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

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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 ($\delta^{13}C$) as a measure of water- use-efficiency (WUE) in seven co-occurring species, *Acacia aneura*, *Acacia bivenosa*, *Corymbia opaca*, *Eucalyptus camaldulensis*, *Erythrina vespertillo*, *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 $\delta^{13}C$ 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. The results of this study demonstrated significant variation in density of intact branches, ranging from 0.38 g cm^{-3} 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.

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.

1. 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 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 y^{-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.

2. Materials and Methods

2.1 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 y^{-1} (Bureau of Meteorology, BoM station 15507), most of which falls in monsoonal summer storms. Mean minimum and maximum annual temperatures are 15°C 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 vespertillo*, *Hakea* sp., and *Psydrax latifolia*. Branches of different species varied in diameter (2 – 7 mm), but we standardized our collection by sampling the terminal 25 cm of each branch.

2.2 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 two days; we repeated this washing process twice. Micro-sections of all species with the exception of *E. vespertillo*, were prepared with a sledge Leica SM2010R microtome. Micro-sections of *E. vespertillo* were pre-treated with ethanol (8 hours), xylene (4 hours) 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):

$$VA = \pi ab 0.25 \quad \text{Eq (1)}$$

$$VD = \frac{\#vessels}{mm^2} \quad \text{Eq (2)}$$

$$CA = VAVD \quad \text{Eq (3)}$$

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.

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

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

$$K_s \propto F^{1.5} S^{0.5} \quad \text{Eq (4)}$$

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):

$$F = VAVD \quad \text{Eq (5)}$$

$$S = \frac{VA}{VD} \quad \text{Eq (6)}$$

Vessel implosion resistance was calculated as:

$$(t/b)^2 \quad \text{Eq (7)}$$

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):

$VI = \frac{D_h}{VD}$ where D_h is, Eq (8)

$D_h = \sqrt{\frac{2a^2b^2}{a^2+b^2}}$ Eq (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).

2.4 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 two 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.

2.5 Analysis of stable carbon isotopes $\delta^{13}C$

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 x 5 mm tin capsules for analysis of $\delta^{13}C$. Analyses were performed with a Picarro G2121-i Analyser (Picarro, Santa Clara, CA, USA) for isotopic $C0_2$. Values of $\delta^{13}C$ were quantified as in Eq (10), where *R* corresponds to the isotopic value $^{13}C0_2/^{12}CO_2$ of the sample (R_a) or the standard (R_b). We used atropine and acetanilide as laboratory standard references. Results were normalized with the international standards sucrose (IAEA-CH-6, $\delta^{13}C_{VPDB}$ = -10.45), cellulose (IAEA-CH-3, $\delta^{13}C_{VPDB}$ = -24.72) and graphite (USGS24, $\delta^{13}C_{VPDB}$ = -16.05).

$$\delta^{13}C = (\frac{R_a}{R_b} - 1) \times 1000 \qquad \text{Eq (10)}$$

2.6 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, $\delta^{13}C$, 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 $\delta^{13}C$ of leaves to calculate water-use-efficiency (WUE) following Eq (11) and Eq (12) (Marshall et al. 2007):

$$WUE_i = \frac{c_a(b - \Delta)}{1.6(b - a)} \qquad \text{Eq (11)}$$

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:

$$\Delta = (\delta^{13}C_{atm} - \delta^{13}C_{plant})/(1 + \frac{\delta^{13}C_{plant}}{1000}) \quad \text{Eq (12)}$$

Where $\delta^{13}C_{atm}$ is −8.1‰ (Carbon Dioxide Information Analysis Center 2014) and $\delta^{13}C$ 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).

3. Results

3.1 Xylem traits

The density of intact branches was significantly lower in *E. vespertillo* 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. vespertillo*, *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. vespertillo* (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. vespertillo*, *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. vespertillo* 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 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. vespertillo* 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).

3.2 Stable carbon isotopes $\delta^{13}C$

Values of $\delta^{13}C$ 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).

4. 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. vespertillo* 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. vespertillo*, *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. vespertillo*) 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

337 weight) than *A. aneura*, that has small conductive areas. In addition, we found
338 that *E. vespertillo*, *C. opaca* and *E. camaldulensis* exhibited larger theoretical
339 hydraulic conductivities compared to *Acacia* species, *Hakea* sp. and *Psydrax*
340 *latifolia*, further suggesting that the three former species will exhibit larger
341 transpiration rates and hence larger growth rates than the three latter species.
342 Given that growth and photosynthetic rate correlate with specific leaf area and in
343 many cases determine plant productivity, these findings and our results of
344 potential hydraulic conductivity (Table 3; Martínez-Cabrera and Estrada-Ruiz
345 2014) support our second hypothesis, that species with larger conductive areas
346 and lower density of intact branch utilize more water and gain more carbon than
347 species with lower density of intact branch (Kriedeman 1986; Enquist et al.
348 2007; Cornelissen et al. 2003).
349
350 We determined a significant negative relationship between intrinsic water-use-
351 efficiency and conductive area. High WUE is associated with slow growth
352 species that exhibit low transpiration rates (Ball 1988; Hasselquist et al. 2010;
353 Craven et al. 2013; Table S1). Our results indicated that *Acacia* species, *Hakea* sp.
354 and *P. latifolia,* characterized by small conductive areas, small potential
355 hydraulic conductivities and larger resistance to vessel implosion exhibited a
356 large WUE. These species are also characterized by their low stature in the field
357 and extreme tolerance of very low soil and foliar water potentials (< -6 MPa).
358 Furthermore, *Acacia* species have shallow roots, which can laterally extend 13 m
359 and can only access shallow soil water in our study site (Dunkerley 2002; Rolf
360 Faux field observation). In contrast, *E. camaldulensis* is known to access the
361 shallow (< 3 m) groundwater at our riparian site in the Ti-Tree (O'Grady et al.
362 2009; Rolf Faux field observation). High WUE and high density of intact
363 branches for *Acacia* spp.*, Hakea* sp. and *P. latifolia* are likely to contribute
364 significantly to their tolerance of aridity in the Ti Tree Basin, where a thick fibre
365 matrix, small conductive areas and large vessel implosion resistance confer a
366 larger resistance to xylem embolism. Our results support our third hypothesis
367 that there is an inverse correlation between theoretical sapwood hydraulic
368 conductivity and vessel implosion resistance. The pre-dawn and mid-day foliar
369 water potential of *Acacia* spp. is much lower than that of several co-occurring

