Size-related variation in physiology, carbon gain, and growth of trees in deciduous and evergreen forests

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
GRADUATE SCHOOL OF
THE UNIVERSITY OF MINNESOTA
BY

Kerrie M. Sendall

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Peter B. Reich and Christopher H. Lusk, advisors

November 2012

Acknowledgements

Abstract

A better understanding of species' light requirements throughout developmental stages is required to improve models of forest dynamics. Although it has recently been shown that light requirements of many species may change as trees increase in size, the traits that underlie these ontogenetic patterns are not well understood. This dissertation characterizes the relationships of tree size with physiological and growth responses of eight tree species varying in leaf habit, biome, and shade tolerance. I examined morphological traits and physiological mechanisms operating at the leaf and whole-plant scale, thus allowing for identification of mechanisms that underlie observed ontogenetic variations in growth rates and shade tolerance.

Size-related variation in leaf nutrients and gas exchange rates of each of the eight species were measured. While the general light conditions under which each of the eight species evolved were similar (i.e. all are trees common in forest gaps and understories and vary in shade tolerance within those environments), four of the eight species were temperate deciduous and four were subtropical evergreens. Therefore, my data are most relevant at the species or local community level and in qualitative comparisons across leaf habit. Generally speaking, shade-tolerant species and species with long-lived leaves expressed low rates of gas exchange and nitrogen content, while light-demanding species and species with shorter leaf lifespans showed higher rates of gas exchange and leaf nitrogen. However, size-related patterns of leaf-level traits were inconsistent in both deciduous and evergreen species, making it difficult to disentangle the factors responsible for these changes.

To further investigate the effect of tree size on shade tolerance, we examined a combination of leaf and whole-plant traits of juvenile trees. Increased sapling size caused significant declines in shade tolerance and relative growth rates (RGR). Among the four evergreens, the slope of the relationship between light availability and RGR was steepest in light-demanding species, leading to crossovers in RGR between shade-tolerant and light-demanding species at low light, independent of sapling size. We found that no single factor can explain reductions in growth and shade tolerance with increased tree size, but that variations in self-shading and net daily carbon gain rates per gram of aboveground tissue were strong predictors of the declines.

My results indicate that work on saplings and mature trees may detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone. These differences could have important implications for modeling the dynamics of forest types.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Appendices

Introduction

Trees are important globally because they provide construction materials, food, fuel, and diverse ecosystem services such as clean air and water, taking up and storing carbon, providing habitat for a diversity of organisms, and synthesizing chemicals found in many pharmaceuticals. Trees extend from the warmest and wettest habitats on earth to some of the coldest and driest, and because they are able to acclimate to short-term environmental changes, they can survive for up to thousands of years. Because they are long-lived and immobile, they must have some degree of plasticity in their range of regeneration and survival strategies, including the ability to alter their structure and function with ontogeny and with variations in biotic and abiotic factors.

Trees typically increase in biomass and height by several orders of magnitude from the seedling stage to the adult stage, and therefore they face a variety of constraints as they grow older and larger. A whole tree can be thought of as a system of coarse roots, stems, and branches that are linked to finer systems of foliage and fine roots that acquire and distribute nutrients and carbon (Korner 1994). During their lifetime, trees go through a multitude of changes in their structure and in how they interact with their surrounding environment, such as changes in allometry and architecture, structural properties of woody tissues, and leaf chemistry and physiology. These types of changes often result in functional trade-offs that could result in the optimal design for transport systems in tall trees, maximizing traits such as growth, total net carbon gain, and efficient use of particular resources (Mencuccini et al. 2011). These may include trade-offs between factors such as growth rate versus longevity or hydraulic and biomechanical safety versus productivity (Zimmerman 1983, Kennedy et al. 2010). Consequently, the form of an individual tree can be thought of as the genetic components that direct architectural structure and function, as well as their interaction with the surrounding biotic and abiotic conditions.

An understanding of variations in plant functional traits and in light requirements throughout developmental stages is required to improve models of forest dynamics (Poorter et al. 2005, Lusk et al. 2008). These models assume that properties of tissues and whole plants do not vary with ontogeny, yet it has been shown that traits change with size in complex patterns that likely reflect variations in water, light, nitrogen, and carbon

availability, storage, and use. For example, the SORTIE forest model incorporates species-specific growth rates of nine tree species to describe local competition within northeastern forests in the US, which can be used to predict large-scale forest dynamics. Growth rates vary within the model depending on light availability, but do not vary as a function of tree size. Models like this one can be improved and made more realistic by incorporating ontogenetic trends as well as tolerance to multiple stresses such as low light and drought.

In recent years, an effort has been made to resolve some of these complex ontogenetic patterns in functional traits, but often it is difficult to determine exactly which factors are driving the changes observed. For example, a decline in growth and productivity with tree size has been well-documented (i.e. Gower et al. 1996, Ryan et al. 1997, Binkley et al. 2002), but the cause of the decline is not yet fully understood. Possible causes include declines in photosynthetic rates at the upper bounds of tree size (Yoder et al. 1994, Bond 2000, Koch et al. 2004), a decreased ratio of leaf area to total plant biomass which causes a decline in the ratio of photosynthetic income to respiratory demand (Gerrish 1990, Delagrange et al. 2004, Lusk 2004, Machado and Reich 2006), or increasing reproductive costs in larger trees (Kozlowshi and Uchmanski 1987, Iwasa and Cohen 1989).

More recently, it has been shown that shade tolerance, like growth and productivity, is also affected by plant size. Most research dealing with size-related changes in shade tolerance and growth has focused on differences between saplings and mature trees, or between young and mature stands of economically valuable species. In comparison, there is relatively little research comparing the seedling and sapling life stages. Additionally, many studies have focused on leaf-level traits, rather than whole-plant traits, when attempting to explain patterns of tree growth and carbon gain, while few have integrated the effects of crown architecture and woody tissue respiration. While traits such as leaf-level gas exchange and total leaf area are predictors of whole-plant carbon gain, crown architecture also influences carbon uptake (Falster and Westoby 2003).

In this dissertation, I examine patterns of leaf, stem, and whole-plant traits as a function of tree size in cold-temperate deciduous and subtropical evergreen species. These studies took place at the Cedar Creek Ecosystem Science Reserve and the St. Croix

Watershed Research Station in east-central Minnesota, USA and at Nightcap National Park in northern New South Wales, Australia. <u>Chapter 1:</u> I, along with Peter Reich, examined functional traits of leaves and fine stems in trees ranging in size from seedling to mature canopy tree of three deciduous species (submitted to *Tree Physiology*). <u>Chapter 2:</u> I, along with Peter Reich and Christopher Lusk, consider the effects of ontogeny again, but as it relates to self-shading, and tissue and whole-plant carbon gain and shade tolerance of shaded *Acer saccharum* seedlings and saplings. <u>Chapter 3:</u> I, along with Peter Reich and Christopher Lusk, consider ontogenetic effects on self-shading, crown carbon gain, and relative growth rates of seedlings and saplings of four subtropical trees species varying in shade tolerance. These chapters provide data regarding the effect of ontogeny across a wide range of size classes, as well as on species that differ in leaf habit and biome. We examine morphological traits and physiological mechanisms operating at the leaf and whole-plant scale, thus allowing for identification of mechanisms that underlie observed variations in growth rates and shade tolerance (Introductory Figure). The results will be useful in explaining these ecological trends that are not well-understood, and will also be useful for implementation into models of forest dynamics.
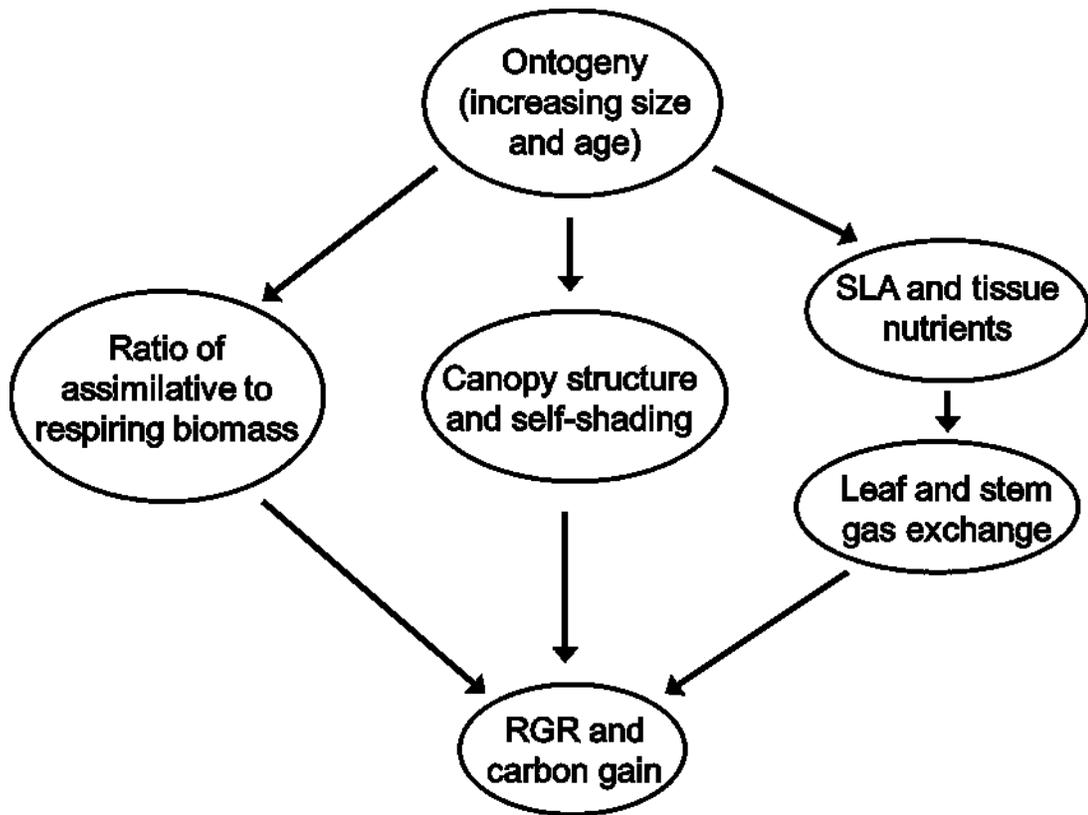
Introductory Figure. Theoretical framework showing the effect of ontogeny on relative growth rate (RGR) and carbon gain, via changes in plant architecture, morphology, and physiology.

CHAPTER 1

Variation in leaf and fine stem CO$_2$ flux as a function of plant size: a comparison of seedlings, saplings, and trees

with P.B. Reich

Rates of tissue level function have often been hypothesized to decline as individual trees grow older and larger, but evidence to assess such changes remains limited, especially for trees larger than sapling size. In this study, we build on and expand the foundation of prior research, by standardizing and accounting for differences in light environments and assessing a wider range of size than typically done in earlier studies. We measured functional traits of leaves and fine stems of three cold-temperate deciduous tree species (*Quercus ellipsoidalis*, *Prunus serotina*, and *Acer rubrum*) at Cedar Creek Ecosystem Science Reserve in Minnesota, USA to assess how these vary with tree height. In all three species, individuals ranging in size from seedling to mature tree were sampled in both high and low light environments to minimize light differences as a potential driver of size-related differences in leaf and stem properties. Maximum photosynthetic rates, leaf and fine stem dark respiration rates and nitrogen concentrations, and leaf mass per area (LMA) were quantified in trees ranging from 0 to 20 m in height. We hypothesized that (H1) gas exchange rates and associated functional traits such as tissue N concentration and LMA would vary with tree size in a pattern reflecting declining function in taller trees, yet maintaining (H2) bivariate trait relations common among species as characterized by the leaf economics spectrum. Taking these two ideas together yielded a third, integrated hypothesis that (H3) nitrogen content and both mass- and nitrogen-based photosynthesis and respiration should decrease monotonically with tree size and leaf mass per area should increase. Our observations partially matched the three hypotheses. We observed increasing LMA and decreasing leaf and stem Rd with increasing size, which matched predictions from H1 or H3. However, leaf and stem N increased with size, the opposite of our prediction from H1 and H3, also rejecting H2, which assumed a parallel size-relation for respiration and N, rather than inverse relations. Finally, we observed a unimodal or "hump-shaped" relationship between photosynthetic capacity and tree height, not mirroring any of the other traits, including those (e.g., %N) it was

expected (H2) to linearly relate to. Results suggest that different traits vary with tree size in different ways that are not consistent with a universal shift towards a lower "return on investment" strategy. Instead, species traits vary with size in patterns that likely reflect complex variation in water, light, nitrogen, and carbon availability, storage, and use; and consequential impacts on the morphology, chemistry and metabolism of leaves and twigs.

Introduction

For a variety of reasons, it has been proposed that metabolic rates vary with organismal size. Size-related changes in metabolic rates have been studied in comparative zoology for over 80 years (Kleiber 1932), but research on the size-dependence of physiological traits in plants began relatively recently (e.g. Cregg et al. 1989). In general, there are both theoretical and empirical grounds to support the hypothesis of declining function at both the tissue and whole plant scale as plants grow larger (Yoder et al. 1994, Gower et al. 1996, Ryan et al. 1997, Bond 2000, Koch et al. 2004), although such results are not always predictive. For woody plants, such variation could exist at the whole plant scale due to variation in proportions of tissues even if tissue level traits were static. Alternatively, variation could exist at the tissue scale but not at the whole plant scale if tissue level variation was offset by changes in the distribution of tissue types.

Variation among taxa in leaf traits has been posited to result from selection for coordinated traits that result in every given taxa being located someplace along a strategy continuum from slow to fast return on investment that is associated with the leaf economics spectrum (Reich et al. 1997, Wright et al. 2004, Falster et al. 2011). Species with long-lived leaves produce a greater lifetime return in carbon fixed per unit nutrient (carbon, nitrogen, or phosphorus) invested relative to species with short-lived leaves (Westoby et al. 2000), which supports the longstanding hypothesis about the benefits of an extended leaf lifespan (Chabot and Hicks 1982). This has been well-supported amongst species within and across sites (Reich et al. 1997, Westoby et al. 2000, Wright et al. 2004), and although some aspects of these patterns also are observed among similar-sized individuals within a species (Williams et al. 1989, Reich et al. 1991, Falster et al. 2011), whether these multi-trait patterns also generally reflect variation among individuals of differing size within a species is not as clear. Many studies have demonstrated changes in leaf traits with increasing tree size consistent with the idea that mass-based leaf function declines with size (Fredericksen et al. 1996, Kolb and Stone 2000, Niinemets 2002, Thomas and Winner 2002), including declines in photosynthetic rates at the upper bounds of tree size (Yoder et al. 1994, Bond 2000, Koch et al. 2004), although not all species measured have followed the same pattern (Thomas 2010). At the

tissue level, one mechanism that may cause decreased photosynthetic rates in taller trees is lower stomatal conductance, which reduces intercellular CO_2 concentrations within leaves and results in a decline in assimilation (Grulke and Miller 1994, Yoder et al. 1994, Fredericksen et al. 1996, Hubbard et al. 1999). However, although several studies demonstrate concurrent declines in foliar stomatal conductance and photosynthetic rates with increasing tree size, the observed decline in stomatal conductance is not always sufficient to explain the reduction in photosynthesis (Niinemets 2002). Even at a constant intercellular CO_2 concentration, size-related declines in photosynthesis have been reported (Miller et al. 1991, Grulke and Miller 1994), indicating that decreases in stomatal conductance are not necessarily the cause of reduced photosynthetic rates in taller trees.

Changes in leaf morphology and chemistry with increasing tree size may also lead to declining assimilation rates. Independent of light environment, leaves of taller trees often exhibit a lower surface area per unit dry mass (Niinemets and Kull 1995, Niinemets 1997b, Rijkers et al. 2000, Thomas 2010); this has been attributed to selection pressures related to differences in ambient light conditions, wind exposure and physical abrasion, and exposure to herbivores and pathogens (Thomas and Winner 2002). Each of these pressures is expected to increase the amount of compounds such as carbohydrates and lignin in leaves in taller trees (Niinemets 1997b, 2002), which may increase tolerance of low water potentials (Niklas 1991, Niinemets et al. 2001). However, these compounds may dilute the amount of nitrogen in foliage, causing a negative association often observed between leaf nitrogen concentration and tree height (Schoettle 1994, Gower et al. 1996, Niinemets 1997a). These declines in leaf nitrogen concentrations may limit the production of photosynthetic compounds in foliage of taller trees, leading to reduced photosynthetic rates. In addition to diluting the nitrogen concentration in leaves, the increased thickness and/or density and amounts of lignin and carbohydrates may also increase CO_2 resistance from intercellular air space to carboxylation sites in the chloroplasts (Parkhurst 1994, Hanba et al. 1999). Therefore, leaves of tall trees may have reduced levels of CO_2 within their chloroplasts relative to smaller trees.

Although the importance of leaf trait variation with tree size and age is increasingly recognized (Yoder et al. 1994, Bond 2000, Kolb and Stone 2000, Thomas and Winner

2002, Koch et al. 2004, Martinez-Vilalta et al. 2007, Merilo et al. 2009), quantitative characterization of changes in leaf traits throughout multiple life stages is still rare (Rijkers et al. 2000, Niinemets 2002, Thomas 2010). Moreover, whereas changes in leaf traits late in tree ontogeny (or comparisons of juvenile to mature trees) have received considerable attention, this is less true for trait changes during the transition from sapling to pole to intermediate-sized to mature trees. Given that some leaf trait changes in early and mid-life may differ from those late in life (Grulke and Miller 1994, Fredericksen et al. 1996, Thomas and Winner 2002, Thomas 2010) both in pattern and in causal agents, it is important to improve our understanding in this area. Furthermore, although most models of forest ecosystem dynamics assume that the properties of leaves do not vary with tree size, if this assumption is untrue, as considerable evidence suggests (Yoder et al. 1994, Bond 2000, Thomas and Winner 2002, Koch et al. 2004, Thomas 2010), the development of generalized relations of leaf traits to tree size for differing tree functional types (conifers, evergreen angiosperms, deciduous angiosperms) will be required before improved models can be advanced.

To advance our understanding, we attempted to build on the foundation of prior studies, many of which did not account for size-related differences in light environments and often made comparisons of few size classes (i.e. seedlings versus adults) or few measured traits. We expanded on these studies by focusing on traits, life history stages, species types, and conditions less studied in prior research of leaf traits and tree size. Specifically this included (a) a wider range of leaf traits than assessed in many prior studies, including size, LMA, photosynthetic capacity, dark respiration and nutrient content (b) fine twig traits (respiration, chemistry) that have rarely if ever been reported in relation to tree size, (c) a focus on the continuous transition from juvenile to reproductive middle-age, (less studied than old age or the two-stage contrast between seedling and adult), (d) evaluating patterns and incident light availability for both sun and shade plants to better address the role of varying light environment among tree sizes, and (e) assessment of whether changes in multiple traits with tree size are consistent with bivariate trait relations common among species as characterized by the leaf economics spectrum (Reich et al. 1997, Wright et al. 2004).

We measured the above chemical, structural, and gas exchange data in three common temperate deciduous forest tree species (*Quercus ellipsoidalis*, *Populus tremuloides* and *Prunus serotina*) in east-central Minnesota, USA. In order to determine whether size-related changes occur independent of light environment, we measured seedlings, saplings and mature trees growing in both dense and sparse trees patches. The following questions were addressed: (i) Do morphological (area, mass, and their ratio) and carbon flux rates and related traits (i.e. photosynthesis, respiration, nitrogen content) of leaves and fine stems vary with increased tree height? (ii) Are such patterns similar or different for different traits, such as for respiration and photosynthesis or for leaves vs. stems? (iii) Do tree-size related patterns of leaf and stem traits reflect coordination among traits? (iv) Are such patterns and coordination similar for the three sympatric tree species? We hypothesized that (H1) gas exchange rates and associated functional traits such as tissue N concentration and LMA would vary with tree size in a pattern reflecting declining function in taller trees, yet maintaining (H2) bivariate trait relations common among species as characterized by the leaf economics spectrum. Taking these two ideas together yielded a third, integrated hypothesis that (H3) nitrogen content and both mass- and nitrogen-based photosynthesis and respiration should decrease monotonically with tree size and leaf mass per area should increase.

Materials and Methods

Field site description

This study was conducted in August 2007 at Cedar Creek Ecosystem Science Reserve (CCESR) in east-central Minnesota, USA (45°24' N, 93°12' W). CCESR is located on the Anoka Sandplain, a glacial outwash area characterized by coarse textured soil low in nitrogen (Pastor et al. 1987). This area is located in the transition zone between the central grasslands and the mixed deciduous forest of eastern North America. Cedar Creek's woodland/savanna habitats contain ten tree species, most commonly northern pin oak (*Quercus ellipsoidalis* E. J. Hill) and bur oak (*Quercus macrocarpa* Michx.), with species such as quaking aspen (*Populus tremuloides* Michx.), black cherry (*Prunus serotina* Ehrh.) and red maple (*Acer rubrum* L.) moderately abundant (Peterson and Reich 2001). We chose to study red maple, northern pin oak and black cherry because they were commonly found growing in both gap and understory microenvironments across a wide range of tree size. Red maple is classified as shade tolerant, while northern pin oak and black cherry are mid-tolerant, with the oak more drought tolerant than the others (Baker 1949).

We determined the height of individuals ranging in size from small seedling to reproductive age growing in an intact forest. Height of saplings <2.0 m tall was measured as the vertical distance between the stem base and the highest live meristem. For individuals taller than 2 m, height was measured to the highest live leaf. To measure height, we used a folding wooden ruler for plants <2 m tall, and an expandable measuring pole for taller individuals. For each species, 4-5 individuals per size class (<0.5, 0.5-1.5, 1.5-3.0, 3.0-6.0, 6.0-10.0 and >10.0 meters) were sampled in both gap (*n* = 30) and understory (*n* = 24) microhabitats to enable the evaluation of possible light differences as a potential driver of size-related differences in leaf and stem properties. Individuals were chosen that were found growing close to one another in dense or sparse tree patches to reduce the amount of environmental heterogeneity (i.e. soil moisture, soil nutrients, etc.) in the measurements. Sampling occurred over the course of four weeks to control for seasonal variation in traits. Representative leaf and stem samples were collected from the upper 1/3 of the canopy of trees to reduce differences in incident light. We measured

fully-expanded leaves with minimal damage from physical abrasion and herbivory, and only current-year stem growth from the apex of branches was measured.

Measuring light availability

We measured the instantaneous percentage of above-canopy photosynthetic photon flux density (%PPFD) above each tree using paired quantum sensors (LI-190s, Li-Cor Inc., Lincoln, Neb.) attached to data-loggers (LI-1000, Li-Cor Inc., Lincoln Neb.). This method provides a reliable and accurate estimate of average canopy openness differences and of integrated quantum flux density (Machado and Reich 1999) and was chosen for practical reasons due to the extended length of the cable on the sensor, which allowed it to be extended into the forest canopy to obtain readings above tall trees. Measurements were made on uniformly overcast days, with one sensor placed in a large, nearby clearcut, and the other placed at the measurement point.

Measuring functional traits

Light saturated photosynthesis (A_{max}) was measured on attached foliage of juvenile trees using an LI-6400 (LI-COR, Lincoln, NE). For mature trees whose leaves could not be reached with a ladder, pole pruners were used to detach branches, which were immediately recut under water and used for photosynthesis measurements. Photosynthetic rates were determined for a subset of leaves prior to and following detachment to ensure that measurements on recently detached foliage were reliable. Environmental conditions were controlled within the leaf cuvette as follows: CO_2 concentration of 380 μmol mol^{-1}, 45-65% relative humidity and block temperature of 25ºC. Irradiance was held at 1500 μmol m^{-2}s^{-1} using the LI-6400 LED light source.

Leaf and stem dark respiration rates (Rd) were measured using samples that were collected at the same time each day (between 08.00 and 09.30 h) to control for potential diurnal variation in leaf carbohydrate status. Immediately following detachment, samples were placed in zip-closure plastic bags with moistened paper towels and stored at 10ºC. In a laboratory, sample bags were transferred to a darkened chamber at 25ºC for 30 minutes prior to Rd measurements. Measurements were completed on the same day as sampling using an LI-6400 with the conifer chamber attachment. Prior research indicates

that respiration rates are similar for detached and attached leaves for up to six hours following cutting (Mitchell et al. 1999, Lee et al. 2005, Machado and Reich 2006). Leaf and stem samples were placed within the conifer chamber, with environmental conditions controlled as above. Samples were kept inside the chamber for approximately 10 minutes, and measurements were recorded only after readings had stabilized.

Following gas exchange measurements, leaves were scanned and the projected area was determined using the image-processing software ImageJ (Abramoff et al. 2004). The basal diameter (i.e. the base of the current-year's growth) and length of each stem segment was measured. All samples were oven-dried at 70ºC for at least 72 hours to determine dry leaf mass for calculation of specific leaf area (SLA, $cm^2 g^{-1}$) and stem dry mass. Samples were ground and analyzed for nitrogen concentrations (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA).

Statistical analysis

Analyses for all relationships were based on log-log transformed data, which was necessary to make the variances equal across tree size and to roughly normalize the data. Variation in functional traits as a function of tree height, %PPFD and their two-way interaction were analyzed by multiple regression analysis. In order to assess the effect of tree height, all traits (gap, understory and pooled groups) were fit with both linear and polynomial functions based on the findings of Thomas 2010, who showed that many traits follow a unimodal, hump-shaped (rather than linear) patterns with tree size. Fits of both model types were compared and that which explained the data best (higher R^2-value, significant parameters) was used. Additional analyses examined the bivariate relationships between all measured functional traits. All statistical analyses were conducted in JMP statistical analysis software (JMP 5.0.1, SAS Institute, Cary, NC).

