

# Dry mass costs of deploying leaf area in relation to leaf size

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## Summary

1. The leaf size–twig size spectrum is an important spectrum of variation between species, although the costs and benefits of larger *vs* smaller leaf size are poorly understood. This study quantified the dry mass costs of deploying leaf area in relation to leaf size, across 70 species from four sites contrasted on the basis of rainfall and soil nutrients in east-temperate Australia.

2. Leaf mass fraction beyond 10 mm<sup>2</sup> of conductive cross-section (LMF<sub>10</sub>) varied threefold and was strongly positively correlated with leaf size, both across all species and within each habitat. This and other key correlations were significant both across species and as evolutionary divergences.

3. An LMF<sub>10</sub> advantage for larger-leaved species should translate into a proportional advantage in dry mass acquisition, yet species with small leaves persist and sustain populations. This raises the question as to what factors might counterbalance an LMF advantage associated with larger leaf size.

4. Within some sites, specific leaf area (SLA) decreased with leaf size, which counterbalanced the LMF<sub>10</sub> advantage. Within other sites, and across all sites, SLA did not decline with leaf size. In these comparisons other factors, such as avoidance of overheating, must counterbalance the LMF<sub>10</sub> advantage to larger-leaved species.

5. Wood density was negatively correlated with leaf size. However, the relationship was weaker within sites in the lower rainfall zone, and species in these sites had generally higher wood density. Sapwood cross-sectional area per unit leaf area (Huber value) varied approximately ninefold across all species, and was correlated with both leaf size and SLA across all species, but not within sites.

*Key-words:* leaf area ratio, leaf mass fraction, leaf size, plant architecture, wood density

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## Introduction

A given total leaf area can be made up of many small or few large leaves. The size of individual leaves is correlated across species with twig cross-sectional area (Corner 1949). Thus there exists a leaf size–twig size spectrum (Westoby *et al.* 2002) that extends from species with small leaves and twigs and close ramification, to species with large leaves and twigs and less frequent branching. In addition to the large spread along the leaf size–twig size spectrum among species coexisting within a habitat, there are well documented trends towards smaller leaves in drier and more nutrient-poor sites (Givnish 1987; Cunningham, Summerhayes & Westoby 1999). Despite these trends, an understanding of the costs and benefits of smaller *vs* larger leaf and twig size remains incomplete.

Allocation of biomass between leaves and stems has a direct influence on plant growth (Evans 1972; Givnish 1995; Reich *et al.* 1998). This paper reports a strong relationship across species between the leaf size–twig size spectrum and the fraction of the leaf-bearing shoot invested as leaf rather than twig. We studied 70 perennial species from four sites chosen to span a range of rainfall and soil nutrients. For this reason we were able to quantify dry mass allocation in leaf-bearing shoots, but not whole-plant growth or allocation.

Dry mass acquisition by a shoot can be broken down into components in a manner analogous to the classical partitioning of relative growth rate:

$$\begin{aligned} \text{Dry mass acquisition/shoot dry mass} &= \text{dry mass} \\ &\text{acquisition/shoot leaf area} \times \text{leaf dry mass}/ \\ &\text{shoot dry mass} \times \text{leaf area/leaf dry mass} \\ &= \text{NAR} \times \text{LMF}_s \times \text{SLA} \end{aligned} \quad \text{eqn 1}$$

Dry mass acquisition is interpreted as the net contribution from the shoot to its own dry mass gain plus any exports contributing to dry mass gain elsewhere in

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the plant. Thus, the first component on the right is the net assimilation rate (NAR) for leaves in the shoot. The second and third components on the right are the leaf mass fraction within the shoot (LMFs, as distinguished from LMF of the whole plant) and the specific leaf area (SLA). The second and third terms on the right can be measured and compared across species, as in the present study, even in the absence of data about the whole plant, or dry mass gain over time.

In equation 1 a doubling in any of the right-hand components would translate directly into doubling of the dry mass acquisition per shoot mass, unless there were correlated changes in other components of the equation. In principle, natural selection should favour architectural shoot designs that yield higher dry mass acquisition per mass of shoot, and thus have a greater return on the investment of biomass. In particular, in terms of growth, designs with higher LMF<sub>s</sub> (lower allocation to stems) should be favoured. However, there is counteracting selection towards higher allocation to stems for purposes of mechanical support, conductance of water to leaves, leaf spacing, and competitiveness through height gain (Evans 1972; Givnish 1995).

Allocation to stems is affected not only by stem diameter and the length of stem between leaves, but also through wood density. Higher wood density represents a higher construction cost for the plant (Loehle & Namkoong 1987; Enquist *et al.* 1999; Hacke, Sperry & Pitterman 2000; Hacke & Sperry 2001), but is associated with greater resistance to drought-induced xylem cavitation (Pockman & Sperry 2000; Hacke *et al.* 2001).

The main purpose of this paper is to report a strong relationship across species between the leaf size–twig size spectrum and LMF within shoots. A secondary aim is to dissect the involvement of SLA and wood density in the deployment of leaf area from a given total shoot mass. We also consider the Huber value, which is the ratio of sapwood cross-sectional area to leaf area. We examine shifts in these variables and their relationship with leaf size across rainfall or soil nutrient contrasts.

Relationships were investigated by cross-species regression and also, for the focal cross-species relationships, as correlations of evolutionary divergences (also known as phylogenetically independent contrasts).

## Materials and methods

### STUDY SITES AND SPECIES

Four study sites were selected on the basis of contrasting rainfall and soil nutrient levels. The two high-rainfall sites were in Strickland State Forest, on the central coast of New South Wales, Australia (mean annual rainfall 1320 mm). One was on relatively high-nutrient and the other on low-nutrient soil. The two low-rainfall sites were in Round Hill Nature Reserve in western NSW, Australia (mean annual rainfall 390 mm). Again, one site was on relatively high-nutrient and one on relatively low-nutrient soils (further site details in Table 1).

