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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Light acclimation optimizes leaf functional traits despite height-related constraints in a canopy shading experiment

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Abstract Within-canopy gradients of leaf functional traits have been linked to both light availability and vertical gradients in leaf water potential. While observational studies can reveal patterns in leaf traits, within-canopy experimental manipulations can provide mechanistic insight to tease apart multiple interacting drivers. Our objectives were to disentangle effects of height and light environment on leaf functional traits by experimentally shading branches along vertical gradients within a sugar maple (*Acer saccharum*) forest. Shading reduced leaf mass per area (LMA), leaf density, area-based leaf nitrogen (N_{area}), and carbon:nitrogen (C:N) ratio, and increased mass-based leaf nitrogen (N_{mass}), highlighting the importance of light availability on leaf morphology and chemistry. Early in the growing season, midday leaf water potential (Ψ_{mid}), LMA, and N_{area} were driven primarily by height; later in the growing season, light became the most important driver for LMA and N_{area}. Carbon isotope composition ($\delta^{13}C$) displayed strong, linear correlations with height throughout the growing season, but did not change with shading, implying that height is more influential than light on water use efficiency and stomatal behavior. LMA, leaf density, N_{mass}, C:N ratio, and $\delta^{13}C$ all changed seasonally, suggesting that leaf ageing effects on leaf functional traits are equally as important as microclimatic conditions. Overall, our results indicate that: (1) stomatal sensitivity to vapor pressure deficit or Ψ_{mid} constrains the supply of CO_2 to leaves at higher heights, independent of light environment, and (2) LMA and N_{area} distributions become functionally optimized through morphological acclimation to light with increasing leaf age despite height-related constraints.

Keywords Foliar morphology · Experimental shading · Leaf mass per area · Light environment · Sugar maple

Introduction

Across biomes, leaf functional traits are important for predicting leaf and ecosystem functioning (Wright et al. 2004; Poorter et al. 2009). Coupled with large-scale relationships developed for plant albedo and leaf nitrogen (Ollinger et al. 2008; Hollinger et al. 2010), these patterns in leaf functional traits [leaf nitrogen per unit leaf area (N_{area}), leaf mass per area (LMA), and photosynthetic capacity] have been used to estimate gross primary productivity from local to global scales (Ryu et al. 2011). Vertical patterns in LMA and carbon:nitrogen (C:N) ratio have been incorporated into canopy process and land surface component models that predict carbon flux and forest response to changes in environmental conditions (Gutschick and Wiegel 1988; Raulier et al. 1999; Hanson et al. 2004; Medlyn 2004; Thornton and Zimmermann 2007). Consequently, leaf functional traits are useful for scaling from leaf- to ecosystem- to global-level processes when modeling carbon, water, and nutrient cycling.

A central theme when modeling forest canopy photosynthesis is the assumption that structural carbon and leaf nitrogen concentrations are optimally distributed with respect to light to maximize carbon gain (Field 1983; Hirose and

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Werger 1987; Sellers et al. 1992; Amthor 1994). During acclimation to high light availability, greater investment of carbon into leaf construction (high LMA) and nitrogen into RUBISCO and thylakoid proteins (high N_{area}) often results in higher rates of light-saturated photosynthesis and greater carbon isotope composition ($\delta^{13}C$) (Evans 1989; Hollinger 1989; Ellsworth and Reich 1993; Berry et al. 1997; Niinemets 1997; Livingston et al. 1998; Bond et al. 1999; Niinemets et al. 1999, 2001; Sack et al. 2006; Duursma and Marshall 2006). Thus, leaf functional traits (LMA, N_{area}, and photosynthetic capacity) and $\delta^{13}C$ are often coordinated and scale with light within tree crowns and forest canopies, corroborating optimal patterns derived from models (Hirose and Werger 1987, Livingston et al. 1998; Ellsworth and Reich 1993; Duursma and Marshall 2006).

However, optimal patterns in photosynthetic capacity with respect to light may be constrained by other environmental factors, resulting in a decline in photosynthetic capacity per unit of irradiance that is often observed in field studies (Hollinger 1996; Bond et al. 1999; Niinemets and Valladares 2004; Buckley et al. 2013). For example, in tall *Sequoia sempervirens* and *Pseudotsuga menziesii* (e.g., up to 113 m; Koch et al. 2004; Woodruff et al. 2004; Burgess and Dawson 2007; Ishii et al. 2008) and in shorter tropical (e.g., up to 45 m; Cavaleri et al. 2010) and temperate deciduous (e.g., up to 18 m; Zwieniecki et al. 2004; Zhang et al. 2011b) trees, gravity and the length of the hydraulic pathway result in water potential gradients with height, potentially limiting leaf development as indicated by LMA. Hydraulic limitation on leaf structure can increase leaf tissue density and cell wall thickness and reduce mesophyll air-space, potentially restricting mesophyll conductance to CO_2 and photosynthesis, as indicated by increasing $\delta^{13}C$ with canopy height (Koch et al. 2004; Niinemets et al. 2004; Mullin et al. 2009; Oldham et al. 2010). The height at which water potential is limiting to leaf development is likely to vary among species depending on their hydraulic characteristics. Leaf N_{area} measured along vertical gradients has been linked to changes in LMA primarily due to the conversion of N_{mass} to N_{area} through LMA and the constant values of N_{mass} (Ellsworth and Reich 1993; Bond et al. 1999). Therefore, any constraints on LMA are likely to have similar constraints on N_{area}. Consistent with these observations, model-based approaches have attributed the discrepancy between theoretical patterns (optimal) and actual patterns (suboptimal) in photosynthetic capacity and leaf nitrogen to hydraulic constraints (Peltoniemi et al. 2012; Buckley et al. 2013). When hydraulic constraints are considered, leaf nitrogen and photosynthetic capacity are not directly proportional to light, likely due to the direct and indirect effects of greater xylem tension on either stomatal or mesophyll conductance to CO_2 (Peltoniemi et al. 2012; Buckley et al. 2013). Evidence also suggests that the discrepancy among theoretical and actual patterns in leaf nitrogen may be an artifact of light models that do not account for diffuse and direct light (Hikosaka 2014).

A major difficulty in understanding the effects of light conditions on leaf functional traits is confounding water potential and light gradients with height. Experimental shading can be a useful approach in teasing apart the effects of light and leaf water potential, testing mechanistic hypotheses, and providing insight into interrelated leaf traits and chemistry. Branch-level shading has shown that leaf function (i.e., photosynthesis, respiration, and leaf nitrogen) can acclimate to shading despite no structural changes (Brooks et al. 1994), and that leaf age has similar effects on photosynthetic capacity as shading (Brooks et al. 1996). Manipulation of branch-level and whole-plant light availability has also provided insight into leaf functional and morphological acclimation to light (Goulet and Bellefleur 1986; Naidu and DeLucia 1998; Bloor and Grubb 2004; Jones and Thomas 2007; Ishii and Ohsugi 2011), branch autonomy (Yamamoto et al. 1999; Brooks et al. 2003; He and Dong 2003; Lacointe et al. 2004; Kawamura 2010), and light effects on branch growth and carbon allocation (Claussen 1996; Henriksson 2001). However, little is known about shading effects on leaves in the presence of gravitational water potential gradients in tall trees.

The main objectives of this study were to identify the effects of shading on leaf functional traits at various heights within a sugar maple (*Acer saccharum*) canopy to tease apart the effects of height (hydraulic limitation) and light environment on leaf functional traits and shoot growth. Using an experimental approach, we tested the following hypotheses for *A. saccharum*: (1) N_{area} is optimally distributed within *A. saccharum* tree crowns as a result of the strong influence of light on LMA (but not on N_{mass}); (2) shading reduces environmental stress on leaves (i.e., reduced light and leaf temperature), resulting in reduced stomatal closure and lower leaf $\delta^{13}C$; (3) leaves growing in higher light availability experience greater increases in N_{area} over time because LMA changes while N_{mass} does not; (4) leaf $\delta^{13}C$ increases over time due to stomatal sensitivity to drier conditions that develop during mid- to late summer.

