

# Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species

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## Summary

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**Key words:** herbivory, leaf functional traits, leaf lifespan, leaf toughness, ontogenetic shifts, sapling shade tolerance, structural equation modeling, tropical trees.

- Leaf toughness is thought to enhance physical defense and leaf lifespan. Here, we evaluated the relative importance of tissue-level leaf traits vs lamina thickness, as well as their ontogenetic changes, for structure-level leaf toughness and regeneration ecology of 19 tropical tree species.
- We measured the fracture toughness of the lamina and veins of sapling leaves with shearing tests, and used principal component analysis and structural equation modeling to evaluate the multivariate relationships among traits that contribute to leaf toughness and their links to ecological performance traits.
- Tissue traits (density and fracture toughness of lamina and vein) were correlated positively with each other, but independent of lamina thickness. The tissue traits and lamina thickness contributed additively to the structure-level toughness (leaf mass per area and work-to-shear). Species with dense and tough leaves as saplings also had dense and tough leaves as seedlings and adults. The patterns of ontogenetic change in trait values differed between the seedling-to-sapling and sapling-to-adult transitions.
- The fracture toughness and tissue density of lamina and veins, but not the lamina thickness, were correlated positively with leaf lifespan and sapling survival, and negatively with herbivory rate and sapling regeneration light requirements, indicating the importance of tissue-level leaf traits.

## Introduction

Leaf toughness is a common and effective means of protecting leaves against a wide array of herbivores and physical disturbances, and thus is expected to enhance leaf lifespan (Edwards, 1989; Lucas *et al.*, 2000; Read & Stokes, 2006; Peeters, 2007). Global analyses have shown that coexisting species often vary substantially with respect to leaf lifespan and associated traits (Reich *et al.*, 1997; Westoby & Wright, 2006). Coley *et al.* (1985) hypothesized that leaves should be well defended when the economic cost of losing a leaf is expensive relative to potential photosynthetic income as a result of resource limitations. In support of this, species that specialize in shaded forest understory or nutrient-poor soils tend to have tough leaves with a long lifespan (Coley, 1983; Turner *et al.*, 1993; Read *et al.*, 2005; Fine *et al.*,

2006). Significant advances have been made during the last 10 yr in the development of material science perspectives of leaf mechanical defense (Aranwela *et al.*, 1999; Lucas *et al.*, 2000; Sanson *et al.*, 2001; Read & Sanson, 2003; Onoda *et al.*, 2008). In this article, we address a question prompted by these advances: how does the mechanistic basis for leaf toughness vary in relation to demography, life history and ecological niches among coexisting species?

Leaf toughness has been defined and measured differently by different researchers using shearing, punching and tearing tests (Read & Sanson, 2003). Fortunately, results are comparable when measurements are normalized properly (Aranwela *et al.*, 1999; Onoda *et al.*, 2008), either per unit fracture area (tissue-level measurement) or per unit fracture length (structure-level measurement that takes the size of the structure into account). For example, fracture toughness

(also known as specific work-to-shear,  $\text{J m}^{-2}$ ) is measured as the work per unit cross-sectional area when a lamina or vein is sheared with a blade (Lucas *et al.*, 2000; Read & Sanson, 2003). By contrast, lamina work-to-shear ( $W_s$ ,  $\text{J m}^{-1}$ ) represents the fracture resistance normalized per unit fracture length, and relates to lamina fracture toughness (LamFT,  $\text{J m}^{-2}$ ) and lamina thickness (LamT, m) as follows:

$$W_s = \text{LamFT} \times \text{LamT} \quad \text{Eqn 1}$$

Likewise, leaf mass per area (LMA,  $\text{g m}^{-2}$ ) is a product of the lamina density (LamD,  $\text{g m}^{-3}$ ) and LamT (m) as:

$$\text{LMA} = \text{LamD} \times \text{LamT} \quad \text{Eqn 2}$$

Many ecological studies have compared leaf mechanical strength at the structure-level only (e.g. Coley, 1983; Poorter, 2009). Decomposing structure-level traits,  $W_s$  and LMA, to tissue-level properties and LamT elucidates the nature of the physical defenses of leaves. For example, the high LMA of inherently slow-growing species from light- or nutrient-limited habitats is mainly attributable to high tissue density rather than the thickness of leaves (Shipley *et al.*, 2006; Poorter *et al.*, 2009). Among eight Neotropical tree species, seedling survival has been found to be correlated positively with high fracture toughness and tissue density, but not with LamT (Alvarez-Clare & Kitajima, 2007). This study also found that tough and dense lamina tissues were associated with tough and dense veins and stems, indicating overall concordance of multiple mechanical strength traits at the leaf and whole-plant levels. Decomposing structure-level leaf toughness to tissue-level toughness and LamT is important because a leaf may be thick solely as a result of thick mesophyll tissues (Onoda *et al.*, 2008), and such leaves may still be heavily attacked by herbivores and pathogens.

Our conceptual framework is summarized graphically in Fig. 1(a), which shows how among-species' variation in matter distribution at the structural level (LMA) can be partitioned to variations in LamD (tissue trait) and LamT (size trait), and how species' differences in  $W_s$  (structure-level leaf toughness) can be attributable to the variation in LamFT (tissue trait) and LamT (size trait). We hypothesize that tissue density, which largely reflects cell wall dry mass per unit volume space (Poorter *et al.*, 2009), should be correlated strongly with fracture toughness, based on the findings by Choong *et al.* (1992) and Alvarez-Clare & Kitajima (2007). Most importantly, we predict that these leaf toughness-related traits influence ecological performance traits (Fig. 1a). If so, they contribute to the functional basis for growth–survival trade-offs found among coexisting tree species (Kitajima, 1994; Kobe, 1999; Walters & Reich, 1999; Wright *et al.*, 2003; Gilbert *et al.*, 2006; Poorter & Bongers, 2006).

In this study we attempt to understand how these components of leaf mechanical strength vary among species in relation to ecological performance traits, including herbivore resistance, leaf lifespan, survival and growth rates of individuals, and regeneration light requirements. We quantified the morphological and biomechanical traits of sapling leaves for 19 species in a tropical moist forest in Bolivia to ask the following questions.

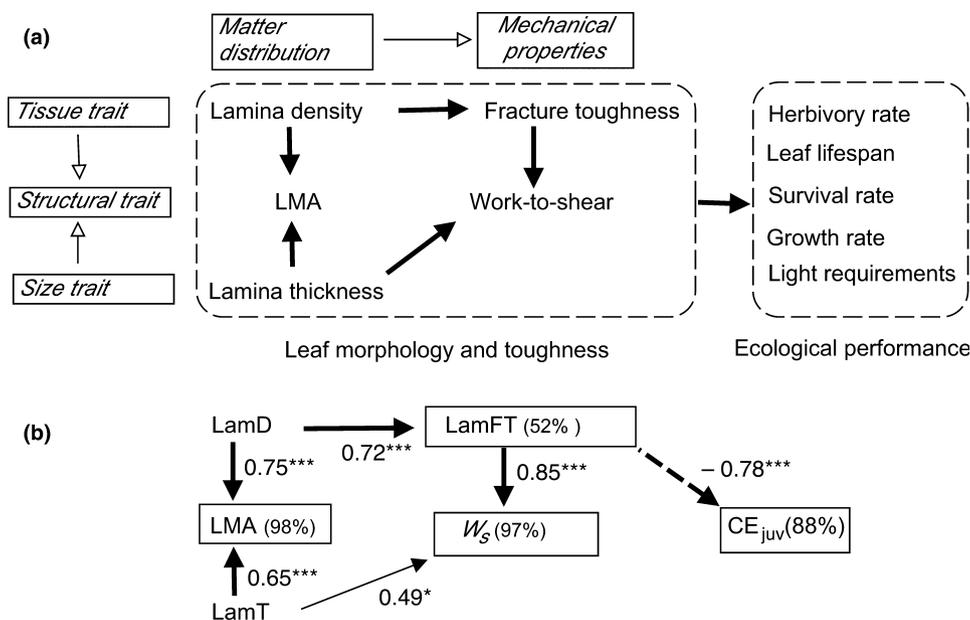
- What is the relative importance of tissue traits (density and fracture toughness) vs size traits (LamT and lamina area) in explaining among-species' variation in leaf mechanical traits at the structure-level (LMA and work-to-shear)?
- How do the morphological and biomechanical traits of juvenile leaves correlate with the ecological performance of leaves (palatability to a generalist herbivore, leaf lifespan) and whole plants (survival and growth rates), as well as the regeneration light requirements of the species?
- How are leaf biomechanical and morphological traits of saplings correlated with those of earlier and later ontogenetic stages?

