

# Large leaves in warm, moist environments confer an advantage in seedling light interception efficiency

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## Summary

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- Leaf size varies conspicuously along environmental gradients. Small leaves help plants cope with drought and frost, because of the effect of leaf size on boundary layer conductance; it is less clear what advantage large leaves confer in benign environments.
- We asked if large leaves give species of warm climates an advantage in seedling light interception efficiency over small-leaved species from colder environments. We measured seedling leaf, architectural and biomass distribution traits of 18 New Zealand temperate rainforest evergreens; we then used a 3-D digitiser and the Y<sub>PLANT</sub> program to model leaf area display and light interception.
- Species associated with mild climates on average had larger leaves and larger specific leaf areas (SLA) than those from cold climates, and displayed larger effective foliage areas per unit of aboveground biomass, indicating higher light interception efficiency at whole-plant level. This reflected differences in total foliage area, rather than in self-shading.
- Our findings advance the understanding of leaf size by showing that large leaves enable seedlings of species with highly conductive (but frost-sensitive) xylem to deploy large foliage areas without increasing self-shading. Leaf size variation along temperature gradients in humid forests may therefore reflect a trade-off between seedling light interception efficiency and susceptibility to frost.

## Introduction

Leaf size is one of the most obvious ways in which plant species differ (Raunkiaer, 1934), ranging over at least five orders of magnitude across the estimated 350 000 extant species of seed plants (Westoby *et al.*, 2002). Notwithstanding the diversity of leaf sizes within some species-rich assemblages (Westoby *et al.*, 2002), conspicuous variation in average leaf size along environmental gradients indicates that this trait must have important implications for plant function (Von Humboldt, 1850; Schimper, 1902; Bailey & Sinnott, 1916; Raunkiaer, 1934; Webb, 1968). However, progress towards a comprehensive theory of the adaptive significance of leaf size has been fitful.

Leaf energy balance theory (Parkhurst & Loucks, 1972) can explain the abundance of small leaves in arid and semiarid regions, and also on sites where subzero temperatures occur throughout much of the year (Wright *et al.*, 2017; Lusk *et al.*, 2018). Because of the effect of leaf width on boundary layer conductance, small leaves are closely coupled to air temperatures by convective exchange with the surrounding air. The thicker boundary layers of large leaves isolate them more from the surrounding air; as a result, they can depart several degrees from ambient air temperature when leaf energy balance is dominated by radiative heating or cooling (Parkhurst & Loucks, 1972). This

occurs if leaves are exposed to radiative heating when stomatal closure due to water shortage prevents evapotranspirative cooling, as is common in arid and semiarid regions. The temperatures of large leaves can also fall several degrees below air temperatures when exposed to radiative cooling on clear nights, making them vulnerable to frost damage (Parkhurst & Loucks, 1972; Leuning, 1988). Both daytime and night-time effects of leaf size on leaf temperatures have been confirmed by recent experimental work (Yates *et al.*, 2010; Leigh *et al.*, 2017; Lusk *et al.*, 2018).

It is less clear what advantages are conferred by the large leaves that are common in warm, moist environments (Wright *et al.*, 2017). Leaf energy balance theory predicts that evapotranspiration in hot, moist, sunny environments will cool large leaves to well below air temperatures, potentially enhancing net carbon gain by reducing respiration (Gates, 1968; Smith, 1978). Conversely, Michaletz *et al.* (2016) showed that large leaves should heat up to optimum temperatures for photosynthesis more rapidly than smaller leaves; but as this mechanism should be of most advantage in cool environments, it seems unable to explain the positive relationship of leaf size with temperature in environments where water is not strongly limiting. Studies of Australian woodland and forest species found evidence that large leaves enhance light interception efficiency at the shoot level: the size of individual leaves was positively correlated with the leaf area ratio

(LAR) of twigs (Wright *et al.*, 2006), and negatively correlated with self-shading (Falster & Westoby, 2003). However, as much of the leaf size variation in the aforementioned Australian datasets is linked to large site differences in water balance, it is unclear whether the patterns they report can be extrapolated to temperature-related variation in leaf size in moist environments.

We asked if leaf size variation along temperature gradients in New Zealand rainforests reflected a trade-off between seedling light interception efficiency and susceptibility to radiative chilling on clear nights. We have previously shown that seedlings of New Zealand evergreen angiosperm trees associated with mild winters have wider vessels, more conductive xylem and more foliage area per unit sapwood area than those native to cold environments, where narrow vessels reduce the risk of freeze–thaw embolism (Lusk *et al.*, 2013a). In this paper we show that large leaves are a vital component of this syndrome, enabling seedlings with highly conductive xylem to deploy large foliage areas without incurring counterproductive levels of self-shading, potentially resulting in a competitive advantage over smaller leaved species in warm, moist environments.

