

# Dark respiration rate increases with plant size in saplings of three temperate tree species despite decreasing tissue nitrogen and nonstructural carbohydrates

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**Summary** In shaded environments, minimizing dark respiration during growth could be an important aspect of maintaining a positive whole-plant net carbon balance. Changes with plant size in both biomass distribution to different tissue types and mass-specific respiration rates ( $R_d$ ) of those tissues would have an impact on whole-plant respiration. In this paper, we evaluated size-related variation in  $R_d$ , biomass distribution, and nitrogen (N) and total nonstructural carbohydrate (TNC) concentrations of leaves, stems and roots of three cold-temperate tree species (*Abies balsamea* (L.) Mill, *Acer rubrum* L. and *Pinus strobus* L.) in a forest understory. We sampled individuals varying in age (6 to 24 years old) and in size (from 2 to 500 g dry mass), and growing across a range of irradiances (from 1 to 13% of full sun) in northern Minnesota, USA. Within each species, we found small changes in  $R_d$ , N and TNC when comparing plants growing across this range of light availability. Consistent with our hypotheses, as plants grew larger, whole-plant N and TNC concentrations in all species declined as a result of a combination of changes in tissue N and shifts in biomass distribution patterns. However, contrary to our hypotheses, whole-plant and tissue  $R_d$  increased with plant size in the three species.

**Keywords:** *Abies balsamea*, *Acer rubrum*, balsam fir, biomass allocation, carbohydrates, deeply shaded, low light, *Pinus strobus*, red maple, root excavation, white pine, whole plant respiration.

## Introduction

A positive balance between photosynthetic gains and respiratory losses allows plants to grow and reproduce. However, even under favorable growth conditions, on average, 40 to 60% of the photosynthates produced daily are lost in respiration (Kraus et al. 1989, Tjoelker et al. 1999a). In deeply shaded environments, dark respiration is likely to be a much greater fraction of total net carbon balance than in high-light conditions (Givnish 1988, Walters et al. 1993a). Therefore, a low mass-specific respiration rate ( $R_d$ ) and associated low car-

bon losses are important components of both adaptation and acclimation to low-light environments (Reich et al. 2003). Reduced carbon losses (per unit mass invested) in low-light environments could occur as a result of decreased concentrations of substrates for respiration, e.g., total nonstructural carbohydrates (TNC), or lower  $R_d$  for a given concentration of substrate.

Tissue and whole-plant  $R_d$  are likely dynamic with changing plant size. We predict that  $R_d$  should decline with increasing plant size for three reasons. First, with increasing plant size, photosynthetic tissues likely decline as a fraction of whole-plant mass. Hence, declining  $R_d$  with increasing size might be required to maintain stable whole-plant carbon balance. Second, as woody plants grow larger, the proportion of fine tissues (fine roots plus foliage), which have high  $R_d$ , should decline in relation to coarser tissues (stems) with lower  $R_d$ . The notion that  $R_d$  should decline with plant size is consistent with data from laboratory studies showing that as young (first-year) plants or organs age and increase in size, their specific growth rates typically decrease and these changes are accompanied by decreases in  $R_d$  (Poorter and Pothmann 1992, Walters et al. 1993b, Tjoelker et al. 1999a). Third, if trees enhance carbon conservation in deeply shaded environments, biomass should be distributed among plant tissues to lessen respiratory loads. Thus, as plants increase in biomass (size), there are likely concomitant changes in: (a) the proportion of respiratory versus photosynthetic tissues; (b) the proportion of tissues with different  $R_d$  (e.g., stem versus leaves); and (c) the  $R_d$  of any given tissue type. These respiratory and biomass distribution patterns are poorly characterized for perennial plants in the field.

In this study, we evaluated the relationship between variation in plant size and tissue or whole-plant  $R_d$  for three species that vary in observed shade tolerance and are common to the boreal and cold temperate forests of North America (Barnes and Wagner 1996): *Abies balsamea* (L.) Mill, *Acer rubrum* L. and *Pinus strobus* L., growing in moderately to deeply shaded understories (less than 13% of full sun) in northern Minnesota, USA. We measured  $R_d$ , biomass distribution and concentrations of nitrogen (N) and TNC of leaves, stems and roots of

different sized saplings. We tested the following hypotheses: (H.1)  $R_d$  declines with increasing shade (H.1A), and with increasing plant size (H.1B); (H.2) tissue N and TNC concentrations decline with increasing shade (H.2A) and with increasing plant size (H.2B); and (H.3) the fraction of whole-plant biomass distributed to foliage (the tissue type with the highest  $R_d$ ) is negatively related to plant size but positively related to increased shading (Walters and Reich 1999).