370 tree species (especially *E. camaldulensis*; O'Grady et al. 2009), indicative of an
371 effective drought tolerant strategy for such species. Additionally, a low specific
372 leaf area in *Acacia* spp. may help them to avoid excessive water loss (O'Grady et
373 al. 2006; O'Grady et al. 2009). In contrast, *E. camaldulensis*, *C. opaca* and *E.*
374 *vespertillo* exhibited low WUE (Table 2) and these species access shallow
375 groundwater (O'Grady et al. 2009). In these phreatophytic species, where water
376 is not a limiting resource, larger conductive areas and transpiration rates do not
377 compromise their hydraulic safety (O'Grady et al. 2009) and support the large
378 growth form of these species.
379
380 Wood density was correlated with density of intact branches (r^2=0.93,
381 p=0.0003). However, wood density was significantly higher than the density of
382 intact branches in most species. These results agree with Santini et al. (2012)
383 that found that bark in small branches is less dense that sapwood and do not
384 contribute to mechanical support. Our results indicate that in small branches (~
385 2 – 7 mm diameter) density of intact branch can be used as a proxy for wood
386 density. In species from the Ti Tree Basin bark may be important in protection
387 from high temperatures; inner bark has also been demonstrated to play a role in
388 reducing water loss and in isolating tree stems from heat (Pausas 2014).
389
390 Wood density has been correlated with resistance to pathogen invasion and
391 higher survival, but this may be at the cost of reduced growth rates and low
392 water storage capacitance of wood, important for maintaining cell turgidity
393 (Bucci et al. 2004; Scholz et al. 2007; Meinzer et al. 2008). Therefore branches of
394 *A. bivenosa* and *A. aneura* which had the highest values of density of intact
395 branches may survive longer than branches of *E. vespertillo*, but this may be at
396 the cost of low growth rates and low water storage capacitance.
397
398 There was a significant negative relationship between vessel area and fibre wall
399 thickness (Fig. 2) that may indicate that vessel area is not crucial in determining
400 the mechanical support within the plant water transport system.
401
402 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 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 West Australia 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.

5. 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 vespertillo*, *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.

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Author contribution

NSS performed the laboratory work, analyzed 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 $\delta^{13}C$ data from leaves and provided laboratory assistance. JC and RF collected the plant material. CL contributed with laboratory work.

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470 **Conflict of interest**
471 The authors declare they have no conflict of interest.
472
473 **References**
474
475 Australian Bureau of Meteorology (2014) Australian Bureau of Meteorology
476 home page. Commonwealth of Australia: Canberra.
477 http://www.bom.gov.au. Accessed 1 November 2014.
478
479 Ball MC (1988) Salinity tolerance in the mangroves Aegiceras corniculatum and
480 Avicennia marina I. Water use in relation to growth, carbon
481 partitioning, and salt balance. Aust J Plant Physiol. 15:447 –
482 464. Doi:10.1071/PP9880447
483
484 Bucci SJ, Goldstein G, Meinzer FC, Scholz FG, Franco AC, Bustamante M (2004)
485 Functional convergence in hydraulic architecture and water
486 relations of tropical savanna trees: from leaf to whole plant.
487 Tree Physiol. 24: 891 – 899. doi: 10.1093/treephys/24.8.891
488
489 Carbon Dioxide Information Analysis Center (2014) http://cdiac.ornl.gov/.
490 Accessed 1 September 2014.
491
492 Carlquist S (1977) Ecological factors in wood evolution: A floristic approach. Am
493 J Bot. 64: 887 – 896.
494
495 Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a
496 worldwide wood economics spectrum. Ecol Lett. 12: 351 –
497 366. doi:10.1111/j.1461-0248.2009.01285.x
498
499 Craven D, Hall JS, Ashton MS, Berlyn GP (2013) Water use efficiency and whole
500 plant performance of nine tropical tree species at two sites

with contrasting water availability in Panama. Trees. 27:639 – 653. doi: 10.1007/s00468-012-0818-0

Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, Steege H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H (2003) A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Aust J Bot. 51: 335 – 380. doi: 10.1071/BT02124

Dunkerley D (2002) Systematic variation of soil infiltration rates within and between the components of the vegetation mosaic in an Australian desert landscape. Hydrol Process. 16: 119 – 131. doi: 10.1001/hyp.357

Eamus D (1991) The interaction of rising CO2 and temperatures with water use efficiency. Plant Cell Environ. 14: 843 – 852. doi: 10.1111/j.1365-3040.1991.tb01447.x

Eamus D (2003) How does ecosystem water balance affect net primary productivity of woody ecosystems? Funct Plant Biol. 30:187–205. doi: 10.1071/FP02084

Enquist B, West G, Charnov E, Brown J (1999) Allometric scaling of production and life-history variation in vascular plants. Nature 401: 909–911. doi:10.1038/44819

Enquist BJ, Kerkhoff AJ, Stark SC, Swenson NG, McCarthy MC, Price CA (2007) A general integrative model for scaling plant growth, carbon flux, and functional trait spectra. Nature. 449:218 – 222. doi: 10.1038/nature06061

