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Interspecific variation in branch and leaf traits among three *Syzygium* tree species from different successional tropical forests

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Abstract. Plant functional traits are closely associated with plant habitats. In this study, we investigated the interspecific variations in stem and leaf hydraulics, xylem and leaf anatomy, gas-exchange rates and leaf pressure–volume relationships among three *Syzygium* tree species in early, mid- and late successional tropical forests. The objective was to understand the response and adaptation of congeneric species, in terms of branch and leaf functional traits, to different environments. A consistent pattern of decline with succession was evident in leaf and sapwood specific hydraulic conductivity (k_s), maximum leaf hydraulic conductance (K_{leaf}), and photosynthetic rates for the three *Syzygium* species. Variations of k_s and K_{leaf} were correlated with changes in vessel anatomy (i.e. vessel density and diameter) and leaf flux-related structure (i.e. stomatal pore index and vein density) respectively. However, specific leaf area and leaf to sapwood area ratio did not significantly differ among the three species. In addition, the mid-successional species had the lowest values of leaf water potential at full turgor and turgor loss point and 50% loss of K_{leaf}, but the greatest value of xylem water potential at 50% loss of k_s. Our results demonstrate that leaf and branch traits associated with photosynthesis and/or hydraulic conductance, rather than those associated with drought tolerance, are the key factors underlying the response and adaptation of the three *Syzygium* tree species along the tropical forest succession.

Additional keywords: cavitation, hydraulic architecture, photosynthesis, tropical secondary succession.

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Introduction

Plant functional traits integrate morphological, physiological, and phenological properties (Violle *et al.* 2007; Pérez-Harguindeguy *et al.* 2013), so they are commonly investigated to predict species distributions from local to global scales (Reich *et al.* 1999; Poorter *et al.* 2010), to assess community composition and function (Garnier *et al.* 2004) and to evaluate plant adaptation to different environments (Westoby *et al.* 2002). For example, leaf economic traits are used to classify plant ecological performance into quick- or slow-return strategies (Wright *et al.* 2004). Several recent studies have revealed that hydraulic-related traits are useful for studying the response and adaptation of plants to environmental changes (McCulloh *et al.* 2011; Vinya *et al.* 2013).

Hydraulic architecture (which mainly concerns terminal branches) includes several important parameters that describe the ability of xylem to supply water to distal leaves and to tolerate drought-induced cavitation (Tyree and Ewers 1991; Pratt *et al.* 2008). These parameters are therefore used to evaluate the adaptation of plants to habitats with variable water availability (Canham *et al.* 2009). For example, *Cordia* species growing at drier sites are more resistant to cavitation than those growing at moister sites (Choat *et al.* 2007). In another study, low leaf specific hydraulic conductivity was considered a main cause of high mortality of *Pinus sylvestris* L. under severe drought events (Martínez-Vilalta and Pinol 2002). Several studies have revealed that xylem hydraulic traits are closely correlated with plant carbon economy (Zhang and Cao 2009), life history (Simonin *et al.* 2012) and shade tolerance (Markesteijn *et al.* 2011*a*). In addition, research has shown that early successional plants usually have greater hydraulic conductivity than late successional species, which helps the early successional

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species cope with the high evaporative demand in early successional habitats (Markesteijn *et al*. 2011*b*; Zhu *et al*. 2013).

Leaves are major hydraulic bottlenecks in plants because they constitute a substantial part (>30%) of the whole-plant hydraulic resistance (Becker *et al*. 1999; Sack and Holbrook 2006). Therefore, leaf hydraulic conductance (K_{leaf}) significantly affects leaf-level function (Brodribb and Holbrook 2003; Sack and Frole 2006). K_{leaf} varies considerably among plant groups. Shade-tolerant species, for example, tend to have much lower K_{leaf} than light-demanding species (Nardini *et al*. 2005; Sack *et al*. 2005). In addition, K_{leaf} is rather sensitive to environmental conditions such as water shortage (Hao *et al*. 2008), temperature fluctuations (Sack *et al*. 2004), and soil nutrient availability (Domec *et al*. 2009). Several studies show that leaves are more vulnerable than terminal branches to drought-induced cavitation (Hao *et al*. 2008; Johnson *et al*. 2011; Bucci *et al*. 2013). Under midday depression, decline of K_{leaf} triggers stomatal closure to prevent further water loss and therefore to reduce xylem tensions in stems. Under extreme drought events, plants may even shed their leaves to protect the functionality of stems (Bucci *et al*. 2012; Pivovaroff *et al*. 2014). Thus, leaf embolism could act as a 'safety valve' that protects plant hydraulic pathway (Chen *et al*. 2009; Bucci *et al*. 2012, 2013).