Methods

Site and experimental design

The study was conducted in closed-canopy sugar maple (*Acer saccharum* Marshall) forest at the Michigan Technological University Ford Forestry Center near Alberta, Michigan, USA (46.65°N, 88.48°W). Mean annual temperature and precipitation at the Ford Forestry Center are 4.8 °C and 810 mm, respectively (NOAA, WS ID 15608). This stand consisted mostly of *Acer saccharum*, but also included *Betula alleghaniensis*, *Ostrya virginiana*, *Tilia americana*, and *Ulmus americana*. In 2009, the mean height of the stand was 23.0 m, the basal area was 33 m^2 ha^{-1}, and the density was 267 trees ha^{-1}. The tree density of *Acer saccharum* was 259 tree ha^{-1} or 97 % of the tree density. A cable zip-line system (Coble and Cavaleri 2014) provided crown access along a two-dimensional plane below three 15-m-high cables, and arborist-style climbing techniques were used to access the canopy above 15 m. More information about the site history and methodology can be found in Coble and Cavaleri (2014).

Prior to bud burst in the spring of 2013, shade structures were installed at four heights (1–3, 7–9, 12–14, and 17–20 m) along three vertical transects (see “Appendix A” in the Electronic supplementary material, ESM). Seven trees were used in the overall design, and each vertical transect contained 2–3 trees. Shade structures were constructed with PVC pipe to form a 0.8 × 0.8 m frame. Shade cloth (50 %) was draped over the frames and tightly fastened using zip ties. The shade structures were suspended from aluminum bars, which were either screwed into or clamped onto large branches with a stainless steel padded repair clamp. Branches below the shade structure and paired branches next to shade structures were flagged for leaf sampling and for branch measurements after leaf senescence.

Light, leaf water potential, and morphology measurements

We measured light conditions as percent photosynthetic photon flux density (%PPFD) for paired shaded and unshaded branches in June and August of 2013 using a ceptometer (Sunfleck PAR ceptometer, Decagon Devices, Pullman, WA, USA). We collected ten measurements above paired shaded and unshaded branches between 12:00 and 15:00 h in June and August. Ten open-sky measurements were collected prior to light measurements in a nearby open field to estimate %PPFD (mean below-canopy PPFD divided by mean above-canopy PPFD × 100). Previous studies have used a similar technique where open-sky measurements were temporally and spatially offset from below-canopy measurements (Martens et al. 1993; Knapp et al. 2008). In order to minimize the error associated with temporal changes in open sky measurements, below-canopy and open-sky measurements were made under uniform overcast or cloudless conditions.

Immediately following light measurements (12:00–15:00 h) in June and August, three leaves from shaded and unshaded branches were cut near the base of the petiole, placed in sealed plastic bags with moist paper towels, and stored in an ice chest until measurements were taken. Midday leaf water potential (Ψ_{mid}) measurements were made using a pressure chamber (PMS Instrument, Co., Corvallis, OR, USA) within an hour of leaf collection. Leaves were then stored at 2 °C until leaf morphology measurements were made.

Leaves were scanned into digital format using an Epson Expression 10000XL flatbed color image scanner (Seiko Epson Corporation, Nagano, Japan), and images were digitally analyzed for one-sided leaf area using ImageJ v1.44j (Schneider et al. 2012). Using Archimede’s principle, we derived leaf volume by immersing fresh leaves in a beaker of water placed on a balance (Coble and Cavaleri 2014). Leaves were dried at 65 °C for 48 h and weighed to the nearest 0.1 mg. LMA was calculated as the leaf dry mass (g) divided by leaf area (m^2), and density was calculated as the leaf dry mass (g) divided by leaf volume (cm^3).

Leaf nitrogen and carbon isotope composition

Leaf samples used to estimate leaf nitrogen and carbon isotope composition were processed at the Michigan Technological University Forest Ecology Stable Isotope Laboratory. The set of three leaves collected from each of the shaded and unshaded branches from each month were combined and ground to a fine powder (8,000 M Mixer/Mill, Spex SamplePrep LLC, Metuchen, NJ, USA). Leaf nitrogen on a mass basis (N_{mass}, mg g^{-1}) was determined using a Costech elemental combustion system 4010 connected to a Thermo Finnigan ConfloIII Interface and Delta+ continuous flow stable isotope ratio mass spectrometer (Thermo Scientific, Waltham, MA, USA). Leaf N_{area} was determined as the product of N_{mass} and LMA divided by 100. Leaf $\delta^{13}C$ was calculated as $\delta^{13}C = 1{,}000\ (R_{sample}/R_{standard}-1)$ (‰), where R_{sample} is the $^{13}C/^{12}C$ ratio for the sample and $R_{standard}$ is the ratio for a standard.

Data analysis

We conducted an ANCOVA for shading, month, and height effects (independent variables) on light (%PPFD), Ψ_{mid}, leaf mass, leaf area, LMA, leaf density, N_{area}, N_{mass}, C:N ratio, and $\delta^{13}C$ (dependent variables). Height (1–21 m) was the covariate, and the categorical variables—shading and month—both had two levels (shaded, unshaded; June, August). Regression analysis was used to determine significant relationships between height and light, morphology, and leaf nitrogen parameters as described above. We used three approaches to separate out the effects of light and height on LMA, N_{area}, and $\delta^{13}C$. First, we plotted LMA, N_{area}, and $\delta^{13}C$ vs. height by month within a narrow band of light conditions (1–3 %). Second, we plotted the residuals of LMA, N_{area}, and $\delta^{13}C$ vs. height against light and the residuals of LMA, N_{area}, and $\delta^{13}C$ vs. light against height. Calculation of residuals accounted for month effects by plotting within each month. Third, we compared the contribution of light and height to the full model ($y = \beta_0 + \beta_1\text{light} + \beta_2\text{height}$) for predicting LMA, N_{area}, and $\delta^{13}C$ using partial R^2 values.

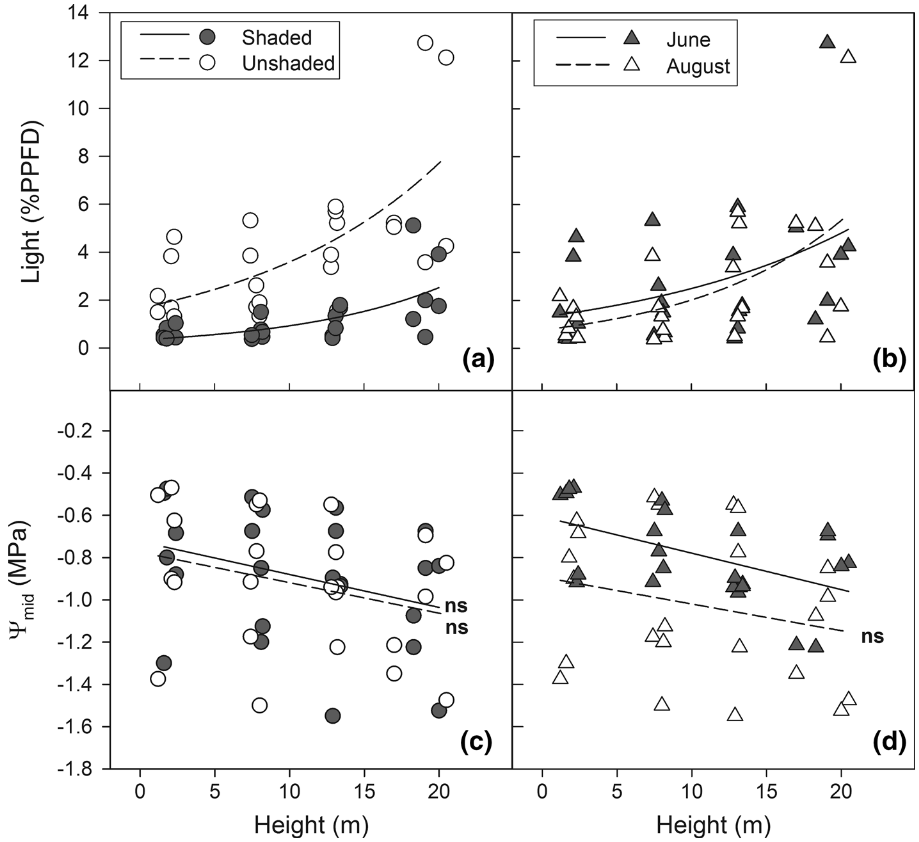


Fig. 1 Relationships between height and light (**a**, **b**) and Ψ_{Mid} (**c**, **d**) for shaded and unshaded leaves (**a**, **c**) and for leaves collected in June and August (**b**, **d**). Nonsignificant relationships ($P > 0.05$) with height are indicated by *ns*

The interaction terms (height × light) were not significant and were not included in the full models. Light values were natural log transformed (ln) for relationships with LMA to satisfy regression assumptions and to develop linear models for ANCOVA and partial R^2 analyses. All statistical analyses were conducted using R statistical software (R Development Core Team 2013). The "lm" and "anova" R functions were used to define the linear model and to produce the ANCOVA output, respectively.

