



## Research paper

# Canopy gradients in leaf functional traits for species that differ in growth strategies and shade tolerance

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Received October 23, 2016; accepted April 27, 2017; handling Editor Hiroaki Ishii

In temperate deciduous forests, vertical gradients in leaf mass per area (LMA) and area-based leaf nitrogen ( $N_{\text{area}}$ ) are strongly controlled by gradients in light availability. While there is evidence that hydrostatic constraints on leaf development may diminish LMA and  $N_{\text{area}}$  responses to light, inherent differences among tree species may also influence leaf developmental and morphological response to light. We investigated vertical gradients in LMA,  $N_{\text{area}}$  and leaf carbon isotope composition ( $\delta^{13}\text{C}$ ) for three temperate deciduous species (*Carpinus caroliniana* Walter, *Fagus grandifolia* Ehrh., *Liriodendron tulipifera* L.) that differed in growth strategy (e.g., indeterminate and determinate growth), shade tolerance and leaf area to sapwood ratio ( $A_l:A_s$ ). Leaves were sampled across a broad range of light conditions within three vertical layers of tree crowns to maximize variation in light availability at each height and to minimize collinearity between light and height. All species displayed similar responses to light with respect to  $N_{\text{area}}$  and  $\delta^{13}\text{C}$ , but not for LMA. Light was more important for gradients in LMA for the shade-tolerant (*C. caroliniana*) and -intolerant (*L. tulipifera*) species with indeterminate growth, and height (e.g., hydrostatic gradients) and light were equally important for the shade-tolerant (*F. grandifolia*) species with determinate growth. *Fagus grandifolia* had a higher morphological plasticity in response to light, which may offer a competitive advantage in occupying a broader range of light conditions throughout the canopy. Differences in responses to light and height for the taller tree species, *L. tulipifera* and *F. grandifolia*, may be attributed to differences in growth strategy or  $A_l:A_s$ , which may alter morphological and functional responses to light availability. While height was important in *F. grandifolia*, height was no more robust in predicting LMA than light in any of the species, confirming the strong role of light availability in determining LMA for temperate deciduous species.

**Keywords:** *Carpinus caroliniana*, *Fagus grandifolia*, leaf carbon isotope composition, leaf mass per area, leaf nitrogen, *Liriodendron tulipifera*.

## Introduction

Within temperate deciduous forests, gradients in light availability are often implicated as a driving factor for two coordinated leaf traits, leaf mass per area (LMA) and leaf nitrogen per area ( $N_{\text{area}}$ ) (Ellsworth and Reich 1993, Niinemets et al. 1998, 1999, Meir et al. 2002, Jones and Thomas 2007, Montpied et al. 2009, Coble and Cavaleri 2014, 2015, Scartazza et al. 2016). In temperate deciduous species, leaves growing in high light conditions with greater LMA often contain a thicker palisade layer that enhances

light penetration to deeper layers of the mesophyll (Jackson 1967, Vogelmann and Martin 1993, Evans 1999, Oguchi et al. 2005) and with greater  $N_{\text{area}}$  partition more nitrogen into ribulose biphosphate carboxylase (RuBisCO) leading to greater photosynthetic capacity (Evans 1989, Hikosaka and Terashima 1995). Due to the strong association among LMA,  $N_{\text{area}}$  and photosynthesis (Ellsworth and Reich 1993, Reich et al. 1999, Wilson et al. 2000, Kitajima et al. 2002, Wright et al. 2004, Montpied et al. 2009), these easy-to-measure leaf traits are necessary for modeling canopy photosynthesis (Aber et al. 1996, Raulier et al. 1999,

Coble et al. 2016) and carbon fluxes at larger scales (Thornton and Zimmerman 2007, Houborg et al. 2009, Ryu et al. 2011).

Height has also been implicated as a strong driver of vertical gradients in LMA in very tall western conifer and tropical trees, and there is growing evidence that height may also influence LMA in shorter temperate deciduous trees. Due to gravity alone, leaf water potential linearly decreases with height ( $-0.01 \text{ MPa m}^{-1}$ ), in both conifer and temperate deciduous species (Scholander et al. 1965, Hellkvist et al. 1974, Bauerle et al. 1999, Woodruff et al. 2004, Coble and Cavaleri 2014). In western conifer trees such as the very tall *Sequoia sempervirens* (~97–113 m) (Koch et al. 2004, Ishii et al. 2008, Mullin et al. 2009, Oldham et al. 2010) and the shorter *Pinus monticola* (45 m), *Pinus ponderosa* (41 m) and *Pseudotsuga menziesii* (40 m) (Marshall and Monserud 2003) the gravitational potential gradient appears to limit leaf development at greater heights. Limited osmotic adjustments and leaf turgor at greater heights may restrict leaf expansion, resulting in leaves with high LMA (Woodruff et al. 2004, Meinzer et al. 2008). Vertical gradients in leaf carbon isotope composition ( $\delta^{13}\text{C}$ ) in tall *S. sempervirens* trees provide further evidence for increasing water stress with height and more frequent stomatal closure of upper canopy leaves (Koch et al. 2004, Ishii et al. 2008). In tall tropical trees (~40 m), LMA was strongly and linearly correlated with height throughout the entire canopy, whereas light appeared to influence LMA only in lower light conditions (Cavaleri et al. 2010). In shorter temperate deciduous trees (~18 m), Zhang et al. (2011, 2012a, 2012b) found that midday leaf water potential values in *Robinia pseudoacacia* seedlings that corresponded with in situ measurements were sufficient to increase LMA through constraints on leaf expansion via reduced cell wall extensibility. Additional evidence for early season height effects on leaf development and LMA were observed during the leaf expansion phase in *Acer saccharum* (~21 m; Coble et al. 2016) and *Quercus rubra* trees (~18 m; Zwieniecki et al. 2004). A potential consequence of morphological adjustments to height is greater investment in carbon-rich compounds (e.g., lignin, cellulose), which improves mechanical strength and tolerance of lower water potential, while simultaneously diluting leaf nitrogen, which leads to sub-optimal distributions in leaf nitrogen (Niinemets et al. 1999, Coble and Cavaleri 2015).

Inherent differences among temperate deciduous species, such as leaf growth strategies, could potentially influence leaf development and their ability to acclimate to the local light environment (Ishii and Ohsugi 2011). Woody plant species can be broadly classified as having indeterminate or determinate growth (Kozlowski 1964, Marks 1975). For indeterminate species, leaves and shoots are formed continuously during the season of growth, whereas leaves of many determinate species are initially formed in buds as leaf primordia in the year prior to leaf growth and experience one flush of leaves at the start of the growing season. Plants with indeterminate growth patterns are capable of

acclimating to light through successive leaf emergence, which may contribute their greater responsiveness to changes in light availability (Naidu and DeLucia 1998, Ishii and Ohsugi 2011). For example, the indeterminate-growth species, *Liriodendron tulipifera* L., had a greater ability in morphological and photosynthetic acclimation to changing light availability compared with the determinate-growth and shade-tolerant species, *A. saccharum* (Naidu and DeLucia 1998). An advantage to indeterminate growth is that species can capitalize on short-term changes in light availability through adjustments in leaf morphology and function in newly formed leaves. In contrast, species with determinate growth patterns are somewhat constrained in acclimation because leaf anatomical structure is predetermined in the year prior to leaf flush and tends to show 'carry-over' effects from previous-year light conditions (Naidu and DeLucia 1998, Jones and Thomas 2007, Ishii and Ohsugi 2011). For example, *Fagus sylvatica* buds growing in full sunlight were found to contain leaf primordia with more cell layers compared with shaded buds, leading to two-layers of palisade cells for sun leaves and one-layer of palisade cells for shade leaves (Eschrich et al. 1989). These growth strategies (indeterminate and determinate) may potentially explain why tall, mature trees of different species may differ in their response to light availability within forest canopies.