A large area of primary tropical forests in south-western China was farmed or logged during the last century, resulting in a large number of tropical forest fragments (Li *et al*. 2009). The resulting landscape consists of open field, secondary forest and patches of mature forest, representing different stages of secondary succession for the region. Species of *Syzygium* (Myrtaceae) are common and widely distributed in tropical forests of south-western China. A recent forest survey in the study site revealed that three tree species belonging to the genus *Syzygium* (*Syzygium szemaoense* Merr. et Perry, *Syzygium cumini* (L.) Skeels and *Syzygium latilimbum* Merr. et Perry) distinctly occur in early, mid- and late successional forests, respectively (SD Zhu, YJ Chen, KF Cao, unpubl. data), the determinants for this association of particular *Syzygium* species with specific successional stages in tropical forests remain unknown. In this work, we investigated the interspecific variations in xylem and leaf anatomy, branch and leaf hydraulic architecture and photosynthetic parameters among the three *Syzygium* species. Our goal was to better understand the response and adaptation of congeneric species to different successional habitats.

Materials and methods

Study site and plant materials

This study was conducted at the Xishuangbanna Tropical Botanical Garden (XTBG, 21°54′N, 101°46′E; 580 m above sea level), Chinese Academy of Sciences, Yunnan Province, south-western China. The study site is significantly influenced by a monsoon climate, with a distinct dry season lasting 6 months (November to April). Mean annual total precipitation is ~1500 mm, of which ~80% occurs in the wet season from May to October. Mean annual temperature is 21.4°C, with monthly mean temperatures ranging from 15.1°C in January to 21.7°C in July (Cao *et al*. 2006).

In the east of XTBG, there is a well protected primary tropical forest with an area over 100 ha (late successional forest). The adjacent forests include heavily disturbed, open forest (early successional forest) and young secondary forest (mid-successional forest). The three studied *Syzygium* tree species (*Syzygium szemaoense* Merr. et Perry, *Syzygium cumini* (L.) Skeels and *Syzygium latilimbum* Merr. et Perry) were selected in forests at different successional stages. Habitat conditions of the three successional forests are summarised in Table 1. For all trait measurements, upper canopy and sun-exposed branches carrying fully expanded and healthy leaves were harvested using long pruners. Five mature individuals for each species were sampled.

Branch hydraulic conductivity

Maximum vessel length (MVL) of the three *Syzygium* species was determined using an air infiltration technique described by Ewers and Fisher (1989). Briefly, branches ~1 m long ($n = 5$–7) from the trees were cut and the cut end was connected with low air pressure (~60 kPa). The distal end of the branch was trimmed back (at a 1 cm intervals) under water until air bubbles emerged. The maximum vessel length was defined as the remaining branch length (Ewers and Fisher 1989).

A total of 10 leaf-bearing branches (5–8 mm in diameter) from five mature individuals (for each of the three species) were cut off in early morning, sealed in black plastic bags with moist towels, and immediately transported to the laboratory. Before measurement, all of the branch samples were re-cut under water and the cut ends were trimmed with a razor blade. The branch segments used for hydraulic measurement were ~10% longer than their MVL. To remove air embolisms, branch segments were flushed with a filtered (diameter: 0.2 µm) 20 mM degassed KCl solution at a pressure of 0.15 MPa for 15–20 min. Each segment was then connected to a hydraulic conductivity-measurement apparatus (Sperry *et al*. 1988). An elevated water reservoir supplied the same flush solution to the segment, with a head pressure of ~5.5 kPa. Water flow through the segment was allowed to equilibrate for ~5–10 min, after which the mass of water flux though the segment over time (in seconds) was measured. Maximum hydraulic conductivity of the segment (k_{h}) was calculated as $k_{\mathrm{h}} = FL/\Delta P$, where F is the flow rate (kg s^{-1}), ΔP is the pressure gradient (MPa) through the segment, and L is the length of the segment (m). Sapwood specific hydraulic conductivity (k_{s}, kg m^{-1} s^{-1} MPa^{-1}) is equivalent to k_{h} divided by

Table 1. Habitat descriptions of the three successional forests where the three *Syzygium* tree species (Myrtaceae) occur

Species	Successional status	Habitat
Syzygium szemaoense Merr. et Perry	Early succession	Heavily disturbed, open forest. Canopy height 8 m, canopy coverage 30−50%
Syzygium cumini (L.) Skeels	Mid- succession	Young secondary forest. Canopy height 10−12 m, canopy coverage 50−60%
Syzygium latilimbum Merr. et Perry	Late succession	Mature forest. Canopy height 25 m, canopy coverage 80−90%

the mean value of sapwood cross-sectional area of both ends of the segment (A_S). Leaf specific hydraulic conductivity (k_l, kg m^{-1} s^{-1} MPa^{-1}) was calculated as the ratio of k_h to total area of leaves distal to the stem segment (A_L). Leaf to sapwood area was calculated as A_L divided by A_S.