Results

%PPFD vs. tree size as a predictor of trait patterns

If traits such as photosynthesis, nitrogen content and leaf mass per unit area (LMA) vary with tree size (Carswell et al. 2000, Rijkers et al. 2000, Niinemets 2002, Kenzo et al. 2006), it is possible that this is due in part to variation in light availability, if leaves sampled from seemingly similar canopy positions are at different (and most likely higher) light levels on taller trees. It is of interest to know both whether leaf traits vary with tree size (regardless of causes) and whether this is due to size per se, to co-variation with light environment, or both. To evaluate this, we measured the instantaneous percentage of above-canopy photosynthetic photon flux density (%PPFD) above each tree (Figure 1.1). In our survey, %PPFD was significantly positively correlated with height in *Acer rubrum* (both gap and understory groups) and in *Prunus serotina* trees in gaps. Light was moderately correlated with height ($p < 0.10$) in *P. serotina* trees in the understory, but there was no correlation in *Quercus ellipsoidalis*. However, these gradients in light were relatively modest across the gradient of tree size, especially for *P. serotina*. As described below, many tissue traits were significantly related to tree height; thus, it is important to clarify whether these relationships were due in part to co-variation with %PPFD and height. To determine this, we conducted multiple regressions for each trait using %PPFD, height and their two-way interaction as explanatory variables (traits in gap and understory groups were run separately, data not shown). The results of these regressions led us to conclude that most of the relationships of tissue traits with tree height are only very modestly or not at all a consequence of co-variation in light and that there are strong effects of tree height once light is accounted for. For example, the strongest light effect involved LMA, which was significantly affected by %PPFD in *Acer rubrum* gap and understory trees and in *Prunus serotina* gaps (where the relationships between %PPFD and height were strongest and thus of most concern). Multiple regression analyses of the relationship between LMA and both height and %PPFD using pooled data (gap and understory) indicate that height is still significant (positively) for all three species once light is accounted for. For other traits such as leaf nitrogen content and most gas exchange rates, %PPFD was not significant in models that include tree height, or was not retained in stepwise regression models that identified tree height as the sole significant

variable. In the remaining presentation of results we do not consider further this co-variation with light, but we do briefly revisit this in the discussion.

Leaf morphology and chemistry

Leaf mass per unit area (LMA) increased log-linearly with height for all three species in both gap and understory groups (Figure 1.1; Appendix Table A.1). LMA is affected by the area and mass of individual leaves, both of which also increased linearly with height for all three species in understory groups (Figure 1.2). In gaps, both leaf area and mass increased in *A. rubrum*, leaf mass increased in *Q. ellipsoidalis*, and no trends were observed in *P. serotina*. Similar to the results for LMA, area-based leaf nitrogen content (N_{area}) increased significantly with height in all species and groups, while mass-based nitrogen content (N_{mass}) increased in both gap and understory groups for *Q. ellipsoidalis* and *A. rubrum*, but not for *P. serotina* (Figure 1.3).

Leaf gas exchange

Area- and mass-based photosynthetic rates (A_{area} and A_{mass}, respectively) varied most significantly with height in *A. rubrum* gap and understory groups, where trait relationships were best explained using polynomial functions with significant 2nd-order terms in all cases (Figure 1.4; Appendix Table A.2). *Q. ellipsoidalis* showed weaker but qualitatively similar patterns in A_{area} and A_{mass} in gaps and when the gap and understory data were pooled, while photosynthetic rates of *P. serotina* did not appear to follow this trend. The ratio of photosynthesis to dark respiration (A_{max}/R_d) showed similar responses across species with the strongest patterns observed in all *A. rubrum* groups, a significant trend in the *Q. ellipsoidalis* pooled data, and no significant pattern for *P. serotina*. Photosynthesis expressed per unit nitrogen (A_{max}/N) showed a significant height relationship and 2nd-order polynomial term for the pooled *A. rubrum* data. In contrast, A_{max}/N declined with tree size in *P. serotina*, as evidenced by the non-significant 2nd-order terms and negative height coefficients in the models.

Both area-based and mass-based leaf dark respiration rates (Rd_{area}) declined with height in most species groups (Figure 1.5). Leaf dark respiration expressed per unit nitrogen (R_d/N) followed a pattern similar to Rd_{mass}, exhibiting significant linear declines

15

with height in all species groups, except for *P. serotina* in gaps where the decline was marginally significant.

Stem traits

Stem diameter showed a linearly increasing relationship with height in *Q. ellipsoidalis* and *A. rubrum* gap and understory groups, but did not vary in *P. serotina* (Figure 1.6). Stem N_{mass} also increased linearly with height in *Q. ellipsoidalis* gaps and *A. rubrum* understories and pooled data, and showed a marginal increase in *P. serotina* gaps. However, stem Rd_{mass} declined with height in *Q. ellipsoidalis* pooled data and *A. rubrum* understories, but did not vary in *P. serotina*. Stem R_d/N showed similar trends, with decreases in *Q. ellipsoidalis* gaps and pooled data and *A. rubrum* understories and pooled data, but no relationship in *P. serotina*.

Bivariate trait relationships

The traits that were most strongly correlated (Appendix Table A.3) for all species in the pooled (per species) data sets were LMA in relation to area-based leaf traits (N_{area}, A_{area} and Rd_{area}). Mass-based leaf traits rarely showed significant correlations with each other or with LMA. Stem traits were also very weakly correlated, with only stem Rd_{mass} and N_{mass} exhibiting a clear positive relationship in pooled *P. serotina* data.

16

Discussion

Our data demonstrate that a number of important leaf and stem traits such as leaf size, leaf mass per unit area, nitrogen content, photosynthetic capacity, and dark respiration rates vary significantly with tree size in our three sampled deciduous tree species. These results support our overarching hypothesis that leaf and stem traits vary with increased tree height, but patterns and coordination among traits were less consistent than we expected or predicted in specific hypotheses 1-3. Both leaf (Rd_{area} and Rd_{mass}) and stem respiration rates and dark respiration per nitrogen content (Rd/N) were found to decline in larger sized individuals and were best described by linear relationships, which was consistent with our predictions from H1 and H3. In contrast, other traits expected to be closely linked to gas exchange (i.e. leaf and stem N) and morphological traits that reflect investment in leaf structure and defense (i.e. LMA) increased monotonically with tree size. The results for LMA were as predicted in H1 and H3, but those for leaf and stem N were the opposite of those predictions, and also rejected H2, which assumed parallel declines with size for respiration and N, rather than inverse relations. We observed a unimodal or "hump-shaped" relationship between photosynthetic capacity (both A_{area} and A_{mass}) and tree height in two of the three temperate deciduous species measured. This pattern did not mirror any of the other traits, including those such as %N that it was expected (H2) to be linearly related to, and also rejected H1 and H3. Although one of the three species (*Prunus serotina*) showed greater variability in most trait relationships than the others, the trends were generally consistent among species.

In our study, individuals of all size classes were measured in both gap and understory microhabitats in order to determine whether differences in light availability have an effect on size-related differences in leaf and stem properties. In other studies it is possible that unaccounted co-variation in ambient light may have occurred and could be at least in part responsible for tree-size patterns when light was not assessed or included as a statistical covariate (Carswell et al. 2000, Kenzo et al. 2006). In our study, tissue measurements were made on the upper leaves of all trees measured so as to maximize their light exposure within each habitat. However, light availability is known to be less for juvenile trees even in very large gaps relative to light availability in the upper canopy (Canham et al. 1990, Sipe and Bazzaz 1994), causing a predictable correlation between tree size and

17

incident light. It is well-established that leaf morphology and physiology are influenced by ambient light conditions (Boardman 1977, Givnish 1988), and that variations in light availability are significant predictors of variation in many leaf traits within forest canopies (Walters et al. 1993a, Kitajima 1994, Rijkers et al. 2000, Kitajima et al. 2005). Therefore, our results (and likely those of many prior studies) are likely to be affected by both tree size as well as ambient light conditions. However, our analyses found that tree size per se was a stronger driver of leaf and stem traits than light differences for similar crown positions of trees differing in size. Our analyses that included %PPFD as a covariate indicated strong effects of tree size independent of %PPFD, and in many cases the effect of %PPFD was not significant. Additionally, if light availability were driving apparent ontogenetic patterns, one might expect to find exclusively monotonic relationships between tree height and leaf and stem traits, as opposed to the unimodal patterns we observed in photosynthetic capacity (and also seen in Thomas 2010).

Most research that has aimed at describing ontogenetic variations in leaf photosynthetic traits across a wide range of tree sizes has focused on conifers, and the majority of these studies have reported declines in photosynthetic rates with tree size and age (Thomas and Winner 2002, Martinez-Vilalta et al. 2007, Merilo et al. 2009, Drake et al. 2010). In contrast, broadleaved species have generally been reported to exhibit increased area-based photosynthetic rates in larger trees (Thomas and Winner 2002, Ishida et al. 2005), while mass-based photosynthesis peaked in sapling-sized individuals (Ishida et al. 2005). Contrary to most of these previously observed patterns, we found that photosynthetic rates exhibited unimodal relationships as a function of tree size, consistent with the recent findings of Thomas (2010). Similar to what we observed, Thomas (2010) found that leaf photosynthetic traits were better described using unimodal relationships than simple linear regressions, and in fact he noted that many prior studies would have benefitted from using this type of fit in their analyses (e.g. Niinemets 2002, Nabeshima and Hiura 2008).

Despite the unimodal patterns observed in photosynthetic capacity, leaf N content exhibited a size-dependent increase, even in the tallest individuals. This was unexpected, given the strong correlation that is often observed between photosynthetic rates and leaf nitrogen (Gulmon and Chu 1981, Chazdon and Field 1987, Reich et al. 1994). The

increase in leaf N in taller individuals may be due to investment in other properties rather than photosynthetic enzymes, such as cell-wall material to strengthen leaf structure. Wind exposure and physical abrasion are likely to be higher in canopy trees than they are in the understory, favoring more robust leaves with higher LMA in the canopy (Thomas and Winner 2002). Increased LMA in taller trees has been observed previously and attributed to a reduction in turgor-driven cellular expansion (Koch et al. 2004, Woodruff et al. 2004). This increase in LMA with height is commonly attributed to physiological differences between sun and shade foliage (Harley et al. 1996, Koch et al. 2004, Ambrose et al. 2009). However, we observed this pattern in leaves measured in both gap and understory environments, implying a physiological difference based on height such as reduced turgor, independent of physiological acclimations to light availability.

Our hypothesis that leaf and stem respiration rates would decline with tree size was based on empirical evidence showing a positive relationship between total Rd (maintenance plus growth respiration) and relative growth rates (Givnish 1988, Poorter et al. 1990, Walters et al. 1993a) and negative relationships of both to plant size. Our observations of respiration rates support this evidence, but contradict the positive relationship that is commonly observed between tissue nitrogen concentration and respiration rates (Merino et al. 1982, Waring et al. 1985, Ryan 1991, Reich et al. 2008). Instead, we found that tissue Rd declined with increasing N concentrations. To further assess whether the decline in Rd could be explained by tree size rather than N, we ran a stepwise backward regression using %PPFD, N_{mass}, and height as factors and found that only height was significant. This indicates that increased tree height, independent of %PPFD and N_{mass}, is driving the observed decline in tissue respiration rates.

Why were the hypothesized parallel changes in various leaf traits so often unsupported by the evidence? It is possible that increasing N, shifts in allocation of N, increasing LMA, and increasing hydraulic limitations (not measured here) with size act to offset each other in terms of effects of net CO_2 exchange rate. All else being equal, increasing N should be associated with increasing photosynthesis and respiration (Gulmon and Chu 1981, Merino et al. 1982, Ryan 1991, Reich et al. 1994), but a lower fraction of that N being allocated to photosynthetic machinery with size could offset that trend. Additionally, as increasing tissue density and/or thickness lead to lower maximum

photosynthetic rates due to internal resistance and/or self-shading (Givnish 1988, Parkhurst 1994, Hanba et al. 1999), increasing LMA with size would "push" net CO_2 exchange rates in an opposite direction than rising tissue N would. Subtle or large size-related differences among species or light habitats in N, its allocation, and LMA, could occur, and result in different impacts on gas exchange rates. For instance, *Prunus serotina* had the largest response of LMA to size and no variation in mass-based leaf N content with size, and consistent with the ideas above, it showed the most negative relationship of mass-based photosynthesis to size. Additionally, if hydraulic influences dampen gas exchange rates in taller trees (Grulke and Miller 1994, Yoder et al. 1994, Fredericksen et al. 1996, Hubbard et al. 1999), this could help explain the plateauing, and sinking, rates of photosynthesis at the tallest tree sizes, despite more linear variation in drivers such as N and LMA.

It is less clear why respiration rates of both leaves and stems should decrease with size, especially as their N levels increase, though a study of *Nothofagus nitida* trees ranging in size from 0.1 to 7 m in height showed that N increased significantly with tree size while respiration rates did not vary (Coopman et al. 2011). It is possible that a greater fraction of the N is allocated to non-metabolic functions with increasing size. It is also possible that respiration in leaves and fine stems higher in canopies are more substrate-limited, but additional research is needed to assess this possibility.

In conclusion, leaf and stem traits vary with increased tree height, independent of variations in light availability, but not in the consistent manner associated with a universal shift towards a lower "return on investment" strategy. Instead, species traits vary with size in patterns that likely reflect complex variation in water, light, nitrogen, and carbon availability, storage, and use; and consequential impacts on the morphology, chemistry and metabolism of leaves and twigs. This conclusion has implications for improving models of forest ecosystem dynamics, which assume that the properties of leaves do not vary with tree size.
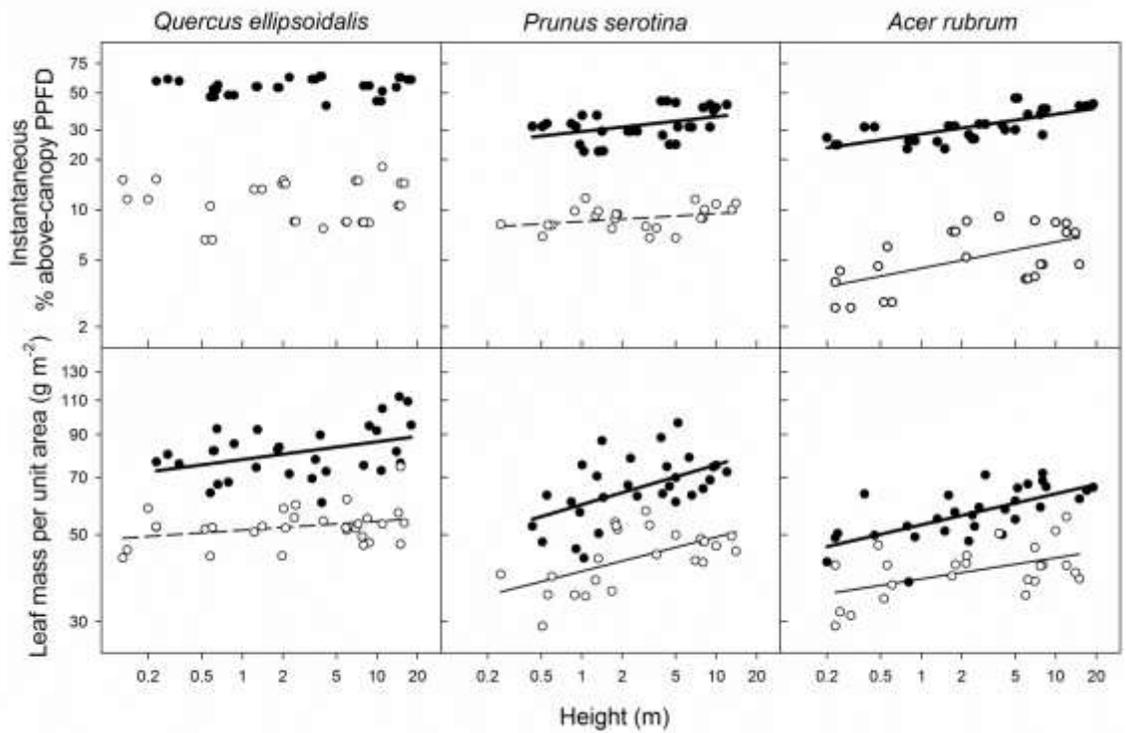
Figure 1.1. Relationships between instantaneous percent above-canopy photosynthetic photon flux density (%PPFD) and tree height, and leaf mass per unit area (LMA) and tree height for three temperate deciduous trees; lines are linear regressions fitted to the log-log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed understory. Solid lines indicate regressions significant at a *p*-value <0.05; dashed lines at a *p*-value of <0.10. R^2 and *p*-values are given in Appendix A.1.
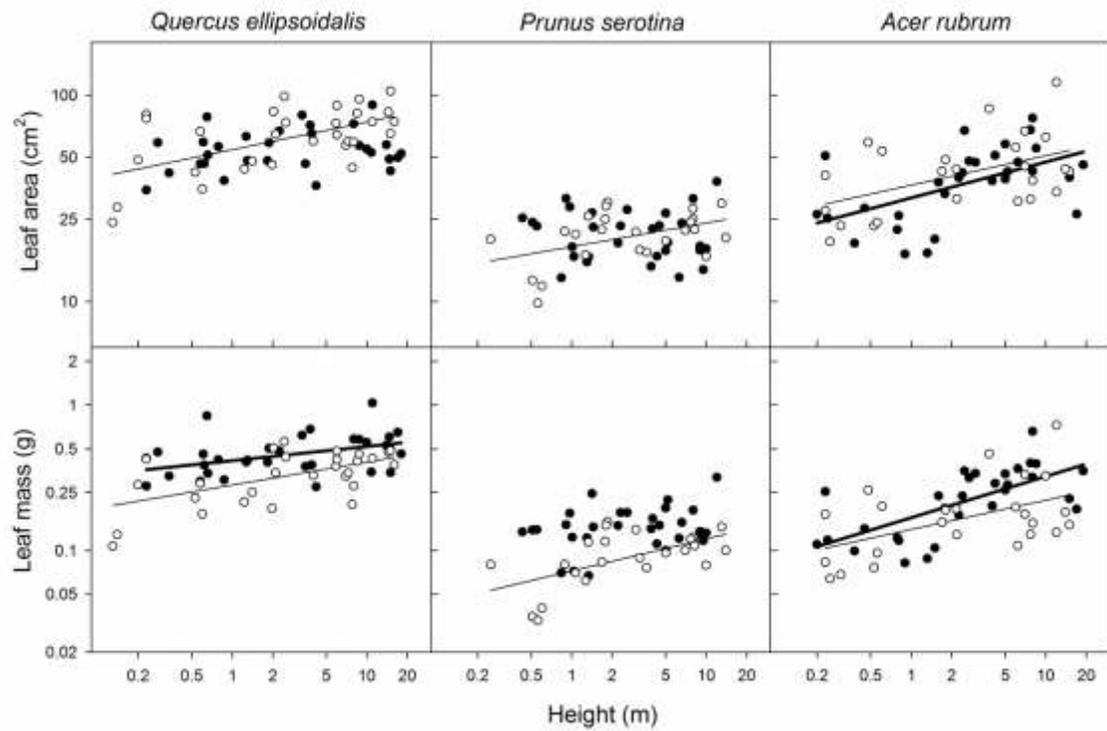
Figure 1.2. Relationships between leaf area and tree height, and leaf mass and tree height for three temperate deciduous trees; lines are significant linear regressions ($p <$ 0.05) fitted to the log-log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed understory. R^2 and *p*-values are given in Appendix A.1.
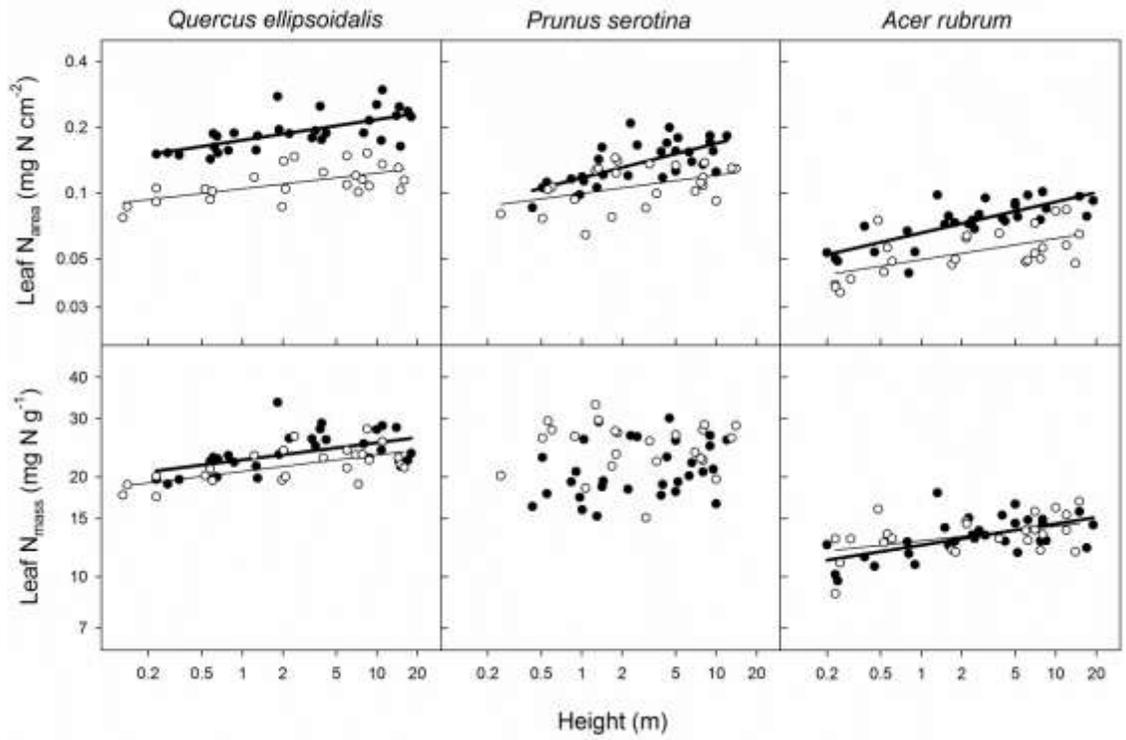
Figure 1.3. Relationships between leaf nitrogen content (expressed on the basis of leaf area (N_{area}) and leaf mass (N_{mass})) and tree height for three temperate deciduous trees; lines are significant linear regressions ($p < 0.05$) fitted to the log-log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed understory. R^2 and p-values are given in Appendix A.1.
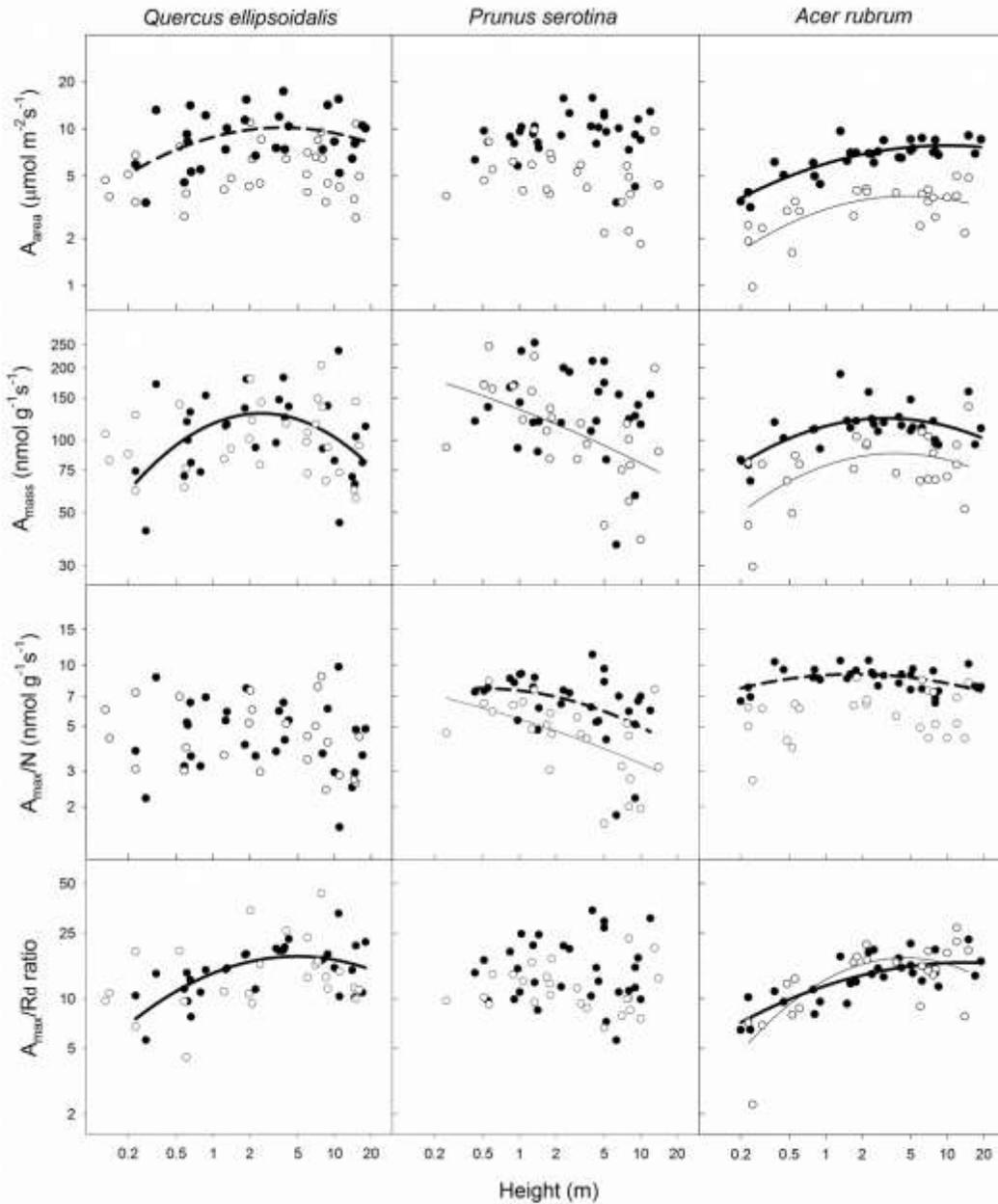
Figure 1.4. Relationships between photosynthetic capacity (expressed on the basis of leaf area (A_{area}) and leaf mass (A_{mass}), photosynthesis expressed per unit nitrogen (A_{max}/N), and the ratio of photosynthesis to dark respiration (A_{max}/Rd) and tree height for three temperate deciduous trees; lines are 2nd order polynomial regressions fitted to the log-log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed understory. Solid lines indicate regressions significant at a p-value <0.05; dashed lines at a p-value of <0.10. R^2 and p-values are given in Appendix A.2.
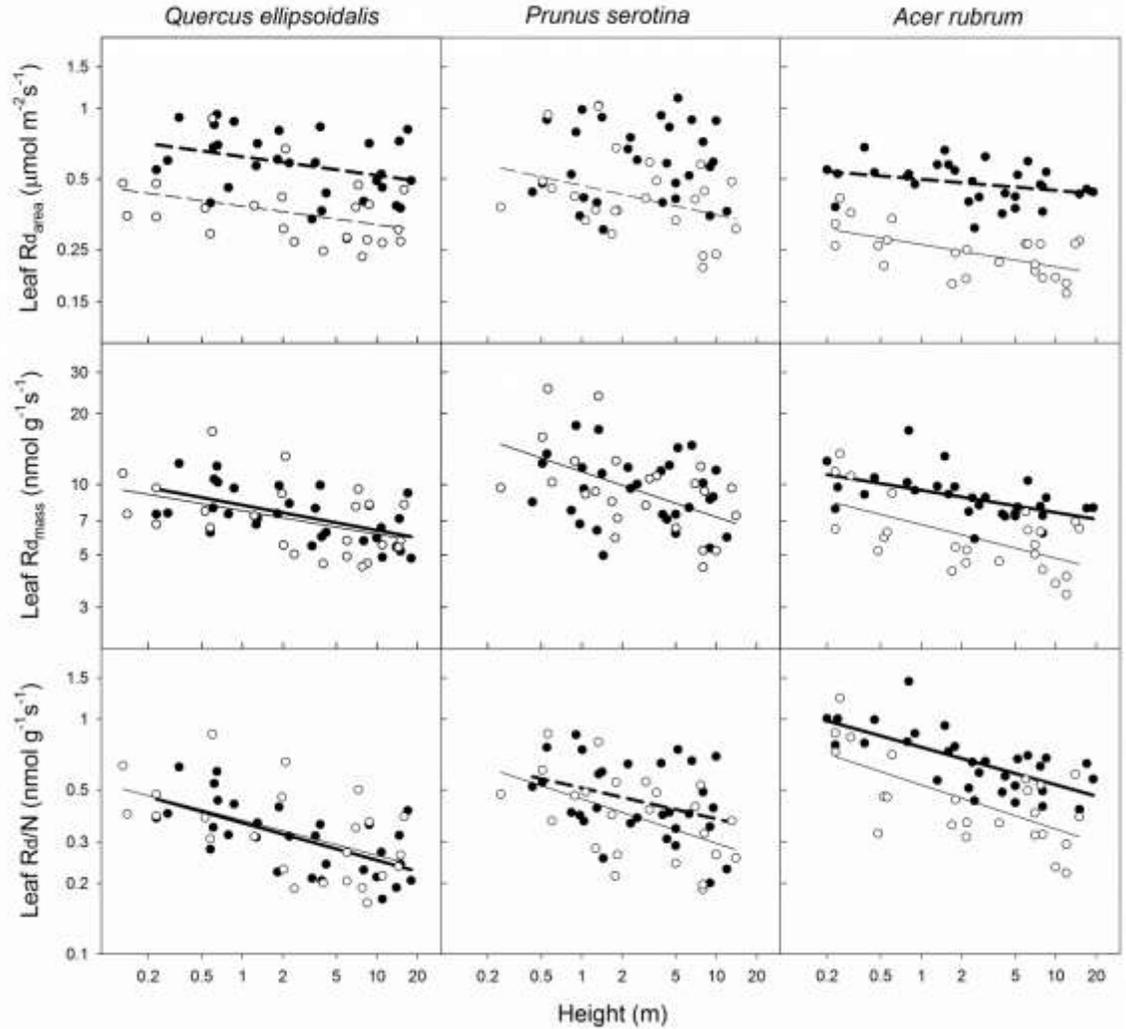
Figure 1.5. Relationships between dark respiration expressed on the basis of leaf area (Rd$_{area}$) and leaf mass (Rd$_{mass}$) and tree height, and dark respiration expressed per unit nitrogen (Rd/N) and tree height for three temperate deciduous trees; lines are linear regressions fitted to the log-log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed understory. Solid lines indicate regressions significant at a *p*-value <0.05; dashed lines at a *p*-value of <0.10. R^2 and *p*-values are given in Appendix A.1.
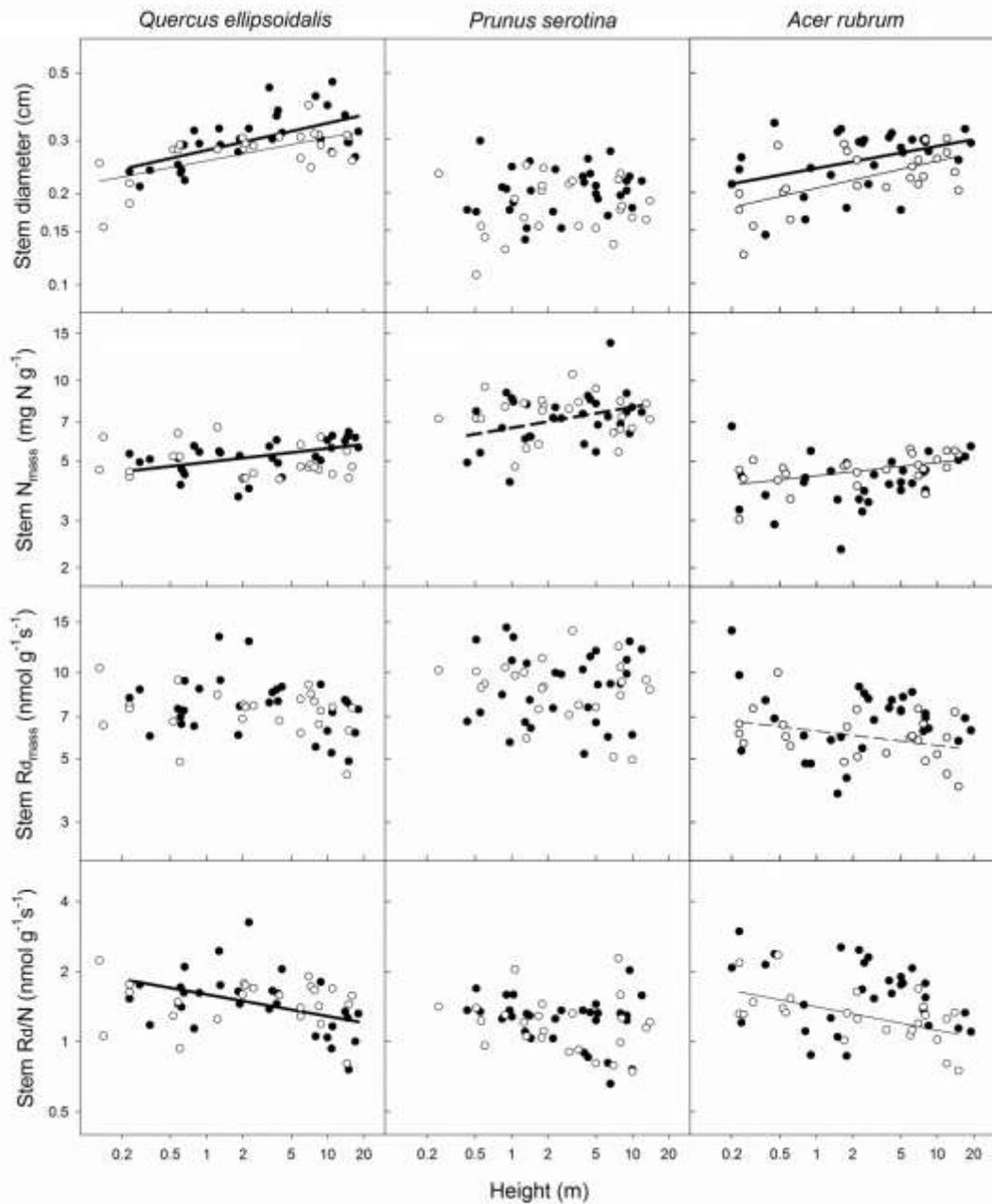
Figure 1.6. Relationships between stem diameter, stem nitrogen content (stem N_{mass}), stem dark respiration (stem Rd_{mass}), and stem respiration expressed per unit nitrogen (stem Rd/N) and tree height for three temperate deciduous trees; lines are linear regressions fitted to the log-log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed understory. Solid lines indicate regressions significant at a *p*-value <0.05; dashed lines at a *p*-value of <0.10. R^2 and *p*-values are given in Appendix A.1.

