stems from heat (Pausas 2015).

Wood density has been correlated with resistance to pathogen invasion and higher survival, but this may be at the cost of reduced growth rates and low water storage capacitance of wood, important for maintaining cell turgidity (Bucci et al. 2004; Scholz et al. 2007; Meinzer et al. 2008). Therefore, branches of *A. bivenosa* and *A. aneura* which had the highest values of density of intact branches may survive longer than branches of *E. vespertilio*, but this may be at the cost of low growth rates and low water storage capacitance.

There was a significant negative relationship between vessel area and fibre wall thickness (Fig. 2) that may indicate that vessel area is not crucial in determining the mechanical support within the plant water transport system.

Although we found a positive trend between density of intact branches and fibre wall thickness, the regression was not significant. Previous studies (Jacobsen et al., Santini et al. 2012; Chave et al. 2009; Zieminska et al. 2013) have found that fibres largely determine wood density. Our study only accounted for fibre wall thickness, but partitioning and arrangement of fibres is also likely to be an important characteristic that determines wood density (Zieminska et al. 2013). *Acacia aneura* and *A. bivenosa,* which exhibited the highest density of intact branches did not have the thickest fibres but these species did form large fibre clusters. *Acacia* species also have little parenchyma tissue and thick vessel walls (Table 1); these characteristics are likely to account for density of intact branches (IAWA 2014).

Carlquist (1977) proposed a drought vulnerability index (VI), calculated as mean vessel diameter divided by vessel density. The correlation of this VI and resistance to drought can be low, such as the correlation of ecosystem average VI with rainfall across a Western Australian rainfall gradient is poor ($r^2 = 0.32$; Carlquist 1977). Poor correlations might be explained by the importance of traits other than vessel diameter and vessel density, including larger vessel wall thickness and larger groupings of vessels into clusters; however, in our species the correlation between vessel wall thickness and VI was not significant. In the present study, the largest VI was observed in the two *Acacia* species. This is contrary to what we know about *Acacia* species, which is that they tolerate aridity extremely well, and transpire even when foliar water potentials are less than −6 MPa (O' Grady et al. 2009) and their density of intact branch is significantly higher than that of *E. camaldulensis* (Table 1). Therefore, we conclude that at least in the current study, the calculation of VI as *per* Carlquist (1977) does not generate a true representation of vulnerability to drought.

Conclusions

This research advances our understanding on how different woody traits play a specific role in driving hydraulic niche separation of co-occurring species. Our results support the hypothesis that density of intact branch negatively correlates with sapwood conductive area. In addition, our results support the idea that xylem hydraulic traits contribute to the set of strategies that allow species co-occurrence under heterogeneous environments. *Erythrina vespertilio*, *C. opaca* and *E. camaldulensis* with their lower density of intact branches, larger conductive areas, thinner fibre walls, small resistance to vessel implosion and deeper root systems (O' Grady et al. 2009, Rolf Faux field observation) are reliant on constantly accessing groundwater resources at the cost of low resistance to cavitation and low WUE but larger growth rates. In contrast, *P. latifolia*, *Hakea* sp. and *Acacia* species that access episodic rain water with their shallow root systems (Rolf Faux field observation), are likely to be less vulnerable to cavitation by having a high density of intact branch, smaller conductive area, thicker fibre walls and high vessel implosion resistance. Associated with these traits is a larger WUE, but possibly, a reduced rate of growth.

Author contribution statement NSS performed the laboratory work, analysed the data, wrote the first manuscript draft and designed the study. DE designed the study, contributed reagents and materials and critically revised the manuscript. RR contributed with δ^{13}C data from leaves and provided laboratory assistance. JC and RF collected the plant material. CL contributed with laboratory work.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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