In the high-rainfall zone, 20 species (four plants per species) were sampled from each site between July and October 2001. In the low-rainfall zone, 14 and 16 species (three plants per species) were sampled from the high- and low-nutrient sites, respectively, in November 2001. Variables were averaged across the three or four plants for each species. For the low-nutrient, low-rainfall site, two subsites several kilometres apart were sampled to ensure sufficient species could be included. The 70 species in this study were all dicots, spanned four orders of magnitude in leaf size, represented 24 families, and included life forms ranging from trees to subshrubs. All species included in this study were evergreen species (Wright *et al.* 2001), thus we would expect no change in leaf phenology over the 5 month sampling period. Within each site, although plants were sampled randomly, the species themselves were chosen to give a maximum spread both phylogenetically and along the leaf size–twig size spectrum. One consequence of this is that they do not represent random samples from each site. Therefore, while interpreting

**Table 1.** Site descriptions and characteristics of the four study sites contrasted on the basis of rainfall and soil nutrient status

	High rainfall*		Low rainfall†	
	High nutrient	Low nutrient	High nutrient	Low nutrient
Latitude (S)	33°22'53"	33°22'26"	32°58'00"	32°58'35"
Longitude (E)	151°19'21"	151°19'12"	146°09'17"	146°08'45"
Vegetation type	Closed forest	Open woodland	Open woodland	Open shrub mallee
Annual rainfall (mm)	1300	1300	390	390
Mean annual temp (°C, max, min)	22.9, 11.0	22.9, 11.0	24.1, 11.1	24.1, 11.1
Mean relative humidity (%; 09:00 h)	73	73	49	49
Total P (µg g <sup>-1</sup> )	335 (47)	98 (6)	250 (33)	132 (14)
Total N (%)	0.370 (0.06)	0.057 (0.008)	0.071 (0.02)	0.031 (0.006)
Total C (%)	5.57 (0.99)	2.15 (0.70)	1.20 (0.36)	0.67 (0.15)

High-rainfall sites were located in Strickland State Forest (Central Coast NSW); low-rainfall sites in Round Hill Nature Reserve (Western NSW). For soil analysis, SD in parentheses after the mean.

\*Chemical analysis completed by State Chemistry Laboratory, Melbourne, Australia.

†Data from Wright *et al.* (2001).

the data we do not emphasize formal significance tests of differences between sites. However, at both low-rainfall sites all available species were sampled, which resulted in a more representative sample of the total species at the site. The full species list and raw data are available on request.

#### PRELIMINARY STUDY: CONSISTENCY OF RELATIONSHIPS ALONG THE BRANCHING SEQUENCE

We initially sampled the 20 species at the low-nutrient, high-rainfall site to test whether the relationships between leaf size (measured as individual leaf area), LMF and leaf area ratio (LAR) were consistent along the branching sequence for each species.

An actively growing branching sequence from the upper canopy of each plant was cut at 80, 160, 320, 640 and 1280 mm along the sequence. At each cut, the long ( $d_1$ ) and short ( $d_2$ ) diameters of the aggregate stem (whole stem including bark) were measured. All biomass above the cut, including side branches, was transferred to a paper bag. Cross-sectional area of the aggregate stem at each distance was calculated as  $\text{area} = (\pi \times d_1 \times d_2)/4$ .

While initial data were collected in terms of aggregate (or whole) stem cross-sectional areas, we subsequently decided to use the conductive tissue cross-section (excluding bark and pith) as a reference diameter. To determine the cross-sectional area of the aggregate (or whole) stem where the cross-section of the conductive tissue was 5, 10, 15, 20 and 30 mm<sup>2</sup> for each species, a ratio of the aggregate stem cross-sectional area to the conductive stem cross-sectional area was measured as follows for each species in the low-nutrient, high-rainfall site. For four individuals per species, two sections of stem 20–30 mm in length, one 3–5 mm and the second 6–8 mm in diameter, were randomly sampled from an actively growing shoot in the upper canopy. Long and short diameters were used to calculate the aggregate cross-sectional area. The stem was then dissected along the vascular cambium and the cross-sectional area of the water-conductive tissue was similarly calculated. Water-conductive tissue was identified as the portion of the stem remaining after dissection along the vascular cambium and removal of the bark (phloem, cortex and epidermis). Examining stained cross-sections of the stem under a light microscope substantiated identification of water-conductive tissue. (Hereafter, the term conductive tissue refers to the water-conductive tissue.) This resulted in two ratios of aggregate to conductive stem cross-sectional area, one at  $\approx 10$  mm<sup>2</sup> and the second at  $\approx 25$  mm<sup>2</sup>. This was done to account for changes in the proportion of conductive and non-conductive tissue along the stem. The first ratio was used to calculate variables at 5, 10 and 15 mm<sup>2</sup> of conductive tissue, and the second ratio was applied at 20 and 30 mm<sup>2</sup>. Average aggregate cross-sectional area of the stem where the conductive tissue was 10 mm<sup>2</sup> ranged from 11.3 mm<sup>2</sup>

in *Epacris pulchella*, which had the thinnest bark, to 18.9 mm<sup>2</sup> in *Angophora costata*, which had the thickest.

Biomass samples were oven dried at 60 °C for 4–5 days. Fruit and flowers were absent in the majority of species. For the few species in which fruit and flowers were present, they were removed and discarded to allow comparison of only the leaf and wood material across all species. Leaves and wood were manually separated and weighed. Dry masses from the successive lengths of the branch were added to give cumulative quantities distal to each diameter, and the following variables were calculated:

- (i) LMF: leaf mass as a proportion of total mass (leaf plus stem)
- (ii) LAR: leaf area per mg dry mass of total mass (leaf plus stem). To estimate total leaf areas, the mass of leaf was multiplied by the average SLA (see below) for the species.

LMF and LAR values at 5, 10, 15, 20 and 30 mm<sup>2</sup> of conductive tissue were estimated by straight-line interpolation between values calculated at points along the branching sequence. For some species the smallest cross-sectional areas were out of the normal size range for that species, while for others the largest cross-sectional areas were not reached within the branching sequence up to 1280 mm. Hence not all species were included at each end of the range of cross-sectional areas.

As relationships between LMF and leaf size were consistent across cross-sectional areas of conductive tissue from 5 to 30 mm<sup>2</sup> (see Results), species at the other three sites were not sampled at such a wide range of stem cross-sections, but at two cross-sections on the stem, chosen to permit interpolation to 10 mm<sup>2</sup> conductive tissue.

