


RESEARCH PAPER

# Linking vein properties to leaf biomechanics across 58 woody species from a subtropical forest

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## Keywords

Leaf mechanical properties; minor vein diameter; subtropical forest; total vein dry mass per leaf area; vein dry mass density.

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## ABSTRACT

- Leaf venations have elements with relatively lower elasticity than other leaf tissue components, which are thought to contribute to leaf biomechanics. A better mechanistic understanding of relationships between vein traits and leaf mechanical properties is essential for ecologically relevant interpretation of leaf structural variations.
- We investigated 13 major (first to third order) and minor (>third order) vein traits, six leaf mechanical properties and other structural traits across 58 woody species from a subtropical forest to elucidate how vein traits contribute to leaf biomechanics.
- Across species, vein dry mass density ( $\rho_v$ ), total vein dry mass per leaf area (VMA) and minor vein diameter ( $VD_{min}$ ), but not the lower-order vein density ( $VLA_{1,2}$ ), were positively correlated with leaf force to punch ( $F_p$ ) and force to tear ( $F_t$ ). Structural equation models showed that  $\rho_v$  and  $VD_{min}$  not only contribute to leaf mechanical properties directly (direct pathway), but also had impacts on leaf biomechanics by influencing leaf thickness and leaf dry mass per area (indirect pathway).
- Our study demonstrated that vein dry mass density and minor vein diameter are the key vein properties for leaf biomechanics. We also suggest that the mechanical characteristics of venations are potential factors influencing leaf mechanical resistance, structure and leaf economics spectrum.

## INTRODUCTION

Leaf mechanical properties include leaf strength, toughness and stiffness, which are key traits for plants to withstand environmental stresses (e.g. wind, rain and/or herbivore pressure) (Lucas *et al.* 2000; Peeters *et al.* 2007; Anten *et al.* 2010; Lim *et al.* 2015). Moreover, leaf biomechanical properties are essential for a species to achieve optimal display angles for optimal light capture and gas exchange (Posada *et al.* 2009). Consequently, to some extent, leaf biomechanics are linked to traits of leaf lifespan and could help to elucidate species longevity (Kitajima & Poorter 2010).

Many previous biomechanical studies have shown that leaf mechanical properties are determined by leaf structural traits. For example, information contained in a global dataset has shown that leaf dry mass per area (LMA) is positively correlated with leaf mechanics (Onoda *et al.* 2011). Kitajima *et al.* (2016) demonstrated the leaf cellulose density plays a key role in variations in leaf toughness in a tropical forest. Through isolating cuticles of 13 evergreen woody species, Onoda *et al.* (2012) found that cuticle thickness improved leaf the resistance to wear and tear. These findings improve our

understanding of the causes of variations in leaf mechanical properties.

Leaf vein networks are not only the transport system supplying water and solutes and exporting carbohydrates, but also a key mechanical system to help maintain leaf position in space (Niklas 1999; Hüve *et al.* 2002; Lambers *et al.* 2008; Scoffoni *et al.* 2016). Veins are mainly composed of xylem tissue, sometimes even sclerenchyma, both of which have low elasticity to help maintain leaf stability (Roth-Nebelsick *et al.* 2001). Vein properties, especially vein density, can be modelled to predict LMA and even the leaf economics spectrum (Blonder *et al.* 2011, 2013, 2014; Sack *et al.* 2013). Because vein traits might quantify vein characteristics such as geometric and compositional features (Blonder *et al.* 2011), one would expect some correlation between vein traits and leaf biomechanics. For example, Kawai & Okada (2016) found that primary and secondary vein density (vein length per leaf area,  $VLA_{1,2}$ ) was positively associated with force required to tear the leaf ( $F_t$ ). The redundancy of the leaf venation system also contributes to tolerance of biotic and abiotic damage (Sack *et al.* 2008; Blonder *et al.* 2014). Not only the arrangement of veins contributes to leaf biomechanics, but also the vein composition, e.g. fibre

content may be important. For instance, cross-sections of leaves of *Tetragonia expansa* showed that sieve elements in the minor veins contained massive fibrous plastids (Esau & Hoefert 1971). Although the primary wall of tracheary elements largely disintegrates, some fibrils still exist in the minor veins (Esau & Hoefert 1971; Zwieniecki *et al.* 2002). A previous study found fibrous plastids and fibrils in minor veins, where these fibrous characteristics play critical roles in leaf biomechanics (Lucas *et al.* 1991).

Although veins tend to influence leaf biomechanics, there are uncertainties in relationships between vein traits and leaf biomechanics. First, results of previous work can be somewhat contradictory. Kawai & Okada (2018) studied 11 temperate deciduous woody species and demonstrated that leaf force to punch ( $F_p$ ; the resistance of leaf tissue to rupture, excluding toughness provided by midrib and main veins) was positively correlated with minor vein diameter ( $VD_{min}$ ), rather than major vein traits such as  $VLA_{1,2}$ ; this was not consistent with results from eight *Fagaceae* species (Kawai & Okada 2016). Second, previous studies have paid little attention to vein mass and vein dry mass density. By separating leaves into various cell and tissue layers, Grace *et al.* (2017) found that vein mass could contribute 11% to variation in LMA. This indicates that vein mass might be related to leaf biomechanics. Moreover, vein dry mass density reflects the compact nature of vein composition. Veins with a higher dry mass density tend to have more fibres and/or other components that enhance leaf mechanical resistance (Roth-Nebelsick *et al.* 2001; Kitajima *et al.* 2016). Furthermore, minor vein diameter could contribute to leaf thickness (Blonder *et al.* 2011), which might consequently influence leaf mechanical properties (Onoda *et al.* 2011).