We predicted that the interspecific variation in sapling leaf toughness at the structural level should reflect species' differences in tissue-level properties as well as LamT (Fig. 1a). We also predicted that shade-tolerant species with higher leaf and plant survival rates should exhibit greater leaf toughness at both the tissue and structure-levels. Following the findings of Alvarez-Clare & Kitajima (2007) for seedlings, fracture toughness and tissue density, rather than LamT, were expected to show positive correlations with leaf and sapling survival. We evaluated these multivariate causal hypotheses by structural equation modeling, which offers a powerful approach for the integration of functional traits and their ecological significance (Shipley, 2000). Leaf size is known to vary dramatically among ontogenetic stages, as well as among species (Ackerly & Donoghue, 1998; Westoby & Wright, 2006; Poorter & Rozendaal, 2008). Hence, our a priori prediction was that large leaves would require greater support at the structure-level by increasing LamT and/or increasing tissue-level toughness. To our knowledge, this is the first study to examine species' differences in mechanical and morphological leaf traits at multiple ontogenetic stages of tree species, linking them directly to their ecological performance.

## Materials and Methods

### Research site and species' selection

Field work was carried out in the lowland tropical moist semi-evergreen forest of La Chonta ( $15^{\circ}47'S$ ,  $62^{\circ}55'W$ ), Bolivia, a long-term research site of the Instituto Boliviano de Investigación Forestal, at an altitude between 300 and 500 m. Annual precipitation in La Chonta is 1580 mm, with a dry season (< 100 mm per month) from April until



**Fig. 1** (a) A path model proposed for the relationships among components of leaf lamina toughness that may influence the ecological performance of leaves (herbivory rate, leaf lifespan) and individuals (survival rates, growth rates, light requirements). Components of leaf toughness are classified to matter distribution and biomechanical properties, which can be measured at the tissue or structure-level. Three causal relationships are depicted in this path model, including the relationship within the tissue level (i.e. tissue density should affect fracture toughness), and how structure-level traits (LMA and  $W_s$ ) should reflect both tissue-level traits (density and fracture toughness) and LamT. (b) Evaluation of the above model with the data from sapling leaves of 19 Neotropical species in this study, using  $CE_{juv}$  as an ecological trait. Numbers by the arrows indicate the path coefficients ( $\beta$ , standardized partial regression coefficients) with their statistical significance ( $*P < 0.05$ ;  $**P < 0.005$ ;  $***P < 0.001$ , also indicated by arrow line thickness), with solid and broken arrows indicating positive and negative effects, respectively. The endogenous (dependent) variables are enclosed in rectangles with the proportion (%) of the total variance explained by the model shown in parentheses. The model fits the data ( $\chi^2 = 15.9$ ,  $df = 9$ ,  $P = 0.068$ ,  $AIC = 39.9$ ), whereas alternative models, including a direct link from LMA to  $W_s$  and/or LamFT, would be rejected ( $P < 0.05$ ). See Table 1 for variable abbreviations, and Fig. S3 for details of model evaluation using structural equation modeling.

October. The forest has an average canopy height of 27 m, stem density of  $367 \text{ ha}^{-1}$ , basal area of  $19.3 \text{ m}^2 \text{ ha}^{-1}$  and species' richness of 59 species  $\text{ha}^{-1}$  (all data for trees > 10 cm in diameter at breast height (DBH); Pena-Claros *et al.*, 2008).

### Study species

We selected 19 common tree species (Table 1), 16 of which reach DBH > 10 cm, comprising 45% of the tree stems at the site. The remaining three species were small subcanopy trees. These species differed in regeneration guilds and other ecological characteristics (Table 1), including regeneration light requirements, leaf lifespan, sapling growth and survival (Poorter & Bongers, 2006), wood density (van Gelder *et al.*, 2006) and maximum adult height (Poorter *et al.*, 2006). We also used an independent, objective and continuous index of the regeneration light requirements of the species (i.e. the inverse of shade tolerance) by analyzing the crown exposure (CE) in relation to the height of individual trees for each species (Poorter & Kitajima, 2007). To this end, on average, 523 individuals per species (median, 263; range, 41–9319) were sampled over their whole size range for the measure-

ment of height and CE (Dawkins & Field, 1978). CE is scored as follows: 1, if the tree does not receive any direct light; 2, if the tree receives lateral light; 3, if the tree receives overhead light on part of the crown (10–90% of the vertical projection of the crown exposed to full overhead light); 4, if the tree receives full overhead light (> 90% of the vertical projection of the crown exposed to vertical light); and 5, if the tree has an emergent crown that has no obstructing foliage over an upward facing cone encompassing the crown, with angles of  $45^\circ$  from the horizontal). For each species, CE was regressed against tree height using a multinomial regression analysis (cf. Poorter *et al.*, 2005; Sheil *et al.*, 2006), and the average population-level CE at a standardized height of 2 m (juvenile CE,  $CE_{juv}$ ) was calculated. Although similar-sized individuals of the same species can be found under a wide range of CEs, we were interested in estimating a population average  $CE_{juv}$  as an index of the regeneration light requirement of the species (Poorter *et al.*, 2005).

### Leaf sampling from saplings, adults and seedlings

For the determination of sapling leaf traits, we sampled typical mature leaves from saplings of each species in May

**Table 1** Study species, their ecological characteristics (means for:  $H_{\max}$ , maximum adult height; Guild, regeneration guild;  $CE_{\text{juv}}$ , average crown exposure of 2 m tall saplings) and key leaf traits of saplings quantified in this study (LamFT, lamina fracture toughness; LamT, lamina thickness; LMA, leaf mass per area measured with 12.9 mm diameter disks;  $W_s$ , work-to-shear of lamina)

Species	Family	$H_{\max}$ (m)	Guild	$CE_{\text{juv}}$	Leaf lifespan (months)	Lamina area (cm <sup>2</sup> )	LamT (mm)	LMA (g m <sup>-2</sup> )	LamFT (J m <sup>-2</sup> )	$W_s$ (mJ m <sup>-1</sup> )
<i>Erythrochiton fallax</i>	Rutaceae	5	ST	1.27	30.7	89	0.34	76.0	360	123
<i>Licaria triandra</i> *	Lauraceae	14	ST	1.35	19.7	63	0.25	77.0	476	118
<i>Casearia</i> sp.	Salicaceae	7	ST	1.39	18.1	33	0.23	74.1	320	73
<i>Pseudolmedia laevis</i> *	Moraceae	35	ST	1.32	15.7	58	0.21	54.2	307	63
<i>Picramnia sellowii</i>	Simaroubaceae	8	ST	1.34	14.8	69	0.25	61.8	297	75
<i>Ampelocera ruizii</i>	Ulmaceae	36	ST	1.35	11.5	49	0.18	49.4	373	66
<i>Stylogyne ambigua</i> *	Myrsinaceae	10	ST	1.46	10.5	85	0.34	71.3	237	81
<i>Pouteria nemorosa</i>	Sapotaceae	35	PST	1.57	8.9	61	0.18	62.7	295	55
<i>Batocarpus amazonicus</i> *	Moraceae	26	PST	1.35	8.4	106	0.23	51.3	263	58
<i>Swietenia macrophylla</i> *	Meliaceae	25	PST	1.62	8.1	105	0.17	52.3	237	40
<i>Gallesia integrifolia</i> *	Phytolaccaceae	42	LLP	1.84	7.7	63	0.24	45.6	203	49
<i>Centrolobium microchaete</i> *	Fabaceae	26	LLP	1.94	7.0	48	0.16	41.1	166	27
<i>Cariniana ianeirensis</i>	Lecythidaceae	44	PST	1.74	6.0	111	0.14	39.9	220	30
<i>Aspidosperma cylindrocarpon</i> *	Apocynaceae	28	PST	1.75	5.7	70	0.28	72.2	213	57
<i>Cecropia concolor</i>	Cecropiaceae	33	P	2.44	4.8	164	0.27	39.9	189	52
<i>Urea caracasana</i>	Urticaceae	13	P	1.99	4.0	437	0.28	29.2	80	22
<i>Jacaratia spinosa</i> *	Caricaceae	21	LLP	2.02	3.5	137	0.18	26.6	113	20
<i>Trema micrantha</i> *	Cannabaceae	31	P	2.52	3.4	30	0.25	44.7	179	44
<i>Heliocarpus americanus</i> *	Malvaceae	22	P	2.36	2.7	229	0.18	31.4	53	10

The species are listed in decreasing order of leaf lifespan. Asterisks indicate the 11 species for which leaves of greenhouse-grown seedlings were also sampled.