## Materials and methods

### Study site

The study area is located at the University of Minnesota's Cloquet Forestry Center in Cloquet, MN, USA (46°40' N, 92°30' W). The stand was planted with white pine (*Pinus strobus*) in about 1930 after being cleared and burned of the original vegetation. The subcanopy and sapling layers are composed primarily of naturally regenerated red maple (*Acer rubrum*) and balsam fir (*Abies balsamea*) with lesser numbers of white pine, whereas the understory is mainly dominated by the shrub *Corylus cornuta* Marsh. Soils in the region are derived from glacial out-wash. The climate is cold-temperate continental with mean January and July temperatures of -12 and 20 °C, respectively, and a growing season of about 120 days. Summer droughts are uncommon. Irradiance at systematic transects in the understory within the study area ranged from 1.0 to 41% of full sun during the summer (mean 11.2%) and 2.8 to 64.5% of full sun during the autumn (mean 26.5%, Machado 1999).

### Species and sapling selection

In 1993, we determined the diameter at the stem base and total height of 1179 tree saplings in a systematic survey of 0.75 ha. In summer 1994, we measured percent canopy openness above each sapling with an LAI-2000 plant canopy analyzer (Li-Cor, Lincoln, NE). Percent canopy openness is a good surrogate of mean daily percent photosynthetic photon flux density for this forest cover (Machado and Reich 1999). Two canopy openness measurements at each plant were taken when the sky was uniformly overcast or 1 h after dawn and 1 h before dusk. We used one LAI-2000 at the measurement point (forest understory) while another paired unit simultaneously measured open sky values in a large clear-cut less than 1 km away. We averaged the two measurements for each plant.

We sought to determine the effects of low-light environments and increases in plant size by selecting saplings ( $n = 35$  for each species) growing in the field across a range of plant heights (25 to 150 cm) and sapling light environments (1 to 13%). The selection of saplings was based on known plant light environments and heights of 1179 tree saplings allowing us to select both large and small saplings of each species growing in similar light environments, typically in close proximity, thus minimizing the possible effects of differences in micro-site fertility or water availability, or both.

To assess whether variation in mass was related to plant age, height or height growth rate and whether any or all of these

varied systematically with light availability, we also assessed the relationships among these variables and their potential implications for interpreting the data.

### Sapling respiration measurements

In 1995, from September 1 to September 18, nine individuals of each species were excavated. The remaining 26 individuals per species were excavated in 1996 between August 28 and September 15. These dates were selected to minimize the effect of active growth on  $R_d$ . By the end of September almost all growth (excluding roots) ceases in conifer species that set bud (Kozłowski and Ward 1957a) and the broadleaf species start to drop their leaves (Kozłowski and Ward 1957b). All excavations were made between 0600 and 0900 h local time and we excavated 2 to 5 individuals per day depending on plant size. Immediately after excavation, the entire root system was covered with wet paper towels and the entire plant was covered with dark plastic bags. All plants were transported to a nearby laboratory at the field station where they were stored in a walk-in refrigerator at 5 °C for no longer than 12 h from the time of excavation to the time of measuring respiration. Every day, plants were divided into leaves, stems and roots, and all the material was kept hydrated in the dark in a temperature-controlled growth chamber at 20 °C for at least 2 h before any respiration measurements were made. All tissues were subsampled based on size and amount. Stems and roots were separated between coarse (more than 5-mm diameter) and moderately fine (less than 5-mm diameter). Rates of CO<sub>2</sub> flux were measured with open configuration, infrared gas analyzers and cuvettes (LCA-3 and PLC-C, Analytical Development, Hoddesdon, U.K.). Rates of net CO<sub>2</sub> efflux from intact roots free of soil and stems and leaves were measured separately in the dark at 20 °C in a temperature-controlled growth chamber. Gas exchange measurements were taken after the readings had stabilized for at least 10 min.