Here, we measured a series of leaf vein properties and mechanical traits across 58 woody species from a subtropical forest, aiming to test the empirical relationships among vein traits and leaf mechanical properties (Fig. 1). Specifically, we hypothesised that: (i) not only VD and VLA, but vein compositional traits such as vein dry mass density, could directly influence leaf mechanical properties; (ii) vein dry mass per area might be associated with leaf biomechanics through its contribution to leaf dry mass per area, while minor vein diameter could affect leaf mechanical properties because of its correlation with leaf thickness.

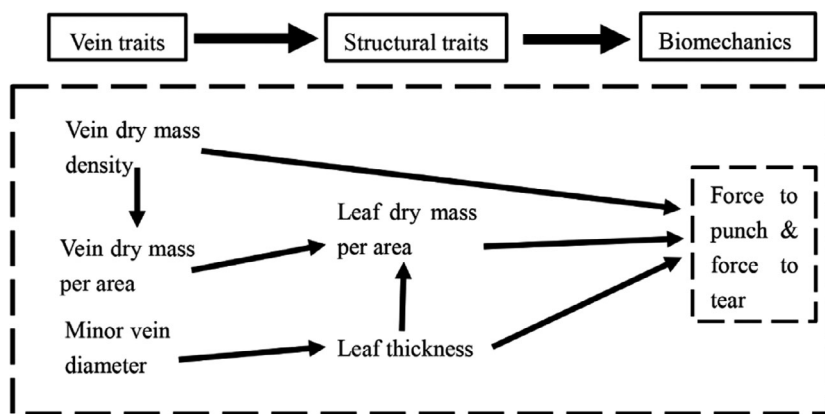
## MATERIAL AND METHODS

### Site and sampling

Leaves were collected from 58 angiosperm woody species in the subtropical forest of Dinghushan Forest Ecosystem Research Station (DFERS;  $21^{\circ}09'21''$ – $21^{\circ}11'30''$  N,  $112^{\circ}30'39''$ – $112^{\circ}33'41''$  E), characterised by a typical subtropical monsoon climate with annual average precipitation of ca. 1900 mm, of which about 80% occurs in the wet season (April to September); mean annual temperature is  $21.4^{\circ}\text{C}$  (Zhu *et al.* 2013). DFERS experiences on average 3.1 typhoons per year (Zhou & Yan 2001). For each species, fully sun-exposed and expanded leaves or shoots were sampled from three to five reproductive mature individuals. All plant material was enclosed in air-tight bags together with wet filter paper and immediately transported to the laboratory for further measurements.

### Vein traits

The vein order was determined according to the conventional method (Leaf Architecture Working Group 1999). First, five leaves per species from three to five individuals were randomly selected and scanned (CanoScan LiDE 100; Canon, Tokyo, Japan) to determine length of the  $1^{\circ}$  and  $2^{\circ}$  vein, as well as the  $3^{\circ}$  vein lengths for large leaves. The diameter of the  $1^{\circ}$  vein was measured from the central part of the mid-vein for each leaf. We chose two secondary veins from top, middle and bottom of leaves, and measured their diameters centrally. The  $2^{\circ}$  vein diameter ( $2^{\circ}VD$ ) was determined by averaging all the measurements. If the leaves were large enough to allow  $3^{\circ}$  veins to be seen clearly in the scanned images with the aid of the scanner, we also measured  $3^{\circ}$  vein diameter ( $3^{\circ}VD$ ) in the same way. Subsequently, three rectangles (at least  $1\text{ cm}^2$ ) centrally located at the top, middle and bottom of the same leaves were obtained. These sections were soaked in 5% w/v NaOH/ $\text{H}_2\text{O}$  solution for 2–10 days (depending on species). Leaves were then rinsed in water and soaked for about 15 min in commercial bleach (6% w/v NaClO/ $\text{H}_2\text{O}$ ). After a second rinse in water, leaves were stained with 1% w/v safranin/ethanol solution for 10 min before being kept for 20 min in 100% ethanol then brought back to water for imaging (Scoffoni & Sack



**Fig. 1.** A conceptual framework for the relationships among traits that contribute to leaf biomechanics. Vein traits (vein dry mass density, vein dry mass per area, minor vein diameter) are considered to first affect leaf structural traits (leaf dry mass per area, leaf thickness) and then reflect the changes in leaf mechanical properties (leaf force to tear and force to punch).

2013). The sections were scanned again for 3° or 4° (in large leaves) vein trait measurements. An optical microscope with digital camera (Leica ICC50 W) was used to take photos of each section (making sure to include at least one 3° vein or 4° vein in each image) for minor vein trait measurements. Vein diameters (VD) and VLA of each order were measured manually with Image J. For 3° vein diameter, at least two central parts of the 3° vein segments in each image were measured. We measured at least ten repetitions from 4° vein to 7° vein, if present, in each image and calculated the mean value as the minor vein diameter ( $VD_{\min}$ ). The major vein density ( $VLA_{\max}$ ) is commonly accepted as the sum of 1st to 3rd VLA, and the remaining higher order vein density is  $VLA_{\min}$ . Since  $VLA_{1,2}$ , the density of primary and secondary veins, is related functionally with leaf mechanical properties (Kawai & Okada 2016), we also calculated  $VLA_{1,2}$  as the lower-order vein density. We estimated vein volume per leaf area (VVA) of each vein order (j) as follows:  $VVA_j = VLA_j \times \pi \times (VD_j/2)^2$ . Total VVA is the sum of each order vein volume per leaf area.

