

# Physiological and morphological correlates of whole-plant light compensation point in temperate deciduous tree seedlings

J. L. Baltzer · S. C. Thomas

Received: 22 February 2006 / Accepted: 7 March 2007 / Published online: 24 April 2007  
© Springer-Verlag 2007

**Abstract** A range of traits, including metabolic costs, biomass allocation and seed reserves, may contribute to interspecific variation in the shade tolerance of tree seedlings. In addition, shade tolerance may be affected by differential responses of species to soil resource availability at low light. We used a custom-built whole-plant gas-exchange chamber to quantify instantaneous whole-plant light compensation point (WPLCP) and to parameterize whole-plant daily C gain models for seedlings of eight temperate deciduous tree species. We examined the relationship of WPLCP to growth, biomass allocation and gas-exchange under high and low light and nutrient availabilities and compared it to WPCLP of naturally recruited saplings. For species showing a response, both increased light and nutrient availability resulted in increased WPLCP. However, species' responses to resource availability did not correspond closely with shade tolerance as has generally been predicted. Variation in WPLCP within species was best predicted by whole-plant dark respiration rates, leaf-level light compensation point and leaf mass per area. Among species, seed size was a strong negative correlate of WPLCP, explaining 66% of the variation. Species with the lowest WPLCP maintained lower growth rates across treatments but greater biomass in the low-light treatment compared with more light-demanding species.

These data suggest that a number of traits, in particular metabolic costs and seed size, contribute to WPLCP. However, gas-exchange-based WPLCP was 1.5–3.5 times lower than corresponding growth-based field estimates of WPLCP, suggesting that other factors such as biotic interactions or ontogenetic shifts in whole-plant light requirements may substantially increase species' WPLCP under natural conditions.

**Keywords** Shade tolerance · Seed size · Photosynthesis · Multiple resource interaction · Growth response

## Introduction

Tree seedlings and saplings experience substantial spatial and temporal variation in both above- and belowground resource availability in the forest understory (Canham et al. 1990; Groffman et al. 1993; Bazzaz and Wayne 1994; Veenendaal et al. 1995). Although regeneration potential in the understory is typically regarded as the ability to survive and grow under low light availability (i.e., shade tolerance), variation in soil resources may also affect juvenile survivorship, growth rates and light requirements. A number of studies have examined multiple resource limitation demonstrating that both soil nutrient and water availability may affect physiological parameters (Abrams and Mostoller 1995; Baltzer and Thomas 2005), growth (Finzi and Canham 2000; Sack 2004; Makana and Thomas 2005) and survivorship (Caspersen and Kobe 2001; Catovsky and Bazzaz 2002) across light availabilities. Comparative studies have also demonstrated differential responses across species to covariation in light and belowground resource availability (e.g., Latham 1992; Coomes and Grubb 1998; Baltzer and Thomas 2005). Such

---

Communicated by Robert Pearcy.

---

J. L. Baltzer · S. C. Thomas  
Faculty of Forestry, University of Toronto,  
33 Willcocks Street, M5S 3B3 Toronto, ON, Canada

J. L. Baltzer (✉)  
Biology Department, Mount Allison University,  
63B York Street, E4L 1G7 Sackville, NB, Canada  
e-mail: jlbaltzer@mta.ca

differences in species' sensitivities to variation in soil resources or covariation in light and soil resources could impact understory growth and survivorship and contribute to inter- and intra-specific differences in shade tolerance.

The contribution of multiple resource interactions to whole-plant gas-exchange has received little attention despite its importance as a mechanistic basis for patterns of growth and survivorship in response to variation in resource availabilities. Lehto and Grace (1994) documented patterns of whole-plant gas-exchange in *Trema guineensis* and *Milicia excelsa*, representative pioneer and climax species from the rain forests of West Africa, demonstrating dramatic differences in growth and maintenance respiration rates between the two species, with *Trema* having greater metabolic costs. However, with nutrient addition, maintenance respiration in shade-tolerant *Milicia* increased to a similar level as *Trema*. Although limited to two species, this result suggests potentially important interspecific differences in whole-plant gas-exchange responses to resource availability. Further investigation with greater species numbers is required to rigorously examine broader patterns of whole-plant responses to resource availability.

Determining the traits of relevance to shade tolerance has long been of interest to plant ecophysiologicalists. Leaf-level traits are frequently assumed to scale up to the more ecologically relevant whole-plant level; however, there is evidence that shade-tolerance patterns cannot necessarily be predicted by leaf-level gas-exchange alone (Kitajima 1994; Kupperts 1994; Sipe and Bazzaz 1994). Although photosynthesis is of primary importance in plants, other factors contribute to the ecological success of a species, since the net C balance per unit biomass at the whole-plant level determines success in the forest understory (Givnish 1988). For example, Phillips and Fahey (2005) demonstrated substantial differences between *Acer saccharum* and *Betula alleghaniensis* in C costs associated with root exudate production (6.9–7.1 and 11.2–13.0%, respectively of total assimilated C). Other traits important to whole-plant C balance may include biomass allocation, leaf longevity, nighttime respiration, biotic factors and physical damage (Givnish 1988; Lusk and Reich 2000; Aiba and Nakashizuka 2005). Another trait of relevance to shade tolerance is seed size, particularly during the first growing season when large interspecific differences may exist in reliance on seed reserves and seedling size and growth rates are strongly correlated with seed size (Walters and Reich 2000). A range of shade tolerance correlates including wood density, minimum naturally occurring light environment and crown illumination/exposure indices, have been used to assess the contribution of physiological and morphological characteristics to shade tolerance (Kitajima 1994; Davies 1998; Lusk and Reich 2000; Aiba and Nakashizuka 2005; Poorter et al. 2005). There is a lack of

agreement among these studies with regards to traits that confer shade tolerance; however, this may be a function of the strength of the relationship of different shade tolerance indices or "proxy measures" to whole-plant C balance.

In this study we compared whole-plant and leaf-level gas-exchange, and growth response of seedlings of eight temperate deciduous tree species ranging in shade tolerance and grown under high and low light and soil nutrient conditions. Seedlings may be expected to be more sensitive to multiple resource gradients than are adult trees given the small area that a single seedling will occupy, and consequently the high heterogeneity of potential resource availabilities experienced by individuals. Understanding changes in species' light requirements in response to resource gradients is therefore crucial to our understanding of forest regeneration dynamics and resulting tree species composition. Specific study goals were: (1) to quantify species' light requirements using both whole-plant gas-exchange measurements and light-growth relationships; (2) to determine how variation in light and soil nutrient availability impact the whole-plant light compensation point (WPLCP); (3) to assess how quantitative measures of light requirements relate to current shade tolerance classifications; and (4) to determine morphological and physiological correlates of WPLCP.

## Materials and methods

### Three definitions of the WPLCP

The broad concept of a WPLCP is straightforward: i.e., WPLCP is defined as the light level at which plants achieve zero C gain. However, WPLCP depends strongly on the temporal scale over which measurements are made, and different measurement techniques are appropriate to different time scales. We define the instantaneous WPLCP (WPLCP<sub>inst</sub>) as the light level at which a plant shows zero net C gain for daytime measurements made over short time scales (seconds to minutes). Here we measure WPLCP<sub>inst</sub> directly as the *x*-intercept of the relationship between net assimilation and incident light using a whole-plant gas-exchange apparatus. We define the diurnal WPLCP (WPLCP<sub>diurnal</sub>) as the average total integrated light level over a 24-h period corresponding to zero net C gain over this same period. Here we estimate WPLCP<sub>diurnal</sub> using a simple model that subtracts estimated nighttime respiration from integrated daytime net C gain under variable light conditions. Due to a variety of processes (e.g., nighttime respiration, mid- and late-day reductions in net assimilation and light saturation) WPLCP<sub>diurnal</sub> is expected to be higher than WPLCP<sub>inst</sub>. Over longer time scales a growth-based estimate of WPLCP (WPLCP<sub>growth</sub>) will generally be the

only feasible method. Here we follow prior studies (Moad 1992; Makana and Thomas 2005) in estimating  $WPLCP_{\text{growth}}$  as the  $x$ -intercept of relationships between incident light integrated over an entire growing season, and aboveground growth.  $WPLCP_{\text{growth}}$  is expected to exceed  $WPLCP_{\text{diurnal}}$  for a variety of reasons, including effects of biotic agents such as herbivores and pathogens, leaf construction costs, and ontogenetic increases in heterotrophic tissue (Givnish 1988; Lusk 2004). In this paper  $WPLCP$  without a modifying subscript denotes the general concept of the light level corresponding to zero C gain; however, in relation to ecological processes, longer time scales (i.e., time scales corresponding to  $WPLCP_{\text{growth}}$ ) are likely to be most relevant.

#### Experimental design and plant growth conditions

Eight temperate deciduous tree species common to forests in the Great Lakes–St. Lawrence region of eastern Canada and ranging in observation-based shade tolerance, were selected (Table 1). Seeds were of local provenance obtained from the National Tree Seed Centre (Canadian Forest Service Atlantic Forestry Centre, Fredericton, NB). Seeds were stratified in moist sand and germination was initiated in early April 2003. Germination light levels correspond to those of the high-light treatment described below. Immediately following germination and prior to the formation of the first true leaves, seedlings were transferred into 17.8-cm-diameter pots (25 cm height) filled with a mixture of approximately two-thirds sand and one-third perlite; at this point the light and nutrient treatments described below were implemented. This porous, sterile potting mixture was used to minimize or eliminate the soil respiration signal during whole-plant gas-exchange measurements while simultaneously allowing for inclusion

of root respiration in the measurements. Blank pots containing only the soil mixture and either high or low nutrient amendments were included in high and low-light treatments for later measurement of soil respiration. The experiment was conducted under controlled glasshouse conditions at the Faculty of Forestry, University of Toronto. Daytime temperatures were maintained between 25 and 30°C. Seedlings and soil blanks were hand-watered daily to maintain soil moisture near field capacity.