The objective of this study was to identify any evidence for height constraints on leaf development for three tree species (*Carpinus caroliniana* Walter, *L. tulipifera*, *Fagus grandifolia* Ehrh.) that differ in growth strategies and shade tolerance. We used a sampling design that maximized the range of light availabilities within each vertical canopy layer to minimize collinearity between light and height when assessing their influence on leaf functional traits (LMA,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$ ). In this study, we also examine differences in leaf area to sapwood area ratio ( $A_i:A_s$ ) because there is evidence that  $A_i:A_s$  plays an important role in whole-tree water relations, and reductions in  $A_i:A_s$  may be a mechanism for tolerating greater declines in leaf water potential and hydraulic constraints (McDowell et al. 2002). We tested the following hypotheses: (i) light is more important than height for determining LMA in temperate deciduous species; and (ii) LMA,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  are strongly coordinated in all species due to the coordination between leaf morphology and leaf function.

## Materials and methods

### Study site

The study was conducted in a mixed deciduous forest at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD (38° 53'N, 76° 33'W). Mean local annual temperature and precipitation is  $12.8 \pm 0.7 \text{ }^\circ\text{C}$  and  $1090 \pm 172 \text{ mm}$ , respectively (NOAA regional climatology 1895–2010). The stand is considered a 'tulip poplar' association (Brush et al. 1980) and originated around 1887 after a large pasture was abandoned. The diverse forest is dominated by *L. tulipifera*, which makes up

49% of total basal area (BA). In order of importance (sum of % density and % BA), other species include *C. caroliniana*, *F. grandifolia*, *Cornus florida*, *Carya tomentosa*, *Liquidambar styraciflua*, *Quercus alba*, *Carya glabra*, *Quercus velutina* and other species. The target species for this study were *C. caroliniana*, *L. tulipifera*, and *F. grandifolia*. *Carpinus caroliniana* has indeterminate growth and is shade-tolerant, *L. tulipifera* has indeterminate growth and is shade-intolerant (Norby and O'Neill 1991, Naidu and DeLucia 1998) and *F. grandifolia* has determinate growth and is shade-tolerant (Marks 1975, Goulet and Bellefleur 1985) (Table 1). Vertical stratification of foliage and phenology for the three species in a stand next to this study site is described in Parker et al. (1989) and Parker and Tibbs (2004). Briefly, *C. caroliniana* is primarily restricted to the lower canopy (<10 m), *L. tulipifera* occupies the mid- (10–25 m) and upper canopies (>25 m) and *F. grandifolia* occupies the mid- to lower canopies with a few large trees [diameter at breast height (dbh) >40 cm] that occupy portions of the upper canopy. The mean ( $\pm$  standard error) height of all *C. caroliniana*, *L. tulipifera* and *F. grandifolia* trees greater than 2.5 cm dbh in a 0.5 ha plot in the same stand in 1997 were  $10.5 \pm 0.01$ ,  $31.2 \pm 0.03$  and  $25.1 \pm 0.02$  m, respectively (unpublished data).

### Study design

Three trees per species were selected, and the crown of each tree was divided into three vertical layers based on crown height. Each layer was divided into five horizontal sections based on horizontal distance from the center of the crown. The division of crown layers into five horizontal sections was made in order to maximize the range of variation in light availability at each sample height for each of three species. The mean ( $\pm$  standard deviation) height of sample trees for *C. caroliniana*, *L. tulipifera* and *F. grandifolia* were  $7.7 \pm 2.2$ ,  $36.4 \pm 1.8$  and  $24.5 \pm 2.4$  m, respectively (Table 1). Light and height measurements and leaf collections were conducted in August 1998 using a personnel hoist suspended from a mobile hydraulic crane.

### Light and height measurements

At each leaf sampling location, hemispherical photographs were taken using a digital imaging system with a gimbaled 180° lens (CI-110 system with LLP Lens, CID, Vancouver, WA, USA). Images were taken in early morning or late evening. Digital hemispherical images were analyzed using Hemiphot software to calculate direct site factor (DSF) (proportion of direct light relative to open conditions), indirect site factor (ISF) (relative

illumination of diffuse light under a standard overcast sky (SOC)) and canopy openness. All of these measures were highly correlated. Here we use the total site factor (TSF), a weighted average of ISF and DSF.

In the summer of 1994 in the same stand, we also measured the photosynthetic photon flux density (PPFD, 400–700 nm) along 29 vertical transects using a cosine-corrected quantum sensor (Model LI-190SB, Li-Cor, Lincoln, NE, USA). The sensor was attached to the top of a balloon and measurements were made at every meter up to 40 m in height. Photosynthetic photon flux density was also measured above the canopy during each transect to serve as the reference. Parker et al. (1996, 2002) provide a full description and application, respectively, of the balloon technique. Light transmittance values were made throughout the canopy, regardless of leaf locations, whereas measurements of TSF were made at the location of leaf samples. While the light transmittance measurements occurred 4 years prior to this study, these data are used to assess the extreme light conditions (5th and 95th percentile) of light transmittance in reference to the TSF values at each height. Furthermore, a tree census of a 0.5 ha plot that began in 1987 (Parker et al. 1989) and continued annually thereafter within the same stand indicated that the stand grew very little in height during the 1994–98 interval. Mean tree height in 1993 for *C. caroliniana*, *L. tulipifera* and *F. grandifolia* was 10.5, 31.2 and 25.1 m, respectively, which changed less than 0.1 m over 4 years. Height at each location and total tree height was measured by dropping a tape measure from the foliage sample to the ground.

### Leaf area to sapwood area ratio ( $A_l:A_s$ )

Leaf area to sapwood area ratio ( $A_l:A_s$ ,  $m^2 cm^{-2}$ ) was estimated for *L. tulipifera* and *F. grandifolia* by first calculating stem diameter at 1.37 m above the ground (dbh) using total tree height from this study and dbh–height allometric relationships reported by Anderson-Teixeira et al. (2015) for the same species at a site ~170 km from the current study location. Diameter at breast height and dbh–leaf area allometric relationships were used to estimate whole-tree leaf area. We estimated the allometric relationship between leaf area and dbh with a non-linear procedure (SAS proc NLIN) based on dataset of concurrent collections of stem diameter and litterfall from an age-series of local plots. Both stem and leaf were identified to the same species classes and leaf masses were converted to leaf area following the procedure described in Parker and Tibbs (2004). The plot total leaf

Table 1. Shade tolerance, growth strategy, mean tree height (m;  $\pm$  standard deviation) and predicted mean leaf area to sapwood area ratio ( $m^2 cm^{-2}$ ) of sample trees. Different letters indicate significant differences between species ( $P < 0.05$ , Tukey's HSD).

Species	Shade tolerance	Growth strategy	Tree height (m)	Predicted $A_l:A_s$ ( $m^2 cm^{-2}$ )
<i>C. caroliniana</i>	Tolerant	Indeterminate	$7.7 \pm 2.2^a$	–
<i>L. tulipifera</i>	Intolerant	Indeterminate	$36.4 \pm 1.8^c$	$0.295 \pm 0.006^a$
<i>F. grandifolia</i>	Tolerant	Determinate	$24.5 \pm 2.4^b$	$0.339 \pm 0.001^b$

area and stem size distributions were used as constraints for fitting the allometric constants for each species. Sapwood area was estimated from dbh using dbh–sapwood area allometric relationships reported by Anderson-Teixeira et al. (2015). Given that sapwood area can change across soil types (Long and Smith 1988), we also estimated the leaf area to basal area ratio ( $\text{m}^2 \text{m}^{-2}$ ) for these species growing within the same stand in order to validate our results. Across a number of temperate deciduous species, basal area and dbh are both strongly correlated with sapwood area (Gebauer et al. 2008). We estimated the leaf area to basal area ratio using stand basal area ( $\text{m}^2 \text{m}^{-2}$ ) and LAI ( $\text{m}^2 \text{m}^{-2}$ ) reported by Parker and Tibbs (2004). Stand-level leaf area per unit basal area was greater for *C. caroliniana* ( $5164 \text{ m}^2 \text{m}^{-2}$ ) and *F. grandifolia* ( $3694 \text{ m}^2 \text{m}^{-2}$ ) compared with *L. tulipifera* ( $487 \text{ m}^2 \text{m}^{-2}$ ).