LLP, long-lived pioneers; P, pioneers; PST, partial shade tolerant; ST, shade tolerant.

2005, at the beginning of the dry season when the humidity and soil water availability were still high. At least four individuals per species were sampled for 17 species, whereas the sample size was three for two species, which is considered to be sufficient for leaf physical strength measurements (Cornelissen *et al.*, 2003). For each leaf, we measured the fresh mass and traced the leaf shape on a sheet of paper immediately following collection and removal of the petiole. The leaves were kept in a plastic bag with a small piece of moist filter paper until shearing tests. The shearing tests (see Biomechanical measurements section) were conducted within 4 h of collection. Adult leaves were sampled from trees with  $CE \geq 3$  and  $DBH \geq 10$  cm, choosing leaves from the outer leaf layer midway between the top and bottom of the crown (Rozendaal *et al.*, 2006). To determine lamina-specific LMA and LamD, two circular disks of 12.9 mm in diameter were collected with a cork borer from each leaf, excluding primary and secondary veins. LamT was measured with a micrometer (SM112; Teclock, Okaya, Japan), avoiding any visible veins. After measurements with fresh leaves, leaves and leaf disks were oven dried at 65°C for a minimum of 3 d to determine the dry mass. LamD was calculated by dividing LMA by LamT. LMA, LamD and the leaf dry matter content (LDMC = ratio of dry mass to fresh mass) were highly correlated between the whole blade and circular disk determinations (Pearson  $r = 0.99$ ,  $P < 0.001$  for all three variables; see Table 1 and Support-

ing Information Table S3 for species' means); thus, we report only the results using values calculated from the circular disks.

Leaves from a minimum of four seedlings were sampled for 11 species (Table 1). The seedlings were grown from seeds in a greenhouse in Santa Cruz, Bolivia (16°30'S, 68°10'W), in 200 ml plastic tubes filled with a 50 : 50 mix of sand and forest soil under 10% of daylight (see Poorter & Markesteijn (2008) for further details). Seedlings were *c.* 2 yr old at the time of this study. As LMA values of greenhouse-grown seedlings are a good approximation of the LMA values of field-sampled seedlings (Bloor, 2003), leaf traits of greenhouse seedlings were likely to approximate seedling leaf traits at the same ontogenetic stage under a similar light environment in the field.

### Biomechanical measurements

We measured the work-to-shear of leaves of seedlings and saplings using a portable universal tester to which a pair of scissors was attached (described in Darvell *et al.* (1996)), following the method described by Lucas *et al.* (2001). A small rectangular section, *c.* 8–9 mm wide and 15–18 mm long, was cut from the central portion of the leaf blade, so that the central vein bordered a long edge of the rectangle. The rectangular section was sheared with the scissors in a single path perpendicular to the central vein, avoiding sec-

ondary and tertiary veins in the lamina portion. The force–displacement curve (i.e. shearing force recorded with a load cell plotted against displacement distance) was distinctive for shearing the vein and lamina tissues, from which the total of shearing each tissue type was determined. The total of shearing the lamina was divided by the rectangular cross-sectional area (=fracture length  $\times$  LamT) to calculate LamFT. The total work to shear of the vein was divided by the vein cross-sectional area ( $=\pi r^2$ ) to calculate the vein fracture toughness. The lamina work-to-shear ( $W_s$ ) was calculated by multiplying LamFT by LamT. When the central vein in the leaf center was wider than 2.5 mm, a rectangular area further towards the tip (where the diameter was between 1.8 and 2.5 mm) was selected for the test. Because the vein was not cut at the same position for all species, we did not analyze the structure-level toughness of the veins (i.e. vein work-to-shear, which is dependent on the diameter). The vein used for the cutting test was separated from the lamina with a razor blade to determine the vein density (VeinD) after drying at 65°C.

For adult leaves of 18 species and sapling leaves of 13 species, we measured the force-to-punch ( $F_p$ ) using a penetrometer constructed with a punch rod with a diameter of 3 mm, mounted on a stationary base with a hole (Rozendaal *et al.*, 2006; Poorter, 2009; L Poorter, unpublished). The leaf was positioned to avoid primary and secondary veins, and the maximum force registered was divided by the rod circumference to normalize per fracture length and to determine  $F_p$ .  $F_p$  was subsequently divided by LamT to calculate the specific force-to-punch ( $F_{ps}$ ). Across species, the results of the shear test and the punch test were correlated linearly with each other (Supporting Information Fig. S1);  $F_{ps}$  was correlated with LamFT (tissue-level correlation,  $r = 0.65$ ,  $P = 0.013$ ,  $n = 18$ ), whereas  $F_p$  was correlated with  $W_s$  (structure-level correlation,  $r = 0.86$ ,  $P < 0.001$ ,  $n = 18$ ).

### Palatability assay

For 18 species, palatability to a generalist herbivore was measured as part of a larger experiment with 40 species. Fresh leaves were collected to test the palatability to a snail species in a cafeteria experiment (cf. Cornelissen *et al.*, 1999). A specialist at the Universidad Nacional de Tucumán, Argentina, was consulted for the taxonomic identification of this locally common snail species. Four square pieces of leaf ( $1 \times 1 \text{ cm}^2$ ) per species were placed at random positions in a plastic tray ( $42 \times 42 \text{ cm}^2$ ) to offer feeding choices to 15 snails per tray. The experiment was replicated three times. The percentage of lamina area remaining was quantified six times during a 5-d period, from which the loss rate per hour was calculated as the absolute value of the slope of  $\log_{10}(\% \text{ area remaining} + 1)$  plotted against time (0–96 h). To achieve normality of the distribution, the loss rate was

$\log_{10}$  transformed after adding a small number (0.000001, which is 1/100 of the lowest nonzero loss rate), and this value was used as the ‘palatability index’, which ranged from  $-7$  for species that were not consumed at all (slope = 0) to  $-0.83$  for *Urera caracasana* which was completely consumed within 72 h. The palatability index was correlated positively with the observed field herbivory rates (% lamina area loss per month) for 12 species whose sapling leaves were marked and monitored in the forest as part of a study with 30 species (L Poorter, unpublished;  $r = 0.58$ ,  $P < 0.05$ , Table S3). The two measures of herbivory exhibited similar trends in relation to leaf traits measured in the study. Thus, we report only the analyses using the palatability index determined for the larger number of species.