## Materials and Methods

### Study area

The three main islands of New Zealand span *c.* 12.5° of latitude, from 34.5° to 47°S. New Zealand climates are broadly described as oceanic temperate (McGlone *et al.*, 2016), with much weaker seasonality than north temperate continental regions. Mean annual temperatures of sites occupied by forest vegetation in New Zealand range from 6 to 7°C at treelines (Cieraad & McGlone, 2014) to 16°C in the far north (NIWA, 2001). Air frosts are rare on northern and western coasts of North Island, but occur throughout most of the year in intermontane basins of eastern South Island and on parts of the volcanic plateau that occupies central North Island (NIWA, 2016). Mean annual precipitation ranges from >4000 mm on high mountains close to the west coast, to <1000 in eastern lowland districts, where potential evapotranspiration often exceeds rainfall (Lusk *et al.*, 2016).

### Plant material

Seedlings of 18 common canopy and subcanopy angiosperm tree species were obtained from commercial nurseries (Table 1). These included species typical of a wide range of forest types throughout New Zealand (Wardle, 1991). Four to five replicate plants of each species were used (Supporting Information Table S1).

Plants were acclimated to a common light environment by growth in a glasshouse for 14 wk (beginning of October 2011 to early January 2012, before trait measurements began; Lusk *et al.*, 2013a). Mean initial height of species ranged from 197 to 316 mm; differences in growth rate resulted in a wider range of mean final heights (274–877 mm; Table 1). Plants were grown in bags filled with a commercial potting mix consisting of

composted pine bark, raw pine bark fibre and 7 mm pumice, plus small amounts of slow-release fertiliser, gypsum, lime, dolomite and a wetting agent. pH was roughly 5.5. Individual plants were randomly assigned to positions on two adjacent benches, and positions were re-randomised every 3 wk. Temperatures were regulated within the approximate range of 5 and 25°C. At temperatures below 5°C, a thermostat activated gas heaters and a forced air ventilation system at temperatures exceeding 25°C. Plants were inspected daily and watered by hand when required. Photosynthetic photofluxes reaching the benches in the glasshouse averaged *c.* 20% of those measured outside. The translucent nature of the roofing material meant that plants received only diffuse light during the middle of the day, but the glass of the upper walls allowed brief periods of direct illumination during the late afternoon.

### Characterising species positions on temperature gradients

We used species' occurrence records and GIS climate surfaces to characterise the average thermal environments occupied by each species. Although mean annual temperature (MAT) is often used to characterise thermal environments, minimum temperatures have more immediate relevance to plant survival and species sorting (Koehler *et al.*, 2012; Kreyling *et al.*, 2015). We obtained occurrence records of each species in national distribution databases (New Zealand Plant Conservation Network, 2016), and then used GIS surfaces to obtain MAT and mean minimum temperatures of the coldest month ( $T_{\min}$ ) of each occurrence from (Landcare Research, 2011). Average MAT and  $T_{\min}$  were then computed for the distribution of each species.

There is an argument for relating seedling performance and functional traits to local climate at the site of origin, as provenance studies often show significant geographical variation in maximum growth rates and/or frost resistance (for example Wilcox & Ledgard, 1983; Hawkins *et al.*, 1991). However, we were able to identify seedling provenances of only 16 of the 18 species we worked with (Table 1). MAT and  $T_{\min}$  at provenance sites of these 16 species were strongly correlated with the equivalent parameters averaged across the recorded distribution of the same species ( $r=0.87$  and  $r=0.93$ , respectively). This indicates that our findings are unlikely to be biased by provenance variation.

### Leaf area display and light interception

We used digital capture of plant architecture to create virtual plants (Hanan & Room, 1997; Falster & Westoby, 2003). The 3-D leaf arrangement of each seedling was recorded using a Fastrak 3D-digitiser (Polhemus, Colchester, VT, USA), in conjunction with the software package FLORADIG (CSIRO Entomology, Brisbane, Qld, Australia). The digitiser includes a magnetic signal receiver and pointer, allowing the user to record the 3-D spatial co-ordinates of the pointer within a hemisphere of 3 m diameter from the receiver. Individual plants are reconstructed virtually by recording a series of point co-ordinates, and the relevant connectivity between points. Stem segments (and petioles, if present) are characterised by their elevation angle, azimuth,

**Table 1** Thermal characterisation of the mean environments occupied by species used for light interception modelling, and average leaf, biomass distribution and foliage display traits of seedlings.