Results

Shading and seasonal effects on environmental drivers and leaf functional traits

Light availability decreased as a result of shading, did not change from June to August, and increased exponentially with height for both shaded and unshaded leaves (Fig. 1a, b). Leaf midday water potential (Ψ_{mid}) was unaffected by shading, was lower in August compared with June, and decreased linearly with height for June (Table 1; Fig. 1c, d). Total cumulative precipitation was 9.0 cm the month prior (May 18 to June 18) to June measurements and 0.4 cm the month prior (July 13 to August 13) to August measurements (NOAA, WS ID 15608).

Leaf mass and area, two components of LMA, both decreased under shading but displayed opposite trends with height (Table 1; Fig. 2). Leaf mass increased linearly with height for unshaded leaves and was greater at higher heights compared with shaded leaves (Table 1; Fig. 2a). Leaf mass also increased linearly with height for leaves collected in June but not for August, and leaf mass was greater for leaves collected in August (Table 1; Fig. 2b). Leaf area was lower for shaded leaves, decreased linearly with height for shaded leaves only, and did not change with height within June and August (Table 1; Fig. 2c, d),

Both LMA and density decreased in response to shading, but shading did not affect branch growth (Table 1; Fig. 2e–h). LMA increased linearly with height among unshaded and shaded leaves, but the slope of the LMA–height relationship was greater for unshaded leaves (Fig. 2e). In contrast, slopes were similar for LMA–height relationships in June and August (Fig. 2f). Similar to leaf mass, leaf density increased linearly with height for unshaded leaves and for leaves collected in June (Table 1; Fig. 2g, h). Finally, height, shade treatment, and the height × shade treatment interaction did not have an effect on 2013 branch growth (cm year^{-1}; Table 1).

Leaf nitrogen among shaded and unshaded branches along vertical gradients was measured to identify potential shading effects at different heights. Mean N_{mass} of shaded

Table 1 Summary of ANCOVA results for tests of height (Ht), shade treatment (ST), month (*M*), and all two-way interaction effects on light (%PPFD), Ψ_{mid}, leaf morphological parameters (mass, area, LMA, density), shoot growth, area- and mass-based leaf nitrogen (N_{mass} and N_{area}), C:N ratio, and $\delta^{13}C$

Response variable	Independent variables						Coefficients ± standard error						
	Ht	ST	*M*	Ht × ST	Ht × *M*	ST × *M*	β_0	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}
(ln)Light	***	***	ns	ns	ns	ns	0.42 ± 0.28	0.07 ± 0.02	−1.42 ± 0.36	0.31 ± 0.35	0.01 ± 0.03	−0.01 ± 0.03	−0.04 ± 0.33
Ψ_{mid}	*	ns	**	ns	ns	ns	−0.92 ± 0.14	−0.01 ± 0.01	0.04 ± 0.18	0.28 ± 0.18	−0.00 ± 0.01	−0.00 ± 0.01	0.01 ± 0.16
Mass (g)	**	***	**	*	ns	ns	0.23 ± 0.03	0.01 ± 0.00	−0.05 ± 0.04	−0.09 ± 0.04	−0.01 ± 0.00	−0.00 ± 0.00	0.06 ± 0.03
Area (cm^2)	*	*	ns	ns	ns	ns	83.06 ± 7.32	−0.55 ± 0.58	−7.28 ± 9.17	−11.8 ± 9.13	−0.66 ± 0.66	0.26 ± 0.66	8.81 ± 8.45
LMA ($g\ m^{-2}$)	***	***	***	**	ns	ns	26.32 ± 1.43	1.15 ± 0.11	−4.00 ± 1.78	−5.82 ± 1.78	−0.40 ± 0.13	−0.19 ± 0.13	3.31 ± 1.64
Density ($g\ cm^{-3}$)	***	***	***	ns	ns	ns	0.27 ± 0.01	0.00 ± 0.00	−0.03 ± 0.02	−0.08 ± 0.02	−0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.02
Shoot growth ($cm\ year^{-1}$)	ns	ns	–	ns	–	–	3.83 ± 0.80	0.05 ± 0.07	−0.44 ± 1.13	–	−0.03 ± 0.09	–	–
N_{mass} ($mg\ g^{-1}$)	ns	***	***	ns	ns	ns	20.00 ± 1.20	0.07 ± 0.09	4.75 ± 1.50	5.41 ± 1.49	−0.20 ± 0.11	0.02 ± 0.11	0.63 ± 1.38
N_{area} ($g\ m^{-2}$)	***	**	ns	**	ns	ns	0.51 ± 0.04	0.03 ± 0.00	0.04 ± 0.06	−0.00 ± 0.05	−0.01 ± 0.00	0.00 ± 0.00	0.05 ± 0.05
C:N	ns	***	***	*	ns	ns	22.87 ± 0.88	−0.08 ± 0.07	−4.12 ± 1.10	−4.34 ± 1.10	0.17 ± 0.08	−0.00 ± 0.08	0.33 ± 1.02
$\delta^{13}C$ (‰)	***	ns	***	ns	ns	ns	−31.54 ± 0.42	0.13 ± 0.03	−0.67 ± 0.52	0.89 ± 0.52	0.02 ± 0.04	0.02 ± 0.04	0.26 ± 0.48

Coefficients (±standard error) for each independent variable in the model are listed in the last seven columns. Model equation: $y = \beta_0 + \beta_1\mathrm{Ht} + \beta_2\mathrm{ST} + \beta_3 M + \beta_{12}\mathrm{Ht} \times \mathrm{ST} + \beta_{13}\mathrm{Ht} \times M + \beta_{23}\mathrm{ST} \times M$, ST = 1 if shaded and −1 if unshaded, *M* = 1 if June and −1 if August; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

leaves was greater than that of unshaded leaves (Table 1; Fig. 3a), and N_{mass} of leaves collected in June was greater than that of leaves collected in August (Table 1; Fig. 3b). N_{area} decreased under shade treatment, particularly at higher heights (Table 1; Fig. 3c). N_{area} increased linearly with height for unshaded and shaded leaves (Fig. 3c) and for leaves collected in June and August (Fig. 3d). The C:N ratio decreased under shade treatment and increased from June to August (Table 1; Fig. 3e, f).

Leaf $\delta^{13}C$ gradients were compared between shaded and unshaded branches to identify acclimation responses to light, but leaf $\delta^{13}C$ did not change under shade treatment (Table 1; Fig. 3g). Leaf $\delta^{13}C$ decreased from June to August at all heights (Table 1; Fig. 3h). Leaf $\delta^{13}C$ increased linearly with height for unshaded and shaded leaves and for leaves collected in June and August (Fig. 3g, h).

Teasing apart the effects of light and height on leaf functional traits

We used a three-step approach (see "Methods" for the full description) to further tease apart the effects of light and height on three important variables: LMA, N_{area}, and $\delta^{13}C$. Within a narrow range of light conditions (1–3 %PPFD), LMA increased with height in June but not in August (Fig. 4a). The residuals of LMA vs. (ln)light increased linearly with height, and the residuals of LMA vs. height increased nonlinearly with height (Fig. 4b, c). The partial regression analysis showed that height contributed more to the full model for predicting LMA in June, but light contributed more to the full model in August (Table 2).

N_{area} increased with height in both June and August within the narrow range of light conditions (Fig. 4d). The residuals of N_{area} vs. light increased linearly with height and the residuals of N_{area} vs. height increased linearly with light (Fig. 4e, f). Partial regression analysis showed that height contributed more to the full model for predicting N_{area} in June, and light contributed more in August (Table 2).

