

# Becoming less tolerant with age: sugar maple, shade, and ontogeny

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**Abstract** Although shade tolerance is often assumed to be a fixed trait, recent work suggests ontogenetic changes in the light requirements of tree species. We determined the influence of gas exchange, biomass distribution, and self-shading on ontogenetic variation in the instantaneous aboveground carbon balance of *Acer saccharum*. We quantified the aboveground biomass distributions of 18 juveniles varying in height and growing in low light in a temperate forest understory in Minnesota, USA. Gas exchange rates of leaf and stem tissues were measured, and the crown architecture of each individual was quantified. The YPLANT program was used to estimate the self-shaded fraction of each crown and to model net leaf-level

carbon gain. Leaf respiration and photosynthesis per gram of leaf tissue increased with plant size. In contrast, stem respiration rates per gram of stem tissue declined, reflecting a shift in the distribution of stem diameter sizes from smaller (with higher respiration) to larger diameter classes. However, these trends were outweighed by ontogenetic increases in self-shading (which reduces the net photosynthesis realized) and stem mass fraction (which increases the proportion of purely respiratory tissue) in terms of influence on net carbon exchange. As a result, net carbon gain per gram of aboveground plant tissue declined with increasing plant size, and the instantaneous aboveground light compensation point increased. When estimates of root respiration were included to model whole-plant carbon gain and light compensation points, relationships with plant size were even more pronounced. Our findings show how an interplay of gas exchange, self-shading, and biomass distribution shapes ontogenetic changes in shade tolerance.

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## Introduction

Species differences in shade tolerance are often invoked to explain successional patterns in forests, with shade-intolerant species being progressively replaced by species that can survive and grow in darker environments (Bazzaz 1979; Givnish 1988; Pacala et al. 1994). This progression is a necessary component of many of the classic theories of succession, in which forests become increasingly dominated by shade-tolerant individuals in the absence of large-scale disturbances (Connell and Slatyer 1977; Whitmore 1989). Although shade tolerance is often assumed to be a

fixed trait of a species, Givnish (1988) predicted that light requirements increase as trees grow larger in size due to rising costs of construction and maintenance of structural support tissues in larger trees. Several studies have supported the prediction of increasing light requirements (i.e., declining shade tolerance) with increased juvenile tree size (Lusk 2004; Kneeshaw et al. 2006; Lusk et al. 2008). Givnish's (1988) assumption of increasing costs of structural support tissues has also found support from many studies. For example, Sterck and Bongers (1998) found that the fractional investment of growth allocated to leaves generally declines with increasing size in tropical trees. Furthermore, leaves turn over faster than woody tissues. As a result, both the leaf area ratio (leaf area/total plant dry mass) and the ratio of photosynthetic to respiratory tissues decrease in larger trees and shrubs. Lusk (2004) reported related patterns in juvenile evergreens in a Chilean temperate forest, as did Delegrange et al. (2004) in a north-temperate deciduous forest. The whole-plant light compensation point (i.e., the light level necessary for zero net carbon gain at the whole-plant level) is clearly related to several components of shade tolerance, including net carbon gain and survival probability under low-light conditions. Based on the whole-plant perspective, Givnish (1988) predicted that light requirements increase as trees grow larger in size. However, there are surprisingly few direct tests of this idea, and none that integrate all of the different components of whole-plant carbon balance (see Online Resource 1 in the Electronic supplementary material, ESM).

Other work indicates that the mechanisms underlying ontogenetic declines in shade tolerance may be more complex than those examined by Givnish (1988). Self-shading of juvenile evergreens has been shown to increase with plant size, reducing light-interception efficiency and net carbon gain at the whole-plant level (Lusk et al. 2011, 2012). Additionally, some previous work (Givnish 1988; Lusk et al. 2011) initially assumed that leaf and stem gas exchange rates do not vary with tree size, but several studies have shown that this is not the case. For instance, stem-specific nitrogen concentrations and respiration rates have been shown to decline with size in seedlings and saplings (Walters et al. 1993; Tjoelker et al. 1999; Reich et al. 2006), as well as in larger trees (Sendall and Reich 2013), congruent with decreasing proportional biomass in leaves and increasing proportional biomass in wood (Reich et al. 2014). Thus, as individuals grow larger, the proportion of tissues with high respiration rates (e.g., leaves and fine twigs) declines in favor of structural support tissues with low respiration rates (e.g., coarse twigs, branches, and boles), potentially causing a decline in stem respiration rates per gram tissue and thus reducing per gram respiration rates at the whole-plant level. Sendall and Reich (2013) also showed that large saplings had higher leaf N content

and photosynthetic rates (see also Thomas 2010) but lower leaf respiration than small saplings, suggesting that carbon gain per gram leaf tissue may remain constant or even increase with size in juvenile trees. Each of these factors likely shapes ontogenetic increases in light requirements, but the effective importance of each remains uncertain.

In the study described in the present paper, we examined the contributions of biomass distribution, self-shading, and gas exchange to ontogenetic variation in the shade tolerance of juvenile *Acer saccharum* (sugar maple). Shade tolerance can be defined in several ways; in this work, we defined it as the minimum light level at which a juvenile tree can maintain a positive carbon balance. This light level, which we refer to from this point forward as the light compensation point, has been shown to correspond with low-light mortality rates (Lusk and Jorgensen 2013), and given that low-light survival is generally related to low-light net carbon balance (e.g., Walters and Reich 1996; Poorter and Bongers 2006), the light compensation point is likely a generally reliable measure of shade tolerance. We have advanced our understanding of the significance of plant size for shade tolerance by addressing the following issues in a single study: (1) we expanded on the range of juvenile tree sizes and ages previously studied; (2) we measured and accounted for size-related changes in the proportions of photosynthetic:respiratory tissues; (3) we employed a well-documented temperature-dependent  $Q_{10}$  to scale daytime and nighttime respiration rates (Tjoelker et al. 2001, Heskell et al., unpublished data); (4) we measured woody tissue respiration rates for multiple stem size classes; and (5) we measured and accounted for both neighbor- and self-shading. We selected juveniles ranging in size from 14 to 272 cm in height, and used hemispherical photography and measurements of plant architecture and gas exchange to model potential summertime net carbon gain of foliage with the YPLANT program (Percy and Yang 1996). We complemented these data with measurements of above-ground woody tissue respiration and estimates of below-ground root respiration obtained from the literature. After harvesting the plants, we quantified sapling age, above-ground biomass distribution, and tissue N concentrations.