CHAPTER 2

Ontogenetic variation in traits and net carbon balance of shaded *Acer saccharum* saplings

with C.H. Lusk and P.B. Reich

Shade tolerance is often assumed to be a fixed trait of a species, though recent work suggests that plant size affects the light requirements and carbon balance of many tree species. We hypothesized that whole-plant light compensation points would be positively related to sapling size while net carbon gain would decline, due in part to increased self-shading and a higher proportion of woody structural support tissues in larger plants. We quantified the aboveground biomass distribution of 18 *Acer saccharum* juveniles (14 to 272 cm tall) growing in low light (6 to 8% of above-canopy PPFD) in the forest understory. Gas exchange rates and nitrogen content of leaf and stem tissues were measured, and the crown architecture of each individual was recorded using a 3-dimensional digitizer. YPLANT was used to estimate the self-shaded fraction of each crown and to model net carbon gain. Both self-shading and stem mass fraction increased with sapling size, which should reduce net carbon gain. However, these were offset to a degree by positive relations of leaf nitrogen and photosynthetic capacity with sapling size and by significant declines in stem nitrogen and dark respiration rates as sapling height increased, reflecting a shift in the distribution of stem diameter sizes from smaller (with higher average N and respiration) to larger diameter classes. Nonetheless, increased proportions of woody biomass had the largest impacts, and net carbon gain per gram plant tissue declined with increasing plant size. As a result, the whole-plant light compensation point increased. Our results suggest that significant ontogenetic variations in light requirements and carbon gain occur even in a shade tolerant species, indicating that work on saplings may detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone.

Introduction

Species differences in shade tolerance are often invoked to explain successional patterns in forests, where shade-intolerant species that require relatively high light levels are replaced by species that can successfully reproduce and grow in darker environments (Bazzaz 1979, Givnish 1988, Pacala et al. 1994). This progression is a necessary component of many of the classic theories of succession, in which forests become increasingly dominated by shade-tolerant individuals in the absence of large-scale disturbances (Connell and Slatyer 1977, Whitmore 1989).

Although shade tolerance is often assumed to be a fixed trait of a species when attempting to explain these patterns, recent work suggests that light requirements of species may change as individuals increase in size. For example, a survey that measured the average light environments inhabited by 47 tropical tree species found that adults of most species grew in areas with greater light availability than saplings (Poorter et al. 2005). Another study of 13 temperate rainforest species that examined juvenile trees less than 120 cm growing in forest understories found similar results, with over half the species measured occupying brighter environments as they grew larger (Lusk et al. 2008), suggesting that whole-plant light compensation points of those species may increase with ontogeny. Alternatively, taller individuals may simply be overtopping neighboring plants and reaching naturally brighter environments within the forest canopy as they grow larger (Poorter et al. 2005). A study of seven southern boreal species indicated that whole-plant light compensation points are directly affected by tree size, with most species having a greater risk of growth-dependent mortality and diminished relative differences in shade tolerance among species in larger trees (Kneeshaw et al. 2006). Lusk et al. (2011) also found that net carbon gain per area foliage declined with tree size in juvenile temperate rainforest species. These studies support the idea that shade tolerance is a dynamic, rather than fixed plant trait, but a lack of quantitative, comparative studies examining low-light carbon balance has limited our ability to evaluate how whole-plant carbon gain is affected by ontogeny.

Ontogenetic variation in light compensation points could be driven by variation in biomass distribution, even if tissue level traits such as leaf respiration remained static throughout all life stages. The fractional investment of annual plant growth allocated to

leaves generally declines with increasing plant size (Sterck and Bongers 1998). As a result, both the leaf area ratio and the ratio of photosynthetic to respiratory tissues decrease in larger trees (Gerrish 1990, Delagrange et al. 2004, Lusk et al. 2004, Machado and Reich 2006). Therefore, trees would exhibit an overall decline in carbon uptake with increasing size even if gas exchange rates remain constant in larger trees. However, many studies have shown that tissue level traits also change with changing tree size. For instance, laboratory studies have shown that as first-year seedlings increase in size, their growth rates typically decrease and this is accompanied by declining dark respiration rates (Walters et al. 1993b, Tjoelker et al. 1999a). Two studies examining cold temperate tree species found that stem and whole-plant nitrogen (N) concentrations declined with increased sapling size, although respiration rates (R_d) of the same tissues increased in one study (Machado and Reich 2006), and declined in the other (Sendall and Reich in prep). Sendall and Reich (in prep) also found that small saplings had higher N content and photosynthetic rates (A), but lower leaf R_d than seedlings, suggesting that carbon gain at the leaf level may remain constant or even increase with size in juvenile trees. Alternatively, in a study comparing seven temperate evergreen species, self-shading increased with size and net carbon gain declined (Lusk et al. 2011), indicating that both the increase in self-shading and declining proportion of photosynthetic tissues may drive the decline in whole-plant carbon gain and shade tolerance.

Few shade tolerance studies have integrated the effects of crown architecture on net carbon gain (but see Lusk et al. 2011). While traits such as leaf-level gas exchange and total leaf area are predictors of whole-plant carbon gain, crown architecture also influences carbon uptake (Falster and Westoby 2003). In this study, we evaluated the relationship between plant size and net daily carbon gain of juvenile sugar maples growing in low light. We used hemispherical photography and measurements of plant architecture and gas exchange to model potential carbon gain with the YPLANT program (Pearcy and Yang 1996). After harvesting the plants, we quantified aboveground biomass distribution, leaf and stem R_d, and tissue N concentrations. We tested the following hypotheses: (H1.1) the fraction of aboveground biomass distributed to foliage declines with increased sapling size, as does the ratio of small diameter to large diameter stem tissue, while (H1.2) self-shading increases with size. (H2.1) Leaf N and related gas

exchange rates (A and R_d) per gram foliage tissue increase with increasing sapling size, but (H2.2) stem N and associated dark respiration rates per gram stem tissue decline. As a result of H1 and H2, (H3.1) whole-plant N and dark respiration rates per gram plant and (H3.2) net daily carbon gain per gram plant will decline in larger saplings, while (H3.3) whole-plant light compensation points will be positively related to plant size (i.e. larger individuals will require higher light levels to maintain positive carbon gain).

Materials and Methods

Study site and species

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

We studied 18 sugar maple seedlings and saplings ranging in height from 14 to 272 cm found growing in the forest understory. Height was measured as the vertical distance between the stem base and the highest live meristem. Individuals were chosen that were found growing within a 20 m^2 area, to reduce the amount of environmental heterogeneity (i.e. soil moisture, soil nutrients, etc.) among different individuals.

Hemispherical photography

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

We were also interested in plant performance under differing light regimes, so in addition to canopy files for ambient light conditions, we developed files representing

31

standardized light regimes (approximately 2%, 4%, 6%, and 8% of above-canopy PPFD) by averaging data from up to five hemispherical photos taken beneath intact deciduous canopies in northern Minnesota (cf. Falster and Westoby 2003).

Modeling carbon gain

The three-dimensional crown arrangement of each tree was recorded using an extended range Liberty 3D-digitzer (Polhemus, Colchester, Vermont, USA) paired with the FLORADIG software package (CSIRO Entomology, Brisbane, Australia) as described by Falster and Westoby (2003). A virtual reconstruction of each plant is achieved by recording a series of point coordinates associated with each leaf or stem segment, as well as the relative connectivity between points. The 3D spatial coordinates are recorded using a magnetic signal receiver and pointer, which can accurately record points within a 2.25m radius from the receiver. Stem and petiole arrangements are described by their length, diameter, elevation angle, and azimuth, while leaves are described by their length, azimuth, and the elevation angles of two vectors on the lamina surface (Falster and Westoby 2003).

The YPLANT software (Pearcy and Yang 1996) was used to model net daily carbon gain of foliage. The 3D crown arrangement recorded for each individual in FLORADIG was converted to the appropriate YPLANT format using a program written in the C programming language (Falster and Westoby 2003). YPLANT inputs are a description of the leaf shape, leaf photosynthetic capacity and respiration rate, the geometry of the crown arrangement, and a description of the canopy structure above the plant which is used to estimate light interception. A solar movement submodel is used to estimate PPFD (μmol photons m^{-2} s^{-1}) intercepted by each leaf surface at different times of day. A photosynthetic submodel estimates the resulting assimilation rate using PPFD response curves generated from measurements of gross photosynthetic capacity and dark respiration rates (Thornley 1976).

YPLANT was used to estimate leaf overlap (i.e. self-shading) since it influences light interception by plant crowns. YPLANT output includes the projected leaf area of each tree, which does not take into account any leaf overlap, as well as the displayed leaf area, which is the effective leaf area for light interception (Pearcy and Yang 1996). The

fraction of leaf area that was self-shaded was calculated as (PA/DA)/PA, where PA is the projected leaf area and DA is the displayed leaf area.

Gas exchange measurements

Estimates of photosynthetic capacity (A_{max}) and dark respiration rates (R_d) are required for simulations of carbon gain in YPLANT. Photosynthetic light response curves were measured on two consecutive mornings (between 09:00 and 12:00) in July, 2008 on attached foliage of all 18 individuals using an LI-6400 (LI-COR, Lincoln, NE). Environmental conditions were controlled within the leaf cuvette as follows: CO_2 concentration of 380 µmol mol^{-1}, 45-65% relative humidity and block temperature of 25ºC. Leaves were exposed to irradiance levels of 1200, 850, 500, 250, 100, 50, and 0 µmol m^{-2}s^{-1} using the LI-6400 LED light source.

Leaf and woody tissue R_d were measured using harvested tissues. In order to minimize the effect of active growth on R_d, these measurements were made late in the growing season. We harvested all aboveground biomass of six individuals per day between 08:00 and 09:30 local time. Immediately following harvest, the aboveground biomass was wrapped in moist paper towels and placed in dark plastic bags. Samples were taken to a nearby laboratory where they were stored in a walk-in refrigerator at 5°C for no longer than five hours from the time of harvesting to the time of measuring respiration. Prior research indicates that respiration rates are similar for detached and attached tissues for up to six hours following cutting (Mitchell et al. 1999, Lee et al. 2005, Machado and Reich 2006). Each day, the plants were separated into leaves and stems, and tissues were subsampled based on size and amount. Stems were separated into diameter classes of < 2.5 mm, 2.5-5.0 mm, 5.0-7.5 mm, 7.5-10.0 mm, and > 10.0 mm. Sample bags were transferred to a darkened chamber at 25°C for 60 minutes prior to R_d measurements. Measurements were completed on the same day as sampling using an LI-6400 with the conifer chamber attachment. Leaf and stem samples were placed within the conifer chamber, with environmental conditions controlled as above. Samples were kept inside the chamber for approximately 10 minutes, and measurements were recorded only after readings had stabilized.

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

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

where $a = 3.22$ and $b = -0.046$ (Kattge et al. in press). These values were multiplied by day or night length to give daily respiration values. For example, to obtain nighttime respiration we used the following formula:

$$R_N \, (\text{mmol m}^{-2}\text{day}^{-1}) = \frac{R \, (\text{mmol m}^{-2}\text{s}^{-1}) * (24 - \text{day length(h)}) * 3600 \, (\text{sh}^{-1})}{10^3 \, (\mu\text{mol mmol}^{-1})}$$

Following gas exchange measurements, leaves were scanned and the projected area was determined using the image-processing software ImageJ (Abramoff et al. 2004). The length and diameter of each stem segment was measured. All samples were oven-dried at 70°C for at least 72 hours to determine dry leaf mass for calculation of specific leaf area (SLA, $\text{cm}^2\text{ g}^{-1}$) and stem dry mass. Samples were ground and analyzed for nitrogen concentrations (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA). Total leaf mass (g) and total stem mass (g) of all plants were measured. These data were used to determine leaf mass fraction (LMF, g leaf g^{-1} total aboveground tissue) and stem mass fraction (SMF, g stem g^{-1} total aboveground tissue). Aboveground R_d (nmol CO_2 g^{-1} plant s^{-1}) was calculated by summing leaf and stem R_d weighted by the proportion of dry mass corresponding to each tissue. Finally, net daily carbon gain (nmol CO_2 g^{-1} plant s^{-1}) of each individual was calculated by subtracting daytime stem R_d and nighttime aboveground R_d from the net daily carbon gain of foliage.

Statistical analysis

To assess the effect of plant height, most traits were log transformed prior to analysis and fit with linear and polynomial functions. Fits of both model types were compared and that which best explained the data (higher R^2-value, significant parameters) was used. Additional analyses examined the bivariate relationship between stem N content and stem R_d. All statistical analyses were conducted in JMP statistical analysis software (JMP 9.0.2, SAS Institute, Cary, NC).

Results

Effect of tree size on biomass distribution and self-shading

Leaf mass fraction of trees was negatively correlated with plant size, while stem mass fraction increased with size (Figure 2.1). On average, over 50% of aboveground tissue in small seedlings was leaf tissue, declining to 27% in 50 cm tall plants and 10-14% in plants over 100 cm in height. A linear increase in self-shading fraction was observed across the tree size gradient (Figure 2.2). Self-shading was 0.12 or less in the smallest seedlings and increased to an average of 0.34 in trees over 200 cm in height.

Tissue chemistry and gas-exchange

Specific leaf area was not affected by tree size, but the area and mass of individual leaves increased with height (Figure 2.3). Maximum photosynthetic rates on both an area- and mass-basis increased significantly with sapling size (Figure 2.4). Mean leaf and stem nitrogen (N) content and associated mass-based dark respiration rates (R_d) varied significantly with tree size, but leaves and stems varied in contrasting ways. Leaf N and leaf R_d increased with plant size (Figure 2.5). In contrast to leaves, mean stem N and R_d declined significantly as sapling height increased (Figure 2.5). This reflects a shift in the distribution of stem diameter sizes with increasing tree size. Across all samples, stem R_d was positively related to stem N (Figure 2.6), with newer growth (i.e. smaller diameter classes) exhibiting higher levels of both relative to older woody tissues, and larger trees having a greater fraction of larger diameter stems.

Average N and R_d of aboveground tissues declined markedly with increased tree size (Figure 2.5). This reflects an ontogenetic increase in the fraction of stem tissues, which had lower N content and R_d than leaves. Per gram aboveground tissue, small seedlings (10-40 cm tall) contained approximately twice the amount of nitrogen found in larger saplings (>150 cm tall), and their respiration rates were also twice as high.

Net daily carbon gain

Under ambient light conditions all plants except a few of the largest individuals were estimated to have a positive aboveground carbon balance, with net carbon gain (nmol CO_2 g^{-1} plant s^{-1}) decreasing significantly with increased sapling size (Figure 2.7).

Individuals in the largest size classes (>100 cm in height) gained significantly less carbon per gram tissue than smaller trees (10-70 cm in height). Moreover, even among the smaller size classes, seedlings less than 20 cm in height exhibited more than twice the rates of carbon gain per gram than 50-70 cm tall individuals.

Under the hypothetical standardized light regimes, brighter environments allowed larger-sized individuals to achieve more positive carbon balances (Figure 2.8). The whole-plant light compensation point (WPLCP) estimated from fitted lines for each light level was reached when saplings were 54 cm, 104 cm, 145 cm, and 230 cm tall for 2%, 4%, 6%, and 8% of above-canopy PPFD, respectively. As light availability decreased, the carbon gain of all individuals declined as expected. However, the effect of size on carbon gain varied between light environments. For instance, the 10-20 cm tall seedlings had predicted rates of carbon gain that were approximately 1.8 times higher than the 50-70 cm tall saplings in 8% light. Under 6% and 4% light, those same seedlings gained approximately 2.3 and 3 times more carbon than the 50-70 cm tall saplings.

Discussion

Our data demonstrate that net carbon gain per gram plant tissue of understory juvenile *Acer saccharum* trees declines with increasing plant size (Figure 2.7). This trend was evident under ambient and standardized light levels. As a result, the whole-plant light compensation point (WPLCP) increased with plant size (Figure 2.8). Additionally, a number of important leaf, stem, and whole-plant traits that are associated with WPLCP such as leaf size, nitrogen content, gas exchange rates, and self-shading also vary significantly with ontogeny in *Acer saccharum* juvenile trees. These results are consistent with our hypothesis that ontogenetic declines in carbon gain with increasing plant size are driven by a higher degree of self-shading (Figure 2.2) and a decline in the fraction of aboveground biomass distributed to foliage (Figure 2.1).

In agreement with results from prior studies of small seedlings (Walters et al. 1993b, Tjoelker et al. 1999a), our study of larger juveniles found that stem and whole-plant specific respiration rates declined with plant size (Figure 2.5). As shoots grow larger, their proportion of metabolically active meristematic tissues with high respiration rates declines, while the proportion of structural tissues with lower respiration rates increases. It is this ontogenetic change that drives the decline in stem respiration rates, while a combination of it and a declining fractional investment of annual plant growth distributed to leaves cause the decline in whole-plant respiration rates. Variation in tissue nitrogen concentrations can also help explain differences in respiration rates since the two are often linearly related (Ryan 1995, Reich et al. 1998, Tjoelker et al. 1999b, Reich et al. 2008). We found that leaf nitrogen content and leaf respiration rates were only weakly correlated (data not shown), which may have been due to the narrow range of light environments in our study. On the other hand, stem nitrogen content and respiration rates were significantly positively correlated (Figure 2.6).

Ontogenetic declines in specific leaf area (SLA) reported in other studies have been attributed to increasing investment in structural support tissue per unit lamina surface area (Niklas and Enquist 2001, Niklas and Cobb 2008). Declines in SLA with tree size were observed both within and across species in these studies, which would help explain the declining growth and carbon balance seen in larger plants due to this added cost of harvesting light (Niklas and Cobb 2008). However, while we did observe a decline in net

carbon gain with *A. saccharum* sapling size, we did not observe a change in SLA. Niklas and Cobb (2008) point out that the variations they observed in SLA may reflect changes in the number of leaves exposed to different light conditions (i.e. sun vs. shade) as the canopy grow larger, as well as the amount of mechanical tissues in leaves at differing positions within the canopy. Our trees were all found growing in low light and our range of sapling size was significantly narrower than in their study, which likely contributed to the lack of variation we saw in SLA. Therefore, other traits such as self-shading and stem respiration appear to be responsible for the decline in net carbon gain per gram plant tissue of juvenile sugar maples in low light.

Compared to the amount of self-shading observed in a study of seven juvenile rainforest evergreens (Lusk et al. 2011), self-shading of *A. saccharum* appears to be similar to two of the species measured (*Aristotelia chilensis* and *Eucryphia cordifolia*) but had lower degrees of shading than the other five species. Self-shading is likely to affect net carbon gain and shade tolerance of trees in forest understories (Valladares et al. 2002, Poorter et al. 2003, Sterck et al. 2005, Falster et al. 2011), causing ontogenetic declines in both as larger saplings produce more leaf tissues (Lusk et al. 2011). It has been suggested that temperate forest trees produce mono-leaf-layered crowns in the shade, in which most leaves are located in the periphery of the crown, either as an adaptive trait (Horn 1971) or as a result of the strong effects of self-shading on trees growing under a closed canopy (Sterck et al. 2005). Since self-shading of *A. saccharum* seems to be low relative to similar sized individuals growing in rainforest sites, this may hold true, with deciduous species distributing their crowns each growing season in a way that maximizes light interception.