#### SAMPLING DRY MASS COSTS AT 10 mm<sup>2</sup> CROSS-SECTIONAL AREA OF CONDUCTIVE TISSUE

At the remaining three sites, a randomly selected actively growing shoot from the upper canopy of each individual was cut at stem diameters of 2–3 mm (3.1–7.1 mm<sup>2</sup> cross-sectional area) and 4–6 mm (12.6–28.3 mm<sup>2</sup> cross-sectional area). In each case the exact long and short diameters and length from the tip to the cut were measured. All leaf and twig material above the cut was transferred to a paper bag, and materials were processed as described above. Similarly to species from the low-nutrient, high-rainfall site, a ratio of the aggregate cross-sectional area to the conductive cross-sectional area was measured to enable calculation of dry mass costs at 10 mm<sup>2</sup> cross-sectional area of conductive tissue. For four individuals per species, a section of stem 20–30 mm in length and 4–6 mm in diameter was randomly sampled from the upper canopy. The ratios of aggregate cross-sectional area of the stem to cross-sectional area of conductive tissue were measured. Values

of LMF and LAR at 10 mm<sup>2</sup> cross-sectional area of conductive tissue were then estimated by interpolation between the two values at the sampled cross-sections.

#### LEAF SIZE (CALCULATED AS INDIVIDUAL LEAF AREA) AND SLA

For the 40 species from the two high-rainfall sites, four sun leaves were collected from each plant (16 leaves per species) to calculate individual leaf area and SLA. Fresh leaves were scanned on a flat-bed scanner and leaf area was measured using DELTA-T SCAN image analysis software (Delta-T, Cambridge, UK). Leaves were dried at 60 °C for 48 h and their mass recorded. For the 30 species from the low-rainfall sites, SLA values were taken as a species average from a previous study that had sampled four leaves per plant and five plants per species (M.P., unpublished data).

#### WOOD DENSITY

The relationship between wood density and leaf size was investigated using four individual plants from each of the 70 species. On a randomly chosen branch from the upper canopy, wood samples 50–70 mm in length were taken firstly where the aggregate stem diameter was 2–3 mm (3.1–7.1 mm<sup>2</sup> cross-sectional area) and secondly at 4–6 mm (12.6–28.3 mm<sup>2</sup> cross-sectional area). Fresh samples were stored on ice and refrigerated until processing. Bark was removed, and wood density (dry mass/volume) at 10 mm<sup>2</sup> conductive tissue estimated by interpolation between the wood density values calculated at the sampled cross-sections. Volume of the wood sample was determined by Archimedes' principle as in Hacke *et al.* (2000a). Using a thin wire, the wood sample was submerged in a water-filled container placed on a balance. The balance and container were sealed to prevent airflow affecting the balance readings, except for a small opening through which the wood sample was lowered into the water. Displacement weight was converted to sample volume by the formula: displacement weight (g)/0.998 (g cm<sup>-3</sup>), where 0.998 g cm<sup>-3</sup> is the density of water at 20 °C. Wood sections were then oven-dried for 4 days at 60 °C before weighing.

#### STATISTICAL ANALYSIS

To examine the relationship between leaf size and LMF, SLA, LAR and wood density, cross-species and phylogenetic analyses were undertaken at two levels. First, for both types of analyses, all species were considered. Second, species within each site were considered separately. The remaining relationships were examined by cross-species analysis for all species, and within each site.

Variance components analysis (Minimum Norm Quadratic Unbiased Estimator, MINQUE; spss for Windows ver. 10) showed that most of the variance lay between, rather than within, species. For all variables

but one, the proportion of variance explained by species ranged from 73 to 93%. The exception was total leaf area in the low-nutrient, high-rainfall site (56%).

All variables except individual leaf area were normally distributed (Kolmogorov–Smirnov test,  $\alpha = 0.05$ ). Individual leaf area was deemed normally distributed after log transformation. LMF data were logit transformed [ $\ln(\text{LMF}/1 - \text{LMF})$ ] for analysis. However, logit transformation of the data did not change the results of the analysis, and thus for clarity untransformed data are presented.

A phylogenetic tree was constructed for the 70 species with ordinal groupings based on Bremer *et al.* (1998); relationships within the Myrtales and Myrtaceae from Johnson & Briggs (1984); and relationships within the Proteaceae from Orchard (1995) and Hoot & Douglas (1998). Whereas for cross-species regressions each data point is a species mean, in correlated divergence analyses each independent divergence contributes a single item of evidence. A data set of divergences was created (Harvey & Pagel 1991), where the divergence was the difference between the values of the trait for the two species or nodes descending from the divergence node. Node values were calculated by averaging the trait values for the two immediately lower species or nodes. In calculating divergences, the direction of subtraction was irrelevant on the condition that the treatment of all traits was consistent. Therefore, in the graphs of divergences in one trait plotted against divergence in another trait, a positive divergence in both traits would be represented as negative divergences if the subtractions were performed the other way around. In addition,  $r^2$  and  $P$  values calculated by linear regression contain no intercept term.

In this study, cross-species analysis was completed on untransformed variables while phylogenetic analyses were done on log-transformed data. Log-transformed data were used for phylogenetic analyses so that the resulting divergences would measure the ratio or multiplication factor across the evolutionary divergence for variables such as LMF. This is an appropriate transformation for these variables, as in accordance with equation 1 a doubling of LMF should result in a doubling of dry mass acquisition per shoot mass unless there are counterbalancing factors.

## Results

#### PRELIMINARY: CONSISTENCY ACROSS A RANGE OF STEM DIAMETERS

At the site where measurements were made along a range of stem diameters, the positive relationship between LMF and leaf size was consistent across all cross-sectional areas of conductive tissue from 5 to 30 mm<sup>2</sup> (Table 2), with  $r^2$  values ranging from 0.49 at 20 mm<sup>2</sup> to 0.63 at 10 mm<sup>2</sup>. The relationship between LAR and leaf size was, to some extent, weaker. It also did not change consistently with increasing cross-

**Table 2.** Summary of correlations between leaf size and leaf mass fraction (LMF) and leaf area ratio (LAR) at a number of cross-sectional areas along the branch sequence for the 20 species from the low-nutrient, higher-rainfall site

Cross-sectional area † (mm <sup>2</sup> )	N	LMF		LAR	
		r <sup>2</sup>	P	r <sup>2</sup>	P
5	19	0.58	<0.0001***	0.16	0.092*
10	20	0.63	<0.0001***	0.14	0.110 <sup>NS</sup>
15	20	0.52	<0.0001***	0.14	0.109 <sup>NS</sup>
20	19	0.49	0.001***	0.28	0.028**
30	14	0.54	0.002***	0.21	0.083*

\*\*\* $P < 0.01$ ; \*\* $P < 0.05$ ; \* $P < 0.1$ ; <sup>NS</sup>Non-significant.