In this work, we address the following questions. (1) How do aboveground biomass distributions and self-shading fractions change as plants grow larger? (2) Does the ratio of small-diameter to large-diameter stem tissue decline with increased plant size? (3) How do leaf and stem nitrogen content and gas exchange rates vary with sapling size? (4) Do net daily carbon gain per gram of aboveground plant tissue and instantaneous aboveground light compensation points vary with plant size? (5) If so, which of the above measures (e.g., biomass distribution, self-shading) drive the ontogenetic changes? (6) Do the slopes of the relationships between light compensation points vs. plant

size and net daily carbon gain vs. plant size differ upon incorporating estimates of belowground respiration?

## Materials and methods

### Study site and species

This study was conducted at the Pine Needles Preserve of the St. Croix Watershed Research Station (45°11'N, 92°49'W) in east-central Minnesota, USA. The general forest cover type is deciduous, dominated by sugar maple (*Acer saccharum* Marsh.) and American basswood (*Tilia americana* L.), with a number of large white pine (*Pinus strobus* L.) scattered throughout. The subcanopy and sapling layers are sparse and are composed primarily of sugar maple. Based on a textural analysis, the soils are a sandy loam, and were formed either from the weathering of the local sandstone or fine windblown sand (Swanson and Meyer 1990). The climate is cold-temperate continental, with mean January and July temperatures of −11 and 23 °C, respectively.

We studied 18 juvenile sugar maples ranging in height from 14 to 272 cm found growing in uniformly low light (6–8 % of above-canopy PPFD) in the forest understory. We chose this range of tree sizes because it includes a

using a magnetic signal receiver and pointer, which can accurately record points within a 2.25-m radius from the receiver. Stem and petiole arrangements are described by their length, diameter, elevation angle, and azimuth, while leaves are described by their length, azimuth, and the elevation angles of two vectors on the lamina surface (Falster and Westoby 2003).

The YPLANT software (Pearcy and Yang 1996) was used to model net daily carbon gain of foliage. The 3D crown arrangement recorded for each individual in FLO-RADIG was converted to the appropriate YPLANT format using a program written in the C programming language (Falster and Westoby 2003). YPLANT inputs are daily minimum and maximum temperatures, the time at which the maximum temperature is reached, a description of the leaf shape, leaf photosynthetic capacity and respiration rate, the geometry of the crown arrangement, and a description of the canopy structure above the plant (described below), which is used to estimate light interception. A solar movement submodel is used to estimate the photosynthetic photon flux density (PPFD,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) intercepted by each leaf surface at different times of the day. YPLANT utilizes the following equation to describe photosynthetic light response curves (Pearcy and Yang):

$$A_N = \frac{\alpha Q + A_{\max} + R_d - \sqrt{((\alpha Q + A_{\max} + R_d)^2 - 4\theta\alpha Q (A_{\max} + R_d))}}{2\theta} - R_d, \quad (1)$$

larger range than seen in many prior studies of juvenile trees, but even the largest trees were small enough to be used for whole-plant measurements. Height was measured as the vertical distance between the stem base and the highest live meristem. Individuals were chosen that were found growing within a 400 m<sup>2</sup> area, to reduce the amount of environmental heterogeneity (i.e., soil moisture, soil nutrients, etc.) among different individuals.

### Modeling carbon gain

The three-dimensional crown arrangement of each tree was recorded using an extended-range Liberty 3D-digitizer (Polhemus, Colchester, VT, USA) paired with the FLO-RADIG software package (CSIRO Entomology, Brisbane, Australia), as described by Falster and Westoby (2003). A virtual reconstruction of each plant is achieved by recording a series of point coordinates associated with each leaf or stem segment, as well as the relative connectivity between points. The 3D spatial coordinates are recorded

where  $A_N$  is the net photosynthetic rate,  $Q$  is the PPFD incident on the leaf surface multiplied by the leaf absorptance,  $A_{\max}$  is the light-saturated photosynthetic rate,  $R_d$  is the dark respiration rate,  $\alpha$  is the quantum yield for CO<sub>2</sub> assimilation, and  $\theta$  is the convexity of the curve.  $A_{\max}$  and  $R_d$  were measured directly (see the “Gas exchange measurements” section below), and the other parameters were assigned their preset values in YPLANT as follows. Leaf absorptance and quantum yield have been shown to vary little between C<sub>3</sub> species (Knapp and Carter 1998; Singaas et al. 2001), so we used the default values of 0.85 and 0.055, respectively. Falster and Westoby (2003) found that their results did not vary substantially when they altered their convexity value, and thus the intermediate value of 0.7 was used. YPLANT calculates  $A_N$  for each leaf separately using output from the fit response to PPFD, adjusting photosynthetic rates based on the amount of light each leaf is receiving. YPLANT output is the daily sum of  $A_N$  for the total leaf area of each plant; i.e., the net daily carbon gain ( $\text{mmol CO}_2 \text{ m}^{-2}$ ) of foliage for each individual.

The photosynthetic calculations in YPLANT also include an equation that estimates self-shading, which influences light interception by plant crowns. YPLANT output includes the projected leaf area (PA) of each tree toward each of 160 sectors of the hemisphere (20 elevation classes  $\times$  8 azimuth classes), which does not take into account any leaf overlap, as well as the displayed leaf area (DA), which is the effective leaf area for light interception (Percy and Yang 1996). The self-shading fraction (i.e., the fraction of leaf area that was self-shaded) was calculated as  $(PA - DA)/PA$ , where a value of zero indicates that no leaf tissues in the canopy overlap in any of the 160 sectors.

