**LETTER**

# Leaf economics and hydraulic traits are decoupled in five species-rich tropical-subtropical forests

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## Abstract
Leaf economics and hydraulic traits are critical to leaf photosynthesis, yet it is debated whether these two sets of traits vary in a fully coordinated manner or there is room for independent variation. Here, we tested the relationship between leaf economics traits, including leaf nitrogen concentration and leaf dry mass per area, and leaf hydraulic traits including stomatal density and vein density in five tropical-subtropical forests. Surprisingly, these two suites of traits were statistically decoupled. This decoupling suggests that independent trait dimensions exist within a leaf, with leaf economics dimension corresponding to light capture and tissue longevity, and the hydraulic dimension to water-use and leaf temperature maintenance. Clearly, leaf economics and hydraulic traits *can* vary independently, thus allowing for more possible plant trait combinations. Compared with a single trait dimension, multiple trait dimensions may better enable species adaptations to multifarious niche dimensions, promote diverse plant strategies and facilitate species coexistence.

## Keywords
Functional divergence, leaf carbon isotope composition ($\delta^{13}C$), leaf dry mass per area, leaf economics spectrum, leaf hydraulics, leaf nitrogen concentration, stomatal density, vein density, woody angiosperms.

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## INTRODUCTION

Studies of plant functional traits across species and biomes have greatly advanced our understanding of plant form and function (Givnish 1987; Reich 2014). From an economics perspective, correlations among plant functional traits have been widely used to identify functional constraints and trade-offs underlying key plant ecological strategies (Westoby *et al.* 2002; Ackerly 2004). These studies have also offered insights into developing global vegetation models that predict ecosystem responses to global environmental change (Soudzilovskaia *et al.* 2013).

Among various plant traits, those related to leaf-level $CO_2$ and water exchange and light capture have probably received most attention, reflecting the critical importance of these processes in global primary production and functioning of the biosphere. One set of leaf-level traits form a single group of leaf economics traits, which include leaf maximum photosynthetic capacity ($A_{max}$), leaf lifespan (LL), leaf dry mass per area (LMA) and leaf nitrogen (N) concentration. These traits are often found to be strongly correlated with each other across species, forming the so-called worldwide leaf economics spectrum, which characterises ecological strategies with quick to slow payback on investments of nutrients and dry mass (Wright *et al.* 2004). The other set of traits group together as leaf hydraulic traits, which consist of traits describing leaf venation structure (Sack & Frole 2006; Brodribb *et al.* 2007; Blonder *et al.* 2011) and stomatal size and distribution (Brodribb & Feild 2010). Venation and stomatal traits are often strongly correlated across species (Sack & Scoffoni 2013), and vary in coordinated manner within species upon plastic adjustment to environmental modifications (Carins Murphy *et al.* 2014), suggesting a balance between water supply (related to venation traits, Sack & Scoffoni 2013) and demand (indicated by stomatal traits, Franks *et al.* 2009).

Since both leaf economics and hydraulic traits are involved in the process of trading water for $CO_2$, a close relationship between these two suites of traits has been proposed. Blonder *et al.* (2011) argued that leaf venation traits underlay the leaf economics spectrum and derived equations to predict leaf economics traits using venation traits. Furthermore, Reich (2014) proposed a plant world view of a single economics spectrum running from fast to slow species, with all traits being 'fast' in 'fast' species and 'slow' in 'slow' species, thus implying a

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strong coordination between leaf economics and hydraulic traits across a wide range of species. By contrast, Sack *et al.* (2013) suggested that the vein origin hypothesis proposed by Blonder *et al.* (2011) is invalid because the equations underlying this hypothesis largely resulted from a circular logic. Sack *et al.* (2014) further showed for a large compiled dataset that LMA was independent of vein density (VD), and Sack *et al.* (2003) showed for a smaller species set that LMA was independent of water flux traits. Moreover, two recent studies (Lloyd *et al.* 2013; Osnas *et al.* 2013) questioned that even within the economics traits, the close linkage between traits such as mass-based leaf N concentration and photosynthetic rate may be a mathematical artefact due to mass normalisation. These studies imply that multifarious traits serving $CO_2$ and water exchange are not necessarily correlated, and using a single spectrum to describe the plant world may be overly simplistic.