### Statistical analyses

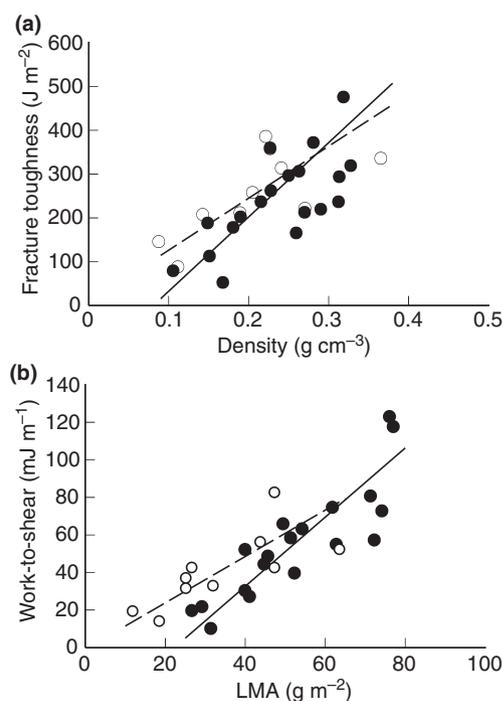
All measured traits varied significantly among species (one-way ANOVA, results not shown). Tests with two-way ANOVA indicated that both species and ontogeny affected significantly all traits measured for multiple ontogenetic stages (results not shown). Hence, in all subsequent analyses, species’ mean values were used at each ontogenetic stage, after  $\log_{10}$  transformations for variables that did not meet the assumption of normality. The survival rate was the only variable that remained non-normal with log or arcsine-square root transformations, and thus correlation analysis involving survival rate was examined using Spearman rank correlation. All other associations between pairs of trait variables were examined using Pearson correlation and standard major axis (SMA) regression with SMATR software (Warton *et al.*, 2006). SMA regression slopes were compared to test whether the relationship changed significantly between ontogenetic stages in terms of regression slope, elevation and shifts along the regression line (Warton *et al.*, 2006). Multivariate associations among lamina and vein traits relevant for mechanical strength and size of sapling leaves were summarized using principal component analysis (PCA). Then, we examined the correlations of the first three PCA axes with sapling traits directly relevant for the regeneration strategies of the species, including palatability index, leaf lifespan, growth rates, survival rates,  $CE_{\text{juv}}$  and wood density. The hypothetical causal structure (Fig. 1a) was evaluated using structural equation modeling (Shipley, 2000; Grace, 2006) with AMOS software (AMOS Development Corporation, Spring House, PA, USA). We compared alternative path models by evaluating the statistical significance of individual paths and the overall model fitting using maximum likelihood chi-squared and the Akaike Information Criterion (AIC). We also used the orthogonal trait axes for leaf traits extracted by PCA as exogenous variables that might influence leaf lifespan, survival, growth rates and light requirements of saplings. Phylogenetic independent con-

traits and their correlations were calculated using Phylocom software, following Webb *et al.* (2008), as described in Supporting Information Table S1. All statistical analyses were conducted using JMP software (SAS Institute, Cary, NC, USA) unless otherwise stated.

## Results

### Mechanical strength of sapling leaves and its determinants

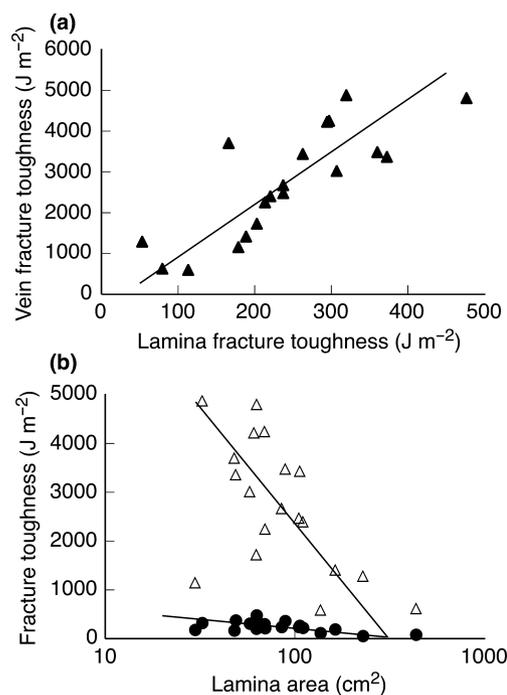
The mechanical strength of sapling leaf lamina varied widely among the 19 tree species (Table 1); LamFT (tissue-level toughness) differed ninefold among species ( $53\text{--}476\text{ J m}^{-2}$ ), whereas  $W_s$  (structure-level toughness) varied 12-fold ( $10\text{--}123\text{ mJ m}^{-1}$ ) and LamT (size trait) varied less than threefold among species ( $0.14\text{--}0.34\text{ mm}$ ). The mechanical strength at the tissue and structure-levels reflected matter distribution within the same level. At the tissue level, LamFT was correlated positively with measures of dry mass density, LamD (Fig. 2a) and LDMC (Table S1). At the structural level,  $W_s$  was correlated positively with LMA



**Fig. 2** Relationships of leaf toughness with dry matter distribution at the tissue level (a) and the structure-level (b) for leaf lamina of saplings (closed symbols, solid line,  $n = 19$  species) and seedlings (open symbols, broken line,  $n = 10$  species). Lines represent the standard major axis (SMA) regressions. (a) Fracture toughness plotted against tissue density;  $r = 0.72$ ,  $P < 0.001$  for saplings;  $r = 0.72$ ,  $P = 0.021$  for seedlings (no significant difference in slopes and elevations between seedlings and saplings). (b) Work-to-shear plotted against leaf mass per area ( $r = 0.87$ ,  $P < 0.001$  for saplings;  $r = 0.74$ ,  $P = 0.014$  for seedlings;  $P > 0.05$  for slope difference,  $P = 0.031$  for elevation difference between saplings and seedlings).

(Fig. 2b). The proposed path model among components of leaf toughness (Fig. 1a) was well supported (Fig. 1b), indicating that the variation in LamFT ( $\beta = 0.85$ ,  $P < 0.001$ ) contributed much more strongly than that of LamT ( $\beta = 0.49$ ,  $P < 0.05$ , Fig. 1b) to the variation in  $W_s$ . Lamina and vein fracture toughness values were correlated strongly with each other across species, even though veins were *c.* 10 times tougher than laminas in all species (Fig. 3a). Unexpectedly, the fracture toughness values of laminas and veins were correlated negatively with the lamina area, indicating that species with large leaves had weak lamina tissues (Fig. 3b). These pair-wise correlations between leaf traits were similar when phylogeny was taken into account (Supporting Information Table S2).

A PCA (Table 2, Supporting Information Fig. S2) revealed that tissue-level leaf traits varied independently of LamT. The fracture toughness of laminas and veins, as well as their dry mass density, loaded positively on PCA1, which explained 65% of the variation, but showed weak or negative correlation with PCA2, which explained an additional 21% of the variation (Table 2). By contrast, LamT contributed little to PCA1, but was the strongest contributor to PCA2. The two structure-level traits that should reflect both tissue properties and LamT (i.e. LMA and  $W_s$ ) showed similar degrees of loading on PCA1 and PCA2 (Table 2). Lamina area was the single strongest contributor to PCA3,



**Fig. 3** Relationships of fracture toughness of primary veins and laminas with each other (a) ( $r = 0.82$ ,  $P < 0.001$ ) and with lamina area (b) (open triangles for veins,  $r = -0.58$ ,  $P = 0.009$ ; closed circles for laminas,  $r = -0.57$ ,  $P = 0.012$ ) for sapling leaves of 19 tree species in Bolivian moist forest. Lines represent standard major axis (SMA) regressions.