### Hemispherical photography

A Nikon D300 digital camera with a 180° fisheye adapter was used to take a hemispherical photograph above each individual, with the top of the camera oriented north. Photos were analyzed using the Gap Light Analyzer software package (Frazer et al. 1999) in conjunction with WinPhot 5 (Ter Steege 1996). Fractional canopy openness (0–1) was calculated for 20 altitude and 8 azimuth classes in each photo. Mean openness in each angle class was calculated for each photo by averaging openness across all azimuth classes. This allowed us to estimate the average time series of direct photosynthetic photon flux density (PPFD) interception for any day of the year. The average summer day length at this latitude is 14.1 h, which occurs on August 7. A file with the average ambient light regime above each individual was generated for this date. This type of file will be referred to as a canopy file from this point forward.

We were also interested in plant performance under differing light regimes, so in addition to canopy files for ambient light conditions, we developed files representing standardized light regimes (approximately 2, 4, 6, and 8 % of above-canopy PPFD) by averaging data from up to five hemispherical photos taken beneath intact deciduous canopies in northern Minnesota (cf. Falster and Westoby 2003). By running model simulations for all individuals at each of these light levels, we were able to estimate how above-ground carbon gain rates would vary under different light conditions, and were thus able to estimate the aboveground light compensation points for each individual.

YPLANT simulates atmospheric light transmission using the ratio of global solar irradiance at the top of the canopy ( $R_s$ ) to the global solar irradiance at the top of the atmosphere ( $R_o$ ) (Roderick et al. 2001). A high value of  $R_s/R_o$  is used to simulate a clear sky, and most light is intercepted as direct beams, while a low value is used to simulate a cloudy sky in which most light is intercepted as diffuse light. We simulated light interception and carbon gain using an  $R_s/R_o$  of 0.6 based on the frequency of cloud cover in the area. Due to possible errors associated

with estimating incoming radiation with hemispherical photography (i.e., overestimation of canopy openness in closed sites when using auto-exposure on digital cameras and assumptions regarding the proportions of diffuse and direct incoming radiation), we performed several sensitivity analyses to evaluate if our results were sensitive to these sources of error. We varied processing thresholds of hemispherical photographs and the transmission coefficient inputs by  $\pm 10$  %, and comparisons of results showed minimal changes in YPLANT model output (less than a 3 % difference on average). Moreover, while these analyses showed some changes in absolute values of carbon gain, they did not alter how these values were affected by tree height and light availability.

### Gas exchange measurements

Estimates of light-saturated photosynthetic rates ( $A_{\max}$ ) and dark respiration rates ( $R_d$ ) are required for simulations of carbon gain in YPLANT.  $A_{\max}$  was measured on fully expanded leaves ( $n = 4$  per individual) in the outer crown on two consecutive mornings (between 09:00 and 12:00) in July 2008 on attached foliage of all 18 individuals using an LI-6400 (LI-COR, Lincoln, NE, USA). Following  $A_{\max}$  measurements, leaves were marked so that the same leaves could later be used for  $R_d$  and SLA measurements. Environmental conditions were controlled within the leaf cuvette as follows: CO<sub>2</sub> concentration of 380  $\mu\text{mol mol}^{-1}$ , 45–65 % relative humidity, and a block temperature of 25 °C. Leaves were exposed to irradiance levels of 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using the LI-6400 LED light source.

Leaf and woody tissue  $R_d$  were measured using harvested tissues of the same 18 individuals used for YPLANT simulations. In order to minimize the effect of active growth on  $R_d$ , these measurements were made late in the growing season (i.e., in the third week of August). By this time in the season, all stem extension growth had ceased and all leaves were fully expanded. Thus, our results are likely more conservative than they would be if we measured earlier in the year and included the respiratory costs of construction and maintenance. We harvested all above-ground biomass of six individuals per day between 08:00 and 09:30 local time. Immediately following harvest, the aboveground biomass was wrapped in moist paper towels and placed in dark plastic bags. Samples were taken to a nearby laboratory where they were stored in a darkened walk-in refrigerator at 5 °C for no longer than five hours from the time of harvesting to the time of measuring respiration. Prior research indicates that respiration rates are similar for detached and attached tissues for up to six hours following cutting (Mitchell et al. 1999; Lee et al. 2005; Machado and Reich 2006). Each day, the plants were separated into leaves and stems, and tissues

were subsampled based on size and amount. Stems were separated into diameter classes of <2.5 mm, 2.5–5.0 mm, 5.0–7.5 mm, 7.5–10.0 mm, and >10.0 mm in order to determine whether stem respiration varies as a function of stem diameter and age. Based on results from Pruyn et al. (2002), it is unlikely that stem cutting markedly affected our respiration measurements. In their study, they found that respiration rates of samples with high surface/volume ratios (i.e., smaller-diameter segment cores) did not differ significantly from those with lower surface/volume ratios, indicating that wounding a higher percentage of active cells (i.e., sapwood cells) did not have a significant effect on respiration. Azcón-Bieto et al. (1983) found that respiration increased for a short period of time after wounding in leaf samples, but they sliced leaves into 1-mm pieces, and thus the fraction of tissues with cut cells was significantly larger than our stem samples, which we generally cut into 5- to 10-cm-long segments. Sample bags were transferred to a darkened chamber at 25 °C for 60 min prior to  $R_d$  measurements. Measurements were completed on the same day as sampling using an LI-6400 with the conifer chamber attachment. Subsamples of leaves and stems of each diameter class were measured individually. The number of stem samples per diameter class measured for each individual differed depending on tissue quantity. For instance, the smallest seedling only had enough stem tissue for one measurement of each of the two smallest size classes, whereas the larger individuals had enough tissue to measure three samples of each diameter class. Samples were placed within the conifer chamber, with environmental conditions controlled as above. Samples were kept inside the chamber for approximately 10 min, and measurements were recorded only after readings had stabilized. Because we were interested in total stem respiration, rather than sapwood or heartwood components, measurements were made on whole-stem segments (i.e., bark and sapwood tissues were not measured separately).