Leaf $\delta^{13}C$ increased linearly with height in June within a narrow range of light conditions, but not in August (Fig. 4g). The residuals of $\delta^{13}C$ vs. light increased linearly with height, and the residuals of $\delta^{13}C$ vs. height did not change with light (Fig. 4h, i). The partial regression analysis showed that height contributed more to the full model for predicting $\delta^{13}C$ in both June and August (Table 2).

Discussion

Optimization of leaf functional traits

Our results provide partial support for our first hypothesis that N_{area}, but not N_{mass}, would be optimally distributed

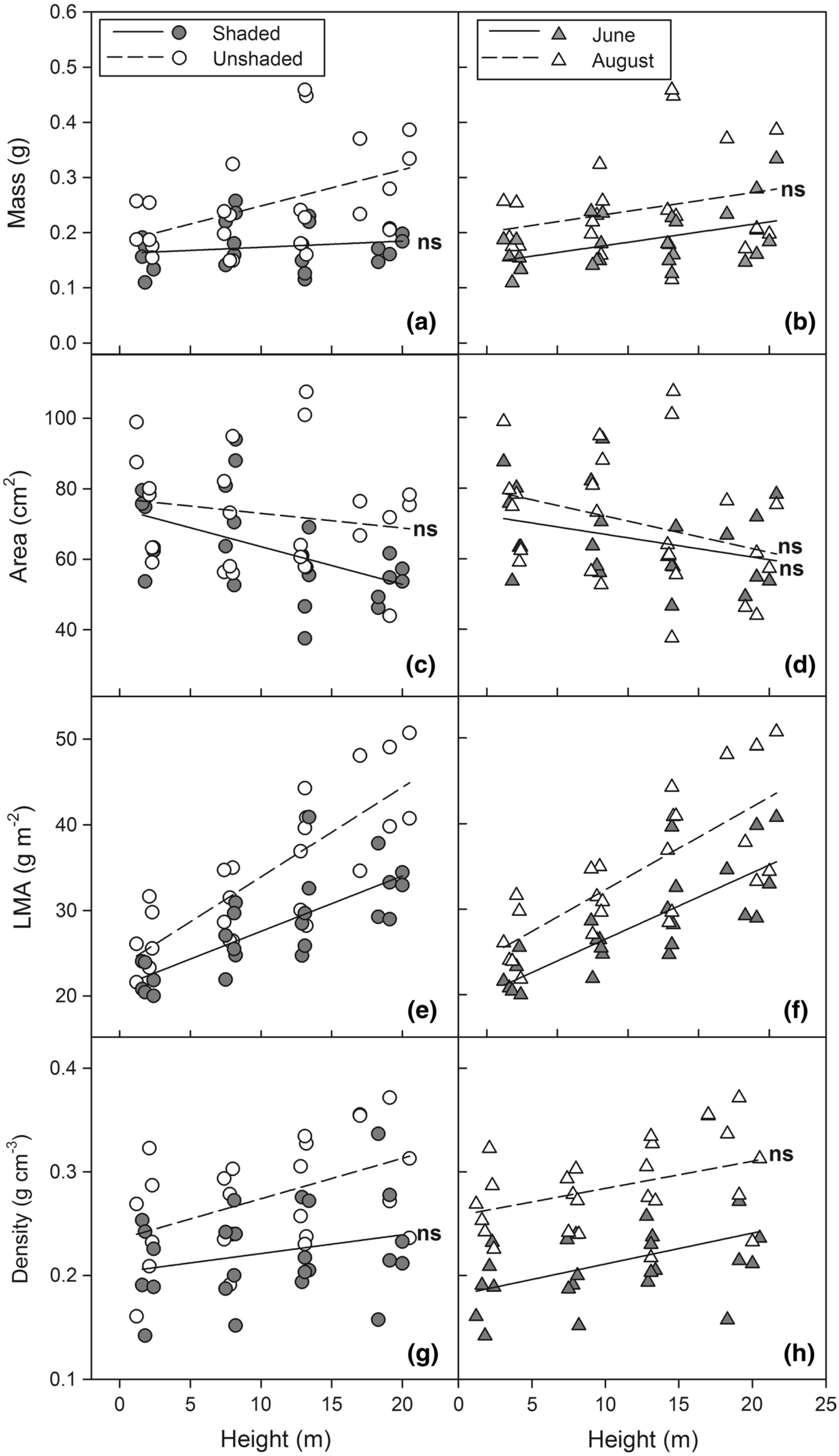
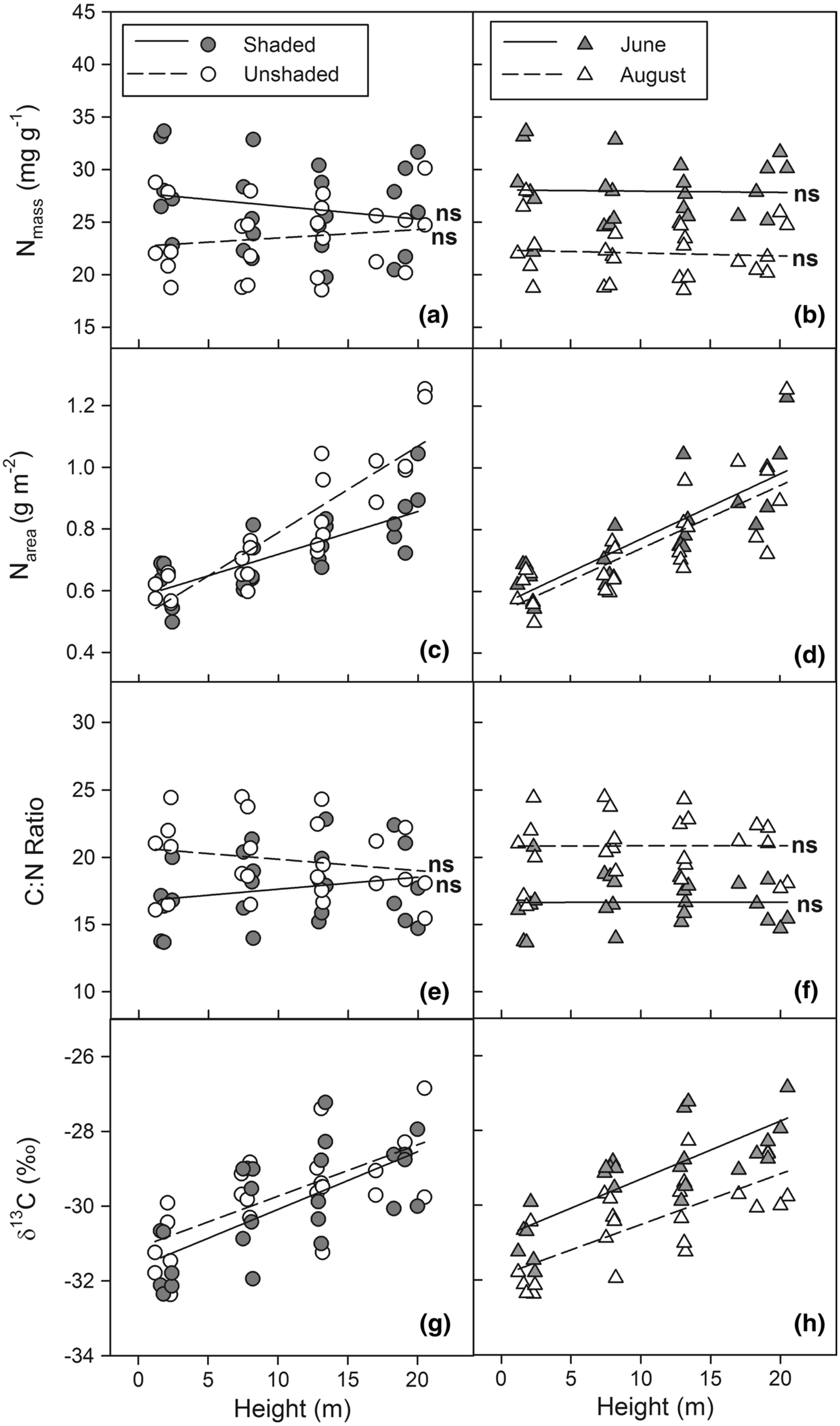