Evidence is accumulating in support of multiple dimensions in plant functional traits, opposing a single spectrum of worldwide plant trait economics. For example, Westoby *et al.* (2002) used several independent leading dimensions to describe plant ecological strategies and Ackerly (2004) showed that life history traits and drought tolerance/avoidance traits were independent. Baraloto *et al.* (2010) also found that leaf economics traits and wood economics traits formed two orthogonal axes, suggesting that plants may combine structural traits of leaves and stems from different parts of leaf and stem economics spectra. This is of paramount significance in understanding vegetation composition, as these independent trait dimensions may be selected by different environmental filters (Laughlin 2014). For example, LMA varies strongly with light availability (Poorter *et al.* 2009; Niinemets *et al.* 2015), leaf N concentration changes with soil nutrient conditions (Richardson *et al.* 2005), and stomatal traits are mainly determined by light, humidity and $CO_2$ (Casson & Hetherington 2010). Taken together, in complex real-world ecosystems where resource space is multi-dimensional and highly heterogeneous (Stewart *et al.* 2000), various combinations among plant traits may be required to adapt to subtle and multifaceted changes in environment to enhance overall plant performance.

Here, we test the hypothesis that leaf hydraulic and economics traits constitute two independent trait syndromes across five species-rich tropical-subtropical forests in China. We analysed 10 key leaf structural, chemical and physiological traits, as well as their phylogenetic signals across 85 woody angiosperms representing 59 genera and 31 families. Specifically, we selected four leaf hydraulic traits including stomatal density (SD), stomatal guard cell length (SL), VD and calculated maximum stomatal conductance to water vapour ($g_{wmax}$), and five traditional leaf economics traits including leaf area-based N concentration ($N_{area}$), leaf mass-based N concentration ($N_{mass}$), LMA, leaf thickness (LT), and leaf tissue density (TD) as well as leaf carbon isotope composition ($\delta^{13}C$) which is an integrated long-term index of water-use efficiency (Farquhar *et al.* 1989). For all the 85 species, we also generated a molecular phylogenetic tree based on two plastid genes (*rbcL* and *matK*) to determine trait phylogenetic signals, using Blomberg's *K*-statistic and Abouheif's test. We focused on tropical-subtropical woody angiosperms because

this plant functional group encompasses high plant diversity (Huang *et al.* 2012), and the use of multiple phylogenetic lineages allows for a better understanding of trait relationships in a phylogenetically informed manner.

## MATERIAL AND METHODS

### Study sites and species

Leaf samples were collected from five Chinese tropical-subtropical forests. The sites covered a latitudinal range from 18°40′ N to 24°32′ N, with the mean annual temperature ranging from 10.7 to 22.4 °C, and mean annual precipitation ranging from 1539 to 2651 mm (Table S1). Fifty-six species were sampled in three subtropical sites, including Mt. Dinghu and Mt. Wutong in Guangdong province and Mt. Ailao in Yunnan province. Twenty-nine species were sampled in two monsoon tropical sites, including Mt. Jianfeng in Hainan province, and Xishuangbanna rainforest in Yunnan province.

All the 85 species were dicots including 60 trees and 25 shrubs, and represented 59 genera and 31 families (see Data S1), covering key clades of common species in southern China, such as magnoliids, fabids and lamiids. All these species are native and dominant in the sub-canopy and canopy layers in the sampled communities.

### Sampling and storage

For each species, at least three individuals were sampled, and from each individual, > 40 mature fully expanded sun-exposed leaves were collected from the upper sunny part of the tree canopy. Once collected, three leaves per individual were immediately put into buffered FAA fixation solution (50% ethanol, 5% glacial acetic acid and 3.7% formaldehyde) for analyses of stomatal and venation traits, and another subsample was immediately put in a sealed envelope with silica gel desiccant for DNA sequencing. The remaining leaf samples were used for the measurements of leaf thickness, LMA and chemical characteristics.

### Trait measurements

Paradermal sections for leaf venation measurement were prepared according to the general protocols described by Carins Murphy *et al.* (2012) and Brodribb *et al.* (2013), with modifications depending on the species. First, the adaxial epidermis and palisade mesophyll were carefully removed using a sharp razor blade or an eyebrow shaver to expose the minor veins. Sections were then placed in bleach (50 g $L^{-1}$ sodium hypochlorite and 13 g $L^{-1}$ sodium hydroxide) for several hours to several days depending on the species until clear. For some species that resisted clearing in bleach alone, sections were first placed in 5% KOH. After clearing, sections were carefully rinsed to remove bleach and then stained in 1% toluidine blue. Finally, 10 fields of view at 10× magnification were photographed for each section using a Leica DFC450 camera (Nussloch, Germany) mounted on a Leica DM 2500 microscope. VD was measured as the total length of leaf venation per leaf area using ImageJ (NIH Image, Bethesda, MD, USA) by manually draw-

ing and counting all veins in the field. For each species, at least four leaves from different individuals were used and 30 fields of view (each field with an area of $1250 \times 937.5$ μm) were selected between the midrib and the margin.