532 Farquhar GD, Richards RA (1983) Isotopic composition of plant carbon
533 correlates with water use efficiency of wheat genotypes. Aust
534 J Plant Physiol. 11: 539 – 552. doi:10.1071/PP9840539
535
536 Froend RH, Drake PL (2006) Defining phreatophyte response to reduced water
537 availability: preliminary investigations on the use of xylem
538 cavitation vulnerability in *Banksia* woodland species. Aus J
539 Bot. 54: 173-179. http://dx.doi.org/10.1071/BT05081.
540
541 Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard RL (2009)
542 Tracing carbon and oxygen isotope signals from newly
543 assimilated sugars in the leaves to the tree-ring archive. Plant
544 Cell Environ. 32:780 – 795. doi: 10.1111/j.1365-
545 3040.2009.01957.x
546
547 Groom PK (2004) Rooting depth and plant water relations explain species
548 distribution patterns within a sandplain landscape. Func
549 Plant Biol. 31: 423-428. doi:10.1071/FP03200.
550
551 Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in
552 wood density and structure are linked to prevention of xylem
553 implosion by negative pressure. Oecologia. 126: 457 – 461.
554 doi: 10.1007/s004420100628
555
556 Harrington GA, Cook PG, Herczeg AL (2002) Spatial and temporal variability of
557 groundwater recharge in Central Australia: A tracer
558 approach. Groundwater. 40: 518-528. doi:10.1111/j.1745-
559 6584.2002.tb02536.x
560
561 Hasselquist N, Allen M, Santiago L (2010) Water relations of evergreen and
562 drought-deciduous trees along a seasonally dry tropical
563 forest chronosequence. Oecologia 164:881–890. doi:
564 10.1007/s00468-012-0818-0

565
566 International Association of Wood Anatomists (IAWA) (2014).
567 http://bio.kuleuven.be/sys/iawa/. Accessed 5 May 2015.
568
569 Jacobsen AL, Ewers FW, Pratt B, Paddock WA, Davis SD (2005) Do xylem fibres
570 affect vessel cavitation resistance? Plant Physiol. 139: 546 –
571 556. doi: http://dx.doi.org/10.1104/pp.104.
572
573 King DA, Davies SJ, Tan S, Noor NSM (2006) The role of wood density and stem
574 support costs in the growth and mortality of tropical trees. J
575 Ecol. 94: 670 – 680. doi:10.1111/j.1365-2745.2006.01112.x
576
577 Kriedemann, PE (1986) Stomatal and photosynthetic limitations to leaf growth.
578 Aust J Plant Physiol. 13, 15-32.
579
580 Lachenbrunch B, McCulloh KA (2014) Traits, properties, and performance: how
581 woody plants combine hydraulic and mechanical functions in
582 a cell, tissue, or whole plant. New Phytol. 204: 747 – 764.
583 doi:10.1111/nph.13035.
584
585 Lewis AM (1992) Measuring the hydraulic diameter of a pore or conduit. Am J
586 Bot. 79: 1158 – 1161.
587
588 Marshall JD, Brooks JR, Lajtha K (2007) Sources of variation in the stable isotopic
589 composition of plants. In: Michener R and Lajtha K (eds)
590 Stable isotopes in ecology and environmental science.
591 Blackwell Publishing. Carlton, Victoria, pp 22 -60
592
593 Martínez-Cabrera HI, Estrada-Ruiz E (2014) Wood anatomy reveals high
594 theoretical hydraulic conductivity and low resistance to
595 vessel implosion in a cretaceous fossil forest from Northern
596 Mexico. PloS One 10:e108866-
597 doi:10.1371/journal.pone.0108866

Meinzer FC, Campanello PI, Domec J, Gatti MG, Goldstein G, Villalobos-Vega R, Woodruff DR (2008) Constraints on physiological function associated with branch architecture and wood density in tropical forest trees. Tree Physiol. 28: 1609-1617.

O' Grady AP, Cook PG, Eamus D, Duguid A, Wischunsen JDH, Fass T, Worldege D (2009) Convergence of tree water use within an arid-zone woodland. Oecologia. 160: 643 – 655. doi: 10.1007/s00442-009-1332-y

O'Grady AP, Eamus D, Cook PG, Lamontagne S (2006) Groundwater use by riparian vegetation in the wet-dry tropics of northern Australia. Aust J Bot. 54:145 – 154. doi:10.1071/BT04164

Pratt R, Jacobsen A, Ewers F, Davis S (2007) Relationships among xylem transport, biomechanics and storage and roots of nine Rhamnaceae species of the California chaparral. New Phytol 174: 787–798. doi: 10.1111/j.1469-8137.2007.02061.x

Pausas JG. Bark thickness and fire regime. Funct Ecol 29:315 – 327.doi:10.1111/1365-2435.12372

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Australia. http://www.R-project.org.

Santini NS, Schmitz N, Bennion V, Lovelock CE (2012) The anatomical basis of the link between density and mechanical strength in mangrove branches. Funct Plant Biol. 40 : 400 – 408.doi: http://dx.doi.org/10.1071/FP12204