A. saccharum is considered to be a shade tolerant species and underwent declines in net carbon gain with ontogeny, but it is difficult to say how these declines may compare with other species in temperate forests. Studies have used various metrics to determine whether shade tolerance and carbon gain vary with juvenile tree size, and whether species with different shade tolerance rankings show distinctive ontogenetic trends. Kneeshaw at al. (2006) found that relative differences in shade tolerance of boreal tree species became slightly less evident in larger sized individuals, while Lusk et al. (2011) observed that rates of carbon gain declined with increasing plant size in evergreen species, but the

degree to which carbon gain declined was not affected by species' shade tolerance rankings. However, another study in a temperate rainforest found that light-demanding species were found in brighter habitats as they grew larger, but that the more shade tolerant species showed little or no variation in light requirements (Lusk et al. 2008). If it is true that shade tolerant species experience minimal changes in light requirements with size, we would expect less tolerant species to exhibit more acute differences across size classes. If the ontogenetic decline is similar across shade tolerance rankings, it is possible that intolerant species simply show greater rates of carbon gain in all size classes, assuming they are growing in light environments above their compensation points.

One caveat to our measurements of net carbon gain is that we did not measure respiration rates of belowground tissues, nor did we include root respiration in our estimates of WPLCP. In a study of similarly sized juvenile temperate trees, root respiration rates per gram of plant tissue were found to be similar to rates of stem tissues (Machado and Reich 2006). While both the fraction of plant tissue distributed to roots, as well as the fraction of whole plant respiration produced by roots tissues declined with ontogeny, a sizeable fraction (up to 25%) of whole-plant respiration was attributed to belowground tissues. This represents an obvious additional respiratory cost for the saplings measured in this study, indicating that WPLCP of juvenile *A. saccharum* trees is likely higher than those reported here. However, it has also been shown that juvenile trees in deciduous forest understories may strongly depend on a window of high light availability in early spring before the overstory canopy leafs out (Augspurger 2008, Lopez et al. 2008). Saplings may gain a large portion of their annual carbon income during this period, which would enable them to survive at light levels below their WPLCP for most of their growing season. Additionally, storage of carbohydrates could allow saplings to survive briefly in light environments in which their rates of carbon gain are negative, and in fact it has been shown that shade-tolerant tropical evergreen species have larger stores of carbohydrates than intolerant species (Poorter and Kitajima 2007). This might indicate that *A. saccharum* saplings should also have relatively large carbon reserves, assuming the pattern of larger reserves in shade tolerant trees holds true for both deciduous and evergreen species.

An understanding of light requirements of species throughout developmental stages is required to improve models of forest dynamics (Poorter et al. 2005, Lusk et al. 2008). This paper shows that significant ontogenetic variations in net carbon gain and WPLCP occurs even in a shade tolerant species, indicating that work on saplings may detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone. These differences could have important effects on successional patterns of all forest types.
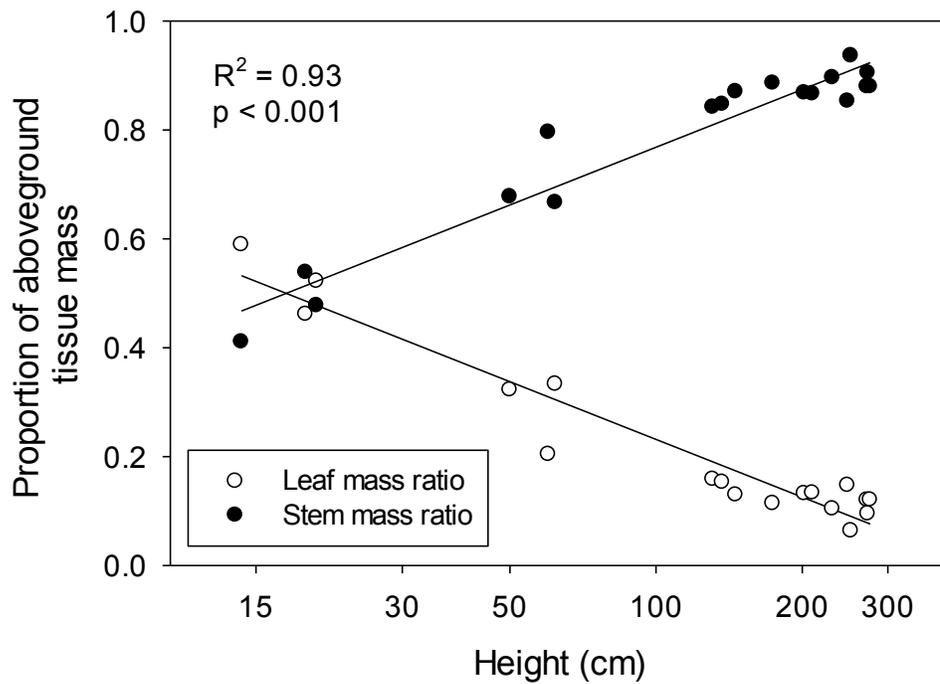
Figure 2.1. Changes in proportions of leaf and stem tissues as a function of *Acer saccharum* juvenile tree height; sapling height was log transformed prior to analysis.
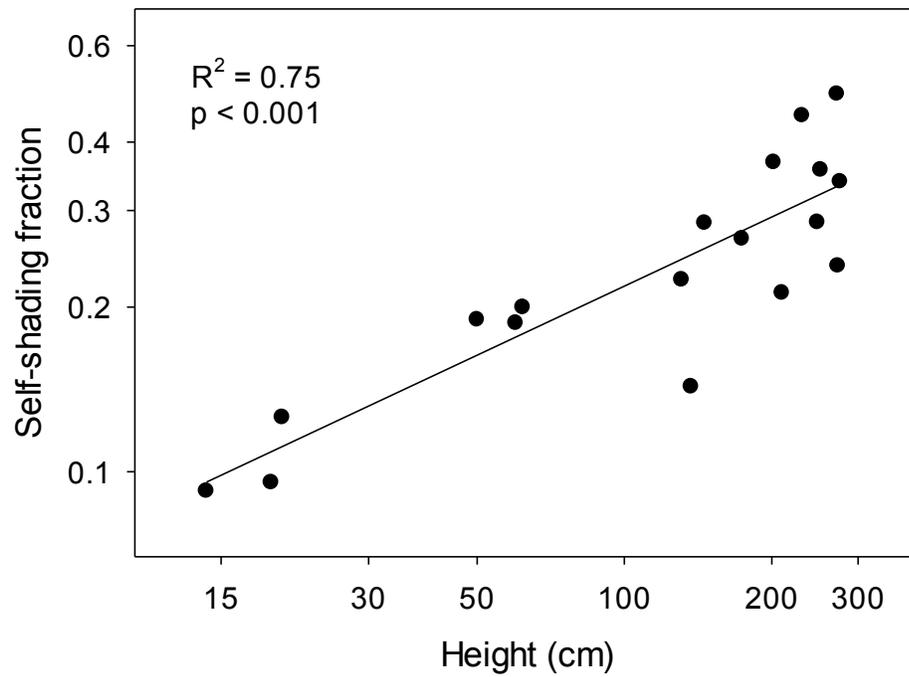
Figure 2.2. Relationship between self-shading and height of juvenile *Acer saccharum* trees; data were log-log transformed prior to analysis.
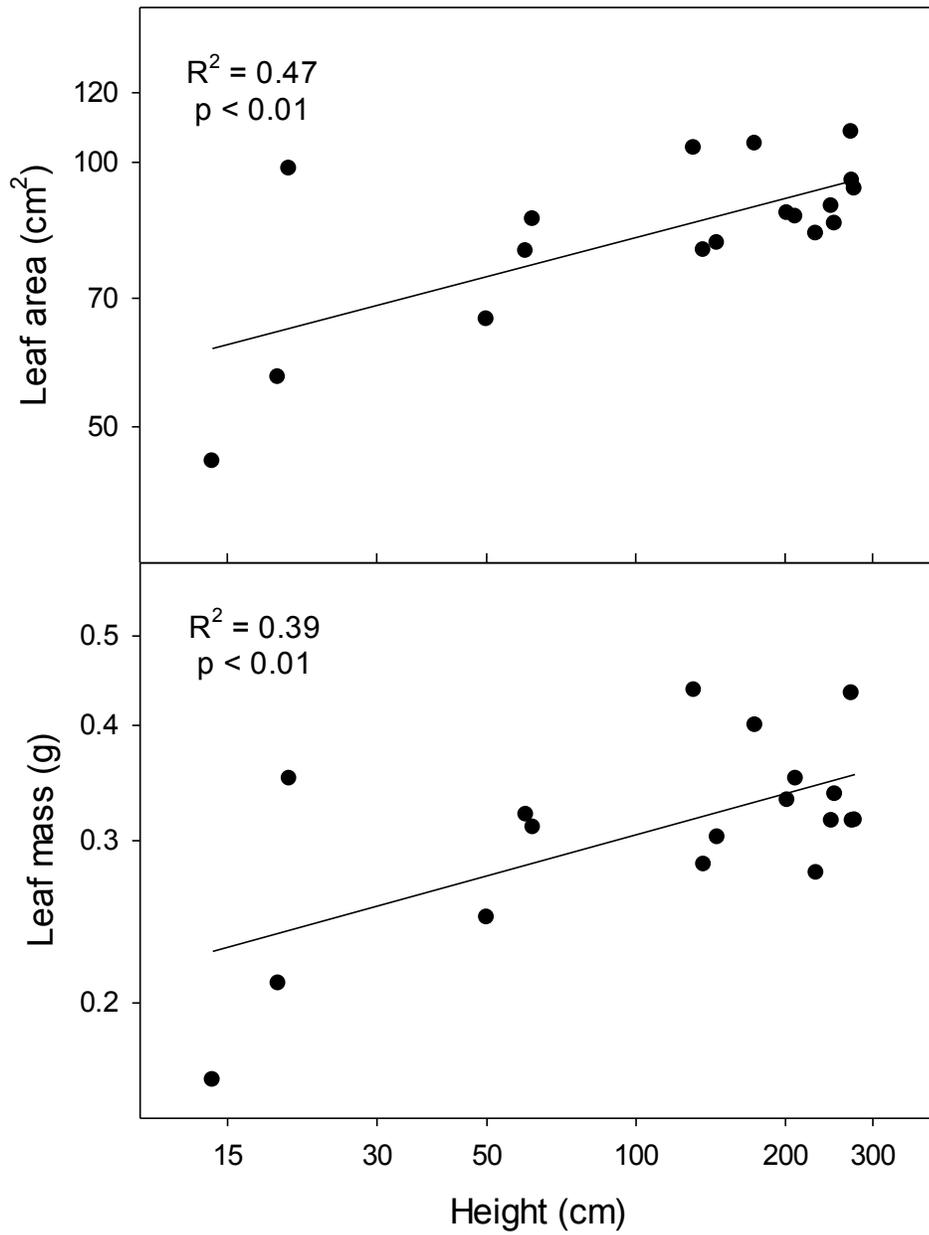
Figure 2.3. Changes in area and mass of individual leaves as a function of *Acer saccharum* juvenile tree height; data were log-log transformed prior to analysis.
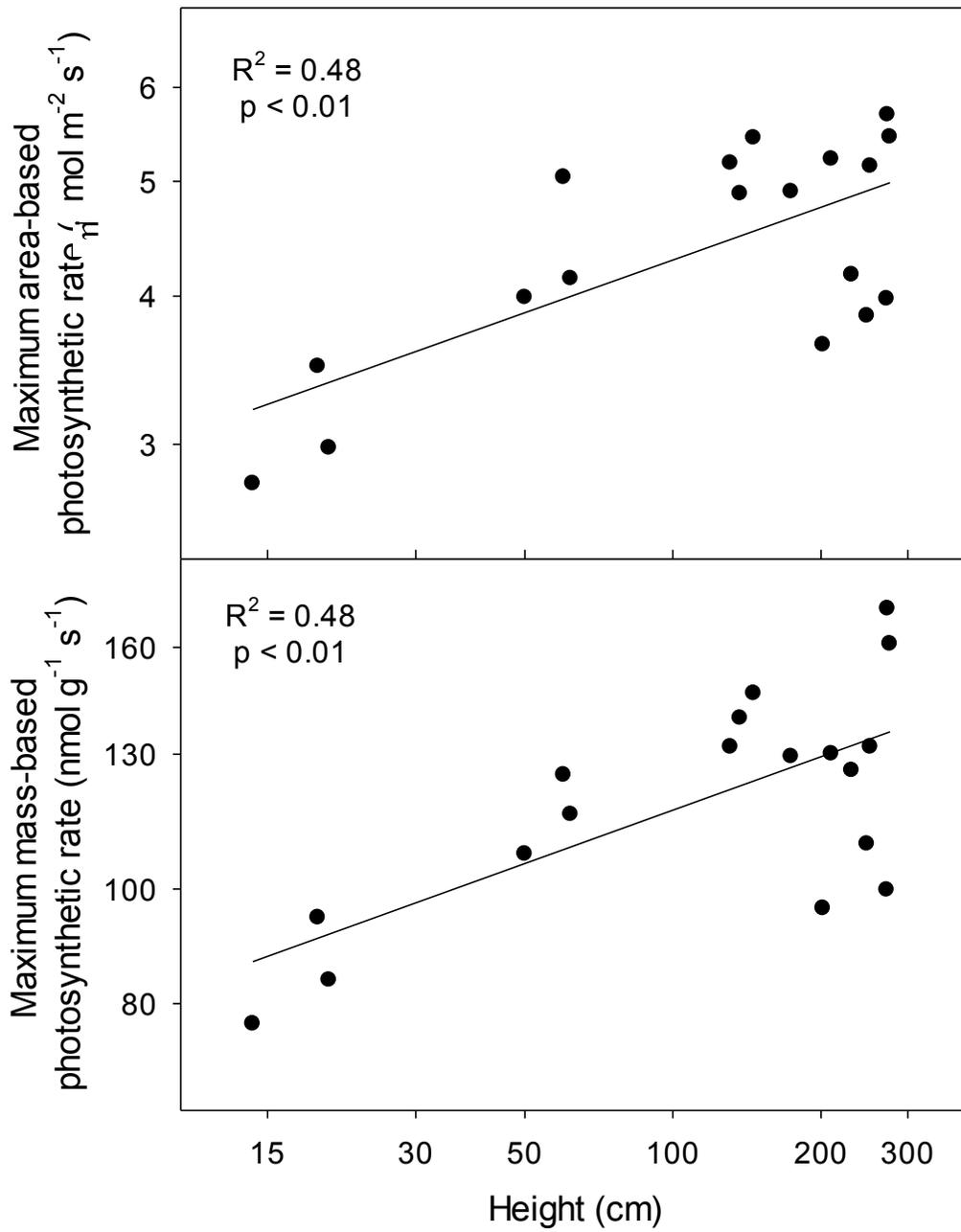
Figure 2.4. Area- and mass-based maximum photosynthetic rates in relation to height of juvenile *Acer saccharum* trees; data were log-log transformed prior to analysis.
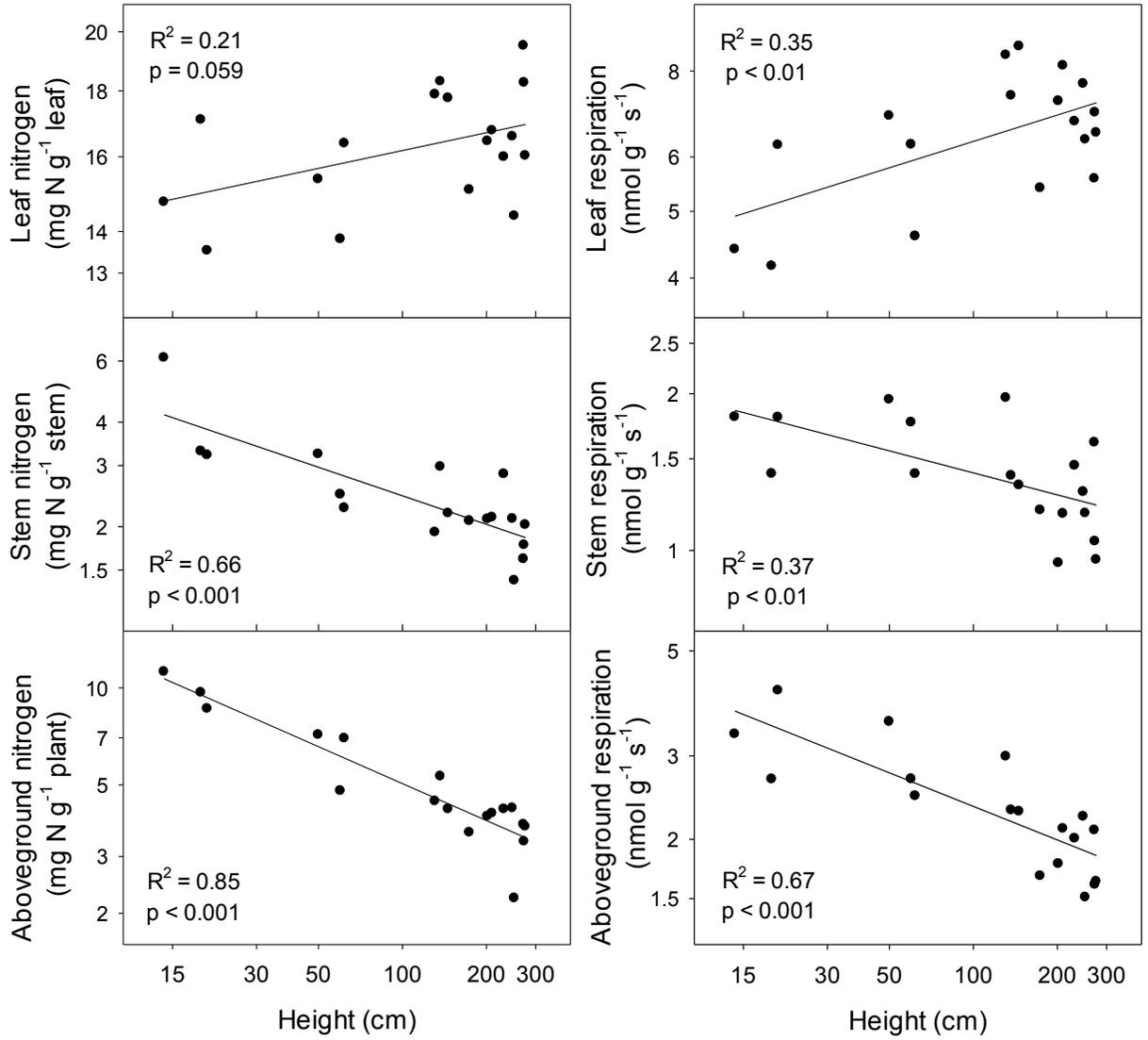
Figure 2.5. Changes in nitrogen concentration and dark respiration rates per gram of leaf, stem, and total aboveground tissues as function of *Acer saccharum* juvenile tree height; data were log-log transformed prior to analysis.
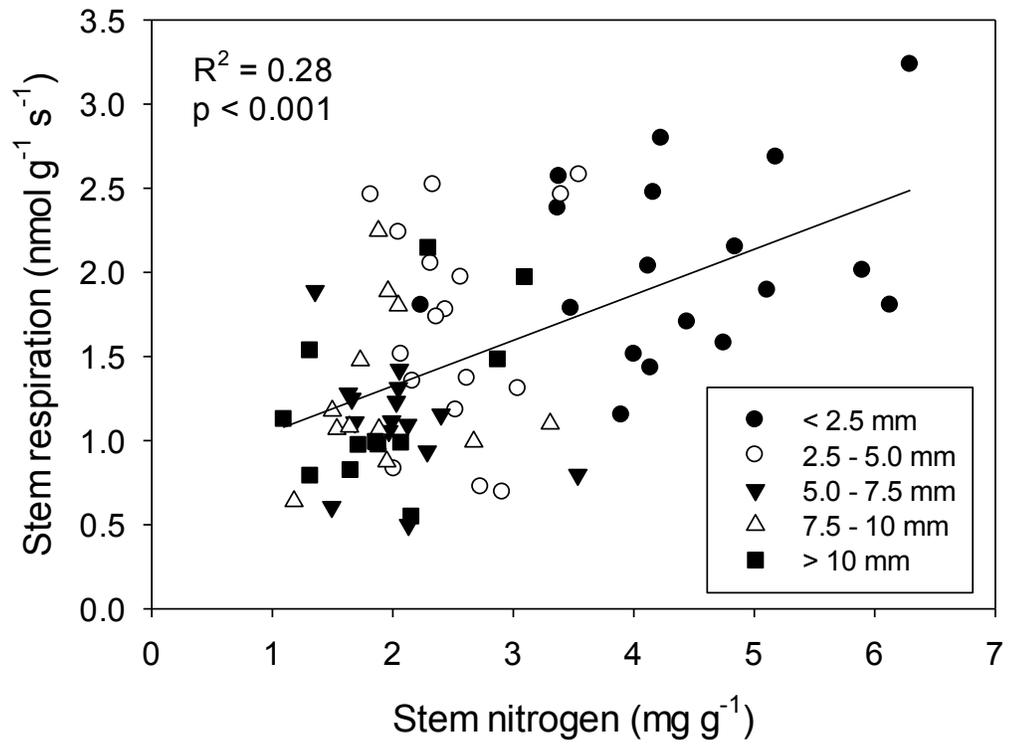
Figure 2.6. Relationship between stem nitrogen content and stem respiration rate per gram tissue for juvenile *Acer saccharum* trees.
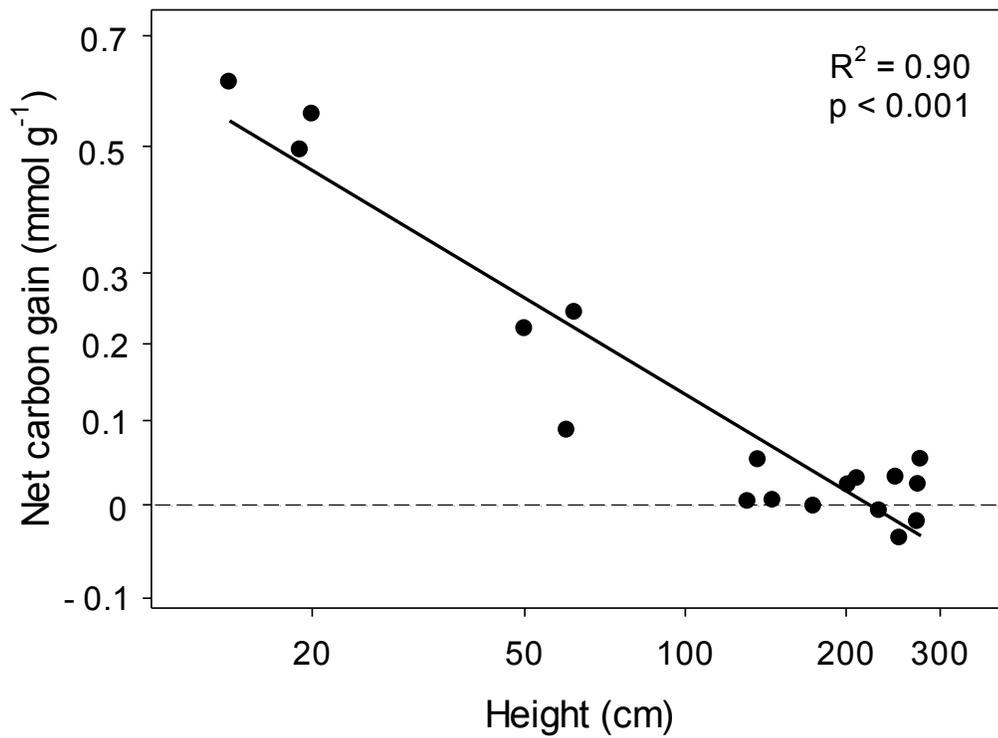
Figure 2.7. Relationship between net daily carbon gain estimated from YPLANT simulations and height of juvenile *Acer saccharum* trees at ambient light levels (6 to 8% of above-canopy PPFD); data were log-log transformed prior to analysis. The dashed line indicates zero net carbon gain.
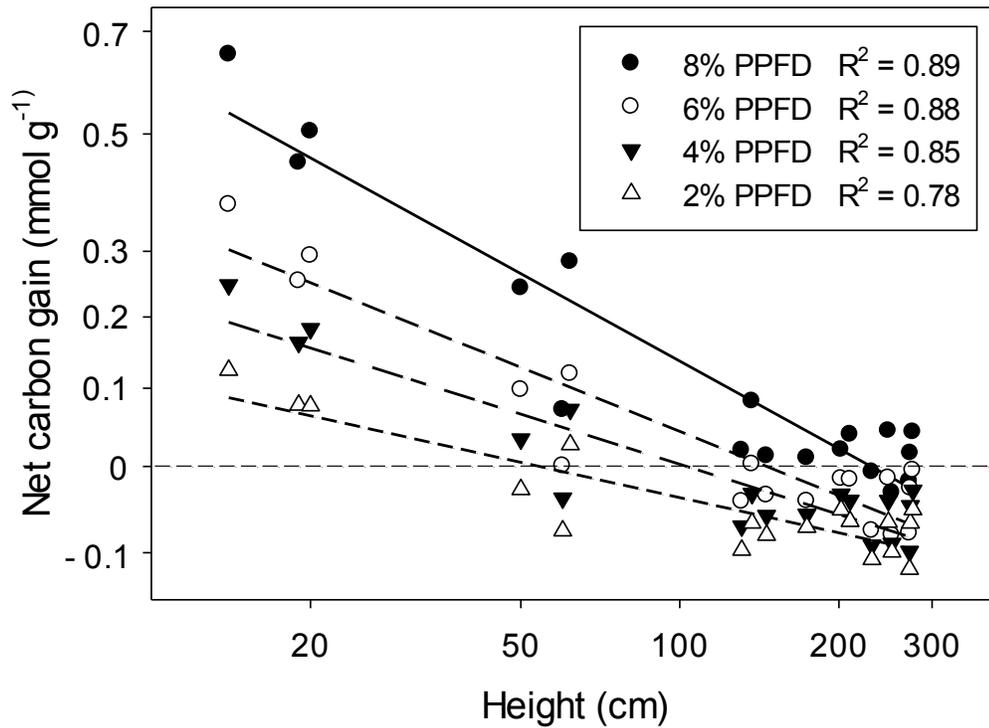
Figure 2.8. Relationships between net daily carbon gain estimated from YPLANT simulations and height of juvenile *Acer saccharum* trees at standardized light levels of 2%, 4%, 6%, and 8% of above-canopy PPFD; data were log-log transformed prior to analysis. Linear regression analyses were significant at a *p*-value of < 0.001; the solid line is the fit at 8% PPFD, the long dashed line is the fit at 6% PPFD, the alternating long-short dashed line is the fit at 4% PPFD, and the short dashed line is the fit at 2% PPFD. The dashed line at a net carbon gain of zero indicates the whole-plant light compensation point.