The experiment examined the influence of variation in light and nutrient availability on foliar and whole-plant gas-exchange, growth and biomass allocation using a  $2 \times 2$  factorial design consisting of high and low light and nutrient conditions. Photon flux density (PFD), relative humidity (RH) and air temperature were measured using Hobo Weather Stations (Onset Computer, Bourne, Mass.). Quantum sensors were positioned at crown height and PFD, temperature and RH measured every 15 min from 30 May to 3 June 2003 for each block. Approximate light levels for high and low light treatments were 1,000/150 and 200/20  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (sunny/cloudy days). Shade was provided by neutral density shade cloth covering a wooden frame. Light quality often has little or no effect on sapling growth and physiology compared to light quantity (Turnbull 1991; Kitajima 1994; but see Lee et al. 1996); therefore simulation of understory light quality was not attempted. Each block contained one replicate per species and nutrient treatment. A 6-month slow-release NPK fertilizer (16-10-10, Nutricote; Plant Products, Brampton, ON) enhanced N availability by 25 and 175  $\text{kg ha}^{-1} \text{year}^{-1}$  in low and high nutrient treatments, respectively. These levels are within the range of N mineralization rates in northern temperate forests (Groffman et al. 2001). Seedlings were randomly allocated to treatment and bench position and rotated monthly to avoid confounding effects of temperature or light gradients.

#### Growth and biomass allocation measurements

Biomass harvests were conducted on 5–6 June, 21–23 July and 3–5 September 2003. *Betula papyrifera* and *Populus grandidentata* showed low germination rates and therefore seedlings were not available for the first harvest. Additionally, both species had 100% mortality in low light and thus data are missing for low-light measurements. For the first two harvests, five seedlings per species and treatment were selected at random. The final harvest included all surviving seedlings. Seedlings were divided into roots, stems and leaves. At the time of first harvest, *Quercus rubra* cotyledons were no longer functional (i.e., were either decaying or no longer attached) and were not included in the biomass measurements. Fresh leaves were measured for area, plant parts were oven dried for 5 days at 60°C and

**Table 1** Study species and their shade tolerance classifications (adapted from Burns and Honkala 1990)

Family	Species	Common name	Shade tolerance
Aceraceae	<i>Acer rubrum</i> L.	Red maple	Mid-tolerant
	<i>Acer saccharum</i> Marsh.	Sugar maple	Tolerant
Betulaceae	<i>Betula alleghaniensis</i> Britt.	Yellow birch	Mid-tolerant
	<i>Betula papyrifera</i> Marsh.	Paper birch	Intolerant
Fagaceae	<i>Quercus rubra</i> L.	Red oak	Mid-tolerant
Oleaceae	<i>Fraxinus americana</i> L.	White ash	Mid-tolerant
Salicaceae	<i>Populus grandidentata</i> Michx.	Bigtooth aspen	Intolerant
Ulmaceae	<i>Ulmus americana</i> L.	American elm	Mid-tolerant

the biomass of each plant part determined. In the final harvest, fresh leaf area of leaves measured for gas-exchange was determined for calculation of leaf mass per area (LMA).

#### Leaf-level gas-exchange

Foliar gas-exchange was measured on recent, fully expanded leaves of plants also measured for whole-plant gas-exchange within 1 day of the whole-plant measurements. All foliar and whole-plant gas-exchange measurements were made in August 2003 over a 2-week period. Measured individuals were selected at random across species the evening prior to measurement. Dark respiration rates ( $R_d$ ), leaf-level light compensation point (LLCP), photosynthetic capacity ( $A_{\max}$ ) and stomatal conductance ( $g_s$ ) were measured using a LI-6400 gas-exchange system (Licor, Lincoln, Neb.). All measurements were made before noon. Sample chamber conditions were maintained at 350 p.p.m.  $\text{CO}_2$  and 50–70% RH. Leaf temperature was maintained between 25 and 30°C. Gas-exchange was measured at eight light levels: 0, 5, 10, 15, 20, 25, 30 and 1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Gas-exchange was not measured until the leaf was fully induced at that irradiance, which was determined visually using graphics available in the LI-6400 programming. At each level five measurements were made, separated by 15-s intervals.  $R_d$  and  $A_{\max}$  were computed as the average of the five measurements made at 0 or 1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. LLCP and  $\phi$  were estimated using linear regression for measurements between 0 and 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (linear in all cases). Measurements of  $g_s$  correspond with those of  $A_{\max}$  and thus are the average of five measurements at 1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Three individuals per species and treatment were measured.

#### Whole-plant gas-exchange

Whole-plant gas-exchange was measured using a custom chamber built to interface with the LI-6400. The chamber was made of 23-cm-diameter Plexiglas tubing coated with Teflon tape to avoid adsorption of gases by the Plexiglas surface. Chamber mixing was achieved using a plenum tubing system (Bev-A-Line IV 1/4" ID). Flow rate within the chamber was great enough that leaf movement was obvious during all measurements thus minimizing boundary layer effects. During measurements, two Plexiglas rings slightly smaller in diameter than the chamber were positioned above and below a ridge in the plant pot lip and separated by a neoprene foam gasket. The rings fitted tightly in the chamber and pressed the foam directly against both the pot exterior and the chamber surfaces forming a seal. A quantum sensor on an adjustable platform was positioned at crown height within the chamber to measure

incident radiation. The quantum sensor was connected to a CR10X datalogger (Campbell Scientific, Edmonton, AB). A temperature probe (model 8100-202, Licor) was positioned adjacent to the seedling to monitor chamber temperature. Steady chamber temperature was important, as pressure changes associated with temperature fluctuations can influence  $\text{CO}_2$  diffusion out of the soil. Cables for both sensors were fed through small holes in the side of the pot and sealed during measurement. The chamber was placed on a shelf covered in shade cloth. When enclosed in the shade cloth, chamber light levels were 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  allowing for whole-plant dark respiration measurements. Illumination was provided with a sodium halide lamp positioned above the chamber. Light levels were varied using a screen containing multiple layers of neutral density shade cloth from which layers could be removed. Heat was dissipated using a water bath beneath the lamp. Motorized fans increased air circulation outside of the chamber further mitigating temperature increases. Chamber conditions were maintained at 350 p.p.m.  $\text{CO}_2$ . To ensure the potting medium was not contributing to measured  $\text{CO}_2$  concentrations, soil respiration was measured using a Licor soil chamber (model 6400-09). Soil respiration was not detected in the soil blanks and therefore no correction was used in whole-plant gas-exchange measurements. Root respiration was equated with rhizosphere respiration as roots, root exudates and microbial associates comprising the rhizosphere are closely associated and rarely considered separately (Andrews et al. 1999).

The evening prior to measurement, seedlings were placed on a shelving unit covered in shade cloth until measurement the following morning. All measurements were made before noon. Whole-plant gas-exchange was measured at 0, 5, 10, 15, 20, 30 and 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to characterize instantaneous  $\text{WPLCP}_{\text{inst}}$ . The chamber design allowed for adjustment of volume according to plant height to maximize the signal to noise ratio.

Diurnal changes in respiration rates have been documented in some species with decreased rates at night (e.g., Penning de Vries et al. 1979). To quantify potential diurnal differences, whole-plant dark respiration was measured in late afternoon after the plant had experienced its respective light treatment for the majority of the day. These plants were then stored in dark conditions and respiration rates measured again between 0600 and 0700 hours the following morning when dark respiration rates should be at their minimum.

#### Calculation of the daily C balance and diurnal $\text{WPLCP}$

Whole-plant C balance was determined by subtracting integrated nighttime respiration rates from daily C gain. C gain ( $C_C$ ) during daylight hours was calculated as the sum

of C gains across all 15-min intervals ( $C_{\text{INT}}$ ). Division by 1,000 converts units to millimoles:

$$C_C = \left( \sum C_{\text{INT}} \right) / 1000$$

$C_{\text{INT}}$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} 15 \text{ min}^{-1}$ ) is calculated as the area-based photosynthetic rate ( $A_{\text{INT}}$  in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of total crown leaf area ( $L_{\text{AC}}$ ) over the 900-s interval:

$$C_{\text{INT}} = A_{\text{INT}} \times L_{\text{AC}} \times 900$$

Instantaneous area-based photosynthesis for time interval INT ( $A_{\text{INT}}$ ) is estimated as:

$$A_{\text{INT}} = \frac{\phi I_{\text{INT}} A_{\text{max}}}{(A_{\text{max}}^2 + [\phi^2 I_{\text{INT}}^2])^{1/2}}$$

where  $\phi$  and  $A_{\text{max}}$  are the mean apparent quantum efficiency and light-saturated photosynthesis, respectively, for the species–treatment combination and  $I_{\text{INT}}$  is the incident radiation at time interval INT for the light treatment in question. Nighttime and afternoon respiration rates did not differ significantly, therefore whole-plant nighttime rates were used as the baseline metabolic costs and converted into a 24-h estimate to approximate daily whole-plant C costs. Whole-plant light response was simulated using the range of light availabilities experienced within the treatment due to variable cloudiness. Whole-plant C balance for days ranging from overcast to sunny were calculated as described above. Daily C gain values were then plotted against total PFD and a whole-plant light-response curve was fitted using a non-rectangular hyperbolic function.