631
632 Silvertown J (2004) Plant coexistence and the niche. Trends Ecol Evol. 19:605-
633 611.doi:10.1016/j.tree.2004.09.003
634
635 Silvertown J, Dodd ME, Gowing DJG, Mountford JO (1999) Hydrologically defined
636 niches reveal a basis for species richness in plant
637 communities. Nature 400:61-63. doi:10.1038/21877
638
639 Sterck F, Markesteijn L, Schieving F, Poorter L (2011) Functional traits
640 determine trade-offs and niches in a tropical forest
641 community. 108:20627 -20632.
642 doi:10.1073/pnas.1106950108
643
644 Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other
645 woody plants. New Phytol. 119:345-360. doi:10.1111/j.1469-
646 8137.1991.tb00035.x
647
648 Terradas J, Peñuelas J, Lloret F (2009) The fluctuation niche in plants.
649 International Journal of Ecology 2009: ID 959702, doi:
650 10.1155/2009/959702.
651
652 Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm (2007)
653 Biophysical properties and functional significance of stem
654 water storage tissues in Neotropical savanna trees. Plant, Cell
655 Environ. 30: 236 – 248. doi :10.1111/j.1365-
656 3040.2006.01623.x
657
658 Zanne A, Westoby M, Falster DS, Ackerly DD, Loarie SR, Arnold SEJ, Coomes DA
659 (2010) Angiosperm wood structure: Global patterns in vessel
660 anatomy and their relation to wood density and potential
661 conductivity. Am J Bot. 97: 207 – 215.
662 doi:10.3732/ajb.0900178
663

Zieminska K, Butler DW, Gleason SM, Wright IJ, Westoby M (2013) Fibre wall and lumen fractions drive wood density variation across 24 Australian angiosperms AoB PLANTS **5**: plt046; doi:10.1093/aobpla/plt046

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Tables

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

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. vespertillo	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)

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. Values are means and standard errors. Different letters among columns indicate means were significantly different $p<0.05$ as tested with a one-way Analysis of Variance.

Species	$\delta^{13}C$ in leaves
E. vespertillo	-27.45 ± 0.07^{A}
C. opaca	-28.43 ± 0.47^{AB}
E. camaldulensis	-29.89 ± 0.41^{B}
P. latifolia	-26.50 ± 0.48^{A}
Hakea sp.	-26.84 ± 0.80^{A}
A. aneura	-26.96 ± 0.27^{A}

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). 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})		**VI**		***n***	**$(t/b)^2$**		***n***
E. vespertillo	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

Table S1. Relative growth rate (RGR) of some of our studied species obtained from the literature. NA indicates that data were not available in the literature.

Species	Relative Growth Rate (RGR, mg g^{-1} day^{-1})	Reference
E. vespertillo	41	Tomlinson et al. (2012)
C. opaca	N.A.	
E. camaldulensis	215	Grotkopp and Reimanek (2007)
P. latifolia	N.A.	
Hakea spp.	Range, 15 - 35	Poot and Lambers (2003)
A. aneura	59.5	Atkin et al. (1999)
A. bivenosa	N.A.	

References

Atkin OK, Schortemeyer M, McFarlane N, Evans JR (1999) The response of fast- and slow-growing Acacia species to elevated atmospheric CO2: an analysis of the underlying components of relative growth rate. Oecologia. 120 : 544 - 554.

Tomlinson KW, Sterck FJ, Bongers F, da Silva DA, Barbosa ERM, Ward D, Bakker FT, van Kaauwen M, Prins HHT, de Bie S, van Langevelde F (2012) Biomass partitioning and root morphology of savanna trees across a water gradient. Journal of Ecology. 100:1113 – 1121.

778 Grotkopp E, Rejmánek M (2007) High seedling relative growth rate and specific leaf area are traits of invasive species: Phylogenetically
779 independent contrasts of woody angiosperms. American Journal of Botany. 94: 526-532.
780
781 Poot P, Lambers H (2003) Growth responses to waterlogging and drainage of woody Hakea (Protaceae) seedlings, originating from
782 contrasting habitats in south-western Australia. Plant and Soil. 253:57-70.

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784 **Figure legends**
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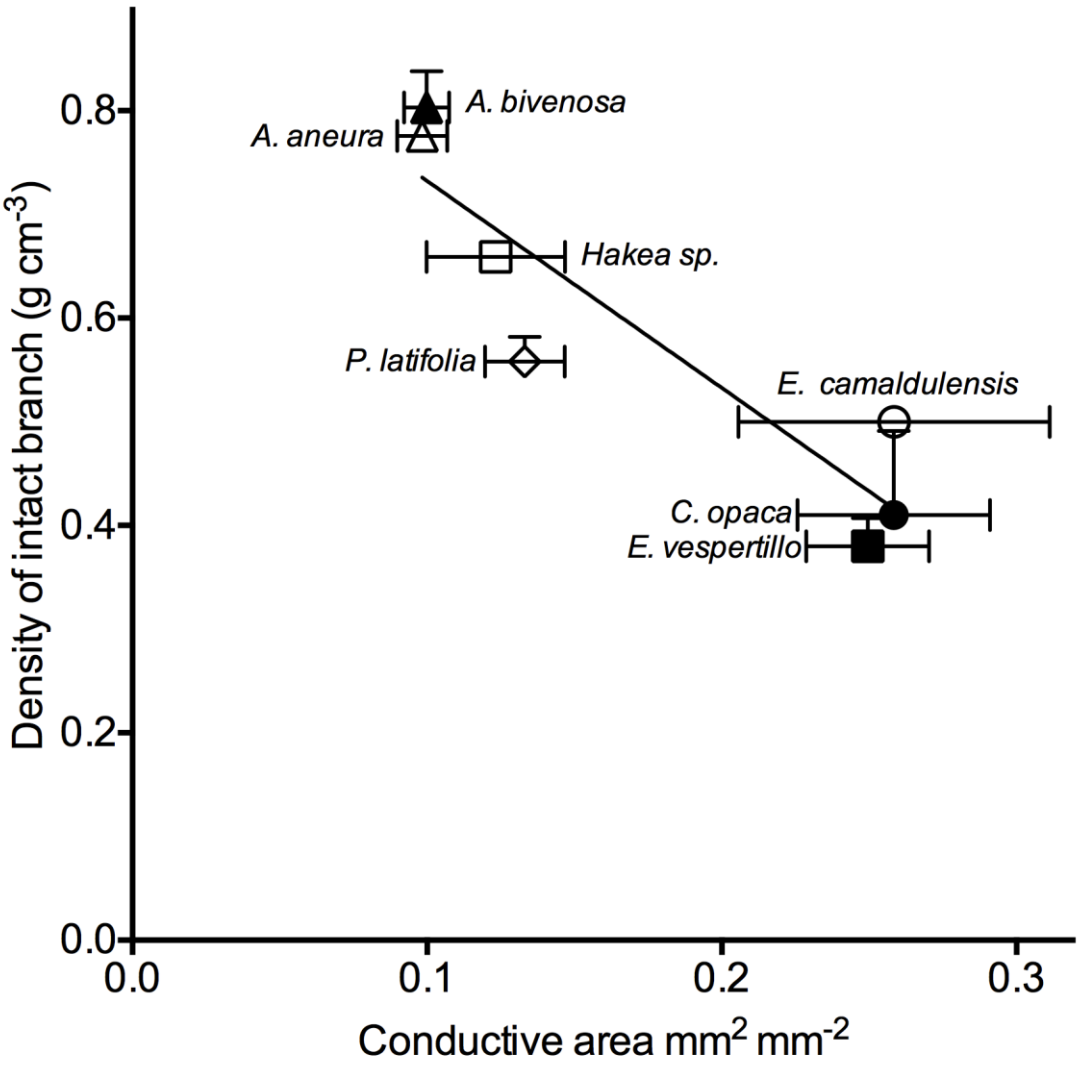