All plant material was dried in a forced air oven (70 °C) and dry mass measured. Total nitrogen (N) concentrations of dried and ground tissues (mg N g<sup>-1</sup> tissue) were measured by the Kjeldahl digestion assay (Research Analytical Laboratory, University of Minnesota). Total nonstructural carbohydrate (TNC) concentrations were determined as the sum of soluble sugar and starch assayed by the methods of Haissig and Dickson (1979) and Hansen and Møller (1975). Sugars were extracted from oven-dried and ground tissue in methanol:chloroform:water and tissue residuals were used for determination of starch content. For all plants, the following parameters were measured: height (cm), total foliage mass (g), total stem mass (g), total root mass (g) and age as number of rings at the base of stem (year). These data were used to determine the following biometric parameters: leaf mass fraction (LMF, g leaf g<sup>-1</sup> total plant mass), stem mass fraction (SMF, g stem g<sup>-1</sup> total plant mass), root mass fraction (RMF, g root g<sup>-1</sup> total plant mass) and relative increase in height for 1993 and 1994 year ((Ln plant height in 1994 - Ln plant height 1993) year<sup>-1</sup>). Finally, whole-plant  $R_d$  (nmol CO<sub>2</sub> g<sup>-1</sup> plant s<sup>-1</sup>) was calculated by summing root, stem and leaf  $R_d$  weighted by the proportion of dry mass corresponding to each tissue.

*Statistical analysis*

We conducted separate analyses for each species. First, variation in  $R_d$ , N concentration and TNC concentration on tissue and whole-plant bases as a function of whole-plant mass, canopy openness and their two-way interaction were analyzed by multiple regression of natural log transformed response variables. These transformations were necessary to make the data approximately normal and to stabilize residual patterns. Second, we calculated the fraction of total plant  $R_d$  and whole-plant mass for each of three tissues: leaves, stems and roots to test the extent to which whole-plant mass and canopy openness influence biomass distribution and respiratory costs. Variation for each tissue in the fraction of whole-plant  $R_d$  and biomass distribution was analyzed as a function of whole-plant mass, canopy openness and their two-way interaction by multiple regression. Only whole-plant mass was natural log transformed. All analyses were conducted with JMP statistical analysis software (JMP 3.2, SAS Institute, Cary, NC).

**Results**

*Relationships among plant height, growth, whole-plant mass and age*

Taller plants had more biomass than shorter plants ( $F$  ratio = 272,  $P < 0.001$  for *Abies*;  $F$  ratio = 135,  $P < 0.001$  for *Acer*; and  $F$  ratio = 205,  $P < 0.001$  for *Pinus*). Plant height showed a significant increase in *Pinus* ( $F$  ratio = 8.8,  $P < 0.006$ ) and a significant decrease in *Acer* ( $F$  ratio = 8.7,  $P < 0.007$ ) in response to increases in light availability, although variation in irradiance explained only a small fraction of total variance (Figure 1). Light environments did not affect plant height in *Abies*. For each species, there were no significant whole-plant mass  $\times$  canopy openness interactions (data not shown). Given the

strong correlation between height and mass, we do not distinguish between height and mass in the following discussion on plant size.

In *Pinus*, the relative increase in height (a surrogate for plant relative growth rate), was negatively related to plant size ( $F$  ratio = 6.7,  $P = 0.005$ ), whereas there was no relationship between relative height growth rate and size in the other species. In contrast, all species showed a significantly higher relative height growth rate with increasing canopy openness ( $F$  ratio = 10.7,  $P = 0.002$  for *Abies*;  $F$  ratio = 6.9,  $P = 0.015$  for *Acer*; and  $F$  ratio = 9.1,  $P = 0.005$  for *Pinus*).

Despite their relatively small sizes, the saplings ranged in age between 7 and 24 years for *Abies* individuals (mean 14 years), 6 to 21 years for *Acer* individuals (mean 12 years) and 8 to 21 years for *Pinus* individuals (mean 14 years). However, larger plants were generally older ( $r^2 = 0.40$ ,  $P = 0.006$  for *Abies*;  $r^2 = 0.49$ ,  $P < 0.001$  for *Pinus*; and not significant for *Acer*).

*Effects of plant size and low-light availability on  $R_d$*

Variations in irradiance had modest effects on  $R_d$  in all species. Light was not significant as a main effect for whole-plant  $R_d$  and was only significant for *Acer* stem  $R_d$  (increase with light,  $F$  ratio = 14.2,  $P < 0.001$ ) and *Pinus* needle  $R_d$  (decrease with light,  $F$  ratio = 6.8,  $P < 0.013$ ), generally refuting H.1A that plants of all species have lower  $R_d$  with increasing shade. Furthermore,  $R_d$  generally increased with plant mass (Figure 2), in direct contradiction to H.1B. Leaf, stem and whole-plant  $R_d$  significantly increased with plant mass in all species, often doubling across the size range in each species (Figure 2). The  $R_d$  of *Abies* roots was also greater in larger saplings ( $F$  ratio = 16.6,  $P < 0.001$ ). The relationships of  $R_d$  with mass were independent of irradiance (no significant interactions).