**Table 2** Principal component analysis (PCA) for lamina and vein traits relevant for leaf toughness, showing the eigenvector values on each of the first three principal component axes

Eigenvectors	PCA1	PCA2	PCA3
Vein fracture toughness	0.38	-0.06	0.19
Lamina fracture toughness	0.38	0.12	0.14
Vein density	0.38	-0.18	-0.04
Lamina density	0.36	-0.28	0.14
Lamina LDMC	0.36	-0.24	0.24
LMA	0.35	0.31	0.00
Lamina work-to-shear	0.31	0.44	0.08
Lamina thickness	0.03	0.71	-0.09
log <sub>10</sub> (lamina area)	-0.29	0.12	0.92
Eigenvalue	5.9	1.9	0.5
Percent	65.3	20.7	6.0
Cumulative percent	65.3	86.1	92.1

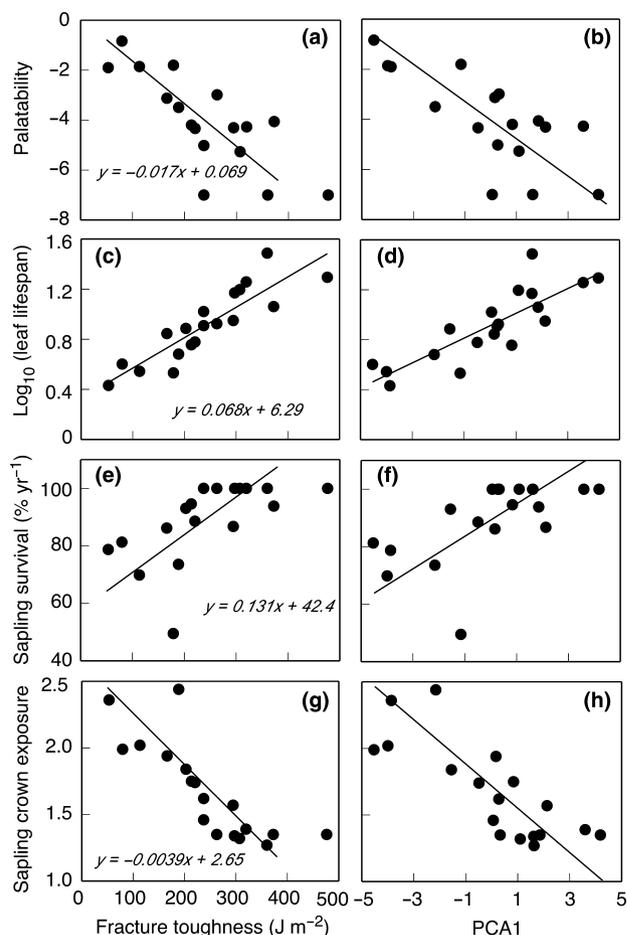
The traits are listed in the order of their contribution (eigenvector value) to the first principal component axis (PCA1). LDMC, leaf dry mass/fresh mass; LMA, leaf mass per area.

which explained 6% of the variance, in addition to the 86% explained by the first two axes. In summary, PCA1 captures the variation in dry mass density and tissue-level mechanical strength, whereas PCA2 and PCA3 summarize two aspects of leaf size, i.e. LamT and lamina area, respectively. The results were identical when all variables were log transformed first (i.e. the same variables contributed to PCA1–3, and together explained 93% of the variance).

### Relationships of leaf mechanical traits to ecological performance traits

The ecological performance traits of the 19 Bolivian tree species exhibited strong correlations with both tissue- and structure-level toughness in the predicted directions, but exhibited no correlations with lamina area and thickness. Species with tougher and denser leaves exhibited lower palatability, longer leaf lifespan, higher sapling survival, slower height growth rates and lower  $CE_{juv}$  (Fig. 4, Table 3). LamFT and PCA1, which summarize tissue toughness and density, showed identical correlations with these ecological performance traits (Fig. 4). The same results were found when phylogeny was taken into account (Table S2).

As expected, mechanically stronger leaves were less likely to be eaten, and more likely to live longer. Palatability by snails was correlated negatively with all leaf traits that contributed to leaf mechanical strength (Table 3, Fig. 4a,b), with fracture toughness and  $W_s$  showing stronger correlation than tissue density and LMA. Likewise, sapling leaf lifespan showed strong positive correlations with LamFT and other traits associated with it (Fig. 4b,c, Table 3). By contrast, LamT contributed little to species' variation in palatability and leaf lifespan (Table 3).



**Fig. 4** Relationships of sapling LamFT (common horizontal axis of panels on the left) and PCA1 from the analysis shown in Table 2 (horizontal axis of panels on the right) with ecological performance traits: (a, b) leaf palatability by snails; (c, d) leaf lifespan; (e, f) sapling survival; (g, h) regeneration light requirements measured as  $CE_{juv}$  at a standardized height of 2 m. All standard major axis (SMA) regressions are significant; see Table 3 for the correlation statistics.

Leaf mechanical properties and matter distribution were also associated strongly with the survival and growth rates of saplings, as well as species' light requirements. Species with higher LamFT survived better (Fig. 4e) even though they tended to grow more slowly (Table 3). The negative correlation of  $CE_{juv}$  with tissue density and toughness of laminas and veins (Table 3) indicates that shade-tolerant species with low  $CE_{juv}$  have dense and tough leaf tissues. The causal hypothesis depicted as the path model (Fig. 1a) was supported by the data (Fig. 1b), which showed that LamFT contributed much more strongly than  $W_s$  to  $CE_{juv}$  in terms of both direct and total effects (see Supporting Information Fig. S3 for details including the evaluation of alternative path models). The PCA1 score, which summarizes tissue-level toughness and matter distribution, exhibited positive and negative relationships with sapling survival and growth (Fig. 4f,h) in similar manners as LamFT did

**Table 3** Pair-wise correlations of lamina and vein traits relevant for leaf biomechanical strength with the ecological performance of saplings, regeneration light requirement ( $CE_{juv}$ ) and wood density of saplings, a stem functional trait

	Palatability index	$\log_{10}$ (leaf lifespan)	Sapling survival rate	$\log_{10}$ (growth rate)	$CE_{juv}$	Wood density
<i>n</i>	17	19	19	19	19	15
Lamina density	<b>-0.61</b> (0.0099)	<b>0.62</b> (0.0048)	<b>0.57</b> (0.010)	<b>-0.75</b> (0.0002)	<b>-0.65</b> (0.0024)	<b>0.82</b> (0.0002)
Lamina LDMC	<b>-0.44</b> (0.076)	<b>0.61</b> (0.0059)	<b>0.55</b> (0.015)	<b>-0.68</b> (0.0013)	<b>-0.61</b> (0.0053)	<b>0.78</b> (0.0005)
Lamina fracture toughness	<b>-0.78</b> (0.0002)	<b>0.88</b> (< 0.0001)	<b>0.78</b> (< 0.0001)	<b>-0.66</b> (0.0022)	<b>-0.78</b> (0.0001)	<b>0.82</b> (0.0002)
Vein density	<b>-0.49</b> (0.049)	<b>0.65</b> (0.0028)	<b>0.54</b> (0.016)	<b>-0.65</b> (0.0026)	<b>-0.60</b> (0.0063)	<b>0.82</b> (0.0002)
Vein fracture toughness	<b>-0.65</b> (0.0051)	<b>0.82</b> (< 0.0001)	<b>0.69</b> (0.0009)	<b>-0.73</b> (0.0004)	<b>-0.76</b> (0.0001)	<b>0.87</b> (< 0.0001)
LMA	<b>-0.83</b> (0.0001)	<b>0.81</b> (< 0.0001)	<b>0.79</b> (< 0.0001)	<b>-0.72</b> (0.0005)	<b>-0.72</b> (0.0005)	<b>0.83</b> (0.0001)
Lamina work-to-shear	<b>-0.83</b> (< 0.0001)	<b>0.87</b> (< 0.0001)	<b>0.77</b> (0.0001)	<b>-0.53</b> (0.019)	<b>-0.67</b> (0.0015)	<b>0.70</b> (0.0004)
Lamina thickness	-0.36 (0.16)	0.30 (0.20)	0.19 (0.44)	-0.04 (0.87)	-0.12 (0.61)	0.09 (0.74)
$\log_{10}$ (lamina area)	0.36 (0.15)	-0.46 (0.049)	-0.20 (0.42)	0.30 (0.21)	0.31 (0.19)	<b>-0.61</b> (0.015)
PCA1	<b>-0.74</b> (0.0008)	<b>0.71</b> (0.0007)	<b>0.69</b> (0.0011)	<b>-0.74</b> (0.0003)	<b>-0.72</b> (0.0005)	<b>0.90</b> (< 0.0001)

Pearson correlation coefficients (bold if  $P < 0.05$ ) are shown for all variables, except that Spearman rank correlation coefficients are shown for sapling survival rate which could not be corrected for normality even with transformation ( $P$  values in parentheses). See Table 2 for the relative contribution of variables to PCA1.