The assumptions underlying the model of whole-plant C gain are as follows: (1) steady state gas-exchange parameters are representative for the course of the day; (2) all leaves within a plant are similar physiologically; (3) induction response is instantaneous and post-illumination C fixation negligible, and (4) illumination through the plant crown is even (i.e., no self-shading occurs within the plant crown). These assumptions are in large part valid for this experiment. First, diurnal differences in measured gas-exchange parameters were negligible (8:00 a.m.–12:00 p.m.). Some studies have reported a midday depression in photosynthetic capacity (e.g., Zotz and Winter 1994), while others have found either no depression or a steady diurnal decrease (e.g., Bassow and Bazzaz 1998). Second, both foliar and whole-plant respiration were measured late in the afternoon and at night with no consistent differences. The assumption that seedling crown leaves are evenly illuminated is reasonable as seedlings were fairly small with limited branching, crown structure and leaf number. Therefore the leaves were likely comparable physiologically as leaves in the seedling crowns

were in similar light environments; likewise, intra-crown shading was negligible.

#### Growth-based field WPLCP estimation

Naturally occurring saplings of *A. rubrum*, *A. saccharum*, *B. papyrifera*, *F. americana*, *P. grandidentata* and *Q. rubra* were selected across a range of light environments at the Koffler Scientific Reserve (44°03'N, 79°29'W), a 348-ha forest reserve in the Oak Ridges Moraine region near Newmarket, Ontario. Seemingly healthy saplings with unbroken stems and initial height of 0.5–1.5 m were selected. Forty individuals of each species were selected and each individual uniquely tagged and measured for height (along the stem from base to apical bud) and basal diameter. Measurements were made from 2001 to 2003 in early May. Diameter<sup>2</sup> × height was used as a non-destructive proxy for whole-plant biomass as the two have been shown to be highly correlated (e.g., Kohyama and Hotta 1990) and above ground relative growth rate (RGR) calculated as:

$$\text{RGR}_{d^2h} = \frac{\ln(d_2^2 h_2) - \ln(d_1^2 h_1)}{(t_2 - t_1)}$$

where  $d_1^2 h_1$  and  $d_2^2 h_2$  are the product of basal diameter squared and height at beginning ( $t_1$ ) and end ( $t_2$ ) of the measurement period, respectively. The use of both diameter and height parameters in calculating growth provides a more robust growth rate measure (in the absence of experiment-specific allometric equations). Since  $d^2 h$  is linearly related to biomass (Kohyama 1991),  $\text{RGR}_{d^2h}$  will closely approximate  $\text{RGR}_{\text{biomass}}$ . Sapling light environment was characterized using hemispherical photographs taken directly above the crown of each sapling using a Nikon Coolpix 900 digital camera with a Nikon FC-E8 fisheye converter. Photograph analysis was conducted using Winscanopy 2001 (Regent Instruments, QC). Above-canopy direct radiation was estimated as a function of site latitude and longitude and growing season (defined as 1 May–1 September). Diffuse radiation was assumed to be 15% of total. Photographs were taken on overcast days or in the early morning following 2001 growth measurements. The light levels of the glasshouse portion of the study correspond with high and low-light individuals of the field component (data not shown). To examine the RGR–light environment relationship, model I regression (species incurring no mortality during the study; PROC REG, SAS) or censored regression (Schmid et al. 1994) (species incurring mortality during the study; PROC LIFEREG, SAS) was conducted with RGR as the dependent variable and daily PFD the predictor. The light level corresponding with zero growth was assumed to characterize WPLCP ( $\text{WPLCP}_{\text{growth}}$ ) (Moad 1992; Makana and Thomas 2005).

Growth-based WPLCP estimates are described in detail elsewhere and correspond closely with light requirements for survival (Baltzer 2005). Estimation of WPLCP<sub>growth</sub> in the glasshouse-grown seedlings was not attempted, therefore WPLCP<sub>growth</sub> from the field-based study and the two gas-exchange-based estimates of WPLCP (WPLCP<sub>inst</sub> and WPLCP<sub>diurnal</sub>) for glasshouse seedlings are compared.

### Statistical analysis

ANOVA (PROC GLM, SAS version 8.1; SAS Institute, Cary, N.C.) was used to test for differences in absolute growth between harvests and treatments across species. Seedling biomass was the dependent variable, and light, nutrients, harvest date and species the predictors. ANOVA was also conducted to examine RGR responses to treatment and species for the entire experiment. Seedling RGR was the dependent variable, and light, nutrients, species and interactions the predictors. Both analyses were limited to the six species with individuals in all treatments (namely *A. rubrum*, *A. saccharum*, *B. alleghaniensis*, *F. americana*, *U. americana* and *Q. rubra*).

Differences in individual physiological parameters ( $A_{\max}$ ,  $R_d$ , LLCP, WPLCP<sub>diurnal</sub>) as well as LMA were tested using ANOVA (PROC GLM, SAS) with  $A_{\max}$ ,  $R_d$ , LLCP, WPLCP<sub>diurnal</sub> or LMA as dependent variables and species, light level, nutrient treatment and interactions as class variables. Only four of the eight study species had sufficient survival and/or size to measure gas-exchange and WPLCP in the low-light treatment (*A. saccharum*, *F. americana*, *Q. rubra* and *U. americana*; see Results). Consequently, two sets of results for each parameter are presented: fertilization and species effects for all eight study species in high light only, followed by light, fertilization and species effects for the four species with replication in all treatments.

Relationships among seed mass, RGR and average high-light WPLCP<sub>diurnal</sub> were examined using type II regression (Falster et al. 2003). Low-light RGR and WPLCP<sub>diurnal</sub> values for a number of species were not available due to poor germination success and plant size constraints on measurements. High-light values were therefore used exclusively to allow inclusion of all study species. Likewise, the relationships of high-light WPLCP<sub>diurnal</sub>, WPLCP<sub>inst</sub> and whole-plant respiration rates ( $R_{WP}$ ) to WPLCP<sub>inst</sub> were characterized using type II regression. To characterize the contribution of physiological and morphological traits to WPLCP, multiple regression analysis was conducted. As  $A_{\max}$  and whole-plant respiration rates were used to calculate the more ecologically relevant WPLCP<sub>diurnal</sub>, we used WPLCP<sub>inst</sub> in this analysis, as its values are measured independently of all physiological and morphological predictors. It should be noted that there is a strong positive

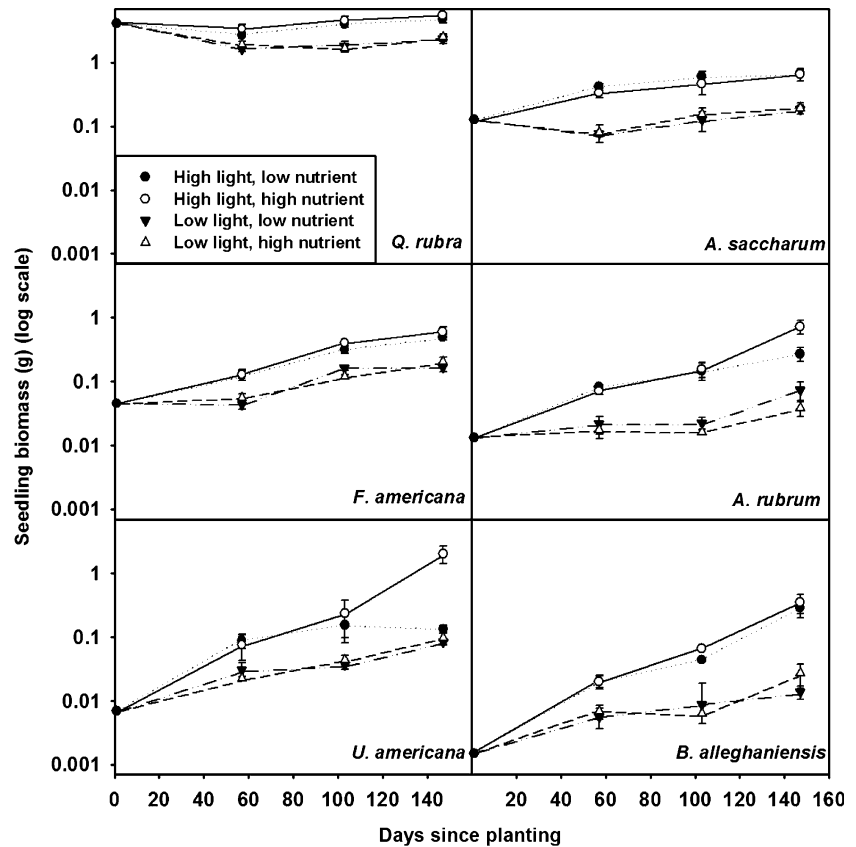
relationship between the two independent measures of WPLCP ( $R^2 = 0.92$ ,  $P = 0.002$ ). Although seed mass was a strong predictor of WPLCP, individual seed masses were not measured. Therefore, to separate interspecific effects from the contribution of physiological and morphological traits to variation in individual WPLCP<sub>inst</sub>, the relationship between WPLCP<sub>inst</sub> and species was determined using a generalized linear model and the residuals of this relationship included as the dependent variable in the multiple regression analysis. To examine biomass partitioning as a predictor of WPLCP<sub>inst</sub>, root–shoot ratio ( $R:S$ , g root tissue g<sup>-1</sup> shoot tissue), leaf area ratio (LAR, leaf area in cm<sup>2</sup> g<sup>-1</sup> plant biomass) and LMA (cm<sup>2</sup> of leaf area g<sup>-1</sup> leaf mass) were included. Physiological predictors included whole-plant respiration rate ( $R_{WP}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), foliar photosynthetic and respiration rates ( $A_{\max}$  and  $R_d$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and LLCP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Model selection was based on the minimum Akaike information criterion (AIC). Lower AIC values indicate a more parsimonious model (Burnham and Anderson 1998) therefore  $\Delta\text{AIC}$  values were calculated for model selection. We also performed a multiple regression on the three significant components of a principal components analysis (PCA) using all predictor variables listed above. Two components (PCA 2 and 3) contributed significantly to the residual variation from the WPLCP<sub>inst</sub>–species relationship. Component 3 contributed most significantly to the model and represented whole-plant respiration rates alone. As the analyses led to similar conclusions, we will only present the results of the multiple regression using the original predictors as this is more meaningful in selecting specific traits contributing to WPLCP.