†Cross-sectional area of conductive tissue.

sectional area, and  $r^2$  values were fairly similar, ranging from 0.14 at 10 mm<sup>2</sup> to 0.28 at 20 mm<sup>2</sup>. In summary, the main relationships of interest were consistent across the range of sapwood cross-sections from 5 to 30 mm<sup>2</sup>. On this basis, results were expressed at a reference sapwood cross-section of 10 mm<sup>2</sup>, and species at the remaining three sites were subsequently sampled at two diameters chosen to permit interpolation to 10 mm<sup>2</sup> conductive tissue.

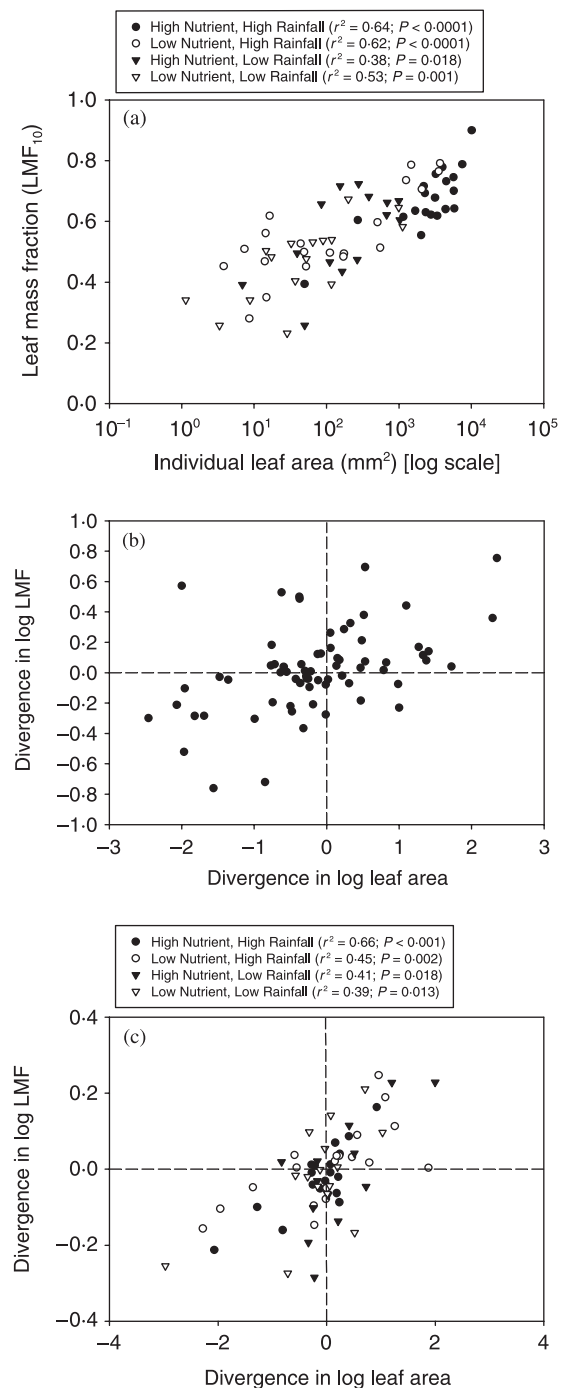
#### RELATIONSHIPS BETWEEN DRY MASS COSTS AND LEAF SIZE (EXPRESSED AS INDIVIDUAL LEAF AREA)

##### LMF beyond 10 mm<sup>2</sup> conductive cross-section (LMF<sub>10</sub>)

LMF<sub>10</sub> was strongly related to leaf size both across the 70 species in the study ( $r^2 = 0.64$ ,  $P < 0.0001$ ; Fig. 1a) and across evolutionary divergences ( $r^2 = 0.21$ ,  $P < 0.0001$ ; Fig. 1b). LMF<sub>10</sub> varied fourfold across all species, ranging from 0.23 to 0.90. The cross-species relationship between leaf size and LMF<sub>10</sub> was also apparent within each site, with the strongest correlation in the high-nutrient, high-rainfall site ( $r^2 = 0.64$ ,  $P < 0.0001$ ; Fig. 1a) and the weakest correlation in the high-nutrient, low-rainfall site ( $r^2 = 0.38$ ,  $P = 0.018$ ). Within-site evolutionary divergence correlations mirrored patterns in the cross-species analyses, with correlations ranging from  $r^2 = 0.39$  ( $P = 0.013$ ) in the low-nutrient, low-rainfall site to  $r^2 = 0.66$  ( $P < 0.0001$ ) in the high-nutrient, high-rainfall site (Fig. 1c).