### Leaf mass per area, leaf nitrogen and carbon isotope composition

At each location, we collected 10 leaves from *C. caroliniana* and 4–6 leaves from *L. tulipifera* and *F. grandifolia* at the ends of branch tips. Leaf collection of *F. grandifolia* and *C. caroliniana* (determinate growth) were from the initial flush of leaves in the early spring, whereas leaf collection of *L. tulipifera* (indeterminate growth) included a mix of older (e.g., from spring flush) and newer leaves. Leaves were temporarily stored in sealed plastic bags and refrigerated. Leaf area was measured using a bench-top leaf area meter (LI-3100 Area Meter, Li-Cor) to the nearest  $0.01 \text{ cm}^2$ . Leaves were then dried at  $60^\circ \text{C}$  for 4 days and weighed to the nearest  $0.1 \text{ mg}$ . Leaf samples used to estimate leaf nitrogen and carbon isotope composition were processed at the SERC. Bulk leaf samples were ground to a fine powder. Leaf nitrogen on a mass basis ( $N_{\text{mass}}$ ,  $\text{mg g}^{-1}$ ) was converted to area-based nitrogen ( $N_{\text{area}}$ ,  $\text{g m}^{-2}$ ) using LMA [ $N_{\text{area}} = (\text{LMA} \times N_{\text{mass}})/1000$ ]. Leaf  $\delta^{13}\text{C}$  was determined using a Carlo Erba Instruments NC 2500 elemental analyzer coupled to a Thermo Scientific Delta V Plus IRMS. Leaf  $\delta^{13}\text{C}$  was calculated as  $^{13}\text{C} = 1000 * (R_{\text{sample}}/R_{\text{standard}} - 1)$  (‰), where  $R_{\text{sample}}$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio for the sample and  $R_{\text{standard}}$  is the ratio for a standard. We note that the leaf carbon isotope data ( $\delta^{13}\text{C}$ ) in this study was used in a previous publication, Graham et al. (2014). However, the primary focus of the data presented by Graham et al. (2014) was to assess canopy openness of ancient canopies from leaf fossil isotopes, and the results in our study are displayed in a different context.

### Data analysis

All statistical analyses were conducted using R software (R Development Core Team 2015). Regression analysis was used to identify the significance and strength of the relationship between height and TSF for each species. Total site factor was natural log-transformed because TSF displayed curved, asymptotic relationships with height and log-transformation of TSF satisfied

regression assumptions. To address collinearity between TSF and height for each species, we calculated the variance inflation factor (VIF) between TSF and height (Ott and Longnecker 2001). The VIF quantifies the extent of inflation of the coefficient variance due to collinearity. A VIF of 1 indicates no collinearity, whereas a VIF of 10 indicates that collinearity is a problem. We used three approaches to separate the effects of TSF and height on LMA. First, we examined the residuals of LMA vs height plotted against TSF and the residuals of LMA vs TSF plotted against height, and compared their  $R^2$  values. Second, we used pairwise partial correlations to separate the effects of TSF and height on LMA. The pairwise partial correlations allowed us to control for light and height when comparing LMA–height and LMA–TSF correlations, respectively. Third, we used partial  $R^2$  analysis to compare the contribution of TSF and height to the full model ( $y = \beta_0 + \beta_1 \text{TSF} + \beta_2 \text{height}$ ) for predicting LMA.

We used ANCOVA for species and covariate (height and TSF) effects on LMA,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$ , as well as the interaction effects (height  $\times$  species, TSF  $\times$  species). An initial analysis that included individual trees as a third factor in the ANCOVA model revealed a non-significant tree effect on LMA and  $N_{\text{area}}$ . Thus, we pooled all data from the three study trees within species. We assessed plasticity for LMA and  $N_{\text{area}}$  by comparing slopes of the LMA–TSF, LMA–height,  $N_{\text{area}}$ –TSF and  $N_{\text{area}}$ –height linear regression models, as well as slopes of the LMA– $N_{\text{area}}$ , LMA– $\delta^{13}\text{C}$  and  $N_{\text{area}}$ – $\delta^{13}\text{C}$  models, among the three species. We tested for differences in slopes by first fitting the ANCOVA model (e.g.,  $\text{LMA} = \beta_0 + \beta_1 \text{TSF} + \beta_2 \text{Species} + \beta_{12} \text{TSF} \times \text{Species}$ ) and assessing significance ( $P < 0.05$ ) of the differences in slopes between the ‘reference’ species and the two remaining species in the summary output in R software. We repeated this analysis after changing the ‘reference’ species so that tests for differences in slopes could be made for all species. R software uses  $t$ -statistics to test whether slopes differ between individual regression models. We found that this approach generates identical results as those observed from the use of dummy variables in multiple regression for comparing slopes (Ott and Longnecker 2001).

## Results

### Tree characteristics and light environment

*Liriodendron tulipifera* had a smaller  $A_i/A_s$  compared with *F. grandifolia* (Table 1), which is consistent with our findings that *L. tulipifera* had a smaller stand-level leaf area to basal area ratio than *F. grandifolia*. Total site factor (TSF) as estimated with hemispherical photography was compared with a more extensive dataset of light transmittance values at the 5th and 95th percentile collected within the same stand (Figure 1). Our results confirm that leaf collections were made from a broad range of light conditions within each height for this stand. Total site factor was weakly correlated with height for all species

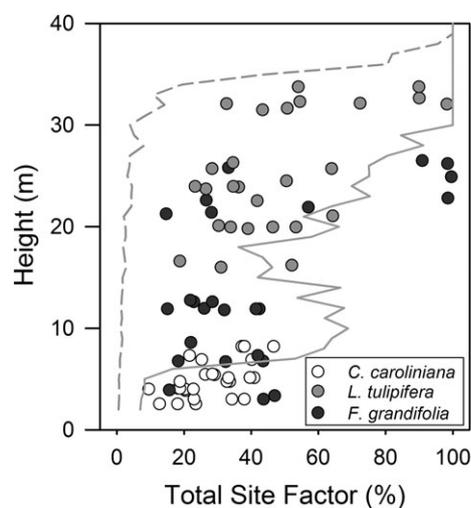


Figure 1. Relationships between height and light (total site factor, %) for *C. caroliniana*, *L. tulipifera* and *F. grandifolia*. The dashed and solid lines represent the 5th and 95th percentile, respectively, of light transmittance (%) values at 1 m height increments.

Table 2. Model parameters for predicting height using total site factor (TSF) including the intercept, slopes, model  $P$ -values,  $R^2$  values and variance inflation factor (VIF) for each species. Model: Height =  $\beta_0 + \beta_1$  (ln) TSF.

Species	Model parameters				
	$\beta_0$	$\beta_1$	Model $P$ -value	$R^2$	VIF
<i>C. caroliniana</i>	-2.78	2.35	$P < 0.01$	0.26	1.35
<i>L. tulipifera</i>	-3.27	7.53	$P < 0.01$	0.29	1.41
<i>F. grandifolia</i>	-7.23	6.05	$P < 0.05$	0.20	1.25

where the  $R^2$  values ranged from 0.20 to 0.29 (Table 2, Figure 1). The range of the VIF between height and (ln)TSF among all species was 1.25–1.41, which indicates that collinearity is not an issue in this study because the values were close to 1.

