

# Photosynthetic capacity peaks at intermediate size in temperate deciduous trees

SEAN C. THOMAS

Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, Ontario, M5S 3B3, Canada

Corresponding author (sc.thomas@utoronto.ca)

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**Summary** Studies of age-related changes in leaf functional biology have generally been based on dichotomous comparisons of young and mature individuals (e.g., saplings and mature canopy trees), with little data available to describe changes through the entire ontogeny of trees, particularly of broadleaf angiosperms. Leaf-level gas-exchange and morphological parameters were quantified in situ in the upper canopy of trees acclimated to high light conditions, spanning a wide range of ontogenetic stages from saplings (~1 cm in stem diameter) to trees >60 cm d.b.h. and nearing their maximum lifespan, in three temperate deciduous tree species in central Ontario, Canada. Traits associated with growth performance, including leaf photosynthetic capacity (expressed on either an area, mass or leaf N basis), stomatal conductance, leaf size and leaf N content, generally showed a unimodal ('hump-shaped') pattern, with peak values at an intermediate ontogenetic stage. In contrast, leaf mass per area (LMA) and related morphological parameters (leaf thickness, leaf tissue density, leaf C content) increased monotonically with tree size, as did water-use efficiency; these monotonic relationships were well described by simple allometric functions of the form  $Y = aX^b$ . For traits showing unimodal patterns, tree size corresponding to the trait maximum differed markedly among traits: all three species showed a similar pattern in which the peak for leaf size occurred in trees ~2–6 cm d.b.h., followed by leaf chemical traits and photosynthetic capacity on a mass or leaf N basis and finally by photosynthetic capacity on a leaf area basis, which peaked approximately at the size of reproductive onset. It is argued that ontogenetic increases in photosynthetic capacity and related traits early in tree ontogeny are general among relatively shade-tolerant tree species that have a low capacity for leaf-level acclimation, as are declines in this set of traits late in tree ontogeny.

**Keywords:** *Acer saccharum*, aging, *Betula alleghaniensis*, leaf morphology, ontogeny, photosynthesis, reproductive onset, senescence, *Tilia americana*.

## Introduction

Physiological processes at the cellular, organ and whole-plant scales of integration show pronounced changes as trees grow and age. Such changes are fundamental to understanding growth patterns of individual trees and stands (Ryan et al. 1997, Bond et al. 2007), tree life-history evolution (Thomas and Bazzaz 1999, Thomas 2003), as well as the responses of trees and forests to pests and pathogens (Kozłowski 1969, Boege and Marquis 2005) and anthropogenic environmental change (Bazzaz et al. 1996, Kolb et al. 1998, Phillips et al. 2008). Since forest management often results in the removal of the largest and oldest trees in a given stand (Franklin et al. 2002), age-related changes in tree physiological processes are also essential in understanding and mitigating management impacts on forest 'ecosystem function'.

Considerable research on ontogenetic changes in leaf gas exchange has focused on the hypothesis that increased hydraulic path length drives reductions in carbon uptake in taller trees via increased stomatal limitation (Ryan and Yoder 1997, Hubbard et al. 1999, Nabeshima and Hiura 2004). However, recent work suggests that a variety of processes impact age-related changes in gas exchange and related aspects of leaf functional biology. Hydraulic limitation has recently been hypothesized to act on many important aspects of leaf function by reducing leaf turgor during expansion rather than through stomatal limitation (Niinemets 2002, Thomas and Winner 2002, Koch et al. 2004, Woodruff et al. 2004, Ryan et al. 2006, Meinzer et al. 2008). Comparative studies have also suggested the importance of size-dependent patterns of biomechanical perturbation and reproductive allocation as correlates of leaf morphology, chemistry and gas exchange in tree canopies (e.g., Thomas and Ickes 1995, Leal and Thomas 2003).

An important aspect of age-related physiological change that has received little attention is the description of quantitative patterns of trait change through the entire course of tree ontogeny. Studies have most often compared mature trees of

canopy stature to seedlings or saplings (e.g., Donovan and Ehleringer 1991, Fredericksen et al. 1996, Cavander-Bares and Bazzaz 2000, Thomas and Winner 2002, Holscher 2004, Nabeshima and Hiura 2004, Reich et al. 2004, Ishida et al. 2005). As a consequence, surprisingly little is known concerning other ontogenetic stages, in particular pre-reproductive trees of intermediate stature and very large, old and potentially senescent trees. Studies that have presented data on gas exchange, leaf morphology and/or chemistry across a wide continuum of tree sizes have focused on conifers (Grulke and Miller 1994, Kolb and Stone 2000, Niinemets 2002, Phillips et al. 2002, Martinez-Vilalta et al. 2007, Greenwood et al. 2008). Conifers appear to diverge from angiosperms in terms of age-related physiological changes, showing a stronger pattern of age-related declines in photosynthetic capacity (Thomas and Winner 2002). Temperate deciduous trees, in contrast, have often been reported to show large increases in photosynthetic capacity in adult trees relative to saplings (e.g., Dawson and Ehleringer 1993, Hanson et al. 1994, Samuelson and Kelly 1997, Cavander-Bares and Bazzaz 2000). Whether such an ontogenetic increase persists throughout the ontogeny of deciduous trees is not clear.

Several indirect lines of evidence suggest that trees of an intermediate ontogenetic stage (e.g., 'pole-sized' trees 5–15 cm in d.b.h. in temperate deciduous forests) may show physiological patterns distinct from either saplings or larger canopy trees. In several tropical species, leaf size peaks at intermediate tree sizes, with the optimum point corresponding approximately to the size at reproductive onset (Alvarez-Buylla and Martinez-Ramos 1992, Thomas and Ickes 1995). This pattern is consistent with effects of reproductive allocation on leaf developmental processes, although changes in leaf morphology in some cases also closely track branching patterns (Alvarez-Buylla and Martinez-Ramos 1992, King 1998, Reich et al. 2004). Large effects of reproductive allocation on leaf chemistry and morphology are similarly suggested by correlative studies of within-canopy variation (e.g., Tappeiner 1969, Hasegawa and Takeda 2001, Leal and Thomas 2003) and by dendrochronological studies that have noted reduced diameter increment or shoot extension growth in years of heavy seed crops (Eis et al. 1965, Woodward et al. 1993, Monks and Kelly 2006).

Very old, potentially senescent trees may also be expected to show physiological patterns distinct from either saplings or younger mature trees. The effects of both pathogens and taxon-specific herbivores are likely to increase cumulatively through tree development (Boege and Marquis 2005). Such increases are expected to have both direct physiological consequence (e.g., decreases in xylem conductance due to woody tissue infection by fungal pathogens) and also to favor increased investment in inducible or constitutive defenses, at least prior to senescence. One possible example of such a constitutive defense is increased leaf lignification, as dramatically exhibited in the production of astrosclerid cells in ancient *Pseudotsuga menziesii* trees (Apple et al. 2002). Induction of herbivore defenses can result in large reductions in leaf

expansion, and in at least one case the same enzyme system involved in herbivore defense appears to itself be a direct regulator of leaf expansion (Moore et al. 2003). In addition to cumulative biotic interactions, very old trees may show disproportionate effects of high reproductive allocation (Thomas 1996a, 1996b, 1996c), changes in hydraulic architecture associated with epicormic branching (Ishii and Ford 2001) and altered leaf function associated with crown thinning and declining within-tree leaf area index (LAI) (Nock et al. 2008).

The present study compares leaf gas-exchange parameters and related aspects of leaf morphology across a wide range of ontogenetic stages in three species of temperate deciduous trees. Access to the upper canopies of a spatially interspersed set of trees of varying size with leaves exposed to high light conditions was made possible through use of a mobile canopy lift system in an uneven-aged managed forest within which very large, old trees were also found. The following questions are addressed: (i) What is the pattern of size dependence in photosynthetic capacity (and related parameters) within a given species? (ii) If a unimodal pattern is found, do peak photosynthetic capacity values correspond to the size at reproductive onset? (iii) How does the ontogenetic pattern for photosynthetic capacity compare to that for leaf size and other aspects of leaf morphology? (iv) How do patterns of size dependence of photosynthetic parameters and leaf morphology vary among tree species?

## Materials and methods

### *Study site and canopy access*

The study was conducted at Haliburton Forest and Wildlife Reserve, located in the Great Lakes–St. Lawrence forest region of Ontario, Canada (45°15' N, 78°34' W). Upland hardwood forests in the region are dominated by *Acer saccharum* Marsh., *Fagus grandifolia* Ehrh., *Tsuga canadensis* L. and *Betula alleghaniensis* Britt. The forest is managed under selection silviculture, following guidelines established by the Ontario Ministry of Natural Resources (OMNR 1998). The sampled area has not been harvested in the last 30 years but was affected by a large windstorm in 1995. This resulted in a highly heterogeneous canopy structure, facilitating the selection of trees with high crown exposure across a wide range of sizes, from saplings to trees >50 cm d.b.h. and showing pronounced crown thinning (Nock et al. 2008) and other signs of senescence.

The three species chosen for study (*A. saccharum*, *B. alleghaniensis* and *Tilia americana*) are common at the study area and range from highly shade tolerant to mid-tolerant (Niinemets and Valladares 2006, Baltzer and Thomas 2007). Foliage for gas exchange and other measurements was sampled from the upper one-third of exposed parts of the canopy of each tree sampled, utilizing a mobile elevating work platform (Scanlift SL240, Kesla Oyj, 2 Metsolantie, Kesälähti, Finland) for accessing heights over 3 m. Trees were chosen for sampling along a skid-trail network, in a

manner to ensure spatial interspersed of trees of a given species among size classes (saplings 1–2 cm d.b.h., and trees 3–10, 10–20, 20–30, 30–40, 40–50 and 50+ cm), with canopy trees including some of those sampled during a study of canopy structure (Nock et al. 2008). Saplings and smaller-sized trees sampled occurred in gaps; larger trees chosen for measurement had relatively exposed crowns.

