

# How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species

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## Summary

- Cell wall fibre and lamina density may interactively affect leaf toughness and leaf lifespan. Here, we tested this with seedlings of 24 neotropical tree species differing in shade tolerance and leaf lifespan under standardized field conditions (140–867 d in gaps; longer in shade). We quantified toughness with a cutting test, explicitly seeking a mechanistic linkage to fibre.
- Lamina density, but not fracture toughness, exhibited a plastic response to gaps vs shade, while neither trait was affected by leaf age. Toughness corrected for lamina density, a recently recognized indicator of material strength per unit mass, was linearly correlated with cellulose content per unit dry mass.
- Leaf lifespan was positively correlated with cellulose and toughness in shade-tolerant species but only weakly in gap-dependent species. Leaf lifespan was uncorrelated with lamina thickness, phenolics and tannin concentrations. In path analysis including all species, leaf lifespan was directly enhanced by density and toughness, and indirectly by cellulose via its effect on toughness. Different suites of leaf traits were correlated with early seedling survival in gaps vs shade.
- In conclusion, cellulose and lamina density jointly enhance leaf fracture toughness, and these carbon-based physical traits, rather than phenolic-based defence, explain species differences in herbivory, leaf lifespan and shade survival.

## Introduction

Forest trees differ widely in demographic, morphological and physiological traits in relation to light environments favourable for juvenile recruitment and persistence (Bazzaz, 1979; Swain & Whitmore, 1988; Welden *et al.*, 1991; Poorter, 2006). The functional basis for such species differences in regeneration light demands may be understood in terms of light utilization strategies (Grime, 1979; Givnish, 1988; Kohyama, 1991) and resistance to natural enemies (Coley, 1983; Augspurger, 1984; Dalling *et al.*, 1997). These differences are often summarized in terms of the contrasts between shade-tolerant and gap-dependent guilds of species. In shaded forest understoreys where light availability limits potential carbon gain, long leaf lifespan is necessary to recoup leaf construction costs, and thus it is critical that leaves are well defended against biotic and abiotic hazards (Bloom *et al.*, 1985; Coley *et al.*, 1985; Endara & Coley, 2011). By contrast, in tree fall gaps, natural selection favours the competitive advantage achieved by rapid height growth and turnover of leaves with high photosynthetic capacity (Bongers & Pompa, 1990). Such contrasting selective regimes are partially responsible for the ubiquitous trait correlations among leaf mass per area (LMA),

nitrogen concentration (N), photosynthetic rates and leaf lifespan (Reich *et al.*, 1992; Wright *et al.*, 2004). These leaf economic spectrum traits, as well as leaf traits that enhance defence against biotic and abiotic hazards, are considered central to the functional trait syndrome associated with the growth-mortality trade-offs observed for juveniles of forest tree species (Kitajima, 1994; Pacala *et al.*, 1996; Gilbert *et al.*, 2006; Sterck *et al.*, 2006; Wright *et al.*, 2010).

Leaf defence can be achieved by physical or chemical properties of molecules that make up leaves, the effectiveness of which may depend on hazard agents and environments. Physical defence traits include those based on toughening or hardening of tissues or a deterrent geometry such as spines and thorns (Grubb, 1986, 1992; Lucas *et al.*, 2000). Toughness-based defence, in particular, reflects quantities, types and arrangements of cell wall components. Chemical defence molecules that operate as toxins, by contrast, may be effective in smaller quantities, but can be circumvented by co-evolved natural enemies (Coley *et al.*, 1985). Comparing multiple putative physical and chemical defence traits across 46 tropical tree species, Coley (1983) found that toughness (measured as punch resistance) and cellulose content were the two traits that best explain species difference in

herbivory rates and leaf lifespan. Although not examined by Coley (1983), LMA and its two components, thickness and lamina density, may also enhance toughness and leaf lifespan (Wright *et al.*, 2004; Kitajima & Poorter, 2010; Onoda *et al.*, 2011). However, mechanistic relationships between these traits remain ambiguous without proper quantification in a manner consistent with conceptual and methodological advances in the understanding of leaf toughness (Lucas *et al.*, 1991; Aranwela *et al.*, 1999; Sanson *et al.*, 2001). Once trait definitions and measurement units are properly formulated, it becomes possible to analyse the relationship between matter distribution and mechanical properties.

In mechanical terms, a leaf lamina is a complex heterogeneous structure containing cells with distinct shapes and cell wall architectures. Wall component molecules (e.g. cellulose microfibrils, hemicelluloses, pectins, lignin and wall proteins) are combined in different ways in different cell types to result in contrasting mechanical properties within (e.g. tough and rigid veins vs elastic mesophylls) and among leaves (e.g. sun vs shade leaves, slow- vs fast-growing species). Here, we recognize this structural hierarchy via *a priori* hypotheses illustrated in Fig. 1. Matter allocation patterns can be quantified per unit dry mass, per unit volume or per unit surface area. These three methods of standardizing matter allocation patterns correspond to three ways of normalizing mechanical measurements of resistance against fracture (Onoda *et al.*, 2011), as summarized in Table 1 for fracture resistance quantified with cutting tests. Work-to-shear ( $W_s$ ) is a measure of structural resistance expressed per unit cut length, and as such it reflects both lamina thickness ( $T$ ) and fracture toughness

( $\tau$ , defined as work to fracture per unit cross sectional area).  $\tau$  is expected to increase with lamina density ( $\rho$ , mass per unit volume), because when  $\rho$  is greater, more dry matter is encountered per cross-sectional area during the cutting operation. Fracture toughness corrected for this density effect (hereafter, density-corrected toughness,  $\gamma$ ) corresponds to the average resistance per unit dry mass of materials comprising the leaf. We predict that values of  $\gamma$  correlate with cell wall fibre content per unit dry mass (Fig. 1, top).

Operationally, the three measures of toughness,  $W_s$ ,  $\tau$  and  $\gamma$  are determined from each other as below (Onoda *et al.*, 2011).

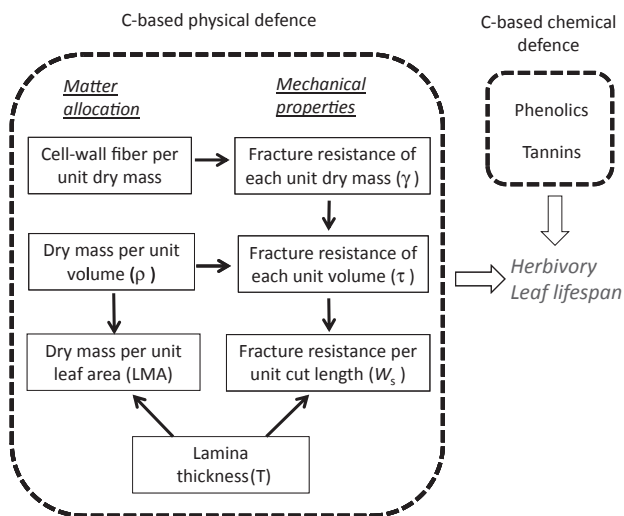
$$W_s = T \cdot \tau \quad \text{Eqn 1}$$

$$\tau = \rho \cdot \gamma \quad \text{Eqn 2}$$

In a given leaf, these two relationships are absolute. But, there is no *a priori* reason to know what proportion of interspecific variation in  $W_s$  is attributable to species differences in  $\tau$  vs  $T$  (Eqn 1), and what proportion of interspecific variation in  $\tau$  is attributable to species differences in  $\gamma$  vs  $\rho$  (Eqn 2). In a global overview, Onoda *et al.* (2011) found that  $\gamma$  explains 60–70% of variation in  $W_s$ , most variation being within sites. However, the mechanistic basis for variation in  $\gamma$  or ecological correlates of  $\gamma$  remains unexplored.