### Light versus height effects on LMA

We found that TSF was more strongly correlated with residuals of the LMA vs height relationships for *C. caroliniana* ( $R^2 = 0.22$ ) and *L. tulipifera* ( $R^2 = 0.37$ ; Figure 2a) than between height and residuals of the LMA vs TSF relationships ( $R^2 = 0.07$ ; Figure 2b). For *F. grandifolia*, the strength of the relationship between TSF and residuals of LMA vs height relationship ( $R^2 = 0.55$ ; Figure 2a) was similar to the relationship between height and residuals of the LMA vs TSF relationship ( $R^2 = 0.52$ ; Figure 2b). The partial  $R^2$  analysis revealed that TSF contributed more to the full model (LMA =  $\beta_0 + \beta_1$ TSF +  $\beta_2$ Height) for *C. caroliniana* and *L. tulipifera*, while the contribution of height and TSF were similar for *F. grandifolia* (Table 3). Consistent with these findings, the pairwise partial correlation analysis showed that TSF was more strongly correlated with LMA when controlling for height for *C. caroliniana* and *L. tulipifera* compared with

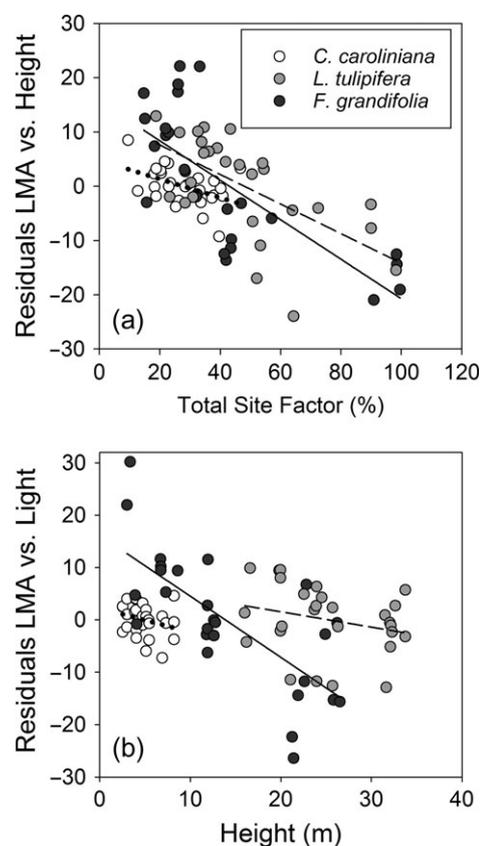


Figure 2. Residuals of LMA vs height (m) plotted against TSF (a) and residuals of LMA vs TSF plotted against height (b) for *C. caroliniana* (dotted lines), *L. tulipifera* (dashed lines) and *F. grandifolia* (solid lines).  $R^2$  values: (a) *C. caroliniana*, 0.22; *L. tulipifera*, 0.37; *F. grandifolia*, 0.55; and (b) *C. caroliniana*, 0.07; *L. tulipifera*, 0.07; *F. grandifolia*, 0.52.

the correlation between LMA and height when controlling for TSF (Table 4). For *F. grandifolia*, the partial correlation also showed that the strengths of the LMA–TSF and LMA–height correlations were similar (Table 4).

### Species responses to light and height

Among the three species, we observed differences in LMA responses to TSF and height, but all three species displayed similar changes in  $N_{area}$  with increasing TSF and height. The ANCOVA indicated significant TSF ( $P < 0.001$ ), species ( $P < 0.001$ ) and TSF  $\times$  species ( $P = 0.003$ ) effects on LMA. Similarly, height ( $P < 0.001$ ), species ( $P < 0.001$ ) and height  $\times$  species ( $P = 0.002$ ) had significant effects on LMA. Leaf mass per area was linearly and positively correlated with TSF and height (Figure 3a and b). The slope of the LMA–TSF relationship for *F. grandifolia* was significantly greater than the slopes for *L. tulipifera* ( $P = 0.004$ ) and *C. caroliniana* ( $P = 0.017$ ), and no difference in slopes were detected between *L. tulipifera* and *C. caroliniana* ( $P = 0.442$ ). The slope of the LMA–height relationship for *F. grandifolia* was significantly greater than the slope for *L. tulipifera* ( $P < 0.001$ ), but not for *C. caroliniana* ( $P = 0.264$ ).

Table 3. For each species, coefficients and  $R^2$  values are displayed for regressions between light (TSF), height and LMA and for multiple regressions between light + height and LMA. Partial  $R^2$  values are shown for adding light or height to the full model and provide an indication of the relative importance of each parameter (height or light) for predicting LMA.

Response variable	Species	N	Light (TSF)			Height			Light and height				Partial $R^2$ for adding:	
			$\beta_0$	$\beta_1$	$R^2$	$\beta_0$	$\beta_1$	$R^2$	$\beta_0$	$\beta_1$	$\beta_2$	$R^2$	Light	Height
LMA	<i>C. caroliniana</i>	27	23.40***	0.31***	0.48	25.30***	1.36**	0.31	22.03***	0.24**	0.64	0.53	0.21	0.05
	<i>L. tulipifera</i>	27	46.28***	0.46***	0.67	37.98***	1.20***	0.36	38.77***	0.39***	0.42	0.71	0.34	0.03
	<i>F. grandifolia</i>	25	24.81***	0.77***	0.71	18.77**	2.68***	0.73	11.16***	0.50***	1.76***	0.94	0.21	0.23

\*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Table 4. Pairwise partial correlations for LMA (response variable) and TSF while controlling for height, and partial correlations for LMA and height controlling for TSF.

Species	Response variable	Independent variable	Controlled variable	N	Coefficient	P-value
<i>C. caroliniana</i>	LMA	TSF	Height	27	0.557	0.001
		Height	TSF	27	0.308	0.113
<i>L. tulipifera</i>	LMA	TSF	Height	27	0.734	<0.001
		Height	TSF	27	0.309	0.112
<i>F. grandifolia</i>	LMA	TSF	Height	25	0.881	<0.001
		Height	TSF	25	0.888	<0.001

*Fagus grandifolia* also had a greater range in LMA than the other species (Figure 3a and b).

For  $N_{\text{area}}$ , we observed significant height ( $P < 0.001$ ), species ( $P = 0.037$ ) and TSF ( $P < 0.001$ ) effects, and the interaction height  $\times$  species ( $P = 0.383$ ) and TSF  $\times$  species ( $P = 0.746$ ) had no effect on  $N_{\text{area}}$ .  $N_{\text{area}}$  was linearly and positively correlated with TSF and height (Figure 3c and d). There were no significant differences in the  $N_{\text{area}}$ -TSF and  $N_{\text{area}}$ -height slopes among the three species ( $P > 0.05$ ).

For  $\delta^{13}\text{C}$ , we observed a significant height ( $P < 0.001$ ), species ( $P = 0.005$ ) and TSF ( $P < 0.001$ ) effect, and the interaction height  $\times$  species ( $P = 0.061$ ) and TSF  $\times$  species ( $P = 0.750$ ) had no effect on  $\delta^{13}\text{C}$ .  $\delta^{13}\text{C}$  was linearly and positively correlated with TSF and height (Figure 3e and f). There were no significant differences in the  $\delta^{13}\text{C}$ -TSF slopes among the three species ( $P > 0.05$ ). Despite the non-significant height  $\times$  species effect on  $\delta^{13}\text{C}$ , the slope of the  $\delta^{13}\text{C}$ -height relationship for *C. caroliniana* was greater than the slopes for *L. tulipifera* ( $P = 0.024$ ) and *F. grandifolia* ( $P = 0.019$ ). No differences in  $\delta^{13}\text{C}$ -height slopes were detected between *L. tulipifera* and *F. grandifolia* ( $P = 0.860$ ).