Vulnerability curves

For the current measurements, we harvested several leaf-bearing branches (nearly 1 m long) for each species at predawn (before sunrise). The cut ends of the branches were immediately immersed in a bucket of water and re-cut. The whole leafy branches were sealed in large black plastic bags (with cut ends remaining in water), and transported to the laboratory within 20 min. Two leaves (at apical and basal of each branch respectively) were sealed with plastic bags before the dehydration. Samples were allowed to dehydrate on the bench in the laboratory at a constant room temperature of 25°C for different periods. Once the desired water potentials were approximately reached, the whole branch was sealed in a plastic bag with a wet paper towel inside allowing equilibration for 1–2 h. Then, we determined stem water potential (ψ_{xylem}) on the two initially bagged leaves using pressure chamber (PMS, Corvallis, OR, USA), cut one stem segment under water and trimmed the cut ends with a razor blade. Initial hydraulic conductivity (k_i) of the segment was measured using the method described above. After air embolisms were flushed out as described earlier, the segment was re-measured to determine maximum hydraulic conductivity (k_{max}). Percentage loss of hydraulic conductivity (PLC$_{stem}$) was calculated as $\text{PLC}_{stem}\ (\%) = 100 \times (k_{max} - k_i)/k_{max}$. The vulnerability curves (VCs) were plotted using PLC$_{stem}$ as a function of ψ_{xylem}, and were fitted using a three-parameter sigmoid model in SigmaPlot 10.0 (SPSS Inc., Chicago, IL, USA). Xylem water potential at 50% loss of hydraulic conductivity (P50$_{stem}$) was used to describe vulnerability to cavitation of terminal branches.

Sapwood density and xylem anatomy

Branch segments that were used for hydraulic conductivity measurements were also used for wood trait analyses. The volume of fresh sapwood (with bark and pith removed) was determined by the water displacement method, and its dry mass was subsequently determined after oven-drying at 70°C for 48 h. The sapwood density (WD, g cm^{-3}) was calculated as the ratio of dry mass to fresh volume.

Transverse sections of xylem tissue (30–50 µm thick) were obtained using a sliding microtome (SM2010R, Leica, Wetzlar, Germany). Images of the slides were scanned using a light microscope equipped with an ocular micrometer (DM2500, Leica) and were analysed with the aid of ImageJ software (http://rsb.info.nih.gov/ij/index.html, accessed 17 January 2014). We randomly chose ~30 vessels from each sample and calculated their diameters (D). The hydraulically weighted vessel diameter (D_H) was calculated as $D_H = (\sum D^4/N)^{1/4}$, where N is the number of vessels (Tyree and Zimmermann 2002). Vessel density (Vde) was calculated as the number of vessels per unit sapwood area (mm^2). Vessel fraction (Vf, %) was determined as the percentage of xylem area occupied by vessels.

Midday leaf water potential in the dry season

We measured the midday leaf water potentials (ψ_{min}) during 1200 to 1400 hours on three continuously sunny days in the mid of the dry season. For each species, we collected 10 sun-exposed branches carrying fully expanded and healthy leaves from five different individuals. After excision, leaf samples were sealed immediately into small plastic bags with moist paper towels inside, and kept in a cool box allowing equilibrium for ~30 min. Leaf water potential was determined using a PMS pressure chamber (PMS, Corvallis, OR, USA).

Leaf pressure-volume relationships

Five leaf-bearing branches from five individuals of each species were harvested and transferred to the laboratory where the basal ends of the branches were immersed in distilled water and re-cut (5 cm removed). The branch samples were rehydrated until leaf water potential was greater than –0.05 MPa. Leaves were first weighed to obtain the initial fresh mass and then immediately placed in a pressure chamber to determine the initial water potential. Leaf mass and water potential were measured periodically during slow desiccation in the laboratory. Finally, leaves were oven-dried for 48 h at 70°C to determine their dry mass. Leaf water potential at full turgor (ψ_{sat}) and turgor loss point (ψ_{tlp}), and bulk modulus of elasticity from full turgor to turgor loss point ($\varepsilon_{sat\text{-}tlp}$) were determined with a pressure-volume relationship analysis program developed by Schulte and Hinckley (1985).

Leaf hydraulic conductance

Leaf hydraulic conductance (K_{leaf}) was determined using the method described by Brodribb and Holbrook (2003). Leaf-bearing canopy branches were collected at predawn and placed in a plastic black bag with moist towels for ~1 h to ensure that all attached leaves were at a similar water potential. After the initial leaf water potential (ψ_{leaf}) of equilibrated branches was determined, neighbouring leaves were cut under water and allowed to rehydrate for 10–30 s. The water potential of the rehydrated leaves was immediately measured using a pressure chamber. Leaf hydraulic conductance was calculated as follows:

$$K_{leaf} = C \times \ln(\psi_0/\psi_f)/t, \tag{1}$$

where C is leaf capacitance, ψ_0 is leaf water potential before rehydration, and ψ_f is leaf water potential after rehydration for t seconds. C of pre- and post-turgor loss was calculated from the slopes of leaf pressure–volume relationships and was expressed in absolute terms and normalised by leaf area:

$$C = \Delta\text{RWC}/\Delta\psi_l \times (\text{DM}/\text{LA}) \times (\text{WM}/\text{DM})/\text{M}, \tag{2}$$

where RWC is relative water content, DM is leaf dry mass (g), LA is leaf area (m^2), WW is mass of leaf water at 100% RWC (g), and M is molar mass of water (g mol^{-1}).