Fig. 2 Relationships between height and leaf mass (**a**, **b**), area (**c**, **d**), LMA (**e**, **f**), and density (**g**, **h**) for shaded and unshaded leaves (**a**, **c**, **e**, **g**) and for leaves collected in June and August (**b**, **d**, **f**, **h**). Nonsignificant relationships ($P > 0.05$) with height are indicated by *ns*

within the canopy as a result of the strong influence of light on LMA. Experimentally reduced light availability resulted in lower leaf mass, density, LMA, and N_{area}, all most apparent in upper canopy positions, whereas N_{mass} showed the opposite trend. Even though N_{mass} increased with shading, a larger decrease in LMA with shading resulted in a decrease in N_{area}. In a previous observational study of *A. saccharum*, Coble and Cavaleri (2014)

Fig. 3 Relationships between height and N_{mass} (**a**, **b**), N_{area} (**c**, **d**), C:N ratio (**e**, **f**), and $\delta^{13}C$ (**g**, **h**) for shaded and unshaded leaves (**a**, **c**, **e**, **g**) and for leaves collected in June and August (**b**, **d**, **f**, **h**). Nonsignificant relationships ($P > 0.05$) with height are indicated by *ns*



concluded that changes in LMA, density, and thickness with height were primarily driven by light. Leaves that develop under high light availability tend to be thicker as a result of thicker palisade mesophyll cell layers, which maximize light capture (Oguchi et al. 2005; Zhang et al. 2011a) and denser leaf tissues (Niinemets et al. 1999; Coble and Cavaleri 2014), both of which lead to greater LMA. For *A. saccharum*, leaves growing in high light with greater LMA also have greater N_{area} and photosynthetic capacity (Ellsworth and Reich 1992a, 1993; Jones and

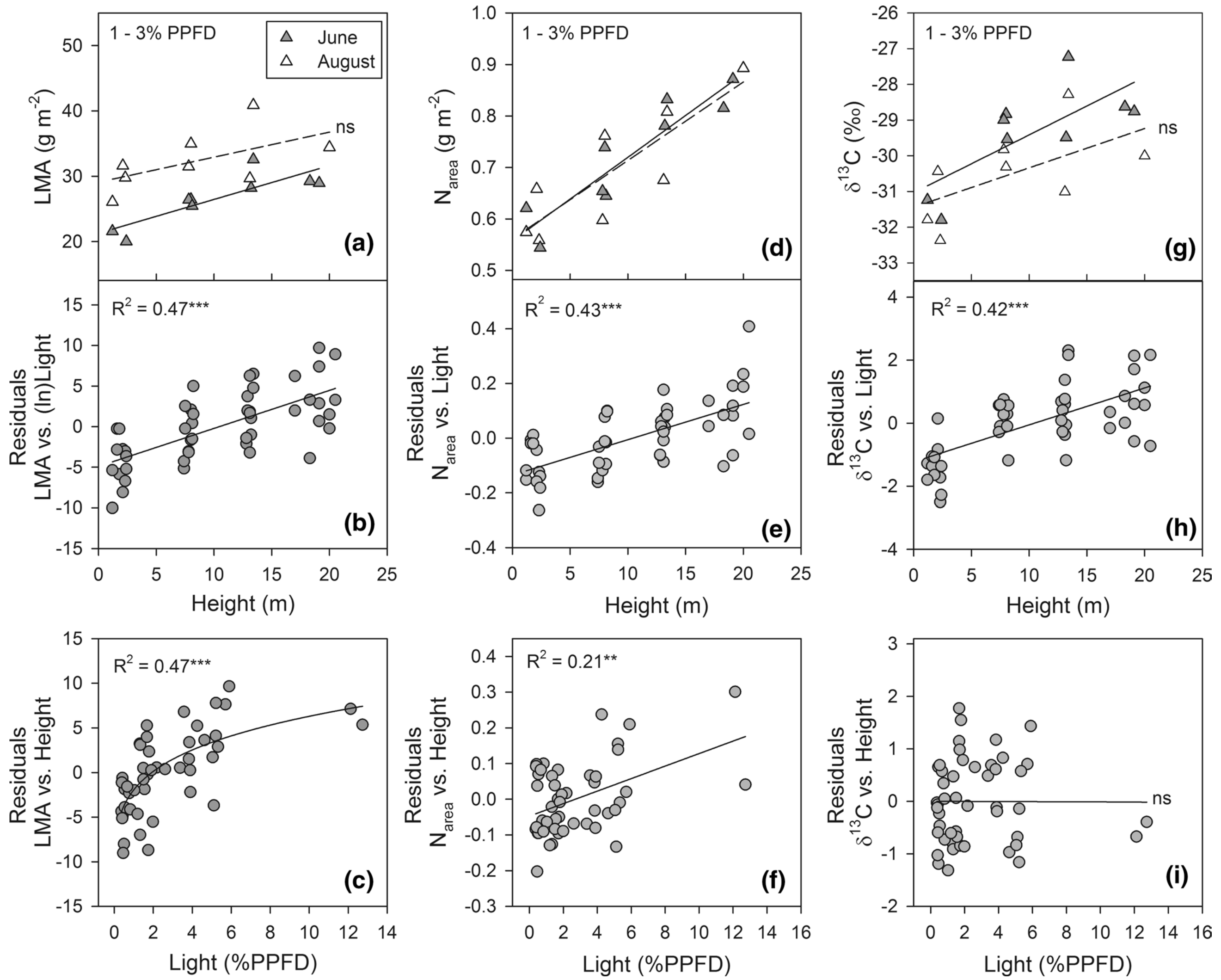


Fig. 4 Relationship between height and LMA, N_{area}, and δ^{13}C for leaves growing within a narrow range of light conditions (1–3 %PPFD; **a**, **d**, **g**). Residuals of LMA, N_{area}, and δ^{13}C vs. light (%PPFD) plotted against height (**b**, **e**, **h**) and residuals of LMA, N_{area}, and δ^{13}C vs. height plotted against light (**c**, **f**, **i**). Note that residuals were calculated from models developed for each month (June and August). Nonsignificant relationships ($P > 0.05$) with height are indicated by *ns*

Thomas 2007), likely due to the critical role of leaf nitrogen in protein-pigment complexes in the thylakoid membrane and in RUBISCO (Evans 1989). Leaves acclimated to high light also maintain higher leaf hydraulic conductance in *A. saccharum* (Sack et al. 2003), other temperate deciduous trees (Aasamaa et al. 2004; Lemoine et al. 2002; Sellin and Kupper 2007; Sellin et al. 2008), and conifer trees (Jerez et al. 2004; Burgess et al. 2006), suggesting that light availability, leaf hydraulic conductance, N_{area}, LMA, and photosynthetic capacity have co-optimal patterns with light. Overall, adjustments in LMA and N_{area} to shading and along light gradients as reported in this study provide evidence that the distributions of LMA and N_{area} become optimized through morphological acclimation to light over the course of the growing season.

Constraints on leaf functional traits

Height effects on LMA and N_{area} were most apparent early in the growing season (June), when leaf water potential showed linear decreases with height. Height has been found to directly drive LMA gradients in forest canopies, where height effects have been detected under saturating light conditions (Burgess and Dawson 2007; Ishii et al. 2008; Cavaleri et al. 2010; Coble et al. 2014). Height-related limitations to leaf development in tall conifer trees (*Pseudotsuga menziesii* and *Sequoia sempervirens*) include water potential gradients (Koch et al. 2004; Burgess and Dawson 2007; Ishii et al. 2008) and subsequent reductions in turgor pressure (Woodruff et al. 2004; Meinzer et al. 2008). Reduced leaf water potential can constrain cell

Table 2 Partial regression analysis of LMA and N_{area} vs height and light

Response variable	Month	n	Light only†			Height only‡			Light and height§				Partial R^2 for adding:	
			β_0	β_1	R^2	β_0	β_1	R^2	β_0	β_1	β_2	R^2	Light	Height
LMA	June	24	25.1***	4.67***	0.54	20.2***	0.751***	0.64	20.1***	3.17***	0.563***	0.85	0.21	0.30
	August	24	31.0***	6.58***	0.65	24.4***	0.936***	0.57	25.7***	4.73***	0.594***	0.83	0.26	0.18
N_{area}	June	24	0.696***	0.029*	0.23	0.557***	0.021***	0.67	0.543***	0.012	0.019***	0.71	0.03	0.48
	August	24	0.615***	0.051***	0.63	0.533***	0.021***	0.59	0.523***	0.035***	0.013***	0.80	0.21	0.17
$\delta^{13}C$	June	24	−29.7***	0.154	0.11	−30.9***	0.155***	0.61	−30.9***	0.019	0.152***	0.62	0.00	0.51
	August	24	−30.8***	0.148	0.11	−31.9***	0.135***	0.53	−31.9***	−0.034	0.143***	0.53	0.00	0.42