786

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

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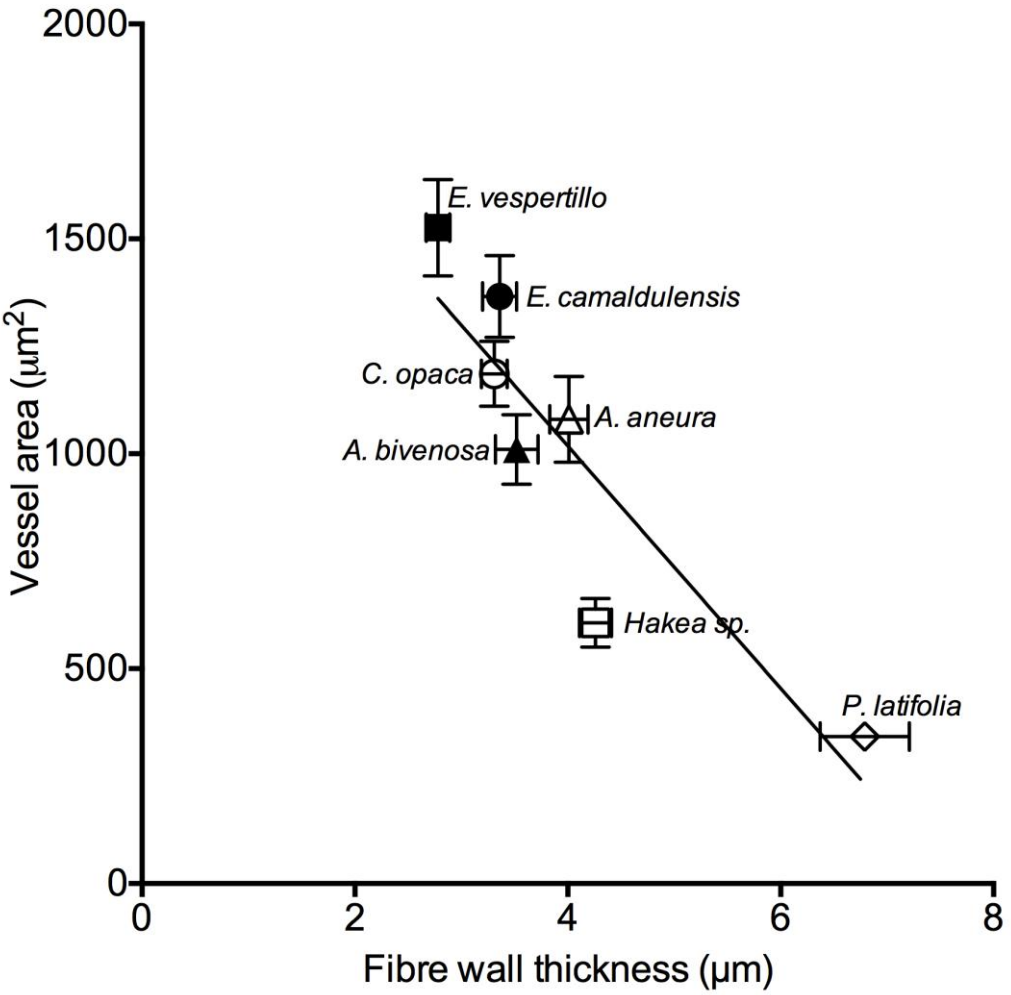