CHAPTER 3

Effects of ontogeny and light availability on rates of net carbon gain and growth of juvenile rainforest evergreens that vary in shade tolerance

with C.H. Lusk and P.B. Reich

Recent work suggests that plant size may affect the light requirements, growth, and carbon balance of many tree species, and that ontogenetic variation in light requirements may be greater in light-demanding species than in their more shade-tolerant competitors. We hypothesized that increased sapling size would lead to significant declines in leaf and whole-plant functional traits, net daily carbon gain, and relative growth rates (RGR) of all species measured, while the magnitude of ontogenetic variation would be more pronounced in light-demanding species. We also predicted that light availability would influence ontogenetic variation in traits and whole-plant performance. We measured transmitted PPFD and RGR of four juvenile subtropical rainforest tree species (13 to 238 cm tall) varying in shade tolerance. A minimum of 111 individuals of each species were measured for growth parameters, and a subset of 36 of these trees per species were measured for leaf gas exchange and nitrogen content, and crown architecture of each individual was recorded using a 3-dimensional digitizer. YPLANT was used to estimate the self-shaded fraction of each crown and to model net carbon gain. Increased sapling size caused a significant increase in self-shading, and significant declines in net daily carbon gain rates and relative growth rates (RGR) of light-demanding species, while ontogenetic variations were minimal or absent in shade-tolerant species. Leaf-level traits such as specific leaf area (SLA) and gas-exchange rates showed some ontogenetic and species specific differences, but patterns among traits were less consistent than expected. The positive relationship between light requirements and the slope of the relationship between light availability and RGR led to crossovers in RGR between shade-tolerant and light-demanding species at low light, independent of sapling size. Our results suggest that significant ontogenetic variations in net daily carbon gain and RGR occur in species that differ widely in shade tolerance, but the magnitude of these changes is dependent on successional status and light availability. These results indicate that work on saplings

may detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone.

Introduction

Light requirements of forest tree species vary considerably, leading to a wide array of shade tolerance levels ranging from intolerant pioneer species to tolerant climax species (King 1994, Poorter 1999, Baltzer and Thomas 2007, Lusk et al. 2008). Growth and survival of juvenile trees in forest gap and understory habitats are important in explaining patterns of succession and species coexistence in forests, and therefore species differences along the light response gradient play a critical role in these patterns.

Ecological studies have yielded inconsistent results regarding the interrelationships of growth, survival, and shade tolerance. A number of studies have suggested that shade-intolerant species have greater growth potential than shade-tolerant species across all light environments, and that shade tolerance is therefore mainly a function of traits that maximize survivorship in low light (Kitajima 1994, Kobe 1999, Kitajima and Bolker 2003). This is consistent with the idea that tolerance to low light is primarily achieved through conservative patterns of carbohydrate storage, enabling saplings to tolerate brief periods of negative carbon balance (Kobe 1997). If shade-intolerant species show consistently higher growth rates across all light levels relative to shade-tolerant species, there should be no crossovers in the relationship between relative growth rate (RGR) and light availability (Kitajima and Bolker 2003). On the other hand, it has been argued that although storage may provide a short-term buffer, negative carbon balance is expected to result in eventual mortality (Baltzer and Thomas 2007) since some degree of growth is necessary to replace leaves and physiologically active roots with finite lifespans (Chabot and Hicks 1982, Eissenstat and Yanai 2002). Accordingly, we should observe species crossovers in growth rates, with shade-tolerant species growing faster than intolerant species at low light, with the latter gaining the advantage in high light (as noted in Walters and Reich 1996, Sack and Grubb 2001). These different results could derive from differences among studies in light levels used and/or in the size and age of juveniles (Walters and Reich 2000, Lusk et al. 2004).

Studies of shade tolerance and growth have often focused on small or young individuals without explicitly testing for the effect of ontogeny (Buchman et al. 1983, Walters et al. 1993b, Lusk et al. 2011). Differences in shade tolerance have often been assumed to be fixed within a species, but recent work suggests that light requirements of

species may change as individuals increase in size and experience shifts in important traits (McConnaughay and Coleman 1999, Messier and Nikinmaa 2000, Lusk et al. 2004, Lusk et al. 2011). For example, the ratio of leaf area to plant biomass generally declines with increasing plant size, because leaves of most species turn over much faster than woody tissues (Sterck and Bongers 1998, Lusk 2002). As a result, the ratio of photosynthetic income to respiratory demands decreases in larger trees (Gerrish 1990, Delagrange et al. 2004, Lusk et al. 2004, Machado and Reich 2006). Due to such ontogenetic changes, seedlings of light-demanding species that may initially outgrow their more shade-tolerant associates across all light environments may undergo declining carbon balance and growth in low light. A study of 20 tropical rainforest species showed that shade-intolerant saplings required higher light levels to maintain positive growth rates relative to shade-tolerant species (Baltzer and Thomas 2007), while work on four temperate evergreen species revealed that shade-intolerant species exhibited steeper ontogenetic declines in growth than shade-tolerant species (Lusk 2004).

In humid evergreen forests, the ontogenetic increase in light requirements may be steeper in light-demanding species than in their more shade-tolerant competitors. Juvenile shade-tolerant evergreens retain their leaves for several years, whereas the most light-demanding species usually turn over their foliage in less than 12 months (Coley 1983, Walters and Reich 1999, Reich et al. 2004, Lusk et al. 2011). As a result, light-demanders undergo the steepest declines in leaf area ratio in low light (Lusk et al. 2004). As might be expected, light-demanders occupy increasingly well-lit environments as they grow larger, whereas this trend is less marked in shade-tolerant species (Lusk et al. 2008). However, to date there are few comparative data on the relative ontogenetic trends in the net carbon gain of species differing in shade tolerance (but see Lusk et al. 2011).

Here we ask how rates of net daily carbon gain and growth of juvenile evergreens relate to plant size and species light requirements. We studied the naturally-occurring juvenile trees of four co-occurring species differing in shade tolerance to determine their light requirements. We selected juveniles ranging in height from 13 to 238 cm growing in old- and second-growth subtropical forest stands, and measured growth rates over a period of 12 to 16 months. We used hemispherical photography and crown architecture

measurements to estimate self-shading, and coupled these measurements with gas exchange rates to model net daily carbon gain using the YPLANT model (Pearcy and Yang 1996). We addressed five questions: (i) Do rates of net daily carbon gain and growth decline significantly with increased sapling size? (ii) Is the magnitude of ontogenetic variation in whole-plant performance more pronounced in light-demanding species? (iii) Do leaf-level traits such as specific leaf area, maximum photosynthesis, dark respiration, and nitrogen content vary in a coordinated fashion with growth and carbon gain? (iv) Is the magnitude of ontogenetic variation in traits and whole-plant performance influenced by light availability, and if so, in similar or different ways among species? (v) Are such changes (from i to iv) reflected in the performance and distribution of species?

Materials and Methods

Study area and species

The study was carried out in Nightcap National Park (NSW, Australia) located at 28°38′S, 153°20′E and at an elevation of 380 meters above sea level. The climate is subtropical with seasonally high rainfall in the summer and autumn; mean annual rainfall at the nearest meteorological station (Whian Whian) is estimated at 2,300 mm and mean annual temperature at 17.6 °C (Bureau of Meteorology, http://www.bom.gov.au/climate/). Our measurements were made in a 20-year-old second growth stand and two nearby old-growth stands, all of which grow on fertile soils derived from basalt (Turner and Kelly 1981).

We aimed to select a suite of species that represented the range of shade tolerance and growth rates present in stands. To this end, we selected one tree species that was well-represented by juveniles in shaded understories (*Argyrodendron trifoliolatum*), as well as two light-demanding species commonly found regenerating in recently-disturbed areas and gaps (*Polyscias murrayi* and *Toona australis*), and one species that was common in intermediate light environments (*Diploglottis australis*) (Table 3.1). All four species have compound leaves, though the number of leaflets varies from three for *A. trifoliolatum* to 30 or more for *P. murrayi*. Small seedlings of *A. trifoliolatum* and *D. australis* have simple leaves, and only develop compound leaves once they become well-established.

Growth measurements

Naturally occurring saplings in four size classes (< 50 cm, 50-100 cm, 100-150 cm, > 150 cm) were selected across as wide a range of light environments as possible. Individuals were tagged, and their heights and basal diameters measured. Height was measured as the vertical distance between the stem base and the highest live meristem, and basal diameter as the average of the diameter on two orthogonal axes. Height and diameter were remeasured at the end of the study period, which ranged from 12 to 16 months.

Basal diameter^2 x height was used as a non-destructive proxy for whole-plant biomass as the two have been shown to be highly correlated (Kohyama and Hotta 1990). Aboveground relative growth rate (RGR) was calculated as:

$$RGR = \frac{\ln(d2^2 h2) - \ln(d1^2 h1)}{(t2 - t1)}$$

where d_1 and d_2 are basal diameter and h_1 and h_2 are height at the beginning (t_1) and end (t_2) of the measurement period, respectively. Using both diameter and height parameters in calculating growth provides a more robust growth rate measure than height alone, as height growth can be highly variable due to leader loss.

Hemispherical photography

A Nikon Coolpix digital camera with a 182° fisheye adapter was used to take a hemispherical photograph above each individual, with the top of the camera oriented north. Photos were analyzed using the Gap Light Analyzer software package (Frazer et al. 1999) to obtain the photosynthetic photon flux density transmittance (PPFD) above each sapling. We estimated the minimum light requirements of species by calculating the 10th percentile of the distribution of a random sample of the measured population of juveniles in relation to the transmitted PPFD (PPFD$_{10}$). Shade tolerant species such as *A. trifoliolatum* have low PPFD$_{10}$ values, while intolerant species such as *P. murrayi* have high values (Table 1).

Modeling carbon gain

We used YPLANT (Pearcy and Yang 1996) and hemispherical photography to model self-shading and carbon gain for a subset of 36 individuals per species, nine per size class across the range of light environments. Hemispherical photos taken above these individuals were analyzed using the Gap Light Analyzer (Frazer et al. 1999) software in conjunction with WinPhot 5 (Ter Steege 1996). Fractional canopy openness (0-1) was calculated for 20 altitude and eight azimuth classes in each photo. Mean openness in each angle class was calculated for each photo by averaging openness across all azimuth classes. This allowed us to estimate the average time series of direct photon

flux density (PFD) interception for any day of the year. Average light regimes were calculated for 4 days equally spaced between the longest (December 21) and shortest (June 21) days of the year.

The three-dimensional crown arrangement of each tree was recorded using an extended range Liberty 3D-digitzer (Polhemus, Colchester, Vermont, USA) paired with the FLORADIG software package (CSIRO Entomology, Brisbane, Australia) as described by Falster and Westoby (2003). A virtual reconstruction of each plant is achieved by recording a series of point coordinates associated with each leaf or stem segment, as well as the relative connectivity between points. The 3D spatial coordinates are recorded using a magnetic signal receiver and pointer, which can accurately record points within a one meter radius from the receiver. Stem and petiole arrangements are described by their length, diameter, elevation angle, and azimuth, while leaves are described by their length, azimuth, and the elevation angles of two vectors on the lamina surface (Falster and Westoby 2003).

YPLANT was then used to model net daily carbon gain. The 3D crown arrangement recorded for each individual in FLORADIG was converted to the appropriate YPLANT format using a program written in the C programming language (Falster and Westoby 2003). YPLANT inputs are a description of the leaf shape, leaf photosynthetic capacity and respiration rate, the geometry of the crown arrangement, and a description of the canopy structure above the plant which is used to estimate light interception. A solar movement submodel is used to estimate PPFD (μmol photons m^{-2} s^{-1}) intercepted by each leaf surface at different times of day. A photosynthetic submodel estimates the resulting assimilation rate using PPFD response curves generated from measurements of gross photosynthetic capacity and dark respiration rates (Thornley 1976).

YPLANT was also used to estimate leaf overlap (i.e. self-shading) since it influences light interception by plant crowns. YPLANT output includes the projected leaf area of each tree, which does not take into account any leaf overlap, as well as the displayed leaf area, which is the effective leaf area for light interception (Pearcy and Yang 1996). The fraction of leaf area that was self-shaded was calculated as (PA/DA)/PA, where PA is the projected leaf area and DA is the displayed leaf area.

Gas exchange measurements

Measurements of photosynthetic capacity (A_{max}) and dark respiration rates (R_d) are required for simulations of carbon gain in YPLANT. A_{max} and R_d were measured for the subset of 36 individuals per species using an LI-6400 (LI-COR, Lincoln, NE). Environmental conditions were controlled within the leaf cuvette as follows: CO_2 concentration of 380 µmol mol^{-1}, 50-70% relative humidity and block temperature of 25°C. Irradiance was held at 1500 µmol m^{-2}s^{-1} using the LI-6400 LED light source for A_{max}, and at 0 µmol m^{-2}s^{-1} for R_d.

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

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

where $a = 3.22$ and $b = -0.046$ (Kattge et al. in press). These values were multiplied by night length to give daily respiration values using the following formula:

$$R_N \text{ (mmol m}^{-2}\text{day}^{-1}) = \frac{R \text{ (mmol m}^{-2}\text{s}^{-1})*(24 - \text{day length(h)})*3600 \text{ (sh}^{-1})}{10^3 \text{ (µmol mmol}^{-1})}$$

Net daily carbon gain (nmol CO_2 g^{-1} plant s^{-1}) of each individual was calculated by subtracting nighttime R_d of foliage from the YPLANT output.

Measured leaf traits

For calculation of specific leaf area (SLA), leaves or leaflets were harvested from every individual tagged for growth measurements. Leaves were scanned and the projected area was determined using the image-processing software ImageJ (Abramoff et al. 2004). All samples were oven-dried at 70°C for at least 72 hours to determine dry leaf

58

mass. A subset of samples corresponding with the 36 digitized trees per species were ground and analyzed for nitrogen concentrations (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA).

Statistical analysis

All traits except for relative growth rate were $\log_{10}$-transformed for all analyses to roughly normalize the data. Ordinary least squares regressions were used to quantify relationships between functional traits and species, log(PPFD), log(Height), and their interactions. Additionally, variation in traits as a function of log(PPFD), log(Height), and their interaction were analyzed. Using the predicted formulas from the latter relationships (excluding the interaction term when not significant), I estimated traits for all species within the range of light environments in which they were commonly found growing. This range of light levels varied among species depending on their light requirements, but fell between 2 and 20 mol m^{-2} day^{-1} transmitted PPFD. For example, the shade-tolerant *A. trifoliolatum* was only found growing in low light, so trait estimates were made at 2, 4, and 5.5 mol m^{-2} day^{-2} PPFD, while the light-demanding *P. murrayi* inhabited brighter microhabitats, so trait estimates for this species were made at 4, 8, 12, and 20 mol m^{-2} day^{-2} PPFD.

Results

Sapling light environment

The species thought to be the more shade tolerant of the four were found in darker locations on average in these forests (Table 3.1), as well as being in darker microsites across all size classes for all individuals selected for the intensive study (Figure 3.1). Additionally, with the exception of *Diploglottis australis*, all species were found growing in significantly brighter microsites on average as they increased in size ($p < 0.05$). Individuals in the largest size class of each of these species were on average found in light environments that were approximately 50% brighter than those of the smallest size class.

Species differences and the effect of ontogeny on whole-plant traits

A linear increase in self-shading was observed across the tree size gradient for all four species, though the trend was strongest in the shade-intolerant *P. murrayi* (Figure 3.2). Self-shading was 0.12 or less in seedlings of the two most shade-tolerant species, and increases were modest with tree height. The more light-demanding species had higher degrees of self-shading in small seedlings (0.10 to 0.22), and while *Toona australis* also exhibited modest increases in larger saplings, self-shading in *P. murrayi* increased to an average of 0.42 in larger plants.

Net daily carbon gain rates were predicted to be positively related to species light requirements, which was the case in smaller size classes (Figure 3.3). However, *D. australis* maintained similar rates of C gain across tree size and *A. trifoliolatum* underwent minor changes with size, while the two light-demanding species experienced significant variations with ontogeny (Table 3.2; Figure 3.4). Consequently, relative differences in C gain rates among species were significantly diminished in larger juveniles (Figure 3.3).

Relative growth rates (RGR) were also predicted to be positively related to species light requirements in high light, with possible rank changes in more shaded environments. Again, *D. australis* maintained similar RGR across size classes (size effect $p = 0.14$) while the other three species had declining RGR in larger juveniles (Table 3.2; Figure 3.5), with the more light-demanding species showing the most

significant ontogenetic declines (Figure 3.6). At low light, RGR of the light-demanding species were significantly diminished compared to growth in brighter environments, and in fact, RGR of the two shade-tolerant species were higher at 2 mol m^{-2} day^{-2} PPFD than that of the shade-intolerant *T. australis*. Additionally, increased sapling size brought about a divergence in RGR among species at low light, while RGR of larger saplings tended to converge in high light (Figure 3.6).

Species differences and the effect of ontogeny on leaf traits

SLA declined significantly with increased sapling size in *A. trifoliolatum* and *T. australis* juveniles, declined marginally with size in *D. australis*, and did not vary with ontogeny in *P. murrayi* (Table 3.2; Figure 3.7). SLA of the light-demanding *T. australis* was over twice that of the other species in smaller size classes, though these differences were diminished in larger saplings (Figure 3.8).

Leaf gas-exchange rates showed some ontogenetic and species specific differences, but patterns and coordination among traits were less consistent than we expected. Photosynthetic rates of *P. murrayi* across all size classes were at least twice those of the other three species (Figure 3.9). Dark respiration rates of *P. murrayi* were also significantly higher in smaller size classes, but rates of all species converged at light levels of 4 mol m^{-2} day^{-1} PPFD or higher (Figure 3.10). These differences were not mirrored in leaf nitrogen concentrations, which did not differ significantly among *P. murrayi*, *T. australis*, and *D. australis*, and were approximately 25% lower in *A. trifoliolatum* (data not shown). There were no significant changes in N with plant size (Appendix Table B.1), and only *T. australis* showed significant variations in photosynthesis with size (Table 3.2; Figure 3.11), while *D. australis* was the only species to exhibit significant ontogenetic changes in dark respiration rates (Table 3.2; Figure 3.12).

Effect of light availability on plant traits

Specific leaf area of all species declined significantly with increasing PPFD, except the most shade-tolerant species, *Argyrodendron trifoliolatum* (Table 3.2; Figure 3.7). Irradiance had significant positive effects on area-based leaf-level photosynthesis and

dark respiration rates of all species except the light-demanding *Toona australis* (Table 3.2; Figures 3.11 and 3.12). Net carbon gain and relative growth rates of all species increased significantly with increasing PPFD (Figures 3.4 and 3.5), though the effect of increased PPFD was stronger in the smaller-sized *Polyscias murrayi* individuals compared to the larger size classes, as evidenced by the significant interaction between log(PPFD) and log(Height) in the model (Table 3.2).

Discussion

Our data suggest that increased sapling size causes a significant decline in net daily carbon gain and relative growth rates (RGR) of light-demanding species, while ontogenetic declines are minimal or absent in shade-tolerant species (Table 3.2; Figures 3.4 and 3.5). These results are consistent with our hypotheses that RGR and net daily C gain rates decline with size, and that the magnitude of ontogenetic variation would be more pronounced in light-demanding species. Variations in self-shading with increased sapling size followed patterns that were consistent with these change in RGR and C gain, with the most light-demanding species, *P. murrayi*, having the steepest slope in the relationship between self-shading and plant size relative to the shade-tolerant species (Figure 3.2). However, leaf-level traits such as specific leaf area (SLA) and gas-exchange rates showed some ontogenetic and species specific differences, but patterns among traits were less consistent than we expected, providing little support for our hypothesis that leaf traits would vary in a coordinated fashion with C gain and RGR.

Self-shading and net daily carbon gain followed similar ontogenetic patterns (Figures 3.2 and 3.3). In both cases, the slopes of the relationships between sapling size and the trait in question were steepest for *P. murrayi*, and were diminished in the more shade-tolerant species. Prior research has shown that self-shading influences net carbon gain and shade tolerance of trees in forest understories (Valladares et al. 2002, Sterck et al. 2005, Falster et al. 2011, Lusk et al. 2011), causing ontogenetic declines in both as larger saplings produce more leaf tissues (Lusk et al. 2011). We hypothesized that this trend would be steeper in light-demanding species compared to shade-tolerant species. Our results support this hypothesis, indicating that species' differences in the relationship between sapling size and self-shading are important drivers of C gain and shade-tolerance.

Ontogenetic trends in RGR were dependent on species' light requirements as well as on light availability (Figure 3.6). In high light, there was a positive relationship between species' light requirements and RGR in the smaller size classes. As trees grew larger, the relationship between successional status and RGR was maintained, but relative differences between species diminished. This reflects the positive relationship between light requirements and the slope of the relationship between light availability and RGR,

63

similar to patterns documented in prior studies (Moad 1992, Sack and Grubb 2001, Montgomery and Chazdon 2002, Baltzer and Thomas 2007). These differences in slope are the cause of the crossovers in growth rate between shade-tolerant and light-demanding species observed at low light in this study. At 2 and 4 mol m^{-2} day^{-2} transmitted PPFD, we found that RGR of *T. australis*, *D. australis*, and *A. trifoliolatum* showed a negative relationship with light requirements, while *P. murrayi* maintained only slightly higher RGR compared to the other species. Comparable findings have been observed among similar-sized individuals varying is shade-tolerance (Sack and Grubb 2001, 2003, Baltzer and Thomas 2007), but to our knowledge, this is one of the first studies to directly test for these differences across size classes (but see Lusk 2004). Our findings contradict the hypothesis that, regardless of light environment, more light-demanding species have consistently higher growth rates than shade-tolerant species (Kitajima 1994, Kobe 1999). Even when comparing species' differences in RGR in the smallest size class, we observed crossovers between light-demanding and shade-tolerant species.

The effect of ontogeny on RGR depended on light availability and tree species (Figure 3.6). At high light, differences among species were most acute in the smallest size classes, and as trees grew larger, the relative differences between species became less marked. These results are supported by Lusk et al. (2004), who also found that differences in RGR between four temperate rainforest species were diminished as saplings grew larger in size, though his study took place in low light. In contrast, we observed that species' differences in RGR in low light were minimized in the smaller size classes and rates diverged as saplings grew larger in size. There are few studies that are directly comparable to these, but Lusk et al. (2008) observed that minimum light requirements (MLRs) of 13 species varying in shade-tolerance were most distinct in larger individuals. This differentiation occurred because the MLRs of shade-tolerant species did not vary significantly with ontogeny, while light-demanding species underwent more significant changes with size, similar to the ontogenetic changes in RGR observed in this study.

The ontogenetic changes we observed in SLA were not always consistent with variations in C gain and RGR (Table 3.2; Figures 3.3, 3.6, and 3.8). For example, SLA

of *P. murrayi* did not vary with increased plant size, while rates of C gain and RGR showed the steepest ontogenetic declines in this species. Conversely, declines in SLA (or lack thereof, in the case of *D. australis*) did correspond with ontogenetic variations in C gain and RGR of the other three species. Ontogenetic declines in SLA reported in other studies have been attributed to increasing investment in structural support tissue per unit lamina surface area (Niklas and Enquist 2001, Niklas and Cobb 2008). Declines in SLA with tree size were observed both within and across species in these studies, which would help explain the declining growth and carbon balance seen in larger plants due to this added cost of harvesting light (Niklas and Cobb 2008). However, our combination of results suggests that different traits may be relatively more important in driving size-related changes in RGR among species depending on their shade-tolerance. Perhaps SLA plays a larger role in species that minimize self-shading within their crowns, while species that experience a higher degree of ontogenetic change in self-shading must maintain high SLA in order to lessen the effect of self-shading.

There was little coordination observed between area-based leaf-level gas exchange rates and C gain or RGR. Maximum photosynthetic rates increased most significantly with sapling size in *T. australis* (Figure 3.11), but both C gain and RGR showed significant declines with increasing size, indicating that variations in SLA and self-shading may play a larger role in the whole-plant performance of this species. Foliar dark respiration rates increased in larger saplings of *A. trifoliolatum* and *D. australis* (Figure 3.12), while these species had minimal or no changes in C gain and RGR with size. Again, the low degree of self-shading observed in these species may help to diminish the effects of increased respiration rates.

One caveat to our measurements of net daily carbon gain is that we did not measure respiration rates of stem or belowground tissues, nor did we include woody tissue respiration in our estimates of C gain. The fractional investment of annual plant growth allocated to leaves generally declines with increasing plant size (Sterck and Bongers 1998), which causes an increase in the ratio of woody respiratory tissues in larger trees (Gerrish 1990, Delagrange et al. 2004, Lusk et al. 2004, Machado and Reich 2006). This represents an obvious additional respiratory cost for the saplings measured in this study,

indicating that whole-plant C gain of all species is likely much lower than reported here, and the slope of the relationship between C gain and sapling size is likely far steeper.

An understanding of species' shade tolerance throughout developmental stages is required to improve models of forest dynamics (Poorter et al. 2005, Lusk et al. 2008). This paper shows that significant ontogenetic variations in net daily carbon gain and RGR occur in species that differ widely in shade tolerance, but the magnitude of these changes is dependent on successional status and light availability. These results indicate that work on saplings may detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone. These differences could have important effects on successional patterns of all forest types.

Table 3.1. Study species with families and the 10th percentile of a random sample of the distribution of juveniles (13 to 238 cm tall) in relation to photosynthetic photon flux density transmittance ($PPFD_{10}$) as an indicator of minimum light requirements for each species.

Species	Family	$PPFD_{10}$	Sample size
Argyrodendron trifoliolatum F.Muell	Malvaceae	1.37	127
Diploglottis australis (G.Don) Radlk.	Sapindaceae	1.62	130
Toona australis (Kuntze) Harms	Meliaceae	3.91	115
Polyscias murrayi (F.Muell) Harms	Araliaceae	4.19	111

Table 3.2. Effects of light availability and juvenile tree size on leaf and whole-plant traits of four subtropical evergreen species. All were traits except for relative growth rate were $\log_{10}$-transformed prior to analysis to roughly normalize the data.