Using these instantaneous dark respiration rates ( $R_{ref}$ ) that were measured at a standard temperature ( $T_{ref}$ , 25 °C), we converted to a rate  $R$  observed at both mean daytime and mean nighttime temperatures ( $T$ ) using a temperature-dependent  $Q_{10}$ :

$$R(T) = R_{ref} \times \exp\left(-\frac{T - T_{ref}}{10}\right) \frac{(a + bT)^{(a+bT)/(10b)}}{(a + bT_{ref})^{(a+bT_{ref})/(10b)}}, \quad (2)$$

where  $a = 3.22$  and  $b = -0.046$  (Tjoelker et al. 2001). This generalized model was developed using  $Q_{10}$  data from 56 species across a wide range of biomes. These values were multiplied by day (stems) or night (stems and leaves) length to give daily respiration values; daytime leaf respiration is included in the net photosynthetic rates from the YPLANT model output, which accounts for temperature

effects, so it was not included here. For example, to obtain nighttime respiration we used the following formula:

$$R_N = (\text{mmol m}^{-2} \text{ day}^{-1}) = R(T)(\text{mmol m}^{-2} \text{ s}^{-1}) \times (24 \text{ h day}^{-1} - \text{day length}) \times 3600 \text{ s h}^{-1}, \quad (3)$$

where  $R(T)$  is the nighttime respiration rate obtained from Eq. 2.

Because respiration rates calculated at mean daytime and nighttime temperatures could yield different results than respiration rates calculated over the diurnal temperature cycle, we also calculated respiration rates at 30- and 60-min intervals. Using these diurnal calculations caused only minor differences (a 2 % change on average) in our estimates of net carbon gain. Thus, for simplicity we present results using the respiration rates calculated at mean daytime and nighttime temperatures.

Following gas exchange measurements, leaves used for  $A_{max}$  and  $R_d$  were scanned and the projected area of leaf lamina was determined using the image-processing software ImageJ (Abramoff et al. 2004). The length and diameter of each stem segment was measured. All samples were oven-dried at 70 °C for at least 72 h to determine dry leaf mass in order to calculate the specific leaf area (SLA,  $\text{cm}^2 \text{ g}^{-1}$ ) and stem dry mass. Each of the leaf and stem samples used for  $A_{max}$  and  $R_d$  measurements were ground separately and analyzed for nitrogen concentrations (ECS 4010 CHNSO analyzer, Costech Analytical Technologies Inc., Valencia, CA, USA). Because we were interested in total stem nitrogen content, rather than sapwood or heartwood components, analyses were run using pooled bark with sapwood tissues. For all plants, the following parameters were measured: total leaf mass (g), total stem mass (g), and age (in years; calculated as the number of rings at the base of the stem). These data were used to determine the leaf mass fraction (LMF,  $\text{g leaf g}^{-1}$  total aboveground tissue) and stem mass fraction (SMF,  $\text{g stem g}^{-1}$  total aboveground tissue). Aboveground  $R_d$  ( $\text{nmol CO}_2 \text{ g}^{-1} \text{ plant s}^{-1}$ ) was calculated by summing leaf and stem  $R_d$  weighted by the proportion of dry mass corresponding to each tissue. Finally, the net daily aboveground carbon gain ( $\text{nmol CO}_2 \text{ g}^{-1} \text{ plant s}^{-1}$ ) of each individual was calculated by subtracting daytime stem  $R_d$  and nighttime aboveground (stem + leaf)  $R_d$  from the YPLANT output (net carbon gain of foliage during the day). To assess how ontogenetic trends in net carbon gain and light compensation points might be affected by root respiration, we estimated root biomass fraction and respiration rates based on those reported in a study of similar-sized juvenile temperate trees (Online Resource 2 in the ESM; Machado and Reich 2006). These added respiratory costs were accounted for in order to estimate whole-plant net carbon gain and whole-plant light compensation points (Online Resource 3 in the ESM).



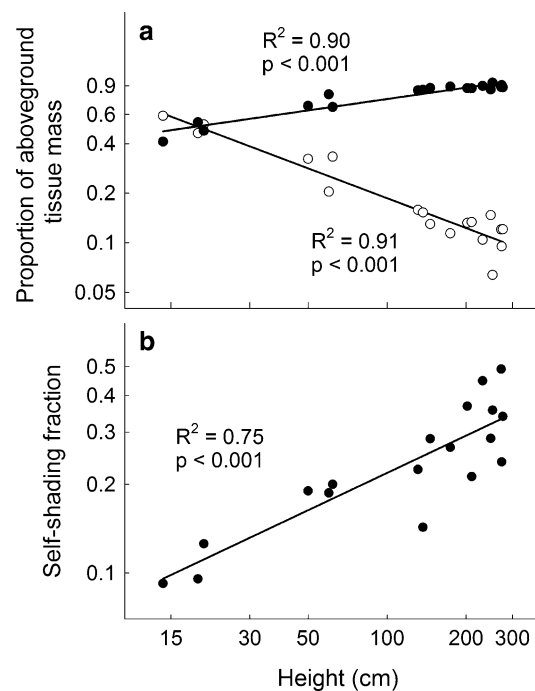
## Statistical analysis

Prior to analyses, most traits were log-transformed in order to better meet the assumptions of the models and to reduce patterns in the residuals. Logarithmic values were more nearly normal than non-transformed values. To test for the potential effect of height on observed relationships, we conducted linear regressions for each trait (i.e., leaf mass fraction, SLA, stem nitrogen). Aboveground net carbon gain under ambient light conditions (6–8 % of above-canopy PPFD) was regressed against tree height to estimate the sapling height at which aboveground net carbon gain is equal to zero. Aboveground net carbon gain under standardized light conditions (2, 4, 6, and 8 % of above-canopy PPFD) was regressed against PPFD to estimate the aboveground light compensation point of each individual. A linear regression of aboveground light compensation point vs. tree height was conducted to determine how the instantaneous aboveground light compensation point is affected by plant size. Similar analyses were performed using whole-plant estimates of carbon gain and light compensation points that incorporated estimates of belowground respiratory costs based on literature values to determine if this added cost affected the strength of the relationships. To determine whether self-shading or biomass fraction had a larger effect on the ontogenetic variation in aboveground net carbon gain and aboveground light compensation points, we ran a general linear model analysis using self-shading, stem biomass fraction, and their interaction as independent variables. Differences in stem dark respiration and stem nitrogen among the five stem diameter classes were analyzed using a one-way ANOVA with tree included as a random effect. The bivariate relationship between stem N content and stem  $R_d$  was analyzed with a linear regression. All statistical analyses were conducted in JMP statistical analysis software (JMP 11, SAS Institute, Cary, NC, USA).