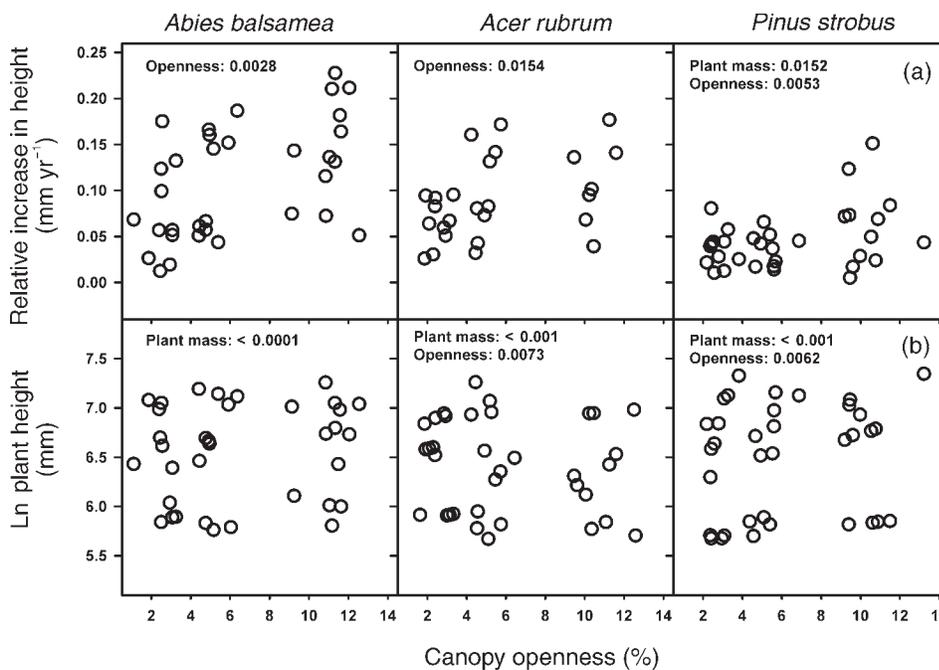


Figure 1. Changes in relative increase in height for 1993 and 1994 year<sup>-1</sup>, Panel a) and plant height (Ln mm, Panel b) as a function of percent canopy openness of naturally grown saplings in forest understories ranging in light from 1 to 13% canopy openness. The model included whole-plant mass (g), canopy openness (%) and two-way interaction. Only  $P$  values less than 0.05 are shown. Values represent measurements on single individuals.