Species	Family	Height at harvest (mm)	MAT	Average $T_{min}$	MAT at site of seedling origin	Leaf size (cm <sup>2</sup> )	Leaf L : W ratio	Leaf angle (°)	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Leaf fraction	aLAR <sub>d</sub>
<i>Fuscospora cliffortioides</i>	Nothofagaceae	540	7.1	-2.0	7.3	0.7	1.26	36.9	116.3	0.41	29.1
<i>Griselinia littoralis</i>	Griselinaceae	470	7.8	1.5	10.8	5.9	1.48	40.7	77.6	0.61	20.2
<i>Lophozonia menziesii</i>	Nothofagaceae	607	8.5	0.1	Unknown	0.9	1.35	41.5	118.7	0.43	27.4
<i>Metrosideros umbellata</i>	Myrtaceae	274	9.2	0.3	10.5	2.2	2.56	43.9	59.1	0.59	14.1
<i>Nestegis cunninghamii</i>	Oleaceae	877	9.3	0.5	9.8	16.4	10.38	60.4	68.0	0.58	19.5
<i>Melicytus ramiiflorus</i>	Violaceae	574	10.7	2.1	11.7	6.9	2.71	47.8	191.1	0.63	43.0
<i>Weinmannia racemosa</i>	Cunoniaceae	360	11.0	1.6	9.8	4.2	2.06	39.7	93.7	0.70	30.7
<i>Elaeocarpus dentatus</i>	Elaeocarpaceae	573	12.2	2.6	11.4	16.0	4.55	42.3	141.0	0.56	38.5
<i>Beilschmiedia tawa</i>	Lauraceae	606	12.2	2.7	11.7	6.9	5.00	54.4	113.3	0.59	31.8
<i>Fuscospora truncata</i>	Nothofagaceae	729	12.2	2.9	10.9	1.7	1.32	31.1	133.4	0.44	32.5
<i>Nightitia excelsa</i>	Proteaceae	628	12.7	4.1	11.7	19.7	5.45	41.0	126.1	0.62	38.4
<i>Corynocarpus laevigatus</i>	Corynocarpaceae	521	12.7	4.3	13.7	49.1	1.82	37.2	120.4	0.66	47.5
<i>Weinmannia silvicola</i>	Cunoniaceae	373	12.8	7.3	Unknown	5.1	1.66	40.9	95.6	0.72	33.6
<i>Laurelia novae-zelandiae</i>	Atherospermataceae	580	13.2	5.1	14.2	7.5	2.14	35.5	169.0	0.5	46.5
<i>Beilschmiedia tarairi</i>	Lauraceae	433	14.0	7.0	14.2	33.0	1.69	34.0	121.2	0.63	38.8
<i>Litsea calicaris</i>	Lauraceae	437	14.2	6.1	13.7	19.6	2.16	37.8	175.4	0.66	51.9
<i>Dysoxylum spectabile</i>	Meliaceae	372	14.5	6.7	14.2	20.6	2.14	48.2	164.0	0.63	48.3
<i>Vitex lucens</i>	Lamiaceae	466	14.8	6.3	14.2	11.9	2.16	32.8	175.9	0.53	38.7

MAT and  $T_{min}$  are mean annual temperatures and mean July minimum temperatures obtained from GIS surfaces (Landcare Research, 2011), averaged across occurrence records of each species in national distribution databases (New Zealand Plant Conservation Network, 2016). 'Leaf fraction' refers here to the contribution of leaves to aboveground dry mass of seedlings. Species are ordered by average MAT and  $T_{min}$  throughout their distributions, starting with those associated with the coldest environments.

length and diameter. Individual leaves were characterised by their length, together with the azimuth and elevation angle of two vectors on the lamina surface. Model leaves, digitised in two dimensions, were used to populate the nodes of each virtual plant. As leaf shape can vary significantly both within and between individuals, we digitised four leaves from each seedling to produce an average leaf shape for each individual seedling. After digitising, aboveground tissues were harvested and separated into leaf and stem fractions, dried for at least 48 h at 65°C, and then weighed for determination of biomass parameters.

The YPLANT software (Pearcy & Yang, 1996) was then used to model seedling leaf display and light interception. The 3-D description of leaf arrangement of each seedling, as recorded in FLORADIG, was converted to the appropriate YPLANT format using a program written in the C programming language (Falster & Westoby, 2003). As light interception by plant crowns is determined by leaf inclination angles as well as overlap among leaves (that is self-shading), we used YPLANT output to estimate both these parameters. YPLANT output includes leaf area projected towards each of 160 sectors of the hemisphere (20 elevation classes × 8 azimuth classes) without taking into account overlap of leaves, and leaf area displayed towards each sector, that is the effective area for light interception (Pearcy & Yang, 1996). The mean leaf elevation angle of a plant crown, weighted by the size of individual leaves, can be estimated as:

$$\text{Angle} = \arccosine(\text{PA}_V/\text{LA}) \quad \text{Eqn 1}$$

where  $\text{PA}_V$  = leaf area projected towards the vertical, and LA = actual leaf area of the plant (Pearcy *et al.*, 2004). The self-shaded

fraction (SS) of the crown leaf area was estimated as  $SS = (\text{PA} - \text{DA})/\text{PA}$ , where PA = projected leaf area and DA = displayed leaf area. This parameter was averaged for the uppermost 80 sectors of the hemisphere (Pearcy *et al.*, 2004; Lusk *et al.*, 2012), as in forest understories most light comes from angles > 45° above the horizontal (Pearcy *et al.*, 2004), because of the effect of solar elevation on optical path length through vegetation.