## Results

### Relationships between tree size and age, biomass distribution, and self-shading

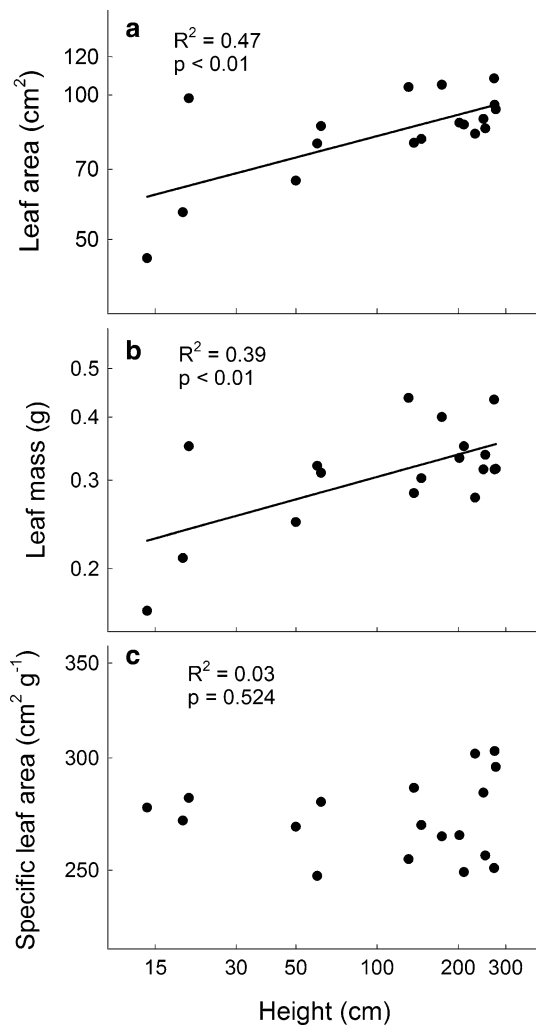
Despite their relatively small sizes, the plants ranged in age from 3 to 28 years old, and taller plants were consistently older ( $R^2 = 0.94$ ,  $p < 0.001$ ). Aboveground leaf mass fraction declined from approximately 50 % of aboveground tissue in small (15 cm tall) juveniles to 10–14 % in plants over 100 cm in height (Fig. 1a). The self-shading fraction increased linearly from <0.12 in the smallest plants to an average of 0.34 in individuals over 200 cm in height (Fig. 1b).



**Fig. 1** Changes in proportions of leaf (*open symbols*) and stem (*filled symbols*) tissues (**a**) and self-shading (**b**) as a function of *Acer saccharum* juvenile tree height; data were log–log-transformed prior to analysis. Regression equations:  $\log(\text{leaf mass fraction}) = 0.48 - 0.60 \times \log(\text{height})$ ;  $\log(\text{stem mass fraction}) = -0.59 + 0.23 \times \log(\text{height})$ ;  $\log(\text{self-shading fraction}) = -1.50 + 0.42 \times \log(\text{height})$

### Tissue chemistry and gas exchange

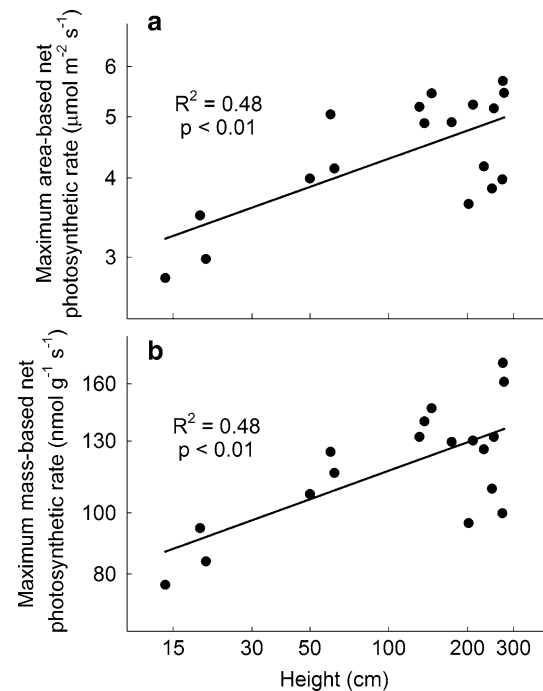
Specific leaf area was not affected by plant size, but the area and mass of individual leaves increased with height (Fig. 2a–c). Maximum photosynthetic rates on both an area and mass basis increased significantly with plant size (Fig. 3a, b). Nitrogen (N) content and mass-based dark respiration rates ( $R_d$ ) show contrasting ontogenetic trends in leaves and stems: leaf N and  $R_d$  increased with plant size (Fig. 4a, Online Resource 4 in the ESM), while stem N and  $R_d$  declined (Fig. 4b, Online Resource 4 in the ESM). This reflects a shift in the distribution of stem diameter sizes with increasing tree size. Across all samples, stem  $R_d$  was positively related to stem N (Online Resource 5 in the ESM), with newer growth (i.e., smaller-diameter classes) exhibiting significantly higher levels of both relative to older woody tissues (Online Resource 6 in the ESM), and larger individuals having a greater fraction of larger-diameter stems. Per gram aboveground N and  $R_d$  declined markedly with increased tree size (Fig. 4c, Online Resource 4 in the ESM), driven by ontogenetic increases in both the fraction of stem tissues and their size.