#### Gas exchange and leaf morphometrics

Gas-exchange measurements focused on light-saturated photosynthesis (i.e., photosynthetic capacity on an area basis,  $A_{\text{area}}$ ), as this parameter can be efficiently surveyed, is closely related to integrated carbon uptake in forest canopies (Zotz and Winter 1993) and has been most widely reported in the related literature (Bond 2000, Thomas and Winner 2002). Representative sun-exposed fully expanded leaves in the upper one-third of the canopy of each tree were chosen for measurement. Gas-exchange measurements were conducted between 0800 and 1300 hours to avoid mid-day stomatal closure, with leaves maintained at a  $\text{CO}_2$  concentration of 350 p.p.m., relative humidity of 50–80%, leaf temperature of 20–25°C (0.5–1.6 kPa vapor pressure difference (VPD)) and photosynthetic photon flux density (PPFD) of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using an LI-6400 photosynthesis system (Li-Cor, Lincoln, NE) equipped with a red/blue light source (6000-02B Red-Blue SI-0951) (Light levels of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  have been found to be saturating for this set of species; Wayne and Bazzaz 1993, Sipe and Bazzaz 1994, Baltzer 2005, S.C. Thomas, unpublished data). Light-saturated photosynthesis ( $A_{\text{area}}$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ) and instantaneous water-use efficiency ( $\text{WUE} = A_{\text{area}}/E$ ) were recorded as the means of triplicate measurements at 20 s intervals on each leaf, after sufficient time for leaf acclimation to chamber conditions (up to 30 min, depending on incident light). Three to seven leaves free of mechanical damage and with minimal herbivore or physical damage or galling were measured for each tree and collected for morphometric measurements on the same day.

Leaf area of entire fresh leaves including petioles was measured using a Li-Cor 1200 leaf area meter. Leaf lamina thickness was measured using a low-force micrometer (No. 227-101, Mitutoyo Co., Japan) as the mean of three replicate measurements dispersed across the lamina of each leaf. Three 6-mm-diameter hole punch samples avoiding major veins were taken from fresh leaf samples, and leaves together with hole punches were dried at 60 °C to constant mass and weighed. Total leaf mass per area ( $\text{LMA}_{\text{total}}$ ) was calculated conventionally as total leaf dry mass/area, and leaf mass per area of lamina tissue ( $\text{LMA}_{\text{lamina}}$ ) was calculated as mass/area ratio of the hole punch samples. Leaf structural allocation (LSA), estimating the proportion of dry mass allocated to all non-lamina structures (i.e., to the leaf petiole, major veins and related structures), was calculated as:

$$\text{LSA} = (1 - \text{LMA}_{\text{lamina}}) / \text{LMA}_{\text{total}} \quad (1)$$

For a subset of leaf samples (39 of 317 total samples), only  $\text{LMA}_{\text{total}}$  was measured, and in these cases  $\text{LMA}_{\text{lamina}}$  was estimated using species-specific linear regression functions (including a y-intercept only where significant). These functions were as follows: for *A. saccharum*,  $\text{LMA}_{\text{lamina}} = 0.80977 \cdot \text{LMA}_{\text{total}}$  ( $r^2_{\text{adj}} = 0.992$ ); for *B. alleghaniensis*,  $\text{LMA}_{\text{lamina}} = 0.83696 \cdot \text{LMA}_{\text{total}}$  ( $r^2_{\text{adj}} = 0.988$ ); for *T. americana*,  $\text{LMA}_{\text{lamina}} = -6.01479 + 0.88807 \cdot \text{LMA}_{\text{total}}$  ( $r^2_{\text{adj}} = 0.954$ ). LSA was only estimated where independent measures of  $\text{LMA}_{\text{total}}$  and  $\text{LMA}_{\text{lamina}}$  were made. Percent leaf carbon and nitrogen were measured using an ECS4010 CHNS analyzer (Costech Analytical Technologies, Valencia, CA) for lamina subsamples of individual leaves (and expressed on a % mass/mass basis). Photosynthetic capacity on a leaf mass basis ( $A_{\text{mass}}$ ) was calculated as  $A_{\text{area}}/\text{LMA}_{\text{lamina}}$ . Potential photosynthetic nitrogen-use efficiency (PPNUE) was calculated as  $A_{\text{mass}} \times \text{percent leaf N}/100$ .

#### Whole-tree measurements and reproductive status

On each sample tree, measurements were made of stem diameter at 1.3 m height (d.b.h.) and crown exposure. The latter was scored qualitatively using a modified crown exposure class assessment (cf. Clark and Clark 1992). The categories were as follows: 1, trees completely overtopped; 1.5, gap edge trees with low lateral light exposure from the side; 2, gap or intermediate crown class with medium light exposure from the side; 2.5, gap or intermediate crown class with high light exposure from the side; 3, co-dominant crown position with <50% of upper crown exposed above; 3.5, co-dominant crown position with >50% of upper crown exposed above; 4, dominant trees receiving some direct light on crown sides, <50% exposed within 90° inverted cone encompassing the crown; 4.5, dominant trees receiving considerable side light, >50% exposed within 90° inverted cone encompassing the crown; 5, emergent trees with crown completely exposed within 90° inverted cone encompassing the crown. All crown class assessments were made by a single individual (the author).

Observations on tree reproductive status were made in August 2006 during a major seeding event at the site. In addition to focal sample trees, observations were made of trees opportunistically sampled along major trails near the study site. Observations for this extended sample of trees consisted of presence/absence of reproductive structures (fruit or pedicels indicating dispersed fruits), d.b.h. and crown exposure class. Trees were exhaustively searched for reproductive structures using 10 × 42 binoculars; cases in which the entire tree crown was not visible from the ground were excluded from the sample. Height and d.b.h. measurements were also made on an additional extended sample of trees near the study site showing no signs of prior stem breakage for allometric analyses. A subset of study trees were cored for age determination, but most larger trees were hollow or displayed heart rot. In the case of *A. saccharum*, there is a relatively close relationship between d.b.h. (in cm) and age at the site (at 0.3 m height):

age = 1.74 + 2.50 × d.b.h.; N = 18;  $r^2 = 0.699$  (data and full methods presented in Nock et al. 2008).

#### *Analysis of reproductive size thresholds and height–diameter allometry*

Reproductive size thresholds were calculated according to Thomas (1996a), with the following modified logistic regression equation used to describe probability of reproduction ( $P$ ) as a function of tree d.b.h.:

$$P = \frac{e^{a + b \ln S}}{1 + e^{a + b \ln S}} \quad (2)$$

where  $a$  and  $b$  are constants,  $e$  is the base of natural logarithms and the size metric ( $S$ ) used is d.b.h.. The inflection point of this function is the size at reproductive onset and, in theory, corresponds to the mode of the probability distribution function for onset of maturity of a set of trees followed through time (Thomas 1996a). This inflection point ( $S_{\text{crit}}$ ) is calculated as:

$$S_{\text{crit}} = e^{b^{-1}[\ln((b-1)/(b+1)) - a]} \quad (3)$$

Equation (2) was fit using maximum likelihood methods, with a binomial error distribution. Possible effects of crown exposure on probability of reproduction were examined by including crown exposure class (treated as a continuous variable) as an additional predictor in the modified logistic regression model (cf. Wright et al. 2005).

Species-specific relationships between height and d.b.h. were described using an exponential generalization of the allometric equation (Thomas 1996b):

$$H = H_{\text{max}} \left( 1 - e^{-aD^b} \right) \quad (4)$$

where  $H$  is tree height,  $D$  is d.b.h.,  $H_{\text{max}}$  is asymptotic maximum height and  $a$  and  $b$  are constants. Equation 4 was estimated using nonlinear least squares regression and was used to calculate relative size at onset of maturity (RSOM), defined as tree height at onset of maturity (estimated by substituting  $S_{\text{crit}}$  (in Eq. 3) into  $D$  in Eq. 4), divided by  $H_{\text{max}}$  (Thomas 1996a) and also average tree height at a reference d.b.h.

#### *Analysis of size-dependent variation in leaf traits*

Preliminary examination of size-dependent patterns indicated that many traits were well described by an allometric relationship of the form  $Y = aD^b$  (where  $D$  is stem diameter,  $Y$  is a given trait and  $a$  and  $b$  are constants) and that variance of trait values increased with tree size (as is nearly universally found in studies of morphological allometry; Huxley 1932, Niklas 1994). Analyses for all relationships, including apparently nonmonotonic ones, were therefore based on log–log transformed data, which greatly improved homoscedasticity of residuals. The analysis of size-dependent patterns followed a two-step procedure. First, tests were performed for the exis-

tence of nonmonotonic ('hump-shaped') relationships for a given trait, based on a pooled analysis of all species. Where the pooled test indicated a significant second-order d.b.h. term, a second-order polynomial function was fit for each species (to log–log transformed data); where the pooled test for significance of the second-order polynomial term was not significant, a linear allometric equation was fit to the data. A significant polynomial term can result from a nonlinear but monotonically increasing (or decreasing) pattern, and therefore a more stringent monotonicity test was also performed (for species × trait combinations considered individually), following Murtaugh (2003). Briefly, this latter test uses the second-order term of a polynomial regression as a test statistic but compares this result to a null model of the best-fitting monotone regression, which describes a step function that is either monotonically increasing or decreasing. The monotone regression is first computed for the data, and the residuals of this regression are resampled using a bootstrapping approach with replacement and added to the monotone regression function; a polynomial regression is then repeatedly fit to the resampled (null) dataset. The  $P$  value is computed as the proportion of second-order coefficients of the resampled data set of greater absolute value than the observed coefficient. In cases where the second-order coefficient is not significant, the  $P$  value is reported for the test for the second-order polynomial term.

Confidence limits for tree d.b.h. corresponding to the location of the peak of putatively hump-shaped relationships were made using a bootstrapping of the polynomial regressions fit to log–log transformed data, with 5000 bootstrap samples used. To test for potential effects of crown exposure on observed relationships, crown exposure was included as a covariate in linear or polynomial models for each trait. Additional analyses examined variation  $A_{\text{area}}$ ,  $A_{\text{mass}}$  and  $g_s$  as a function of leaf structure and chemistry in addition to tree size. Explanatory variables included d.b.h., (d.b.h.)<sup>2</sup>,  $LMA_{\text{lamina}}$ , leaf thickness, leaf N and species as a categorical variable (with all continuous variables log transformed prior to analysis), followed by stepwise model selection based on Akaike's Information Criterion (AICc) (Burnham and Anderson 2002). All statistical analyses were conducted in R v.1.13 (R Development Core Team 2008).

## Results

### *Reproductive size thresholds*

All three species exhibited pronounced size dependence of reproduction consistent with a 'threshold' pattern ( $b > 1$  in Eq. 2: see Thomas 1996a). *Betula alleghaniensis* showed a more gradual increase in probability of reproduction with size ( $b = 3.7$ ) than did *A. saccharum* ( $b = 5.2$ ) or *T. americana* ( $b = 4.9$ ) (Table 1). *Betula alleghaniensis* also showed the lowest estimated reproductive size threshold ( $S_{\text{crit}} = 12.9$  cm d.b.h.) though not significantly different from that for *A. saccharum*

Table 1. Parameter estimates for modified logistic regression equation describing probability of reproduction as a function of tree size (d.b.h.) in three temperate deciduous trees:  $a$  and  $b$  are the intercept and slope parameters of Eq. (2), and  $S_{\text{crit}}$  is the size (d.b.h.) at reproductive onset, corresponding to the inflection point of Eq. (2). Sample sizes are given in terms of total trees in the sample ( $N$ ) and total reproductive trees ( $N_r$ ).