After harvesting plants, we calculated a new variable that integrates the effects of aboveground biomass distribution and architectural traits on the effective leaf area that plants actually display, that is the displayed aboveground LAR (aLAR<sub>d</sub>) (cf. Lusk *et al.*, 2012). This was computed as DA/aboveground plant dry mass, after averaging DA for the uppermost 80 sectors of the hemisphere, that is sectors > 45° above the horizon. aLAR<sub>d</sub> was used as an indicator of the relative light interception potential of each of our study species, per unit of aboveground biomass.

### Statistical analysis

ANOVA was used to test for species' differences in leaf, architectural and biomass distribution traits; this was carried out using STATISTICA (Stat Soft Inc., Tulsa, OK, USA). As well as least squares regression, we carried out phylogenetic generalised least squares regressions (PGLS: Symonds & Blomberg, 2014) using COMPARE (Martins, 2004) to examine correlations of biomass and leaf display traits with each other, and with species' average positions on temperate gradients (Table 1). A phylogenetic tree (Fig. S1) was generated according to relationships indicated by Stevens (2001).

Structural equation modelling was then used to test a multivariate model predicting direct and indirect influences of traits on  $aLAR_d$ . We used the 'PIECEWISESEM' R package v.2.1 (Lefcheck, 2016) to fit the phylogenetic SEM. We used phylogenetic generalised least squares regression models by way of the 'gls' function from the 'NLME' R package (Pinheiro *et al.*, 2011) for each regression model. Our initial expectations (Fig. 1) were based on relationships reported in previous empirical studies. Our initial hypothesis predicted that the relationship between climate and  $aLAR_d$  would be indirect, and would be mediated through leaf size and specific leaf area (SLA). Leaf length-to-width ratio (L : W) was also included as a variable known to influence self-shading and hence light interception efficiency (Takenaka, 1994) although, as far as we are aware, it has not been linked to temperature.

## Results

### Trait correlations

ANOVA showed highly significant interspecific variation in all leaf, biomass distribution, architectural and foliage display variables ( $P < 0.001$  in all cases: Table 2). Average seedling leaf size spanned 70-fold variation across the 18 species, ranging from  $0.7 \text{ cm}^2$  in *Fuscospora cliffortioides* to  $49.1 \text{ cm}^2$  in *Corynocarpus laevigata*; this far exceeded interspecific variation in any of the other measured traits (Table 1).  $aLAR_d$  varied 3.7-fold across the 18 species, ranging from  $14.1 \text{ cm}^2 \text{ g}^{-1}$  in *Metrosideros umbellata* to  $51.9 \text{ cm}^2 \text{ g}^{-1}$  in *Litsea calicularis* (Table 1).

Both parameters of species' average thermal environments (MAT and  $T_{\min}$ ) were positively correlated with leaf size, SLA and  $aLAR_d$ ; and these relationships were all at least as strong under PGLS as under cross-species correlations (Table 3). MAT was in general more strongly correlated with these traits than was  $T_{\min}$ . Species native to warm environments therefore on average had larger, thinner and/or less dense leaves than species native to cold environments, as well as displaying more leaf area per unit aboveground biomass (Fig. 2). Leaf fraction was also significantly positively correlated with  $T_{\min}$  averaged throughout species' distributions, but not significantly so with MAT (Table 3). Neither leaf L : W, nor leaf angle, nor self-shading

was significantly related to species' average thermal environments.

Both cross-species correlations and PGLS showed SLA to be the strongest correlate of interspecific variation in  $aLAR_d$ , followed by leaf size (Table 3).