We took midrib dry mass density as the estimate of vein dry mass density, and modelled the midrib as a cone with basal diameter measured at the petiole insertion (Grace *et al.* 2017). The first, three to ten leaves of each species from three to five individuals were chosen to measure basal diameter and length of the midrib. Second, we separated the midribs from the leaves, using a double-edge razor blade, and dried them for 72 h at 65 °C before weighing. The vein dry mass density ( $\rho_v$ ) was determined as midrib dry mass/midrib volume. Vein dry mass per leaf area (VMA) of each vein order (j) was used to multiply  $\rho$  by  $VVA_j$ . VMA is the sum of each order vein dry mass per leaf area.

### Leaf structural traits

Twenty fully expanded and sun-exposed leaves were collected from each individual. Total areas were measured with a leaf area meter (Li-3000A; Li-Cor, Lincoln, NE, USA) to determine leaf area (LA). The leaves were then oven-dried for 72 h at 65 °C to determine dry mass. Leaf mass per area (LMA) was calculated as dry mass per leaf area. Leaf thickness (LT) was measured from leaf cross-sections using Image J. Leaf density (LD) was calculated by dividing LMA by LT.

### Leaf mechanical properties

We used the 'tearing or tensile test' method described by Perez-Harguindeguy *et al.* (2013) with a digital force gauge (precision 0.001N; HADPI, Leqing, China). For each species, 20 leaves were selected and cut into strip sections from the central part of the leaves (5 × 25 mm), avoiding the midrib. The maximum force per unit width of each specimen was defined as 'force to tear' ( $F_t$ ,  $\text{kN}\cdot\text{m}^{-1}$ ) (Onoda *et al.* 2011). We used a punch-and-die test to measure the maximum force required for the punch rod to penetrate a leaf. Such measurements were made on leaf laminae (excluding midribs), and the diameter of the flat-end punch rod was 0.6 mm. Leaf force to punch ( $F_p$ ,  $\text{kN}\cdot\text{m}^{-1}$ ) was determined as the maximum force per fractional circumference along the leaf lamina surface (Onoda *et al.* 2011). Leaf tearing strength ( $S_t$ ,  $\text{MN}\cdot\text{m}^{-2}$ ) and leaf punch strength ( $S_p$ ,  $\text{MN}\cdot\text{m}^{-2}$ ) were calculated as  $F_t/LT$  and  $F_p/LT$ , respectively. We also considered leaf biomechanics on a dry

mass basis. This is because these two expressions have different meanings: the leaf light capture process is area-related, while leaf structural carbon and nutrient investment are naturally considered on a mass basis (He *et al.* 2019), which can be inter-converted with each other through LMA (Reich *et al.* 1998; Poorter *et al.* 2009). Specific leaf tear strength ( $S_t/LD$  or  $F_t/LMA$ ,  $\text{N}\cdot\text{g}^{-1}\cdot\text{m}$ ) and specific leaf punch strength ( $S_p/LD$  or  $F_p/LMA$ ,  $\text{N}\cdot\text{g}^{-1}\cdot\text{m}$ ), which are measures of strength of leaf mass, were calculated as  $S_t/LD$  and  $S_p/LD$ , respectively.

We quantified the biomechanical properties of leaves using force to tear ( $F_t$ ) and force to punch ( $F_p$ ). A punch (or penetrometer) test is used to evaluate fracture resistance vertically against the leaf surface (Choong *et al.* 1992), whereas a tensile (or tearing) test is used to evaluate tensile resistance along the leaf surface (Aranwela *et al.* 1999). Hence, these two tests evaluate leaf mechanical properties in orthogonal directions, *i.e.* the punch test may be more closely related to resistance to insect herbivory (Peeters *et al.* 2007), and the tear test to resistance to animal grazing and/or wind damage (Anten *et al.* 2010). In fact, the research site of the present study is located in a subtropical evergreen monsoon forest with heavy insect herbivory. For instance, a previous study by Schuldt *et al.* (2010) reported that species-specific mean herbivory damage to leaf area ranged from 3% to 16% in a subtropical forest ecosystem in China. Also, the study site is hit by typhoons 3.1 times (on average) each year (Zhou & Yan 2001). Therefore, both insect herbivory and frequent typhoons might have had a significant influence on leaf mechanical properties in this forest community.

### Statistics

We log-transformed the mean values of all measured data to improve normality and heteroscedasticity. All analyses were carried out with the log-transformed mean values. All associations between pairs of trait variables were analysed with SPSS version 18.0 software (SPSS, Chicago, IL, USA) using Pearson correlation and standard major axis (SMA) regression with SMATR software (Warton *et al.* 2006) to find which vein traits make contributions to leaf biomechanics.

The hypothetical conceptual framework (Fig. 1) was evaluated using structural equation modelling (Grace 2006) with AMOS software (AMOS Development, Spring House, PA, USA). We compared alternative path models by evaluating the statistical significance of individual paths, and path coefficients ( $\beta$ ) were estimated with log-ratio maximum likelihood. The total effect of one variable on another is the total of path coefficients for direct paths plus the products of path coefficients for indirect paths *via* other variables. The model was tested with a  $\chi^2$  test and evaluated with the Akaike Information Criterion (AIC). Including the latent variable 'biomechanics', which combines both  $F_t$  and  $F_p$ , and increased the overall model fit ( $R^2$ ) (data not shown).