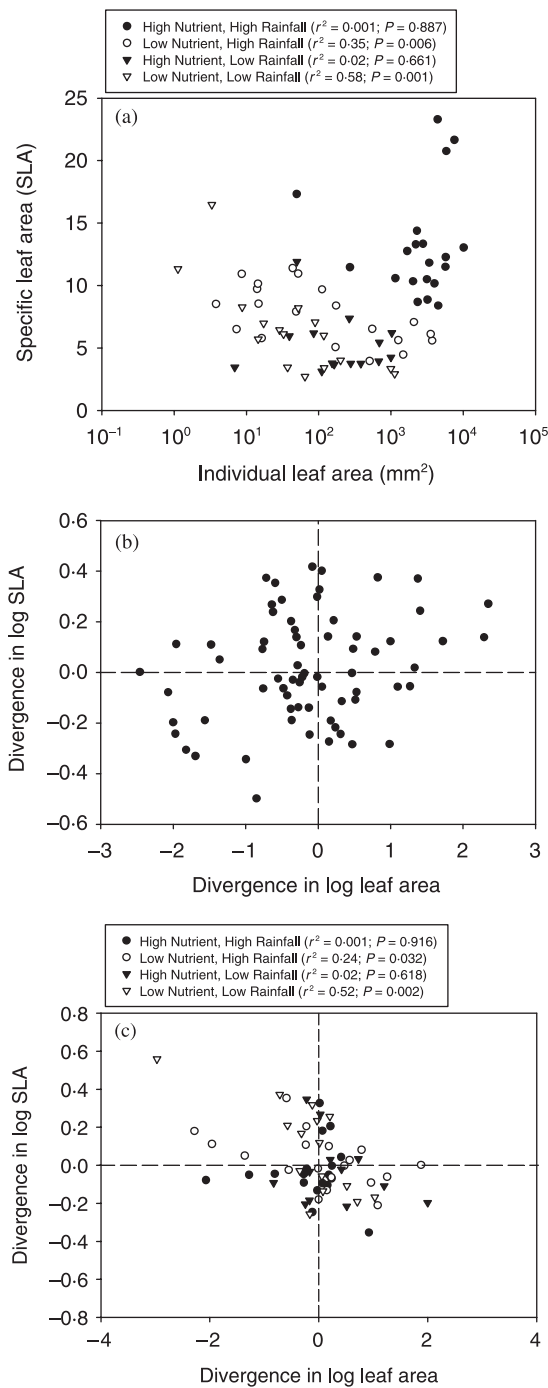
##### Specific leaf area

Across all species, SLA varied tenfold, ranging from 2.7 to 23.3 mm<sup>2</sup> mg<sup>-1</sup>, and was weakly positively correlated with leaf size in both cross-species ( $r^2 = 0.06$ ,  $P = 0.05$ ; Fig. 2a) and phylogenetic analyses ( $r^2 = 0.07$ ,  $P = 0.03$ ; Fig. 2b). The positive correlation across all species was driven primarily by differences between sites, especially the high SLA values of species at the high-nutrient,



**Fig. 1.** Cross-species and correlated divergence regressions between leaf mass fraction and leaf size. (a) Cross-species regression for all species and within each site; (b) correlated divergence regression for all species; (c) within-site correlated divergence regressions.

high-rainfall site (Fig. 2a). Within each of the low-nutrient sites there were strong negative relationships between leaf size and SLA ( $r^2 = 0.35$ ,  $P = 0.006$  at high rainfall;  $r^2 = 0.58$ ,  $P = 0.001$  at low rainfall), whereas within the high-nutrient sites there were no relationships ( $P > 0.05$  in both rainfall zones). Similarly, for the within-site correlated divergence analyses, leaf size was negatively correlated with SLA in the low-nutrient sites at high and low rainfall ( $r^2 = 0.24$ ,  $P = 0.032$ ;  $r^2 = 0.52$ ,

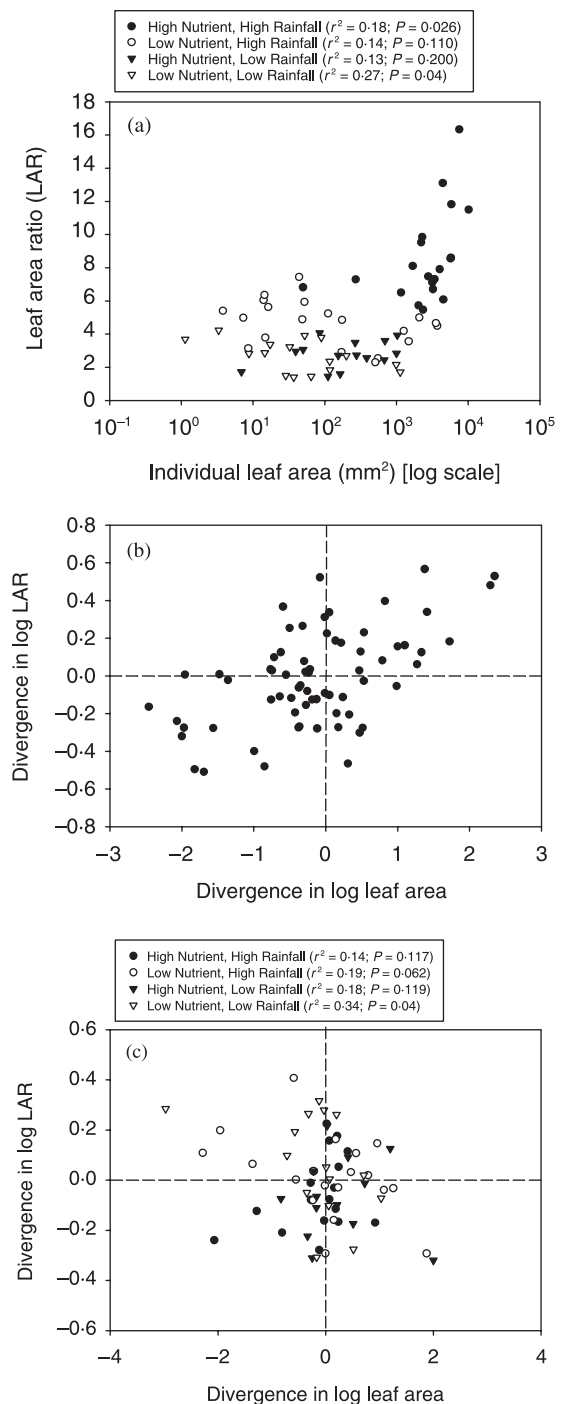


**Fig. 2.** Cross-species and correlated divergence regressions between specific leaf area and leaf size. (a) Cross-species regression for all species and within each site; (b) correlated divergence regression for all species; (c) within-site correlated divergence regressions.

$P = 0.002$ , respectively; Fig. 2c), but not in either of the high-nutrient sites ( $P > 0.05$ ).