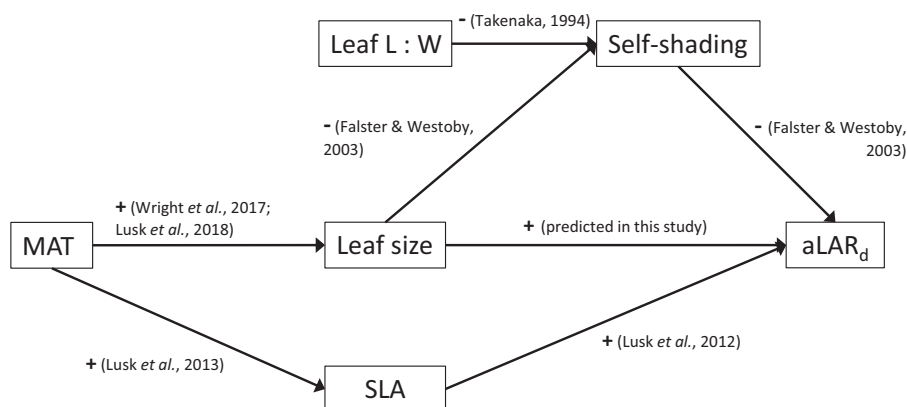
### Structural equation modelling

The initial structural equation model fitted the data reasonably well (Fisher's  $C = 22.77$  with  $P$ -value = 0.064,  $df = 14$ ). However, the pathway from leaf size to self-shading was not significant, and tests of directed separation indicated that there was a missing pathway directly between leaf L : W and  $aLAR_d$ . We modified the original model by removing the pathway from leaf size to self-shading and adding the pathway from leaf L : W to  $aLAR_d$ . This modification substantially improved the model fit to the data (Fisher's  $C = 17.895$ ,  $df = 14$ ,  $P = 0.212$ ) and reduced the Akaike information criterion (AIC) from 52.8 to 47.9 (Fig. 3).

The final model explained significant variation in  $aLAR_d$  ( $R^2 = 0.89$ ), Self-shading ( $R^2 = 0.70$ ), leaf L : W ( $R^2 = 0.13$ ), leaf size ( $R^2 = 0.29$ ), and SLA ( $R^2 = 0.50$ ). Self-shading was negatively related to leaf L : W.  $aLAR_d$  was negatively related to self-shading and positively related to leaf size and SLA. The relationship between leaf L : W and  $aLAR_d$  was more complicated, because a negative direct effect of leaf L : W on  $aLAR_d$  was offset by a positive indirect effect mediated through self-shading.  $aLAR_d$  and self-shading were unrelated to MAT, whereas MAT was positively related to leaf size and SLA (Fig. 3).

## Discussion

Although our hypothesis emphasises frost as a selective pressure on leaf size, MAT was a slightly better predictor of leaf size and foliage display than were winter minimum temperatures (Table 3). This situation might be an artefact resulting from the current limitations of minimum temperature GIS surfaces, which do not capture local topographic effects on cold air drainage (Wright *et al.*, 2006), and instead assume that temperatures in general are controlled by regional adiabatic lapse rates. Alternatively, the slightly greater predictive power of MAT may reflect the relevance of leaf dimensions to minimum air temperatures during



**Fig. 1** Our initial structural equation model. This model fit the data reasonably well (Fisher's  $C = 22.77$   $df = 14$ ,  $P = 0.064$ ,  $AIC = 52.8$ ), but the path from leaf size to self-shading was not significant, and there was a large residual covariance between leaf L : W and  $aLAR_d$ ; accordingly, we modified this model to obtain one that fitted the data better (Fig. 3). Citations indicated studies in which the various pathways have been reported. MAT = mean annual temperature; L : W = length-to-width ratio; SLA = specific leaf area;  $aLAR_d$  = displayed aboveground leaf area ratio (average displayed leaf area/aboveground biomass).

**Table 2** ANOVA testing for interspecific variation in leaf, architectural and biomass distribution traits of temperate evergreen seedlings.

Effect	SS	df	MS	F	P
<b>(log) Leaf size</b>					
Intercept	54.64	1	54.64	5601	< 0.00001
Species	19.260	17	1.132	116.1	< 0.00001
Error	0.5561	57	0.00976		
<b>(log) Leaf length : width</b>					
Intercept	10.84	1	10.84	7488	< 0.00001
Species	4.305	17	0.2532	174.9	< 0.00001
Error	0.0825	57	0.00145		
<b>Leaf angle</b>					
Intercept	127 814	1	127814	4321	< 0.00001
Species	4025	17	236.8	8.004	< 0.00001
Error	1686	57	29.58		
<b>(log) Specific leaf area</b>					
Intercept	320.7	1	320.7	117 300	< 0.00001
Species	1.449	17	0.0852	31.17	< 0.00001
Error	0.1558	57	0.0027		
<b>Leaf fraction</b>					
Intercept	24.95	1	24.95	11 511.91	< 0.00001
Species	0.6059	17	0.0356	16.45	< 0.00001
Error	0.1235	57	0.00217		
<b>Self-shaded %</b>					
Intercept	38 874	1	38 874	1391	< 0.00001
Species	3329	17	195.8	7.008	< 0.00001
Error	1593	57	27.94		
<b>(log) aLAR<sub>d</sub></b>					
Intercept	171.1	1	171.1	27 120	< 0.00001
Species	1.601	17	0.0942	14.93	< 0.00001
Error	0.3596	57	0.0063		

**Table 3** Correlations among temperature parameters of New Zealand evergreen tree species' distributions and seedling traits.