## Results

### Seedling growth rates

*B. papyrifera* and *P. grandidentata* showed low establishment rates, requiring replanting through the first harvest; growth and biomass data are therefore not presented for these species. The remaining species differed substantially in seedling biomass with *Q. rubra* having the largest seedlings and *B. alleghaniensis* the smallest ( $F_{71,460} = 179.01$ ,  $P < 0.0001$ ; Fig. 1). Similarly, species RGRs differed with *A. saccharum* and *Q. rubra* having the lowest RGR and *B. alleghaniensis* the highest (Table 2). Both seedling biomass ( $F_{71,460} = 73.12$ ,  $P < 0.0001$ ; Fig. 1) and RGR (Table 2) were greater in the high-light treatment although the magnitude of this response was species-specific (Table 2; Fig. 1). *A. rubrum* and *B. alleghaniensis* showed the strongest RGR responses to increased light while all other species showed little or no growth rate

**Fig. 1** Species' growth curves (mean  $\pm$  SE) for temperate tree species grown under glasshouse conditions in high- and low-light and nutrient treatments. The initial mass corresponds to mean seed mass. Planting occurred on 10 April 2003 and subsequent harvest dates were 5–6 June, 21–23 July and 3–5 September 2003. Panels are ordered on the basis of seed mass



difference between high and low light. *U. americana* showed a strong growth response to nutrient availability in high light. Specifically, growth rate in the final harvest in the high light–low nutrient combination were comparable to those of low-light-grown plants whereas high light–high nutrient-grown plants had substantially higher growth rates than any other treatment combination (Fig. 1). Across species, increased nutrient availability resulted in a marginally higher biomass; however, when compared with light or species terms, the nutrient term had little effect ( $F_{71,460} = 3.18$ ,  $P = 0.0751$ ; Fig. 1). RGR was, however, strongly influenced by both nutrient and light treatments; RGR increased with increased availability of both resources (Table 2). There were marginally significant species  $\times$  nutrient and light  $\times$  nutrient interactions (Table 2). *A. rubrum*, *B. alleghaniensis* and *U. americana* showed positive RGR responses to increased nutrients while the remaining species responded minimally (Fig. 1). Likewise, nutrient effects tended to be much more pronounced in high light (Fig. 1).

#### Leaf traits

For the eight study species measured in high light,  $A_{\max}$  differed significantly ( $F_{15,28} = 11.08$ ,  $P < 0.0001$ ) ranging from  $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  (*A. saccharum*) to  $11.1 \mu\text{mol m}^{-2} \text{s}^{-1}$

(*P. grandidentata*) (Table 3). Fertilization increased  $A_{\max}$  ( $F_{15,28} = 6.30$ ,  $P = 0.0182$ ) although this response was species-specific ( $F_{15,28} = 2.43$ ,  $P = 0.0446$ ). In high light, *A. rubrum* was most responsive to increases in soil nutrients showing a nearly fourfold increase in  $A_{\max}$  in high-compared with low-nutrient treatments. Similarly, *B. papyrifera*, *A. saccharum* and *F. americana* approximately doubled  $A_{\max}$  with increased nutrient availability. *B. alleghaniensis*, *P. grandidentata*, *Q. rubra* and *U. americana* were not responsive to the nutrient treatment in high light (Table 3). The four species with individuals in all treatments also differed significantly in  $A_{\max}$  ( $F_{15,32} = 24.20$ ,  $P < 0.0001$ ).  $A_{\max}$  was greater in high than low light for these species ( $F_{15,32} = 7.29$ ,  $P = 0.0110$ ), while there was no main effect of fertilization ( $F_{15,32} = 0.86$ ,  $P = 0.3611$ ). Individual species responses to light treatment differed ( $F_{15,32} = 9.81$ ,  $P < 0.0001$ ); *U. americana* reduced  $A_{\max}$  by 50% when grown in low light, *F. americana* and *A. saccharum* both down-regulated  $A_{\max}$  in low light, but less so than *U. americana*, while *Q. rubra* was unresponsive to light availability (Table 3). There was a significant species  $\times$  nutrient treatment effect ( $F_{15,32} = 3.01$ ,  $P = 0.0447$ ); all species with the exception of *U. americana* increased  $A_{\max}$  with fertilization (Table 3).

Species differed significantly in their foliar respiration rates ( $R_d$ ;  $F_{15,28} = 9.45$ ,  $P < 0.0001$ ) ranging from

**Table 2** Three-way ANOVA results for the effects of species (*Spp.*)<sup>a</sup>, light treatment (*Light*), nutrient treatment (*Fert.*) and interactions on glasshouse-grown seedling relative growth rate ( $\text{g g}^{-1} \text{month}^{-1}$ ) from 4 June to 15 September 2003. *MS* Mean squares

Source	<i>df</i> <sup>b</sup>	<i>MS</i>	<i>F</i>	<i>P</i>
<i>Spp.</i>	5	0.92	9.77	<b>&lt;0.0001</b>
<i>Light</i>	1	0.72	7.63	<b>0.0062</b>
<i>Fert.</i>	1	1.26	13.45	<b>0.0003</b>
<i>Spp.</i> × <i>Light</i>	5	0.31	3.26	<b>0.0072</b>
<i>Spp.</i> × <i>Fert.</i>	5	0.19	2.06	0.0709
<i>Light</i> × <i>Fert.</i>	1	0.26	2.82	0.0940
<i>Light</i> × <i>Fert.</i> × <i>Spp.</i>	5	0.06	0.68	0.6406

*P* < 0.05 in bold

<sup>a</sup> Species included *A. rubrum*, *A. saccharum*, *B. alleghaniensis*, *F. americana*, *Q. rubra* and *U. americana*

<sup>b</sup> Model and error *df* were 23 and 263, respectively

1.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in *P. grandidentata* to 0.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in *F. americana*. *U. americana* had surprisingly low  $R_d$  given that it maintained the second highest  $A_{\text{max}}$  across species. Fertilization had no detectable effect on  $R_d$  for the eight study species in high light (main effect,  $F_{15,28} = 0.63$ ,  $P = 0.4331$ ; interaction term,  $F_{15,28} = 1.82$ ,  $P = 0.1230$ ). In the four species measured in both light treatments,  $R_d$

differed significantly with *Q. rubra* having the greatest and *F. americana* the lowest ( $F_{15,32} = 4.99$ ,  $P = 0.0060$ ; Table 3).  $R_d$  increased in high light ( $F_{15,32} = 39.67$ ,  $P < 0.0001$ ), whereas nutrient availability had no detectable effect ( $F_{15,32} = 1.71$ ,  $P = 0.1998$ ; Table 3). Species' responses to soil nutrients differed ( $F_{15,32} = 3.68$ ,  $P = 0.0221$ ), with *U. americana* showing the greatest increase in  $R_d$  under high nutrients and *A. saccharum* showing a slight reduction (Table 3).

Leaf-level light compensation point did not differ significantly among species ( $F_{15,28} = 0.99$ ,  $P = 0.4612$ ) and there was no detectable nutrient treatment ( $F_{15,28} = 0.79$ ,  $P = 0.3832$ ) or interaction effect ( $F_{15,28} = 0.60$ ,  $P = 0.7541$ ) across the eight study species in high light (Table 3). For the four species with individuals surviving in all treatments, neither species ( $F_{15,32} = 1.50$ ,  $P = 0.2321$ ) nor nutrient treatment ( $F_{15,32} = 0.90$ ,  $P = 0.3490$ ) affected LLCP (Table 3); however, in low-light LLCP was on average ~50% of that in high light ( $F_{15,32} = 43.12$ ,  $P < 0.0001$ ; Table 3).