Species	$N$	$N_r$	$a$	95% CI	$b$	95% CI	$S_{\text{crit}}$ (cm)	95% CI
<i>Acer saccharum</i>	72	43	-14.09	(-22.5, -8.03)	5.16	(2.96, 8.24)	14.2	(11.8, 14.9)
<i>Betula alleghaniensis</i>	61	36	-10.00	(-16.4, -5.20)	3.69	(1.99, 5.98)	12.9	(7.9, 14.7)
<i>Tilia americana</i>	53	19	-14.98	(-24.8, -8.58)	4.95	(2.81, 8.22)	19.0	(16.2, 19.8)

Table 2. Parameter estimates for asymptotic height–diameter allometries in three temperate deciduous trees, the relative size at onset of maturity (RSOM) and the proportion of asymptotic height reached at 30 cm d.b.h. (P30cm):  $H_{\text{max}}$  is asymptotic height, and  $a$  and  $b$  are the intercept and slope parameters of Eq. (4); RSOM is height at onset of maturity divided by asymptotic height. Parameter estimates for Eq. (4) are given  $\pm 1$  SE.

Species	$N$	$H_{\text{max}}$ (m)	$a$	$b$	RSOM	P30cm
<i>Acer saccharum</i>	57	24.0 $\pm$ 0.4	0.053 $\pm$ 0.009	1.10 $\pm$ 0.07	0.622	0.890
<i>Betula alleghaniensis</i>	51	25.1 $\pm$ 1.1	0.037 $\pm$ 0.011	1.18 $\pm$ 0.13	0.525	0.866
<i>Tilia americana</i>	20	26.3 $\pm$ 3.1	0.047 $\pm$ 0.013	1.03 $\pm$ 0.15	0.623	0.790

( $S_{\text{crit}} = 14.2$  cm d.b.h.); *T. americana* showed a significantly higher reproductive size threshold than the other two species ( $S_{\text{crit}} = 19.0$  cm d.b.h.) (Table 1). Inclusion of crown exposure class as a predictor did not result in increased predictive power in explaining reproductive status, as evaluated using an AIC criterion approach (results not shown). Site-specific height–diameter allometries indicate similar asymptotic heights among the three species, ranging from 24.0–26.3 m (Table 2). The height–diameter allometries also permit calculation of the RSOM, defined as height at onset of reproduction divided by asymptotic height (Thomas 1996a); RSOM was nearly identical in *A. saccharum* and *T. americana* ( $\sim 0.62$ ) but lower in *B. alleghaniensis* (0.52).

#### Leaf morphology

Leaf size showed, qualitatively, a hump-shaped pattern for all three species sampled, with a highly significant second-order polynomial term and significant monotonicity result for *A. saccharum* and *T. americana* (Figure 1; Table 3). The monotonicity test was not significant for *B. alleghaniensis*, which showed particularly high variability in leaf size among larger trees.

In contrast to the leaf size results, leaf mass per area ( $\text{LMA}_{\text{lamina}}$ ) showed a linearly increasing relationship with d.b.h. for all three species (Figure 2; Table 3); neither the second-order polynomial or monotonicity tests were significant for any species, but linear allometric relationships were high-

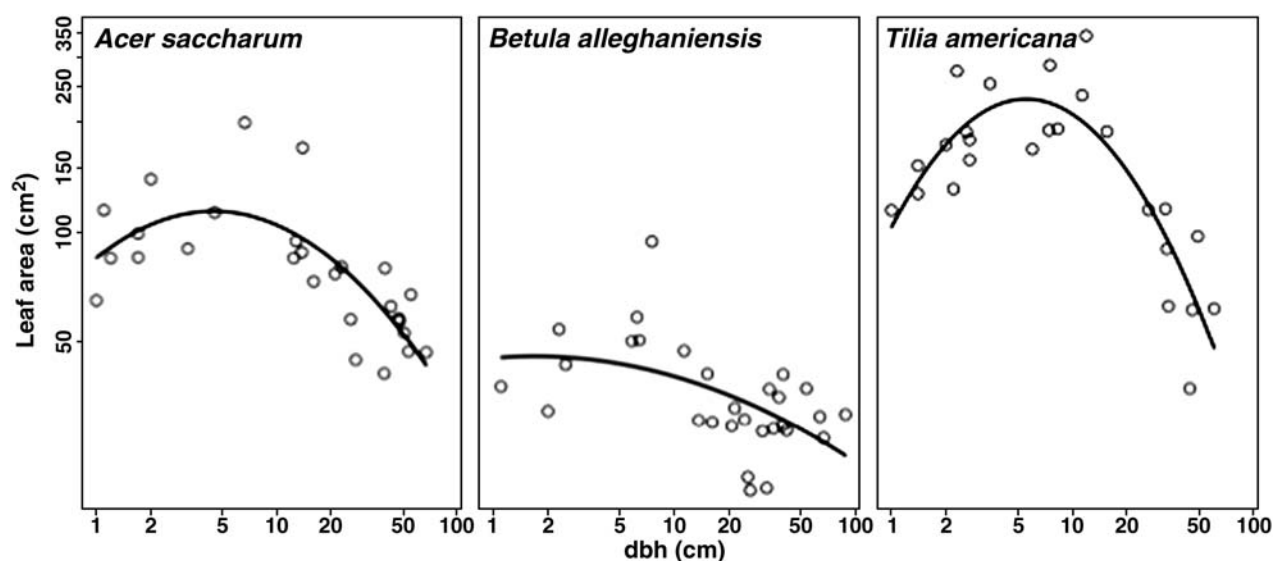


Figure 1. Relationships between leaf size (area/leaf) and d.b.h. for three temperate deciduous trees; both x- and y-axes are log transformed. Each point represents a mean of three to seven measurements of leaves from the upper canopy of trees sampled under high light conditions (trees with exposed canopies or within-canopy gaps). Lines are second-order polynomials fitted to the log–log transformed data. Corresponding  $P$  values and parameters and test results for regression analyses and monotonicity tests are given in Table 3.

Table 3. Results of regression analyses, monotonicity tests and species-pooled tests of relationships between leaf physiological parameters and tree size shown in Figures 1, 2, 3, 4, 5, 6 and 7. The 'pooled tests' give results for species-pooled analyses (of log–log transformed data) that include main effect terms for d.b.h., species, diameter × species (d × s) interaction and a second-order polynomial term for d.b.h. [(d.b.h.)<sup>2</sup>]. Where the (d.b.h.)<sup>2</sup> term is significant for the pooled analysis, second-order polynomials are fit for all three species ( $\log X = a + b \cdot \log D + c \cdot (\log D)^2$ ); otherwise a linear (allometric) equation was fit ( $\log X = a + b \cdot \log D$ ). Corresponding parameter values are given for each species, with the adjusted  $r^2$  value and  $P$  values for the regression ( $P$  (reg)) and Murtaugh (2003) test for monotonicity ( $P$  (mono)).  $P$  values are given in bold where  $P < 0.05$ .

Variable	Parameter	Species			Pooled tests	
		<i>A. saccharum</i>	<i>B. alleghaniensis</i>	<i>T. americana</i>	Term	$P$
Area/leaf (Figure 1)	$a$	1.930	1.655	2.014	d.b.h.	<b>&lt;0.001</b>
	$b$	0.394	0.042	0.940	species	<b>&lt;0.001</b>
	$c$	0.305	−0.092	−0.632	d × s	0.122
	$r_{\text{adj}}^2$	0.533	0.225	0.724	(d.b.h.) <sup>2</sup>	<b>&lt;0.001</b>
	$P$ (reg)	<b>&lt;0.001</b>	<b>0.014</b>	<b>&lt;0.001</b>		
	$P$ (mono)	<b>0.004</b>	0.185	<b>0.001</b>		
LMA (Figure 2)	$a$	1.601	1.487	1.376	d.b.h.	<b>&lt;0.001</b>
	$b$	0.167	0.220	0.289	species	<b>&lt;0.001</b>
	$r_{\text{adj}}^2$	0.763	0.623	0.732	d × s	<b>0.037</b>
	$P$ (reg)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	(d.b.h.) <sup>2</sup>	0.852
	$P$ (mono)	0.979	0.967	0.222		
Leaf thickness (Figure 2)	$a$	1.096	1.132	1.201	d.b.h.	<b>&lt;0.001</b>
	$b$	0.112	0.136	0.079	species	<b>&lt;0.001</b>
	$r_{\text{adj}}^2$	0.685	0.362	0.290	d × s	0.306
	$P$ (reg)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.003</b>	(d.b.h.) <sup>2</sup>	0.865
	$P$ (mono)	0.382	0.300	0.557		
Leaf tissue density (Figure 2)	$a$	−0.495	−0.632	−0.798	d.b.h.	<b>&lt;0.001</b>
	$b$	0.056	0.066	0.196	species	<b>&lt;0.001</b>
	$r_{\text{adj}}^2$	0.511	0.142	0.637	d × s	<b>&lt;0.001</b>
	$P$ (reg)	<b>&lt;0.001</b>	<b>0.025</b>	<b>&lt;0.001</b>	(d.b.h.) <sup>2</sup>	0.660
	$P$ (mono)	0.122	0.421	0.263		
Leaf structural allocation (Figure 3)	$a$	−0.641	0.784	−0.509	d.b.h.	<b>0.008</b>
	$b$	−0.061	0.0095	0.159	species	<b>0.026</b>
	$r_{\text{adj}}^2$	0.102	0	0.542	d × s	0.089
	$P$ (reg)	0.087	0.883	<b>&lt;0.001</b>	(d.b.h.) <sup>2</sup>	0.108
	$P$ (mono)	0.991	0.847	0.631		
$A_{\text{area}}$ (Figure 4)	$a$	0.547	0.837	0.553	d.b.h.	<b>&lt;0.001</b>
	$b$	0.689	0.373	1.041	species	<b>&lt;0.001</b>
	$c$	−0.306	−0.180	−0.443	d × s	0.147
	$r_{\text{adj}}^2$	0.492	0	0.541	(d.b.h.) <sup>2</sup>	<b>&lt;0.001</b>
	$P$ (reg)	<b>&lt;0.001</b>	0.428	<b>&lt;0.001</b>		
$A_{\text{mass}}$ (Figure 4)	$a$	1.957	2.280	2.248	d.b.h.	<b>0.001</b>
	$b$	0.493	0.338	0.577	species	<b>&lt;0.001</b>
	$c$	−0.291	−0.264	−0.359	d × s	0.451
	$r_{\text{adj}}^2$	0.229	0.264	0.377	(d.b.h.) <sup>2</sup>	<b>&lt;0.001</b>
	$P$ (reg)	<b>0.017</b>	<b>0.007</b>	<b>0.002</b>		
PPNUE (Figure 4)	$a$	0.275	0.676	0.731	d.b.h.	<b>&lt;0.001</b>
	$b$	0.615	0.635	0.755	species	<b>&lt;0.001</b>
	$c$	−0.362	−0.45	−0.473	d × s	0.287
	$r_{\text{adj}}^2$	0.300	0.338	0.460	(d.b.h.) <sup>2</sup>	<b>&lt;0.001</b>
	$P$ (reg)	<b>0.005</b>	<b>0.002</b>	<b>&lt;0.001</b>		
$g_s$ (Figure 5)	$a$	1.308	−0.950	−1.299	d.b.h.	<b>0.012</b>
	$b$	0.584	0.126	1.125	species	<b>&lt;0.001</b>
	$c$	−0.305	−0.058	−0.467	d × s	<b>&lt;0.001</b>
	$r_{\text{adj}}^2$	0.146	0	0.483	(d.b.h.) <sup>2</sup>	0.021
	$P$ (reg)	0.058	0.909	<b>&lt;0.001</b>		
	$P$ (mono)	<b>0.004</b>	0.260	<b>0.018</b>		