The response of K_{leaf} to declining ψ_{leaf} was determined for each species according to Blackman *et al.* (2010). Leaf-bearing branches were collected in the early morning and allowed to dehydrate to obtain a range of water potentials through slow drying on the bench. K_{leaf} was then measured on leaves from each of the equilibrated branches (at different water potentials) as described earlier. Leaf vulnerability curves were plotted using percentage loss of hydraulic conductivity (PLC_{leaf}) as a function of ψ_{leaf} before rehydration, and were fitted using a three-parameter sigmoid model in SigmaPlot 10.0 (SPSS Inc.). Leaf water potential at 50% loss of maximum K_{leaf} ($P50_{leaf}$) was used to estimate the vulnerability of leaves to hydraulic dysfunction (Blackman *et al*. 2010). Hydraulic safety margin (HSM) was defined as the difference between ψ_{min} and $P50_{leaf}$.

Gas-exchange rate

Maximum CO_2 assimilation rates (A_{max}) and stomatal conductance (g_s) were measured between 0800 and 1130 hours on sunny days using a Li-6400 portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE, USA). Leaf-bearing branches at similar heights as those taken for hydraulic measurements (~20 mm in diameter) were harvested. The cut ends of the detached branches were immediately immersed in water and re-cut. Photosynthetic measurements were conducted within 1 h after branches were harvested (Wyka *et al*. 2012). Based on preliminary trials, photosynthetic photon flux density was set at 1500 $\mu mol\ m^{-2}\ s^{-1}$ to ensure that light-saturated photosynthetic rates were measured for all species (SD Zhu, YJ Chen, KF Cao, unpubl. data). Ambient CO_2 and air temperature were maintained at 390 $\mu mol\ mol^{-1}$ and 28°C. The leaf to air vapor pressure deficit was maintained at 1.0–1.5 kPa. Before data were recorded, leaves were exposed to the above conditions for 5–10 min to allow the stabilisation of photosynthetic parameter. Five individual trees per species were chosen, and five leaves from each individual tree were measured.

Leaf morphology and anatomy

For specific leaf area (SLA) determinations, 10 fully expanded leaves were collected from each individual. Leaf areas were measured with a leaf area meter (Li-3000A; Li-Cor), and then leaves were oven-dried for 48 h at 70°C to determine their dry mass. SLA was calculated as leaf area per unit dry mass.

Free-hand transverse sections were made from the middle parts of the leaves; the sections were wet mounted and immediately examined with a light microscope (YS100, Nikon, Tokyo, Japan). The thicknesses of the leaf lamina, palisade and spongy mesophyll were measured.

Stomatal density (SD) and guard cell length (GCL) were obtained by microscopic examination and measurement of imprints from abaxial nail varnish peels, which were taken centrally in the leaf midway between the midrib and the margin. Total stomatal pore area index (SPI) was calculated as: $SPI = SD \times GCL^2$.

To determine leaf vein density (VD), we followed the method by Sack and Frole (2006). Leaf regions were selected from the middle of the right-hand side of leaves. Regions of ~2 mm^2 were imaged using a light microscope (DM2500, Leica), and total vein length (1–3 order) was analysed using ImageJ (http://rsb.info.nih.gov/ij/index.html, accessed 17 January 2014). VD was defined as total vein length per unit leaf area. A total of 10 leaves from five individuals for each species were measured to determine leaf anatomical traits.

Statistical analysis

Branch and leaf traits were compared among the three *Syzygium* species with one-way ANOVAs. All statistical analyses were performed using SPSS ver. 13.0 software (SPSS).

Results

The three *Syzygium* species displayed non-significant differences in SLA (Table 2). The early and mid-successional species had similar leaf dry mass contents and palisade to spongy ratios, which were significantly higher than those of the late successional species. The mid-successional species had the thickest palisade among the three species. Along the successional sere, there was a significant increase in leaf thickness but a significant decrease in guard cell length, stomatal density, stomatal pore index, leaf vein density and vessel density (Table 2). The early and mid-successional species had similar values of maximum vessel length and D_H, which were higher than those of the late successional species. The three species had similar A_L/A_S values (Table 2). WD tended to increase along the succession,

Table 2. Leaf and branch traits of the three *Syzygium* species in this study

Values are means ± s.e. Different letters indicate significant differences at $P < 0.05$