### Coordination in leaf traits among the three species

Relationships between the coordinated leaf traits LMA,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  were investigated to identify potential explanations for differences in leaf function and morphology among the three species (Figure 4). We found that LMA was positively correlated with  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  in all three species—the relation was linear in *C. caroliniana* and *L. tulipifera*, but non-linearly (quadratic in shape) correlated with  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  for *F. grandifolia*

(Figure 4a and b). The interaction (LMA  $\times$  Species) had a significant effect on both  $N_{\text{area}}$  ( $F = 5.76$ ,  $P = 0.005$ ) and  $\delta^{13}\text{C}$  ( $F = 3.17$ ,  $P = 0.048$ ), indicating that the slopes were significantly different among species. In contrast,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  were linearly correlated among all three species (Figure 4c), and the slopes of these relationships were similar among the three species ( $F = 0.20$ ,  $P = 0.818$ ).

## Discussion

### Light and height effects on LMA

Consistent with our first hypothesis, we found that light was more important than height in determining LMA for the indeterminate species, *L. tulipifera* and *C. caroliniana*. Light was also important in predicting LMA in *F. grandifolia*, but height was equally important. For *L. tulipifera*, *F. grandifolia* and other temperate deciduous species, both leaf thickness and LMA increase with increasing light availability (Goulet and Bellefleur 1985, Niinemets and Fleck 2002), suggesting that changes in LMA along vertical gradients in *L. tulipifera* and *F. grandifolia* are likely influenced by leaf thickness. Thicker leaves in temperate deciduous species tend to have thicker palisade layers (Eschrich et al. 1989, Oguchi et al. 2005), which facilitate greater light penetration to deeper layers of the mesophyll (Vogelmann and Martin 1993, Evans 1999). Leaf thicknesses of *Fagus crenata*, *Fagus japonica* and *F. sylvatica* leaves are determined during bud development in the summer of the previous year and during leaf expansion (Eschrich et al. 1989, Kimura et al. 1998, Uemura et al. 2000), which may explain why light was not considerably more important than height in regulating LMA for *F. grandifolia*.

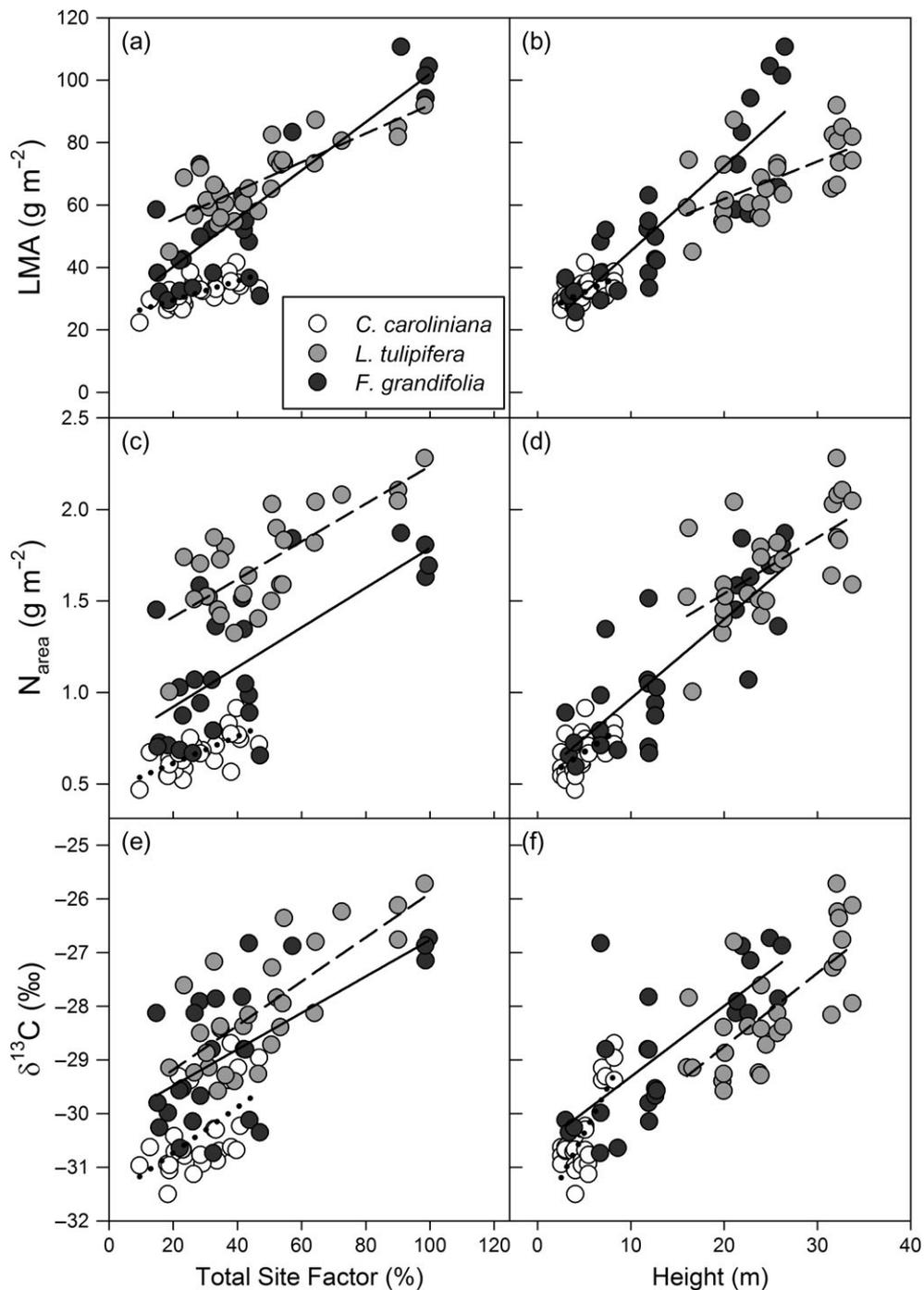


Figure 3. Relationships between LMA and total site factor (TSF) (a), LMA and height (b),  $N_{\text{area}}$  and TSF (c),  $N_{\text{area}}$  and height (d),  $\delta^{13}\text{C}$  and TSF (e), and  $\delta^{13}\text{C}$  and height for *C. caroliniana* (dotted lines), *L. tulipifera* (dashed lines) and *F. grandifolia* (solid lines).

Over the lifespan of deciduous leaves, the light environment can change considerably, particularly during leaf expansion when total crown leaf area rapidly increases from zero to a near-maximum value for the growing season (Parker and Tibbs 2004).