Continued

Table 3. Continued

Variable	Parameter	Species			Pooled tests	
		<i>A. saccharum</i>	<i>B. alleghaniensis</i>	<i>T. americana</i>	Term	<i>P</i>
WUE (Figure 6)	a	-2.345	-2.471	-2.454	d.b.h.	<0.001
	b	0.115	0.138	0.166	species	0.031
	$r_{\text{adj}}^2$	0.181	0.186	0.389	d × s	0.670
	P (reg)	<b>0.016</b>	<b>0.011</b>	<0.001	(d.b.h.) <sup>2</sup>	0.213
	P (mono)	0.163	<b>0.045</b>	0.828		
Leaf N (Figure 7)	a	0.319	0.397	0.485	d.b.h.	<0.001
	b	0.121	0.294	0.175	species	<0.001
	c	-0.07	-0.186	-0.114	d × s	0.139
	$r_{\text{adj}}^2$	0.094	0.478	0.401	(d.b.h.) <sup>2</sup>	<0.001
	P (reg)	0.117	<0.001	<0.001		
Leaf C (Figure 7)	a	1.684	1.678	3.812	d.b.h.	<0.001
	b	0.002	0.003	0.014	species	<0.001
	$r_{\text{adj}}^2$	0.015	0.007	0.311	d × s	0.009
	P (reg)	0.251	0.282	<b>0.002</b>	(d.b.h.) <sup>2</sup>	0.165
	P (mono)	0.407	0.104	0.148		
Leaf C:N ratio <sup>-1</sup> (Figure 7)	a	1.368	1.275	1.166	d.b.h.	<0.001
	b	-0.124	-0.274	-0.141	species	<0.001
	c	0.072	0.178	0.104	d × s	0.100
	$r_{\text{adj}}^2$	0.090	0.479	0.456	(d.b.h.) <sup>2</sup>	<0.001
	P (reg)	0.124	<0.001	<0.001		
P (mono)	<b>0.019</b>	<b>0.046</b>	<b>0.007</b>			

ly significant in each case.  $LMA_{\text{lamina}}$  can be thought of as consisting of two components: leaf thickness and leaf tissue density (mass per volume) (Witkowski and Lamont 1991). Both of these components also showed strong linear allometric relationships with d.b.h. in all three species (Figure 2). Slopes for the relationship between LMA and d.b.h. and for leaf tissue density and d.b.h. varied significantly among species, as indicated by significant d.b.h. × species terms in the pooled model.

Leaf structural allocation (LSA; Eq. 1), measuring the proportion of leaf dry mass in the petiole, midrib, major veins, and associated structures, varied from ~10–35% among all samples. In *T. americana*, there was a highly significant decline in LSA with tree size, which averaged ~30% in saplings, falling to ~15–20% in large mature trees (Figure 3). A weaker, marginally significant trend in this direction was also found for *A. saccharum*, and no trend was apparent in *B. alleghaniensis* (Figure 3; Table 3). In the latter species, major secondary veins are very closely spaced, particularly in upper-canopy leaves of many large trees, making it difficult to obtain lamina samples that entirely avoid secondary veins during sampling. This source of bias, in addition to high variability among individual trees, may contribute to the apparent lack of size dependence in LSA in this species.

#### Leaf gas exchange and chemistry

Photosynthetic capacity on a leaf area basis ( $A_{\text{area}}$ ) showed unimodal, hump-shaped patterns relative to tree size, with a highly significant monotonicity result for *A. saccharum* and

*T. americana* (Figure 4; Table 3). *Betula alleghaniensis* showed a weaker but qualitatively similar pattern, with a marginal monotonicity result. Photosynthetic capacity expressed on a leaf mass basis ( $A_{\text{mass}}$ ) and leaf N basis (PPNUE) also showed unimodal patterns in all three species (Figure 4), with significant second-order polynomial term and monotonicity results in all cases ( $P = 0.054$  in the case of the  $A_{\text{mass}}$  monotonicity result for *B. alleghaniensis*). Similar to the result for  $A_{\text{area}}$ ,  $g_s$  showed a unimodal, hump-shaped pattern relative to tree size in all three species, with significant monotonicity results for *A. saccharum* and *T. americana* and a much weaker pattern for *B. alleghaniensis* (Figure 5; Table 3). Results for  $g_s$  also indicate a highly significant d.b.h. by species interaction (Table 3), corresponding to a very steep initial rise in  $g_s$  with d.b.h. for *T. americana* and an essentially flat pattern for *B. alleghaniensis* (Figure 5). Instantaneous WUE was relatively weakly correlated with tree size and was described by a linear allometric function for all three species (Figure 6; Table 3).

Among leaf chemistry traits, leaf N showed a unimodal pattern relative to tree size in all three species (Figure 7; Table 3), with significant second-order polynomial term and monotonicity results in two of three cases. In contrast, leaf C showed a trend toward linear allometric increases with tree size, significant in the case of *T. americana* but not other species, corresponding to a significant d.b.h. × species interaction (Figure 7; Table 3). Leaf C:N ratio showed a U-shaped pattern: the second-order polynomial term and monotonicity tests (on the reciprocal of leaf C:N) were significant in two of three cases (Figure 7; Table 3).

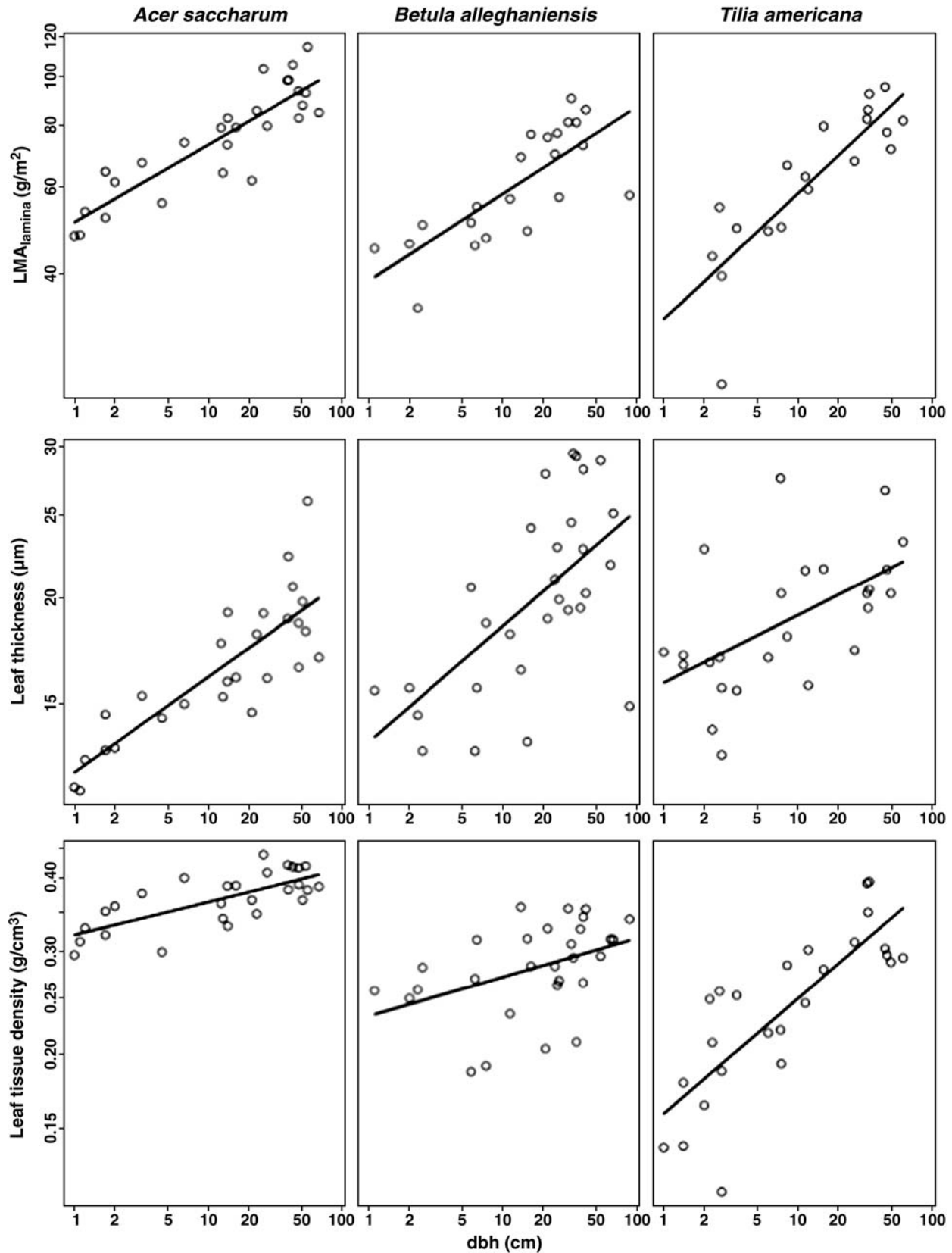


Figure 2. Relationships between leaf mass per area and d.b.h., leaf thickness and d.b.h. and leaf tissue density and d.b.h. for three temperate deciduous trees. Lines are linear regressions fitted to the log–log transformed data. Statistically significant species  $\times$  d.b.h. interactions were found for LMA ( $P = 0.037$ ) and leaf tissue density ( $P < 0.001$ ).  $P$  values for the monotonicity test and parameters and test results for regression analyses are given in Table 3.