Trait	Abbreviation (units)	*S. szemaoense*	*S. cumini*	*S. latilimbum*
Specific leaf area	SLA ($cm^2\ g^{-1}$)	156.69 ± 15.04a	143.23 ± 3.25a	130.01 ± 2.04a
Leaf dry mass content	LDMC ($g\ g^{-1}$)	0.33 ± 0.01a	0.35 ± 0.02a	0.26 ± 0.01b
Leaf thickness	LT (µm)	204.90 ± 12.29a	230.30 ± 7.69b	311.70 ± 12.91c
Palisade tissue thickness	PT (µm)	62.30 ± 5.15b	75.37 ± 2.83a	58.67 ± 1.33b
Spongy tissue thickness	ST (µm)	112.76 ± 5.29a	123.75 ± 1.93a	216.00 ± 5.81b
Palisade to spongy ratio	P/S	0.61 ± 0.03a	0.61 ± 0.03a	0.27 ± 0.01b
Guard cell length	GCL (µm)	20.29 ± 0.26a	17.38 ± 0.26b	13.65 ± 0.06c
Stomatal density	s.d. (no. mm^{-2})	596 ± 32a	524 ± 24b	341 ± 19c
Stomatal pore index	SPI	0.25 ± 0.02a	0.16 ± 0.01b	0.06 ± 0.01c
Leaf vein density	VD ($mm\ mm^{-2}$)	9.14 ± 0.39a	8.37 ± 0.13b	7.07 ± 0.22c
Maximum vessel length	MVL (mm)	70.6 ± 9.89a	69.0 ± 7.0a	62.8 ± 6.2b
Vessel density	Vde (no. mm^{-2})	97 ± 8a	69 ± 4b	44 ± 3c
Hydraulically weighted vessel diameter	D_H (µm)	52.23 ± 1.02a	54.67 ± 0.77a	42.27 ± 0.41b
Vessel fraction	Vf (%)	15.74 ± 1.59a	12.45 ± 0.64b	5.05 ± 0.90c
Sapwood density	WD ($g\ cm^{-3}$)	0.53 ± 0.01a	0.61 ± 0.01b	0.63 ± 0.02b
Leaf to sapwood area ratio	A_L/A_S ($m^2\ mm^{-2}$)	1.19 ± 0.07a	1.19 ± 0.10a	1.14 ± 0.09a

although values were not significantly different for the mid and late successional species (Table 2).

The specific hydraulic conductivity of both stem (k_s) and leaf (k_l) significantly decreased along the succession (Fig. 1*a*, *b*); the same was true for K_{leaf}, A_{max}, and g_s (Fig. 1*c*–*e*). There were no consistent changes in pressure–volume traits along the succession in that the mid-successional species had the lowest ψ_{sat} and ψ_{tlp} values and the highest bulk modulus of elasticity from full turgor to turgor loss point ($\varepsilon_{sat\text{-}tlp}$) value (Fig. 2).

Regarding plant resistance to embolism in stems and leaves, the early and mid-successional species had similar $P50_{stem}$ values (–1.15 and –0.96 MPa respectively), which were higher than that of the late successional species (–2.04 MPa) (Fig. 3*a*–*c*). The early successional species had the least negative $P50_{leaf}$ value (–1.03 MPa), while the mid-successional species had the most negative value (–2.17 MPa) (Fig. 3*d*–*f*). In the middle of the dry season, although the mid- and late successional species had larger hydraulic safety margin (HSM) than the early successional species, all of the three species showed positive values of HSM (Fig. 3*d*–*f*).

Discussion

In studies of adaptations of plant functional traits to environmental conditions, the comparison of congeneric species may be especially useful, because it minimises phylogenetic effects (Lens *et al.* 2011). Our results clearly demonstrate that early successional *Syzygium* species (*S. szemaoense*) had greater xylem hydraulic conductivity (k_s) than late successional *Syzygium* species (*S. latilimbum*) (Fig. 1): k_s is determined by both vessel diameter and vessel number (Tyree and Zimmermann 2002). In this study, although vessel diameter was similar for the early and mid-successional species, vessel density and vessel fraction were greater for the early successional species and contributed to its higher k_s. The late successional species had the lowest k_s, because its xylem was occupied by the thinnest and fewest vessels among the three species (Table 2).

In agreement with previous findings (Nardini *et al.* 2005; Sack *et al.* 2005), K_{leaf} significantly decreased from the early, to the mid-, to the late successional *Syzygium* species (Fig. 1). Although the associations between hydraulic conductance and structural traits of leaves have been studied, the results have been controversial. For example, Aasamaa *et al.* (2005) found that K_{leaf} was correlated with the width of xylem conduits in the midrib, vein density, and SLA. Other researchers, however, reported that K_{leaf} was correlated with stomatal pore area, palisade thickness, and palisade/spongy ratio but not with SLA (Sack *et al.* 2004, 2005; Sack and Frole 2006). In the present study, the variation in K_{leaf} was consistent with the changes in stomatal traits (GCL, SD and SPI) and vein density but not with the changes in SLA and leaf mesophyll thickness (palisade, spongy and ratio of palisade to spongy mesophyll (P/S)), indicating that K_{leaf} is closely correlated with water flux-related traits of leaves (Sack and Holbrook 2006).

The early successional species, which had the greatest hydraulic conductivity (i.e. k_s, k_l, and K_{leaf}) also had the highest photosynthetic rate (i.e. A_{area} and g_s), indicating a strong coordination between hydraulic and photosynthetic