	$T_{min}$	MAT	Leaf size	Leaf L : W	Leaf angle	SLA	Leaf fraction	Self-shading	aLAR <sub>d</sub>
$T_{min}$		0.92**	0.60**	-0.59**	-0.54*	0.66**	0.34	0.47*	0.76**
MAT	0.92**		0.62**	-0.49*	-0.55*	0.77**	0.25	0.46	0.82**
Leaf size	0.66**	0.67**		-0.02	-0.11	0.40	0.56*	0.07	0.64**
Leaf L : W	-0.09	0.07	0.42		0.87**	-0.49*	-0.01	-0.89**	-0.44
Leaf angle	-0.30	-0.27	0.12	0.74**		-0.44	0.08	-0.91**	-0.36
SLA	0.48*	0.60**	0.25	-0.19	-0.36		-0.11	0.53*	0.93**
Leaf fraction	0.49*	0.38	0.62**	0.22	0.23	-0.14		-0.05	0.17
Self-shading	0.21	0.15	-0.10	-0.77**	-0.81**	0.39	-0.02		0.42
aLAR <sub>d</sub>	0.67**	0.75**	0.51*	-0.14	-0.34	0.89**	0.18	0.35	

Figures to lower left of diagonal show Pearson correlation coefficients of cross-species relationships; figures to the upper right show phylogenetic least squares regressions.

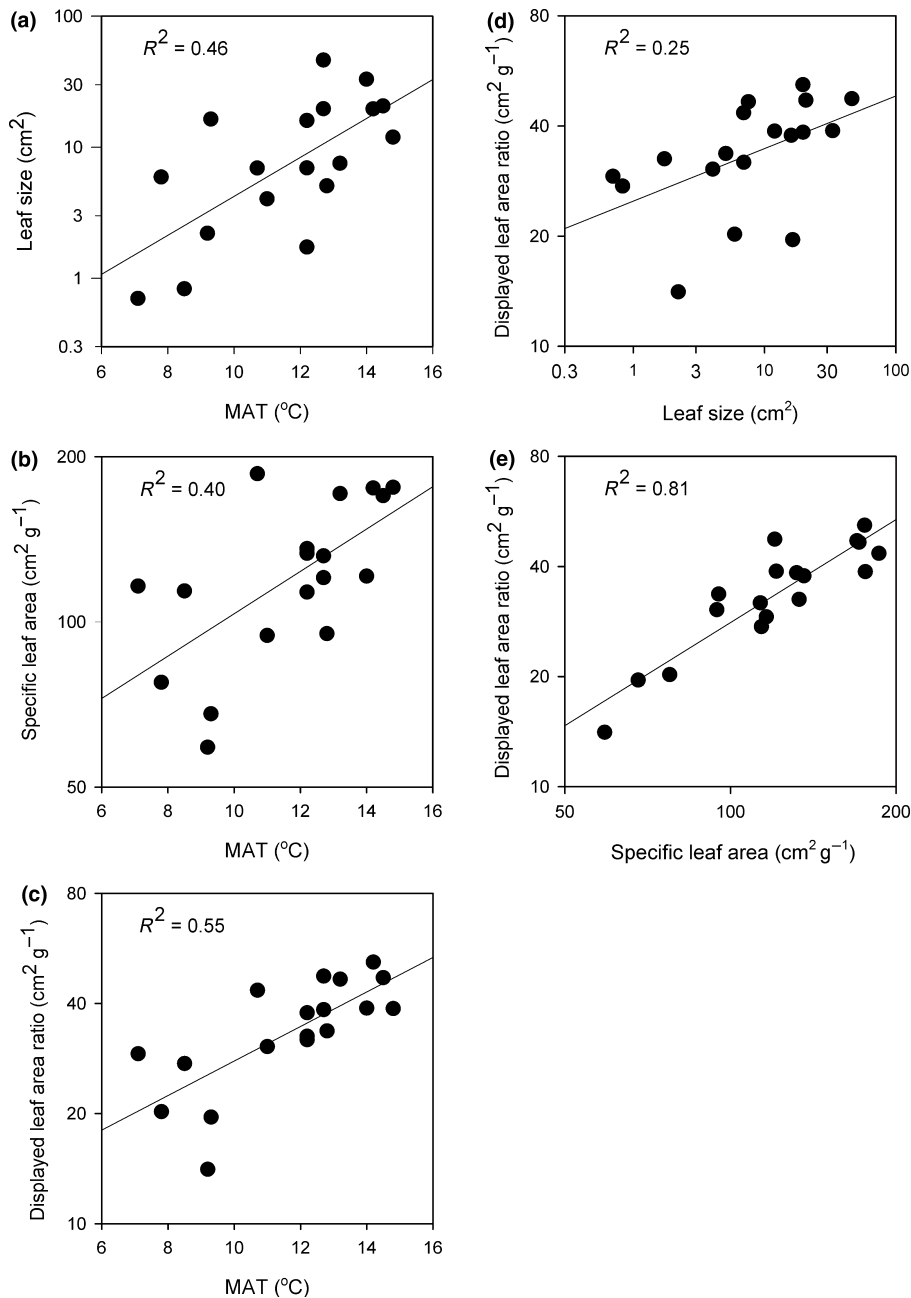
$T_{min}$  = mean July minimum temperatures; MAT = mean annual temperature; L : W = length-to-width ratio; SLA = specific leaf area; aLAR<sub>d</sub> = displayed aboveground leaf area ratio (average displayed leaf area/aboveground biomass).