*Fagus grandifolia* is also more shade tolerant and has a greater  $A_i:A_s$  than *L. tulipifera*, which is consistent with other studies that observed greater  $A_i:A_s$  in *F. grandifolia* than other species (Matheny et al. 2014) and greater  $A_i:A_s$  in more shade-tolerant

species (Waring et al. 1982). There is strong evidence that  $A_i:A_s$  plays an important role in whole-tree water relations (Whitehead et al. 1984, McDowell et al. 2002, Simonin et al. 2006). Within species, lower  $A_i:A_s$  at drier sites (Mencuccini and Grace 1994) and in taller trees (McDowell et al. 2002) may act to compensate high evaporative demand and reduce gradients in leaf water potential. Similarly, the lower  $A_i:A_s$  of the taller *L. tulipifera* may reduce gradients in leaf water potential thereby reducing height

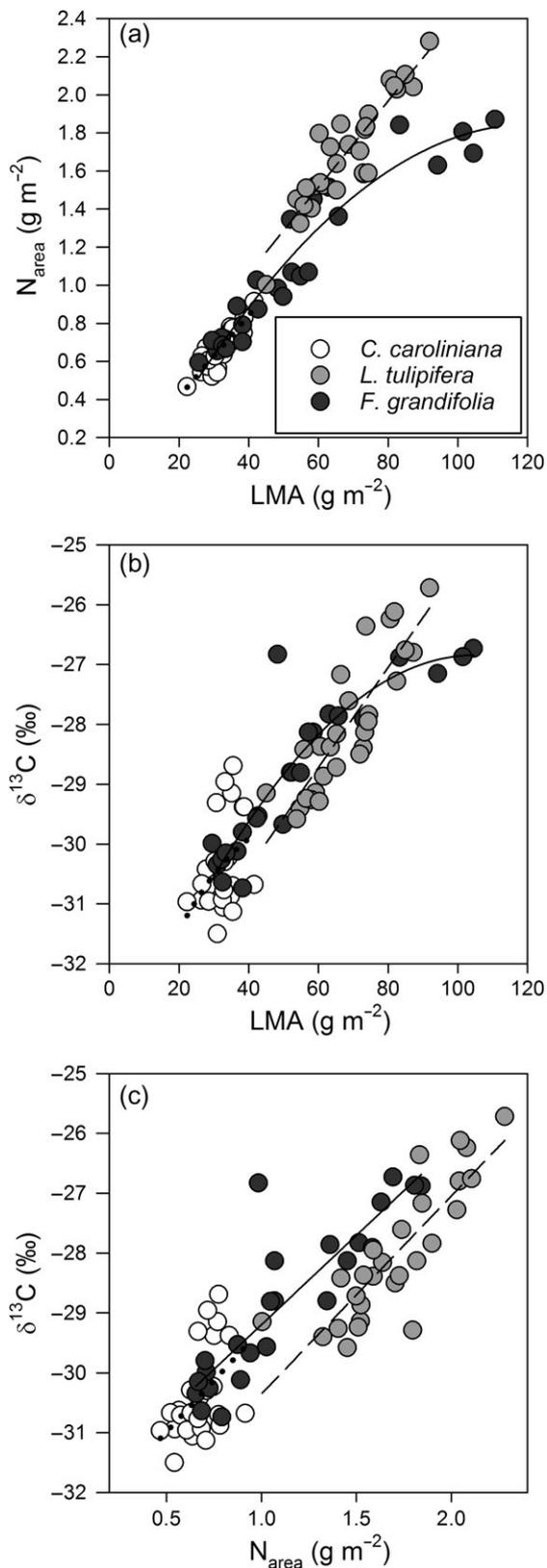


Figure 4. Relationships between  $N_{\text{area}}$  and LMA (a),  $\delta^{13}\text{C}$  and LMA (b),  $\delta^{13}\text{C}$  and  $N_{\text{area}}$  (c) for *C. caroliniana* (dotted lines), *L. tulipifera* (dashed lines) and *F. grandifolia* (solid lines). A linear model was fit to all data except for *F. grandifolia* in (a) and (b), which displayed a quadratic function.

effects on leaf development and LMA, whereas the high  $A_i:A_s$  in *F. grandifolia* may experience larger gradients in leaf water potential and height effects on LMA. Shoot  $A_i:A_s$  of *L. tulipifera* decreases with increasing height and light availability within tree crowns (Sack et al. 2006), which may also reduce gradients in leaf water potential brought about by height or greater exposure. Furthermore, greater investment in vascular tissue in leaves of *L. tulipifera* growing at high light may enhance water supply to leaves (Niinemets and Fleck 2002), potentially minimizing effects of height on LMA. It is worth noting that  $A_i:A_s$  may also compensate for changes in other factors such as leaf specific hydraulic conductivity without compensating for changes in gradients in leaf water potential. Furthermore, leaf water potential was not measured in this study. Thus, further work in identifying consequences of lower  $A_i:A_s$  on gradients in leaf water potential and leaf morphology among temperate deciduous species is required.

Regardless of the mechanistic explanations of LMA gradients for *F. grandifolia*, this species displayed a greater morphological plasticity in response to light and greater range of LMA compared with *L. tulipifera* and *C. caroliniana*. The high plasticity of *F. grandifolia* may partially explain why this species is successful in persisting in the understory of dense canopies for a long period of time (45–50 years), while occupying high-light, upper canopy positions at maturity (Canham 1990).

#### Vertical gradients in $\delta^{13}\text{C}$

Within forest canopies, the pattern of increasing leaf  $\delta^{13}\text{C}$  with height has been attributed to both reduced stomatal conductance with height as a result of gradients in leaf water potential (e.g., decline in  $\text{CO}_2$  supply; Koch et al. 2004, Ishii et al. 2008, Coble and Cavaleri 2015) and greater photosynthetic capacity with increasing light availability (e.g., increase in photosynthetic demand of  $\text{CO}_2$  and greater RuBisCO; Berry et al. 1997, Duursma and Marshall 2006). Gradients of  $\delta^{13}\text{C}$  of canopy air have been observed near the soil surface, but  $\delta^{13}\text{C}$  of air has been found to be constant above 3 m (Berry et al. 1997) and contributes little to the vertical change in leaf  $\delta^{13}\text{C}$  (Buchmann et al. 1997). In this study, there appears to be more evidence to support the hypothesis that gradients in  $\delta^{13}\text{C}$  are associated with increasing  $\text{CO}_2$  demand as a result of greater photosynthetic capacity in the upper canopy.  $N_{\text{area}}$  is strongly correlated with photosynthetic capacity in many temperate deciduous tree species (Ellsworth and Reich 1993, Wilson et al. 2000), including the European beech (*F. sylvatica*; Scartazza et al. 2016), a congener of one of our species.  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  were linearly and strongly correlated, providing evidence that greater  $\delta^{13}\text{C}$  in the upper canopy was primarily a result of higher photosynthetic demand of  $\text{CO}_2$  (e.g., higher RuBisCO activity) in the photosynthetic tissue. Similar to the results found in this study, increasing photosynthetic capacity (high  $\text{CO}_2$  demand) was primarily

attributed to increasing  $\delta^{13}\text{C}$  with canopy height in a conifer-dominated stand that displayed strong correlations between  $\delta^{13}\text{C}$  and  $N_{\text{area}}$  (Duursma and Marshall 2006).