Because of the statistical nonindependence of the three measure of toughness as described above, it is not possible to test the full conceptual model illustrated in Fig. 1, but several recent studies shed light on the predicted causal relationships. In juvenile leaves of evergreen tree species,  $W_s$ ,  $\tau$  and  $\rho$ , but not  $T$ , correlate with palatability, leaf lifespan, growth, survival and regeneration light requirements (Kitajima & Poorter, 2010; Lusk *et al.*, 2010). By contrast, Westbrook *et al.* (2011) asked what makes a leaf tough from an evolutionary perspective by comparing phylogenetic independent contrasts for leaf toughness and fibre contents of subcanopy individuals of 197 species of trees and shrubs. Their results show that  $\tau$  correlates with  $\rho$  and %cellulose, and that  $\tau$ ,  $\rho$  and %cellulose, but not  $T$ , covary with juvenile survival in shade. None of these three studies explicitly asked how density-corrected toughness,  $\gamma$ , might correlate with leaf lifespan, growth, survival and habitat preference. Phenotypic plasticity of toughness-related traits in response to light environment and leaf age also remains unexplored. It is well known that young expanding leaves are less tough than mature leaves (Coley, 1983; Choong, 1996; Grubb *et al.*, 2008), but leaf age effects after full expansion are unknown even though leaf lifespan may be exceedingly long for juveniles of shade-tolerant trees.

In order to evaluate interspecific variation and phenotypic plasticity of leaf toughness-related traits, we planted 24 tree species in replicate gap and understorey common garden experiments in a Panamanian moist forest. We examined correlations among functional and demographic traits in relation to their regeneration light demands, following the conceptual framework illustrated in Fig. 1 that relates carbon-based leaf defences including mechanical ( $W_s$ ,  $\tau$ ,  $\gamma$ ,  $T$  and  $\rho$ ) and chemical (phenols and



**Fig. 1** A conceptual framework for the relationships among traits that contribute to carbon-based physical and chemical defence of leaves. No *a priori* association is expected between physical and chemical defence traits, while strong *a priori* relationships are expected among physical defence traits (see text). We developed alternative path models (e.g. Fig. 4, Supporting Information Fig. S2) based on these *a priori* expectations on direct and indirect effects of mechanical defence traits on leaf demography. For example, we predicted that lamina density and cell wall fibre content may reduce herbivory and enhance leaf lifespan, but their effects may be direct as well as indirect through their effects on toughness (i.e.  $\tau$  and  $\gamma$ ).

**Table 1** Summary of material distribution traits expected to influence leaf mechanical properties, in terms of leaf fracture resistance measured with a cutting test

Matter distribution traits		Corresponding fracture resistance traits	
Matter distribution standardization	Traits examined in this study	Mechanical measurement normalization	Traits examined in this study
Quantity per unit dry mass	%cellulose, %lignin, %ADF, %NDF	Fracture toughness divided by density	$\gamma$ : density-corrected toughness ( $\text{mJ g}^{-1} \text{m}$ )
Quantity per unit volume	$\rho$ : Lamina density ( $\text{g cm}^{-3}$ ), cellulose per volume ( $\text{g cm}^{-3}$ )	Work to fracture per unit cross-sectional area of cut	$\tau$ : fracture toughness ( $\text{J m}^{-2}$ )
Quantity per unit surface area	LMA ( $\text{g m}^{-2}$ )	Work to fracture per unit cut length	$W_s$ : work-to-shear ( $\text{J m}^{-1}$ )

Material distribution traits are grouped by their standardization schemes in the left side, whereas the fracture resistance trait that should be compared with each type of matter distribution trait is listed in the right within the same row. See Fig. 1 for the expected interrelationships among these traits. ADF, acid detergent fibre; NDF, neutral detergent fibre; LMA, leaf mass per area.

tannins) defences. We compared alternative path models to evaluate predicted multivariate relationships among toughness-related traits and leaf lifespan as illustrated in Fig. 1. By doing so, we could test whether fibre and lamina density had direct effects on leaf lifespan in addition to their indirect effects mediated through toughness. Based on the result of Westbrook *et al.* (2011), we expected that %cellulose, but not other fibre components, would correlate with density-corrected toughness, and that this cellulose-based toughness should correlate positively with leaf lifespan and juvenile survival in shade, both within and across gap- and shade-tolerant guilds. The results addressed the following questions:

- How do  $W_s$  and its underlying components,  $T$ ,  $\tau$ ,  $\rho$ , and  $\gamma$ , and cell wall fibre contents, covary across species, leaf ages, and light environments (gap vs shaded understorey)?
- Which aspects of carbon-based defence correlate with herbivory level and leaf lifespan across species? More specifically, we were interested in comparing carbon-based chemical defence (phenolics and tannins) with fibre content and mechanical resistance ( $W_s$ ,  $\tau$  and  $\gamma$ ).
- How do these leaf-level traits differ between regeneration guilds (gap-dependent vs shade-tolerant) and in relation to seedling survival in gaps and shade?

## Materials and Methods

### Site and species

The study was conducted in the Barro Colorado Nature Monument (BCNM) in Panama. Leigh *et al.* (1982) describe the flora, ecology and environment of the seasonal moist tropical forests of the BCNM. Annual rainfall averages 2600 mm, 90% of which falls between May and December. We established six common gardens in 20-m tall secondary forest on the Buena Vista Peninsula in May 2002. Each garden was approximately rectangular (7–8 m  $\times$  8–10 m) and was enclosed by a metal-mesh fence to exclude ground-dwelling vertebrates. Three gardens were established in natural treefall gaps whose edges were cleared and widened to maintain the same-sized opening throughout the

experiment. The remaining three gardens were located in shaded understorey. We took canopy photos at five positions per garden, and calibrated them against photosynthetic active radiation (PAR) measured with quantum sensors (LI-185; LICOR, Lincoln, NE, USA) for a minimum of 3 d in the centre of each garden. The mean PAR during the wet season was 0.5–0.8% of above-canopy PAR (0.14–0.18  $\text{mol d}^{-1}$ ) in understorey shade gardens and 23.4%, 37.7% and 50.7% of above-canopy PAR (6.7, 11.3 and 11.5  $\text{mol d}^{-1}$ ) in gap gardens.

The 24 study species (Table 2) were common tree species, including gap-dependent species rarely observed in the shaded understorey and shade-tolerant species whose seedlings and saplings were frequent in the shaded understorey of old growth forest in BCNM (Condit *et al.*, 2006; Comita *et al.*, 2010; K. Kitajima, A.-M. Llorens, C. Stefanescu *et al.* unpublished data and field observations; see Supporting Information Notes S1 for more information). The 24 species ranged broadly in leaf lifespan and seedling survival in shade (Table 2). Seedlings were transplanted into the six common gardens between April 2002 and May 2003. Seeds were collected from forests within the BCNM, and germinated under 30% or 1% light availability in plastic trays filled with washed sand and forest soil from the top 30-cm of the soil profile. After radicle emergence and before full expansion of the first leaf, 6–12 seedlings of each species germinated in high and low light were transplanted to two randomly chosen sections of each gap and understorey garden, respectively. Seedlings were planted at least 30 cm from one another. Mortality within the first week post-transplant was negligible as seedlings were transplanted during the rainy season.