#### *LAR beyond 10 mm<sup>2</sup> conductive cross-section ( $LAR_{10}$ )*

LAR (leaf area per shoot mass) is the product of LMF and SLA. As described above, LMF consistently increased with leaf size; SLA decreased with leaf size within the low-nutrient sites, was unrelated within the



**Fig. 3.** Cross-species and correlated divergence regressions between leaf area ratio and leaf size. (a) Cross-species regression for all species and within each site; (b) correlated divergence regression for all species; (c) within-site correlated divergence regressions.

high-nutrient sites, and increased weakly with leaf size between sites. Considering first the low-nutrient sites, the decrease in SLA counteracted the effect of the LMF increase with leaf size, with the result that  $LAR_{10}$  was uncorrelated with leaf size (at the high-rainfall site ( $P > 0.05$  both across species and as evolutionary divergences) and negatively correlated at the low rainfall site ( $r^2 = 0.27$ ,  $P = 0.04$  across species;  $r^2 = 0.34$ ,  $P = 0.04$  as evolutionary divergences) (Fig. 3a,c). At

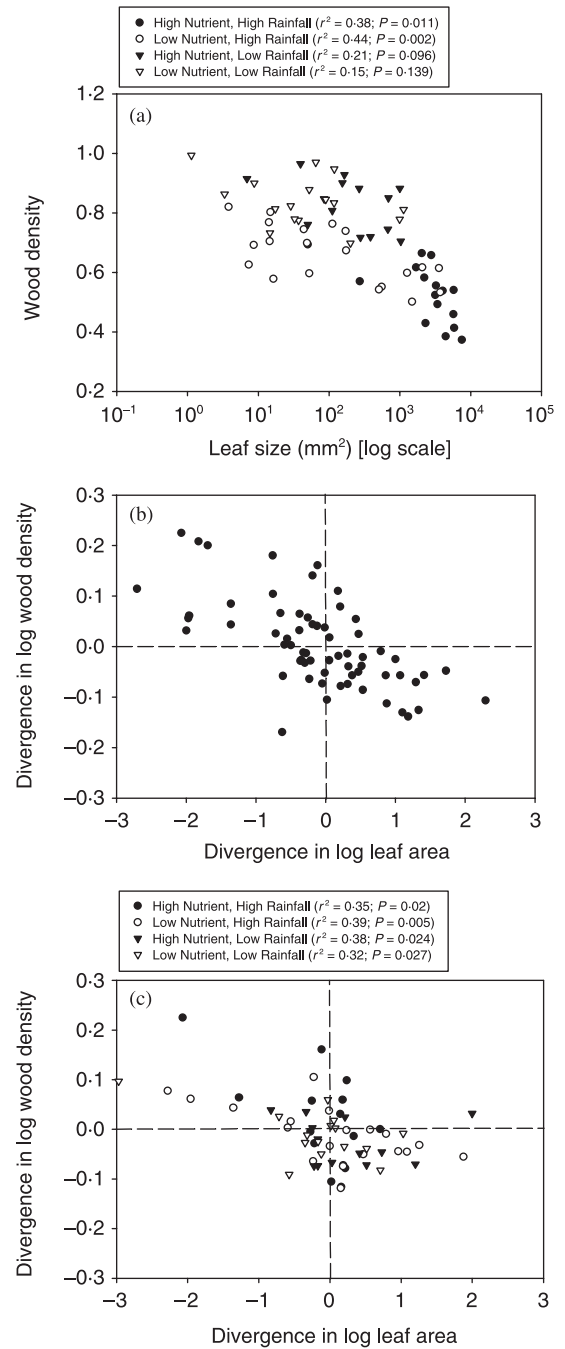
the high-nutrient sites SLA did not counteract the effect of LMF increasing with leaf size in the same way. At the high-nutrient, high-rainfall site LAR was weakly positively correlated with leaf size across species ( $r^2 = 0.18$ ,  $P = 0.026$ ), and uncorrelated as evolutionary divergences ( $r^2 = 0.14$ ,  $P = 0.12$ ). At the high-nutrient, low-rainfall site LAR and leaf size were uncorrelated ( $P > 0.05$  both across species and as evolutionary divergences). Over all sites, LAR was positively associated with leaf size cross-species ( $r^2 = 0.26$ ,  $P < 0.0001$ ; Fig. 3a), and as evolutionary divergences ( $r^2 = 0.30$ ,  $P < 0.0001$ ; Fig. 3b). This overall effect was driven mainly by species at the high-nutrient, high-rainfall site, which had larger leaves and higher LAR than at the other three sites (Fig. 3a).

The LAR was distinctly lower for most species at low-rainfall than at high-rainfall sites, across the range of leaf sizes (Fig. 3a). This was partly because of lower SLA (Fig. 2a), and partly because of lower LMF<sub>10</sub>, especially among the smaller leaf sizes (Fig. 1a).

#### Wood density

Across all sites, wood density ranged from 0.99 g cm<sup>-3</sup> at 1.13 mm<sup>2</sup> leaf size to 0.37 g cm<sup>-3</sup> at 7542 mm<sup>2</sup>. It was negatively correlated with leaf size both across species ( $r^2 = 0.44$ ,  $P < 0.0001$ ; Fig. 4a) and across evolutionary divergences ( $r^2 = 0.42$ ,  $P < 0.0001$ ; Fig. 4b). In particular, species with leaf sizes above 1000 mm<sup>2</sup>, all of which were from high-rainfall sites, had wood density below 0.7 g cm<sup>-3</sup> and ranged as low as 0.4 g cm<sup>-3</sup>. For within-site cross-species and phylogenetic analyses, there was a strong negative relationship between wood density and leaf size at high-rainfall sites (high-nutrient site: cross-species  $r^2 = 0.38$ ,  $P = 0.011$ , evolutionary divergences  $r^2 = 0.35$ ,  $P = 0.02$ ; low-nutrient site: cross-species  $r^2 = 0.44$ ,  $P = 0.002$ , evolutionary divergences  $r^2 = 0.39$ ,  $P = 0.005$ ; Fig. 4a,c). For cross-species analysis in the low rainfall zone, the relationship was not significant but still suggested the same trend at both high-nutrient ( $r^2 = 0.21$ ,  $P = 0.096$ ) and low-nutrient sites ( $r^2 = 0.15$ ,  $P = 0.139$ ). For both sites within the low-rainfall zone, the within-site evolutionary divergence correlation was stronger than the within-site cross-species correlation (high-nutrient site  $r^2 = 0.38$ ,  $P = 0.024$ ; low-nutrient site  $r^2 = 0.32$ ,  $P = 0.027$ ; Fig. 4c).