### Coordination of LMA, $N_{\text{area}}$ and $\delta^{13}\text{C}$

Our results support our second hypothesis that LMA,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  are inter-related due to the link between leaf morphology and function. While  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  displayed a similar response to light among the three species (e.g., similar slopes), we found that LMA responded differently among the three species. These results are unexpected for LMA and  $N_{\text{area}}$ . Due to the use of LMA in calculating  $N_{\text{area}}$  ( $N_{\text{area}} = \text{LMA} \times N_{\text{mass}}$ ) and the fact that  $N_{\text{mass}}$  tends to be constant throughout the canopy, changes in  $N_{\text{area}}$  typically follow changes in LMA. While  $N_{\text{area}}$  generally followed changes in LMA in this study, we also showed that for *F. grandifolia*,  $N_{\text{area}}$  and LMA were non-linearly correlated, particularly at higher LMA where increases in LMA corresponded with diminishing increases in  $N_{\text{area}}$ . This is likely due to lower  $N_{\text{mass}}$  in the upper canopy for *F. grandifolia* (data not shown), leading to a non-linear relationship between  $N_{\text{area}}$  and LMA. From a physiological viewpoint, this may be due to greater investment in nitrogen-poor compounds to leaves such as cell walls or cuticular waxes leading to a dilution effect on leaf nitrogen. In another tall, shade-tolerant tree (*Tilia cordata*), there was evidence that high LMA and denser leaves contained greater lignin concentrations and lower nitrogen concentrations, which likely improved foliar mechanical strength and tolerance to low leaf water potential while constraining photosynthetic capacity (Niinemets et al. 1999). In this study, structural adjustments to tolerate saturating light conditions and higher evaporative demand at greater heights for *F. grandifolia* appeared to have functional consequences. More specifically,  $N_{\text{area}}$  (a proxy for photosynthetic capacity) may have been constrained due to greater investment in carbon-rich compounds to offset low water potential and increase mechanical strength for exposed leaves growing at greater heights. This may explain why both height and light were equally important for explaining LMA for *F. grandifolia*. While height appeared to be important in explaining LMA gradients and constraints on leaf function in *F. grandifolia*, leaf area above 20 m for *F. grandifolia* is relatively low compared with lower portions of the canopy and with other species at the same height in the same stand (Parker et al. 1989, Parker and Tibbs 2004).

### Conclusions

This study compared differences in LMA,  $N_{\text{area}}$  and  $\delta^{13}\text{C}$  along canopy gradients in light availability among tree species with very different growth strategies and shade tolerance. Our study highlights the important finding that three species displayed differing LMA responses to light, but the  $N_{\text{area}}$  response to light was consistent among all three species. Leaf mass per area of *C. caroliniana* and *L. tulipifera*, both indeterminate growth species,

were finely tuned with light availability along the canopy profile, while that of *F. grandifolia*, a determinate growth species, was affected by both light and height. *Fagus grandifolia* also displayed the greatest morphological plasticity in response to light. The equal importance of light and height in *F. grandifolia* may be associated with the determinate growth strategy and/or a greater  $A_i/A_s$  compared with the other tall tree, *L. tulipifera*. There also appeared to be consequences of maintaining a higher LMA in the upper canopy for *F. grandifolia*, such as constraints on  $N_{\text{area}}$ , compared with *L. tulipifera*. While there was evidence for height effects in *F. grandifolia*, light appeared to be most important for explaining vertical gradients in LMA when considering all three species.

### Acknowledgments

We thank Glenn Piercey (then at the Carnegie Institution of Washington), George Rasberry, Melanie Moses, Claire Dacey and numerous SERC Forest Canopy Lab volunteers who helped with field work and laboratory sample processing.

### Conflict of interest

None declared.

### Funding

This project was supported by the Smithsonian Institution Loeb Fund, the Smithsonian Work-Study Fellowship Program, and the Carnegie Institution of Washington, DC.

### References

- Aber JD, Reich PB, Goulden ML (1996) Extrapolating leaf  $\text{CO}_2$  exchange to the canopy: a generalized model of forest photosynthesis compared with measurements by eddy correlation. *Oecologia* 106:257–265.
- Anderson-Teixeira KJ, McGarvey JC, Muller-Landau HC et al. (2015) Size-related scaling of tree form and function in a mixed-age forest. *Funct Ecol* 29:1587–1602.
- Bauerle WL, Hinkley TM, Cermak J, Kucera J, Bible K (1999) The canopy water relations of old-growth Douglas-fir trees. *Trees Struct Funct* 13: 211–217.
- Berry SC, Varney GT, Flanagan LB (1997) Leaf  $\delta^{13}\text{C}$  in *Pinus resinosa* trees and understory plants: variation associated with light and  $\text{CO}_2$  gradients. *Oecologia* 109:499–506.
- Brush GS, Lenk C, Smith J (1980) The natural forests of Maryland: an explanation of the vegetation map of Maryland. *Ecol Monogr* 50: 77–92.
- Buchmann N, Kao WY, Ehleringer J (1997) Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia* 110: 109–119.
- Canham CD (1990) Suppression and release during canopy recruitment in *Fagus grandifolia*. *Bull Torrey Bot Club* 117:1–7.
- Cavaleri MA, Oberbauer SF, Clark DB, Clark DA, Ryan MG (2010) Height is more important than light in determining leaf morphology in a tropical forest. *Ecology* 91:1730–1739.