### Survival census for leaves and seedlings

Survival of all transplanted seedlings was recorded by weekly census. Production and survival of leaves were monitored on a subset of seedlings ( $\geq 3$  individuals per garden per species). At each monthly census, we numbered newly expanded leaves sequentially with a nontoxic waterproof marker, and recorded presence/absence of previously marked leaves. Both censuses continued until July 2006. For early seedling survival, we

**Table 2** Study species and their ecological characteristics, including regeneration guild (T, shade-tolerant; G, gap-dependent), median leaf lifespan and seedling survival in gap and shade common gardens, and mean %leaf-damage (proportion of leaves showing herbivory damage) in gap gardens

Species	Family	Regeneration guild	Median leaf lifespan (d) <sup>1</sup>		Seedling survival <sup>2</sup>		%leaf-damage
			Gap	Shade	Gap	Shade	
<i>Aspidosperma spruceanum</i>	Apocynaceae	T	867	> 1074	0.96	0.96	23.0
<i>Calophyllum longifolium</i>	Clusiaceae	T	701	1253	0.71	0.79	45.8
<i>Tetragastris panamensis</i>	Burseraceae	T	672	1044	0.85	1.00	72.7
<i>Gustavia superba</i>	Lecythidaceae	T	643	> 1170	0.92	0.75	47.0
<i>Trichilia tuberculata</i>	Meliaceae	T	643	> 1260	1.00	0.94	19.5
<i>Brosimum alicastrum</i>	Moraceae	T	614	1028	0.67	0.90	45.8
<i>Randia armata</i>	Rubiaceae	T	531	957	0.97	0.97	29.0
<i>Posoqueria latifolia</i>	Rubiaceae	T	523	1104	0.91	1.00	63.3
<i>Virola surinamensis</i>	Myristicaceae	T	504	1091	0.93	1.00	74.8
<i>Pouteria reticulata</i>	Sapotaceae	T	447	> 991	0.96	0.95	21.6
<i>Beilschmiedia pendula</i>	Lauraceae	T	420	1119	0.83	0.85	54.4
<i>Lacistema aggregatum</i>	Salicaceae	G	391	584	1.00	0.88	27.7
<i>Pachira sessilis</i>	Malvaceae	T	336	553	0.88	0.97	78.2
<i>Eugenia nesiotica</i>	Myrtaceae	T	307	636	0.94	0.56	32.4
<i>Dipteryx oleifera</i>	Fabaceae	G	280	na	0.94	na	95.0
<i>Vochysia ferruginea</i>	Vochysiaceae	G	280	728	0.93	0.63	50.2
<i>Tabernaemontana arborea</i>	Apocynaceae	T	252	441	1.00	0.97	65.8
<i>Pentagonia macrophylla</i>	Rubiaceae	G	252	483	0.83	0.67	73.8
<i>Tocoyena pittieri</i>	Rubiaceae	T	252	593	1.00	0.96	23.6
<i>Genipa americana</i>	Rubiaceae	T	224	532	0.89	0.94	76.2
<i>Hybanthus prunifolius</i>	Violaceae	T	224	350	1.00	0.88	52.3
<i>Jacaranda copaia</i>	Bignoniaceae	G	224	na	0.71	0.18	81.5
<i>Anacardium excelsum</i>	Anacardiaceae	G	161	167	0.94	0.61	77.3
<i>Ceiba pentandra</i>	Malvaceae	G	140	na	0.87	0.00	66.2

Species are listed in decreasing order of median leaf lifespan in gaps. See the Materials and Methods section for details.

<sup>1</sup>For species with long leaf lifespan, > x d, where x indicates the maximum leaf age recorded at the end of the study (proportion of leaves alive at the indicated age: *A. spruceanum*: 87%, *G. superba*: 55%, *T. tuberculata*: 61%, and *P. reticulata*: 81%, according to Kaplan–Meier estimates of survivorship curves); na, not available due to high seedling mortality in shade.

<sup>2</sup>Proportion of seedlings that survived for 90 d after planting to gaps and shade common gardens.

calculated the proportion of seedlings alive 90 d post-transplant, pooling conspecific seedlings across gardens within each light environment (Table 2). The proportion of seedlings surviving 90 d in shade gardens was positively correlated with the shade tolerance index of saplings (1–4 cm diameter at breast height) used in the community-wide analysis of Comita *et al.* (2010) ( $n = 22$ ,  $r = 0.67$ ,  $P = 0.008$ ). The age at leaf death was analysed using the Kaplan–Meier method, which accounts for censored leaves that were removed from the census due to harvest or death of the plant. Table S1 presents sample sizes for leaf demography, which ranged from 125 to 1273 leaves per species.

### Measurements of herbivory and leaf disks across leaf age gradients

Leaf traits were determined between May and July 2004 in the BCNM at the middle of the field campaign (June 2004), when seedling age ranged from 272 to 749 d among species (average = 572 d). Because leaf traits may change with leaf age (e.g. Kitajima *et al.*, 1997, 2002; Mediavilla *et al.*, 2008), we preselected five pairs of leaves of similar age per plant to cover the full range of leaf ages within each individual. For each preselected leaf, the areal proportion removed by herbivores was measured

nondestructively with a gridded transparent sheet. Levels of herbivory were represented by two variables – the average percentage of area missing (henceforth, %area) and the percentage of leaves with holes and/or missing edges (henceforth, % leaf damage).

One leaf disk (1.41 cm<sup>2</sup> each) was collected from each leaf using a cork borer, avoiding major veins as much as possible. Disks were placed between moist filter paper in plastic bags in a cooler chilled with ice and brought back to the laboratory. Subsequent monitoring of leaf survival detected no significant difference in leaf demography between intact leaves and sampled leaves. After determining fresh mass, one disk from each pair of same-aged leaves was used for measurements of toughness, lamina thickness and dry mass contents, while a second disk was used for methanol extraction to quantify phenolics and tannin. In gaps, all 24 species were sampled. In shade, seedlings were too small for many species and only four species with sufficient seedling size and number could be included.

Fracture toughness was measured with the cutting method developed by Lucas & Pereira (1990), using a pair of scissors mounted on a portable universal tester (Darvell *et al.*, 1996). Lamina work-to-shear ( $W_s$ , J m<sup>-1</sup>) was obtained by dividing total work by the length of the cutting pass. Lamina thickness ( $T$ ) was measured using an analogue thickness gauge (Teclock SM112,



Nagano, Japan). Lamina fracture toughness ( $\tau$ ,  $\text{J m}^{-2}$ ) was calculated by dividing  $W_s$  by  $T$ . All cut pieces were collected and dried at  $65^\circ\text{C}$  to determine dry mass. Leaf mass per area (LMA) was determined by dividing disk dry mass by disk area. Leaf tissue density ( $\rho$ ) was determined by dividing LMA by  $T$ . Density-corrected toughness was calculated by dividing  $\tau$  by  $\rho$  (Eqn 2). Analysis of phenolics and tannin followed the micro-analytical method described in detail by Lucas *et al.* (2001) and Dominy *et al.* (2003), after combining leaf disks to obtain 0.1 g fresh sample per plant (see Notes S1 for further details).

### Nitrogen and fibre contents

Leaf nitrogen concentrations were determined from a separate set of leaf disks sampled from 10 species with sufficiently large plants in gaps as part of a study focusing on leaf age effects on photosynthetic physiology (Stefanescu, 2006). After drying at  $60^\circ\text{C}$  to constant weight, nitrogen content per unit mass (%N) was determined using an elemental analyzer (Costech Analytical Model 4010, Valencia, CA, USA). Because of strong negative effects of leaf age, %N was estimated for a standardized age of 30 d from species-specific regressions of %N vs leaf age for comparison across species.

Cell wall fibre contents were determined for 21 species with a method modified from Van Soest (1963) to determine neutral detergent fibre (%NDF), acid detergent fibre (%ADF) and %lignin on an ash-free mass basis. This used dried leaf samples from 3- to 4-yr-old plants that were destructively harvested in 2005–2006 from the same common garden experiment (see Notes S1 for further details). The %hemicellulose (= %NDF – %ADF) value represents the water-soluble fibre fraction consisting mostly of hemicelluloses and pectin, which provide pliable cross-links between cellulose microfibrils (Carpita & Gibeau, 1993). The %cellulose (= %ADF – %lignin) value is a measure of the relative abundance of cellulose microfibrils which influence the directional tensile strength of primary and secondary cell walls (Carpita & Gibeau, 1993). By contrast, large chemically recalcitrant lignin molecules consist of irregular conjugations of many phenolic molecules and provide permanent and rigid cross-linkages within the secondary cell wall (Taiz & Zeiger, 2002). The structural combination of these heterogeneous materials with contrasting mechanical properties determines the toughness of leaf tissues (Jeronimidis, 1980; Lucas *et al.*, 2000). Fibre contents per unit volume (e.g.  $\text{g cellulose cm}^{-3}$ ) were also examined for potential correlation with nonfibre traits.