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800 **Fig. 2.** Relationship between vessel area and fibre wall thickness (r^2=0.80;
801 p=0.006) for seven co occurring species from the Ti Tree basin, Northern
802 Territory. The regression was: Vessel area= -282 Fibre wall thickness + 2145.
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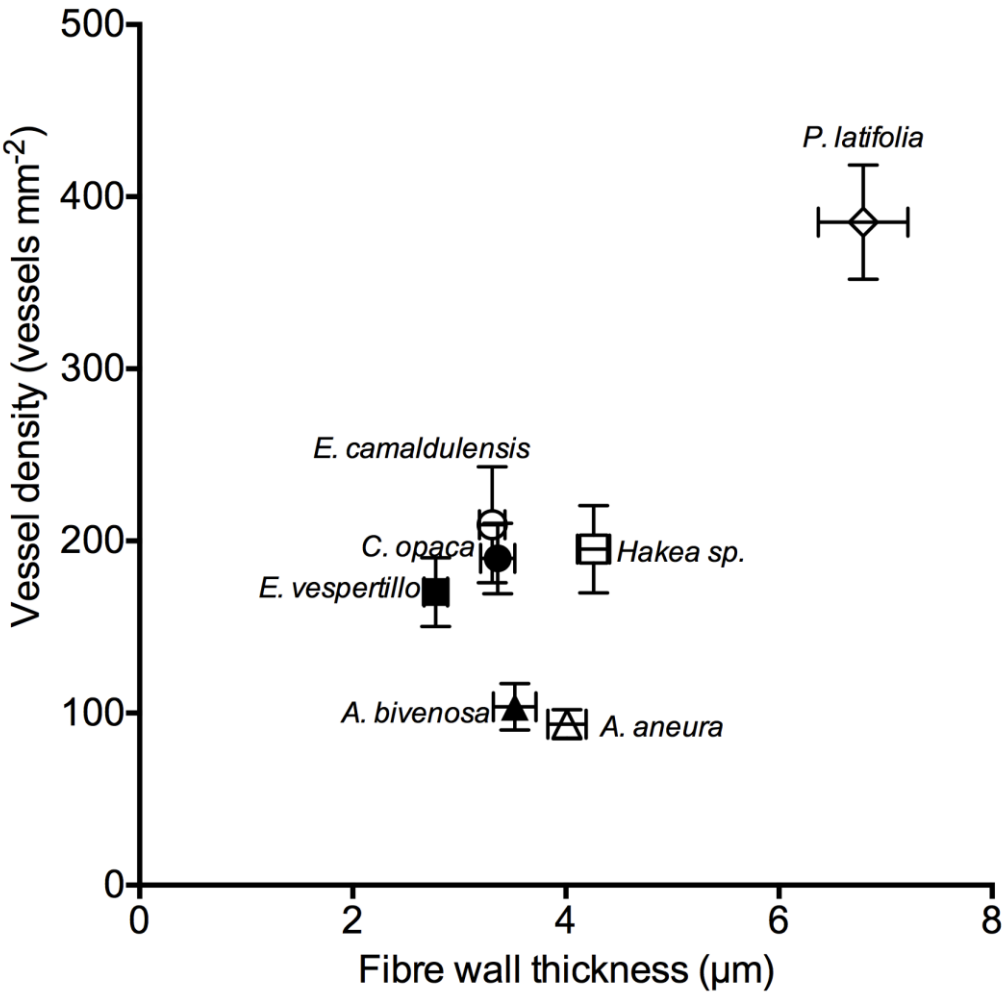