- Coble AP, Cavaleri MA (2014) Light drives vertical gradients of leaf morphology in a sugar maple (*Acer saccharum*) forest. *Tree Physiol* 34:146–158.
- Coble AP, Cavaleri MA (2015) Light acclimation optimizes leaf functional traits despite height-related constraints in a canopy shading experiment. *Oecologia* 177:1131–1143.
- Coble AP, VanderWall B, Mau A, Cavaleri M (2016) How vertical patterns in leaf traits shift seasonally and the implications for modeling canopy photosynthesis in a temperate deciduous forest. *Tree Physiol* 36:1077–1091.
- Duursma RA, Marshall JD (2006) Vertical canopy gradients in  $\delta^{13}\text{C}$  correspond with leaf nitrogen content in a mixed-species conifer forest. *Trees* 20:496–506.
- Ellsworth DS, Reich PB (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* 96:169–178.
- Eschrich W, Burchardt R, Essiamah S (1989) The induction of sun and shade leaves of the European beech (*Fagus sylvatica* L.): anatomical studies. *Trees* 3:1–10.
- Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of  $\text{C}_3$  plants. *Oecologia* 78:9–19.
- Evans JR (1999) Leaf anatomy enables more equal access to light and  $\text{CO}_2$  between chloroplasts. *New Phytol* 143:93–104.
- Gebauer T, Horna V, Leuschner C (2008) Variability in radial sap flux density patterns and sapwood area among seven co-occurring temperate broad-leaved species. *Tree Physiol* 28:1821–1830.
- Goulet F, Bellefleur P (1985) Leaf morphology plasticity in response to light environment in deciduous tree species and its implication on forest succession. *Can J For Res* 16:1192–1195.
- Graham HV, Patzkowsky ME, Wing SL, Parker GG, Fogel ML, Freeman KH (2014) Isotopic characteristics of canopies in simulated leaf assemblages. *Geochim Cosmochim Acta* 144:82–95.
- Hellkvist J, Richards GP, Jarvis PG (1974) Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. *J Appl Ecol* 11:637–667.
- Hikosaka K, Terashima I (1995) A model of the acclimation of photosynthesis in the leaves of  $\text{C}_3$  plants to sun and shade with respect to nitrogen use. *Plant Cell Environ* 18:605–618.
- Houborg R, Anderson MC, Norman JM, Wilson T, Meyers T (2009) Intercomparison of a 'bottom-up' and 'top-down' modeling paradigm for estimating carbon and energy fluxes over a variety of vegetative regimes across the U.S. *Agric For Meteorol* 149:1875–1895.
- Ishii H, Ohsugi Y (2011) Light acclimation potential and carry-over effects vary among three evergreen tree species with contrasting patterns of leaf emergence and maturation. *Tree Physiol* 31:819–830.
- Ishii HT, Jennings GM, Sillett SC, Koch GW (2008) Hydrostatic constraints on morphological exploitation of light in tall *Sequoia sempervirens* trees. *Oecologia* 156:751–763.
- Kimura K, Ishida A, Uemura A, Matsumoto Y, Terashima I (1998) Effects of current-year and previous-year PFDs on shoot gross morphology and leaf properties in *Fagus japonica*. *Tree Physiol* 18:459–466.
- Kitajima K, Mulkey SS, Samaniego M, Wright SJ (2002) Decline of photosynthetic capacity with leaf age and position in two tropical pioneer tree species. *Am J Bot* 89:1925–1932.
- Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. *Nature* 428:851–854.
- Kozlowski TT (1964) Shoot growth in woody plants. *Bot Rev* 30:335–392.
- Jackson LWR (1967) Effect of shade on leaf structure of deciduous tree species. *Ecology* 48:498–499.
- Jones TA, Thomas SC (2007) Leaf-level acclimation to gap creation in mature *Acer saccharum* trees. *Tree Physiol* 27:281–290.
- Long JN, Smith FW (1988) Leaf area-sapwood area relations of lodgepole pine as influenced by stand density and site index. *Can J For Res* 18:247–250.
- Marks PL (1975) On the relation between extension growth and successional status of deciduous trees of the Northeastern United States. *Bull Torrey Bot Club* 102:172–177.
- Marshall JD, Monserud RA (2003) Foliage height influences specific leaf area of three conifer species. *Can J For Res* 33:164–170.
- Matheny AM, Bohrer G, Vogel CS et al. (2014) Species-specific transpiration responses to intermediate disturbance in a northern hardwood forest. *J Geophys Res* 119:2292–2311.
- McDowell N, Barnard H, Bond BJ et al. (2002) The relationship between tree height and leaf area: sapwood area ratio. *Oecologia* 132:12–20.
- Meinzer FC, Bond BJ, Karanian JA (2008) Biophysical constraints on leaf expansion in a tall conifer. *Tree Physiol* 28:197–206.
- Meir P, Kruijt B, Broadmeadow M, Barbosa E, Kull O, Carswell F, Nobre A, Jarvis PG (2002) Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen concentration and leaf mass per unit area. *Plant Cell Environ* 25:343–357.
- Mencuccini M, Grace J (1994) Climate influences the leaf area/sapwood area ratio in Scots pine. *Tree Physiol* 15:1–10.
- Montpied P, Granier A, Dreyer E (2009) Seasonal time-course of gradients of photosynthetic capacity and mesophyll conductance to  $\text{CO}_2$  across a beech (*Fagus sylvatica* L.) canopy. *J Exp Bot* 60:2407–2418.
- Mullin LP, Sillett SC, Koch GW, Tu KP, Antoine ME (2009) Physiological consequences of height-related morphological variation in *Sequoia sempervirens* foliage. *Tree Physiol* 29:999–1010.
- Naidu SL, DeLucia EH (1998) Physiological and morphological acclimation of shade-grown tree seedlings to late-season canopy gap formation. *Plant Ecol* 138:27–40.
- Niinemets Ü, Fleck S (2002) Petiole mechanics, leaf inclination, morphology, and investment in support in relation to light availability in the canopy of *Liriodendron tulipifera*. *Oecologia* 132:21–33.
- Niinemets Ü, Kull O, Tenhunen JD (1998) An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiol* 18:681–696.
- Niinemets Ü, Kull O, Tenhunen JD (1999) Variability in leaf morphology and chemical composition as a function of canopy light environment in coexisting deciduous trees. *Int J Plant Sci* 160:837–848.
- Norby RJ, O'Neill EG (1991) Leaf area compensation and nutrient interactions in  $\text{CO}_2$ -enriched seedlings of yellow poplar (*Liriodendron tulipifera* L.). *New Phytol* 117:515–528.
- Oguchi R, Hikosaka K, Hirose T (2005) Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant Cell Environ* 28:916–927.
- Oldham AR, Sillett SC, Tomescu AMF, Koch GW (2010) The hydrostatic gradient, not light availability, drives height-related variation in *Sequoia sempervirens* (Cupressaceae) leaf anatomy. *Am J Bot* 97:1087–1097.
- Ott RL, Longnecker M (2001) An introduction to statistical methods and data analysis, 5th edn. Duxbury, Pacific Grove, CA.
- Parker GG, Tibbs DJ (2004) Structural phenology of the leaf community in the canopy of a *Liriodendron tulipifera* L. forest in Maryland, USA. *For Sci* 50:387–397.
- Parker GG, O'Neill JP, Higman D (1989) Vertical profile and canopy organization in a mixed deciduous forest. *Vegetatio* 85:1–11.
- Parker GG, Stone PJ, Bowers D (1996) A balloon for microclimate observations within the forest canopy. *J Appl Ecol* 33:173–177.
- Parker GG, Davis MM, Chapotin SM (2002) Canopy light transmittance in Douglas-fir-western hemlock stands. *Tree Physiol* 22:147–157.
- Raulier F, Bernier PY, Ung C-H (1999) Canopy photosynthesis of sugar maple (*Acer saccharum*): comparing big-leaf and multilayer extrapolations of leaf-level measurements. *Tree Physiol* 19:407–420.

- R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org> (4 March 2016, date last accessed).
- Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology* 80:1955–1969.
- Ryu Y, Baldocchi DD, Kobayashi H et al. (2011) Integration of MODIS land and atmosphere products with a coupled-process model to estimate gross primary productivity and evapotranspiration from 1 km to global scales. *Glob Biogeochem Cycles* 25:Gb4017 .
- Sack L, Melcher PJ, Liu WH, Middleton E, Pardee T (2006) How strong is intracopy leaf plasticity in temperate deciduous trees? *Am J Bot* 93: 829–839.
- Scartazza A, Baccio DD, Bertolotto P, Gavrichkova O, Matteucci G (2016) Investigating the European beech (*Fagus sylvatica* L.) leaf characteristics along the vertical canopy profile: leaf structure, photosynthetic capacity, light energy dissipation and photoprotection mechanisms. *Tree Physiol* 31:1–17.
- Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* 148:339–346.
- Simonin K, Kolb TE, Montes-Helu M, Koch GW (2006) Restoration thinning and influence of tree size and leaf area to sapwood area ratio on water relations of *Pinus ponderosa*. *Tree Physiol* 26:493–503.
- Thornton PE, Zimmerman NE (2007) An improved canopy integration scheme for a land surface model with prognostic canopy structure. *J Clim* 20:3902–3923.
- Uemura A, Ishida A, Nakano T, Terashima I, Tanabe H, Matsumoto Y (2000) Acclimation of leaf characteristics of *Fagus* species to previous-year and current-year solar irradiances. *Tree Physiol* 20:945–951.
- Vogelmann TC, Martin G (1993) The functional significance of palisade tissue: penetration of directional versus diffuse light. *Plant Cell Environ* 16:65–72.
- Waring RH, Schroeder PE, Oren R (1982) Application of the pipe model theory to predict canopy leaf area. *Can J For Res* 12:556–560.
- Whitehead D, Edwards WRN, Jarvis PG (1984) Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. *Can J For Res* 14:940–947.
- Wilson KB, Baldocchi DD, Hanson PJ (2000) Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. *Tree Physiol* 20:565–578.
- Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? *Plant Cell Environ* 27:229–236.
- Wright IJ, Reich PB, Westoby M et al. (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Zhang Y, Equiza MA, Zheng Q, Tyree MT (2011) Factors controlling plasticity of leaf morphology in *Robinia pseudoacacia*: III. biophysical constraints on leaf expansion under long-term water stress. *Physiol Plantarum* 143:367–374.
- Zhang Y, Zheng Q, Tyree MT (2012a) Factors controlling plasticity of leaf morphology in *Robinia pseudoacacia* L. I: height-associated variation in leaf structure. *Ann For Sci* 69:29–37.
- Zhang Y, Equiza MA, Zheng Q, Tyree MT (2012b) Factors controlling plasticity of leaf morphology in *Robinia pseudoacacia* L. II: the impact of water stress on leaf morphology of seedlings grown in a controlled environment chamber. *Ann For Sci* 69:39–47.
- Zwieniecki MA, Boyce CK, Holbrook NM (2004) Hydraulic limitations imposed by crown placement determine final size and shape of *Quercus rubra* L. leaves. *Plant Cell Environ* 27:357–365.