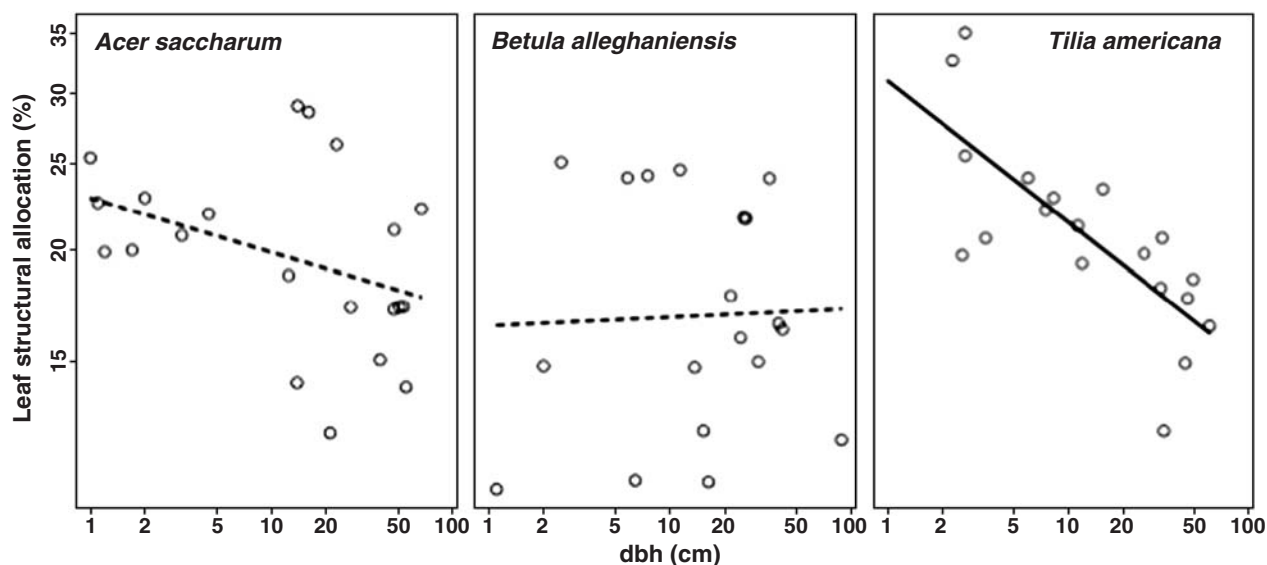


Figure 3. Relationships between leaf structural allocation (defined as dry mass allocation to petiole, midrib, and major veins: Eq. (1)) and d.b.h. for three temperate deciduous trees. Solid lines indicate regressions significant ( $P < 0.05$ ) for species considered individually, and dashed lines indicate regressions where the species-pooled analysis, but not the individual species analysis, is significant. Corresponding  $P$  values for the monotonicity test and parameters and test results for regression analyses are given in Table 3.

#### Potential physiological drivers of gas-exchange patterns

Additional linear model analyses were performed to determine the extent to which size-dependent patterns in  $A_{\text{area}}$ ,  $A_{\text{mass}}$  and  $g_s$  could be explained by changes in leaf structure and chemistry. In each case,  $\text{LMA}_{\text{lamina}}$ , leaf thickness, and leaf N were included as potential explanatory variables, in addition to tree species and the linear and quadratic terms for d.b.h.. In the pooled data set,  $A_{\text{area}}$  showed a moderate correlation with leaf N ( $r = 0.487$ ;  $P < 0.001$ ) and a weak correlation with leaf thickness ( $r = 0.226$ ;  $P = 0.043$ );  $g_s$  was likewise correlated within leaf N ( $r = 0.466$ ;  $P < 0.001$ ). However, in linear model analyses, only the d.b.h. terms (linear and quadratic) and species terms were significant ( $P < 0.001$ ). Correspondingly, AIC-selected models excluded  $\text{LMA}_{\text{lamina}}$ , leaf thickness and leaf N as predictors. Similar analyses for  $A_{\text{mass}}$  indicated a high positive correlation with leaf N ( $r = 0.811$ ;  $P < 0.001$ ) and negative correlation with  $\text{LMA}_{\text{lamina}}$  ( $r = -0.636$ ;  $P < 0.001$ ). The linear model analyses had significant terms for  $\text{LMA}_{\text{lamina}}$  in addition to the linear and quadratic terms for d.b.h. and the species, and this set of terms was retained in the AIC-selected model. The overall results thus indicate strong size-dependent trends in  $A_{\text{area}}$ ,  $A_{\text{mass}}$  and  $g_s$  that are not entirely explained by changes in leaf N,  $\text{LMA}_{\text{lamina}}$  or leaf thickness.

#### Position of trait maxima relative to reproductive onset

The d.b.h. corresponding to trait maxima were calculated on the basis of the quadratic allometric model, with confidence limits estimated using a bootstrap approach (see Materials and methods). For all traits in each of the study species, the estimated d.b.h. corresponding to the trait maxima preceded the estimated size at reproductive onset (Figure 8), in

many cases significantly so (as judged by lack of overlap of 95% confidence limits). The only traits that showed maxima close to the size at reproductive onset were  $A_{\text{area}}$  and corresponding values of  $g_s$ . Tree size corresponding to the estimated maxima for leaf size,  $A_{\text{mass}}$ , PPNU, the reciprocal of leaf C:N and leaf N fell in the range of 1.6–7.4 cm across traits and species (averaging 5.7 cm), well below the size of reproductive onset. Although estimates of the maximum points had much broader limits for *B. alleghaniensis* than the other two species, all three species showed a similar pattern of trait maxima. Pairwise correlations for the estimated d.b.h. maxima of traits among the three species ranged from 0.93–0.98 and were highly significant in each case ( $P < 0.01$ ).

#### Crown exposure versus tree size as predictor of trait patterns

Sampling was conducted to reduce differences in incident light by choosing trees under relatively open conditions (i.e., saplings in gaps and canopy trees with dominant or co-dominant crown positions) and by sampling foliage only in the upper crown positions. However, light acclimation effects are still likely to contribute to apparent ontogenetic patterns. To statistically control for such effects, a set of analyses were conducted in which canopy position (scored as a modified crown exposure class index; see Materials and methods) was included as a predictor variable, together with d.b.h., in a multiple linear regression. For traits that showed unimodal patterns (i.e., leaf size,  $A_{\text{area}}$ ,  $g_s$  and leaf N, C:N ratio), a corresponding quadratic model was used. In only two of 39 cases was the crown exposure term significant ( $P < 0.05$ ;  $A_{\text{area}}$  and conductance for *T. americana*), and in both of these cases the linear and quadratic terms for d.b.h. in the model remained highly significant (Table 4).

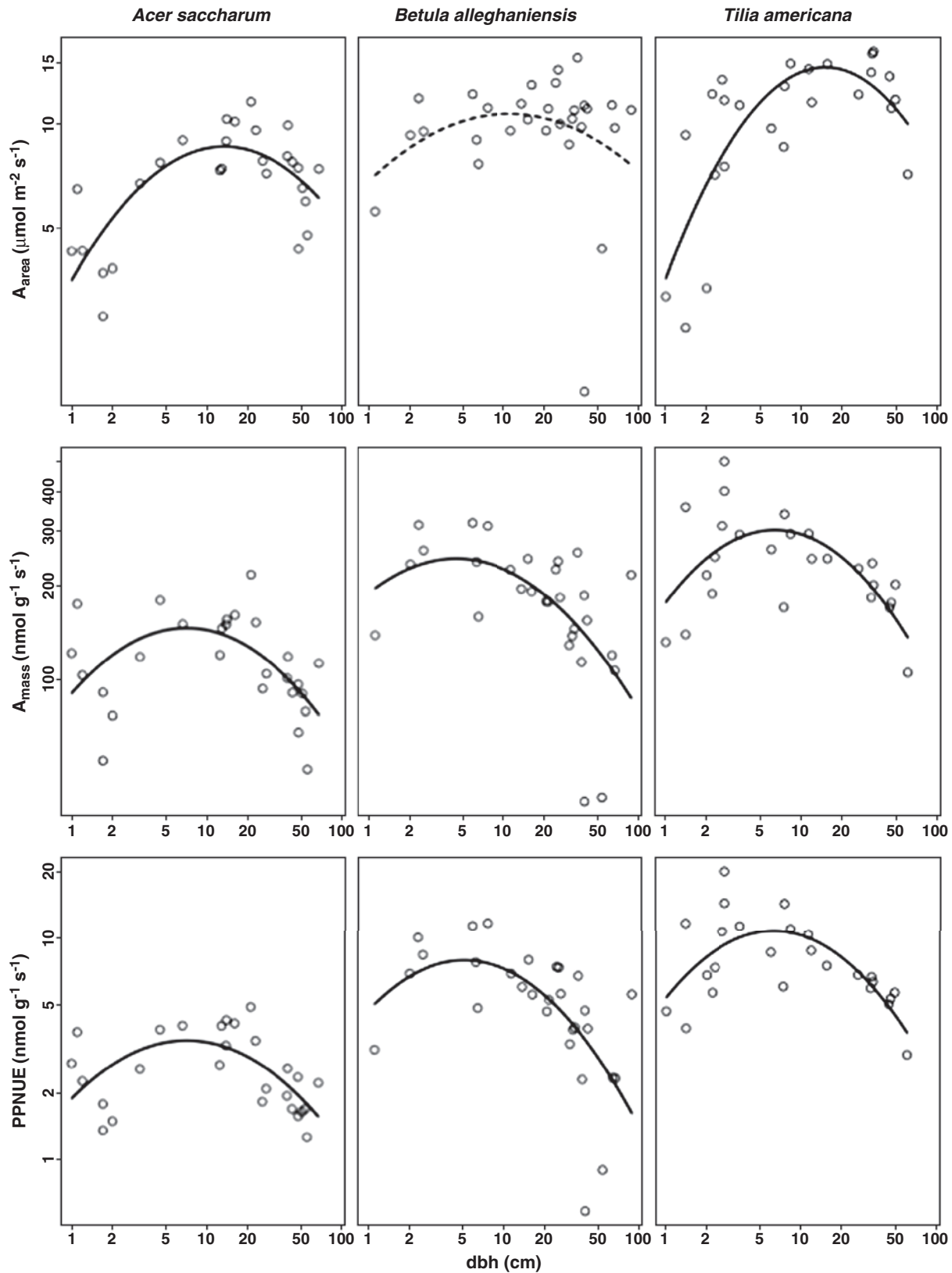


Figure 4. Relationships between photosynthetic capacity (expressed on the basis of leaf area ( $A_{\text{area}}$ ), leaf mass ( $A_{\text{mass}}$ ) and leaf N (PPNUE)) and d.b.h. for three temperate deciduous trees. Solid lines indicate regressions (second-order polynomial fitted to log–log transformed data) significant ( $P < 0.05$ ) for species considered individually, and dashed lines indicate cases where the species-pooled analysis, but not the individual species analysis, is significant. Corresponding  $P$  values for the monotonicity test and parameters and test results for regression analyses are given in Table 3.