Samples for stomatal observations were made using the nail polish impression method as described in Franks *et al.* (2009). Given that the 85 dicotyledonous species used in this study are hypostomatous (having stomata on the lower side of leaves), stomatal traits were therefore calculated based on the adaxial surface. Stomatal density (mm$^{-2}$) was calculated as the number of stomata per unit epidermal area and SL (μm) as the length between the outer junctions of guard cells at the proximal ends of the stoma. Both SD and SL were measured using a light microscope (Motic B5, Xiamen, China) at 10–20$\times$ magnification and 20–40$\times$ magnification respectively. For each species, at least six leaves from different individuals were used for these measurements and 20 fields of view were randomly selected. Potential maximum stomatal conductance to water vapour ($g_{wmax}$, mol m$^{-2}$ s$^{-1}$) was calculated according to eqn 2 in Franks *et al.* (2009).

Leaf surface area was scanned in the field immediately after sampling using a portable scanner (Canon LiDE 110, Tokyo, Japan) and then measured using ImageJ software (NIH Image). The leaf samples were then oven-dried at 65 °C for 48 h to a constant mass. The mass of dried leaf samples was estimated with a precision of 0.1 mg. Leaf dry mass per unit area (LMA, g m$^{-2}$) was calculated as the ratio of leaf dry mass to projected leaf area. Leaf thickness (LT, mm) was also measured immediately after sampling using digital calipers (SMCTW Company, Shanghai, China), avoiding the influence of leaf major veins. Leaf tissue density (TD, g cm$^{-3}$) was then calculated as LMA divided by LT. The oven-dried leaf samples were ground to fine powder using a SPEX 8000-D mixer mill (SPEX, Edison, NJ, USA). Leaf nitrogen (N) concentration was determined using an elemental analyser (Vario EL III, Elementar, Hanau, Germany). Around 1 mg of ground material was then weighed and leaf stable carbon isotope composition was analysed by the isotopic Cavity-Ring Down Spectrometer equipped with the Combustion Module (CM-CRDS system, Picarro, CA, USA). The precision of the CM-CRDS system is 0.2–0.3‰.

### Phylogenetic tree construction

Plant genomic DNA was extracted from silica gel-dried leaves. The 693-bp fragment of the Rubisco large subunit (*rbcL*) and 756-bp fragment of maturase K (*matK*) were amplified (Table S2 for details). The polymerase chain reaction (PCR) program was 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 50 °C for 45 s, 72 °C for 45 s; followed by a final extension of 72 °C for 8 min. Unpurified PCR products were sequenced bidirectionally by an ABI-PRISM3730XL Genetic Analyzer (Foster City, CA, USA) with BigDye terminator v3.1. The accession numbers of these two sequences of all sampled species can be found in Table S3.

After model selection using jModelTest v2.1.1 (Posada 2008), the phylogenetic tree was constructed using maximum likelihood (ML) and Bayesian approaches. In the phylogenetic analyses, the tree branch length was set to be proportional to

the difference of divergence time between neighbour clades. Divergence time was estimated by BEAST1.7.1 (Drummond *et al.* 2012) with eight fossil calibration nodes (Table S4). Detailed information is in the Appendix S1.

### Data analysis

For each leaf trait, we calculated mean values for each individual species, and then calculated the trait minimum, maximum and coefficient of variation across all 85 species. To meet the assumption of normality, all original data were log10-transformed before calculating Pearson correlations for pairwise combinations of traits using PASW Statistics 18.0 (IBM, Chicago, IL, USA). Multiple-trait relationships were analysed by principal component analysis (PCA) in CANOCO software for Windows 5.0 (Microcomputer Power, Ithaca, NY, USA), and species were then ordered by their scores on the first two principal components. All trait data were standardised using the zero-mean approach prior to PCA. Furthermore, we conducted three PCAs for three different groups of variables (coloured differently in Fig. 1a), created a sampling distribution using 100,000 permutations of PC1 scores of each PCA, and then tested the independence between leaf economics and hydraulic traits using R 3.0.1 (R Core Team 2013).