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807 **Fig. 3.** Relationship between vessel density and fibre wall thickness (r^2=0.05;
808 p=0.67) for six co occurring species from the Ti Tree basin, Northern Territory.
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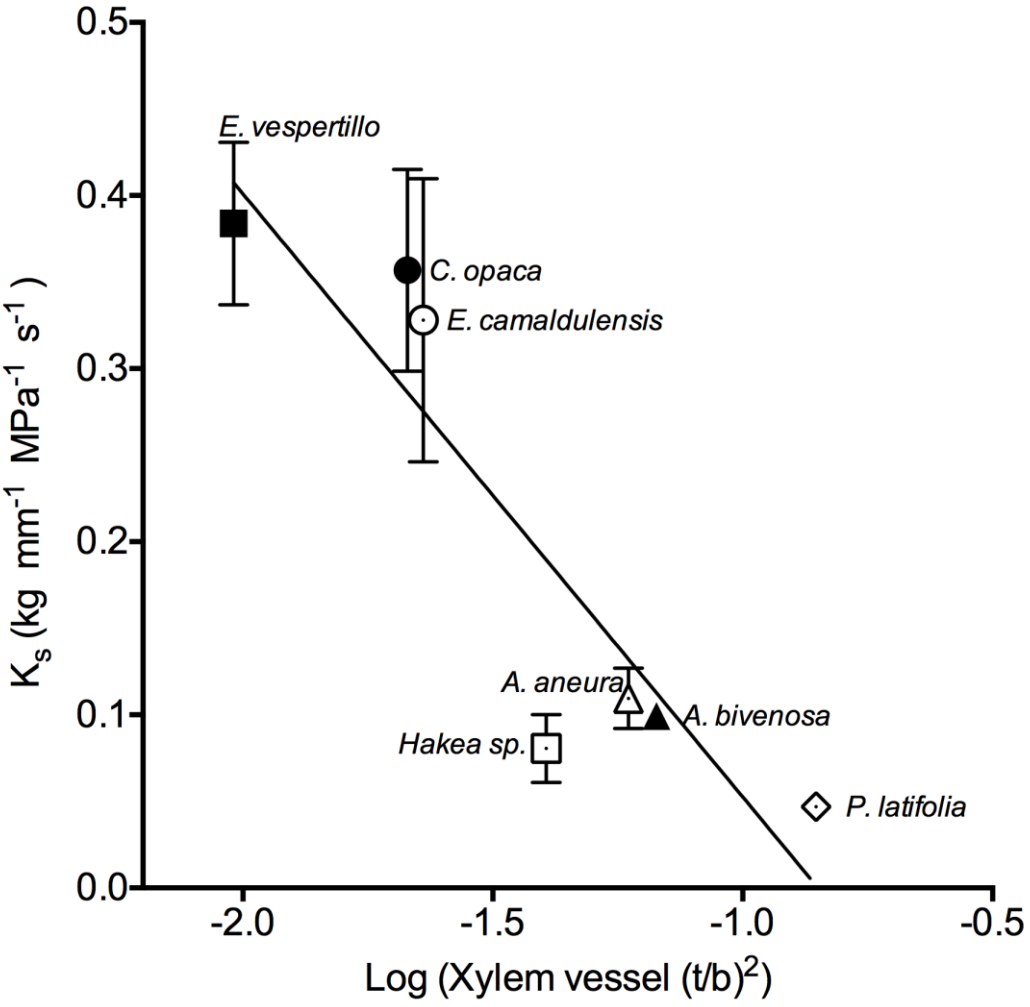
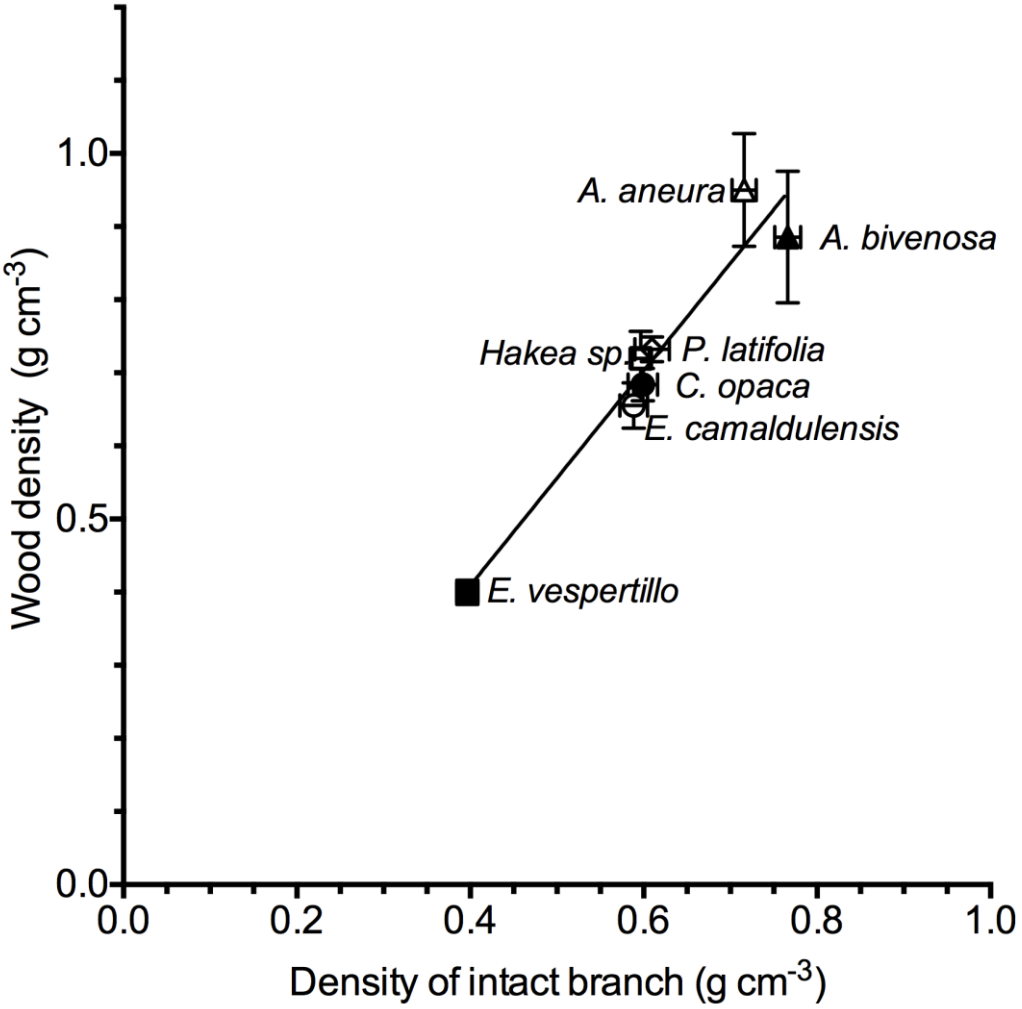


Fig.4. Relationship between theoretical hydraulic conductivity (K_s) and log xylem vessel implosion resistance $(t/b)^2$, the regression was K_s = -0.3484 xylem vessel $(t/b)^2$ – 0.2958 (r^2=0.83, p=0.004).



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831 **Fig. 5.** Relationship between wood density and density of intact branch (r^2=0.93;
832 p=0.0003) for seven co-occurring species from the Ti Tree basin, Northern
833 Territory. The regression was: Wood density = 1.47 Density of intact branch -
834 0.18.