† LMA = $\beta_0 + \beta_1$ln(light), $N_{area} = \beta_0 + \beta_1$light, $\delta^{13}C = \beta_0 + \beta_1$light; ‡LMA = $\beta_0 + \beta_1$height, $N_{area} = \beta_0 + \beta_1$height, $\delta^{13}C = \beta_0 + \beta_1$height; §LMA = $\beta_0 + \beta_1$ln(light) + $\beta_2$height, $N_{area} = \beta_0 + \beta_1$light + $\beta_2$height, $\delta^{13}C = \beta_0 + \beta_1$light + $\beta_2$height. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

turgor pressure if no osmotic adjustments are made, which may result in denser leaf tissue because turgor pressure is necessary for cell expansion and division (Lockhart 1965; Hsiao 1973). However, recent evidence suggests that leaf water storage in tall trees may compensate for the limitations of hydraulic transport (Ishii et al. 2014). In temperate deciduous species such as *Robinia pseudoacacia*, greater turgor pressure in water-stressed leaves of seedlings can be achieved by osmotic adjustment through most of the day, but midday depressions in turgor pressure that fall below the yield pressure of cell wall extension can lead to reduced leaf expansion (Zhang et al. 2011b). If hydraulic constraints are accounted for (e.g., low leaf hydraulic conductance), the simulated optimal N_{area} distribution is not proportional to light (Peltoniemi et al. 2012), which may explain why field-based observations of photosynthetic capacity indicate that it saturates at high light (Buckley et al. 2013). Decreasing leaf hydraulic conductance with height often occurs in conifer or evergreen species (e.g., Ryan et al. 2006), but it has been found to increase with height in the deciduous species *Tilia cordata* and *Betula penula* (Sellin and Kupper 2007; Sellin et al. 2008). Also, sun leaves in *A. saccharum* can maintain higher leaf hydraulic conductance than shade leaves (Sack et al. 2003). Thus, biophysical limitations on leaf growth associated with lower leaf water potentials early in the growing season may constrain the optimal distributions of LMA which, in turn, likely constrain the optimal N_{area} distributions given the relative insensitivity of N_{mass} to vertical environmental gradients.

Our results do not support our second hypothesis that shading reduces environmental stress, resulting in lower $\delta^{13}C$. In the same stand as used in this study, Coble and Cavaleri (2014) found that predawn water potential decreased linearly with height, which was likely due to the gravitational component of water potential (Scholander 1965), since transpiration is negligible at night. Thus, leaves at the top of the canopy maintain greater tension in the water column due to height alone. When trees are transpiring, however, this underlying gravitational tension in the water column is amplified by a combination of frictional resistances and greater evaporative demand, potentially leading to decreases in stomatal aperture (Bauerle et al. 1999; Koch et al. 2004; Niinemets et al. 2004; Ishii et al. 2008). In addition to gravitational potential gradients observed in this stand, VPD increased with height during the same study period (data not shown). The stomatal conductance of *A. saccharum* is particularly sensitive to leaf water potential and high VPD under high light conditions (Ellsworth and Reich 1992b). Collectively, these studies indicate that gradients in leaf water potential and/or VPD may impose constraints on stomatal conductance, resulting in greater $\delta^{13}C$ higher in the canopy. In contrast, Duursma and Marshall (2006) attributed vertical gradients in $\delta^{13}C$

to fractionation in the conductance from intercellular air space to the chloroplast, which generally scales with photosynthetic capacity. We suspect that this was not the case in our study because N_{area} and LMA both strongly correlate with photosynthetic capacity in *A. saccharum* (Ellsworth and Reich 1993; Jones and Thomas 2007), and N_{area} and LMA both responded to shading whereas $\delta^{13}C$ did not.

Seasonal effects on optimization and constraints on leaf function

Our results do not fully support our third hypothesis that leaves growing in higher light would experience a greater increase in N_{area} over time due to changes in LMA. Due to a simultaneous increase in LMA and decrease in N_{mass}, the combination of opposite changes over time neutralized any effects of time on N_{area}. Although there was no effect of time on N_{area} at any height, we found that height was more important earlier in the growing season whereas light was more important later in the growing season. Migita et al. (2007) suggested that N_{area} in *Quercus serrata* is optimized both spatially and temporally where optimal distributions occurred later in the growing season. However, these conclusions were based on data collected only during the late growing season (September) through leaf senescence (November). Extending the work of Migita et al. (2007), we present multiple lines of evidence indicating that the N_{area} distribution was constrained by height early in the growing season and became functionally optimized later in the growing season through the acclimation of LMA to the light conditions.

Seasonal increases in leaf mass, LMA, leaf density, and C:N and decreases in N_{mass} and $\delta^{13}C$ suggest that leaves accumulate carbon-rich structural compounds or other compounds low in nitrogen, which may also indirectly affect the seasonal progression toward N_{area} optimization with light. First, studies have suggested that seasonal changes in LMA correspond with an accumulation of structural proteins (Yasumura et al. 2006) and calcium and silicon (Kitajima et al. 2002), indicating that seasonal patterns in LMA may be due to changes in cell wall structure and chemical composition. Calcium is particularly important in the deposition of lignin and non-cellulosic polysaccharides in cell walls (Eklund and Eliasson 1990). Second, a decrease in N_{mass} over time has been observed in other studies and was attributed to the accumulation of carbon (Reich and Walters 1994), which is further supported by the increase in the C:N ratio over time in this study. Previous investigations into N_{mass} have found that, across and within species, thicker, denser leaves tend to have lower N_{mass} (Reich and Walters 1994; Niinemets 1999; Wright et al. 2004). Consistent with this finding, LMA and density in this study were both negatively correlated with N_{mass}; however, density explained 54 % of the variation in N_{mass}, whereas LMA explained only 23 % of the variation in N_{mass} (data not shown). These studies and our experiment indicate a greater investment in cell wall structure or lignification over time, possibly allowing leaves to tolerate low Ψ_{mid} later in the growing season. We hypothesize that early-season constraints of leaf water potential on leaf morphology and nitrogen are more apparent because leaf cell wall thickening and lignification are not fully developed. Later in the growing season, however, leaves in high-light conditions are able to invest more into cell wall structure, thus offsetting constraints associated with gradients in leaf water potential later in the growing season (i.e., increasing importance of light over time).

Contrary to our expectations (hypothesis 4), we observed a decrease in $\delta^{13}C$ from June to August despite drier conditions in July and August. Seasonal declines in $\delta^{13}C$ have been reported in other studies and generally showed similar patterns among upper and lower canopy leaves (Damesin et al. 1997; Niinemets et al. 1999; Helle and Schleser 2004; Damesin and Lelarge 2003). During drier conditions, leaves tend to become more enriched in ^{13}C as stomatal aperture decreases and as $^{12}CO_2$ becomes depleted in substomatal chambers (Farquhar et al. 1989). However, the declines in $\delta^{13}C$ and precipitation during the growing season indicate that soil water availability was not a limitation on photosynthesis in July and August, since $\delta^{13}C$ represents the integrated photosynthetic activity (Dawson et al. 2002). We would also expect midday declines in stomatal conductance with decreasing leaf water potential as previously observed in *A. saccharum* seedlings growing in high light (Ellsworth and Reich 1992b). However, $\delta^{13}C$ values were lower in August despite the lower Ψ_{mid} observed in August compared with June, suggesting that the Ψ_{mid} was not low enough to initiate midday stomatal closure. Overall, we speculate that leaf acclimation to light during leaf maturation reduced stomatal sensitivity to reduced leaf water potential.