Leaf mass per area differed significantly among the eight study species occurring in the high-light treatment ( $F_{15,28} = 7.58$ ,  $P < 0.0001$ ) with *A. saccharum* and *Q. rubra* having the highest LMA and *B. papyrifera* and *P. grandidentata* the lowest (Table 3). There was no effect of nutrient treatment ( $F_{15,28} = 0.33$ ,  $P = 0.5695$ ) or the

**Table 3** Leaf traits for high (*L*) and low (*l*) light and high (*N*) and low (*n*) nutrient treatments for glasshouse-grown seedlings of eight temperate deciduous tree species. Means ± SEs of untransformed data are shown ( $n = 3$ ).  $A_{\text{max}}$  Photosynthetic capacity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $R_d$  foliar respiration ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), LLCP leaf-level light compen-

sation point ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), LMA leaf mass per area ( $\text{g cm}^{-2}$ ), AR *A. rubrum*, AS *A. saccharum*, BA *B. alleghaniensis*, BP *B. papyrifera*, FA *F. americana*, PG *P. grandidentata*, UA *U. americana*, n.d. no data in a given treatment

Variable	Species								
	Treatment	AR	AS	BA	BP	FA	PG	QR	UA
$A_{\text{max}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Ln	2.1 ± 0.3	1.5 ± 0.5	6.2 ± 1.1	3.6 ± 0.8	2.9 ± 0.9	11.7 ± 1.9	4.7 ± 0.8	9.4 ± 0.9
	LN	8.1 ± 1.9	3.4 ± 0.9	5.4 ± 0.8	6.8 ± 0.9	5.5 ± 0.7	10.4 ± 1.2	4.0 ± 0.6	8.7 ± 1.6
	ln	2.5 ± 0.5	2.3 ± 0.4	5.6 ± 0.6	n.d.	3.1 ± 0.6	n.d.	4.9 ± 0.6	5.4 ± 0.2
	IN	n.d.	1.2 ± 0.1	n.d.	n.d.	4.8 ± 0.6	n.d.	6.1 ± 0.4	3.6 ± 1.1
$R_d$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Ln	0.3 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	1.0 ± 0.2	0.5 ± 0.1	0.3 ± 0.0
	LN	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	1.3 ± 0.2	0.7 ± 0.0	0.6 ± 0.1
	ln	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	n.d.	0.2 ± 0.0	n.d.	0.3 ± 0.0	0.2 ± 0.0
	IN	n.d.	0.3 ± 0.1	n.d.	n.d.	0.3 ± 0.0	n.d.	0.3 ± 0.0	0.3 ± 0.0
LLCP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Ln	7.0 ± 1.4	7.9 ± 2.4	9.5 ± 1.8	10.8 ± 3.1	7.4 ± 1.4	10.3 ± 3.6	9.3 ± 1.6	8.0 ± 3.1
	LN	9.5 ± 1.9	7.8 ± 1.5	7.4 ± 1.9	9.9 ± 2.0	7.4 ± 0.6	16.9 ± 3.2	11.2 ± 0.1	9.2 ± 0.6
	ln	4.6 ± 1.0	3.9 ± 0.4	4.4 ± 1.0	n.d.	4.3 ± 0.3	n.d.	5.3 ± 0.6	3.6 ± 0.5
	IN	n.d.	4.8 ± 0.6	n.d.	n.d.	4.3 ± 0.5	n.d.	4.5 ± 0.1	5.1 ± 0.1
LMA ( $\text{g m}^{-2}$ )	Ln	38.9 ± 5.8	63.9 ± 6.2	35.8 ± 3.2	16.5 ± 8.1	44.2 ± 3.5	30.3 ± 1.2	60.0 ± 5.8	49.3 ± 3.7
	LN	37.0 ± 3.1	57.3 ± 6.5	28.7 ± 6.1	35.5 ± 4.1	39.1 ± 1.0	22.8 ± 1.7	62.3 ± 2.1	39.9 ± 9.9
	ln	24.6 ± 2.7	32.3 ± 1.4	23.4 ± 4.7	n.d.	18.5 ± 1.9	n.d.	43.5 ± 1.6	24.7 ± 2.8
	IN	n.d.	31.3 ± 1.4	n.d.	n.d.	17.8 ± 1.0	n.d.	41.3 ± 1.6	18.5 ± 1.0

interaction term ( $F_{15,28} = 0.88$ ,  $P = 0.5369$ ) on LMA in high light. For the four species with individuals in all treatments, LMA varied with species ( $F_{15,32} = 14.04$ ,  $P < 0.0001$ ) and light treatment ( $F_{15,32} = 72.60$ ,  $P < 0.0001$ ). All species reduced LMA substantially in the low-light treatment (Table 3).

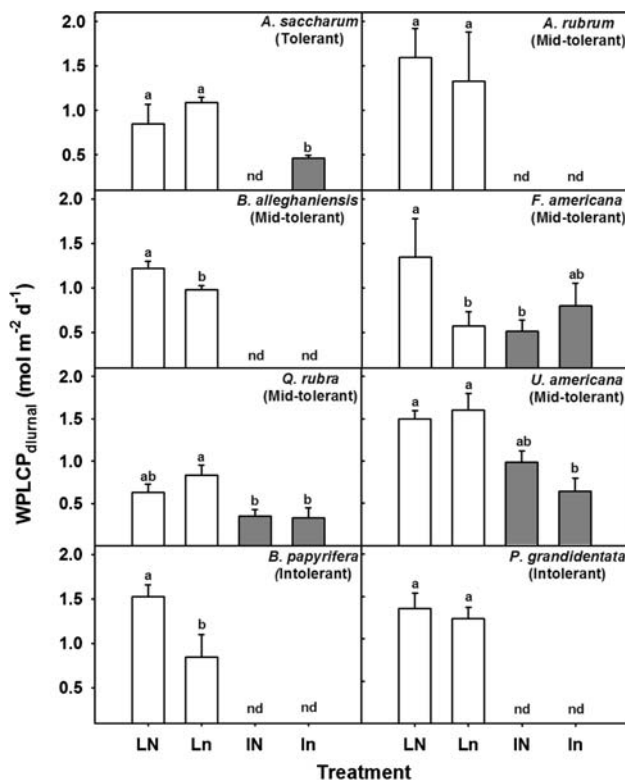
Responses of WPLCP to resource availability

There were significant differences among species in  $WPLCP_{diurnal}$  ( $F_{15,25} = 2.84$ ,  $P = 0.0253$ ; Fig. 2) for the eight species with individuals in high light. High-light-grown plants generally had higher  $WPLCP_{diurnal}$  in the high nutrient treatment ( $F_{15,25} = 8.16$ ,  $P = 0.0085$ ). The interaction term was not significant ( $F_{15,25} = 1.81$ ,  $P = 0.1292$ ). For the four species with individuals in all treatments, high-light-grown plants generally had higher  $WPLCP_{diurnal}$  ( $F_{14,24} = 15.20$ ,  $P = 0.0007$ ; Fig. 2). Although there was no main effect of nutrient availability ( $F_{14,24} = 0.32$ ,  $P = 0.5787$ ), the three-way interaction term (light  $\times$  fertilization  $\times$  species) was significant ( $F_{14,24} = 4.09$ ,  $P = 0.0296$ ). In high light, *F. americana* increased

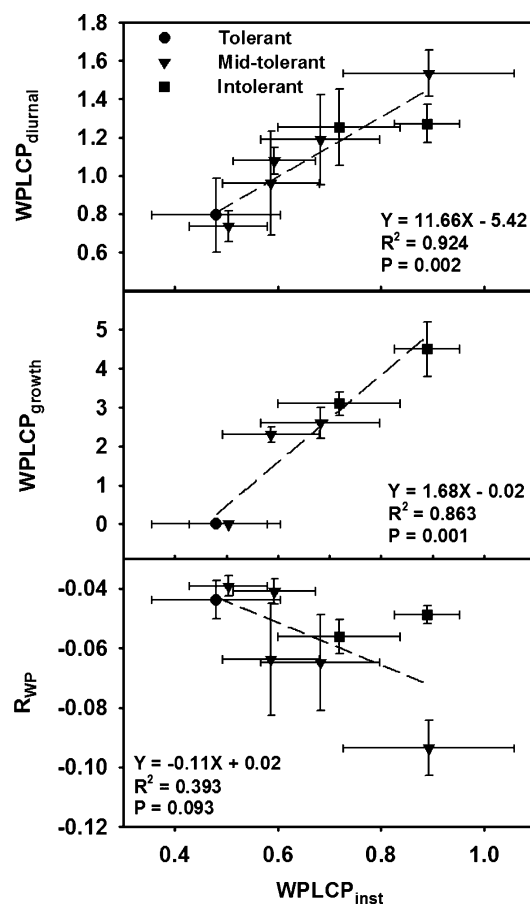
$WPLCP_{diurnal}$  with nutrient addition, a response not evident in low light, or even marginally reversed (Fig. 2). Conversely, *U. americana* showed a non-significant increase in  $WPLCP_{diurnal}$  with enhanced nutrient availability in low light. *Q. rubra* responded only to light availability (Fig. 2).

Contribution of physiological and morphological traits to WPLCP

Species differed in WPLCP regardless of the metric used (instantaneous, diurnal or growth-based; Fig. 3). There was a continuum of gas-exchange-based WPLCP values with *Q. rubra* and *A. saccharum* maintaining the lowest



**Fig. 2** High (L) and low (l) light and high (N) and low (n) nutrient treatment effects on mean ( $\pm$ SE) gas-exchange-based diurnal whole-plant light compensation point ( $WPLCP_{diurnal}$ ) for glasshouse-grown seedlings. Letters indicate post-hoc pairwise comparisons within species. Treatments in which  $WPLCP_{diurnal}$  estimates were not available are indicated by nd (no data)



**Fig. 3** Standardized major axis relationships of instantaneous  $WPLCP$  ( $WPLCP_{inst}$ ) with  $WPLCP_{diurnal}$  and growth-based  $WPLCP$  ( $WPLCP_{growth}$ ) and whole-plant dark respiration ( $R_{WP}$ ). Observational rankings of tolerant (circles), mid-tolerant (triangles) and intolerant (squares) species (adapted from Burns and Honkala 1990).  $WPLCP_{inst}$ ,  $WPLCP_{diurnal}$  and  $R_{WP}$  were measured on glasshouse-grown seedlings while  $WPLCP_{growth}$  measurements were made on field-grown saplings. All  $WPLCP$  values are mean light levels in  $mol\ m^{-2}\ d^{-1} \pm SE$  while  $R_{WP}$  values are  $mol\ of\ CO_2\ m^{-2}\ d^{-1} \pm SE$ . Values for high-light-grown individuals were used for  $WPLCP_{inst}$ ,  $WPLCP_{diurnal}$  and  $R_{WP}$