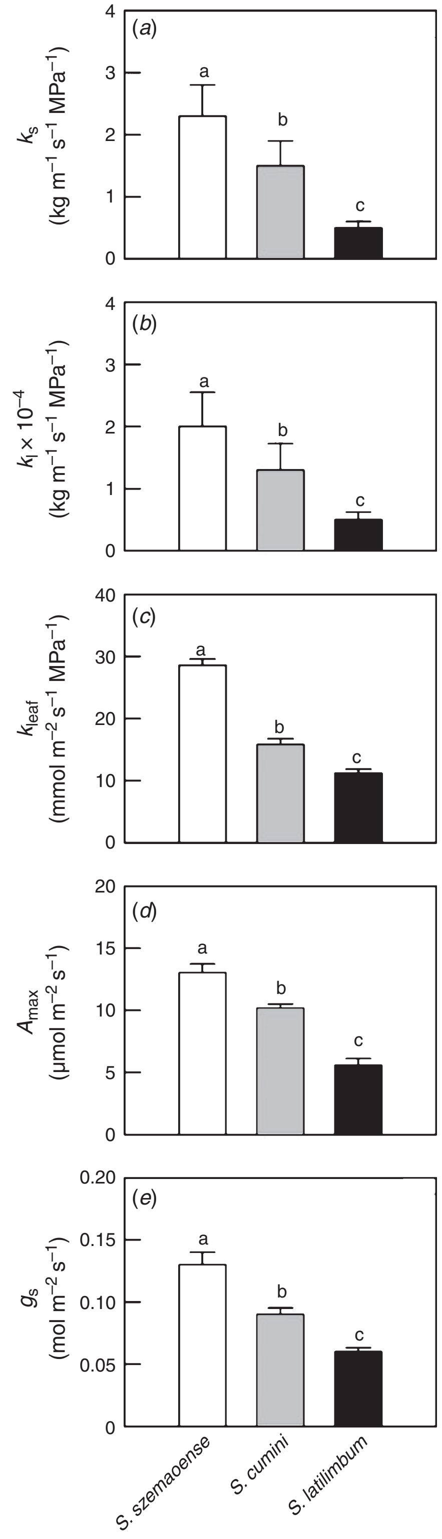

Fig. 1. Branch hydraulic conductivity, leaf hydraulic conductance, and photosynthetic traits of the three *Syzygium* species. Abbreviaions: k_s, sapwood specific hydraulic conductivity; k_l, leaf specific hydraulic conductivity; K_{leaf}, maximum leaf hydraulic conductance; A_{max}, maximum net photosynthetic rate; g_s, stomatal conductance. Values are means ± s.e. Different letters indicate significant difference at $P < 0.05$.

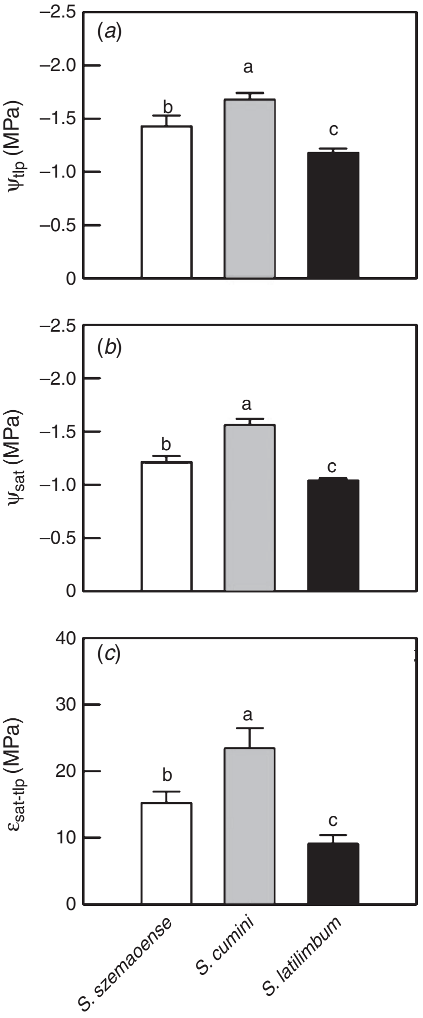

Fig. 2. Leaf pressure-volume traits of the three *Syzygium* species. Abbreviations: ψ_{tlp}, water potential at the turgor loss point; ψ_{sat}, water potential at full turgor; $\varepsilon_{sat\text{-}tlp}$, bulk modulus of elasticity from full turgor to turgor loss. Values are means ± s.e. Different letters indicate significant difference at $P < 0.05$.

traits, which has previously been observed in a number of studies (Brodribb *et al.* 2002; Santiago *et al.* 2004; Zhu *et al.* 2013). High hydraulic conductivity and photosynthetic rate contribute to rapid growth and may help the early successional *Syzygium* species dominate in resource-rich habitats including the disturbed, open sites of tropical forests (Navas *et al.* 2003; Poorter and Bongers 2006). In contrast, conservative features (higher WD, narrower D_H, and more negative $P50_{stem}$) may facilitate the survival of the late successional *Syzygium* species in the shaded understory (Poorter 2009; Poorter *et al.* 2010).

Regulation of the leaf : sapwood area ratio (A_L/A_S) is an important hydraulic response that enables plants to maintain water balance under different moisture conditions. For instance, species growing at dry sites or exposed to high vapour pressure deficits tend to have a low value of A_L/A_S (Addington *et al.* 2006; Gotsch *et al.* 2010). However, Markesteijn *et al.* (2011*a*) found no correlations between A_L/A_S and indices of drought and shade tolerance across 40 tropical tree species from different habitats. These authors suggested that plants adjust their leaf-level water supply by altering xylem properties (i.e. vessel length, pith pores) or by altering hydraulic resistance (i.e. ion concentration of the sap), without changing A_L/A_S. This might explain the lack of significant difference in A_L/A_S among the three *Syzygium* species (Table 2), even though they occur in different successional forests with different light and water conditions.