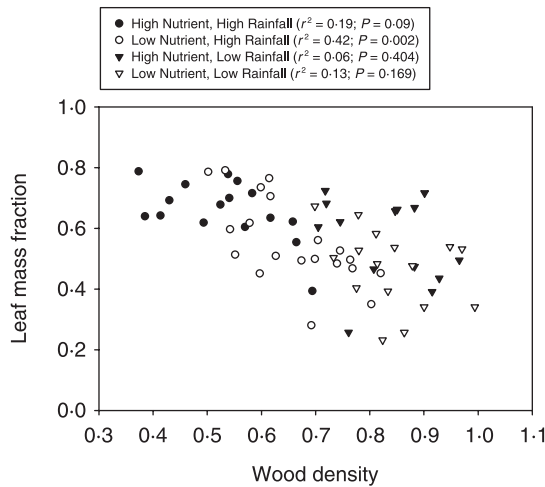
Across all species, LMF was strongly negatively correlated with wood density ( $r^2 = 0.59$ ,  $P < 0.0001$ ; Fig. 5). Similar to the leaf size–wood density relationship, the pattern across all species was mainly due to differences between sites, especially species from the high-nutrient, high-rainfall site which tended to have high LMF and low wood density. Within sites, LMF and wood density were somewhat related at high rainfall (low nutrient  $r^2 = 0.42$ ,  $P = 0.002$ ; high nutrient  $r^2 = 0.19$ ,  $P = 0.09$ ), but unrelated at low rainfall (high nutrient  $r^2 = 0.06$ ,  $P = 0.404$ ; low nutrient  $r^2 = 0.13$ ,  $P = 0.169$ ).



**Fig. 4.** Cross-species and correlated divergence regressions between wood density and leaf size; (a) cross-species regression for all species and within each site; (b) correlated divergence regression for all species; (c) within-site correlated divergence regressions.

#### Huber value at 10 mm<sup>2</sup> cross-section

The Huber value, conductive cross-sectional area per unit leaf area supported, varied approximately nine-fold from  $1.1 \times 10^{-4}$  to  $9.8 \times 10^{-4}$  across all species. The Huber value was not correlated with leaf size across all species ( $P > 0.05$ ; Fig. 6a). There was a negative relationship between Huber value and SLA across all species ( $r^2 = 0.19$ ,  $P < 0.001$ ), but no relationship between Huber value and SLA within sites ( $P > 0.05$ ; Fig. 6b),



**Fig. 5.** Relationship between leaf mass fraction and wood density both within and between sites.

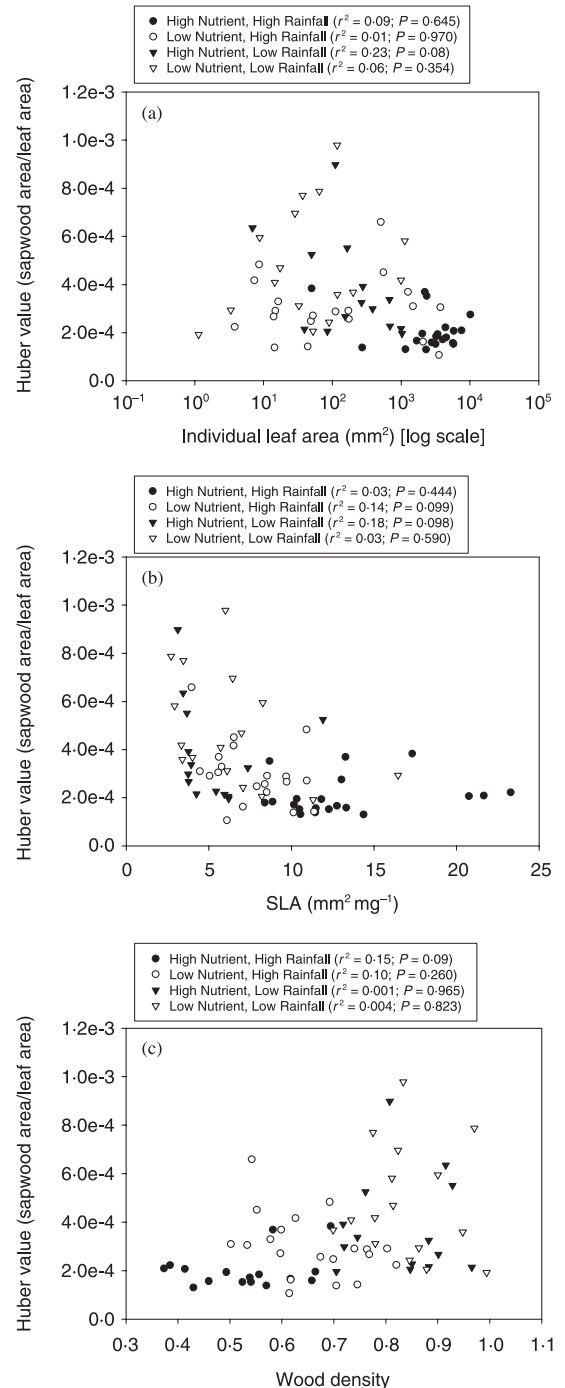
although no species exhibited both high SLA and high Huber values. Huber value was correlated with wood density across all species ( $r^2 = 0.13$ ,  $P = 0.003$ ), but not within sites ( $P > 0.05$ ; Fig. 6c). There was an absence of species with low wood density and high Huber values (Fig. 6c), and a distinct separation of sites along the wood density dimension. The separation of sites along the wood density and Huber value dimensions was more apparent in relation to rainfall than in relation to soil nutrients.

## Discussion

In this study the main relationships between leaf size and other traits were similar whether measured as correlations across present-day species or as correlations of evolutionary divergences. This is a common outcome for traits of ecological importance (Ackerly 1999). The cross-species correlation indicates combinations of traits that are successful in maintaining populations in the present day. Concordance with evolutionary divergence correlation indicates that through evolutionary time, clades have diverged along similar correlation patterns, probably for similar reasons of ecological competence (Westoby 1999).

The most striking and potentially important pattern found in this work was the strong relationship between leaf size and LMF within the shoot ( $LMF_s$ ). In accordance with the relationships in equation 1, the  $LMF_s$  advantage of larger over smaller leaves, which was up to threefold (Fig. 1a), should translate directly into a proportional dry mass acquisition advantage, unless it is counteracted by other factors.