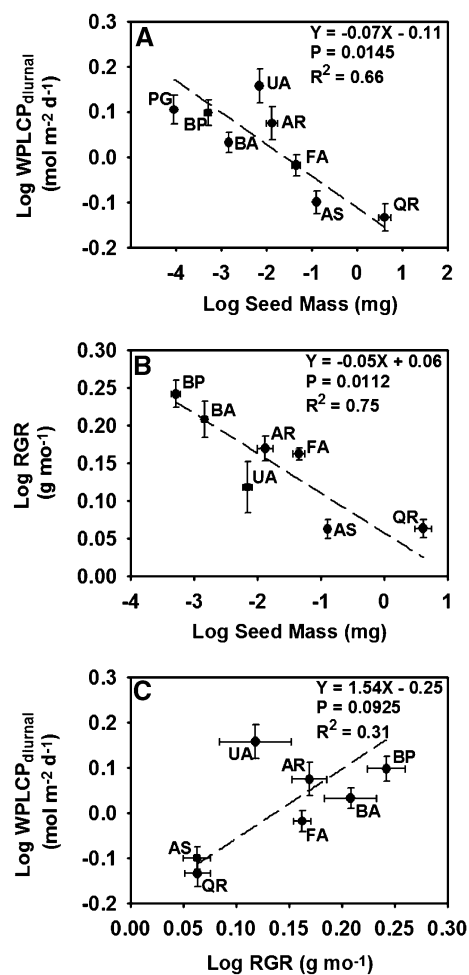
whole-plant light requirements and *U. americana* the highest (Fig. 2). There was no clear association between shade tolerance-based species rankings and WPLCP measured in this study (Fig. 3). It is noteworthy that gas-exchange-based measures of WPLCP for species considered intermediate in shade tolerance range from the highest (*U. americana*) to the lowest (*Q. rubra*) among the study species (Fig. 2). Quantitative values of WPLCP<sub>diurnal</sub> were higher than WPLCP<sub>inst</sub> and lower than WPLCP<sub>growth</sub>; however, all measures of WPLCP correlate strongly with one another (Fig. 3).

Interspecific differences in WPLCP<sub>diurnal</sub> in the high-light treatment were closely related to seed size, which explained 66% of variation in WPLCP<sub>diurnal</sub> (Fig. 4a). A tight negative relationship existed between seed size and RGR in high light ( $P = 0.0112$ ,  $r^2 = 0.75$ ; Fig. 4b) and RGR in high light correlated positively with WPLCP<sub>diurnal</sub> (Fig. 4c).

Several traits of individual seedlings showed weak to moderately strong correlations with individual WPLCP<sub>inst</sub>.  $R_{WP}$ ,  $R_d$ ,  $A_{max}$  and LLCP had significant positive correlations with WPLCP<sub>inst</sub> ( $r^2 = 0.06$ – $0.40$ ;  $P = 0.042$ – $<0.0001$ ; Fig. 3c for the WPLCP<sub>inst</sub>– $R_{WP}$  relationship). Root–shoot ratio showed a weak negative correlation with WPLCP<sub>inst</sub> ( $r^2 = 0.09$ ,  $P = 0.0145$ ) indicating that species with greater C costs invested more heavily in aboveground organs. The AIC comparisons in the multiple regression analysis showed that the model including  $R_{WP}$ , LLCP and LMA as predictors of WPLCP<sub>inst</sub> was more parsimonious than all other models tested (Table 4). Of these three variables,  $R_{WP}$  contributed most to the model (Table 5), which was corroborated by the PCA analysis (results not shown; see Statistical analysis).

## Discussion

Our results suggest that light requirements of temperate deciduous tree seedlings, as quantified by gas-exchange-based WPLCP (WPLCP<sub>diurnal</sub>), are related to seed size, with physiological traits and responses to environmental conditions modulating individual WPLCP<sub>inst</sub>. WPLCP<sub>diurnal</sub> responded strongly to light availability while effects of nutrient availability were limited to the high-light treatment alone. These results imply that spatial variation in soil nutrient availability will have limited impact on seedling success in the understory aside from areas under recent canopy openings. The best predictor of variation in WPLCP<sub>inst</sub>, when the effect of species was accounted for, was whole-plant dark respiration rate, while morphological variables typically attributed to shade tolerance such as root–shoot ratio and LARs did not contribute significantly to variation in individual light requirements. None of the



**Fig. 4** Standardized major axis relationships among log diurnal whole-plant light compensation point in high light (WPLCP<sub>diurnal</sub>; mol m<sup>-2</sup> day<sup>-1</sup>), log seed mass (mg) and log relative growth rates (RGR; g g<sup>-1</sup> month<sup>-1</sup>) for high-light grown seedlings. All values are for glasshouse-grown seedlings. Species initials adjacent to data points are as follows: AR *Acer rubrum*, AS *A. saccharum*, BA *Betula alleghaniensis*, BP *B. papyrifera*, FA *Fraxinus americana*, PG *Populus grandidentata*, QR *Quercus rubra*, UA *Ulmus americana*

three quantitative estimates of whole-plant light requirements correlated well with observational shade tolerance rankings.

### Physiological and morphological determinants of WPLCP

Three traits contributed significantly to variation in estimates of individual WPLCP<sub>inst</sub>. These included two gas-exchange parameters ( $R_{WP}$  and LLCP), which correlated positively with WPLCP<sub>inst</sub>, and one morphological parameter (LMA), which on its own showed no significant relationship with WPLCP<sub>inst</sub>. Whole-plant respiration rates were the strongest predictor of individual WPLCP<sub>inst</sub> in the

**Table 4** Multiple regression models examining physiological and morphological traits as predictors of instantaneous WPLCP ( $WPLCP_{inst}$ ) for glasshouse-grown seedlings. AIC Akaike information criterion,  $R_{WP}$  whole-plant respiration rates ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), LAR leaf area ratio ( $\text{m}^{-2} \text{g}^{-1}$ ),  $g_s$  stomatal conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ), R:S root–shoot ratio ( $\text{g g}^{-1}$ ); for other abbreviations, see Table 3

Predictors	$r^2$	AIC	$\Delta\text{AIC}$
$R_{WP}$ , LLCP, LMA, LAR, $g_s$ , $A_{max}$ , R:d, R:S	0.4568	385.75	0
$R_{WP}$ , LLCP, LMA, LAR, $A_{max}$ , R:S	0.4558	383.87	–1.88
$R_{WP}$ , LLCP, LMA, LAR, $A_{max}$	0.454	382.57	–3.18
$R_{WP}$ , LLCP, LMA, $A_{max}$	0.4501	380.52	–5.23
$R_{WP}$ , LLCP, LMA	0.4342	379.35	–6.40

**Table 5** Most parsimonious AIC-selected multiple regression model examining physiological and morphological traits as predictors of  $WPLCP_{inst}$  for glasshouse-grown seedlings; for abbreviations, see Tables 3 and 4

Model <sup>a</sup>	Parameter estimate	SE	<i>t</i> -value	<i>Pr</i> >  <i>t</i>
Intercept	–10.81	1.98	–5.46	<0.0001
$R_{WP}$	7.82	1.36	5.73	<0.0001
LLCP	0.36	0.17	2.12	0.0383
LMA	827.79	386.9	2.14	0.0365

<sup>a</sup> Model and error *df* are 3 and 60, respectively ( $r^2 = 0.43$ ,  $F_{3,60} = 15.35$ ,  $P < 0.0001$ )

multiple regression analysis. This supports the idea that maintenance of positive net C balance is likely achieved through minimizing energy expenditure rather than maximizing energy gain rates as the latter is constrained in light-limited environments (Grime and Jeffery 1965; Reich et al. 2003). Previous studies have similarly demonstrated greater whole-plant or foliar respiration rates in light-demanding species (Walters et al. 1993; Reich et al. 1998b; Lusk and Reich 2000). Respiration rates should be better predictors of WPLCP than other physiological attributes as metabolic costs are incurred both day and night and regardless of irradiance. This has important consequences in light-limited environments as a given respiration rate will consume more of the energy available for growth or storage when light availability and therefore C gain are low. Both LLCP and LMA were also maintained as significant predictors in the AIC-selected model. LLCP corresponds to the foliar physiological break-even point and has been suggested to be a good proxy for shade tolerance. Species with lower LLCP should be better able to tolerate low light as they will less frequently experience leaf-level C deficits, again corroborating the role of C conservation in shade tolerance (Walters and Reich 1999; Reich et al.

2003). It should be noted, however, that LLCP only predicted about 11% of the variation in  $WPLCP_{inst}$  and its role as a proxy measure for species' light requirements should thus be considered carefully. LMA was a significant predictor in the most parsimonious AIC-selected model, which confirms other studies examining correlates of shade tolerance (Kitajima 1994; Davies 1998). LMA is considered important in stress tolerance generally as thicker, more durable leaves are thought to confer resistance to physical damage, herbivory and pathogens and LMA typically correlates with leaf nutrient concentrations and lifespan (Reich et al. 2003), traits aimed at resource conservation as opposed to enhanced C gain in low light.

Traits conferring resource conservation thus contributed strongly to  $WPLCP_{inst}$ ; however, the best-fit model explained only approximately half of the variation in  $WPLCP_{inst}$ . This result suggests that direct measures of WPLCP (either gas-exchange- or growth-based) are needed to quantify and determine shifts in species' light requirements or rankings as a function of factors such as resource availability, ontogeny and climate. In contrast, a recent study examining  $WPLCP_{growth}$  in Bornean trees determined that foliar respiration alone explained 79% of variation in growth-based WPLCP, and thus may make a suitable proxy measure for WPLCP in tropical tree saplings (J. L. Baltzer and S. C. Thomas, submitted).