SLA was higher for early than for late successional species in some studies (Garnier *et al.* 2004; Navas *et al.* 2010) but were similar for early and late successional species in other studies (Nogueira *et al.* 2004; Han *et al.* 2010; Zhu *et al.* 2013). Because SLA is closely associated with leaf structural investments (Poorter and Bongers 2006), the lack of significant difference in SLA among the three *Syzygium* species in this study (Table 2) was consistent with Zhu and Cao (2010), who reported that *S. latilimbum* in a mature forest had similar construction costs as the pioneer tree species *S. szemaoense* (125 and 135 g glucose m^{-2} respectively).

Xylem vulnerabilty curves (VCs) show the variations of the degree of cavitation as a function of xylem pressure, which can be measured by different methods including bench dehydration, centrifugation and air injection (Cochard *et al.* 2013). Recently, there are accumulating concerns about methodological artefacts in measuring VCs (Cochard *et al.* 2013; Rockwell *et al.* 2014). That is, centrifugation or air-injection VCs obtained on stem segments shorter than maximum vessel length usually show an 'exponential' type, which might be caused by open-vessel artefact leading to overestimation of vulnerability of cavitation, especially for species with long vessels (Ennajeh *et al.* 2011; Martin-StPaul *et al.* 2014). In contrast, most bench-drying VCs were 'sigmoidal' type, which is in agreement with native embolism levels and water potential values measured in the field (Torres-Ruiz *et al.* 2014; Wang *et al.* 2014). In the present study, VCs of the three *Syzygium* species were obtained using the bench-drying method. In contrast to 'sigmoidal' type VC of *S. latilimbum*, VCs of *S. szemaoense* and *S. cumini* were prone to be an 'exponential' type which might be realistic, because relatively large vessels were observed for these two species (Table 2), thus, giving them potentially high vulnerable xylem and cavitation could be induced at low xylem tensions (Hacke *et al.* 2006; Chen *et al.* 2009). This result is in consistent with previous findings in some woody species from miombo woodlands (Vinya *et al.* 2013) and Mediterranean region (Iovi *et al.* 2009), showing that species with relatively large vessels are inclined to have 'exponential' type VCs.

Recently, Wheeler *et al.* (2013) claimed an 'excision artefact' in conventional bench drying method, which might lead to overestimation of PLC_{stem}, because the act of cutting stems under water while the xylem is under large tensions (e.g. after stem dehydration) may result in additional embolism. To avoid this artefact, the authors conducted a rehydration treatment before sample excision, during which the xylem tensions are relaxed. According to Wheeler *et al.* (2013), branches 2–4 times of the maximum vessel length should be initially sampled. Once desired xylem tension was achieved during the process of bench drying, the base of the original branch (about a maximum vessel

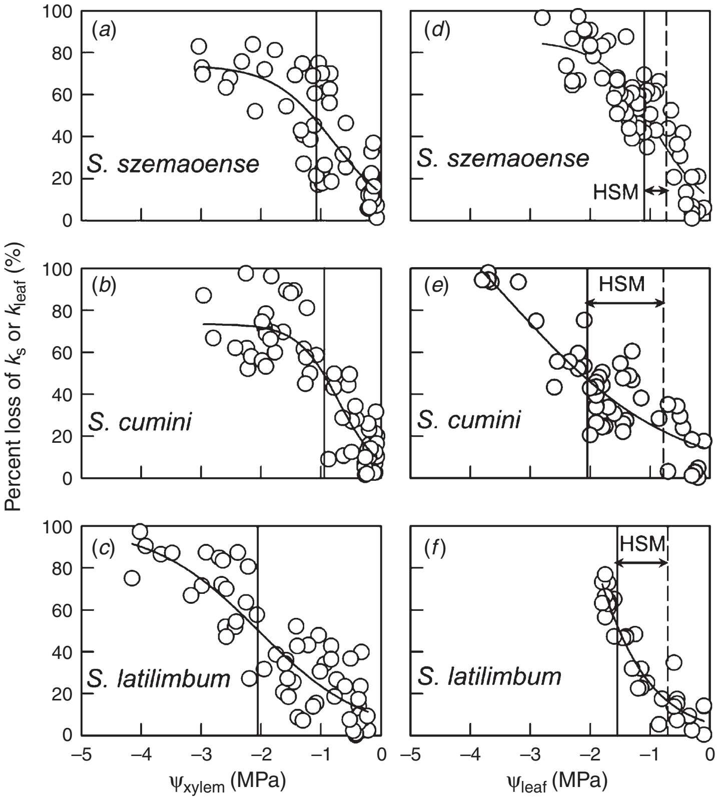

Fig. 3. Branch xylem and leaf vulnerability curves of the three *Syzygium* species. Abbreviations: ψ_{xylem}, xylem water potential; ψ_{leaf}, leaf water potential; vertical solid lines indicate xylem water potential inducing 50% loss of hydraulic conductivity (*a–c*) and leaf water potential at 50% loss of leaf hydraulic conductance ($P50_{leaf}$; *d–f*). Vertical dashed lines show minimum midday leaf water potential in the dry season (ψ_{min}). Hydraulic safety margin (HSM) is defined as the differences between ψ_{min} and $P50_{leaf}$.