Conclusions

We show that multiple interacting effects (light, height, and time) play roles in both optimizing and constraining distributions of leaf functional traits in *A. saccharum*. An underlying assumption behind current "big leaf" models that integrate leaf photosynthesis and functional traits over the canopy is that leaf nitrogen distribution is optimal with respect to light. Based on our results, we contend that constraints on leaf functional traits should be accounted for when integrating leaf functional traits with these models. Early-season constraints on leaf functional traits appear to be associated with gradients in leaf water potential. We show that LMA, N_{mass}, C:N ratio, and $\delta^{13}C$

can change substantially over the growing season, suggesting that the highly regulated processes inherent to leaf maturation involve a combination of cell wall thickening and carbon and calcium accumulation. We speculate that cell wall thickening or lignification associated with leaf maturation contributes to the optimization of N_{area} and LMA with respect to light. Overall, our results indicate that light acclimation with increasing leaf age optimized the leaf functional traits of a broadleaf deciduous tree, despite the underlying height-related constraints that were more pronounced in the early growing season.

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References

Aasamaa K, Sõber A, Hartung W, Niinemets U (2004) Drought acclimation of two deciduous tree species of different layers in a temperate forest canopy. Trees 18:93–101. doi:10.1007/s00468-003-0285-8

Amthor JS (1994) Scaling CO_2–photosynthesis relationships from the leaf to the canopy. Photosynth Res 39:321–350

Bauerle WL, Hinckley TM, Cermak J, Kucera J, Bible K (1999) The canopy water relations of old-growth Douglas-fir trees. Trees 13:211–217

Berry SC, Varney GT, Flanagan LB (1997) Leaf $\delta^{13}C$ in *Pinus resinosa* trees and understory plants: variation associated with light and CO_2 gradients. Oecologia 109:499–506

Bloor JMG, Grubb PJ (2004) Morphological plasticity of shade-tolerant tropical rainforest tree seedlings exposed to light changes. Funct Ecol 18:337–348. doi:10.1111/j.0269-8463.2004.00831.x

Bond BJ, Farnsworth BT, Coulombe RA, Winner WE (1999) Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. Oecologia 120:183–192. doi:10.1007/s004420050847

Brooks JR, Hinckley TM, Sprugel DG (1994) Acclimation responses of mature *Abies amabilis* sun foliage to shading. Oecologia 100:316–324. doi:10.1007/Bf00316960

Brooks JR, Sprugel DG, Hinckley TM (1996) The effects of light acclimation during and after foliage expansion on photosynthesis of *Abies amabilis* foliage within the canopy. Oecologia 107:21–32. doi:10.1007/BF00582231

Brooks JR, Schulte PJ, Bond BJ, Coulombe R, Domec JC, Hinckley TM, McDowell N, Phillips N (2003) Does foliage on the same branch compete for the same water? Experiments on Douglas-fir trees. Trees 17:101–108. doi:10.1007/s00468-002-0207-1

Buckley TN, Cescatti A, Farquhar GD (2013) What does optimization theory actually predict about crown profiles of photosynthetic capacity when models incorporate greater realism? Plant Cell Environ 36:1547–1563. doi:10.1111/pce.12091

Burgess SSO, Dawson TE (2007) Predicting the limits to tree height using statistical regressions of leaf traits. New Phytol 174:626–636. doi:10.1111/j.1469-8137.2007.02017.x

Burgess SSO, Pittermann J, Dawson TE (2006) Hydraulic efficiency and safety of branch xylem increases with height in *Sequoia sempervirens* (D. Don) crowns. Plant Cell Environ 29:229–239. doi:10.1111/j.1365-3040.2005.01415.x

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. doi:10.1890/09-1326.1

Claussen JW (1996) Acclimation abilities of three tropical rainforest seedlings to an increase in light intensity. Forest Ecol Manag 80:245–255. doi:10.1016/0378-1127(95)03606-7

Coble AP, Cavaleri MA (2014) Light drives vertical gradients of leaf morphology in a sugar maple (*Acer saccharum*) forest. Tree Physiol 34:146–158. doi:10.1093/treephys/tpt126

Coble AP, Autio A, Cavaleri MA, Binkley D, Ryan MG (2014) Converging patterns of vertical variability in leaf morphology and nitrogen across seven *Eucalyptus* plantations in Brazil and Hawaii, USA. Trees 28:1–15. doi:10.1007/s00468-013-0925-6

Damesin C, Lelarge C (2003) Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves. Plant Cell Environ 26:207–219. doi:10.1046/j.1365-3040.2003.00951.x

Damesin C, Rambal S, Joffre R (1997) Between-tree variations in leaf $\delta^{13}C$ of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability. Oecologia 111:26–35. doi:10.1007/s004420050204

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–559. doi:10.1146/annurev.ecolsys.33.020602.095451

Duursma RA, Marshall JD (2006) Vertical canopy gradients in $\delta^{13}C$ correspond with leaf nitrogen content in a mixed-species conifer forest. Trees 20:496–506. doi:10.1007/s00468-006-0065-3

Eklund L, Eliasson L (1990) Effects of calcium ion concentration on cell wall synthesis. J Exp Bot 41:863–867. doi:10.1093/jxb/41.7.863

Ellsworth DS, Reich PB (1992a) Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer saccharum* seedlings in contrasting forest light environments. Funct Ecol 6:423–435. doi:10.2307/2389280

Ellsworth DS, Reich PB (1992b) Water relations and gas exchange of *Acer saccharum* seedlings in contrasting natural light and water regimes. Tree Physiol 10:1–20

Ellsworth DS, Reich PB (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. Oecologia 96:169–178

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Field C (1983) Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. Oecologia 56:341–347. doi:10.1007/BF00379710

Goulet F, Bellefleur P (1986) Leaf morphology plasticity in response to light environment in deciduous tree species and its implication on forest succession. Can J Forest Res 16:1192–1195. doi:10.1139/x86-212

Gutschick VP, Wiegel FW (1988) Optimizing the canopy photosynthetic rate by patterns of investment in specific leaf mass. Am Nat 132:67–86. doi:10.1086/284838

Hanson PJ, Amthor JS, Wullschleger SD, Wilson KB, Grant RF, Hartley A, Hui D, Hunt ER, Johnson DW, Kimball JS, King AW, Luo Y, McNulty SG, Sun G, Thornton PE, Wang S, Williams M, Baldocchi DD, Cushman RM (2004) Oak forest carbon and water simulations: model intercomparisons and evaluations against independent data. Ecol Monogr 74:443–489. doi:10.1890/03-4049

He WM, Dong M (2003) Physiological acclimation and growth response to partial shading in *Salix matsudana* in the Mu Us Sandland in China. Trees 17:87–93

Helle G, Schleser GH (2004) Beyond CO_2-fixation by Rubisco—an interpretation of $^{13}C/^{12}C$ variations in tree rings from novel intra-seasonal studies on broad-leaf trees. Plant Cell Environ 27:367–380

Henriksson J (2001) Differential shading of branches or whole trees: survival, growth, and reproduction. Oecologia 126:482–486. doi:10.1007/s004420000547

Hikosaka K (2014) Optimal nitrogen distribution within a leaf canopy under direct and diffuse light. Plant Cell Environ 37:2077–2085

Hirose T, Werger MJA (1987) Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. Oecologia 72:520–526

Hollinger DY (1989) Canopy organization and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. Funct Ecol 3:53–62. doi:10.2307/2389675

Hollinger DY (1996) Optimality and nitrogen allocation in a tree canopy. Tree Physiol 16:627–634

Hollinger DY, Ollinger SV, Richardson AD, Meyers TP, Dail DB, Martin ME, Scott NA, Arkebauer TJ, Baldocchi DD, Clark KL, Curtis PS, Davis KJ, Desai AR, Dragoni D, Goulden ML, Gu L, Katul GG, Pallardy SG, Paw UKT, Schmid HP, Stoy PC, Suyker AE, Verma SB (2010) Albedo estimates for land surface models and support for a new paradigm based on foliage nitrogen concentration. Glob Change Biol 16:696–710

Hsiao TC (1973) Plant responses to water stress. Annu Rev Plant Physiol 24:519–570

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. doi:10.1093/treephys/tpr079

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. doi:10.1007/s00442-008-1032-z

Ishii HR, Azuma W, Kuroda K, Sillett S (2014) Pushing the limits to tree height: could foliar water storage compensate for hydraulic constraints in *Sequoia sempervirens*. Funct Ecol 28:1087–1093. doi:10.1111/1365-2435.12284