#### Whole-plant gas-exchange responses to resource availability

Increases in light availability generally resulted in higher  $WPLCP_{diurnal}$ . The best-fit model indicated that  $R_{WP}$ , LLCP and LMA are the best predictors of  $WPLCP_{inst}$ . All three traits show strong plastic responses to light availability regardless of species, therefore a similar response of WPLCP should be expected. Nutrient availability also increased  $WPLCP_{diurnal}$ , a response that was, however, limited to the high-light treatment. This is in contrast with some studies which have shown nutrient additions to reduce seedling survival in low-light environments (Grubb et al. 1996; Catovsky and Bazzaz 2002). However, our findings are supported by a number of other related studies. Lehto and Grace (1994) examined whole-plant gas-exchange of two tropical forest species and demonstrated increases in whole-plant respiration with increased nutrient availability in shade-tolerant *M. excelsa*, while light-demanding *T. guineensis* was unaffected. This increase, however, occurred only in the high-light treatment. Likewise, Kobe (2006) demonstrated that soil N exerted little influence on the growth of saplings of four temperate forest tree species in the understory but that in high-light conditions where photosynthetic potential could be achieved that N played an important role. Our results demonstrate vari-

ability in species' WPLCP<sub>diurnal</sub> response to nutrient availability in high light conditions with little apparent relationship between species response and whole-plant light requirements. This finding suggests that responsiveness to nutrient availability is not simply a function of shade tolerance. This could have important consequences with respect to regeneration dynamics, as independent responses of species to gradients of nutrient and light will increase the potential for differentiation of species across this multiple resource gradient (Bazzaz and Miao 1993; Sack and Grubb 2002; Sack 2004).

#### Interspecific variation in WPLCP

Species differed substantially in both gas-exchange-based measures of WPLCP (Fig. 3). Those typically classified as shade tolerant (*A. saccharum*) or light demanding (*B. papyrifera* and *P. grandidentata*) did not rank lowest or highest, respectively, along the WPLCP continuum as would be expected if observational shade tolerance rankings correspond with quantitative values of WPLCP (Fig. 2). Likewise, among the "mid-tolerant" species (*A. rubrum*, *B. alleghaniensis*, *F. americana*, *Q. rubra* and *U. americana*) there were much larger differences in WPLCP<sub>diurnal</sub> and WPLCP<sub>inst</sub> than would have been anticipated given their similar ranking. For example, patterns of growth and WPLCP<sub>diurnal</sub> of mid-tolerant *Q. rubra* mirror those of *A. saccharum*, the most shade-tolerant study species according to observational classifications. Kaelke et al. (2001) also ranked *Q. rubra* and *A. saccharum* similarly with respect to shade tolerance based on growth, morphology, resource allocation and physiology.

Seed size was a strong predictor of both WPLCP<sub>diurnal</sub> and RGR in the present study, explaining 66 and 76% of variation, respectively (Fig. 4a, b). The relationship between WPLCP<sub>diurnal</sub> and RGR was significant but much weaker, with high-light RGR explaining only 30% of variation in high-light WPLCP<sub>diurnal</sub> (Fig. 4c). These results are consistent with other studies examining RGR or shade tolerance as a function of seed size (Kitajima 1994; Saverimuttu and Westoby 1996; Reich et al. 1998a; Sack et al. 2003; Poorter and Rose 2005). A number of explanations for the relationship between shade tolerance and seed size have been proposed; however, the maintenance of the predicted relationship when a gas-exchange-based metric is used supports the idea that inherent metabolic differences are contributing to this relationship. Myers and Kitajima (2007) recently examined the seed size–shade tolerance relationship as a function of carbohydrate storage and found a strong relationship between survival following defoliation or extreme low-light treatments, carbohydrate storage in stems and roots and seed size. It is reasonable to speculate that shade tolerance is achieved through main-

tenance of low metabolic demands and slow growth as illustrated in the present study but that stored carbohydrates in the stems and roots enable larger-seeded species to endure periods of extreme biotic or abiotic stress.

The strong relationships among seed size, RGR and WPLCP are likely to hold only for seedlings per se (e.g., Westoby et al. 1996). Wide variation observed among mid-tolerant species may thus indicate divergent ontogenetic trends in WPLCP among this set of species as traits other than seed size assume greater importance in later stages (e.g., Lusk 2004). It should be noted, however, that for the species that could be compared there was good correlation between WPLCP<sub>inst</sub> and WPLCP<sub>growth</sub>, measured at the seedling and sapling stages, respectively (Fig. 3a). Regardless, these early stages of regeneration have profound consequences, as mortality rates are extremely high in tree seedlings during the first year of growth (George and Bazzaz 1999; Catovsky and Bazzaz 2002). Therefore, even relatively short-lived differences in whole-plant light requirements among seedlings during these early stages will influence regeneration dynamics.

How well do gas-exchange-based measures predict realized light requirements?

In the glasshouse component of the study, WPLCP was estimated as the light availability at which gas-exchange-based whole-plant C balance is zero. In comparison to growth-based WPLCP measured in the natural forest, these levels tended to be lower, particularly for light-demanding and mid-tolerant species (Fig. 3b). Beaudet et al. (2000) noted that although their estimates of daily whole-plant C gain were lower in both high and low light for *A. saccharum* than for either *B. alleghaniensis* or *Fagus grandifolia*, *A. saccharum* was the dominant species in the forest. There are a few factors that could contribute to the discrepancies noted by Beaudet et al. (2000) and quantified in the present study. Light-demanding species tend to be more susceptible to herbivory as a result of lower investment in leaf defenses and higher leaf protein concentrations (Coley 1983; Coley and Barone 1996). Tissue replacement in the understory is costly and may increase WPLCP substantially. Similarly, species differ in their ability to tolerate fungal pathogens (Augspurger 1984; O'Hanlon-Manners and Kotanen 2004). Glasshouse estimates of WPLCP were made using 5-month-old seedlings while field-based estimates depicted light requirements of saplings within a narrow size range but of undetermined age and certainly older than the glasshouse seedlings. As previously mentioned, ontogenetic differences in light requirements may thus contribute to quantitative differences (though in this case not rankings) in measured WPLCP between the field and

glasshouse studies. Lusk (2004) demonstrated ontogenetic rank reversals in patterns of biomass allocation in relation to shade tolerance: specifically, more shade-tolerant species increased LAR and decreased root mass fraction with age/size when compared with more light-demanding species, which was equated to a healthier C balance in shade-tolerant species at these light levels (Lusk 2004). In the present study, differences in light requirements of glasshouse seedlings vs. field-grown saplings could also arise from errors in the estimation of individual sapling light environment made in the field study, or from the amenable growing conditions provided in the glasshouse study (e.g., stable temperatures, low exposure to wind and UV radiation, etc.). Glasshouse and common garden studies that have successfully grown light-demanding species in light conditions at which they are not found in natural forests provides additional (though anecdotal) support for the role of biotic and abiotic stress agents in determining shade tolerance (e.g., Strauss-Debenedetti and Bazzaz 1991; Dalling et al. 1999).

## Conclusions

Prior comparative studies that have explicitly examined a range of physiological and morphological traits as correlates of variation in tree shade tolerance, while also including a sufficient number of species to provide a statistical basis for inference have frequently shown seed size to be a strong correlate of shade tolerance or related indices (e.g., Kitajima 1994; Reich et al. 1998a). Data in the present study demonstrate a strong negative correlation between physiological WPLCP, which was largely determined by metabolic costs, and seed size. Likewise, RGR correlated with both WPLCP and seed size. As the measure of shade tolerance quantified in the present study was entirely gas-exchange based, this implies that metabolic costs and physiological C balance are key components of the widely recognized relationship between shade tolerance and seed size. In the present study, two independent quantitative measures of WPLCP are presented. Poor correlation exists between either growth- or gas-exchange-based WPLCP and current observational shade-tolerance rankings. This may be due to regional differences in species shade tolerance due to any number of climatic or geologic factors that change across a species range (e.g., Kobe 1996). Alternatively, observational rankings could be based upon later ontogenetic stages. WPLCP likely shows ontogenetic changes, and as it does so, traits contributing to variation in shade tolerance may also change systematically (e.g., Delagrangue et al. 2004; Aiba and Nakashizuka 2005). It would be informative, if also technically challenging, to extend the whole-plant gas-exchange approach in the present study to later stages

of tree development. However, adaptation of growth-based methods for estimating WPLCP may be a more tractable field method for assessing ontogenetic shifts in WPLCP, as well as for broader quantitative surveys of tree light requirements.

**Acknowledgements** We thank Liora Zimmerman, Andrew Spring, Trevor Jones and Mike Bendszak for assistance in experimental establishment and measurements, Art Fredeen for input on chamber design, and John Caspersen and Rowan Sage for comments on earlier versions of the manuscript. Thanks to Robert Percy and two anonymous referees for thorough and helpful reviews that greatly improved the manuscript. Research was funded by the Natural Sciences and Engineering Research Council of Canada and complied with current Canadian laws.