length) was removed under water, and then the branch was allowed to rehydrate for 30 min to 2 h with the shoot end sealed inside a plastic bag (negative pressure in the xylem should be relaxed during this procedure). Then the pressure relaxed branches were used for PLC_{stem} determination. In the present study, due to long vessels possessed by the *Syzygium* species (Table 2), it is a difficult challenge to obtain stems that are long enough (2–3 m) from the studied species, as too many lateral branches emerged along the stem, making it difficult to sample suitable stems and follow the procedure by Wheeler *et al.* (2013). In addition, the studied *Syzygium* species showed large variation in stem diameter from the most distal end to the basal end along a long stem, for instance, the basal diameter of a 2 m long-branch of *S. latilimbum* (~3 times of its maximum vessel length) is over 30 mm (difficult to cut off with long pruners). Nevertheless, we agree that it is worthy extending what reported by Wheeler *et al.* (2013), i.e. the influence of 'excision artefact' on stem VCs, particularly with diverse tropical species possessing long vessels (e.g. woody vines in tropical forests).

Among the three *Syzygium* species in the current study, the late successional species showed the highest resistance to cavitation (with the highest WD and narrowest D_H). Interestingly, the other two *Syzygium* species differed significantly in WD and k_s, but had relative high and similar $P50_{stem}$ values, indicating the lack of a trade-off between hydraulic safety and efficiency across the *Syzygium* species tested in this study. There was no directional change in leaf pressure–volume traits for the three *Syzygium* species along the successional gradient in that the mid-successional species had the lowest ψ_{sat} and ψ_{tlp} values and the highest $\varepsilon_{sat\text{-}tlp}$ value (Fig. 2). Presumably, the observed variation of these traits might be partly due to the changes in LDMC (Table 2), because leaves with higher LDMC tend to have thicker and more rigid cell walls, which could enable the maintenance of cell turgor at lower leaf water potential (Bartlett *et al.* 2012). Additionally, $P50_{leaf}$ has been linked to leaf traits associated with drought resistance, particularly pressure-volume traits (Blackman *et al.* 2010), and this would explain the low ψ_{sat}, ψ_{tlp}, and $P50_{leaf}$ values for the mid-successional species.

During the dry season, all of the three *Syzygium* species maintained their midday leaf water potential above $P50_{leaf}$ (Fig. 3*d–f*), i.e. the minimum water potential of these species in the dry season did not exceed their hydraulic limits. In addition, the three species showed positive values of hydraulic safety margins in the same period (Fig. 3*d–f*), these results implied that plants in the study site might not suffer from severe drought stress in the dry season. Among the three *Syzygium* species, the early successional species had the narrowest hydraulic safety margin (Fig. 3*d*). This is in consistent with the findings by Markesteijn *et al*. (2011*b*) that pioneer tree species operate $\psi_{\min}$ close to their hydraulic limits in a tropical forest. The authors claimed that narrow safety margin indicated a great risk of hydraulic failure, but deep rooting and high wood water storage could secure water supply from stem to terminal leaves (Carter and White 2009).

Contrary to studies showing that leaves are more vulnerable to drought-induced cavitation than terminal branches (Hao *et al*. 2008; Johnson *et al*. 2011; Bucci *et al*. 2013), we found in the present study that $P50_{\text{leaf}}$ was either close to $P50_{\text{stem}}$ (for *S. latilimbum*) or more negative than it (for *S. cumini*) (Fig. 3). Vulnerability segmentation has been widely identified in woody plants occurred in drought habitats, particularly for those with narrow hydraulic safety margins (Bucci *et al*. 2013; Pivovaroff *et al*. 2014). We noted that leaves showing similar (or even higher) resistance to cavitation as compared with stems were also observed at least in some species occurred in moist habitats (present study; Chen *et al*. 2009; Bucci *et al*. 2012), leading to a lack of vulnerability to segmentation in these species. Nevertheless, it is not necessarily that species displaying a lack of vulnerability to segmentation are more vulnerable to drought stress, because several compensatory mechanisms such as high water storage, great hydraulic efficiency and reduction of leaf area could effectively reduce the risk of embolism formation in stems (Bucci *et al*. 2012; Pineda-García *et al*. 2013).

In conclusion, our results demonstrate that the three *Syzygium* species displayed a consistent decline in hydraulic efficiency (k_s, k_l, and K_{leaf}) and photosynthetic rate (area and g_s) along the succession sere. However, no directional changes in traits related to cavitation resistance ($P50_{\text{stem}}$ and $P50_{\text{leaf}}$) and pressure–volume traits (ψ_{sat}, ψ_{tlp} and $\varepsilon_{\text{sat-tlp}}$) were observed, although the three congeneric species occur in different successional forests and experience different environmental conditions. Our findings highlight that leaf and branch traits related to photosynthesis and/or hydraulics, rather than to drought tolerance, are the key factors underlying the response and adaptation of the three *Syzygium* species along the tropical secondary succession.

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