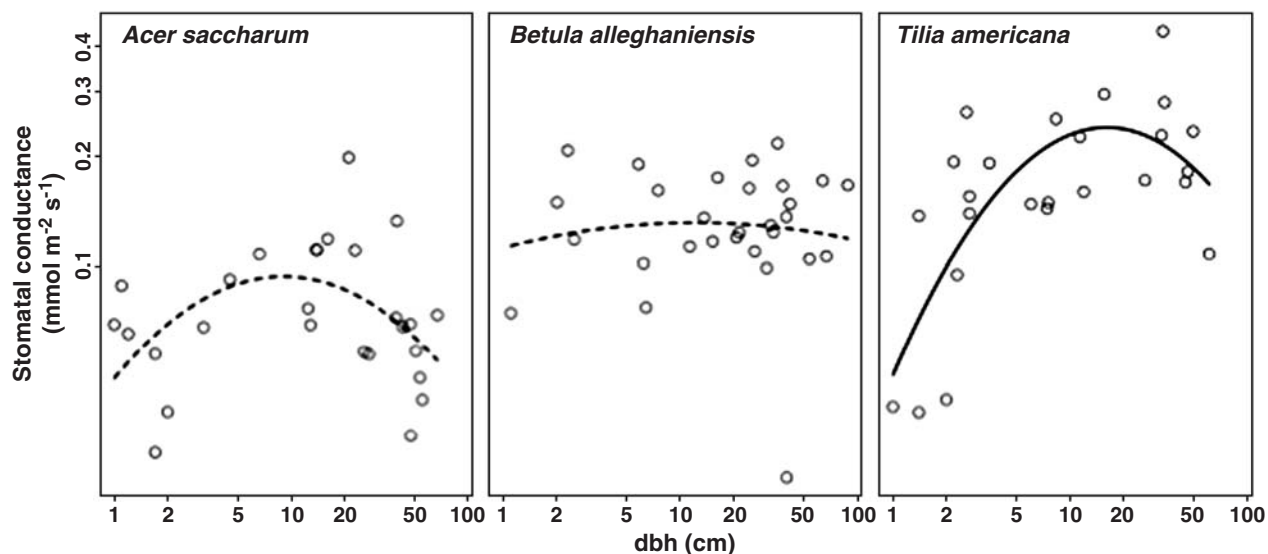


Figure 5. Relationships between stomatal conductance ( $g_s$ ) and d.b.h. for three temperate deciduous trees. Solid lines indicate regressions (second-order polynomial fitted to log–log transformed data) significant ( $P < 0.05$ ) for species considered individually, and dashed lines indicate cases where the species-pooled analysis, but not the individual species analysis, is significant. Corresponding  $P$  values for the monotonicity test and parameters and test results for regression analyses are given in Table 3.

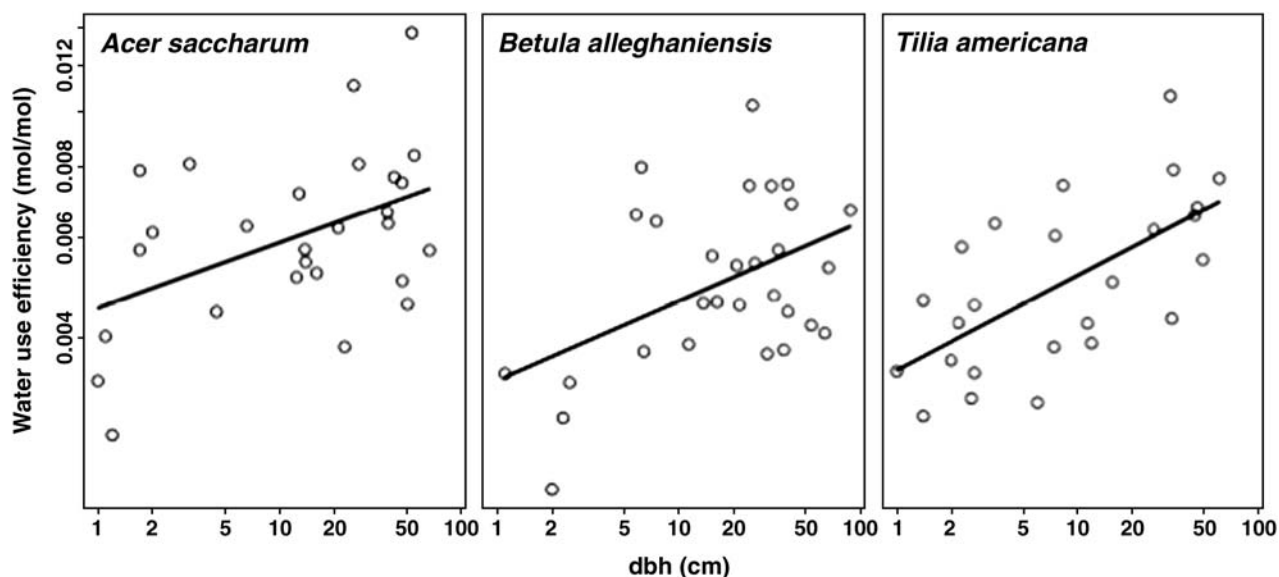


Figure 6. Relationships between instantaneous water-use efficiency (WUE) and d.b.h. for three temperate deciduous trees. Solid lines are linear regressions fitted to the log–log transformed data. Corresponding  $P$  values for the monotonicity test and parameters and test results for regression analyses are given in Table 3.

## Discussion

The present study documents a unimodal or ‘hump-shaped’ relationship between photosynthetic capacity (measured as either  $A_{\text{area}}$  or  $A_{\text{mass}}$ ) and stem diameter that is similar in each of the three species of temperate deciduous trees characterized. Other traits expected to be positively related to tree growth and closely linked to photosynthetic capacity (i.e., PPNUe,  $g_s$ , leaf N and the reciprocal of foliar C:N) generally showed a similar unimodal pattern, as did leaf

size. In contrast, most leaf morphological and chemical traits that reflect investment in leaf structure or defense, including  $LMA_{\text{lamina}}$ , leaf thickness, leaf tissue density and leaf C content, increased monotonically with tree size and were well described by linear allometric relationships. Although one of the three species (*B. alleghaniensis*) showed greater variability in most trait patterns than the others, the form of these relationships were generally consistent among species, as were the relative positions of trait maxima during ontogeny (Figure 8).

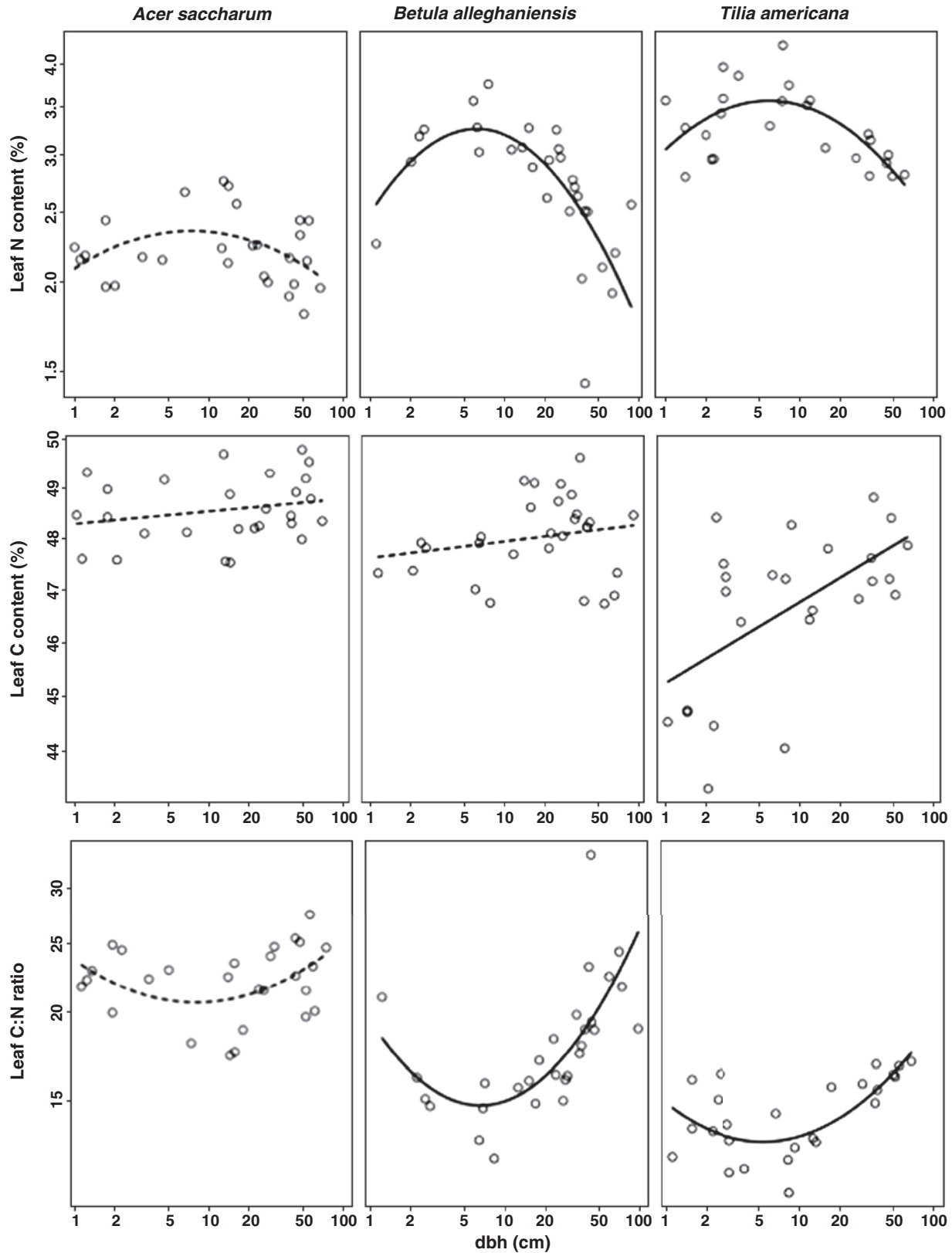


Figure 7. Relationships between leaf N content and d.b.h., leaf C content and d.b.h., and leaf C:N ratio and d.b.h. for three temperate deciduous trees. Solid lines indicate regressions (second-order polynomials for leaf N and C:N ratio, linear regression for leaf C, fitted to log–log transformed data) significant ( $P < 0.05$ ) for species considered individually, and dashed lines indicate cases where the species-pooled analysis, but not the individual species analysis, is significant. Corresponding  $P$  values for the monotonicity test and parameters and test results for regression analyses are given in Table 3.