## References

- Abrams MD, Mostoller SA (1995) Gas exchange, leaf structure and nitrogen in contrasting successional tree species growing in open and understory sites during a drought. *Tree Physiol* 15:361–370
- Aiba M, Nakashizuka T (2005) Sapling structure and regeneration strategy in 18 *Shorea* species co-occurring in a tropical rainforest. *Ann Bot* 96:313–321
- Andrews JA, Harrison KG, Matamala R, Schlesinger WH (1999) Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). *Soil Sci Soc Am J* 63:1429–1435
- Augsburger CK (1984) Seedling survival of tropical tree species—interactions of dispersal distance, light-gaps, and pathogens. *Ecology* 65:1705–1712
- Baltzer JL (2005) The importance of multiple resource limitation to shade tolerance and habitat preferences in tropical and temperate tree saplings. PhD dissertation, University of Toronto, Toronto
- Baltzer JL, Thomas SC (2005) Leaf optical responses to light and soil nutrient availability in temperate deciduous trees. *Am J Bot* 92:214–223
- Bassow SL, Bazzaz FA (1998) How environmental conditions affect canopy leaf-level photosynthesis in four deciduous tree species. *Ecology* 79:2660–2675
- Bazzaz FA, Miao SL (1993) Successional status, seed size and responses of tree seedlings to CO<sub>2</sub>, light and nutrients. *Ecology* 74:104–112
- Bazzaz FA, Wayne PM (1994) Coping with environmental heterogeneity: the physiological ecology of tree seedling regeneration across the gap-understory continuum. In: Caldwell MM, Pearcy RW (eds) *Exploitation of environmental heterogeneity by plants*. Academic Press, San Diego, Calif., pp 349–390
- Beaudet M, Messier C, Hilbert DW, Lo E, Wang ZM, Lechowicz MJ (2000) Leaf- and plant-level carbon gain in yellow birch, sugar maple, and beech seedlings from contrasting forest light environments. *Can J For Res* 30:390–404
- Burnham KP, Anderson DR (1998) *Model selection and inference: a practical information theoretic approach*. Springer, Berlin Heidelberg New York
- Burns RM, Honkala BH (1990) *Silvics of North America*. Hardwoods, vol 2. US Department of Agriculture, Washington, D.C.
- Canham CD, Denslow JS, Platt WJ, Runkle JR, Spies TA, White PS (1990) Light regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. *Can J For Res* 20:620–631
- Caspersen JP, Kobe RK (2001) Interspecific variation in sapling mortality in relation to growth and soil moisture. *Oikos* 92:160–168

- Catovsky S, Bazzaz FA (2002) Nitrogen availability influences regeneration of temperate tree species in the understory seedling bank. *Ecol Appl* 12:1056–1070
- Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol Monogr* 53:209–233
- Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forests. *Annu Rev Ecol Syst* 27:305–335
- Coomes DA, Grubb PJ (1998) Responses of juvenile trees to above- and belowground competition in nutrient-starved Amazonian rain forest. *Ecology* 79:768–782
- Dalling JW, Lovelock CE, Hubbell SP (1999) Growth responses of seedlings of two neotropical pioneer species to simulated forest gap environments. *J Trop Ecol* 15:827–839
- Davies SJ (1998) Photosynthesis of nine pioneer *Macaranga* species from Borneo in relation to life history. *Ecology* 79:2292–2308
- Delagrangé S, Messier C, Lechowicz MJ, Dizengremel P (2004) Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. *Tree Physiol* 24:775–784
- Falster DS, Warton DI, Wright IJ (2003) (S)MATR: standardised major axis tests and routines. <http://www.bio.mq.edu.au/ecology/SMATR/>
- Finzi AC, Canham CD (2000) Sapling growth in response to light and nutrient availability in a southern New England forest. *For Ecol Manage* 131:153–165
- George LO, Bazzaz FA (1999) The fern understory as an ecological filter: growth and survival of canopy-tree seedlings. *Ecology* 80:846–856
- Givnish TJ (1988) Adaptation to sun and shade—a whole plant perspective. *Aust J Plant Physiol* 15:63–92
- Grime JP, Jeffery DW (1965) Seedling establishment in vertical gradients of sunlight. *J Ecol* 53:621–642
- Groffman PM, Zak DR, Christensen S, Mosier A, Tiedje JM (1993) Early spring nitrogen dynamics in a temperate forest landscape. *Ecology* 74:1579–1585
- Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL (2001) Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry* 56:191–213
- Grubb PJ, Lee WG, Kollmann J, Wilson JB (1996) Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*. *J Ecol* 84:827–840
- Kaelke CM, Kruger EL, Reich PB (2001) Trade-offs in seedling survival, growth, and physiology among hardwood species of contrasting successional status along a light-availability gradient. *Can J For Res* 31:1602–1616
- Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98:419–428
- Kobe RK (1996) Intraspecific variation in sapling mortality and growth predicts geographic variation in forest composition. *Ecol Monogr* 66:181–201
- Kobe RK (2006) Sapling growth as a function of light and landscape-level variation in soil water and foliar nitrogen in northern Michigan. *Oecologia* 147:119–133
- Kohyama T (1991) A functional model describing sapling growth under a tropical forest canopy. *Funct Ecol* 5:83–90
- Kohyama T, Hotta M (1990) Significance of allometry in tropical saplings. *Funct Ecol* 4:515–521
- Koppers M (1994) Canopy gaps: competitive light interception and economic space filling—a matter of whole-plant allocation. In: Caldwell MM, Pearcy RW (eds) *Exploitation of environmental heterogeneity by plants*. Academic Press, San Diego, Calif., pp 111–144
- Latham RE (1992) Co-occurring tree species change rank in seedling performance with resources varied experimentally. *Ecology* 73:2129–2144
- Lee DW, Baskaran K, Mansor M, Mohamad H, Yap SK (1996) Irradiance and spectral quality affect Asian tropical rain forest tree seedling development. *Ecology* 77:568–580
- Lehto T, Grace J (1994) Carbon balance of tropical tree seedlings: a comparison of two species. *New Phytol* 127:455–463
- Lusk CH (2004) Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. *Funct Ecol* 18:820–828
- Lusk CH, Reich PB (2000) Relationships of leaf dark respiration with light environment and tissue nitrogen content in juveniles of 11 cold-temperate tree species. *Oecologia* 123:318–329
- Makana J-R, Thomas SC (2005) Effects of light-gaps and litter removal on seedling performance in six African timber tree species. *Biotropica* 37:227–237
- Moad AS (1992) Dipterocarp juvenile growth and understory light availability in Malaysian tropical forest. PhD dissertation, Harvard University, Cambridge, Mass.
- Myers JA, Kitajima K (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *J Ecol* 95:383–395
- O'Hanlon-Manners DL, Kotanen PM (2004) Evidence that fungal pathogens inhibit recruitment of a shade intolerant tree, white birch (*Betula papyrifera*), in understory habitats. *Oecologia* 140:650–653
- Penning de Vries FWT, Witlage JM, Kremer D (1979) Rates of respiration and of increase in structural dry matter in young wheat, ryegrass, and maize plants in relation to temperature, to water stress, and to their sugar content. *Ann Bot* 44:595–609
- Phillips RP, Fahey TJ (2005) Patterns of rhizosphere carbon flux in sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*) saplings. *Global Change Biol* 11:983–995
- Poorter L, Rose S (2005) Light-dependent changes in the relationship between seed mass and seedling traits: a meta-analysis for rain forest tree species. *Oecologia* 142:378–387
- Poorter L, Bongers F, Sterck FJ, Woll H (2005) Beyond the regeneration phase: differentiation of height-light trajectories among tropical tree species. *J Ecol* 93:256–267
- Reich PB, Tjoelker MG, Walters MB, Vanderklein DW, Buschena C (1998a) Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Funct Ecol* 12:327–338
- Reich PB, Walters MB, Tjoelker MG, Vanderklein DW, Buschena C (1998b) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. *Funct Ecol* 12:395–405
- Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategies. *Int J Plant Sci* 164:S143–S164
- Sack L (2004) Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos* 107:110–127
- Sack L, Grubb PJ (2002) The combined impacts of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. *Oecologia* 131:175–185
- Sack L, Grubb PJ, Maranon T (2003) The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecol* 168:139–163
- Saverimuttu T, Westoby M (1996) Seedling longevity under deep shade in relation to seed size. *J Ecol* 84:681–689
- Schmid B, Polasek W, Weiner J, Krause A, Stoll P (1994) Modeling of discontinuous relationships in biology with censored regression. *Am Nat* 143:494–507

- Sipe TW, Bazzaz FA (1994) Gap partitioning among maples in Central New England: shoot architecture and photosynthesis. *Ecology* 75:2318–2332
- Strauss-Debenedetti S, Bazzaz FA (1991) Plasticity and acclimation to light in tropical Moraceae of different successional positions. *Oecologia* 87:377–387
- Turnbull MH (1991) The effect of light quantity and quality during development on the photosynthetic characteristics of six Australian rainforest tree species. *Oecologia* 87:110–117
- Veenendaal EM, Swaine MD, Agyeman VK, Blay D, Abebrese IK, Mullins CE (1995) Differences in plant and soil water relations in and around a forest gap in West Africa during the dry season may influence seedling establishment and survival. *J Ecol* 83:83–90
- Walters MB, Reich PB (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytol* 143:143–154
- Walters MB, Reich PB (2000) Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. *Ecology* 81:1887–1901
- Walters MB, Kruger EL, Reich PB (1993) Growth, biomass distribution and CO<sub>2</sub> exchange of northern hardwood seedlings in high and low light—relationships with successional status and shade tolerance. *Oecologia* 94:7–16
- Westoby M, Leishman M, Lord J (1996) Comparative ecology of seed size and dispersal. *Philos Trans R Soc Lond Ser B Biol Sci* 351:1309–1318
- Zotz G, Winter K (1994) Photosynthesis of a tropical canopy tree, *Ceiba pentandra*, in a lowland forest in Panama. *Tree Physiol* 14:1291–1301