We also used phylogenetic analyses on all the single traits and trait pairs to find out whether evolutionary history might influence leaf mechanical properties and vein traits, and their relationships. We generated a phylogenetic tree using the Phylocom program (Webb & Donoghue 2005), based on the Angiosperm Phylogeny Group III (Figure S1). **Phylogenetic signals for all traits were calculated using Pagel's  $\lambda$**  (Pagel 1999). We used the *pgls* function in the *caper* package (Orme

**Table 1.** Leaf traits measured in this study, including minimum species mean, mean of species means, maximum species mean.

Traits	Symbol	Unit	Min–mean–max
Vein traits			
Primary vein density	1°VLA	10 <sup>-1</sup> mm·mm <sup>-2</sup>	0.11–0.39–0.96
Secondary vein density	2°VLA	mm·mm <sup>-2</sup>	0.04–0.19–0.75
Tertiary vein density	3°VLA	mm·mm <sup>-2</sup>	0.12–0.63–1.78
Minor vein density	VLA <sub>min</sub>	mm·mm <sup>-2</sup>	1.78–5.12–13.02
Major vein density	VLA <sub>maj</sub>	mm·mm <sup>-2</sup>	0.19–0.86–2.13
Vein density of primary and secondary vein	VLA <sub>1+2</sub>	mm·mm <sup>-2</sup>	0.05–0.23–0.81
Primary vein diameter	1°VD	mm	0.39–0.92–3.41
Secondary vein diameter	2°VD	mm	0.11–0.29–1.07
Tertiary vein diameter	3°VD	mm	0.03–0.10–0.49
Minor vein diameter	VD <sub>min</sub>	10 <sup>-1</sup> mm	0.07–0.30–0.54
Vein dry mass density	$\rho_v$	g·cm <sup>-3</sup>	0.16–0.54–1.51
Total vein mass per leaf area	VMA	g·m <sup>-2</sup>	2.80–21.89–76.55
Total vein volume per leaf area	VVA	10 <sup>-1</sup> mm <sup>3</sup> ·mm <sup>-2</sup>	0.09–0.43–1.37
Leaf mechanical properties			
Force to tear	$F_t$	kN·m <sup>-1</sup>	0.18–0.41–0.83
Force to punch	$F_p$	kN·m <sup>-1</sup>	0.14–0.28–0.46
Leaf tearing strength	$S_t$	MN·m <sup>-2</sup>	0.79–2.45–4.94
Leaf punching strength	$S_p$	MN·m <sup>-2</sup>	0.57–1.70–2.69
Specific leaf tearing strength	$S_t/LD$	N·g <sup>-1</sup> ·m	2.40–6.03–10.14
Specific leaf punching strength	$S_p/LD$	N·g <sup>-1</sup> ·m	1.86–4.20–8.89
Other structural traits			
Leaf dry mass per area	LMA	g·m <sup>-2</sup>	35.63–70.25–141.97
Leaf thickness	LT	μm	78.08–178.22–378.42
Single leaf area	LA	cm <sup>2</sup>	10.91–54.00–554.52
Leaf density	LD	g·cm <sup>-3</sup>	0.16–0.42–0.77

*et al.* 2013) to run  $\lambda$  tests to detect phylogenetic signals and to calculate phylogenetically corrected correlation coefficients for trait pairs (phylogenetic generalised least square, PGLS). This gives  $\lambda$  values between zero and one, where  $\lambda = 0$  indicates no phylogenetic signal and  $\lambda = 1$  suggests that the trait distribution conforms to Brownian motion (BM).

## RESULTS

Thirteen vein traits, including length, diameter, volume and mass, six leaf mechanical properties and three other structural traits of the 58 woody angiosperm species were measured (Table 1; Table S1). There was a large range among the measured traits. Species varied about 9.4-fold in  $\rho_v$ , from 0.16 to 1.51 g·cm<sup>-3</sup>. The VD<sub>min</sub> varied about 7.7-fold across species, from 0.07 to 0.54·10<sup>-1</sup> mm. And there was a surprisingly large range in VMA, which varied about 27.3-fold across species, ranging from 2.80 to 76.55 g·m<sup>-2</sup>. Mechanical properties also differed significantly across species. The  $F_t$  varied about 4.6-fold, from 0.18 to 0.83 kN·m<sup>-1</sup>, and the  $F_p$  varied about 3.3-fold, from 0.14 to 0.46 kN·m<sup>-1</sup>.

### Vein properties and their relationships to leaf biomechanics

There were significant relationships between vein traits and leaf mechanical properties across the 58 subtropical tree species, even though their leaf morphology and venation architectures were different (Table 1). Among measured traits,  $\rho_v$  was linearly correlated with both  $F_p$  ( $r = 0.45$ ,  $P < 0.001$ ; Fig. 2a) and  $F_t$  ( $r = 0.41$ ,  $P < 0.001$ ; Fig. 2e). VD<sub>min</sub> also

showed strong positive correlations with  $F_p$  ( $r = 0.46$ ,  $P < 0.001$ ; Fig. 2b) and  $F_t$  ( $r = 0.42$ ,  $P < 0.001$ ; Fig. 2f). The VMA co-varied with  $F_p$  ( $r = 0.35$ ,  $P < 0.01$ ; Fig. 2c) and  $F_t$  ( $r = 0.39$ ,  $P < 0.01$ ; Fig. 2g). However, other measured vein traits were independent of leaf mechanical properties, including VLA<sub>1+2</sub> (Fig. 2d, h; Table S2). In our results, VLA<sub>1+2</sub> showed no correlations with either  $F_t$  or  $F_p$ .