\*,  $P < 0.05$ , \*\*,  $P < 0.01$ .

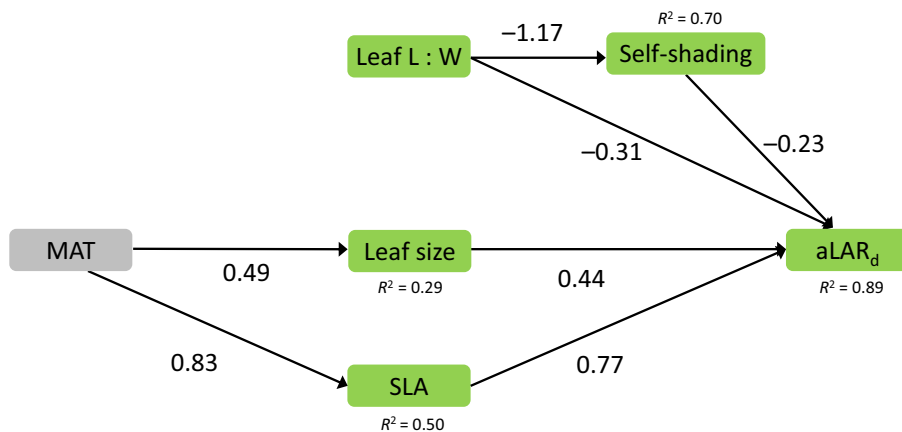
spring and autumn, more than winter. The leaf tissues of most evergreens will tolerate lower temperatures in winter than during the rest of the year (Bannister, 2007), and the effect of leaf size on leaf-to-air temperature differentials may be more important in protecting active leaves from frost damage during spring and autumn (Wright *et al.*, 2017; Lusk *et al.*, 2018).

SLA was the single most important contributor to the higher seedling light interception efficiency of species associated with warm climates (Figs 2, 3). The strong correlation of SLA with species' average thermal environments matches a pattern reported

from an elevational gradient in southern China, spanning a range of temperatures equivalent to subtropical to cool-temperate environments (Bai *et al.*, 2015); leaf lifespans also increased with increasing elevation, as SLA decreased. This trend to slower leaf economics in cool climates may reflect declining annual growth rates with increasing elevation (Malhi *et al.*, 2017); the resulting slowdown of foliage turnover in cold environments probably selects for robust leaf construction, enabling leaves to achieve their potential lifetimes. This relationship of leaf economics with temperature is not apparent in deciduous assemblages from more



**Fig. 2** Relationships among leaf, seedling biomass distribution and light interception traits of 18 temperate evergreens; as well as relationships with mean annual temperature averaged across each species' reported distribution. Significant ( $P < 0.05$ ) shown with solid lines.



**Fig. 3** Final phylogenetic structural equation model illustrating determinants of seedling light interception among 18 temperate evergreens (Fisher's  $C = 17.895$ ,  $df = 14$ ,  $P = 0.212$ ,  $AIC = 47.9$ ). All regression paths were significant ( $P < 0.05$ ). Parameters are reported as standardised regression coefficients, and  $R^2$  for each response variable are shown next to the boxes. MAT = mean annual temperature; L : W = length-to-width ratio; SLA = specific leaf area;  $aLAR_d$  = displayed aboveground leaf area ratio (average displayed leaf area/aboveground biomass).

strongly seasonal temperate climates (van Ommen Kloeke *et al.*, 2012), or in more heterogeneous datasets that include many deciduous species (Ordoñez *et al.*, 2009).

Leaf size also contributed significantly to the higher light interception efficiency (as measured by  $aLAR_d$ ) of species of warm climates (Figs 2, 3). Large-leaved species developed large foliage areas, paralleling the findings of a study of the foliage display in distal ends of canopy branches (rather than seedlings) cut from adult trees of 38 Australian woodland species (Falster & Westoby, 2003). As self-shading in our dataset was not related to leaf size (Table 3), this relationship of leaf size with total foliage area resulted in  $aLAR_d$  being largest in species from warm climates, for example *Corynocarpus laevigatus*, *Dysoxylum spectabile* and *Litsea calicaris*. A study of Chilean and New Zealand temperate forest evergreens also reported a relationship between leaf size and the light interception efficiency of seedlings (Lusk *et al.*, 2012), although no climatic relationships were analysed in that study. Leaf size also has implications for the ability of juvenile trees to suppress competitors: large leaves cast longer umbrae than small leaves (Horn, 1971; Valladares, 1999), and may therefore be indirectly advantageous in denying light to shorter juvenile trees and vines that could potentially overtop them (McGlone *et al.*, 2010). The advantage of large leaves in enabling juvenile trees to display a large foliage area in warm environments can only be realised if accompanied by large-diameter conduits that permit narrow stems to conduct water efficiently (Gleason *et al.*, 2012). Hydraulic mean conduit diameters published in a companion paper (Lusk *et al.*, 2013a) are positively correlated with species' mean leaf sizes reported in the present study ( $r = 0.53$ ,  $P = 0.029$ ), confirming a degree of integration of leaf and stem traits along temperature gradients in New Zealand humid forests.