### Statistical analysis

We evaluated path models based on the *a priori* relationships shown in Fig. 1, but because  $W_s$ ,  $\tau$  and  $\gamma$  were not measured independently (Eqns 1 and 2), we compared alternative models, each including only one of these three traits. The overall model fit and the significance of each causal path were examined using structural equation modelling software (AMOS v5, AMOS Development Corporation; Grace, 2006). Path coefficients ( $\beta$ ) were estimated with log-ratio maximum likelihood. The total

effect of one variable on another is the total of path coefficients for direct paths plus the products of path coefficients for indirect paths via other variables. Model fit was assessed with a  $\chi^2$ -test that determines whether hypothesized trait correlations deviate significantly from observed correlations (Grace, 2006). Non-significance ( $P > 0.05$ ) meant failure to reject the path model. Alternative causally plausible models were evaluated through addition and subtraction of individual paths and variables, and evaluated with the Akaike Information Criterion (AIC).

Phylogenetically independent contrasts (PICs) were obtained with Phylomatic software available online (<http://www.phylodiversity.net/phyloomatic/>), based on the APG3-derived megatree. One polytomy reduced the total number of PICs that could be analysed to 22 for the 24 species. Pairwise PIC correlations were calculated with the Phylocom v. 4.1, Analysis of Traits module (Webb *et al.*, 2008), assuming uniform branch lengths. Because observed variation in leaf toughness and its relationship with leaf lifespan may be largely due to variations between gap-dependent and shade-tolerant guilds rather than within each guild (Dominy *et al.*, 2008), we used ANCOVA to evaluate how trait correlations may be influenced differently for the two regeneration guilds. All standard statistical analyses (Pearson correlations, ANOVA and ANCOVA) were performed with JMP v8.0 (SAS Institute, Cary, NC, USA).

## Results

### Species differences in leaf lifespan, herbivory and leaf traits

The 24 species varied widely in leaf lifespan, herbivory damage and seedling survival (Table 2). The median leaf lifespan varied from 160 to 867 d and from 167 to  $> 1260$  d for seedlings growing in gaps and in shade, respectively. In all species, leaf lifespan was always longer for shade-grown seedlings than gap-grown seedlings (Table 2, paired  $t = 8.0$ ,  $P < 0.001$ ). For 17 species for which median leaf lifespan could be determined in both environments, there was a strong correlation between the two environments ( $r = 0.90$ ,  $P < 0.001$ , Fig. S1). Shade-tolerant species tended to have longer leaf lifespan than gap-dependent species within a given environment, but there was substantial variation within each guild and overlap between guilds (Table 2, Fig. S1). Herbivory also exhibited wide ranges (minimum to maximum of species means), both in terms of proportion of leaves showing damage (%leaf-damage, 19.5–95%, Table 2) and proportion of area missing (%area, 0.3–41.3%, Table 3).

Leaf functional traits measured for gap-grown seedlings varied widely among species with negligible effects of leaf age except for %N (see Tables 3 and S1 for species mean values and ANCOVA results for the effects of species, leaf age and their interaction). For toughness-related traits, species means ranged two- to eight-fold as follows: lamina thickness (0.13–0.29 mm), density (0.24–0.42  $\text{g cm}^{-3}$ ), LMA (32.8–86.4  $\text{g m}^{-2}$ ),  $W_s$  (0.019–0.155  $\text{J m}^{-1}$ ),  $\tau$  (83.5–525.5  $\text{J m}^{-2}$ ), %cellulose (9.7–33.6%), and %N (1.38–3.07%, at 30 d after leaf full expansion). Many of the predicted correlations amongst these traits (Fig. 1) were

**Table 3** Species means for selected leaf traits relevant for toughness for gap-grown juveniles (listed in the order of median leaf lifespan as in Table 2)

Species	%herbivory	Lamina thickness ( <i>T</i> , mm)	Lamina density ( $\rho$ , g cm <sup>-3</sup> )	LMA (g m <sup>-2</sup> )	Work-to-shear ( $W_s$ , J m <sup>-1</sup> )	Fracture toughness ( $\tau$ , J m <sup>-2</sup> )	Density-corrected toughness ( $\gamma$ , mJ g <sup>-1</sup> m)	%Cellulose	%Nitrogen
<i>A. spruceanum</i>	0.3	0.22	0.36	78.1	0.097	438.6	1.22	28.5	1.71
<i>C. longifolium</i>	5.8	0.29	0.29	86.4	0.155	525.5	1.80	33.6	1.38
<i>T. panamensis</i>	6.9	0.16	0.37	57.5	0.069	429.0	1.15	21.3	1.71
<i>G. superba</i>	10.4	0.15	0.37	52.9	0.050	349.7	0.95	23.2	2.89
<i>T. tuberculata</i>	6.8	0.16	0.42	68.7	0.048	289.4	0.69	13.2	2.17
<i>B. alicastrum</i>	4.4	0.21	0.32	66.6	0.052	253.4	0.79	16.3	
<i>R. armata</i>	3.1	0.19	0.33	61.0	0.039	209.3	0.63		
<i>P. latifolia</i>	18.3	0.21	0.34	71.6	0.060	284.3	0.84	21.2	
<i>V. surinamensis</i>	11.1	0.15	0.31	47.9	0.035	223.8	0.71	17.6	1.88
<i>P. reticulata</i>	6.0	0.14	0.38	52.4	0.038	267.4	0.70	21.1	
<i>B. pendula</i>	5.7	0.21	0.38	76.6	0.070	345.8	0.91	16.7	
<i>L. aggregatum</i>	8.2	0.15	0.25	35.5	0.040	273.8	1.11	30.4	
<i>P. sessilis</i>	9.2	0.18	0.29	53.2	0.050	273.7	0.95	22.5	
<i>E. nesiotica</i>	15.4	0.13	0.35	42.6	0.035	268.6	0.77	19.8	
<i>D. oleifera</i>	41.3	0.17	0.24	41.1	0.040	244.4	1.01	23.5	
<i>V. ferruginea</i>	4.8	0.18	0.23	42.3	0.045	252.6	1.11	17.9	2.96
<i>T. arborea</i>	11.6	0.13	0.28	32.8	0.019	147.8	0.53	14.2	2.93
<i>P. macrophylla</i>	7.2	0.23	0.24	54.4	0.074	321.9	1.34	27.1	
<i>T. pittieri</i>	1.6	0.15	0.33	47.7	0.026	189.7	0.57		
<i>G. americana</i>	23.9	0.17	0.27	42.6	0.029	170.8	0.63	12.5	2.08
<i>H. prunifolius</i>	10.0	0.13	0.27	34.9	0.013	101.8	0.37	18.8	
<i>J. copaia</i>	4.6	0.14	0.35	46.7	0.011	83.5	0.24	9.7	
<i>A. excelsum</i>	9.4	0.18	0.28	47.2	0.054	301.8	1.07		1.83
<i>C. pentandra</i>	7.6	0.15	0.24	36.5	0.019	122.0	0.51	13.5	3.07
Sp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Leaf age	0.77	0.10	0.15	0.52	0.92	0.74	0.26		<0.001
Interaction	0.04	0.45	0.63	0.06	0.47	0.44	0.03		<0.001

Shown at the bottom are *P*-values for ANCOVAs used to evaluate species differences, effects of leaf age, and species × leaf age interactions for traits determined with leaves of known age and for ANOVA for %cellulose, which was determined at plant level. See Supporting Information Table S1 for additional trait values.

LMA, leaf mass per area.

significant and similar in strength and direction for species mean and PIC values (Table 4), as explained further in the next section. For traits that were not related to toughness, species means also ranged widely (Table S1) as follows: NDF (37.1–68.5%), lignin (8.1–25.9%), phenolics (1.2–23.1 mg gallic acid equivalent g<sup>-1</sup> dry mass), tannins (0–18.7 quebracho tannin equivalent g<sup>-1</sup> dry mass; 15 species had no detectable tannin with the radial diffusion method).