Variable	Effect		Species			
			A. trifoliolatum	*D. australis*	*T. australis*	*P. murrayi*
SLA ($cm^2 g^{-1}$)	log (PPFD)	F-value	0.08	37.58	15.76	136.42
		p	0.819	**< 0.001**	**< 0.001**	**< 0.001**
	log (Height)	F-value	6.30	3.42	28.41	0.00
		p	**0.026**	*0.087*	**< 0.001**	0.849
	log (PPFD) x log (Height)	F-value	0.48	1.90	0.48	0.53
		p	0.481	0.294	0.887	0.673
		R^2	0.05	0.27	0.38	0.62
		n	118	126	106	90
A_{area} ($\mu mol\ CO_2\ m^{-2}s^{-1}$)	log (PPFD)	F-value	5.06	16.32	1.59	42.25
		p	**0.031**	**< 0.001**	0.140	**< 0.001**
	log (Height)	F-value	3.96	0.10	4.28	0.08
		p	*0.073*	0.782	**0.048**	0.656
	log (PPFD) x log (Height)	F-value	0.96	0.01	0.16	3.39
		p	0.729	0.766	0.792	0.329
		R^2	0.27	0.30	0.23	0.53
		n	37	38	32	41

Rd_{area} (μmol CO_2 m^{-2}s^{-1})	log (PPFD)	F-value	12.60	2.50	0.32	11.36
		p	**0.001**	**0.037**	0.500	**0.001**
	log (Height)	F-value	5.02	7.13	2.82	4.28
		p	*0.085*	**0.009**	0.145	0.135
	log (PPFD) x log (Height)	F-value	0.04	0.03	0.09	0.01
		p	0.801	0.413	0.677	0.809
		R^2	0.39	0.27	0.11	0.27
		n	38	37	32	41
Net C gain (mmol m^{-2}day^{-1})	log (PPFD)	F-value	14.52	40.70	28.09	59.60
		p	**0.002**	**< 0.001**	**< 0.001**	**< 0.001**
	log (Height)	F-value	1.88	1.74	3.96	22.47
		p	0.116	0.172	**0.033**	**< 0.001**
	log (PPFD) x log (Height)	F-value	1.74	0.06	0.07	0.30
		p	0.217	0.928	0.732	0.942
		R^2	0.29	0.53	0.50	0.70
		n	35	37	36	32
Relative growth rate	log (PPFD)	F-value	43.56	27.67	53.88	173.71
		p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	log (Height)	F-value	19.10	2.13	13.40	144.00
		p	**< 0.001**	0.141	**< 0.001**	**< 0.001**
	log (PPFD) x log (Height)	F-value	1.42	0.25	0.85	28.62
		p	0.269	0.597	0.525	**< 0.001**
		R^2	0.29	0.19	0.36	0.74
		n	122	127	108	84
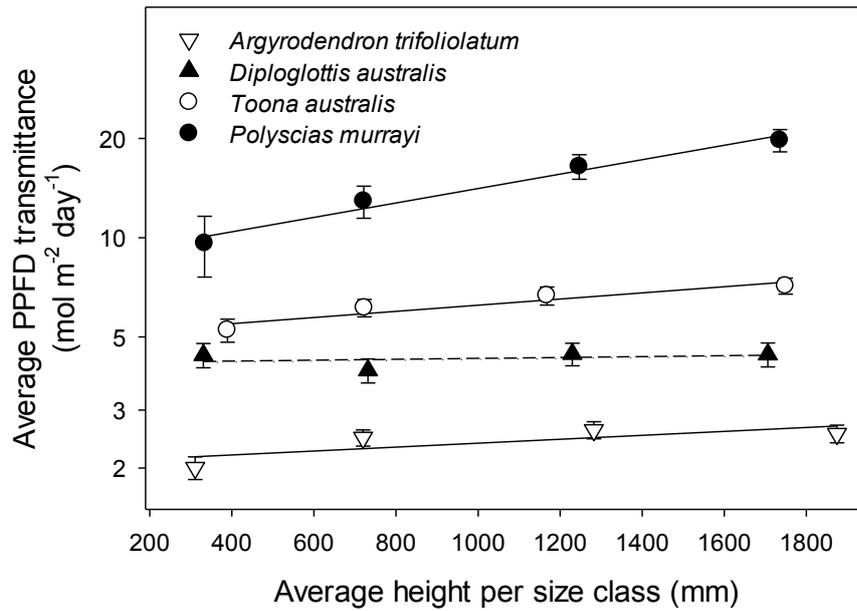
Figure 3.1. Average light environment of four juvenile size classes of four tree species in a subtropical forest. Solid lines denote species with statistically significant increases ($p < 0.05$) in light environment with increased sapling size; dashed line indicates no significant change across the size range.
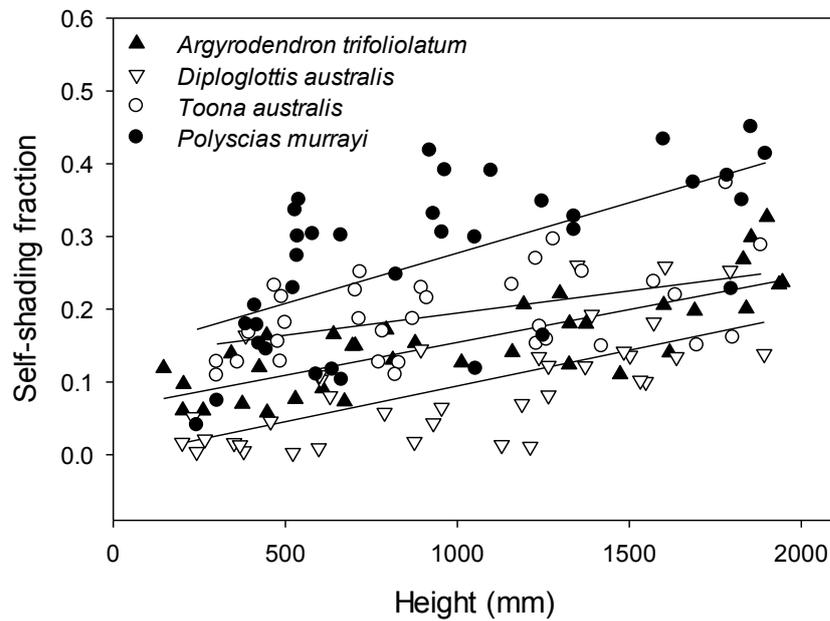
Figure 3.2. Relationships between crown self-shading and height of juvenile subtropical tree species. Lines denote statistically significant ($p < 0.05$) increases with increased sapling size. R^2-values were 0.63, 0.45, 0.23, and 0.36 for *A. trifoliolatum*, *D. australis*, *P. murrayi*, and *T. australis*, respectively.
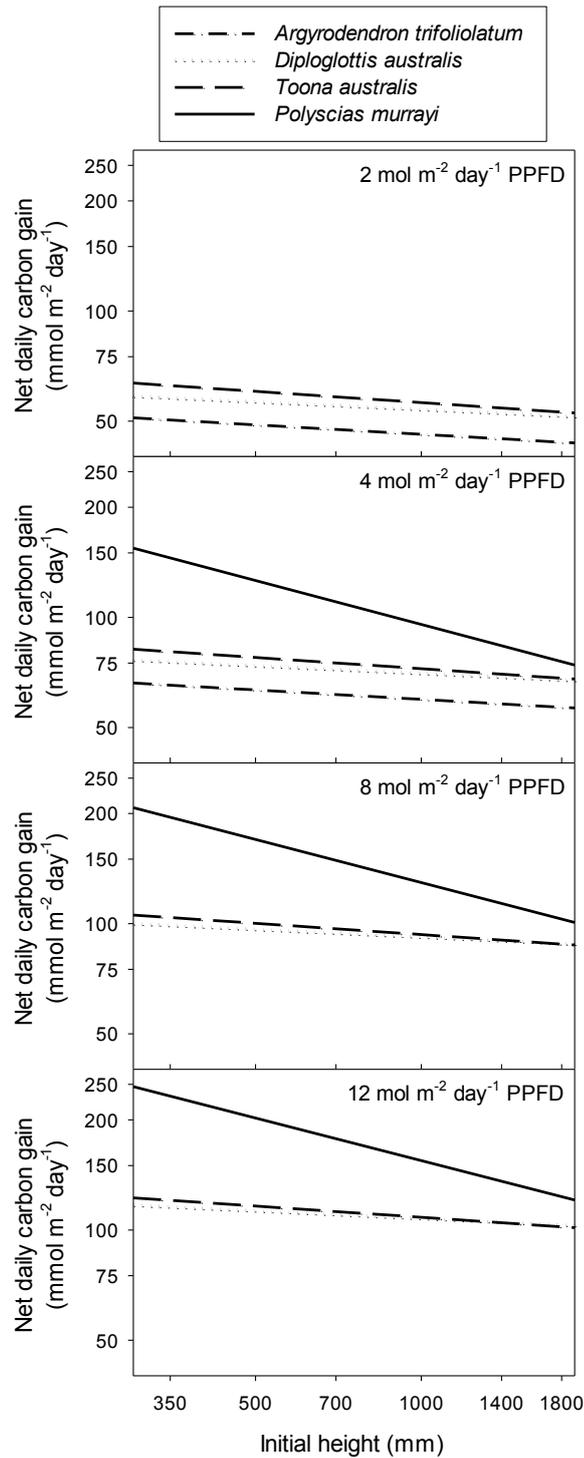
Figure 3.3. Variation in net daily carbon gain of juvenile subtropical tree species as a function of sapling size. Using the predicted formulas from the relationships between C gain and log(PPFD), log(Height), and their interaction for each species (excluding the interaction term when it was not significant), we estimated C gain for all species across the range of light levels in which they naturally occurred. Panels represent estimates from statistical models at assigned values of 2, 4, 8, and 12 mol m^{-2} day^{-1} transmitted PPFD.
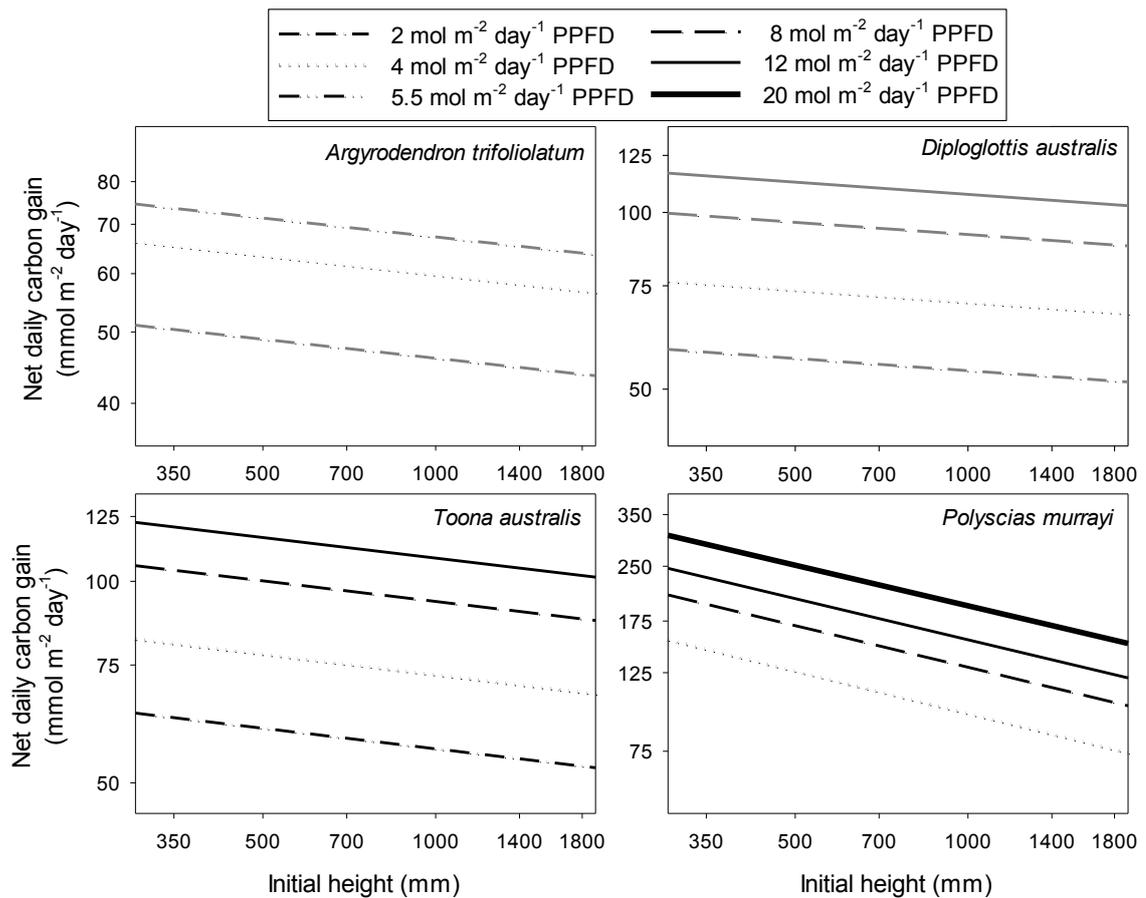
Figure 3.4. Variation in net daily carbon gain of juvenile subtropical tree species as a function of log(PPFD), log(Height), and their interaction. Using the predicted formulas from these relationships (excluding the interaction term when it was not significant), we estimated C gain for all species across the range of light levels in which they naturally occurred. Lines represent estimates from statistical models at assigned values of 2, 4, 5.5, 8, 12, and 20 mol m^{-2} day^{-1} transmitted PPFD. Significant ontogenetic changes ($p < 0.05$) are shown with black lines; non-significant relationships are shown in gray.
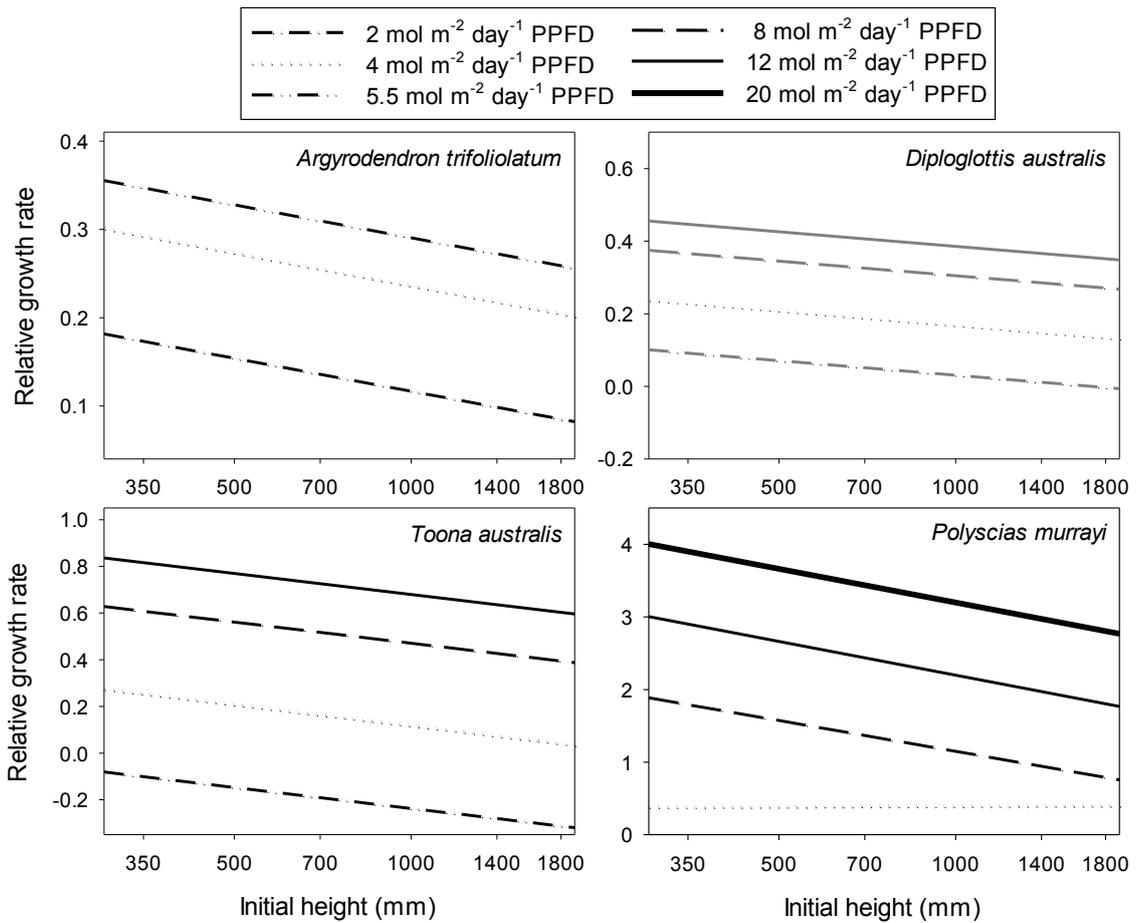
Figure 3.5. Variation in relative growth rates (RGR) of juvenile subtropical tree species as a function of log(PPFD), log(Height), and their interaction. Using the predicted formulas from these relationships (excluding the interaction term when it was not significant), we estimated RGR for all species across the range of light levels in which they naturally occurred. Lines represent estimates from statistical models at assigned values of 2, 4, 5.5, 8, 12, and 20 mol m^{-2} day^{-1} transmitted PPFD. Significant ontogenetic changes ($p < 0.001$) are shown with black lines; non-significant relationships are shown in gray.
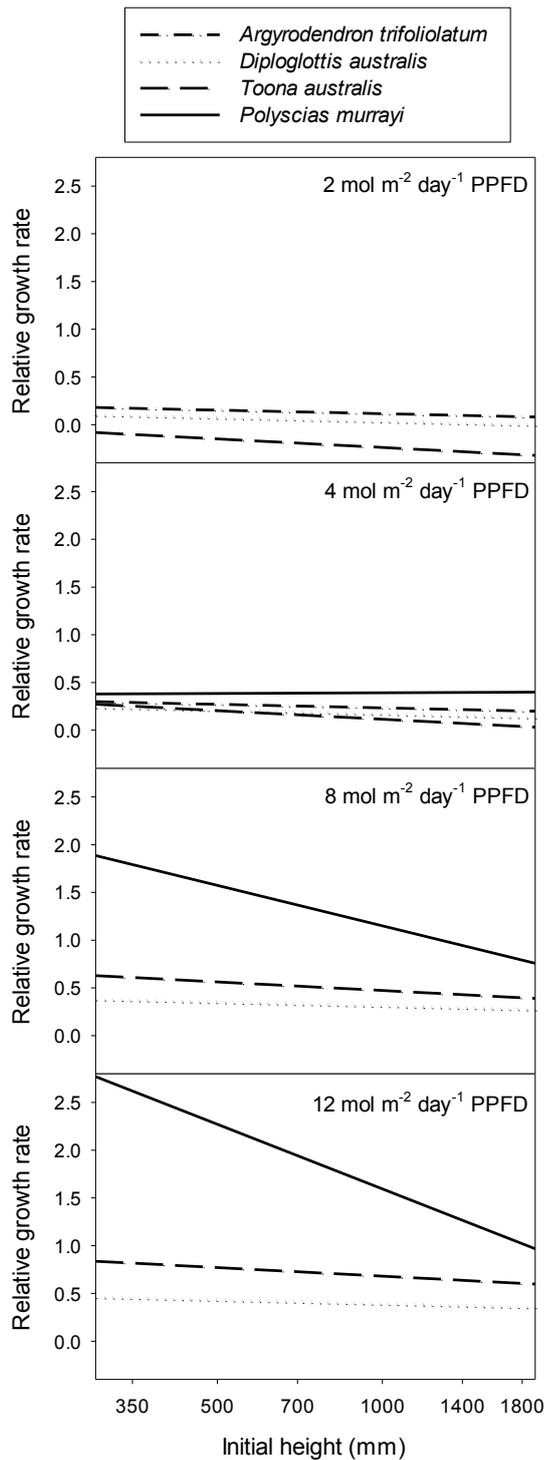
Figure 3.6. Variation in relative growth rates (RGR) of juvenile subtropical tree species as a function of sapling size. Using the predicted formulas from the relationships between RGR and log(PPFD), log(Height), and their interaction for each species (excluding the interaction term when it was not significant), we estimated RGR for all species across the range of light levels in which they naturally occurred. Panels represent estimates from statistical models at assigned values of 2, 4, 8, and 12 mol m^{-2} day^{-1} transmitted PPFD.
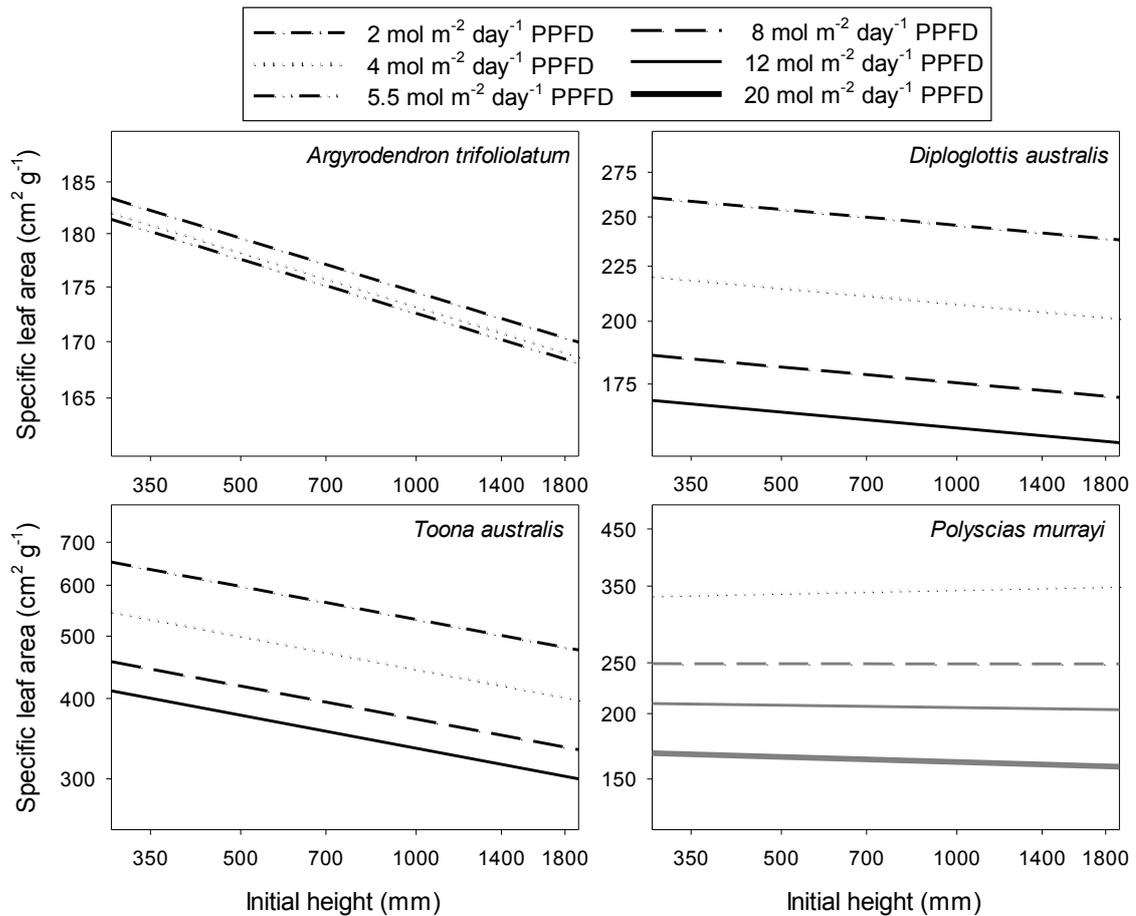
Figure 3.7. Variation in specific leaf area (SLA) of juvenile subtropical tree species as a function of log(PPFD), log(Height), and their interaction. Using the predicted formulas from these relationships (excluding the interaction term when it was not significant), we estimated SLA for all species across the range of light levels in which they naturally occurred. Lines represent estimates from statistical models at assigned values of 2, 4, 5.5, 8, 12, and 20 mol m^{-2} day^{-1} transmitted PPFD. Significant ontogenetic changes ($p < 0.10$) are shown with black lines; non-significant relationships are shown in gray.
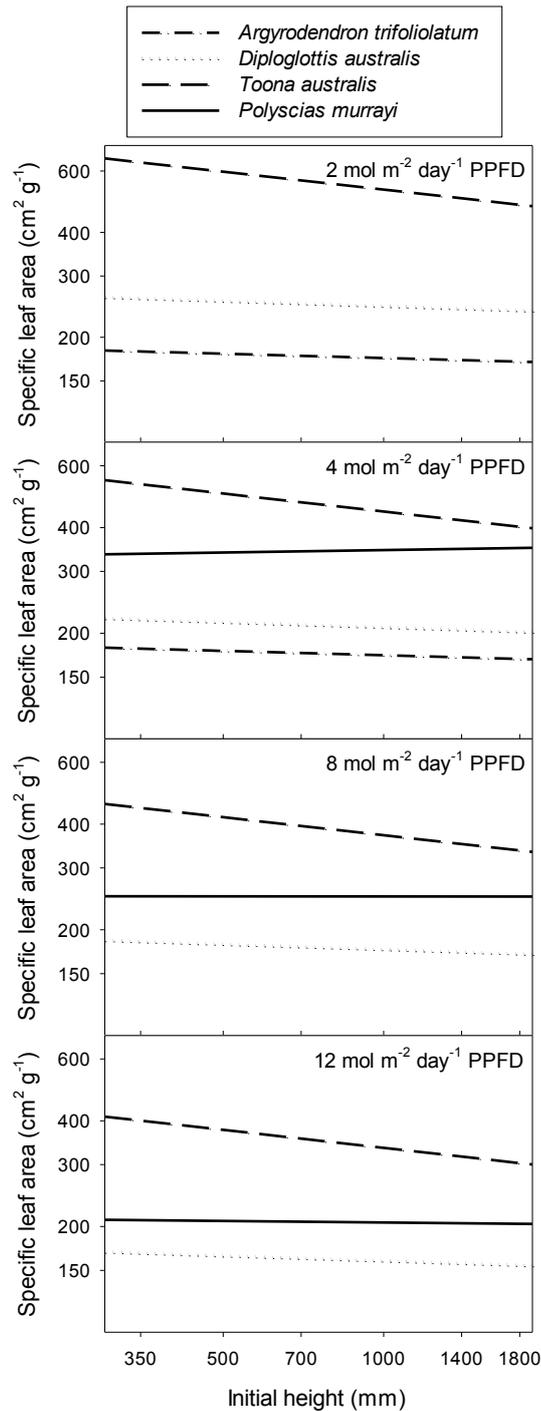
Figure 3.8. Variation in specific leaf area (SLA) of juvenile subtropical tree species as a function of sapling size. Using the predicted formulas from the relationships between SLA and log(PPFD), log(Height), and their interaction for each species (excluding the interaction term when it was not significant), we estimated SLA for all species across the range of light levels in which they naturally occurred. Panels represent estimates from statistical models at assigned values of 2, 4, 8, and 12 mol m^{-2} day^{-1} transmitted PPFD.
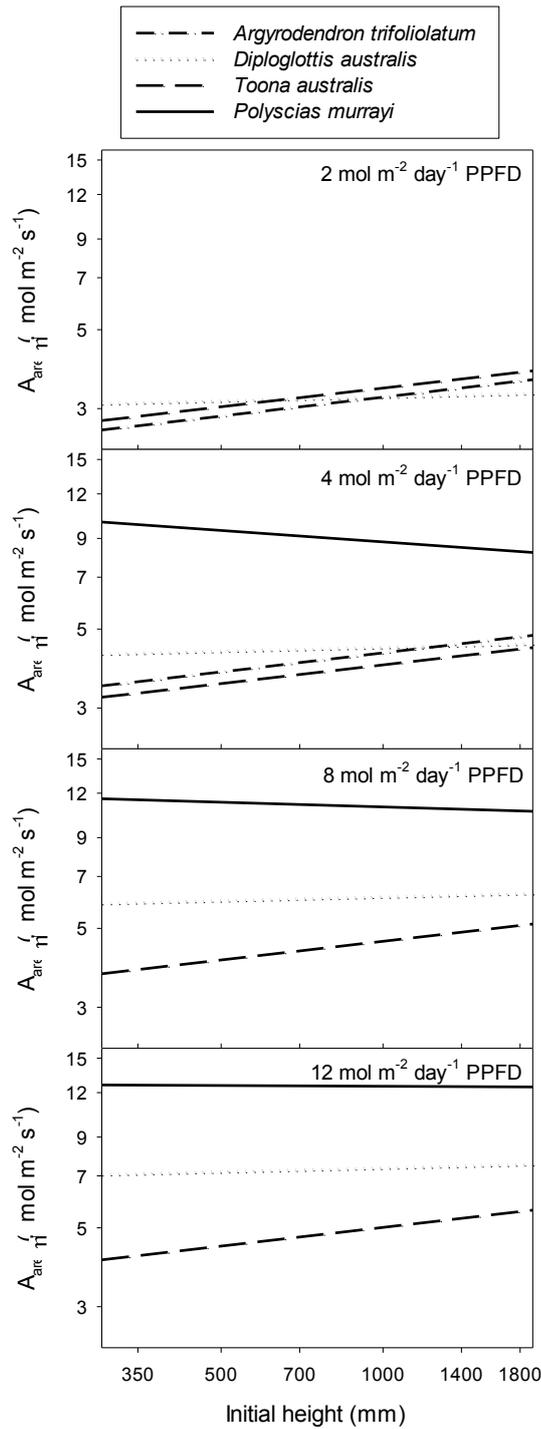
Figure 3.9. Variation in area-based maximum photosynthetic rates (A_{area}) of juvenile subtropical tree species as a function of sapling size. Using the predicted formulas from the relationships between A_{area} and log(PPFD), log(Height), and their interaction for each species (excluding the interaction term when it was not significant), we estimated A_{area} for all species across the range of light levels in which they naturally occurred. Panels represent estimates from statistical models at assigned values of 2, 4, 8, and 12 mol m^{-2} day^{-1} transmitted PPFD.