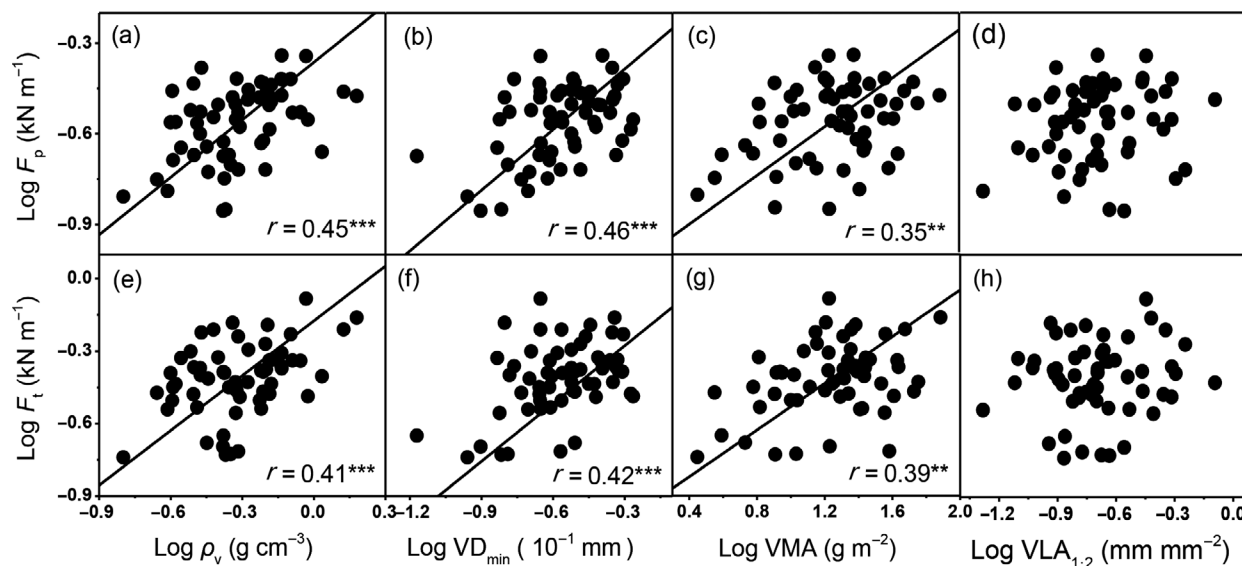
### Vein properties and their relationships to other leaf structural traits

The VD<sub>min</sub> was correlated positively with LT ( $r = 0.22$ ,  $P < 0.05$ ; Fig. 3b). The  $\rho_v$  had a significant negative correlation with LA ( $r = -0.47$ ,  $P < 0.001$ ; Fig. 3d) and positive correlation with LMA ( $r = 0.43$ ,  $P < 0.001$ ; Fig. 3g). Additionally, both VD<sub>min</sub> ( $r = 0.45$ ,  $P < 0.001$ ; Fig. 3h) and VMA ( $r = 0.36$ ,  $P < 0.001$ ; Fig. 3i) were also positively correlated with LMA. The LD was not correlated with  $\rho_v$ , VD<sub>min</sub> or VMA.

### Structural equation model of vein properties and leaf biomechanics

Our conceptual model was well supported by our empirical data (Fig. 4). There were three key patterns for vein traits that affect leaf biomechanics. First, the variation in  $\rho_v$  would directly contribute to variations in leaf biomechanics ( $\beta = 0.33$ ,  $P < 0.05$ ). Second, both the variation in  $\rho_v$  ( $\beta = 0.49$ ,  $P < 0.001$ ) and VD<sub>min</sub> ( $\beta = 0.29$ ,  $P < 0.01$ ) contributed to the variation in VMA, and the latter affected the variation in LMA ( $\beta = 0.31$ ,  $P < 0.01$ ), then LMA contributed the variation in leaf





**Fig. 2.** The correlations of vein traits with leaf mechanical properties for 58 woody species. Relationships of (a, b, c, d) force to punch ( $F_p$ ) and (e, f, g, h) force to tear ( $F_t$ ) with vein dry mass density ( $\rho_v$ ), minor vein diameter ( $VD_{min}$ ), vein mass per leaf area (VMA) and vein density of primary and secondary vein ( $VLA_{1,2}$ ). All data are log-transformed to improve normality and heteroscedasticity. The regressions are obtained from fitted standardised major axes. Correlations are based on Pearson correlation analyses with correlation coefficients ( $r$ ) and  $P$ -values across 58 species reported. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

mechanical properties ( $\beta = 0.41$ ,  $P < 0.01$ ). Third, the variation in  $VD_{min}$  also contributed to the variation in LT ( $\beta = 0.30$ ,  $P < 0.05$ ), and the latter not only affected the variation in LMA ( $\beta = 0.28$ ,  $P < 0.05$ ) but also contributed to leaf biomechanics ( $\beta = 0.24$ ,  $P = 0.059$ ). The leaf biomechanics here was evaluated by  $F_t$  ( $\beta = 0.84$ ,  $P < 0.001$ ) and  $F_p$  ( $\beta = 0.72$ ,  $P < 0.001$ ).

#### Phylogenetic independence of leaf mechanical properties, vein and structural traits

For phylogenetic signals,  $\lambda$  values of all four vein traits did not differ from zero ( $\lambda < 0.0001$ ,  $P_{(\lambda=0)} = 1$ ). For leaf mechanical properties, both  $F_t$  and  $F_p$  also had weak phylogenetic signals ( $P_{(\lambda=0)} = 0.25$  and  $0.30$ , respectively). Moreover, all three structural traits also had no phylogenetic signal (Table S3). This indicated that the 58 woody species in our study had no phylogenetic signals for these 13 traits.