### Correlates of leaf toughness at material, tissue and structural levels

Cell wall fibre contents per unit mass, including %hemicellulose, %cellulose and %lignin, varied independently of each other (Table S2). As expected (Fig. 1), density-corrected toughness ( $\gamma$ ) correlated strongly and positively with %cellulose ( $r = 0.84$ ,  $P < 0.001$ , Fig. 2a). By contrast,  $\gamma$  had a marginally significant, negative correlation with %hemicellulose ( $r = -0.44$ ,  $P = 0.04$ ) and no significant correlation with the other fibre contents (Tables 4, S2). Although %NDF is often considered to be a good approximation of cell wall dry mass fraction (approximately, %NDF = %hemicellulose + %cellulose + %lignin), %NDF was not correlated with  $\gamma$  ( $r = -0.33$ ,  $P = 0.60$ ).

As expected (Fig. 1), fracture toughness ( $\tau$ ) was correlated with  $\gamma$ , %cellulose, cellulose : N ratio, and cellulose per unit volume (Table 4). Unexpectedly,  $\tau$  did not have a significant pairwise correlation with lamina density,  $\rho$  ( $r = 0.33$ ,  $P = 0.11$ ). Yet,  $\tau$  was more strongly correlated with cellulose per unit volume than with %cellulose (Table 4, Fig. 2b), presumably because the former incorporates the joint effects of  $\rho$  and %cellulose.  $\rho$  varied independently of  $T$  and %cellulose (Table 4,  $P > 0.4$ ), while  $T$  and %cellulose were weakly correlated (Table 4,  $P = 0.02$ ). As expected (Fig. 1), work-to-shear ( $W_s$ ) was correlated with both  $\tau$  ( $r = 0.94$ ) and  $T$  ( $r = 0.80$ ).

### Phenotypic plasticity of toughness-related traits with leaf age and light environment

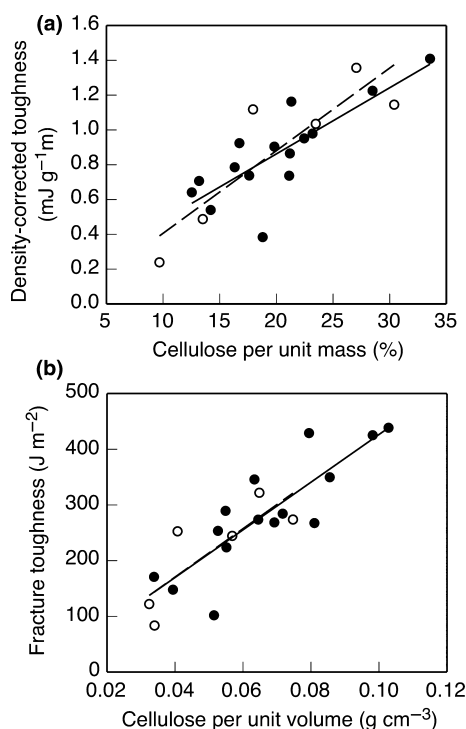
Leaf age had a nonsignificant or negligibly small effect on all leaf traits of gap-grown seedlings except for %N (which declined with leaf age, Table 3) and leaf size (which was smaller for older leaves that had developed when seedlings were smaller, Table S1). Light environment, on the other hand, affected several toughness-related traits in four to seven shade-tolerant species for which leaf traits were also determined in shade (see Table S3 for species means for shade-grown plants and the results of two-way

**Table 4** Pairwise correlations for leaf traits relevant for leaf toughness and nutritional quality, using species mean values for gap-grown plants

	%Cellulose	%lignin	Cellulose : N	Density-corrected tough. ( $\gamma$ )	Lamina density ( $\rho$ )	Cellulose per volume	Fracture tough. ( $\tau$ )	Lamina thick ( $T$ ) <sup>1</sup>	Leaf mass per area <sup>1</sup>	Work-to-shear <sup>1</sup>
<i>n</i>	21	21	10	24	24	21	24	24	24	24
%Cellulose		0.39		<b>0.92</b>		<b>0.90</b>	<b>0.86</b>	<b>0.59</b>	0.45	<b>0.83</b>
%lignin	0.28			0.34	-0.12	0.25	0.21	0.27	0.16	0.28
Cellulose : N	<b>0.93</b>	0.34								
Density-corrected toughness ( $\gamma$ )	<b>0.84</b>	0.33	<b>0.86</b>		-0.13	<b>0.82</b>	<b>0.90</b>	<b>0.71</b>	0.48	<b>0.91</b>
Lamina Density ( $\rho$ )	-0.16	-0.01	0.34	-0.15		0.46	0.33	0.07	<b>0.65</b>	0.23
Cellulose per volume	<b>0.83</b>	-0.24	<b>0.90</b>	<b>0.71</b>	0.40		<b>0.92</b>	<b>0.58</b>	<b>0.69</b>	<b>0.85</b>
Fracture toughness ( $\tau$ )	<b>0.69</b>	0.29	<b>0.87</b>	<b>0.87</b>	0.33	<b>0.86</b>		<b>0.73</b>	<b>0.76</b>	<b>0.96</b>
Lamina thickness ( $T$ ) <sup>1</sup>	0.49	0.31	<b>0.80</b>	<b>0.64</b>	-0.07	0.41	<b>0.61</b>		<b>0.84</b>	<b>0.85</b>
Leaf mass per area <sup>1</sup>	0.31	0.25	<b>0.85</b>	0.41	<b>0.58</b>	<b>0.60</b>	<b>0.70</b>	<b>0.77</b>		<b>0.79</b>
Work-to-shear <sup>1</sup>	<b>0.68</b>	0.36	<b>0.91</b>	<b>0.88</b>	0.19	<b>0.75</b>	<b>0.94</b>	<b>0.80</b>	<b>0.76</b>	

Pearson correlation coefficients are shown below the diagonal for species-level correlations (number of species, *n*, indicated in the first row), and above the diagonal for phylogenetically independent contrast correlations. Significance of correlation is indicated by bold italic ( $P < 0.001$ ), bold non-italic ( $P < 0.01$ ) and italic ( $P < 0.05$ ). For cellulose : N ratio, PIC correlation cells are blank because nitrogen-leaf age relationship was determined only for 10 species.

<sup>1</sup>log-transformed prior to analysis.



**Fig. 2** The relationship of toughness with cellulose contents for leaves of seedlings of 21 tropical tree species: (a) density-corrected toughness against cellulose per unit mass, and (b) fracture toughness against cellulose per unit volume. Open and closed symbols represent species mean values for gap-dependent species and shade-tolerant species, respectively. ANCOVA shows no significant effects of guilds on the slope and elevation of either relationship. See Table 4 for correlations across gap and shade guilds.

ANOVA on the effects of light environment and species). LMA,  $\rho$ , %cellulose and %lignin were greater for gap-grown seedlings, while  $\gamma$  and %phenolics were greater for shade-grown seedlings ( $P < 0.001$  for all). Despite these plastic changes, neither  $T$ ,  $\tau$  nor  $W_s$  differed significantly between gap- and shade-grown

seedlings ( $P > 0.05$ ). In terms of two measures of herbivory, %leaf-damage was greater in gaps than shade ( $P = 0.03$ ), while %area did not differ significantly between gaps and shade. When species–environment interactions were significant ( $\gamma$ , %cellulose, %phenolics, %lignin), the direction of phenotypic changes between gap and shade was consistent across species but the magnitudes of change differed among species.

#### Correlations of leaf lifespan with toughness-related traits

Median leaf lifespan of gap-grown seedlings was positively correlated with all measures of toughness and cellulose, but not with phenolics and tannin (Table 5). Across 24 species, leaf lifespan was strongly correlated with  $\tau$  ( $r = 0.73$ ) and  $W_s$  ( $r = 0.73$ ), but only marginally with  $\gamma$  ( $r = 0.40$ ) and  $T$  ( $r = 0.40$ ). Leaf lifespan was also positively correlated with  $\rho$  ( $r = 0.60$ ) and cellulose per volume ( $r = 0.77$ ), which were also the only two traits correlated with %leaf-damage ( $r = -0.46$  and  $-0.48$ , respectively; Table 5). Median leaf lifespan was negatively correlated with %leaf-damage ( $r = -0.45$ ,  $P = 0.027$ ). Fibre fractions other than cellulose (NPE, lignin, ash) were not significantly correlated with leaf lifespan or %leaf-damage.