To test for the phylogenetic signal of each trait, Blomberg's *K*-statistic was calculated, and a larger *K* value indicates a greater phylogenetic conservatism for the given trait (Blomberg *et al.* 2003). We also performed the Abouheif's test (Abouheif 1999) to detect phylogenetic signals of all traits. To test for the effect of phylogeny on interspecific comparisons, we conducted species-level analysis via phylogenic independent contrasts (PIC). All these phylogenetic analyses were conducted in R (R Core Team 2013).

### RESULTS

Among leaf hydraulic traits, a positive correlation was found between SD and VD ($r = 0.35$, $P < 0.01$) (Table 1; Fig. S1, Table S5), whereas negative correlations were found between SL and SD ($r = -0.70$, $P < 0.01$) (Table 1; Fig. S2). Among leaf economics traits, a positive correlation was found between leaf area-based nitrogen concentration (N$_{area}$) and LMA ($r = 0.34$, $P < 0.01$), whereas a negative correlation was found between leaf mass-based nitrogen concentration (N$_{mass}$) and LMA ($r = -0.56$, $P < 0.01$) (Table 1). Pearson correlation analysis using PIC showed consistent results with the analysis using original data, except that the correlation between LMA and N$_{area}$ was not significant according to PIC (Table 1), suggesting that this interspecific coordinated pattern was strongly influenced by plant phylogeny.

Contrary to these significant correlations, leaf hydraulic traits were uncorrelated with LMA and N$_{area}$ ($P > 0.05$; Table 1). Specifically, $g_{wmax}$ was not correlated with N$_{area}$ ($P > 0.05$) and $\delta^{13}$C was not correlated with either N$_{area}$ or $g_{wmax}$ (though $\delta^{13}$C was positively correlated with LT, Table 1). PCA using the original data further confirmed that leaf hydraulic traits were independent of leaf economics traits (Fig. 1a). The first PCA axis accounted for 30.2% of total variation and showed strong loadings on LT, SD, SL, VD
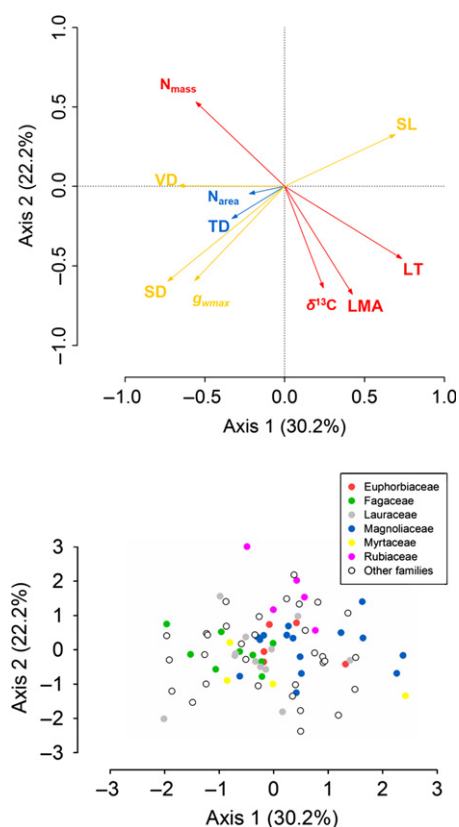
**Figure 1** Principal component analysis (PCA) highlighting independent axes of leaf functional traits among 85 woody angiosperms in subtropical forests. Trait abbreviations are provided in Table 3. The trait loading plot in the upper panel shows leaf hydraulic traits as yellow lines, the bulk of leaf economics traits as red lines and the remaining economics traits as blue lines. The PCA ordination using all species in the lower panel demonstrates various combinations of leaf hydraulic and economics traits corresponding to multiple water-use and light capture strategies among species from different families.

and $g_{wmax}$ (in the descending order) (Table 2). The second axis, which accounted for 22.2% of total variation, had strong loadings on LMA and $\delta^{13}C$ (in the descending order) (Table 2). Further simulation analyses of three correlations among PC1 scores of three groups of traits (leaf hydraulic traits, leaf economics traits together with $N_{area}$ and TD,

coloured differently in Fig. 1a) had *P* values of 0.1591, 0.6542 and 0.179, which confirmed that leaf hydraulic traits were orthogonal to leaf economics traits (Fig. S3). Additional PCA analyses performed across different sites and families showed similar independence between leaf hydraulic traits and leaf economics traits (detailed results not shown). PCA ordination using all species demonstrated variable combinations of leaf hydraulic and economics traits among 85 woody angiosperms from different families (Fig. 1b) and from different subtropical forests (Fig. S4).