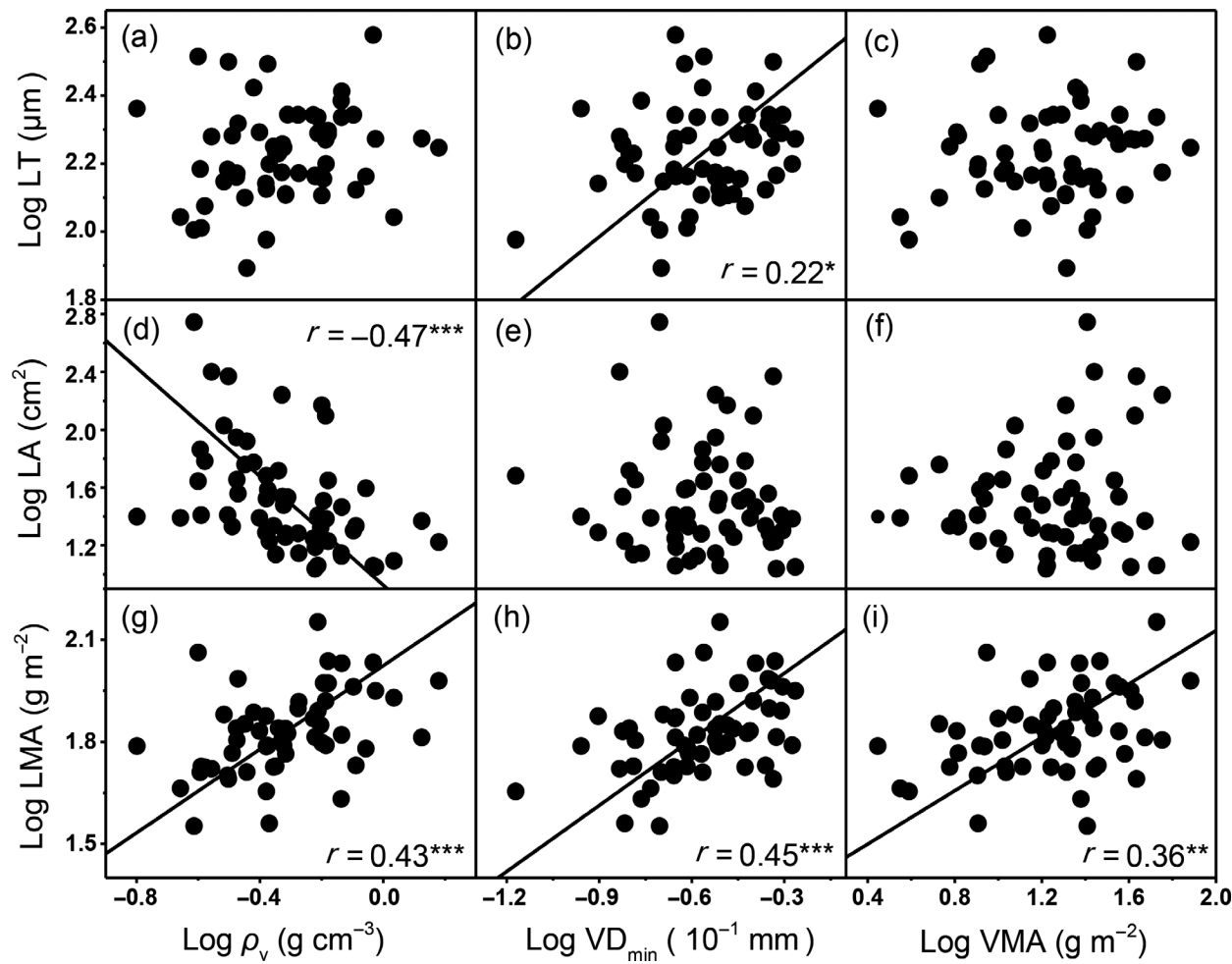
For the relationships between trait pairs, we also tested their phylogenetic independence (Table S3). The  $P$ -values of all the PGLS models and the  $R^2$  were similar to those based on Pearson correlations, but all the  $\lambda$  values did not differ from zero ( $\lambda < 0.0001$ ,  $P_{(\lambda=0)} = 1$ ; for  $\rho_v \sim LT$ ,  $\lambda = 0.098$ ,  $P_{(\lambda=0)} = 0.462$  and for  $\rho_v \sim LA$ ,  $\lambda < 0.0001$ ,  $P_{(\lambda=0)} = 0.066$ ), suggesting that these relationships were not influenced by the evolutionary history. The phylogenetic influences on our results could be neglected, probably due to trait convergence of cohort species in the stable environment of a subtropical forest. Therefore, we will not advance further discussion on the phylogenetic models.