Cellulose provided the material-basis for leaf toughness, as indicated by the strong linear relationships after appropriate normalization (Fig. 2). Shade-tolerant and gap-dependent guilds exhibited an identical relationship between %cellulose and density-corrected toughness (Fig. 2a) and between cellulose per unit volume and fracture toughness (Fig. 2b). However, the relationships of leaf lifespan with cellulose and fracture toughness differed between shade-tolerant and gap-dependent guilds (Fig. 3). This was true whether cellulose content was expressed per unit dry mass or volume (Fig. 3a,b) and whether fracture toughness was corrected for density or not (Fig. 3c,d). The positive effect of cellulose contents and fracture toughness on leaf lifespan was steeper for shade-tolerant species than for gap-dependent species. Gap-dependent species may have moderately tough leaves, but

**Table 5** Pairwise correlations of ecological traits (median leaf lifespan, percentage of leaves damaged by herbivory, seedling survival in shade and gaps) with leaf traits

	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i> <sub>PIC</sub>	<i>r</i> <sub>PIC</sub>
Correlation of median leaf lifespan with					
Density-corrected toughness	24	0.40	0.053	22	0.49
Cellulose per mass	21	0.44	0.045	19	<b>0.60</b>
Cellulose : nitrogen ratio	10	<b>0.80</b>	0.005		
Fracture toughness	24	<b>0.72</b>	<0.001	22	<b>0.77</b>
Lamina density	24	<b>0.60</b>	0.002	22	<b>0.64</b>
Cellulose per volume	21	<b>0.77</b>	<0.001	19	<b>0.60</b>
Log (work-to-shear)	24	<b>0.66</b>	<0.001	22	<b>0.72</b>
Log (LMA)	24	<b>0.76</b>	<0.001	22	<b>0.80</b>
Log (Lamina thickness)	24	0.43	0.054	22	<b>0.57</b>
%Phenolics	23	0.01	NS	22	-0.18
%Tannin	23	0.24	NS	22	0.17
%Leaf-damage	24	-0.45	0.027	22	<b>-0.53</b>
Correlation of %leaf-damage with					
Cellulose per volume	21	-0.48	0.028	19	-0.38
Lamina density	24	-0.46	0.024	22	<b>-0.62</b>
Correlation of shade seedling survival with					
Median leaf lifespan	23	0.49	0.017	22	0.44
Cellulose per volume	21	0.40	0.080	19	0.18
Fracture toughness	23	0.35	0.100	22	0.33
Correlation of gap seedling survival with					
Log (lamina thickness)	24	<b>-0.52</b>	0.010	22	<b>-0.55</b>
Log (LMA)	24	-0.44	0.030	22	-0.46
%leaf-damage	24	-0.42	0.039	22	-0.20

Shown are Pearson correlation coefficients (*r*, followed by level of significance *P*) and for phylogenetic independent contrasts (*r*<sub>PIC</sub>). Only correlations with *r* > 0.35 are shown, except for correlation of leaf lifespan with phenolics and tannin. *n*, number of species; *n*<sub>PIC</sub>, number of PICs. Three traits that reflect lamina thickness (work-to-shear, LMA, lamina thickness) are log-transformed to improve normality. Significance of correlation is indicated by bold italic (*P* < 0.001), bold non-italic (*P* < 0.01) and italic (*P* ≤ 0.05).

their leaf lifespan was shorter than shade-tolerant species with similarly tough leaves (Fig. 3c,d).

### Correlations of leaf traits with seedling survival

Different leaf traits were correlated with early seedling survival in understorey vs gaps. Shade survival of seedlings was positively correlated with leaf lifespan, cellulose per unit volume and fracture toughness (Table 5). Gap survival of seedlings was negatively correlated with lamina thickness, LMA and %leaf-damage. These pairwise correlations were also supported by PICs (Table 5). The positive relationship of shade survival with leaf lifespan was steeper for gap-dependent species than for shade-tolerant species (ANCOVA, guild main effect = ns, lifespan as covariate significant at *P* = 0.02, guild × lifespan interaction significant at *P* = 0.02).

### Direct and indirect effects of toughness-related traits on leaf lifespan

We compared alternative path models (Fig. 4) to evaluate direct and indirect effects of cellulose content, lamina density and toughness on median leaf lifespan. Each alternative model included only one measure of toughness  $\gamma$ ,  $\tau$  or  $W_s$ , because they were not determined independently of each other. Figure 4 shows the best-fit model that included  $\gamma$  (Model A),  $\tau$  (Model B) and  $W_s$  (Model C) in which the linkage between cellulose and toughness were evaluated.

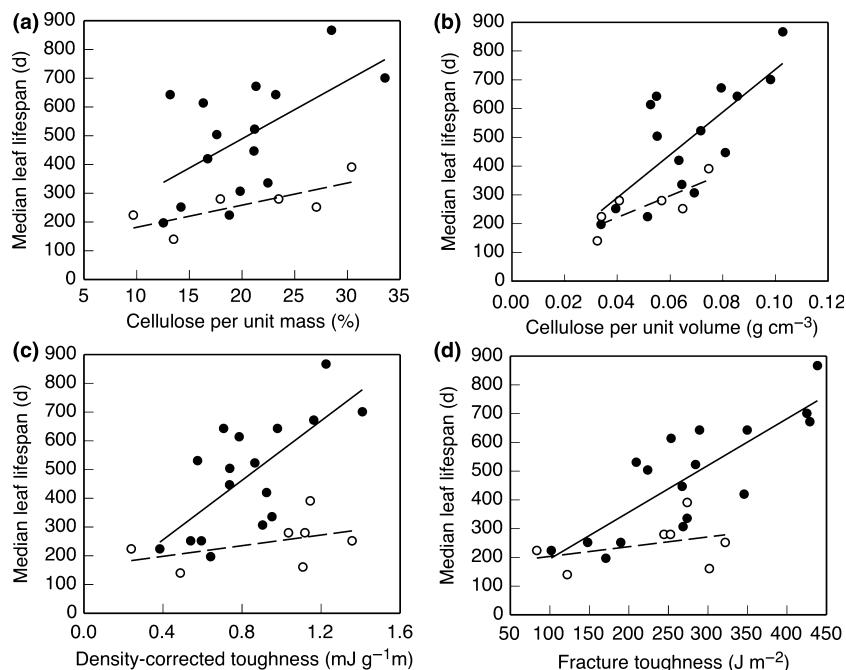
Model A predicts that cellulose per unit mass directly enhances  $\gamma$ , but only enhances leaf lifespan indirectly via its effect on  $\gamma$ . Adding a direct and nonsignificant path from cellulose to leaf lifespan ( $\beta = 0.28$ , *P* = 0.14) to Model A would minimally affect the support ( $\Delta$ AIC = 0.1; Fig. S2a). Adding lamina thickness (*T*) to Model A, with its covariance with cellulose per unit mass (*r* = 0.49, *P* = 0.04) and an insignificant direct path to leaf lifespan would significantly reduce the model fit ( $\chi^2 = 10.0$ , *P* = 0.08,  $\Delta$ AIC = 13.3).

In Model B,  $\tau$  is included instead of  $\gamma$ . As hypothesized in Fig. 1,  $\tau$  depended on lamina density and cellulose per unit mass. Lamina density also had a significant direct effect on leaf lifespan in addition to its indirect positive effect via  $\tau$ . The data did not support the addition of *T* to Model B (*P* < 0.001). If cellulose per unit mass was replaced with cellulose per unit volume – because the latter incorporates the effect of lamina density – there was no significant path from lamina density to  $\tau$  (Fig. S2b). Models A and B were similarly supported by the data ( $\Delta$ AIC = 1.4), although Model B explained a slightly greater proportion of interspecific variation in leaf lifespan than Model A.