These two trait axes showed contrasting phylogenetic signals and different ranges of variation. For the first axis, traits such as SD had low Blomberg's *K*, indicating little phylogenetic conservatism across 85 woody species sampled here (Table 3; Table S3). Additionally, SD and VD showed large variation of 18.8-fold and 5.7-fold, whereas SL showed relatively small variation of 3.5-fold (Table 3; Fig. S5). These results indicate that *areal densities* of leaf hydraulic structures are highly variable (Fig. S6), whereas *cell sizes* of leaf hydraulic structures varied within a smaller range. By contrast, for the second axis, leaf economics traits (such as LMA) showed significant, but relatively weak phylogenetic signals as indicated by both Blomberg's *K* values (Table 3) and Abouheif's test (Fig. S7).

## DISCUSSION

Leaf hydraulic traits (such as SD and VD) and leaf economics traits (such as leaf N concentration and LMA) are critical to leaf functioning. It has been proposed that these two suites of traits co-vary tightly because they both serve photosynthesis (Blonder *et al.* 2011; Reich 2014). However, in this study, we found that leaf hydraulic and economics traits were statistically decoupled in five tropical-subtropical forests (Fig. 1; Fig. S3). This surprising result suggests that the relationship between these two trait syndromes may not be as simple as previously thought. The independence between leaf hydraulic and economics traits may be explained from structural, evolutionary and ecological perspectives.

First, this independence pattern may be a result of the physical separation of leaf structures that correspond with leaf hydraulic and economics traits, and this physical separation alone may have an impact on leaf functioning. We argue that

**Table 1** Pearson correlation coefficients (lower diagonal) and phylogenetically independent contrasts (upper diagonal) among 10 leaf functional traits for 85 subtropical woody angiosperms

| | LT | TD | LMA | $N_{mass}$ | $N_{area}$ | $\delta^{13}C$ | VD | SL | SD | $g_{wmax}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| LT | | −0.16 | **0.75** | **−0.50** | −0.08 | **0.43** | **−0.68** | −0.07 | −0.01 | −0.11 |
| TD | **−0.45** | | **0.47** | −0.03 | **0.32** | −0.17 | 0.16 | 0.08 | −0.04 | −0.08 |
| LMA | **0.54** | **0.51** | | **−0.44** | 0.18 | **0.25** | **−0.62** | 0.04 | −0.07 | −0.20 |
| $N_{mass}$ | **−0.50** | −0.09 | **−0.56** | | **0.78** | 0.09 | **0.41** | −0.03 | 0.13 | 0.16 |
| $N_{area}$ | −0.05 | **0.40** | **0.34** | **0.59** | | 0.18 | 0.05 | 0.05 | 0.13 | 0.11 |
| $\delta^{13}C$ | **0.51** | −0.17 | **0.34** | −0.18 | 0.13 | | −0.21 | **−0.43** | 0.19 | 0.07 |
| VD | **−0.38** | 0.20 | −0.19 | **0.30** | 0.17 | −0.13 | | −0.23 | **0.34** | **0.46** |
| SL | **0.26** | −0.18 | 0.08 | −0.11 | −0.05 | 0.02 | **−0.47** | | **−0.62** | **−0.45** |
| SD | **−0.22** | 0.18 | −0.03 | 0.05 | 0.03 | 0.16 | **0.35** | **−0.70** | | **0.94** |
| $g_{wmax}$ | −0.14 | 0.14 | 0.01 | 0.00 | 0.01 | 0.21 | 0.20 | **−0.34** | **0.91** | |

All traits were log-transformed to meet the normality assumption. Significant correlations are indicated in bold. Trait abbreviations are provided in Table 3.

**Table 2** Loading scores of 10 functional traits in the PCA among 85 subtropical woody angiosperms

| Component | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| % of variance | 30.2 | 22.2 | 17.1 |
| *Loadings of traits* | | | |
| LT | 0.7339 | −0.4542 | −0.064 |
| SD | −0.7322 | −0.5963 | −0.2697 |
| SL | 0.6921 | 0.3264 | 0.1354 |
| VD | −0.6642 | 0.0042 | 0.1173 |
| $g_{wmax}$ | −0.5637 | −0.5935 | −0.2863 |
| $N_{mass}$ | −0.556 | 0.5309 | 0.2621 |
| LMA | 0.4243 | −0.6791 | 0.5415 |
| TD | −0.3304 | −0.2024 | 0.6919 |
| $\delta^{13}C$ | 0.2418 | −0.6394 | −0.0028 |
| $N_{area}$ | −0.2221 | −0.0458 | 0.8214 |