Jerez M, Dean TJ, Roberts SD, Evans DL (2004) Patterns of branch permeability with crown depth among loblolly pine families differing in growth rate and crown size. Trees 18:145–150. doi:10.1007/s00468-003-0288-5

Jones TA, Thomas SC (2007) Leaf-level acclimation to gap creation in mature *Acer saccharum* trees. Tree Physiol 27:281–290. doi:10.1093/treephys/27.2.281

Kawamura K (2010) A conceptual framework for the study of modular responses to local environmental heterogeneity within the plant crown and a review of related concepts. Ecol Res 25:733–744. doi:10.1007/s11284-009-0688-0

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

Knapp BO, Wang GG, Walker JL (2008) Relating the survival and growth of planted longleaf pine seedlings to microsite conditions altered by site preparation treatments. Forest Ecol Manag 255:3768–3777

Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. Nature 428:851–854. doi:10.1038/Nature02417

Lacointe A, Deleens E, Ameglio T, Saint-Joanis B, Lelarge C, Vandame M, Song GC, Daudet FA (2004) Testing the branch autonomy theory: a $^{13}C/^{14}C$ double-labelling experiment on differentially shaded branches. Plant Cell Environ 27:1159–1168. doi:10.1111/j.1365-3040.2004.01221.x

Lemoine D, Cochard H, Granier A (2002) Within crown variation in hydraulic architecture in beech (*Fagus sylvatica* L): evidence for a stomatal control of xylem embolism. Ann For Sci 59:19–27. doi:10.1051/forest:2001002

Livingston NJ, Whitehead D, Kelliher FM, Wang Y-P, Grace JC, Walcroft AS, Byers JN, McSeveny TM, Millard P (1998) Nitrogen allocation and carbon isotope fractionation in relation to intercepted radiation and position in a young *Pinus radiata* D. Don tree. Plant Cell Environ 21:795–803. doi:10.1046/j.1365-3040.1998.00314.x

Lockhart JA (1965) An analysis of irreversible plant cell elongation. J Theor Biol 8:264–275

Martens SN, Ustin SL, Rousseau RA (1993) Estimation of tree canopy leaf area index by gap fraction analysis. Forest Ecol Manag 61:91–108

Medlyn B (2004) A MAESTRO retrospective. In: Mencuccini M GJ, Moncrieff J, McNaughton KG (eds) Forests at the land–atmosphere interface. Oxford University Press, Oxford

Meinzer FC, Bond BJ, Karanian JA (2008) Biophysical constraints on leaf expansion in a tall conifer. Tree Physiol 28:197–206. doi:10.1093/treephys/28.2.197

Migita C, Chiba Y, Tange T (2007) Seasonal and spatial variations in leaf nitrogen content and resorption in a *Quercus serrata* canopy. Tree Physiol 27:63–70

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 U (1997) Distribution patterns of foliar carbon and nitrogen as affected by tree dimensions and relative light conditions in the canopy of *Picea abies*. Trees 11:144–154

Niinemets U (1999) Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. New Phytol 144:35–47

Niinemets U, Valladares F (2004) Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. Plant Biol 6:254–268. doi:10.1055/s-2004-817881

Niinemets U, 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. doi:10.1086/314180

Niinemets U, Ellsworth DS, Lukjanova A, Tobias M (2001) Site fertility and the morphological and photosynthetic acclimation of *Pinus sylvestris* needles to light. Tree Physiol 21:1231–1244. doi:10.1093/treephys/21.17.1231

Niinemets U, Sonninen E, Tobias M (2004) Canopy gradients in leaf intercellular CO_2 mole fractions revisited: interactions between leaf irradiance and water stress need consideration. Plant Cell Environ 27:569–583. doi:10.1111/j.1365-3040.2003.01168.x

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. doi:10.1111/j.1365-3040.2005.01344.x

Oldham AR, Sillett SC, Tomescu AMF, Koch GW (2010) The hydrostatic gradients, not light availability, drive height-related variation in *Sequoia sempervirens* (Cupressaceae) leaf anatomy. Am J Bot 97:1087–1097

Ollinger SV, Richardson AD, Martin ME, Hollinger DY, Frolking SE, Reich PB, Plourde LC, Katul GG, Munger JW, Oren R, Smith ML, Paw UKT, Bolstad PV, Cook BD, Day MC, Martin TA, Monson RK, Schmid HP (2008) Canopy nitrogen, carbon assimilation, and albedo in temperate and boreal forests: functional relations and potential climate feedbacks. Proc Natl Acad Sci USA 49:19336–19341

Peltoniemi MS, Duursma RA, Medlyn BE (2012) Co-optimal distribution of leaf nitrogen and hydraulic conductance in plant canopies. Tree Physiol 32:510–519. doi:10.1093/treephys/tps023

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 182:565–588

R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org. Accessed 8 June 2011

Raulier F, Bernier PY, Ung CH (1999) Canopy photosynthesis of sugar maple (*Acer saccharum*): comparing big-leaf and multilayer extrapolations of leaf-level measurements. Tree Physiol 19:407–420. doi:10.1093/treephys/19.7.407

Reich PB, Walters MB (1994) Photosynthesis-nitrogen relations in Amazonian tree species. II. Variation in nitrogen vis-à-vis specific leaf area influences mass- and area-based expressions. Oecologia 97:73–81

Ryan MG, Phillips N, Bond BJ (2006) The hydraulic limitation hypothesis revisited. Plant Cell Environ 29:367–381

Ryu Y, Baldocchi DD, Kobayashi H, van Ingen C, Li J, Black TA, Beringer J, van Gorsel E, Knohl A, Law BE, Roupsard O (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. Global Biogeochem Cy 25:Gb4017

Sack L, Cowan PD, Jaikumar N, Holbrook NM (2003) The "hydrology" of leaves: co-ordination of structure and function in temperate woody species. Plant Cell Environ 26:1343–1356

Sack L, Melcher PJ, Liu WH, Middleton E, Pardee T (2006) How strong is intracanopy leaf plasticity in temperate deciduous trees? Am J Bot 93:829–839. doi:10.3732/ajb.93.6.829

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. Science 148:339–346

Sellers PJ, Berry JA, Collatz GJ, Field CB, Hall FG (1992) Canopy reflectance, photosynthesis, and transpiration. III. A reanalysis using improved leaf models and a new canopy integration scheme. Remote Sens Environ 42:187–216

Sellin A, Kupper P (2007) Effects of enhanced hydraulic supply for foliage on stomatal responses in little-leaf linden (*Tilia cordata* Mill.). Eur J Forest Res 126:241–251

Sellin A, Õunapuu E, Kupper P (2008) Effects of light intensity and duration on leaf hydraulic conductance and distribution of resistance in shoots of silver birch (*Betula pendula*). Physiol Plant 134:412–420. doi:10.1111/j.1399-3054.2008.01142.x

Thornton PE, Zimmermann NE (2007) An improved canopy integration scheme for a land surface model with prognostic canopy structure. J Clim 20:3902–3923. doi:10.1175/Jcli4222.1

Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? Plant Cell Environ 27:229–236. doi:10.1111/j.1365-3040.2003.01141.x

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827. doi:10.1038/Nature02403

Yamamoto T, Nobori H, Sasaki H, Hayasaka K (1999) The effects of shading on translocation of ^{13}C-photosynthates between lateral branches during the rapid growth period of cherry, pear, and Japanese persimmon fruit. J Japan Soc Hort Sci 68:302–311

Yasumura Y, Hikosaka K, Hirose T (2006) Seasonal changes in photosynthesis, nitrogen content and nitrogen partitioning in *Lindera umbellata* leaves grown in high or low irradiance. Tree Physiol 26:1315–1323. doi:10.1093/treephys/26.10.1315

Zhang Y, Zheng Q, Tyree MT (2011a) Factors controlling plasticity of leaf morphology in *Robinia psuedoacacia* L. I: Height-associated variation in leaf structure. Ann Forest Sci 69:1–9. doi:10.1007/s13595-011-0133-8

Zhang Y, Equia MA, Zheng Q, Tyree MT (2011b) 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

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. doi:10.1111/j.1365-3040.2003.01153.x