LDMC, leaf dry mass/fresh mass; LMA, leaf mass per area.

(Fig. 4e,g, Table 3). By contrast, PCA2 and PCA3 scores, which largely reflect leaf thickness and lamina area, exhibited no significant pair-wise correlations with any ecological performance traits ( $P > 0.2$ ).

#### Ontogenetic correlations and shifts of leaf biomechanical traits

Species' means of many leaf traits changed during seedling-to-sapling and sapling-to-adult transitions, but species' ranks in these traits were concordant across ontogenetic stages. Species with tough and dense leaves as seedlings had tough and dense leaves as saplings ( $n = 11$  species,  $r = 0.59$ – $0.91$ , Table 4), and those with tough and dense leaves as saplings had tough and dense leaves as adults ( $n = 18$  species,  $r = 0.72$ – $0.89$ , Table 4) (see Supporting Information

Table S4 for species' means for seedlings and adults). Tissue density, LMA and LamT were also correlated positively between ontogenetic stages. Species' ranks in leaf traits were therefore concordant across ontogenetic stages (Table 4). Lamina area was the only trait that did not exhibit significant ontogenetic concordance ( $r = 0.01$  for the seedling-to-sapling transition and  $r = 0.45$  for the sapling-to-adult transition, Table 4).

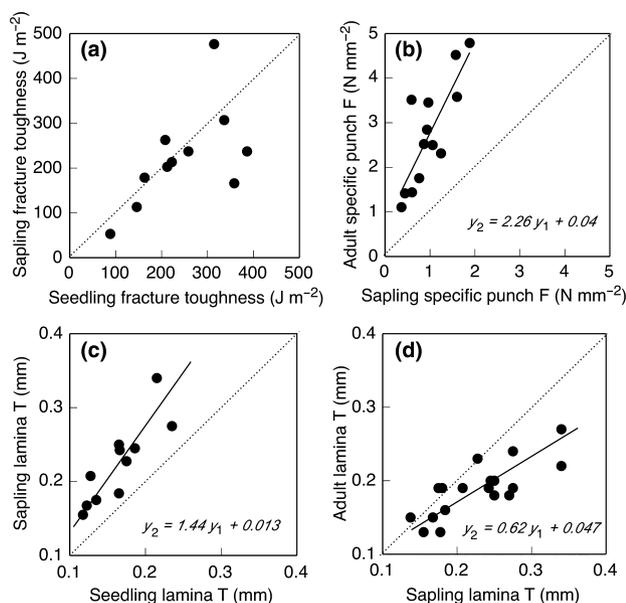
Many species showed significantly increased LMA and structure-level toughness ( $W_s$  and  $F_p$ ) with ontogenetic development ( $P < 0.05$  according to paired  $t$ -test, Table 4), but the mechanistic reasons underlining these structure-level increases differed between the two transitions. In the seedling-to-sapling transition, there was no general pattern for changes in tissue density and toughness across species ( $P > 0.05$ , paired  $t$ -test, Table 4); when sapling LamFT was

**Table 4** Ontogenetic shifts of species' mean values for lamina and vein traits relevant for leaf toughness from seedlings to saplings and from saplings to adults

Ontogenetic transition	Seedling-to-sapling					Sapling-to-adult				
	<i>n</i>	Paired <i>t</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>n</i>	Paired <i>t</i>	<i>P</i>	<i>r</i>	<i>P</i>
Lamina tissue density	10	1.6	ns	0.66	0.040	18	7.6	< 0.0001	0.78	0.0001
Lamina tissue-level toughness <sup>a</sup>	11	-0.8	ns	0.59	0.055	13	7.6	< 0.0001	0.82	0.0006
Vein tissue-level toughness <sup>a</sup>	11	4.4	0.0014	0.91	0.0001	na				
LMA	10	6.6	0.0001	0.86	0.0014	18	4.4	0.0004	0.72	0.0008
Lamina structure-level toughness <sup>b</sup>	11	1.9	0.042	0.75	0.0081	13	6.2	< 0.0001	0.89	< 0.0001
$\log_{10}$ (lamina area)	11	8.3	< 0.0001	0.01	0.97	18	-0.6	0.55	0.45	0.059
Lamina thickness	11	6.7	0.0001	0.85	< 0.001	18	-4.4	0.0004	0.79	< 0.0001

*n*, number of species. A positive paired *t*-value means that trait values increase with ontogenetic stages at the indicated *P* level with a two-tailed test. Also shown are Pearson correlation coefficients (*r*) for ontogenetic concordance between the two stages. ns,  $P \gg 0.05$ ; na, data not available. LMA, leaf mass per area.

<sup>a</sup>Tissue-level toughness was quantified as the fracture toughness determined by the shearing test for seedlings and saplings, and as specific force-to-punch determined with a penetrometer for saplings and adults. <sup>b</sup>Structure-level toughness was quantified as work-to-shear for seedlings and saplings, and as force-to-punch for saplings and adults.



**Fig. 5** Ontogenetic correlations for LamFT between seedlings and saplings ( $r = 0.59$ ,  $P = 0.055$ ; slope not significantly different from 1.0) (a), specific force-to-punch ( $F_{sp}$ ) between saplings and adults (slope  $> 1.0$  at  $P = 0.0001$ ) (b), lamina thickness ( $T$ ) between seedlings and saplings (slope  $> 1.0$  at  $P = 0.059$ ) (c) and lamina thickness between saplings and adults (slope  $< 1.0$  at  $P = 0.006$ ) (d). Dotted lines indicate 1 : 1 line, and solid lines indicate significant standard major axis (SMA) regressions ( $P < 0.05$ ). See Table 4 for correlation statistics.

plotted against seedling LamFT (Fig. 5a), most points fell near the 1 : 1 line. By contrast, LamT increased significantly from seedlings to saplings (with all points above the 1 : 1 line in Fig. 5c; paired  $t$ -test  $P < 0.001$ , Table 4). Thus, the increase in  $W_s$  from seedlings to saplings (Table 4) was solely a result of an increase in LamT (Fig. 5c). By contrast, during the sapling-to-adult transition,  $F_{ps}$  (Fig. 5b) and LamD increased significantly (paired  $t$ -tests, Table 4), whereas LamT actually decreased in many species (Fig. 5d). Thus, significant increases in structure-level traits, LMA and  $F_p$ , from saplings to adults were solely a result of the increases in LamD and tissue toughness.

The lamina area increased significantly from seedlings to saplings (paired  $t$ -test,  $P < 0.001$ ), but the degrees and directions of the changes differed so much among species that there was no interspecific correlation in lamina area between seedling and sapling stages ( $r = 0.01$ ,  $P = 0.97$ , Table 4). During the sapling-to-adult transition, there was neither a general pattern of lamina area change across species (paired  $t = -0.6$ ,  $P = 0.55$ ) nor significant ontogenetic concordance ( $r = 0.45$ ,  $P = 0.059$ , Table 4). In summary, ontogenetic shifts of individual leaf traits may switch directions between the seedling-to-sapling transition and the sapling-to-adult transition (Fig. 6), even though overall species' ranks were maintained across ontogenetic stages (with the exception of the lamina area).

	Seedling	Sapling	Adult
Tissue-level density and toughness	→	↗	↗
LMA and structural strength	↗	↗	↗
Lamina thickness	↗	↘	↘
Lamina area	↗	↗	↗

**Fig. 6** Directions of ontogenetic changes for components of leaf toughness supported by paired  $t$ -test in Table 4. Between seedling and sapling stages, tissue-level traits (tissue density and fracture toughness) did not change significantly, whereas lamina thickness and area increased; thus, the increase in structure-level toughness (work-to-shear) was solely a result of an increase in lamina thickness. By contrast, between sapling and adult stage, tissue density and fracture toughness increased, even though the lamina thickness and lamina area decreased; hence, the increase in structure-level toughness (force-to-punch) was solely a result of an increase in tissue density and lamina toughness.