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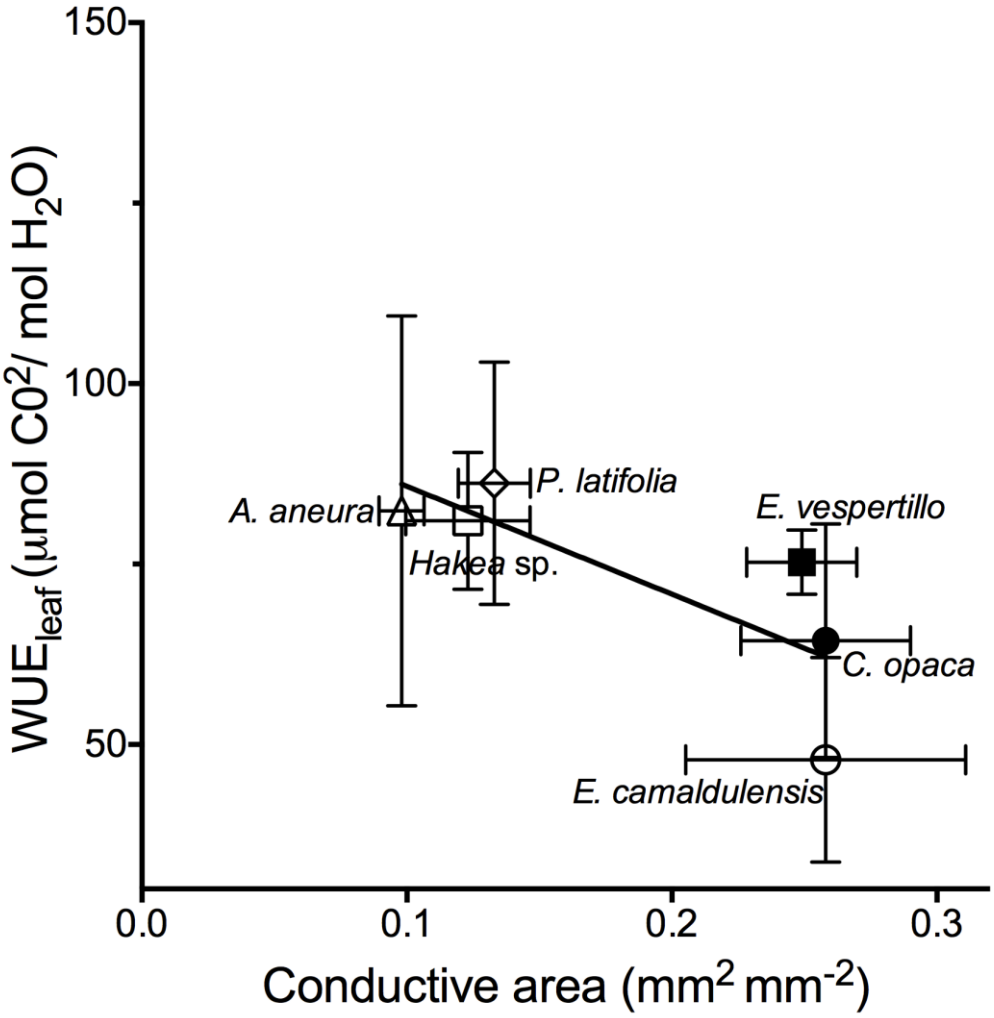

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838 **Fig. 6.** Correlation between water use efficiency (WUE, obtained with $\delta^{13}C$ from

839 leaves) and conductive area (r^2=0.81, $p<0.05$).

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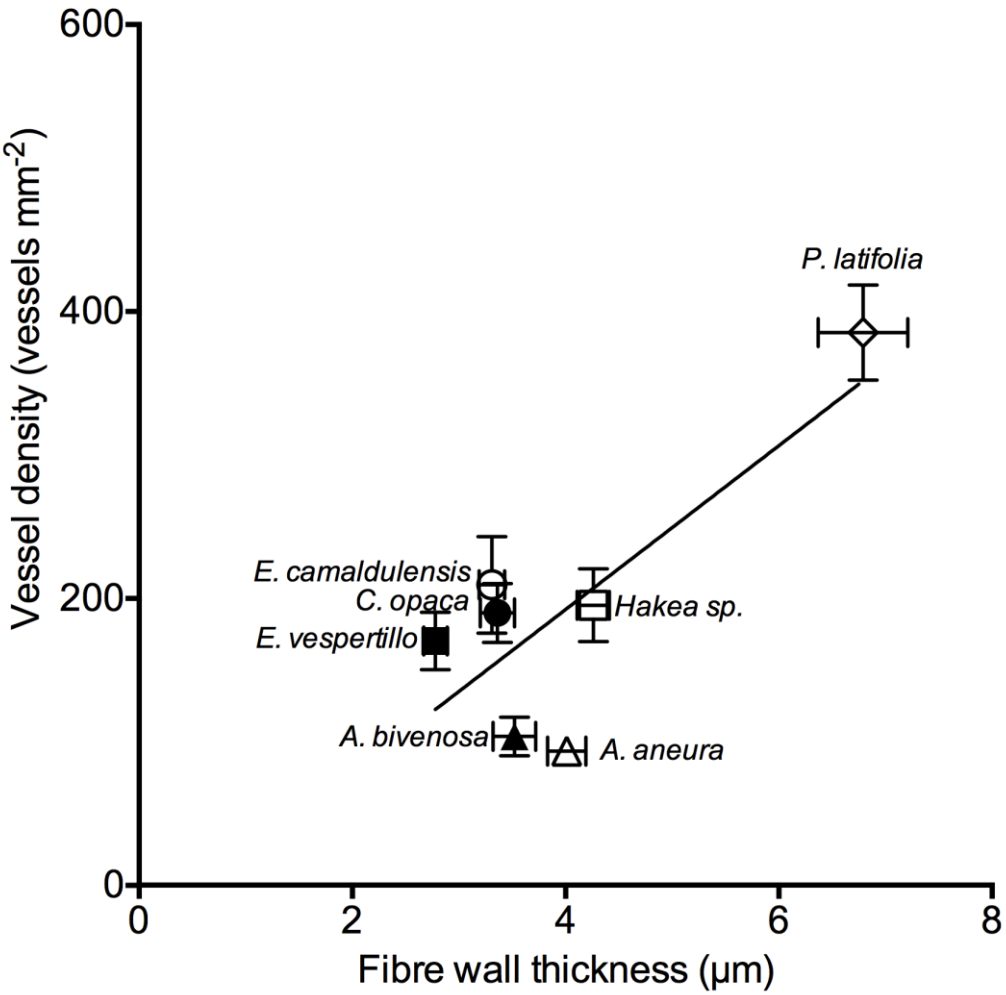

841

842 **Fig. S1.** Relationship between vessel density and fibre wall thickness (r^2=0.61;
843 p=0.037) for seven co occurring species from the Ti Tree basin, Northern
844 Territory. The regression was: Vessel density= 57.1 Fibre wall thickness – 36.23.

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