In Model C,  $W_s$  is chosen as the mechanical measure. As hypothesized in Fig. 1,  $W_s$  was influenced by both cellulose and *T*, covariance of which must be included for the model fit. Alternative models modified from Model C by dropping either *T* or cellulose (Fig. S2c,d, respectively) also fit the data.

In summary, comparisons across the three alternative models in Fig. 4 show that lamina density always had a significant direct





**Fig. 3** The effects of cellulose contents and toughness on median leaf lifespan for the two regeneration guilds of tropical trees, gap-dependent (open symbols with broken trend line) and shade-tolerant (closed symbols with solid line). The physical defence traits (ANCOVA results associated with each) are: (a) cellulose per unit dry mass (guild  $P = 0.006$ ; covariate  $P = 0.02$ ; interaction, not significant (ns)), (b) cellulose per unit volume (guild  $P = 0.05$ ; covariate  $P = 0.006$ ; interaction, ns), (c) density-corrected toughness (guild  $P < 0.001$ ; covariate  $P = 0.003$ ; interaction  $P = 0.03$ ), and (d) fracture toughness (guild  $P = 0.001$ ; covariate  $P = 0.003$ ; interaction  $P = 0.03$ ).

positive effect on leaf lifespan, while cellulose had a significant indirect effect via toughness regardless of how the latter was normalized ( $\gamma$ ,  $\tau$  or  $W_s$ ). Lamina thickness, by contrast, had no direct effect on leaf lifespan, but did have a significant positive effect on  $W_s$  (Fig. 4c).

## Discussion

### Leaf mechanical properties and structural carbon allocation

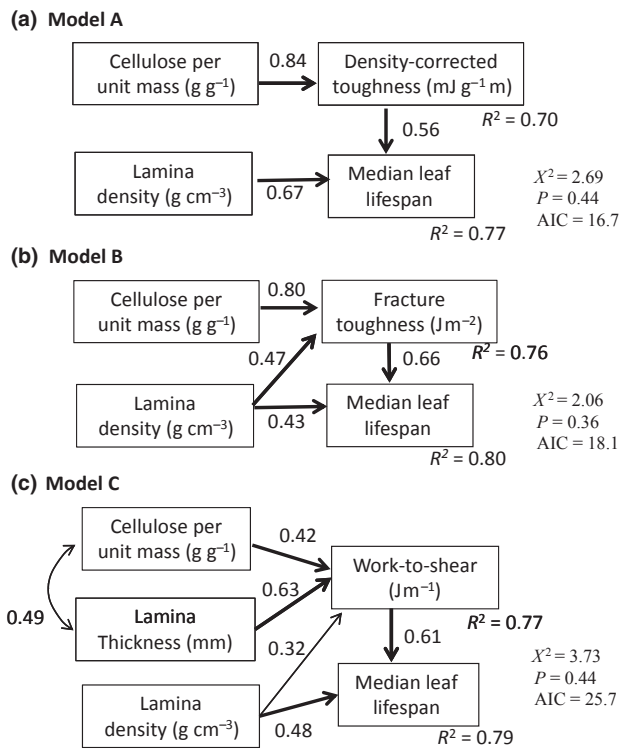
Both cellulose and lamina density contributed to leaf toughness and enhanced leaf lifespan (Figs 2–4), especially when their effects were considered together. Lamina density was a significant determinant of fracture toughness, but only when cellulose effect was simultaneously taken into account (Table 4, Fig. 4). Furthermore, the direct positive effect of lamina density on leaf lifespan was stronger when its indirect effect through toughness was simultaneously accounted for (Fig. 4), compared to a simple pairwise correlation (Table 5). The densest material component, cellulose, seems of overwhelming importance in setting the level of density-corrected toughness (Fig. 2a; Table 4), consistent with the results of Alvarez-Clare & Kitajima (2007) and Westbrook *et al.* (2011). Although all cell wall components are probably brittle in isolation, our results show that in the composite construction of cell walls, cellulose provides the indispensable skeleton. By contrast, density-corrected toughness was uncorrelated with lignin and hemicellulose (Table S2) and only weakly with %ADF (= %cellulose + %lignin) ( $r = 0.47$ ,  $P = 0.03$ ). This contrasts with a commonly held view in ecology that lignin contributes to toughness (Cornelissen *et al.*, 1999). Lignin has a role in stiffening the cell wall via water exclusion (increasing bending resistance; Alvarez-Clare & Kitajima, 2007) and is vital for ecosystem processes via its chemical properties, variously slowing digestion in animal guts, litter decomposition and nutrient

cycling (Aerts, 1997; Cornelissen *et al.*, 2004; Kurokawa & Nakashizuka, 2008). However, the lack of correlation between %lignin and toughness, also reported from Malaysian tropical forest (Kurokawa & Nakashizuka, 2008), means that toughness may not be an easy-to-measure ‘soft trait’ to substitute for ‘hard traits’ relevant to nutrient cycling (Diaz *et al.*, 2004).

### Phenotypic plasticity of leaf lifespan and carbon-based defence

Many leaf traits exhibit substantial phenotypic plasticity in response to leaf age, ontogenetic stages and the environmental conditions in which they develop, although intraspecific variation is generally believed to be smaller than interspecific variations (Gotsch *et al.*, 2010; Fajardo & Piper, 2011). We found that lamina density and LMA increased significantly with leaf age in some species, while toughness did not (Tables 3, S1). The leaf age gradient was unfortunately confounded with the light gradient within the crown and by ontogeny in the current study (i.e. older leaves were produced by much smaller seedlings). However, a previous study of seedling leaves at the same position 6 months apart (Alvarez-Clare & Kitajima, 2007) did not find any effects of leaf age on toughness either.

This common garden study avoided the confounding of *in situ* environment and habitat preference of the species. For example, gap phenotypes of gap-dependent species are often compared with shade phenotypes of shade-tolerant species in field-based samplings (e.g. Gotsch *et al.*, 2010; Onoda *et al.*, 2011), but in such comparisons it is ambiguous whether observed trait variations reflect evolutionary or plastic responses (Endara & Coley, 2011). Both the theory of optimal leaf lifespan and empirical studies predict that LMA, lamina thickness, and phenolic and tannin contents increase with higher light availability, while leaf lifespan decreases with light availability (Kikuzawa, 1984;



**Fig. 4** Three alternative path models supported by interspecific correlations among 21 tropical tree species. Each model tests a subset of the conceptual model (Fig. 1), including only one measure of mechanical resistance against fracture, either (a) density-corrected toughness (Model A), (b) fracture toughness (Model B), or (c) work-to-shear (Model C). The overall model fit is indicated by likelihood  $\chi^2$  (i.e. the data do not reject the illustrated models when  $P > 0.1$ ), and selected from alternative models by AIC comparisons (see Supporting Information Fig. S2 for alternative models with similar levels of overall fits).  $R^2$  values represent the proportion of interspecific variance in toughness and median leaf lifespan explained by each model. Unidirectional arrows indicate positive direct paths, with numbers indicating path coefficients (standardized partial regression coefficients,  $\beta$ ). Bidirectional arrows indicate significant correlations between two exogenous variables ( $P < 0.05$ ). All paths are supported at  $P < 0.001$  except for a thin arrow in Model C (lamina density to work-to-shear,  $P = 0.02$ ). See Tables 4 and 5 for pairwise correlations.

Ellsworth & Reich, 1992; Shure & Wilson, 1993; Ackerly & Bazzaz, 1995; Oguchi *et al.*, 2006; Barbehenn & Constabel, 2011). Our results matched most of these expectations, with shorter leaf lifespan, but greater LMA and leaf density in gap-grown than shade-grown plants.