### Effects of ontogeny on trait-to-trait and trait-to-performance relationships

The relationship between fracture toughness and tissue density was identical for seedlings and saplings (Fig. 2a). By contrast, the relationship between two structure-level traits,  $W_s$  and LMA, differed slightly between seedling and sapling stages, showing that seedlings tend to have higher  $W_s$  at a given LMA (Fig. 2b). LamT was independent of tissue-level traits at all ontogenetic stages ( $P > 0.05$ ), and ontogenetic changes in lamina area were not necessarily accompanied by changes in LamT.

The relationships of lamina toughness with performance traits were maintained across ontogenetic stages, indicating that leaf toughness values of seedlings and adults can predict the ecological performance of saplings. Seedling LamFT was correlated positively with sapling leaf lifespan ( $n = 11$  species,  $r = 0.80$ ,  $P = 0.003$ ) and survival ( $n = 11$  species,  $r_s = 0.63$ ,  $P = 0.038$ ). Adult lamina  $F_p$  was correlated with the palatability index ( $n = 16$  species,  $r = -0.68$ ,  $P = 0.002$ ), leaf lifespan ( $n = 18$  species,  $r = 0.74$ ,  $P < 0.001$ ), survival ( $n = 18$ ,  $r_s = 0.50$ ,  $P = 0.03$ ) and height growth rate ( $n = 18$  species,  $r = -0.57$ ,  $P = 0.013$ ) of saplings.

### Discussion

The results of this study strongly suggest that tissue-level leaf toughness is the essence of the link between structure-level leaf toughness and the ecological performance of juvenile trees. The same conclusion was reached by Grubb *et al.* (2008) in their reanalysis of the 46-species dataset of Coley (1983), as well as in a study of young seedlings of eight Pan-

amanian tree species by Alvarez-Clare & Kitajima (2007). Our study supports this conclusion more convincingly by demonstrating the link between leaf mechanical strength and multiple measures of ecological performance and the regeneration light requirements of tree species.

### Tissue density and toughness as the functional basis of tough leaves

LMA is widely recognized as a trait that correlates positively with structure-level leaf toughness, such as  $W_s$  (Wright & Westoby, 2002; Read & Sanson, 2003; Hanley *et al.*, 2007; Mediavilla *et al.*, 2008), and negatively with photosynthetic rates and nitrogen contents per unit mass (Wright *et al.*, 2004). These correlations exist because the contents of metabolically active mesophyll cells, rather than cell walls and cuticles, occupy large proportions of leaf volume space in species with low LMA (Niinemets, 1999; Shipley *et al.*, 2006). We found that LMA predicted  $W_s$  ( $r = 0.87$ ), although LamFT alone predicted  $W_s$  equally well ( $r = 0.87$ , Table S1). The evaluation of the proposed path model (Fig. 1a) demonstrated that tissue density and toughness played a greater role than LamT in linking LMA to  $W_s$  (Figs 1b, S3).

Anatomically, leaf tissue density reflects the abundance of vascular tissues (Choong *et al.*, 1992), which have thick cell walls and much higher tissue density ( $1.40 \text{ g cm}^{-3}$ ) than mesophyll ( $0.31 \text{ g cm}^{-3}$ ) and epidermis ( $0.08 \text{ g cm}^{-3}$ ) (Poorter *et al.*, 2009). Hence, a greater proportional representation of vascular tissues indicates increases in cell wall volume fraction (Shipley *et al.*, 2006; Poorter *et al.*, 2009), fiber contents per unit volume and fracture toughness (Choong *et al.*, 1992; Lucas *et al.*, 2000). Veins were *c.* 10 times tougher than lamina, as found in other studies of tropical eudicot trees (Choong *et al.*, 1992; Dominy *et al.*, 2003; Alvarez-Clare & Kitajima, 2007). Although we carefully avoided any obvious veins, many fine vascular tissues were probably included in the leaf laminas sampled.

Surprisingly, fracture toughness values of both laminas and veins were correlated negatively with lamina area among the study species (Fig. 3b). This was the opposite of our a priori expectation that larger leaves should require greater mechanical support to maintain optimal leaf display. LamT did not compensate for weak laminas of large leaves, because LamT and lamina area were not correlated with each other ( $r = 0.08$ , Table S1). However, the central vein diameter was correlated positively with leaf size ( $r = 0.80$ ,  $P < 0.01$ ;  $\log_{10}(\text{vein diameter}) = 0.35 \log_{10}(\text{lamina area}) - 0.67$ ). The four species with the largest leaves were light-demanding pioneers of *Urera*, *Heliocarpus*, *Cecropia* and *Jacaratia*. Visual observations suggest that these large-leaved species employ 'kite'-type leaves common among pioneers (Grubb, 1986), in which thin and soft laminas are supported by large-diameter veins. These mesophyll-rich

large leaves may also depend on turgor for self-support, as suggested by frequent wilting later in the day.

### Importance of tissue density and fracture toughness for ecological strategies of trees

The results of this study strongly suggest that the tissue density and toughness of laminas and veins are important as part of the shade tolerance strategy characterized by a long leaf lifespan, high survival rates and slow growth rates during the regeneration phase (Kitajima & Poorter, 2008). The optimal leaf lifespan should be longer in shady and other resource-limited environments, where the carbon cost of leaf construction is high relative to the potential photosynthetic income (Coley *et al.*, 1985; Williams *et al.*, 1989; Kikuzawa, 1991). In general, shade-tolerant evergreen species tend to have long-lived leaves with high LMA, low nitrogen contents and low photosynthetic capacity per unit mass (Walters & Reich, 2000; Niinemets, 2006; Poorter & Bongers, 2006; Lusk *et al.*, 2008). Compiling data for 597 woody species, Niinemets (1999) showed that leaf tissue density, rather than LamT, underpins this trade-off between photosynthetic capacity per unit mass and LMA. Parallel to this, the results reported here demonstrate that tissue density, rather than LamT, is the aspect of LMA that enables leaves to achieve a long lifespan against biotic hazards. The central role of tissue density and toughness for physical defense is evident in the slower rates of consumption of tougher leaves by a generalist snail (Fig. 4) and the community of herbivores in the field ( $n = 14$  species,  $r = -0.74$ ,  $P < 0.003$ , L Poorter, unpublished).

The negative correlation between components of tissue toughness and  $CE_{\text{juv}}$  (Table 3, Fig. 4g,h) supports directly the predicted link of physical defense with regeneration light requirements. By contrast, LamT and leaf size showed no correlation with  $CE_{\text{juv}}$ . A previous study also found that  $CE_{\text{juv}}$  was correlated with punch strength ( $r = -0.46$ ,  $P = 0.004$ ) and tissue density ( $r = -0.53$ ,  $P = 0.001$ ) of lamina, but not with LamT ( $r = -0.05$ ,  $P = 0.75$ ), for adult leaves of 39 tree species at the same site (sampled from 10–20 cm DBH individuals; Poorter, 2009). The associations of leaf toughness-related traits with ecological performance are evolutionarily convergent, as phylogenetic independent contrasts show trait correlations that are of a similar strength to the nonphylogenetic cross-species' correlations (Table S2).