A correlation between leaf size and  $LMF_s$  does not prove a functional relationship. Nevertheless, the growth benefits of high  $LMF_s$  suggest that, if possible, it would be advantageous for small-leaved species to evolve towards the higher  $LMF_s$  achieved by larger-leaved species. However, a wide range of leaf sizes persists in plant communities. This strongly suggests there are counter-



**Fig. 6.** Cross-species relationship between Huber value (sapwood area/leaf area) and leaf and wood attributes: (a) leaf size; (b) specific leaf area (SLA); (c) wood density.

balancing factors favouring smaller leaves. What disadvantages of large leaves might counterbalance their  $LMF_s$  advantage, and lead to the range of leaf sizes actually observed? One possible counterbalancing factor is NAR (equation 1). Although NAR was not measured in this study, there is no obvious reason why smaller leaves should systematically achieve higher NAR.

Alternatively, a decrease in SLA with leaf size did counteract the increase in  $LMF_s$  in comparisons within the two low-nutrient sites, and thus there was no relationship between leaf size and LAR in these



sites (Fig. 3a). Conversely, larger-leaved species still had an  $LAR_s$  advantage within the high-rainfall, high-nutrient site, and between that site and others.

Grubb (1995) and Shipley (1995) both found that among species within the same habitat and with similar ecology, larger-leaved species had lower SLA. A need for greater mechanical support in larger leaves was suggested as an explanation for this pattern. Patterns within our low-nutrient sites were similar to this, and could be interpreted as larger-leaved species having less mechanical support in the twig (higher  $LMF_s$ ), but requiring more reinforcing structure in the leaf itself (which results in lower SLA).

On the other hand Niinemets (1996, 1998) and Niinemets & Kull (1994) found higher SLA in larger-leaved species, and thus a decline in SLA with leaf size is not found within all vegetation types. Across sites, SLA and leaf size both tend to decline towards lower rainfall and lower soil nutrients (Givnish 1987; Cunningham *et al.* 1999; Fonseca *et al.* 2000; Niinemets 2001; Ackerly *et al.* 2002). This was also found in the present study, and suggests that in data sets spanning a range of habitats, within- and between-habitat patterns may cancel, leaving little or no overall relationship between leaf size and SLA (Wilson, Thompson & Hodgson 1999; Fonseca *et al.* 2000; Ackerly *et al.* 2002).

Factors other than dry mass allocation can also favour smaller leaf sizes, and may counteract the growth advantage of higher  $LMF_s$  in particular comparisons. The best established counterbalance is that smaller leaves shed heat by convection more efficiently (Givnish 1987; Ackerly *et al.* 2002). This advantage should be more important in stronger radiation streams, and where water is in short supply (as transpiration is also effective in shedding heat). This presumably is a factor favouring smaller leaf sizes at low rainfall and in less shaded vegetation. A second factor could be that smaller-leaved species may suffer less herbivory during leaf expansion, as the duration of expansion is shorter (Moles & Westoby 2000).

As well as the dry mass of twigs we investigated wood density, a property that has important implications for both dry mass costs and hydraulic function of stems. Stems constructed from higher density wood represent a higher construction cost per unit volume for the plant, and may result in a reduced growth rate for the plant and lower storage capacity in stems (Givnish 1995; Enquist *et al.* 1999; Hacke & Sperry 2001; Hacke *et al.* 2001). However, the mechanical strength of the wood is directly proportional to its density (Niklas 1992; Hacke *et al.* 2001) and higher wood density is associated with greater resistance to drought-induced xylem cavitation (Pockman & Sperry 2000; Hacke & Sperry 2001). Hacke *et al.* (2000) recently explored the relationship between wood density and resistance to xylem cavitation. The xylem pressure required to induce 50% embolism was strongly correlated with wood density, and was related to minimum seasonal water potential, indicating a def-

inite trade-off between stem safety and construction cost. In the present study, wood density was negatively correlated with leaf size across all habitats. There was, however, a separation according to site, with species at the low-rainfall sites having, on average, higher wood densities, suggesting a greater need for resistance to embolism in species at these sites.

The amount of leaf area supported should scale with cross-sectional area of the stem for hydraulic and mechanical reasons (Shinozaki *et al.* 1964; Carlquist 1975; Niklas 1992; Mencuccini & Grace 1995). The simplest expectation for the relationship between leaf area and stem cross-sectional area is that across species in a given environment, a given cross-sectional area of conductive tissue supports approximately the same amount of leaf area, while differences in evaporative conditions modulate the conductive cross-section required per unit leaf area (the Huber value). This idea is based on the pipe-model theory presented by Shinozaki *et al.* (1964), in which they demonstrated a relationship between the weight of foliage and weight of non-photosynthetic tissue in the live canopy. In the present study Huber values ranged approximately ninefold across all sites, and were not related to leaf size. However, Huber value was negatively correlated with SLA. Across all species, Huber values were positively correlated with wood density, and there was a distinct separation of sites, with species from the low-rainfall zone having high wood density but a large spread of Huber values, while species from the high-rainfall zone had lower wood density and low Huber values only (Fig. 6c). Cavender-Bares & Holbrook (2001) found similarly that leaf area per shoot increased and Huber values decreased with increasing soil moisture of species habitats. The triangular relationship in Fig. 6(c) suggests that species with low wood density (higher conductance and less resistance to xylem cavitation) and with low amounts of leaf area per conductive cross-section are uncompetitive or inviable.

Quantification of the relationship between leaf size and the dry mass costs of deploying leaf area in the distal canopy of mature plants is important in relation to the growth economics of plants, and in understanding the relative costs and benefits of larger *vs* smaller leaves. The finding that  $LMF$  increased substantially with leaf size, both within and between habitats, may represent a significant growth advantage to plant architectures with larger leaves and twigs. In some comparisons within sites, the  $LMF$  advantage was counterbalanced by a decrease in SLA, leaving no overall relationship between leaf size and  $LAR$ . In other comparisons, however, there was an  $LAR$  advantage as well as an  $LMF$  advantage to larger leaf size. To improve understanding about the costs and benefits of larger *vs* smaller leaf size, we need to broaden our knowledge about the generality of the  $LMF$  advantage, to clarify the range of situations where it is offset by a decrease in SLA, and to identify counteracting factors in other situations.

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