Plastic responses to light environments may not coincide with adaptive responses to light environments (Shure & Wilson, 1993; Lusk *et al.*, 2008; Endara & Coley, 2011). Hence, adaptation to shade with longer leaf lifespan was accompanied by an increase in tissue density and toughness (Figs 3, 4), while acclimation to shade resulted in a decrease in tissue density and %cellulose, and inconsistent and small changes in fracture toughness (Table S3). Perhaps, the plastic response of fracture toughness ( $\tau = \rho \cdot \gamma$ ) to light is difficult to predict because density ( $\rho$ ) generally decreases with shading (Niinemets, 2001; Oguchi *et al.*, 2006; Kitajima & Poorter, 2010), while density-corrected toughness ( $\gamma$ ) tends to increase with shading (thus ‘shade leaves punch above their weight’; Lusk *et al.*, 2010; Onoda *et al.*, 2011).

### Importance of cellulose-based toughness for long leaf lifespan

Despite recognition of the ecological importance of leaf toughness for 40 years or more (Feeny, 1970), few studies have explicitly examined toughness at different levels of the leaf structural organization. Being so heterogeneous, with different toughness values for different tissues, toughness at different levels may not show the same ecological trend. Density-corrected toughness ( $\gamma$ ) correlated closely with %cellulose, and  $\gamma$  and tissue density together contribute to fracture toughness and leaf lifespan (Fig. 4). It appears that fracture toughness and work-to-shear are similarly related to herbivory and leaf lifespan (Table 4). A similar conclusion was reached by Dominy *et al.* (2008) who re-analysed the leaf lifespan data of Coley (1983) with new measurements of fracture toughness, instead of the penetrometry in Coley’s original study. Although lamina thickness contributes to  $W_s$ , it shows little relationship with the demographic characteristics of species (Table 5, also Wright *et al.*, 2001; Kitajima & Poorter, 2010; Westbrook *et al.*, 2011; but see Funk & Throop, 2010).

Carbon investment in cellulose microfibrils and its resulting toughness predicted species differences in herbivory damage and leaf lifespan, while lignin, hemicellulose and nonstructural carbon-based defence such as phenolics and tannins did not. Only four species overlapped between the current study and 46 species studied by Coley (1983). In both studies, leaf toughness and cellulose per unit mass strongly correlated with leaf lifespan, while lignin and phenolics per unit mass did not. Greater importance of toughness or cellulose on herbivory and leaf lifespan, compared to phenolics or lignin, is also reported for temperate tree species (Matsuki & Koike, 2006; Mediavilla *et al.*, 2008; Pearse, 2011). A recent meta-analysis (Endara & Coley, 2011) also found that phenolics and tannin show no generalizable relationships with toughness, herbivory level or leaf lifespan, perhaps because individual tannins and phenolic compounds vary greatly in their properties and functions (Close & McArthur, 2002; Lokvam & Kursar, 2005; Barbehenn & Constabel, 2011).

Cell wall fibre may reduce herbivory not just because fibre increases toughness, but also because it reduces nutritional values, which are also affected by protein and water contents (Peeters, 2007; Clissold *et al.*, 2009). But, %NDF (total nondigestive fibre contents) were uncorrelated with herbivory and leaf lifespan. Other studies also found that herbivore choice and level are often influenced more by toughness than by fibre contents and digestibility (Hill & Lucas (1996) for primates, Clissold *et al.* (2004) for insects, Kurokawa & Nakashizuka (2008) for field herbivory rates). One interesting idea is that nutritional values may correlate better with herbivory when expressed per unit work to fracture (Clissold *et al.*, 2009). Hence, Lusk *et al.* (2010) proposed %cell content (=  $100 - \%NDF$ ) divided by density-corrected toughness as an indicator of the relative nutritional value per bite effort. In three of our four shade-tolerant species, this nutritional value index was higher for gap-grown seedlings than shade-grown seedlings similar to the finding by Lusk *et al.* (2010). However, this index was not correlated with %leaf-damage within the gap,

and only weakly with median leaf lifespan across 21 species ( $r = -0.46$ ,  $P = 0.03$ ).

### Leaf trait syndromes adaptive to gaps vs shade

Leaf lifespan and seedling survival were associated with leaf functional traits, but two unexpected results emerged. First, different sets of leaf traits were correlated with seedling survival in gaps vs shade (Table 5). Leaf lifespan, leaf toughness and lamina density were positively correlated with seedling survival in shade, but none of these traits were significant for seedling survival in gaps. Not surprisingly, a higher level of herbivory meant lower survival in gaps (Table 5). Lamina thickness and LMA, which might be associated with sclerophylly (Grubb, 1986; Wright *et al.*, 2001), had no correlation with %leaf-damage and unexpectedly negative effects on gap survival. We determined seedling survival for the first 3 months after germination, during which we observed that species with high mortality in gaps exhibited stunted growth suggestive of stress from excessive light and heat. Perhaps, constitutively thick leaves with high lamina density may limit efficient CO<sub>2</sub> exchange (Niinemets *et al.*, 2009) and safe channelling of excess radiation energy.

Second, the relationship of leaf lifespan with toughness differed between gap-dependent and shade-tolerant guilds (Fig. 3), even though the mechanistic link between cellulose and toughness was identical across the two groups (Fig. 2). Re-analysing the data of Coley (1983), Dominy *et al.* (2008) observed that the positive correlation of toughness with leaf lifespan and herbivory reflects species differences between shade-tolerant and pioneer guilds, but not within the shade-tolerant guild. By contrast, we found that leaf toughness was associated with leaf lifespan within the latter guild, similar to findings by Lusk *et al.* (2010) and Numata *et al.* (2003). Density-corrected toughness, the demographic implication of which was examined explicitly for the first time, had a strong positive effect on leaf lifespan for shade-tolerant species, but this was much weaker for gap-dependent species (Fig. 3c). Thus, at intermediate-to-high levels of biomass investment to cellulose (Fig. 3a), shade-tolerant species had greater leaf lifespans. This is perhaps because shade-tolerant and gap-dependent species may differ in anatomical arrangements of thick-walled cells and the ratio of mesophyll, vein and epidermal tissues. Such shifts in the relationship between leaf lifespan and leaf toughness between ecological guilds can also be found in relation to adaptation to other environmental factors. Wright & Westoby (2002) found that the relationship of leaf lifespan with both LMA and work-to-shear differed between species from low- and high-rainfall regions. It is interesting that longer leaf lifespan and toughness were only weakly associated within gap guild (Fig. 3). Perhaps, gap-dependent species are selected to have similarly rapid turnover of short-lived leaves to achieve fast growth (Kikuzawa, 1991; Ackerly, 1999; Portsmouth & Niinemets, 2007), while species difference in cellulose contents among gap-dependent species may reflect differences in leaf display and architecture. Future studies that integrate anatomy with mechanical properties, such as Onoda *et al.*'s (2008) approach for acclimation to light and nutrient, may reveal important

anatomical traits responsible for adaptive and acclimation responses of leaf traits to environments.

### Conclusion

Carbon allocation to cellulose, but not other cell wall components or phenolics and tannin, contributed to leaf toughness. Cellulose contents and density-corrected toughness were strongly associated with leaf lifespan within the shade-tolerant guild, but less so in gap-dependent species, which tended to possess shorter-lived leaves at the same %cellulose content and density-corrected toughness. Lamina density had both direct and indirect effects via toughness on herbivory level (negative) and leaf lifespan (positive). Toughness and long leaf lifespan were associated with better survival in shade, but not in gaps. Lamina thickness was uncorrelated with shade tolerance or leaf lifespan, but a combination of thick lamina and high LMA negatively influenced gap survival. Overall, our results place greater importance on cellulose-based physical defence than phenolic-based defence or nutritional values as correlates of species differences in herbivory and leaf lifespan associated with life history strategies and regeneration light requirements.

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## Supporting Information

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

**Fig. S1** Relationship of median leaf lifespan of shade-grown seedlings with leaf lifespan and toughness of gap grown seedlings.

**Fig. S2** Alternative models to the path models shown in Fig. 4 with adequate fit.

**Table S1** Species means for additional leaf traits measured with gap-grown seedlings

**Table S2** Correlations between cell wall fibre components and density-corrected toughness

**Table S3** Leaf traits measured with seedlings grown in understory shade gardens

**Notes S1** Additional methodological details on regeneration guild classification and trait measurements.

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