**Fig. 2** Changes in area (a) and mass (b) of individual leaves and specific leaf area (c) as a function of *Acer saccharum* juvenile tree height; data were log–log-transformed prior to analysis. Regression equations:  $\log(\text{leaf area}) = 1.61 + 0.15 \times \log(\text{height})$ ;  $\log(\text{leaf mass}) = -0.81 + 0.15 \times \log(\text{height})$ ;  $\log(\text{SLA}) = 2.43 + 0.002 \times \log(\text{height})$

### Simulated net daily (24-h) carbon gain and light compensation points

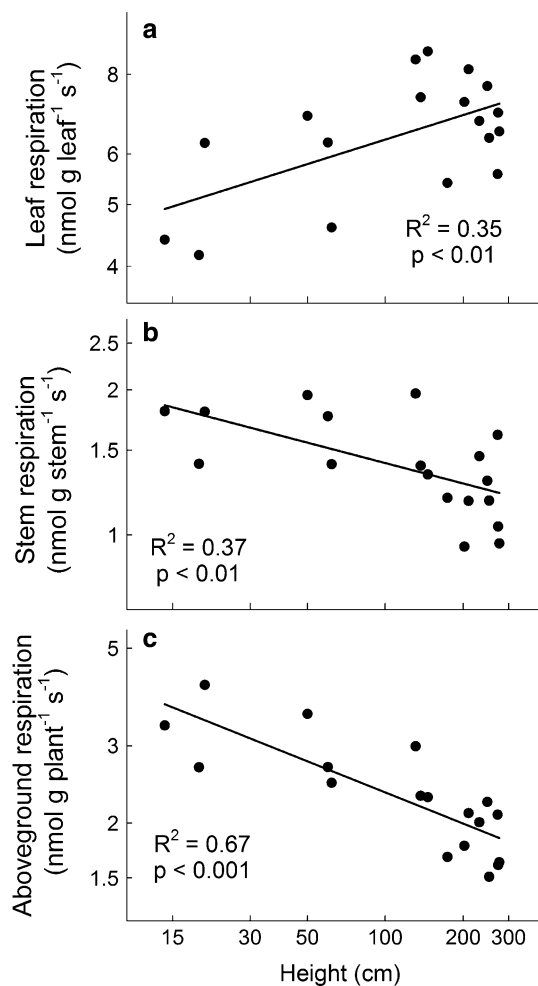
Under ambient light conditions, all plants except for one of the largest individuals were estimated to have a positive aboveground carbon balance, with simulated aboveground net carbon gain ( $\text{mmol CO}_2 \text{ g plant}^{-1} \text{ s}^{-1}$ ) decreasing significantly with increased plant size (Fig. 5). Individuals in the largest size classes (>100 cm in height) gained significantly less carbon per gram of aboveground tissue than smaller trees (10–70 cm in height). Moreover, even among the smaller size classes, seedlings less than 20 cm in height exhibited more than twice the rates of net carbon gain per gram of tissue than 50- to 70-cm-tall individuals. Under



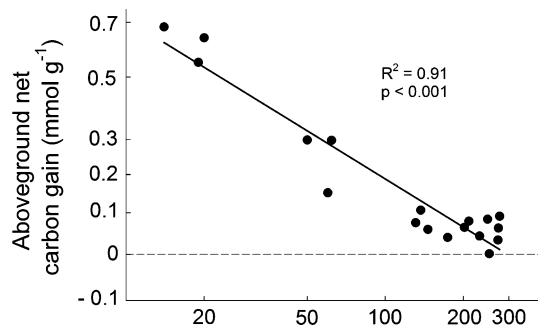
**Fig. 3** Area-based (a) and mass-based (b) maximum net photosynthetic rates in relation to height of juvenile *Acer saccharum* trees; data were log–log-transformed prior to analysis. Regression equations:  $\log(A_{\text{area}}) = 0.34 + 0.15 \times \log(\text{height})$ ;  $\log(A_{\text{mass}}) = 1.77 + 0.15 \times \log(\text{height})$

hypothetical standardized light regimes, brighter environments resulted in more positive carbon balances for individuals of all sizes (Fig. 6a). The maximum height for positive aboveground carbon gain estimated from fitted lines for light levels of 2, 4, 6, and 8 % of above-canopy PPFD was reached when saplings were 132, 178, 222, and 305 tall, respectively (Fig. 6b). In other words, taller saplings required more light to maintain positive rates of carbon gain, and thus had higher aboveground light compensation points (Fig. 6c). Aboveground light compensation points ranged from 0.53 to 2.73  $\text{mol PPFD m}^{-2} \text{ day}^{-1}$ , which are comparable with findings for shade-tolerant species in prior studies (e.g., Baltzer and Thomas 2007; Lusk and Jorgensen 2013). These values reflect a range of  $\approx 1.5$ –6.5 % of above-canopy PPFD. Both aboveground carbon gain and light compensation points were more significantly affected by variations in stem biomass fraction than by self-shading (Table 1). However, the significant interaction term in both models indicates that the degree of self-shading influences the strength of the biomass fraction effect.