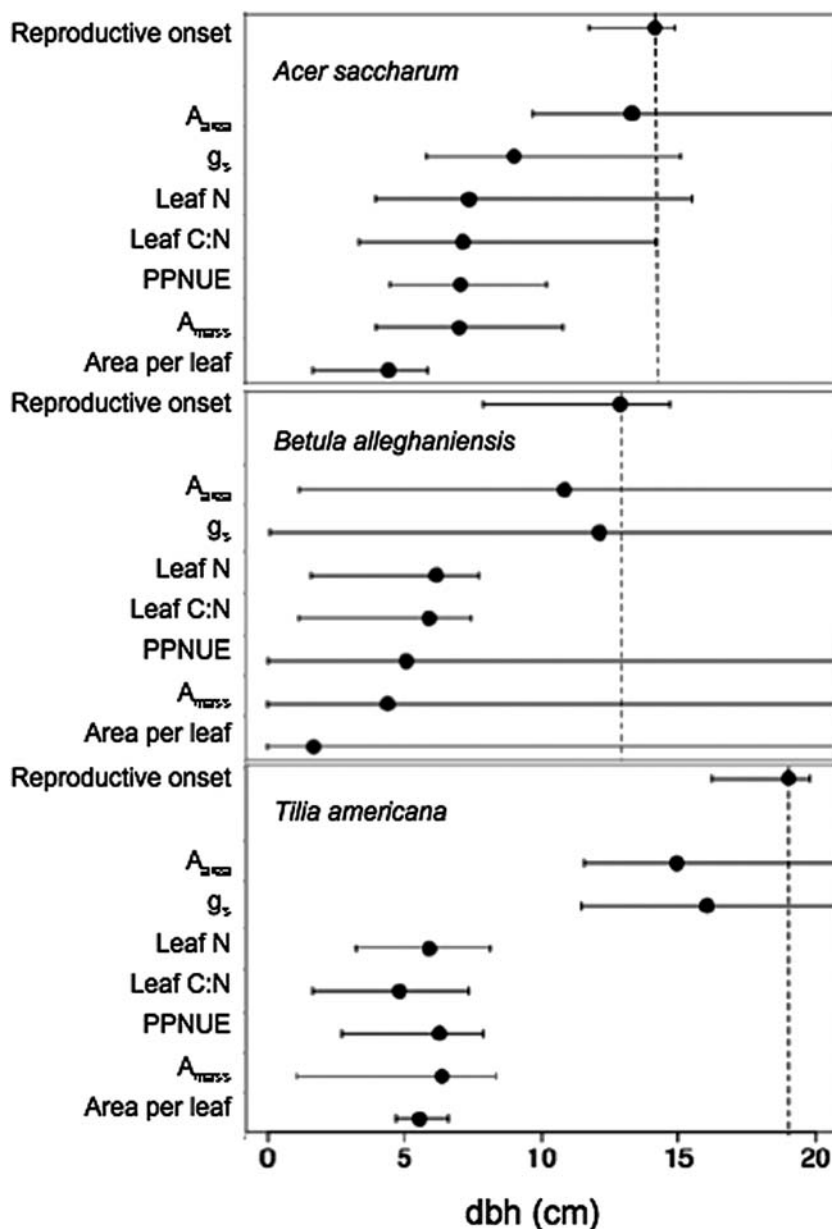


Figure 8. Location and 95% confidence limits for reproductive size thresholds and tree size corresponding to trait maxima for traits showing unimodal patterns of size dependence in three species of temperate deciduous trees. Confidence limits for trait maxima were estimated using a bootstrap resampling approach (see Materials and methods); in the case of leaf C:N ratio, the reciprocal value is used in analyses. The dotted lines indicate the reproductive size threshold.

Prior studies describing patterns of leaf photosynthetic traits across a wide range of tree sizes have mainly focused on conifers. While these studies have not suggested any increase in  $A_{area}$  with tree size among smaller size classes, explicit tests for a unimodal relationship have not been made, and sampling effort may not have been sufficient to detect such a trend if present. Niinemets (2002) pooled data from studies across northern Europe to examine size-related trends in a range of leaf physiological traits in *Picea abies* and *Pinus sylvestris*. Although linear allometric relationships were used to describe these patterns, there are a number of cases where a unimodal pattern would almost certainly provide a better de-

scription of the data presented (e.g., needle length as a function of tree height for *P. abies* and  $A_{area}$ ,  $g_s$  and  $V_{cmax}$  as a function of tree height for *P. sylvestris*; Figures 3E, 4C, 5C and 6C, respectively, in Niinemets 2002). Similarly, a recent study of three Asian *Acer* species shows data for  $A_{area}$  and  $g_s$  that strongly suggest unimodal patterns, but simple linear regressions were fitted (Nabeshima and Hiura 2008). Several other studies have compared gas-exchange parameters across age cohorts in conifers (Grulke and Miller 1994, Kolb and Stone 2000, Phillips et al. 2002, Greenwood et al. 2008); however, a common pattern in these studies is high variability among age classes that is at least partially confounded with

Table 4. Results of multiple regression analyses including crown exposure in addition to d.b.h. as predictors of leaf traits examined. In all cases, crown exposure was added as an additive covariate; thus, for variables described by a linear allometric relationship, the overall function fitted was  $\log V = a \cdot \log(D) + b \cdot E$  (where  $V$  is the variable in question,  $D$  is d.b.h. and  $E$  is crown exposure); for variables described by a quadratic allometric pattern, the overall function fitted was  $\log V = a \cdot \log(D) + b \cdot (\log(D))^2 + c \cdot E$ .  $P$  values  $<0.05$  are indicated in bold.

Variable	Term	<i>A. saccharum</i>		<i>B. alleghaniensis</i>		<i>T. americana</i>	
		Parameter	$P$ value	Parameter	$P$ value	Parameter	$P$ value
LMA	d.b.h.	0.18819*	<b>&lt;0.001</b>	0.17654*	<b>0.002</b>	0.24167*	<b>&lt;0.001</b>
	exposure	-0.12080	0.459	0.20804	0.275	0.22634	0.218
Leaf thickness	d.b.h.	0.10926*	<b>&lt;0.001</b>	0.08994*	0.096	0.09181*	<b>0.018</b>
	exposure	0.01418	0.915	0.21781	0.268	-0.05953	0.639
Leaf tissue density	d.b.h.	0.07862*	<b>&lt;0.001</b>	0.08576*	0.065	0.17287*	<b>&lt;0.001</b>
	exposure	-0.13125	0.158	-0.09471	0.569	0.11050	0.479
Leaf structural allocation	d.b.h.	-0.12862*	0.040	0.00637	0.948	-0.12794*	<b>0.044</b>
	exposure	0.13653	0.173	0.00658	0.965	-0.04182	0.522
Leaf C	d.b.h.	0.00095	0.773	0.00912*	<b>0.036</b>	0.013464*	<b>0.047</b>
	exposure	0.00254	0.629	-0.01023	0.066	0.001672	0.837
WUE	d.b.h.	0.14955*	0.060	0.25642*	<b>0.003</b>	0.12123*	0.060
	exposure	-0.06923	0.572	-0.19577	0.063	0.07535	0.338
Area/leaf	d.b.h.	0.39012*	<b>0.028</b>	0.05989	0.773	0.82270*	<b>&lt;0.001</b>
	(d.b.h.) <sup>2</sup>	-0.13262*	<b>0.002</b>	-0.05429	0.251	-0.22437*	<b>&lt;0.001</b>
	exposure	0.01133	0.914	0.08157	0.478	-0.16262	0.111
$A_{\text{area}}$	d.b.h.	0.69723*	<b>&lt;0.001</b>	0.32067	0.269	1.28218*	<b>&lt;0.001</b>
	(d.b.h.) <sup>2</sup>	-0.13223*	<b>0.001</b>	-0.03721	0.565	-0.29545*	<b>&lt;0.001</b>
	exposure	-0.02212	0.827	-0.23499	0.146	0.33465	<b>0.003</b>
$A_{\text{mass}}$	d.b.h.	0.48327*	<b>0.019</b>	0.27899*	0.334	0.68588*	<b>0.002</b>
	(d.b.h.) <sup>2</sup>	-0.12684*	<b>0.007</b>	-0.06794*	0.297	-0.20253*	<b>&lt;0.001</b>
	exposure	0.02425	0.842	-0.26711	0.100	0.15159	0.135
PPNUE	d.b.h.	0.59850*	<b>0.008</b>	0.55206*	0.144	0.86715*	<b>0.001</b>
	(d.b.h.) <sup>2</sup>	-0.15816*	<b>0.002</b>	-0.13038*	0.126	-0.25358*	<b>&lt;0.001</b>
	exposure	0.04225	0.745	-0.37298	0.077	0.15618	0.180
$g_s$	d.b.h.	0.60969*	<b>0.019</b>	0.08404	0.773	1.40204*	<b>&lt;0.001</b>
	(d.b.h.) <sup>2</sup>	-0.13090*	<b>0.024</b>	0.00781	0.905	-0.32109*	<b>&lt;0.001</b>
	exposure	-0.06491	0.673	-0.18975	0.244	0.38506	<b>0.008</b>
Leaf N	d.b.h.	0.11581*	0.088	0.27173*	<b>0.010</b>	0.17616*	<b>0.018</b>
	(d.b.h.) <sup>2</sup>	-0.03074*	<b>0.046</b>	-0.06295*	<b>0.009</b>	-0.04979*	<b>0.011</b>
	exposure	0.01454	0.724	-0.10269	0.070	0.00111	0.974
Leaf C:N ratio <sup>-1</sup>	d.b.h.	0.11883*	0.100	0.11883*	0.100	0.13515*	0.063
	(d.b.h.) <sup>2</sup>	-0.03184*	0.052	-0.03184*	0.052	-0.04243*	<b>0.028</b>
	exposure	0.01330	0.762	0.01330	0.762	-0.00829	0.808

\* $P < 0.05$ , indicates cases for which the corresponding term without crown exposure included as a covariate is significant.

site-level variation in soils and other factors. In the present study, use of a mobile aerial work platform in an uneven-aged forest facilitated a sampling regime in which in situ measurements of gas exchange could be made in the upper canopies of trees of varying age that were spatially interspersed at a single site, thus mitigating against such confounding effects.