78
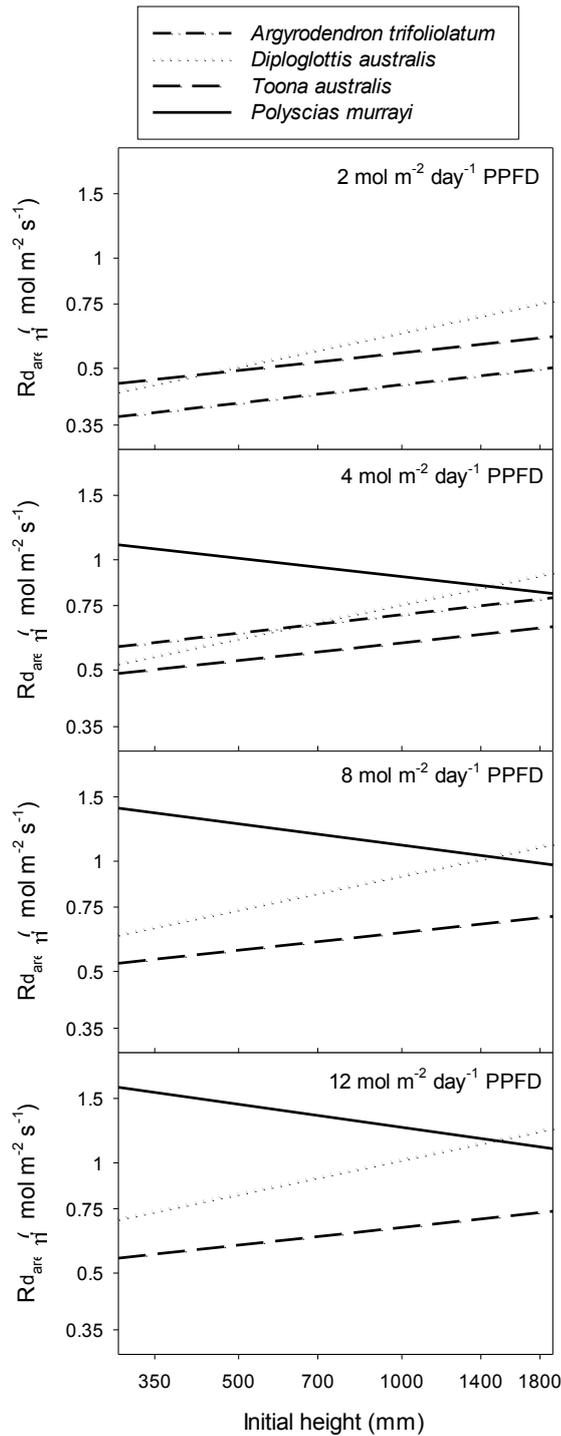
Figure 3.10. Variation in area-based dark respiration rates (Rd_{area}) of juvenile subtropical tree species as a function of sapling size. Using the predicted formulas from the relationships between Rd_{area} and log(PPFD), log(Height), and their interaction for each species (excluding the interaction term when it was not significant), we estimated Rd_{area} for all species across the range of light levels in which they naturally occurred. Panels represent estimates from statistical models at assigned values of 2, 4, 8, and 12 mol m^{-2} day^{-1} transmitted PPFD.
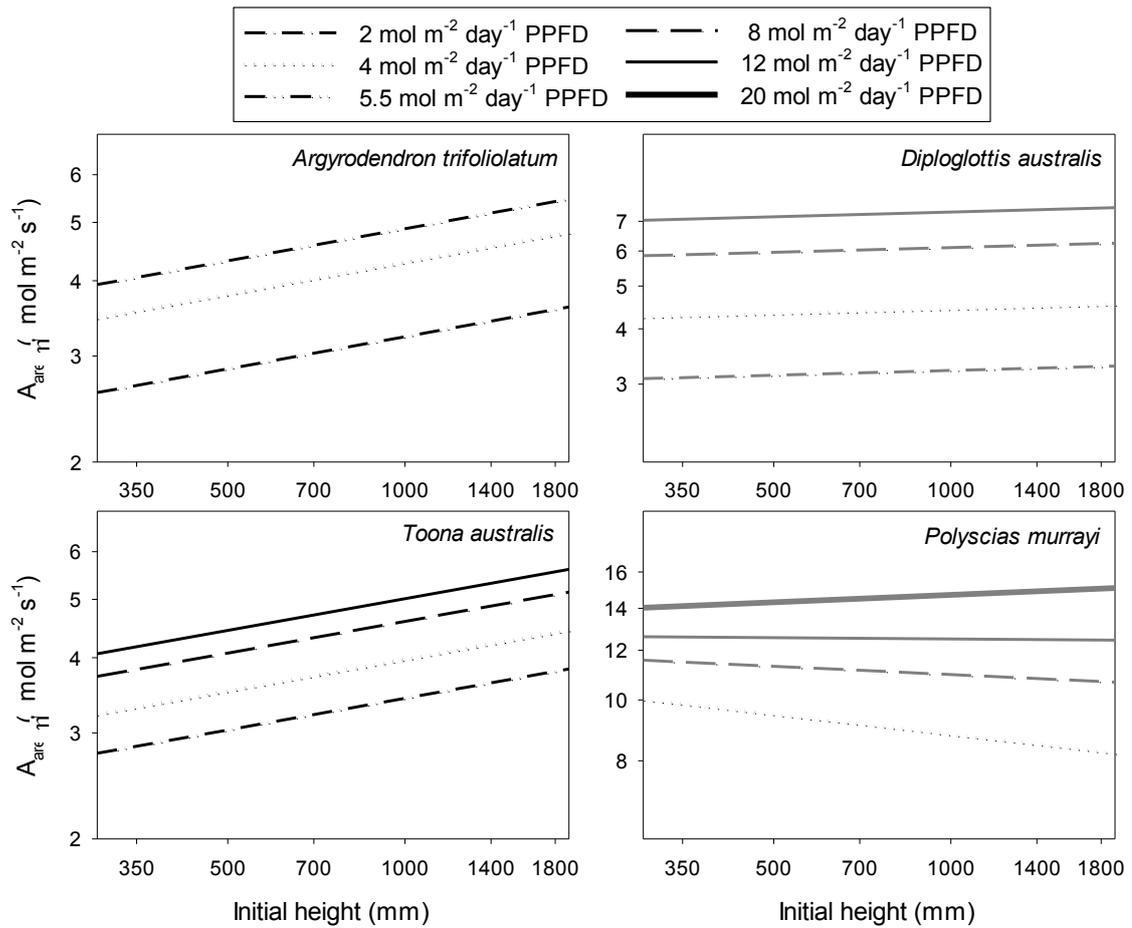
Figure 3.11. Variation in area-based maximum photosynthetic rates (A_{area}) of juvenile subtropical tree species as a function of log(PPFD), log(Height), and their interaction. Using the predicted formulas from these relationships (excluding the interaction term when it was not significant), we estimated (A_{area}) for all species across the range of light levels in which they naturally occurred. Lines represent estimates from statistical models at assigned values of 2, 4, 5.5, 8, 12, and 20 mol m^{-2} day^{-1} transmitted PPFD. Significant ontogenetic changes ($p < 0.10$) are shown with black lines; non-significant relationships are shown in gray.

Figure 3.12. Variation in area-based dark respiration rates (Rd$_{area}$) of juvenile subtropical tree species as a function of log(PPFD), log(Height), and their interaction. Using the predicted formulas from these relationships (excluding the interaction term when it was not significant), we estimated (Rd$_{area}$) for all species across the range of light levels in which they naturally occurred. Lines represent estimates from statistical models at assigned values of 2, 4, 5.5, 8, 12, and 20 mol m^{-2} day^{-1} transmitted PPFD. Significant ontogenetic changes ($p < 0.10$) are shown with black lines; non-significant relationships are shown in gray.

Conclusions

A better understanding of variations in plant functional traits and in light requirements throughout developmental stages is required to improve models of forest dynamics, which assume that properties of tissues and whole plants do not vary with ontogeny. It has been shown that traits change with tree size in complex patterns, and an effort has been made in recent years to resolve some of these patterns, though it is often difficult to determine exactly which factors are driving the changes observed. Here, I investigated patterns of leaf, stem, and whole-plant traits as a function of tree size in cold-temperate deciduous and subtropical evergreen species. I examined the effect of ontogeny across a wide range of size classes, as well as on species that differ in leaf habit and biome. I looked for patterns of morphological traits and physiological mechanisms operating at the leaf and whole-plant scale, thus allowing for identification of mechanisms that underlie observed variations in growth rates and shade tolerance. I learned the following:

Chapter 1: I asked how leaf and stem traits of three widespread North American deciduous tree species varied with tree size. Leaf size, leaf mass per unit area, nitrogen content, photosynthetic capacity, and dark respiration rates were all found to vary significantly with tree size. These results supported our overarching hypothesis that leaf and stem traits vary with increased tree height, but patterns and coordination among traits were less consistent than we predicted, and traits did not vary in a manner associated with a universal shift towards a lower "return on investment" strategy. This strategy posits that variation among taxa in leaf traits result from selection along a strategy continuum from slow to fast return on investment associated with the leaf economics spectrum. Instead, species traits varied with size in patterns that likely reflect complex variation in water, light, nitrogen, and carbon availability, storage, and use; with consequential impacts on the morphology, chemistry and metabolism of leaves and twigs.

Chapter 2: I measured gas exchange and biomass distribution traits of juvenile *Acer saccharum* growing in a shaded understory in Minnesota, USA, digitized and calculated the light environment of all 18 plants, and used the simulation model YPLANT to determine how tree size influenced net daily carbon gain. Net carbon gain per gram plant tissue of trees declined with increasing plant size, causing an increase in light requirements. A number of tissue and whole-plant traits varied with

size. However, the most important drivers of this decline in net carbon gain with sapling size were a higher degree of self-shading and a decline in the fraction of aboveground biomass distributed to foliage, while leaf-level traits were not strong predictors of the decline. These results suggest that significant ontogenetic variations in light requirements and carbon gain occur even in some shade-tolerant species, indicating that work on saplings may detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone.

Chapter 3: I measured gas exchange, biomass distribution traits, and growth rates of juveniles of four evergreen rainforest tree species differing in shade tolerance in Nightcap National Park, New Shout Wales, Australia, and used the YPLANT model to determine how tree size influenced net daily carbon gain. Increased sapling size caused a significant decline in net daily carbon gain and relative growth rates (RGR) of light-demanding species, while ontogenetic declines were minimal or absent in shade-tolerant species. Variations in self-shading in larger saplings followed patterns that were consistent with these change in RGR and C gain, while leaf-level traits such as specific leaf area and respiration rates were not strong predictors of these ontogenetic patterns. The slope of the relationship between light availability and RGR was steepest in light-demanding species, leading to crossovers in RGR between shade-tolerant and light-demanding species at low light, in both small seedlings and larger juveniles. Our results indicate that increased sapling size causes a convergence among species in growth and shade tolerance at high light levels, but a divergence at low light. Again, this highlights the importance of comparing traits at different life stages, since this work can detect differences in shade tolerance and associated traits that are not evident in studies utilizing seedlings alone.

While the general light conditions under which each of my eight measured species evolved were similar (i.e. all are trees common in forest gaps and understories and vary in shade tolerance within those environments), four of the eight species were temperate deciduous and four were subtropical evergreens. Therefore, my data are most relevant at the species or local community level and in qualitative comparisons across leaf habit. Generally speaking, shade-tolerant species and species with long-lived leaves expressed low rates of gas exchange and nitrogen content, while light-demanding species and species with shorter leaf lifespans showed higher rates of gas exchange and leaf nitrogen. However, size-related patterns of leaf-level traits differed among species, making it difficult to disentangle the factors responsible for these

changes. In the case of *A. saccharum*, leaf nitrogen and gas-exchange rates increased with sapling size, but the bivariate relationships between the traits were weak relative to other studies. In the other seven deciduous and evergreen species measured, traits often varied in conflicting patterns, such as increased nitrogen content in leaves that showed little or no variability in photosynthetic rates. It is possible that, in cases like this one, a greater fraction of leaf nitrogen is allocated to non-metabolic functions with increasing size rather than to photosynthetic machinery. Hydraulic influences, rather than leaf nitrogen content, could also be driving variations in leaf gas exchange rates in taller trees. Therefore, future research utilizing leaf-level traits should consider integrating leaf structure, chemistry and stomatal and mesophyll factors to help resolve some of these mechanistic questions.

Variations in self-shading and net daily carbon gain rates per gram of aboveground tissue taken together explained most of the increase in light requirements observed in juvenile *Acer saccharum* trees. Self-shading and C gain were also predictors of declining growth and shade tolerance in subtropical evergreens, though these estimates of C gain did not include woody tissue respiration rates, so the relationship was weaker than that seen for *A. saccharum.* The importance of woody tissue biomass fraction and respiration rates were quite apparent in Chapter 2, though measuring these traits required destructive harvesting of all aboveground tissues of these juvenile trees. More studies like this one that incorporate crown architecture measurements with total respiration rates of harvested tissues, or approaches using allometric equations to estimate woody respiration rates in trees that vary in size, are necessary to gain better estimates of net carbon gain. While this dissertation did not find clear relationships between leaf traits and plant size or net carbon gain, it is still important to investigate these trait variations further. They are still useful measurements in their own right, even if their integration into models of whole-plant traits is also useful.

Although the slopes of the relationship between light availability and RGR of evergreens correlated with species' light requirements, it remains unclear whether this relationship will hold true for deciduous species. Deciduous species face an additional constraint in having to replace all of their leaf tissue each year in order to survive, so relative differences in shade tolerance among these species may not be as distinct as those in evergreen species. Further studies of the relationship between

growth and light availability of deciduous species are required to determine how leaf habit affects ontogenetic changes in shade tolerance.

Literature cited

Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image processing with Image J. Biophotonics International **11**:36-42.

Ambrose, A. R., S. C. Sillett, and T. E. Dawson. 2009. Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. Plant Cell and Environment **32**:743-757.

Augspurger, C. K. 2008. Early spring leaf out enhances growth and survival of saplings in a temperate deciduous forest. Oecologia **156**:281-286.

Baker, F. S. 1949. A Revised Tolerance Table. Journal of Forestry **47**:179-181.

Baltzer, J. L. and S. C. Thomas. 2007. Determinants of whole-plant light requirements in Bornean rain forest tree saplings. Journal of Ecology **95**:1208-1221.

Bazzaz, F. A. 1979. Physiological ecology of plant succession. Annual Review of Ecology and Systematics **10**:351-371.

Binkley, D., M. G. Ryan, J. L. Stape, H. Barnard, and J. H. Fownes. 2002. Age-related decline in forest ecosystem growth, an individual-tree, stand-structure hypothesis. Ecosystems **5**:58-67.

Boardman, N. K. 1977. Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology **28**:355-377.

Bond, B. J. 2000. Age-related changes in photosynthesis of woody plants. Trends in Plant Science **5**:349-353.

Buchman, R. G., S. P. Pederson, and N. R. Walters. 1983. A tree survival model with application to species of the Great Lakes region. Canadian Journal of Forest Research **13**:601-608.

Canham, C. D., J. S. Denslow, W. J. Platt, J. R. Runkle, T. A. Spies, and P. S. White. 1990. Light regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. Canadian Journal of Forest Research **20**:620-631.

Carswell, F. E., P. Meir, E. V. Wandelli, L. C. M. Bonates, B. Kruijt, E. M. Barbosa, A. D. Nobre, J. Grace, and P. G. Jarvis. 2000. Photosynthetic capacity in a central Amazonian rain forest. Tree Physiology **20**:179-186.

Chabot, B. F. and D. J. Hicks. 1982. The ecology of leaf lifespans. Annual Review of Ecology and Systematics **13**:229-259.

Chazdon, R. L. and C. B. Field. 1987. Determinants of photosynthetic capacity in six rainforest Piper species. Oecologia **73**:222-230.

Coley, P. D. 1983. Herbivory and defense characteristics of tree species in a lowland tropical forest. Ecological Monographs **53**:209-233.

Connell, J. H. and R. O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. The American Naturalist **111**:1119-1144.

Coopman, R. E., V. F. Briceno, L. J. Corcuera, M. Reyes-Diaz, D. Alvarez, K. Saez, J. I. Garcia-Plazaola, M. Alberdi, and L. A. Bravo. 2011. Tree size and light availability increase photochemical instead of non-photochemical capacities of *Nothofagus nitida* trees growing in an evergreen temperate rain forest. Tree Physiology **31**:1128-1141.

Cregg, B. M., J. E. Halpin, P. M. Dougherty, and R. O. Teskey. 1989. Comparitive physiology and morphology of seedling and mature forest trees. Page 331 *in* R. D. Noble, J. L. Martin, and K. L. Jensen, editors. Air Pollution Effects on Vegetation. USDA Forest Service, Broomall, PA.

Delagrange, S., C. Messier, M. J. Lechowicz, and P. Dizengremel. 2004. Physiological, morphological and allocational plasticity in understory

deciduous trees: importance of plant size and light availability. Tree Physiology **22**:775-784.

Drake, J. E., L. M. Raetz, S. C. Davis, and E. H. DeLucia. 2010. Hydraulic limitation not declining nitrogen availability causes the age-related photosynthetic decline in loblolly pine (*Pinus taeda* L.). Plant Cell and Environment **33**:1756-1766.

Eissenstat, D. M. and R. D. Yanai. 2002. Root life span, efficiency, and turnover. Pages 221-238 *in* Y. Waisel, A. Eshel, and U. Kafkafi, editors. Plant Roots: The Hidden Half. Marcel Dekker, Inc., New York.

Falster, D. S., P. B. Reich, D. S. Ellsworth, I. J. Wright, M. Westoby, J. Oleksyn, and T. Lee. 2011. Lifetime return on investment increases with leaf lifespan among 10 Australian woodland species. New Phytologist **193**:409-419.

Falster, D. S. and M. Westoby. 2003. Leaf size and angle vary widely across species: what consequences for light interception? New Phytologist **158**:509-525.

Frazer, G. W., C. D. Canham, and K. P. Lertzman. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-color fisheye photographs, users manual and program documentation. Simon Frazer University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York.

Fredericksen, T. S., K. C. Steiner, J. M. Skelley, B. J. Joyce, T. E. Kolb, K. B. Kouterick, and J. R. Ferdinand. 1996. Diel and seasonal patterns of leaf gas exchange and xylem water potentials of different sized *Prunus serotina* Ehrh. trees. Forest Science **42**:359-365.

Gerrish, G. 1990. Relating carbon allocation patterns to tree senescence in *Metrosideros* forests. Ecology **71**:1176-1184.

Givnish, T. J. 1988. Adaptation to Sun and Shade: A Whole-plant Perspective. Australian Journal of Plant Physiology **15**:63-92.

Gower, S. T., R. E. McMurtrie, and D. Murty. 1996. Aboveground net primary production decline with stand age: potential causes. Trends in Ecology and Evolutionary Research **11**:378-382.

Grulke, N. E. and P. R. Miller. 1994. Changes in gas exchange characteristics during the life span of the giant sequoia: implications for response to current and future concentrations of atmospheric ozone. Tree Physiology **14**:658-668.

Gulmon, S. L. and C. C. Chu. 1981. The effect of light and nitrogen on photosynthesis, leaf characteristics and dry matter allocation in the Chaparral shrub *Diplacus aurantiacus*. Oecologia **49**:207-212.

Hanba, Y. T., S. Miyazawa, and I. Terashima. 1999. The influence of leaf thickness on the CO_2 transfer conductance and leaf stable carbon isotope ratio for some evergreen tree species in Japanese warm-temperate forests. Functional Ecology **13**:632-639.

Harley, P., A. Guenther, and P. Zimmerman. 1996. Effects of light, temperature, and canopy position on net photosynthesis and isoprene emission from sweetgum leaves. Tree Physiology **16**:25-32.

Horn, H. S., editor. 1971. The Adaptive Geometry of Trees. Princeton University Press, Princeton, NJ, USA.

Hubbard, R. M., B. J. Bond, and M. G. Ryan. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiology **19**:165-172.

Ishida, A., K. Yazaki, and A. L. Hoe. 2005. Ontogenetic transition of leaf physiology and anatomy from seedlings to mature trees of a rain forest pioneer tree, *Macaranga gigantea*. Tree Physiology **25**:513-522.

Iwasa, Y. and D. Cohen. 1989. Optimal growth schedule of a a perennial plant. American Naturalist **133**:480-505.

Kattge, J., W. Knorr, P. B. Reich, M. G. Tjoelker, O. K. Atkin, T. Ziehn, and C. Wirth. in press. Modelling respiration of vegetation: Mathematical requirements of a general temperature-dependent Q_{10}.

Kennedy, M., T. M. Hinckley, and E. D. Ford. 2010. Defining how aging *Pseudotsuga* and *Abies* compensate for mulitple stresses through multi-criteria assessment of a functional-structural model. Tree Physiology **30**:3-22.

Kenzo, T., T. Ichie, Y. Watanabe, R. Yoneda, I. Ninomiya, and T. Koike. 2006. Changes in photosynthesis and leaf characteristics with tree height in five dipterocarp species in a tropical rain forest. Tree Physiology **26**:865-873.

King, D. A. 1994. Influence of Light Level on the Growth and Morphology of Saplings in a Panamanian Forest. American Journal of Botany **81**:948-957.

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.

Kitajima, K. and B. Bolker. 2003. Testing performance rank-reversals among coexisting species: crossover point innradiance analysis. Functional Ecology **17**:276-281.

Kitajima, K., S. S. Mulkey, and S. J. Wright. 2005. Variation in crown light utilization characteristics among tropical canopy trees. Annals of Botany **95**:535-547.

Kleiber, M. 1932. Body size and metabolism. Hilgardia **6**:315-353.

Kneeshaw, D. D., R. K. Kobe, K. D. Coates, and C. Messier. 2006. Sapling size influences shade tolerance ranking among southern boreal tree species. Journal of Ecology **94**:471-480.

Kobe, R. K. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos **80**:226-233.

Kobe, R. K. 1999. Light gradient partitioning among tropical tree species through differential seedling mortality and growth. Ecology **80**:187-201.

Koch, G. W., S. C. Sillett, G. M. Jennings, and S. D. Davis. 2004. The limits to tree height. Nature **428**:851-854.

Kohyama, T. and M. Hotta. 1990. Significance of Allometry in Tropical Saplings. Functional Ecology **4**:515-521.

Kolb, T. E. and J. E. Stone. 2000. Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. Tree Physiology **20**:1-12.

Korner, C. 1994. Biomass fractionation in plants: a reconsideration of definitions based on plant functions.*in* J. Roy and E. Garneir, editors. A Whole Plant Perspective on Carbon-Nitrogen Interactions. SPB Academic Publishing, The Hague.

Kozlowshi, J. and J. Uchmanski. 1987. Optimal individual growth and reproduction in perennial species with indeterminate growth. Evolutionary Ecology **1**:214-230.