#### DISCUSSION

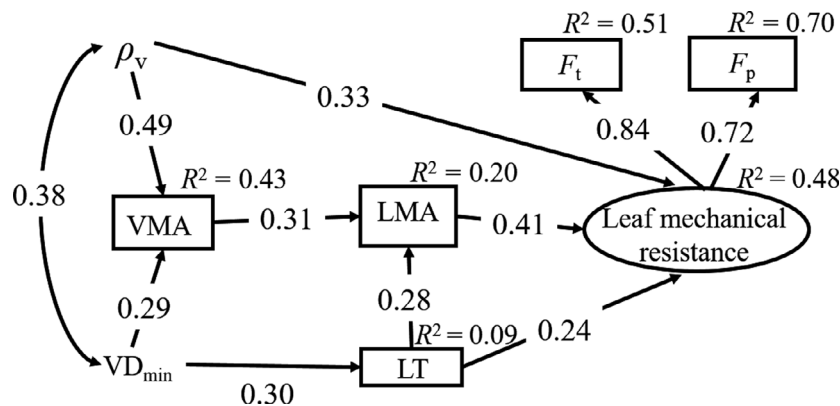
The significant positive correlations of both  $\rho_v$  and  $VD_{min}$  with  $F_t$  and  $F_p$  across the 58 woody species highlight the functional link between vein structural properties and the leaf mechanical

resistance. Our species group spanned a wide range of leaf biomechanics, especially for  $F_p$ , with a maximum value of  $0.83 \text{ kN} \cdot \text{m}^{-1}$  in *Syzygium rehderianum* and a minimum value of  $0.18 \text{ kN} \cdot \text{m}^{-1}$  in *Clerodendrum fortunatum*, which to the best of our knowledge, is among the smallest values recorded for woody species globally (Onoda *et al.* 2011). Other traits measured here also spanned a wide range of values, which increased the probability of verifying significant trait–trait relationships across species. We also noted that species with very high leaf biomechanics ( $F_t > 0.83 \text{ kN} \cdot \text{m}^{-1}$  and  $F_p > 0.46 \text{ kN} \cdot \text{m}^{-1}$ ) were not present in our study. These results were consistent with the findings that both  $F_t$  and  $F_p$  of woody species under mean annual precipitation about  $1800 \text{ mm} \cdot \text{year}^{-1}$  were less than  $1 \text{ kN} \cdot \text{m}^{-1}$  (Onoda *et al.* 2011). It has been reported that plants with high leaf biomechanics are often species such as sclerophyllous plants, which have adapted to stressful conditions like low-nutrient soils and/or water deficits (Oertli *et al.* 1990; Turner 1994; Read *et al.* 2005). At our study site, *i.e.* a subtropical broadleaved forest, there is relatively high nutrient content in the soil and water supply (Zhu *et al.* 2013), which might result in no species with very high leaf biomechanics in this forest community.

Vein traits did affect leaf mechanical properties, as we found  $\rho_v$ ,  $VD_{min}$  and VMA were correlated with both  $F_t$  and  $F_p$  (Fig. 2; Table S2). As shown in structural equation modelling (Fig. 4), we found three ways to explain the effects of vein properties on leaf biomechanics. First, the  $\rho_v$  could directly influence leaf mechanical resistance. The  $\rho_v$  reflects the components of the leaf venation (Choong *et al.* 1992). Cellulose is one of the densest material elements (Lucas *et al.* 1991, 2000; Kitajima *et al.* 2012), higher  $\rho_v$  implies higher content of cellulose, lignin and hemicellulose. Additionally,  $\rho_v$  was reported to be correlated with leaf lamina density (Poorter *et al.* 2009; Kitajima & Poorter 2010), which enhances leaf biomechanics together with cellulose (Kitajima *et al.* 2012). Moreover, Song



**Fig. 3.** The correlations of vein traits with leaf structural traits for 58 woody species. Relationships of (a, b, c) leaf thickness (LT), (d, e, f) leaf area (LA) and (g, h, i) leaf dry mass per area (LMA) with vein dry mass density ( $\rho_v$ ), minor vein diameter ( $VD_{min}$ ) and vein mass per leaf area (VMA). All data are log-transformed to improve normality and heteroscedasticity. The regressions are obtained from fitted standardised major axes. Correlations are based on Pearson correlation analyses with correlation coefficients ( $r$ ) and  $P$ -values across 58 species reported.  $^*P < 0.05$ ;  $^{**}P < 0.01$ ;  $^{***}P < 0.001$ .



**Fig. 4.** Evaluation of the conceptual model in Fig. 1 with the data from 58 woody species using structural equation modelling analysis ( $\chi^2 = 13.891$ ,  $P = 0.178$ ,  $df = 10$ ). Numbers by the arrows indicate the path coefficients ( $\beta$ , standardised partial regression coefficients). Exogenous (independent) variables are the vein traits. The endogenous (dependent) variables are enclosed in rectangles and the latent variables are enclosed in an ellipse, both with the proportion ( $R^2$ ) of the total variance explained by the model shown at the upper right corner.

*et al.* (2017) indicated that leaf area could have a positive relationship with leaf net photosynthesis rate. According to the leaf economics spectrum, species with relatively low photosynthesis rate tend to have slow-growth patterns and high LMA (Wright *et al.* 2004). Therefore, plants with smaller leaf area would invest more carbon into the constructions of veins and other tissues so as to maintain longer life cycles, which makes denser elements in the veins, reflected in higher  $\rho_v$  (Fig. 3). Kull & Herbig (1994) indicated that with the vein type unchanged, reducing leaf area will strengthen leaf mechanical properties. Thus,  $\rho_v$  could contribute to leaf mechanical properties directly, and also affect VMA. Second, a large vein lumen provides the possibility to contain more dense materials (see the interaction between  $\rho_v$  and  $VD_{\min}$  in Fig. 4). And  $VD_{\min}$  could also affect VMA mathematically. As a key impact factor for LMA, the increase in VMA certainly will cause the increase in LMA (Grace *et al.* 2017), and LMA is widely accepted to have a positively relationship with leaf mechanical properties (Onoda *et al.* 2008; Lusk *et al.* 2010; Méndez-Alonzo *et al.* 2013). The low-mass mesophyll cell, not cell walls and other structures, contain large proportions of volume space in leaves (Shipley *et al.* 2006). We observed that LMA contributed most to leaf biomechanics (Fig. 4). Third, minor veins are embedded in the lamina like a sandwich, thus, if the thickness of other tissues remains unchanged, the growth of leaf diameter will increase the leaf thickness (Turrell 1942; Blonder *et al.* 2011). Leaves with larger thickness tend to have higher mechanical resistance (Wright *et al.* 2001; Kitajima *et al.* 2012). However, the increase of  $VD_{\min}$  will result in a decrease of the distance between the nearest minor veins (Sack *et al.* 2013). While Noblin *et al.* (2008) suggested that the distance between the nearest minor veins was almost equal to half of the leaf thickness across 32 different species. That means that larger  $VD_{\min}$  will be correlated with thinner leaves, which is contrary to our results.