### Functional integration of traits with structural equation modeling

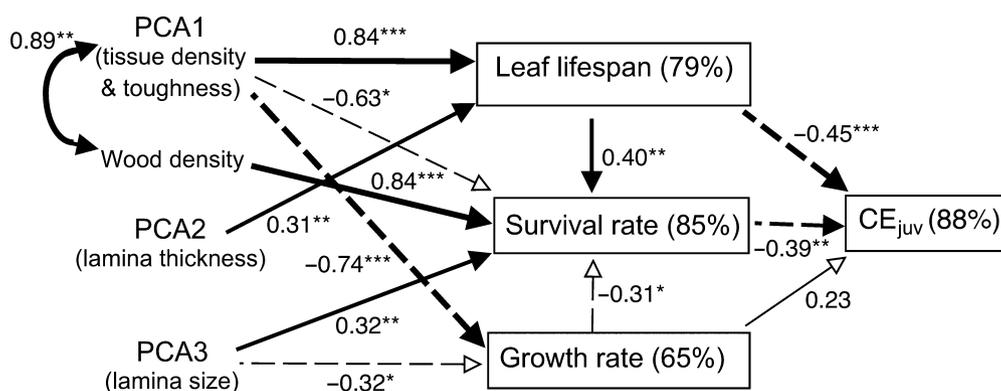
The predicted causal links among traits that contribute to leaf mechanical strength, ecological performance and regeneration strategy are well supported by structural equation modeling using the results of this study. The best-supported

model among the alternatives (Figs 7, S3) showed that a higher score of PCA1 (an integrative trait index representing high tissue density and toughness of laminae and veins, and smaller lamina area, Table 2) indicated a longer leaf lifespan and better sapling survival (via leaf lifespan), but slower sapling growth rates (Fig. 7). By contrast, PCA2 (reflecting LamT and  $W_s$ ) and PCA3 (reflecting lamina area) showed no pair-wise correlation with ecological performance (Table 3), and had much more limited partial effects when PCA1 was taken into account in the path model (Fig. 7). The result of this analysis is consistent with the generalization from the literature (reviewed by Kitajima & Poorter, 2008); shade tolerance is achieved through a long leaf lifespan and a high juvenile survival rate, whereas specialization to high-light environments is achieved through faster growth. The model shown in Fig. 7 also included wood density, which is widely recognized as a key functional trait associated with shade tolerance and the life history strategy of tropical trees (Augsburger, 1984; van Gelder *et al.*, 2006; Wright *et al.*, 2007; Poorter *et al.*, 2010). In the best-fit path model, wood density had a strong direct effect on sapling survival, as well as an indirect effect through its association with tissue-level toughness. It is interesting that PCA1 had a negative direct effect on sapling survival ( $\beta = -0.63$ ,  $P = 0.02$ ), even though its indirect positive effect through leaf lifespan ( $\beta = 0.84 \times 0.40 = 0.34$ ) and its correlation with wood density ( $r = 0.89$ ,  $P < 0.001$ ) resulted in a strong positive pair-wise correlation with sapling survival ( $r = 0.69$ ,  $P = 0.001$ , Table 3).

### Ontogenetic concordance and shifts in leaf biomechanical traits

Each leaf trait showed strong positive interspecific correlations between seedling, sapling and adult stages (Table 4). The only exception was leaf size, which typically became larger from seedling to sapling, but to various degrees amongst species. Seedlings of small-seeded species were constrained from having large leaves because large leaves cannot be built from small seed reserves. The leaves of larger saplings may be associated with longer internodes (Ackerly & Donoghue, 1998; Poorter & Rozendaal, 2008) that are expected to contribute to rapid height growth, especially in treefall gaps (Kitajima & Poorter, 2008). However, the negative direct effect of PCA3 (representing lamina area) on sapling growth rates suggests that more studies are necessary for a better understanding of the ecological significance of leaf size. The overall concordance of leaf toughness at both tissue and structural levels means that the leaf toughness of adults is a good predictor of the leaf traits and ecological performance of juveniles. This ontogenetic concordance may reflect a strong natural selection on leaf toughness at the juvenile stage, as has been found for other leaf traits (Poorter, 2007).

The ontogenetic concordance stemmed from the common patterns of trait value shifts, as summarized in Fig. 6. From seedlings to saplings, tissue density and tissue-level toughness did not change significantly. The increase in LamT, therefore, was apparently the solution to increase the structure-level toughness in order to support larger sapling leaves. The sapling-to-adult transition exhibited strikingly



**Fig. 7** Functional integration of traits using structural equation modeling, linking orthogonal axes of sapling leaf traits with the ecological performance of 19 Bolivian tree species ( $\chi^2 = 11.4$ ,  $P = 0.72$ ,  $df = 15$ ,  $AIC = 69.4$ ). Exogenous (independent) variables are the orthogonal axes extracted by the principal component analysis (PCA1 largely contributed by tissue density and toughness, PCA2 mainly reflecting lamina thickness and PCA3 reflecting lamina area; see Table 2 for details) and sapling wood density. The statistical support for this model means that the four exogenous variables directly and indirectly influence demographic traits, including leaf lifespan, sapling survival rate and height growth rate, which, in turn, influence  $CE_{juv}$  (an index of regeneration light requirements). The endogenous (dependent) variables are enclosed in rectangles. The proportion of the total variance (%) of each endogenous variable explained by the model is shown in parentheses. Numbers by the arrows indicate the path coefficients, with asterisks indicating statistical significance (\* $P < 0.05$ ; \*\* $P < 0.005$ ; \*\*\* $P < 0.001$ ). Solid and broken arrows indicate positive and negative effects, respectively. The double-headed arrow between PCA1 and wood density indicates the correlation between these two exogenous variables ( $r = 0.89$ ). See Fig. S3 for details of model evaluation with structural equation modeling.

different patterns compared with the seedling-to-sapling transition (Fig. 6). In most species, LamT decreased from saplings to adults; yet LMA and  $W_s$  increased because the increase in tissue density and toughness overcompensated for the decrease in LamT (Table 4). A meta-analysis by Thomas & Winner (2002) also found that LMA typically increased from saplings to adult trees, although it is not clear whether changes in LamT or lamina density were responsible in the studies included in their analysis. Gras *et al.* (2005) found that, in four *Eucalyptus* species, LamT increased without a change in tissue-level toughness from juveniles to adults, which differs from the pattern summarized in Fig. 6. The decrease in LamT observed in the current study could be partly explained by a reduction in leaf size in some species whose saplings had large leaves (e.g. *Stylogene*, *Erythrochiton*, *Aspidosperma*, *Urera* and *Heliocarpus*). However, leaf size did not decrease in all species. Leaf traits, including tissue density and mechanical strength, can exhibit phenotypic plasticity in response to a light environment (Terashima *et al.*, 2001; Onoda *et al.*, 2008), and species differ in the ontogenetic trajectories of light availability measured as CE (Poorter *et al.*, 2005). Greater vapour pressure deficit and wind in the upper canopy than in the understory may also affect the optimal leaf morphology. These observations suggest that light and other microenvironmental factors need to be documented in order to better understand the mechanistic reasons underlying the ontogenetic shifts of leaf mechanical traits.

## Conclusions

A hierarchical approach that distinguishes between tissue-level traits and size-related traits, as well as matter distribution from mechanical strength, can elucidate the mechanistic links between leaf traits and their functional significance. Structural equation modeling supported the path models that depicted multivariate causal relationships among traits contributing to leaf toughness, as well as their links to ecological performance (Figs 1, 7). Tissue properties, including tissue density and fracture toughness of laminae and veins, were central to the structure-level leaf toughness linked to antiherbivore defense, leaf lifespan, sapling survival and growth rates, and regeneration light requirements of tropical tree species. LamT was a significant contributor to lamina work-to-shear, but was unrelated to ecological performance and regeneration strategies of the species. The leaf traits that contributed to leaf toughness showed ontogenetic shifts that changed directions and magnitudes between seedling-to-sapling and sapling-to-adult transitions (Fig. 6). Yet, the species' rank order remained such that tissue-level toughness and the density of adult leaves predicted the regeneration ecological characteristics of juveniles. These results suggest the importance of

distinguishing between tissue-level and structure-level toughness; otherwise, one may draw the erroneous conclusion that thickness *per se* has a defensive role, partly because thickness is often viewed as an essential aspect of sclerophyllous leaves.

## Acknowledgements

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Table S1** Pair-wise correlations between traits relevant for leaf mechanical properties, using species' mean values and phylogenetic independent contrasts.

**Table S2** Correlations of phylogenetic independent contrasts of ecological performance with traits relevant for mechanical strength and size of leaves.

**Table S3** Species' means for additional sapling leaf traits used in the analysis.

**Table S4** Species' means for leaf mechanical traits of adults and seedlings.

**Fig. S1** Relationship between force-to-punch measured with a penetrometer and work-to-shear determined by the shearing test.

**Fig. S2** Plot showing loading of each trait on PCA1 and PCA2 for the results shown in Table 2.

**Fig. S3** Evaluation of alternative path models by structural equation modeling for the relationships among traits contributing to leaf mechanical strength and their relationship with ecological performance.

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