Traits are arranged in descending order of their loadings on the PC1. Trait abbreviations are provided in Table 3.

these two sets of traits are features of different structural modules (Fig. 2) and represent two functional subsystems within the leaf. For dicotyledonous species, leaf mesophyll is primarily partitioned between palisade and spongy mesophyll tissues (Parkhurst & Givnish 1986) and can thus be divided into an upper and a lower subsystem. Leaf hydraulic structures are located in the lower compartment of the leaf (Fig. 2) with stomatal pores at the lower epidermis and minor veins in the middle of the leaf (Brodribb & Feild 2010). Conversely, a greater fraction of leaf N on an area basis is found in the palisade tissue of the leaf (Fig. 2; Osnas *et al.* 2013). It is well known that leaf hydraulic traits are responsible for stomatal conductance ($g_{max}$ in Fig. 2) and water transpiration (Franks & Beerling 2009), whereas leaf $N_{area}$ is closely related to leaf $CO_2$ carboxylation (Field & Mooney 1986) ($V_{cmax}$ in Fig. 2), and the two processes do not necessarily vary simultaneously or with the same rates (as discussed below). We further argue that because spatial organisation between upper and lower layers of a leaf may influence $CO_2$ gradients and $CO_2$–water exchange within the leaf (Zwieniecki & Boyce 2014), different combinations of leaf lower and upper layers will likely result in different leaf functioning. Other leaf economics traits, such

as LMA and leaf thickness, characterise features of the entire leaf (Fig. 2), and will likely influence these physiological processes in ways different from upper and lower layers of the leaf individually.

Second, the independence of these two sets of leaf traits may be related to the contrasting evolutionary trajectories of hydraulic vs. non-hydraulic structures. Beerling (2005) proposed that a webbing of photosynthetic mesophyll tissues (more closely related to leaf economics traits) may have appeared long after the dichotomising vascular axis (more closely related to leaf hydraulic traits) in the leaf blades, and this time sequence might be related to the decline of global atmospheric $CO_2$ concentrations in Earth history. Our observed independence between hydraulic traits and economics traits may in part be an imprint of these separate ancient plant evolutionary steps.

Last but not the least, independence between hydraulic and economics traits give a certain leaf much more *freedom* to adjust its functioning to a local environment (environment surrounding a leaf, a tree canopy, or a forest). For example, with increasing tree height in *Pinus densiflora*, stomatal conductance of needles may decrease as a response to greater water stress at greater tree height, but leaf $N_{mass}$ may increase to compensate for increased $CO_2$ diffusive resistance at greater tree height and thus to minimise the decline in light-saturated photosynthesis (Han 2011). In this case, stomatal conductance and leaf $N_{mass}$ varied in opposite ways (this contrasts with the predictions of theories of single fast-slow trait dimension that stomatal conductance and leaf $N_{mass}$ should vary in the same direction), which gives plants greater flexibility to improve leaf photosynthetic performance under water stress. Tanaka *et al.* (2013) also found that when comparing different *Arabidopsis thaliana* genotypes, the genotype with higher leaf photosynthetic rates had higher SD with no change in maximum carboxylation capacity. In this case, increased SD affects photosynthetic capacity by modulating the $CO_2$ gas diffusion process rather than carboxylation activity. Clearly, the independence among different traits allows for greater plasticity in maintaining or improving photosynthetic performance, enhances overall canopy plasticity, and thus may increase

**Table 3** Variation in 10 leaf functional traits measured in 85 woody species

| Leaf functional traits | Abbrev | Unit | Mean | SE* | CV† | Max | Min | Blomberg's $K$ |
|---|---|---|---|---|---|---|---|---|
| Stomatal density | SD | $mm^{-2}$ | 394 | 0.027 | 67% | 1716 | 91 | **0.06** |
| Maximum stomatal conductance to water vapor | $g_{wmax}$ | $mol\ m^{-2}\ s^{-1}$ | 1.4 | 0.021 | 47% | 4.8 | 0.4 | 0.05 |
| Leaf mass-based nitrogen concentration | $N_{mass}$ | $mg\ g^{-1}$ | 18.05 | 0.018 | 39% | 40.69 | 5.40 | 0.03 |
| Leaf area-based nitrogen concentration | $N_{area}$ | $g\ m^{-2}$ | 1.34 | 0.016 | 37% | 3.38 | 0.54 | 0.03 |
| Leaf dry mass per area | LMA | $g\ m^{-2}$ | 80 | 0.016 | 34% | 159 | 33 | **0.06** |
| Leaf thickness | LT | mm | 0.24 | 0.015 | 34% | 0.56 | 0.09 | **0.07** |
| Leaf tissue density | TD | $g\ cm^{-3}$ | 0.34 | 0.015 | 33% | 0.86 | 0.14 | **0.10** |
| Vein density | VD | $mm\ mm^{-2}$ | 6.13 | 0.015 | 31% | 13.01 | 2.28 | 0.04 |
| Stomatal guard cell length | SL | μm | 21 | 0.012 | 24% | 35 | 10 | **0.11** |
| Leaf carbon isotope composition | $\delta^{13}C$ | ‰ | −32.0 | 0.003 | 6% | −27.4 | −36.7 | **0.10** |