However, we found no correlations between  $VLA_{1-2}$  and leaf mechanical properties (both  $F_t$  and  $F_p$ ; Fig. 2), which was quite different from the positive result of Kawai & Okada (2016). We speculate two possible reasons to explain this discrepancy: (i) we carefully avoided any obvious veins, many minor veins were probably included, but the primary and secondary veins were excluded when we measured the  $F_t$  and  $F_p$ ; hence,  $VLA_{1-2}$  is not correlated with leaf biomechanics inevitably even though it has tougher structure and high mechanical resistance (Méndez-Alonzo *et al.* 2013); (ii) We obtained most vein traits together to research their contributions to leaf mechanical properties among woody species from different families in a subtropical forest. The vein traits of species from different families in various habitats vary to a large extent (Sack & Scoffoni 2013; Blackman *et al.* 2016; Schneider *et al.* 2017). This relationship was only found in *Fagaceae* species from temperate forests. Because evolutionary history had no impacts on this relationship (Table S3), environmental differences should be the main contributors to explain trait variances. Studies have indicated that VLA and other leaf structures varied widely across different habitats. Zhu *et al.* (2012) investigated *Quercus variabilis* from ten *in situ* and seven garden populations, and suggested that the  $VLA_{\min}$  were negatively related to latitude, and positively coordinated with mean annual temperature. In addition, irradiance is also a vital factor for leaf venation and anatomical plasticity: plants grown in high light have high VLA (Carins Murphy *et al.* 2012; Scoffoni *et al.* 2015). Similarly, leaf

mechanical properties are also influenced by light intensities and nutrient availability (Onoda *et al.* 2008, 2011) and habitat (Méndez-Alonzo *et al.* 2013). Therefore, different habitats (temperate forests *versus* subtropical forest) with discrepant irradiance and soil conditions could be the real reason for the diversity of the relationships between leaf venation and biomechanics.

We found no relationships between tissue density and leaf biomechanics (Table S5), which is inconsistent with the study in tropical moist semi-evergreen forest of Bolivia (Kitajima & Poorter 2010). Our study site is a lower montane forest with distinct topographic variations, and suffers frequent typhoons every year (Zhou & Yan 2001), resulting in a large variation in light environment for the studied species, which might, in turn, cause greater plasticity for leaf density through the accumulation of photosynthate (He *et al.* 2019). In contrast, leaves of different ecological strategies under different light environments could exhibit similar tissue quality, for example, leaves of light-demanding species in the sun and those of shade-tolerant species in the shade could have thick and dense leaves (Lusk *et al.* 2008, 2010), which might reduce the tissue density variations among species. We also found no relationships among vein traits and any material strength measurement ( $S_t$ ,  $S_p$ ,  $S_t/LD$  and  $S_p/LD$ ) (Table S2). It has been shown that material strength can explain most of the variation in structural strength (*e.g.*  $F_t$ ,  $F_p$ ) across species (Onoda *et al.* 2011; Kitajima *et al.* 2012). These results suggest that the LT and LD accounted for the contributions of veins to leaf mechanical resistance, while LT might be the major contributor to leaf biomechanics for species at our study site (Fig. 4). The  $\rho_v$  was significantly negatively correlated with lamina size (Fig. 3d), indicating that there might be fewer fibres in veins of large leaves. This might, in turn, reduce vein fracture toughness in large leaves, consistent with the findings of Kitajima & Poorter (2010).

Although lower-order veins are likely to be more lignified than higher-order veins, the degree of lignification in primary and secondary veins is similar (Zwieniecki *et al.* 2002; Ohtsuka *et al.* 2018). In addition, we found that primary and secondary vein volume accounts for 79% (on average) of total vein volume in our study (Table S4), thus, the effect of applying the same  $\rho_v$  for all vein orders to estimate VMA would be minor. Since primary and secondary veins often taper greatly around the leaf tip, their volumes per area here might be overestimated. Moreover, it becomes increasingly difficult to separate the primary vein from the rest of the lamina, particularly for species with small leaves. Therefore, corrections should be applied in future to obtain more accurate primary vein mass to calculate the vein dry mass density (Niinemets *et al.* 2007). Besides, more work is needed under different climates and environments to test whether such correlations still exist at a larger scale. According to current research, vein traits affect leaf hydraulics (Blackman *et al.* 2010; Sommerville *et al.* 2012; Scoffoni *et al.* 2016), leaf mechanical properties and even leaf economics spectrum (Walls 2011). Further research is needed to combine all these leaf functional traits to improve understanding of the ecophysiological mechanisms of plant adaptability at a global scale.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Phylogenetic relationships of the 58 woody species in this study. Families are indicated by different segments on the right side of the figure.

**Table S1.** Characteristics of 58 woody species tested in the present study.

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