The advantage of light interception efficiency conferred by large leaves is probably confined to juvenile trees, as penumbral effects within crowns of larger trees may enable small-leaved species to harvest light efficiently by developing large leaf area indices. Stenberg (1995) simulated the impact of penumbral effects on light interception and photosynthesis of *Pinus sylvestris* L. canopies. She found that penumbral effects within shoots <250 mm long were minimal. By contrast, shading from another *P. sylvestris* shoot situated >250 mm from the target could be better characterised as diffuse shading because of the prevalence of penumbra, resulting in higher carbon gain than that predicted by a model assuming parallel solar beam geometry (as does YPLANT). Umbral lengths within canopies are proportional to leaf width (Horn, 1971; Valladares, 1999) and, as leaves of all species in the present study are considerably broader than those of *P. sylvestris*, penumbral effects should not be a significant influence on light interception and carbon gain over the range of seedling sizes that we studied. However, penumbral effects will increasingly dominate light environments within crowns of small-leaved species as they grow taller (Stenberg, 1995) and probably explain the high leaf area indices and deep crowns developed by adult trees of some shade-tolerant conifers (for example Bolstad & Gower, 1990; Leverenz & Hinckley, 1990). Nonetheless,

juvenile tree growth and survival are good predictors of forest composition (Pacala *et al.*, 1993; Kobe, 1996), as a result of the critical demographic bottlenecks occurring during the seedling and sapling stages (Grubb, 1977; Bond, 1989; Poorter, 2007); the resulting environmental filtering in both tree-fall gaps and understories in humid forests has been shown to favour traits promoting light pre-emption and/or efficient light interception by juvenile plants (Saldaña *et al.*, 2007; Lusk & Laughlin, 2017).

As well as minimising chilling on clear nights, small leaf dimensions may also favour nutrient acquisition in the cold, wet environments typical of montane and subalpine sites in New Zealand. Yates *et al.* (2010) pointed out that the shallow boundary layers associated with small leaf dimensions confer a capacity for high transpiration when evaporative demand is low and water is plentiful. This effect may therefore be important for driving mass flow to the roots of small-leaved species such as *Fuscospora cliffortioides* and *Lophozonia menziesii* in upland New Zealand, where precipitation, as is often applied, exceeds evapotranspiration, and where fog and waterlogging are common (Veblen & Stewart, 1982; Jane & Green, 1986).

In conclusion, this study provides the most conclusive empirical demonstration so far of a mechanism that can explain the prevalence of large leaves in environments where there is little exposure to frost or drought. Our results are consistent with the hypothesis that inherent variation in leaf size along temperature gradients in humid evergreen forests reflects a trade-off between light interception efficiency and susceptibility to frost damage. Although small leaves are less vulnerable to night-time chilling (and hence to frost damage on sites with short frost-free periods: Lusk *et al.*, 2018), this study shows for the first time that they limit the juvenile trees' abilities to display large foliage areas effectively and hence to maximise light capture per unit of biomass (Figs 2, 3). This trade-off appears to be bound up with a parallel trade-off in xylem form and function: narrow conduits are resistant to freeze–thaw embolism (Davis *et al.*, 1999), but also result in low sapwood conductivity, limiting seedlings to small foliage areas (Gleason *et al.*, 2012; Lusk *et al.*, 2013a) and/or low photosynthetic capacity (Brodribb & Feild, 2000), in turn probably reducing their competitiveness in less frost-prone environments (Koehler *et al.*, 2012; Lusk *et al.*, 2013b). Leaf size therefore appears to be one of a suite of traits with physiologically plausible links to species sorting along temperature gradients in humid forests. Our findings add to a body of evidence showing that carbon gain potential trades off with resistance to various types of environmental stress (Lambers *et al.*, 2008).


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## Author contributions

CHL developed the idea, supervised the research, and wrote most of the paper. ERPG gathered most of the data. DCL carried out structural equation modelling, wrote the corresponding parts of the methods and results sections, created Fig. S1, and edited the manuscript.

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## Supporting Information

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

**Fig. S1** Phylogeny used in the phylogenetic least squares regression (derived from Stevens, 2001), showing colour-coded trait values.

**Table S1** Replicate seedlings of 18 New Zealand rainforest evergreens, showing leaf, biomass distribution and light interception traits.

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