*SE, standard error.
†CV, coefficient of variance.
The traits are listed in order of CV, from largest to smallest. Mean values for all traits were calculated on the original scale, while SE is provided on log-scale for comparability. Blomberg's $K$ values in bold indicate they are significant at $P < 0.05$. Blomberg's $K$ characterises the phylogenetic signal in the data ($K = 1$ corresponds to Brownian motion evolution, while $K < 1$ to more divergence in trait space than for a random walk evolution) (Blomberg *et al.* 2003).
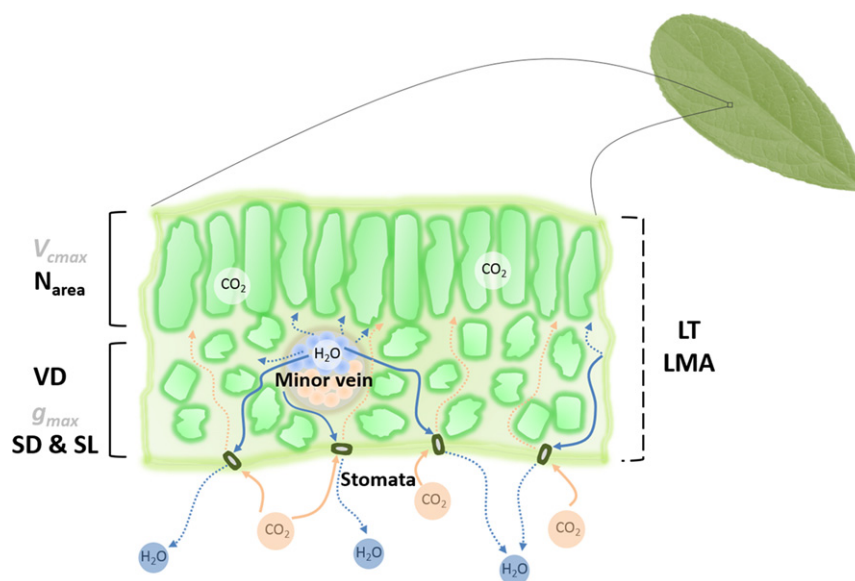
**Figure 2** Conceptual diagram of an expanded leaf section highlighting leaf-level modular organisation of key leaf functions. The traits related to the maximum carboxylation rate in photosynthesis ($V_{cmax}$) vs. stomatal conductance ($g_{max}$) are spatially separated into two hypothetical functional modules within the leaf. The first module is primarily located in the palisade mesophyll, while the second module is located in the bottom half of the leaf in the spongy mesophyll. The first module is primarily responsible for $V_{cmax}$ and its activity is dependent on leaf nitrogen concentration ($N_{area}$) and associated traits. The second module determines $g_{max}$ and the transpiration flux, and the activity of this second leaf component is dependent on stomatal density (SD) and stomatal guard cell length (SL). Leaf dry mass per area (LMA) and leaf thickness (LT) are integrated traits characterising different facets of whole leaf constitution.

growth of each plant individual in their particular set of environmental conditions (light, water, temperature, etc.).

Despite the independence between these two suites of trait syndromes, traits within each suite were closely associated, further indicating the two-subsystem phenomenon. Among hydraulic traits, SD was positively correlated with VD (Table 1; Fig. S1), indicating a coordination between water demand (e.g. leaf gas exchange) and water supply (transport) capacity (Zhang *et al.* 2012; Sack & Scoffoni 2013). This coordination between stomata and venation is likely to be driven by fundamental differences in cell size (Brodribb *et al.* 2013). Among economics traits, LMA was negatively correlated with leaf $N_{mass}$ and positively correlated with $N_{area}$ (Table 1), consistent with previous studies (Wright *et al.* 2004), as both LMA and N concentration are related to light capture (Niinemets & Sack 2006; Sterck *et al.* 2011; Soudzilovskaia *et al.* 2013). Thus, leaf hydraulic traits constitute a continuum in gas exchange and water transport capacities, whereas leaf economics traits represent a continuum in light capture capacity and carbon economy.