As expected, accounting for root respiration decreased the maximum height a juvenile could be before experiencing negative whole-plant carbon balance (at a standardized light level; Online Resource 7 in the ESM) and carbon gain per gram of plant (Online Resource 3 in the ESM), while



**Fig. 4** Changes in dark respiration rates per gram of leaf (a), stem (b), and total aboveground (c) tissues as function of *Acer saccharum* juvenile tree height; data were log–log-transformed prior to analysis. Regression equations:  $\log(\text{leaf } R_d) = 0.55 + 0.13 \times \log(\text{height})$ ;  $\log(\text{stem } R_d) = 0.43 - 0.14 \times \log(\text{height})$ ;  $\log(\text{aboveground } R_d) = 0.84 - 0.24 \times \log(\text{height})$



**Fig. 5** Relationship between aboveground net daily carbon gain estimated from YPLANT simulations and height of juvenile *Acer saccharum* trees at ambient light levels (6–8 % of above-canopy PPFD); data were log–log-transformed prior to analysis. The dashed line indicates zero net carbon gain. Regression equation:  $\log(\text{carbon gain}) = 0.16 - 0.16 \times \log(\text{height})$

increasing the light compensation point (Online Resource 3 in the ESM). However, perhaps the most notable finding was the difference in the way aboveground and whole-plant light compensation points varied with height, with this relationship becoming considerably steeper when whole plants were considered (Online Resource 3 in the ESM).

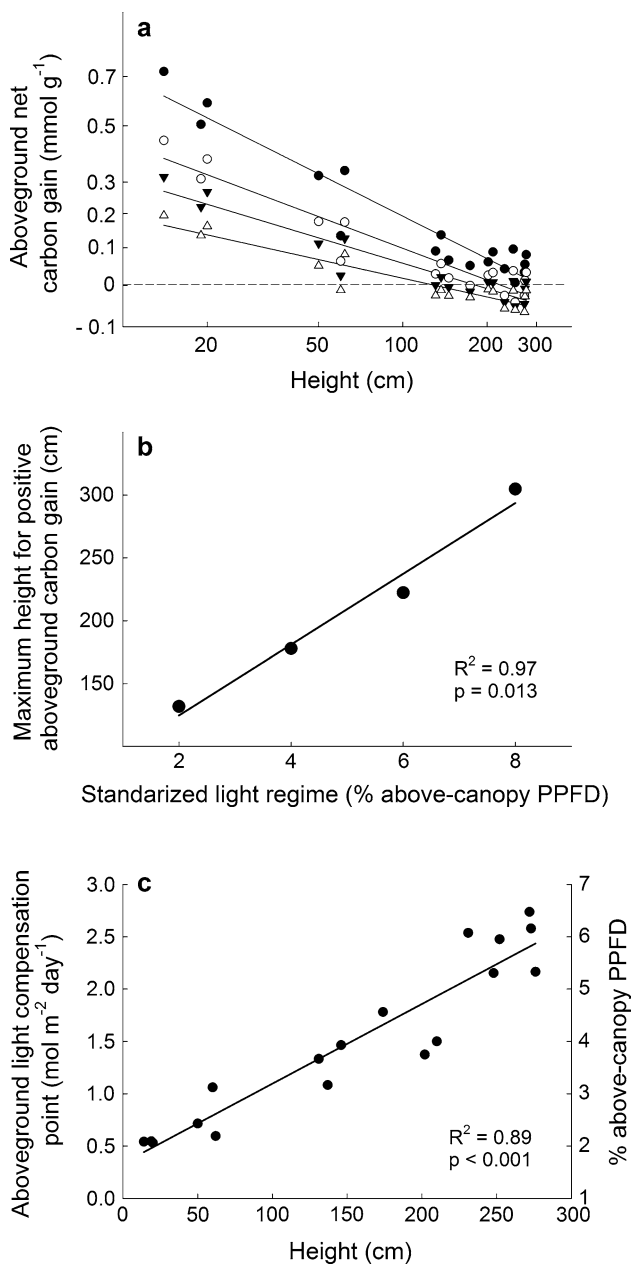
## Discussion

Our data demonstrate that daily (24-h) net carbon gain per gram of aboveground plant tissue of understory juvenile *Acer saccharum* trees declines with increasing plant size in summertime (Fig. 5). This trend was evident under ambient and standardized light levels. Correspondingly, the aboveground instantaneous light compensation point increased with plant size (Fig. 6c). Additionally, a number of important leaf, stem, and whole-plant traits that are associated with light requirements such as leaf size, nitrogen content, gas exchange rates, and self-shading also varied significantly with ontogeny in *Acer saccharum* juvenile trees. Our results suggest that ontogenetic increases in light compensation points and declines in carbon gain are largely driven by an increase in the fraction of live nonphotosynthetic tissues, but that the degree of self-shading influences the strength of these relationships (Table 1). Given that survival in low-light understory conditions is enhanced by greater net carbon gain or growth (Walters and Reich 1996; Poorter and Bongers 2006; Lusk and Jorgenson 2013), these results strongly support the idea that several aspects of sugar maple shade tolerance (net carbon gain, growth, and survival at a given light level) diminish as individuals grow larger in size.

In agreement with results from prior studies of small seedlings (Walters et al. 1993; Tjoelker et al. 1999), our study of larger juveniles found that stem- and aboveground-specific respiration rates declined with plant size (Fig. 4b, c). As juvenile trees grow larger, their proportion of metabolically active meristematic tissues with higher respiration rates (i.e., small-diameter twigs) declines, while the proportion of structural support tissues with lower respiration rates (i.e., large-diameter stems) increases. It is this ontogenetic change that drives the decline in stem respiration rates, while a combination of declining stem respiration and a declining fractional investment of annual plant growth distributed to leaves cause the decline in aboveground respiration per unit total plant biomass.