Prior studies also support the generality of a unimodal pattern of leaf size variation with tree size and/or age. Leaf size appears to peak approximately at the size at onset of maturity in several tropical species (Alvarez-Buylla and Martinez-Ramos 1992, Thomas and Ickes 1995), but in others the peak in leaf size falls very early in ontogeny, presumably prior to reproductive onset (Reich et al. 2004, Panditharathna et al. 2008). One tropical study has also documented an apparent peak in both leaf size and photosynthetic capacity in saplings (Ishida et al. 2005), but only three age classes (seedlings, saplings and adult trees) were examined. Results to

date thus suggest that temperate deciduous trees may show a delayed onset of reproduction relative to the peak in leaf size as compared to tropical evergreen species; however, quantitative analyses strictly comparable to those presented here (i.e., Figure 8) for additional species, both temperate and tropical, are necessary to clarify this point. The finding of a peak in leaf size well before reproductive onset is not consistent with the hypothesis that allocation of C or N to reproductive structures directly drives declines in leaf size late in tree ontogeny (Thomas and Ickes 1995), since the decline commences prior to any reproductive allocation.

In the present study, individuals of all size classes were selected to maximize light exposure, with samples collected in the upper leaves of saplings in gaps and larger trees with dominant or co-dominant canopy positions. However, integrated light levels are markedly attenuated for saplings even in very large gaps relative to light incident at the upper can-

opy (e.g., Sipe and Bazzaz 1994), giving rise to an inevitable correlation of incident light with tree size. Ambient light conditions are known to influence both leaf morphology and photosynthetic traits generally (Boardman 1977, Givnish 1988), and variability in light conditions and/or canopy openness has been found to be a significant predictor of variation in many leaf traits within forest canopies (e.g., Rijkers et al. 2000, Leal and Thomas 2003). Thus, the trends shown here are likely to be influenced by both leaf acclimation to ambient light conditions as well as tree size, particularly across the range of tree size between saplings and trees entering the main forest canopy (~1–20 cm d.b.h.). Nevertheless, the results presented and prior observations indicate that light acclimation responses are of secondary importance. First, analyses that explicitly included canopy exposure class as a covariate revealed strong effects of tree size independent of crown exposure; in most cases, effects of crown exposure were nonsignificant (Table 4). Second, the magnitude of ontogenetic changes in traits were generally larger than those documented in studies of light acclimation responses of the same tree species. For example, in *A. saccharum*, a number of studies (Ellsworth and Reich 1992a, 1992b, Sipe and Bazzaz 1994, Beaudet et al. 2000, Baltzer 2005) have quantified modest differences in  $A_{\text{area}}$  between understory and gap environments (an average 39% increase; range –3–80%) and smaller differences between gaps and large clearings (an average 19% increase; range –6–47%). By comparison, the peak  $A_{\text{area}}$  for intermediate-sized *A. saccharum* trees (15 cm d.b.h., estimated as  $8.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  by the fitted equation) was more than double that of 1 cm d.b.h. saplings ( $3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) measured in gaps (Figure 5; Table 3). Third, if ambient light conditions were determining apparent ontogenetic patterns, one would expect to find exclusively monotonic relationships between tree size and all leaf traits, not the unimodal patterns described here.

While declines in  $A_{\text{area}}$  and other performance-related traits late in tree ontogeny have received much attention, the possible causes (proximate and ultimate) for increases in  $A_{\text{area}}$  from saplings to intermediate-sized trees have not. Some prior reviews have suggested that ontogenetic increases in  $A_{\text{area}}$  or  $g_s$  are restricted to cases in which larger trees are able to access ground water not accessible to smaller trees (Bond 2000, Munné-Bosch 2007). In the present study, soils were shallow (~30–70 cm in depth; Peng and Thomas 2006), and even the smallest saplings measured would generally have roots extending to bedrock. Size-dependent access to water is therefore very unlikely to influence observed patterns. A consistent unimodal pattern of trait variation would seem to necessarily involve true ontogenetic patterns—i.e., age- or size-related changes in genetic expression (Day et al. 2002)—rather than any simple biophysical process. In this regard, it is instructive to consider the broader literature on the physiology of leaf expansion. Through the 1970s, it was common to postulate that changes in leaf growth were driven directly by changes in turgor (e.g., Boyer 1968, Hsiao 1973, Wenkert et al. 1978). However, a large body of re-

search since the 1980s has demonstrated that leaf expansion can change dramatically without any change in turgor (e.g., Van Volkenburgh and Boyer 1985) and that variation in leaf expansion is in general driven mainly by expression of membrane-intrinsic proton pumps, cell wall peroxidases and other highly regulated cellular processes (Fry 1995, Van Volkenburgh 1999). Any general explanation of age-related patterns of leaf size should thus consider ultimate causation—the action of natural selection—on developmental changes in gene expression that are strictly ontogenetic rather than only biophysical processes or phenotypically plastic responses to the environment.

What is a plausible ‘ultimate’ explanation for ontogenetic increases in  $A_{\text{area}}$  and related traits? In shade-tolerant tree species, it is common to find either very limited capacity to acclimate to high (i.e., full-sun) conditions or pronounced declines in  $A_{\text{area}}$  and related traits in high light conditions (e.g., Strauss-Debenedetti and Bazzaz 1991, 1996, Mitamura et al. 2009). In theory, this pattern is expected only if there is an appreciable ‘cost’ inherent in the ability to acclimate. For example, the capacity to synthesize carotenoid pigments involved in photoprotection is energetically expensive (Demmig-Adams and Adams 1992, Long et al. 1994) and may not be favored in a species that will typically be out-competed as a sapling under high light conditions. Another type of cost, specific to deciduous trees, stems from the fact that saplings typically show earlier leaf-out in the spring compared with canopy trees (Augspurger and Bartlett 2003). Production of energetically expensive high-light-adapted leaves during this time would compromise leaf performance following canopy closure. However, competitively successful tall-statured trees will eventually reach the canopy and must therefore produce leaves that can function effectively in and take advantage of full-sun conditions. This combination of factors: predictable low light early in ontogeny, predictable high light late in ontogeny and an appreciable cost to light acclimation should favor the evolution of an ontogenetic pattern involving progressive development of sun-leaf traits. A prediction of this hypothesis is that increases in  $A_{\text{area}}$  and related traits early in tree ontogeny should not occur in extreme early-successional species that regenerate only under very high light conditions. It is intriguing that some conifers considered ‘paradigmatic’ examples in studies of age-related changes in growth and physiology (e.g., *Pseudotsuga menziesii*) fall in this category (Niinemets and Valladares 2006).

The present study, by restricting measurements to trees >1 cm d.b.h., did not address one important aspect of the entire ontogenetic trajectory of trees: namely, the seedling–sapling transition, defined by the loss of functional importance of seed reserves (Fenner 1987, Kitajima 2002). Leaf functional traits of seedlings are likely to differ from those of saplings due to effects of the mobilization and utilization of seed reserves as well as seedling-specific adaptations related to emergence, desiccation tolerance and defense against seedling-specific pathogens and herbivores, among other factors. It thus seems

likely that the size-related patterns of leaf traits documented in the present study do not extrapolate to those of seedlings. In particular, seed N reserves will almost certainly result in a pattern of declining leaf N near the seedling–sapling transition (e.g., Kitajima 2002), with concomitant effects on other physiological functions.

The present study also indicates that traits examined showed consistent patterns of decline or increase late in tree ontogeny, when height growth has largely ceased. Height–diameter relationships for the three species at this site indicate that 79–89% of asymptotic maximum height has been obtained at 30 cm d.b.h. (Table 2). However, fitted equations predict proportionately greater  $A_{\text{mass}}$  decreases for a tree growing from 30 to 70 cm of 33% in *A. saccharum* (from 112 to 75  $\text{nmol g}^{-1} \text{s}^{-1}$ ), 37% in *B. alleghaniensis* (from 160 to 101  $\text{nmol g}^{-1} \text{s}^{-1}$ ) and 41% in *T. americana* (from 207 to 123  $\text{nmol g}^{-1} \text{s}^{-1}$ ). These results suggest that the direct effects of either hydraulic path length or of the gravitational component of leaf water potential on leaf turgor are insufficient to explain the observed changes in net photosynthesis late in ontogeny. Regression analyses also showed that size-related changes in  $A_{\text{area}}$ ,  $A_{\text{mass}}$  and  $g_s$  were not driven solely by changes in leaf N,  $\text{LMA}_{\text{lamina}}$  or leaf thickness.

In the case of *A. saccharum*, large effects on leaf gas exchange of the ubiquitous gall-forming mite *Vasates aceriscrumena* on mature canopy trees have recently been documented: galling increases with tree size (Thomas et al. 2010), and the presence of even a few minute galls drastically reduces  $A_{\text{area}}$  (Rajit Patankar, personal communication). Although heavily galled leaves were specifically avoided during sampling in the present study, even low levels of galling may have contributed to observed patterns. Speculatively, the strong effects of galling on canopy photosynthesis may provide an evolutionary explanation for changes in leaf morphology and chemistry late in tree ontogeny. Specifically, increases in  $\text{LMA}_{\text{lamina}}$ , leaf C and leaf tissue density are consistent with an increase in leaf lignification which could possibly offer some protection against effects of galling arthropods while contributing to progressive declines in  $A_{\text{area}}$ .

The existence of a hump-shaped relationship between  $A_{\text{area}}$  and tree size helps reconcile some apparently contradictory results in the literature. A pattern of decreasing  $A_{\text{area}}$  with tree size has widely been reported in studies of conifers, while studies of temperate deciduous trees have commonly noted a higher  $A_{\text{area}}$  in adult trees relative to saplings (Thomas and Winner 2002). In light of the unimodal patterns presented here, it seems likely that prior studies reporting ontogenetic increases in  $A_{\text{area}}$  and related traits in angiosperms (Jurik et al. 1988, Stickan and Zhang 1992, Dawson and Ehleringer 1993, Hanson et al. 1994, Dawson 1996, Samuelson and Kelly 1997, Cavander-Bares and Bazzaz 2000) have effectively compared saplings to relatively young mature trees near the ontogenetic peak but have failed to detect or characterize the long phase of decline following reproductive onset.

Hump-shaped relationships are also of considerable interest from the perspective of understanding ecophysiological

correlates of tree growth patterns (Weiner and Thomas 2001). Tree growth curves generally show a sigmoidal shape that is asymmetric, with an inflection point falling early in tree ontogeny and a much more gradual reduction in growth among older trees (Assmann 1970, Vanclay 1994, Bond et al. 2007). The back-transformed log–log polynomial regression equations used here to describe unimodal relationships with  $A_{\text{area}}$  and related parameters show a similar asymmetric pattern to the differential form of tree growth curves. Understanding the linkages between these patterns throughout tree ontogeny seems essential to developing a comprehensive theory of age-related change in growth and physiology of trees. Such a theory seems certain to require an understanding of both true ontogenetic effects involving progressive changes in gene expression before and after the onset of reproduction, in addition to the direct biophysical consequences of increased size and acclimation responses to environmental gradients.

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