**Implications for understanding diverse plant strategies and species diversity**

Leaf hydraulic traits and economics traits are supposed to be strongly correlated based on several theories, such as 'single trait dimension' argued by Reich (2014), or 'venation networks as origin of the leaf economics spectrum' argued by Blonder *et al.* (2011). Here, our results from five species-rich subtropical forests clearly showed that these two suites of traits *can* vary independently. These results are consistent with reports that area-based vein traits (such as VD) and gas exchange traits (such as stomatal pore area) may be uncorrelated with

LMA across six woody temperate species in Harvard Forest (Sack *et al.* 2003) or for a much larger set of species compiled from the literature (Sack *et al.* 2013). Our results are also in line with individual experimental studies showing that leaf hydraulic traits and economics traits may not vary simultaneously (Tanaka *et al.* 2013) or even vary in opposite directions (Han 2011). Thus, greater freedom in leaf trait variation seems to be the rule and tightly constrained variation to form a single trait axis seems to be the special cases, particularly in species-rich biomes where species differences are often gradual (Fig. 1b) and environmental gradients may be finely divided.

The existence of these independent trait axes has several implications for understanding plant strategies in resource capture and in adaptation to multifarious environmental gradients (or stresses) in these species-rich forests and in other biomes. Among the species we studied, those with high gas exchange and water transport capacities (many small stomata with dense minor veins) can have either low costs (low LMA; e.g. *Osmanthus didymopetalus*) or high carbon costs (high LMA; e.g. *Millettia leptobotrya*) for light capture. Thus, plant functional traits have freedom to vary independently (although within the limitations imposed by cell size or genome size as suggested by Feild & Brodribb 2013), and we believe this freedom is the basis for multiple alternative strategies so frequently observed in nature (Marks & Lechowicz 2006). If one trait combination and thus one species can be viewed as one ecological strategy, more possible combinations of independent plant trait dimensions can allow greater varieties of strategies (Laughlin 2014). More combinations of trait dimensions in turn may allow species to adapt to multifarious niche dimensions (Nosil *et al.* 2009), thus facilitating species coexistence (Adler *et al.* 2013), enhancing species diversity (Kraft *et al.* 2008) and contributing

to ecosystem stability (Pillar *et al.* 2013). Clearly, a single trait dimension of critical traits would severely constrain the freedom of trait variations, leading to a plant world much poorer and less complex than we see today. In the future, identifying additional independent plant trait axes should lead to better understanding of species diversity in diverse ecosystems.

The extent to which leaf hydraulic and economics traits are decoupled also has implications for developing predictive global vegetation models. Our results strongly suggest that leaf hydraulic and economics traits should be considered separately in modelling biogeochemical processes, if the decoupling of these two sets of traits is broadly applicable across different biomes. This appears likely as multiple trait dimensions (even at the leaf level) may be required if multiple species were to coexist within a community as predicted theoretically (Laughlin 2014). Indeed, when we added our data to the compilation by Sack *et al.* (2013), we found that the independence between LMA and leaf VD occurred not only in the subtropics we studied, but also in the temperate biome ($r = 0.144$, $P = 0.140$, $n = 106$), whereas patterns within other biomes differed somewhat (Fig. S8a). In general, no broad relationship between LMA and VD was found across different biomes and in the entire global dataset (Fig. S8a), and subdividing the dataset into different plant functional types further supported overall independence of these traits (Fig. S8b). At the global scale, Osnas *et al.* (2013) also suggested that we should search for plant trait dimensions that are independent of leaf economics traits to better represent biodiversity in global models. Multiple independent leaf trait dimensions found in our study may represent a valuable step in clarifying the future path for this endeavour.

## CONCLUSION

We now have clear evidence from five diverse tropical-subtropical forests to indicate that leaf economics and hydraulic traits are not tightly constrained onto a single axis, but have *freedom* to form independent axes. These independent trait axes may correspond to upper and lower compartments within the leaf, which represent different functional modules for leaf functioning. Appreciation of decoupled leaf economics and hydraulic traits offers a new perspective for understanding diverse plant strategies and species coexistence, and for better incorporating biodiversity into global models.

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