Unlike several previous studies, we found no ontogenetic declines in specific leaf area (SLA). Such patterns have been attributed to increasing investment in structural support tissue per unit lamina surface area (Niklas and Enquist 2001; Niklas and Cobb 2008). Declines in SLA with tree size were observed both within and across species





**Fig. 6** Relationship between aboveground net daily carbon gain estimated from YPLANT simulations and height of juvenile *Acer saccharum* trees at standardized light levels of 2, 4, 6, and 8 % of above-canopy PPFD (**a**); data were log–log-transformed prior to analysis. Linear regression analyses were significant at a  $p$  value of  $<0.001$ ; the filled circles represent simulations at 8 % PPFD [ $\log(\text{carbon gain}) = 0.16 - 0.16 \times \log(\text{height})$ ,  $R^2 = 0.91$ ], the open circles represent simulations at 6 % PPFD [ $\log(\text{carbon gain}) = -0.06 - 0.12 \times \log(\text{height})$ ,  $R^2 = 0.90$ ], the filled triangles represent simulations from 4 % PPFD [ $\log(\text{carbon gain}) = -0.20 - 0.09 \times \log(\text{height})$ ,  $R^2 = 0.89$ ], and the open triangles represent simulations at 2 % PPFD [ $\log(\text{carbon gain}) = -0.40 - 0.07 \times \log(\text{height})$ ,  $R^2 = 0.87$ ]. The dashed line at a net carbon gain of zero indicates the maximum height for aboveground carbon gain. **b** shows the relationship between maximum height for aboveground carbon gain and standardized light availability (% of above-canopy PPFD). **c** shows the relationships between aboveground light compensation points (on the left axis) and % above-canopy PPFD (on the right axis) vs. juvenile tree height

in those studies, which would help explain the declining growth and carbon balance seen in larger plants due to this added cost of harvesting light (Niklas and Cobb 2008). However, while we did observe a decline in net carbon gain with *A. saccharum* sapling size, we did not observe a change in SLA. Niklas and Cobb (2010) point out that variation in specific leaf area may be driven more by association with short vs. long shoots than by tree size, with long shoots producing leaves with higher SLA on average compared to short shoots. Given that smaller trees generally have proportionally higher numbers of long shoots, it is possible that this is why SLA did not vary with size in our study. Niklas and Cobb (2008) also point out that the variations they observed in SLA may reflect changes in the number of leaves exposed to different light conditions (i.e., sun vs. shade) as the canopy grows larger, as well as the amount of mechanical tissues in leaves at differing positions within the canopy (Sendall and Reich 2013). Our juvenile sugar maples were all found growing in low light, and our range of sapling size was significantly narrower than in their study, which likely contributed to the lack of variation we saw in SLA. Therefore, other traits such as biomass fractions and stem respiration appear to be responsible for the decline in net carbon gain per gram of plant tissue of juvenile sugar maples in low light.

The magnitude of self-shading for *A. saccharum* was similar to those of two juvenile temperate rainforest evergreens with relatively short leaf lifespans (*Aristotelia chilensis* (Molina) Stunz and *Eucryphia cordifolia* Cav.), but less than those of five species with longer leaf lifespans (Lusk et al. 2011). Self-shading affects net carbon gain and shade tolerance of trees in forest understories (Valladares et al. 2002; Poorter et al. 2003; Sterck et al. 2005; Falster et al. 2012), causing ontogenetic declines in both, as larger saplings produce more leaves (Duursma et al. 2012). We found that self-shading doubled as juveniles grew from  $\approx 15$  cm to  $\approx 50$  cm, while net carbon gain declined by approximately 60 %. However, our results show that changes in the proportion of structural support tissues play a more significant role than self-shading in the decline in net carbon gain in larger juveniles, which is evident in comparisons of individuals 50 and  $>200$  cm tall. This comparison shows a 77 % decline in net carbon gain, while self-shading increases by only 36 %.

A number of assumptions and simplifications were involved in our measurements and simulations, and therefore our results include considerable uncertainty. These include averaging information from several hemispherical photos to obtain standardized light environments for model simulations, conversion of instantaneous  $R_d$  to daytime and nighttime  $R_d$  using a temperature-dependent  $Q_{10}$ , and the assumptions built into the YPLANT model, such as no reflection of light from the ground to the plant (Pearcy

**Table 1** GLM testing effects of self-shading and stem biomass fraction on aboveground net daily carbon gain ( $\text{mmol g}^{-1}$ ) and aboveground light compensation points ( $\text{mol m}^{-2} \text{day}^{-1}$ )

Variable	Aboveground net daily carbon gain			Aboveground light compensation point		
	Coefficient	<i>F</i> value	<i>P</i>	Coefficient	<i>F</i> value	<i>P</i>
Self-shading	−0.1	0.01	0.937	1.1	2.02	0.177
Stem biomass fraction	−7.8	9.92	0.007	8.5	12.25	0.004
Self-shading × stem biomass fraction	−15.1	5.71	0.032	20.8	11.22	0.005

and Yang 1996). It should also be noted that our method of measuring stem respiration rates ( $\text{CO}_2$  efflux), while commonly used to estimate stem respiration, does not account for  $\text{CO}_2$  absorbed internally in xylem sap (Teskey et al. 2008). Nonetheless, our results suggest that *A. saccharum* likely experiences significantly lower midsummer daily net carbon gain with increased size, at a standardized light level.

Our results are also limited in that we assessed instantaneous aboveground light compensation points during the middle of the growing season and did not estimate seasonal or annual light compensation points. Measurements of construction costs and growth respiration, “dormant” season respiration rates, and early spring photosynthesis would be necessary to extend our findings to whole years. Construction costs associated with replacing leaf and fine root tissues as well as dormant season respiration represent obvious additional respiratory costs for the maple saplings measured in our current study, indicating that annual light compensation points of juvenile *A. saccharum* trees are likely higher than those reported here. However, it has also been shown that juvenile trees in deciduous forest understories may strongly depend on a window of high light availability in early spring before the overstory canopy leafs out (Harrington et al. 1989; Augspurger 2008; Lopez et al. 2008). Juvenile trees may gain a large portion of their annual carbon income during this period, which would enable them to survive at light levels below their light compensation point for most of their growing season.

In conclusion, our study shows how the interplay of gas exchange rates, biomass distribution, and self-shading shapes ontogenetic declines in the shade tolerance of a deciduous tree species. We also show that, despite having a higher proportion of large-diameter stems with low respiration rates per gram of tissue, larger individuals are less shade tolerant than smaller individuals. Integrating these effects helps clarify the mechanisms underlying size-related changes in shade tolerance.

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**Author contribution statement** KMS and PBR conceived and designed the project. KMS conducted fieldwork, ran model simulations and analyzed the data. KMS, PBR, and CHL wrote the manuscript.

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