Lee, T. D., P. B. Reich, and P. V. Bolstad. 2005. Acclimation of leaf respiration to temperature is rapid and related to specific leaf area, soluble sugars and leaf nitrogen across three temperate deciduous tree species. Functional Ecology **19**:640-647.

Lopez, O. R., K. Farris-Lopez, R. A. Montgomery, and T. J. Givnish. 2008. Leaf phenology in relation to canopy closure in southern Appalachian trees. American Journal of Botany **95**:1395-1407.

Lusk, C. 2002. Leaf area accumulation helps juvenile evergreen trees tolerate shade in a temperate rainforest. Oecologia **132**:188-196.

Lusk, C. H. 2004. Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. Functional Ecology **18**:820-828.

Lusk, C. H., D. S. Falster, C. K. Jara-Vergara, M. Jimenez-Castillo, and A. Saldana-Mendoza. 2008. Ontogenetic variation in light requirements of juvenile rainforest evergreens. Functional Ecology **22**:454-459.

Lusk, C. H., D. S. Falster, M. Perez-Millaqueo, and A. Saldana. 2004. Ontogenetic variation in light interception, self-shading and biomass distribution of seedlings of the conifer *Araucaria araucana* (Molina) K. Koch. Revista Chilena de Historia Natural **79**:321-328.

Lusk, C. H., M. M. Perez-Millaqueo, F. I. Piper, and A. Saldana. 2011. Ontogeny, understory light interception and simulated carbon gain of juvenile rainforest evergreens differing in shade tolerance. Annals of Botany **108**:419-428.

Machado, J. L. and P. B. Reich. 1999. Evaluation of several measures of canopy openness as predictors of photosynthetic photon flux density in deeply shaded conifer-dominated forest understory. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **29**:1438-1444.

Machado, J. L. and P. B. Reich. 2006. Dark respiration rate increases with plant size in saplings of three temperate tree species despite decreasing tissue nitrogen and nonstructural carbohydrates. Tree Physiology **26**:915-923.

Martinez-Vilalta, J., D. Vanderklein, and M. Mencuccini. 2007. Tree height and age-related decline in growth in Scots pine (*Pinus sylvestris* L.). Oecologia **150**:529-544.

McConnaughay, K. D. M. and J. S. Coleman. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology **80**:2581-2593.

Mencuccini, M., T. Holtta, and J. Martinez-Vilalta. 2011. Comparative criteria for models of the vascular transport systems of tall trees.*in* F. C. Meinzer, B. Lachenbruck, and T. E. Dawson, editors. Size- and Age-Related Changes in Tree Structure and Function. Springer, New York.

Merilo, E., I. Tulva, O. Raim, A. Kukit, A. Sellin, and O. Kull. 2009. Changes in needle nitrogen partitioning and photosynthesis during 80 years of tree ontogeny in *Picea abies*. Trees **23**:951-958.

Merino, J., C. Field, and H. A. Mooney. 1982. Construction and maintenance costs of Mediterranean-climate evergreen and deciduous leaves. I. Growth and CO_2 exchange analysis. Oecologia **53**:208-213.

Messier, C. and E. Nikinmaa. 2000. Effects of light availability and sapling size on the growth, biomass allocation, and crown morphology of understory sugar maple, yellow birch, and beech. Ecoscience **7**:345-356.

Miller, P. R., L. E. Eddleman, and J. M. Miller. 1991. The response of western juniper (*Juniperus occidentalis*) to reductions in above- and below-ground tissue. Canadian Journal of Forest Research **21**:207-216.

Mitchell, K. A., P. V. Bolstad, and J. M. Vose. 1999. Interspecific and environmentally induced variation in foliar dark respiration among eighteen southeastern deciduous tree species. Tree Physiology **19**:861-870.

Moad, A. S. 1992. Dipterocarp juvenile growth and understroy light availability in Malaysian tropical forest. PhD Dissertation. University of Aberdeen, Aberdeen, UK.

Montgomery, R. A. and R. L. Chazdon. 2002. Light gradient partitioning by tropical tree seedlings in the absence of canopy gaps. Oecologia **131**:165-174.

Nabeshima, E. and H. Hiura. 2008. Size-dependency in hydraulic and photosynthetic properties of three *Acer* species having different maximum sizes. Ecological Research **23**:281-288.

Niinemets, U. 1997a. Distribution patterns of carbon and nitrogen as affected by tree dimensions and relative light conditions in the canopy of *Picea abies*. Trees **11**:144-154.

Niinemets, U. 1997b. Energy requirements for foliage construction depends on tree size in young *Picea abies* trees. Trees **11**:420-431.

Niinemets, U. 2002. Stomatal condictance alone does not explain the decline in foliar photosynthetic rate with increasing tree age and size in *Picea abies* and *Pinus sylvestris*. Tree Physiology **22**:515-535.

Niinemets, U., D. S. Ellsworth, A. Lukjanova, and M. Tobias. 2001. Site fertility and the morphological and photosynthetic acclimation of *Pinus sylvestris* needles to light. Tree Physiology **21**:1231-1244.

Niinemets, U. and O. Kull. 1995. Effects of light availability and tree size on the architecture and assimilative surface in the canopy of *Picea abies*: variation in needle morphology. Tree Physiology **15**:307-315.

Niklas, K. J. 1991. Effects of tissue volume and location on the mechanical consequences of dehydration of petioles. American Journal of Botany **78**:361-369.

Niklas, K. J. and E. D. Cobb. 2008. Evidence for "diminishing returns" from the scaling of stem diameter and specific leaf area. American Journal of Botany **95**:549-557.

Niklas, K. J. and B. J. Enquist. 2001. Invariant scaling relationships for interspecific plant biomass production rates and body size. Proceedings of the National Academy of Sciences **98**:2922-2927.

Pacala, S. W., C. D. Canham, J. Silander, J. A., and R. K. Kobe. 1994. Sapling growth as a function of resources in a north temperate forest. Canadian Journal of Forest Research **24**:2172-2183.

Parkhurst, D. F. 1994. Diffusion of CO_2 and other gases inside leaves. New Phytologist **126**:449-479.

Pastor, J., M. A. Stillwell, and D. Tilman. 1987. Nitrogen mineralization and nitrification in four Minnesota old fields. Oecologia **71**:481-485.

Pearcy, R. W. and W. M. Yang. 1996. A three-dimensional crown architecture model for assessment of light capture and carbon gain by understory plants. Oecologia **108**:1-12.

Peterson, D. and P. B. Reich. 2001. Prescribed fire in oak savanna: Fire frequency effects on stand structure and dynamics. Ecological Applications **11**:914-927.

Poorter, H., C. Remkes, and H. Lambers. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. Plant Physiology **94**:621-627.

Poorter, L. 1999. Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. Functional Ecology **13**:396-410.

Poorter, L., F. Bongers, F. J. Sterck, and H. Woll. 2003. Architecture of 53 rain forest tree species differing in adult stature and shade tolerance. Ecology **84**:602-608.

Poorter, L., F. Bongers, F. J. Sterck, and H. Woll. 2005. Beyond the regeneration phase: differentiation of height-light trajectories among tropical tree species. Journal of Ecology **93**:256-267.

Poorter, L. and K. Kitajima. 2007. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. Ecology **88**:1000-1011.

Reich, P. B., D. S. Ellsworth, and M. B. Walters. 1991. Leaf development and season influence the relationships between leaf nitrogen, leaf mass per area, and photosynthesis in maple and oak trees. Plant Cell and Environment **14**:251-259.

Reich, P. B., M. G. Tjoelker, K. S. Pregitzer, I. J. Wright, J. Oleksyn, and J. L. Machado. 2008. Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. Ecology Letters **11**:793-801.

Reich, P. B., C. Uhl, M. B. Walters, L. Prugh, and D. S. Ellsworth. 2004. Leaf demography and phenology in Amazonian rain forest: a census of 40,000 leaves of 23 tree species. Ecological Monographs **74**:3-23.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: global convergence in plant functioning. Proceedings of the National Academy of Sciences **94**:13730-13734.

Reich, P. B., M. B. Walters, D. S. Ellsworth, and C. Uhl. 1994. Photosynthesis-Nitrogen Relationships in Amazonian Tree Species. I. Patterns among Species and Communities. Oecologia **97**:62-72.

Reich, P. B., M. B. Walters, D. S. Ellsworth, J. M. Vose, J. C. Volin, C. Gresham, and W. D. Bowman. 1998. Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. Oecologia **114**:471-482.

Rijkers, T., T. L. Pons, and F. Bongers. 2000. The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. Functional Ecology **14**:77-86.

Ryan, M. G. 1991. Effects of climate change on plant respiration. Ecological Applications **1**:157-167.

Ryan, M. G. 1995. Foliar Maintenance Respiration of Sub-Alpine and Boreal Trees and Shrubs in Relation to Nitrogen-Content. Plant Cell and Environment **18**:765-772.

Ryan, M. G., D. Binkley, and J. H. Fownes. 1997. Age-related declines in forest productivity: pattern and process. Advances in Ecological Research **27**:213-262.

Sack, L. and P. J. Grubb. 2001. Why do species of woody seedlings change rank in relative growth rate between low and high irradiance? Functional Ecology **15**:145-154.

Sack, L. and P. J. Grubb. 2003. Crossovers in seedling relative growth rates between high and low irradiance: analyses and ecological potential. Functional Ecology **17**:281-287.

Schoettle, A. W. 1994. Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. Tree Physiology **14**:1055-1068.

Sendall, K. M. and P. B. Reich. in prep. Variation in leaf and fine stem CO_2 flux as a function of plant size: a comparison of seedlings, saplings, and trees. Tree Physiology.

Sipe, T. W. and F. A. Bazzaz. 1994. Gap partitioning among maples (*Acer*) in central New England - shoot architecture and photosynthesis. Ecology **75**:2318-2332.

Sterck, F. J. and F. Bongers. 1998. Ontogenetic chnages in size, allometry, and mechanical design of tropical rain forest trees. American Journal of Botany **85**:266-272.

Sterck, F. J., F. Schieving, A. Lemmens, and T. L. Pons. 2005. Performance of trees in forest canopies: explorations with a bottom-up functional-structural plant growth model. New Phytologist **166**:827-843.

Swanson, L. and G. N. Meyer, editors. 1990. Geologic Atlas of Washington County, Minnesota.

Ter Steege, H. 1996. Wonphot 5: a programme to analyze vegetation indices, light and light quality from hemispherical photographs. Tropenbos Guyana Reports. Georgetown, Guyana: Tropenbos Guyana Programme.

Thomas, S. C. 2010. Photosynthetic capacity peaks at intermediate size in temperate deciduous trees. Tree Physiology **30**:555-573.

Thomas, S. C. and W. E. Winner. 2002. Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. Tree Physiology **22**:117-127.

Thornley, J. H. M. 1976. Mathematical Models in Plant Physiology. Academic Press, London.

Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 1999a. Acclimation of respiration to temperature and CO_2 in seedlings of boreal tree species in relation to plant size and relative growth rate. Global Change Biology **5**:679-691.

Tjoelker, M. G., P. B. Reich, and J. Oleksyn. 1999b. Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. Plant Cell and Environment **22**:767-778.

Turner, J. and J. Kelly. 1981. Relationships between soil nutrients and vegetation in a north coast forest, New South Wales. Australian Foresty Journal **11**:201-208.

Valladares, F., J. B. Stillman, and R. W. Pearcy. 2002. Convergence in light capture efficiencies among tropical forest understory plants with contrasting crown architecture: a case of morphological compensation. American Journal of Botany **89**:1275-1284.

Walters, M. B., E. L. Kruger, and P. B. Reich. 1993a. Growth, biomass distribution and CO_2 exchangeof northern hardwood seedlings in high and low light: relationships with successional status and shade tolerance. Oecologia **94**:7-16.

Walters, M. B., E. L. Kruger, and P. B. Reich. 1993b. Relative Growth-Rate in Relation to Physiological and Morphological Traits for Northern Hardwood Tree Seedlings - Species, Light Environment and Ontogenic Considerations. Oecologia **96**:219-231.

Walters, M. B. and P. B. Reich. 1996. Are shade tolerance, survival, and growth linked? Low light and, nitrogen effects on hardwood seedlings. Ecology **77**:841-853.

Walters, M. B. and P. B. Reich. 1999. Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? New Phytologist **143**:143-154.

Walters, M. B. and P. B. Reich. 2000. Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. Ecology **81**:1887-1901.

Waring, R. H., A. J. S. McDonald, S. Larsson, T. Ericsson, A. Wiren, E. Arwidsson, A. Ericsson, and T. Lohammar. 1985. Differences in chemical composition of

plants grown at constant relative growth rates with stable mineral nutrition. Oecologia **66**:157-160.

Westoby, M., D. Warton, and P. B. Reich. 2000. The time value of leaf area. American Naturalist **155**:649-656.

Whitmore, T. C. 1989. Canopy gaps and the two major groups of trees. Ecology **70**:536-538.

Williams, K., C. Field, and H. A. Mooney. 1989. Relationships among leaf construction cost, leaf longevity, and light environment in rain-forst plants of the genus *Piper*. The American Naturalist **133**:198-211.

Woodruff, D. R., B. J. Bond, and F. C. Meinzer. 2004. Does turgor limit growth in tall trees? Plant Cell and Environment **27**:229-236.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.

Yoder, B. J., M. G. Ryan, R. H. Waring, A. W. Schoettle, and M. R. Kaufmann. 1994. Evidence of reduced photosynthetic rates in old trees. Forest Science **40**:513-527.

Zimmerman, M. H. 1983. Xylem structure and the ascent of sap. Springer, Berlin.

Appendix A. Supplementary information for chapter 1 results.

Appendix Table A.1. Results of linear regression analyses (of log-log transformed data) using tree height as the predictor of all traits examined. %PPFD was added as a covariate, but in most cases was not significant or was not retained in the model after stepwise regression analyses. Results are shown for trees growing in canopy gaps, in forest understories, and for pooled data. *P*-values <0.10 are indicated in bold.

		Gap			Understory			Pooled		
		R^2	p	Slope	R^2	p	Slope	R^2	p	Slope
Quercus ellipsoidalis	Leaf mass	**0.17**	**0.024**	**0.10**	**0.33**	**<0.001**	**0.16**	**0.22**	**<0.001**	**0.13**
	Leaf area	0.05	0.249	0.04	**0.33**	**<0.001**	**0.13**	**0.18**	**<0.001**	**0.09**
	LMA	**0.16**	**0.026**	**0.05**	**0.10**	**0.091**	**0.02**	0.04	0.138	0.03
	Leaf N_{area}	**0.44**	**<0.001**	**0.09**	**0.38**	**0.001**	**0.07**	**0.15**	**0.004**	**0.09**
	Leaf N_{mass}	**0.26**	**0.004**	**0.05**	**0.40**	**<0.001**	**0.05**	**0.29**	**<0.001**	**0.05**
	Leaf Rd_{area}	**0.13**	**0.052**	**-0.08**	**0.14**	**0.070**	**-0.07**	**0.08**	**0.044**	**-0.07**
	Leaf Rd_{mass}	**0.32**	**0.001**	**-0.11**	**0.20**	**0.029**	**-0.10**	**0.25**	**<0.001**	**-0.10**
	Leaf Rd/N	**0.41**	**<0.001**	**-0.16**	**0.27**	**0.008**	**-0.15**	**0.34**	**<0.001**	**-0.15**
	Stem diameter	**0.36**	**<0.001**	**0.09**	**0.40**	**<0.001**	**0.09**	**0.36**	**<0.001**	**0.08**

	Stem N$_{mass}$	**0.25**	**0.005**	**0.05**	0.03	0.470	-0.01	0.04	0.157	0.02
	Stem Rd$_{mass}$	0.06	0.177	-0.04	0.05	0.303	-0.03	**0.05**	**0.092**	**-0.03**
	Stem Rd/N	**0.19**	**0.017**	**-0.09**	0.01	0.995	-0.01	**0.08**	**0.036**	**-0.05**
Prunus serotina	Leaf mass	0.09	0.117	0.10	**0.35**	**0.003**	**0.22**	**0.15**	**0.003**	**0.17**
	Leaf area	0.01	0.701	-0.02	**0.21**	**0.026**	**0.11**	0.03	0.187	0.05
	LMA	**0.29**	**0.002**	**0.10**	**0.34**	**0.003**	**0.09**	**0.14**	**0.005**	**0.10**
	Leaf N$_{area}$	**0.46**	**<0.001**	**0.16**	**0.18**	**0.040**	**0.08**	**0.25**	**<0.001**	**0.12**
	Leaf N$_{mass}$	0.08	0.111	0.06	0.01	0.881	-0.01	0.02	0.363	0.02
	Leaf Rd$_{area}$	0.00	0.911	0.01	**0.13**	**0.081**	**-0.12**	0.02	0.312	-0.05
	Leaf Rd$_{mass}$	0.05	0.236	-0.07	**0.27**	**0.010**	**-0.19**	**0.14**	**0.005**	**-0.14**
	Leaf Rd/N	**0.12**	**0.058**	**-0.13**	**0.26**	**0.010**	**-0.19**	**0.18**	**0.002**	**-0.16**
	Stem diameter	0.02	0.459	0.03	0.02	0.548	0.02	0.02	0.338	0.03
	Stem N$_{mass}$	**0.12**	**0.060**	**0.08**	0.00	0.976	0.00	0.04	0.166	0.04
	Stem Rd$_{mass}$	0.00	0.805	0.01	0.01	0.288	-0.05	0.01	0.602	-0.02
	Stem Rd/N	0.07	0.992	-0.06	0.04	0.935	-0.05	0.05	0.910	-0.06

Acer rubrum	Leaf mass	**0.46**	**<0.001**	**0.28**	0.25	0.012	0.20	0.33	<0.001	0.24
	Leaf area	**0.29**	**0.002**	**0.17**	0.24	0.015	0.14	0.26	<0.001	0.16
	LMA	**0.44**	**<0.001**	**0.08**	0.26	0.011	0.05	0.16	0.003	0.07
	Leaf N_{area}	**0.61**	**<0.001**	**0.14**	0.35	0.002	0.10	0.33	<0.001	0.12
	Leaf N_{mass}	**0.35**	**<0.001**	**0.06**	0.22	0.020	0.04	0.28	<0.001	0.05
	Leaf Rd_{area}	**0.10**	**0.087**	**-0.05**	0.35	0.002	-0.09	0.06	0.084	-0.07
	Leaf Rd_{mass}	**0.30**	**0.002**	**-0.10**	0.37	0.002	-0.14	0.22	<0.001	-0.12
	Leaf Rd/N	**0.47**	**<0.001**	**-0.16**	0.43	<0.001	-0.19	0.36	<0.001	-0.17
	Stem diameter	**0.18**	**0.021**	**0.07**	0.36	0.002	0.09	0.23	<0.001	0.08
	Stem N_{mass}	0.06	0.193	0.04	**0.27**	**0.010**	**0.05**	0.10	0.017	0.05
	Stem Rd_{mass}	0.01	0.635	-0.02	**0.14**	**0.069**	**-0.05**	0.04	0.159	-0.03
	Stem Rd/N	0.05	0.996	-0.06	**0.33**	**0.004**	**-0.10**	0.12	0.010	-0.08

Appendix Table A.2. Results of 2nd order polynomial regression analyses (of log-log transformed data) using tree height as the predictor of all traits examined. %PPFD was added as a covariate, but in most cases was not significant or was not retained in the model after stepwise regression analyses. Results are shown for trees growing in canopy gaps, in forest understories, and for pooled data. *P*-values of 0.10 or less are indicated in bold.

		Gap					Understory				
		R^2	p	a	b	c	R^2	p	a	b	c
Quercus ellipsoidalis	A_{area}	**0.15**	**0.104**	**0.980**	**0.061**	**-0.186**	0.07	0.392	0.758	0.006	-0.091
	A_{mass}	**0.21**	**0.043**	**2.111**	-0.003	**-0.272**	0.06	0.204	2.041	-0.017	-0.089
	A_{max}/N	0.10	0.239	0.725	-0.051	-0.174	0.08	0.408	0.711	-0.084	-0.080
	A_{max}/Rd	**0.40**	**0.001**	**1.190**	**0.124**	**-0.215**	0.12	0.250	1.164	0.057	-0.108
Prunus serotina	A_{area}	0.08	0.344	1.002	0.002	-0.242	0.10	0.332	0.746	-0.119	-0.097
	A_{mass}	0.08	0.338	2.203	-0.111	-0.158	**0.27**	**0.036**	**2.134**	**-0.219**	-0.028
	A_{max}/N	**0.17**	**0.081**	**0.895**	**-0.163**	-0.110	**0.30**	**0.025**	**0.740**	**-0.214**	-0.031
	A_{max}/Rd	0.01	0.932	1.190	-0.011	-0.089	0.00	0.999	1.057	-0.005	0.004

		R^2	p	a	b	c	R^2	p	a	b	c
Acer rubrum	A_{area}	**0.72**	**<0.001**	**0.795**	**0.145**	**-0.117**	**0.47**	**0.001**	**0.512**	**0.115**	**-0.185**
	A_{mass}	**0.36**	**0.002**	**2.078**	0.033	**-0.132**	**0.28**	**0.031**	**1.920**	0.064	-0.152
	A_{max}/N	**0.19**	**0.054**	**0.962**	-0.022	**-0.078**	0.16	0.153	0.795	0.025	-0.128
	A_{max}/Rd	**0.59**	**<0.001**	**1.094**	**0.164**	**-0.106**	**0.58**	**<0.001**	**1.158**	**0.178**	**-0.312**

		Pooled				
		R^2	p	a	b	c
Quercus ellipsoidalis	A_{area}	**0.09**	**0.070**	**0.876**	0.023	**-0.140**
	A_{mass}	**0.11**	**0.040**	**2.072**	-0.021	**-0.146**
	A_{max}/N	0.07	0.138	0.712	-0.071	-0.102
	A_{max}/Rd	**0.21**	**0.003**	**1.168**	**0.088**	**-0.127**
Prunus serotina	A_{area}	0.05	0.261	0.906	-0.061	-0.233
	A_{mass}	**0.15**	**0.018**	**2.176**	**-0.164**	-0.096
	A_{max}/N	**0.19**	**0.005**	**0.836**	**-0.190**	-0.109
	A_{max}/Rd	0.01	0.870	1.138	-0.008	-0.067

Acer rubrum	A_{area}	**0.29**	**<0.001**	**0.697**	**0.118**	**-0.219**
	A_{mass}	**0.25**	**<0.001**	**2.020**	0.042	**-0.179**
	A_{max}/N	**0.13**	**0.031**	**0.901**	-0.004	**-0.139**
	A_{max}/Rd	**0.55**	**<0.001**	**1.111**	**0.177**	**-0.185**

Appendix Table A.3. A Pearson correlation matrix, where correlation coefficients are given between leaf and stem traits. Results are for pooled data from both understory and gap environments.

Quercus ellipsoidalis

	A_{area}	Rd_{area}	LMA	N_{area}	Stem N	Stem Rd
A_{area}	1.00					
Rd_{area}	0.53	1.00				
LMA	0.64	0.63	1.00			
N_{area}	0.60	0.42	0.92	1.00		
Stem N					1.00	NS
Stem Rd						1.00

Prunus serotina

	A_{area}	Rd_{area}	LMA	N_{area}	Stem N	Stem Rd
A_{area}	1.00					
Rd_{area}	0.54	1.00				
LMA	0.84	0.53	1.00			
N_{area}	0.86	0.33	0.88	1.00		
Stem N					1.00	NS
Stem Rd						1.00

Acer rubrum

	A_{area}	Rd_{area}	LMA	N_{area}	Stem N	Stem Rd
A_{area}	1.00					
Rd_{area}	0.58	1.00				
LMA	0.50	0.42	1.00			
N_{area}	0.43	0.37	0.71	1.00		
Stem N					1.00	0.44
Stem Rd						1.00

Appendix B. Supplementary information for chapter 3 results.

Appendix Table B.1. Effects of species, light availability, and juvenile tree size on leaf and whole-plant traits of four subtropical evergreen species. All were traits except for relative growth rate were log_{10}-transformed prior to analysis to roughly normalize the data.

Variable	Effect	F-value	p
SLA ($cm^2 g^{-1}$)	Species	231.92	**< 0.001**
	log (PPFD)	99.07	**< 0.001**
	log (Height)	5.70	**0.017**
	Species * log (PPFD)	17.51	**< 0.001**
	Species * log (Height)	3.38	**0.018**
	log (PPFD) * log (Height)	0.01	0.951
	Species * log (PPFD) * log (Height)	0.54	0.652
Leaf nitrogen (%)	Species	7.60	**< 0.001**
	log (PPFD)	1.21	0.274
	log (Height)	0.17	0.683
	Species * log (PPFD)	3.07	**0.029**
	Species * log (Height)	0.14	0.934
	log (PPFD) * log (Height)	0.01	0.927
	Species * log (PPFD) * log (Height)	0.96	0.413
A_{area} ($\mu mol\ CO_2\ m^{-2}s^{-1}$)	Species	20.73	**< 0.001**
	log (PPFD)	25.07	**< 0.001**
	log (Height)	1.14	0.289
	Species * log (PPFD)	*0.74*	0.531
	Species * log (Height)	0.77	0.513
	log (PPFD) * log (Height)	0.15	0.696
	Species * log (PPFD) * log (Height)	0.20	0.897

Rd_{area} (μmol CO_2 m^{-2}s^{-1})	Species	5.05	**0.002**
	log (PPFD)	18.74	**< 0.001**
	log (Height)	2.22	0.138
	Species * log (PPFD)	1.56	0.201
	Species * log (Height)	1.72	0.165
	log (PPFD) * log (Height)	0.24	0.626
	Species * log (PPFD) * log (Height)	0.21	0.886
Net C gain (mmol m^{-2}day^{-1})	Species	11.28	**< 0.001**
	log (PPFD)	82.28	**< 0.001**
	log (Height)	16.83	**< 0.001**
	Species * log (PPFD)	0.17	0.915
	Species * log (Height)	2.67	**0.051**
	log (PPFD) * log (Height)	0.59	0.446
	Species * log (PPFD) * log (Height)	0.63	0.597
Relative growth rate	Species	12.71	**< 0.001**
	log (PPFD)	298.61	**< 0.001**
	log (Height)	5.56	**0.019**
	Species * log (PPFD)	88.45	**< 0.001**
	Species * log (Height)	0.68	0.562
	log (PPFD) * log (Height)	22.62	**< 0.001**
	Species * log (PPFD) * log (Height)	19.54	**< 0.001**