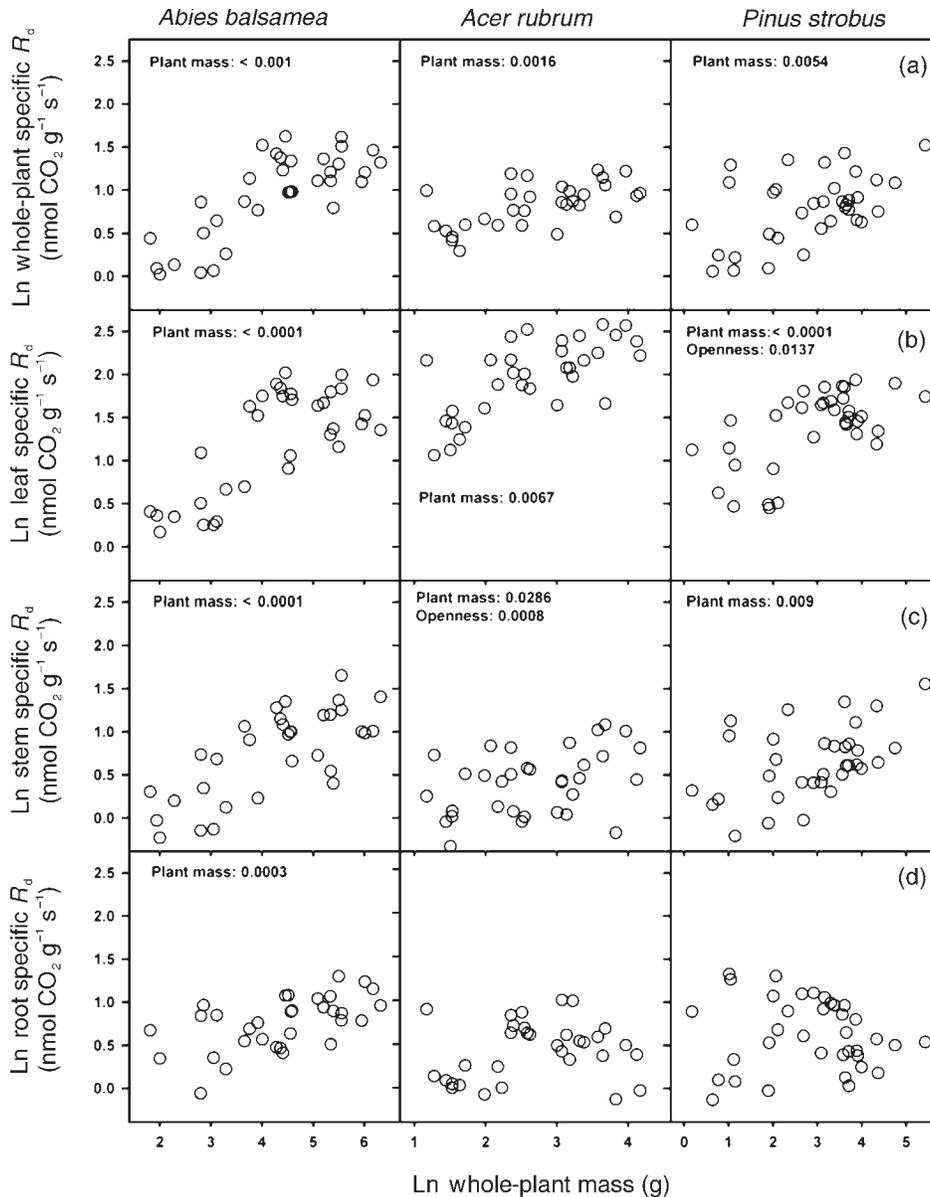


Figure 2. Changes in mass-specific dark respiration rate ( $R_d$ ,  $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ ) for whole plant (Panel a), leaf (Panel b), stem (Panel c) and root (Panel d) as a function of whole-plant mass (g) of naturally grown saplings in forest understories ranging in light from 1 to 13% canopy openness. The model included whole-plant mass (g), canopy openness (%) and two-way interaction. Only  $P$  values less than 0.05 are shown. Values represent measurements on single individuals.

#### Effects of plant size and low-light environment on N and TNC concentrations

Increased shading generally did not lead to lower concentrations of whole-plant or tissue N or TNC concentrations, refuting H.2A. The exceptions included a decrease in whole-plant ( $F$  ratio = 8.2,  $P < 0.001$ ) and stem N concentrations with increasing shade in *Pinus* ( $F$  ratio = 9.7,  $P < 0.004$ ) and an increase in root TNC concentration with increasing shade in *Acer* ( $F$  ratio = 12.5,  $P < 0.004$ ). There were also several interactions of light with plant mass (data not shown).

Whole-plant N concentration decreased with increasing plant mass in all species (Figure 3), supporting H.2B. This response was mainly driven by consistent decreases in stem and root N concentrations with increases in plant size (Figure 3). For TNC, there was some, but inconsistent, support for H.2B in all species (Figure 4). Among species, *Acer* had higher fo-

liage N concentrations compared with the two conifers. However, whole-plant N concentration was not higher in *Acer*, because of the effects of species-specific differences in biomass distribution among leaves, stems and roots on whole-plant N concentration (Figure 3). We found large differences in tissue TNC concentrations among species. For example, foliage TNC concentration was 3 times higher in *Abies* than in *Acer* or *Pinus*, but TNC concentrations in stems and especially roots of *Acer* were 2 and 8 times higher, respectively, than those of the two conifer species (Figure 4).

#### Distribution of biomass and whole-plant $R_d$

The distribution of biomass to different tissues varied with plant size in all species (Table 1 and Figure 5, Panel a). For *Acer*, leaf mass fraction (LMF) and root mass fraction (RMF) significantly decreased and stem mass fraction (SMF) in-

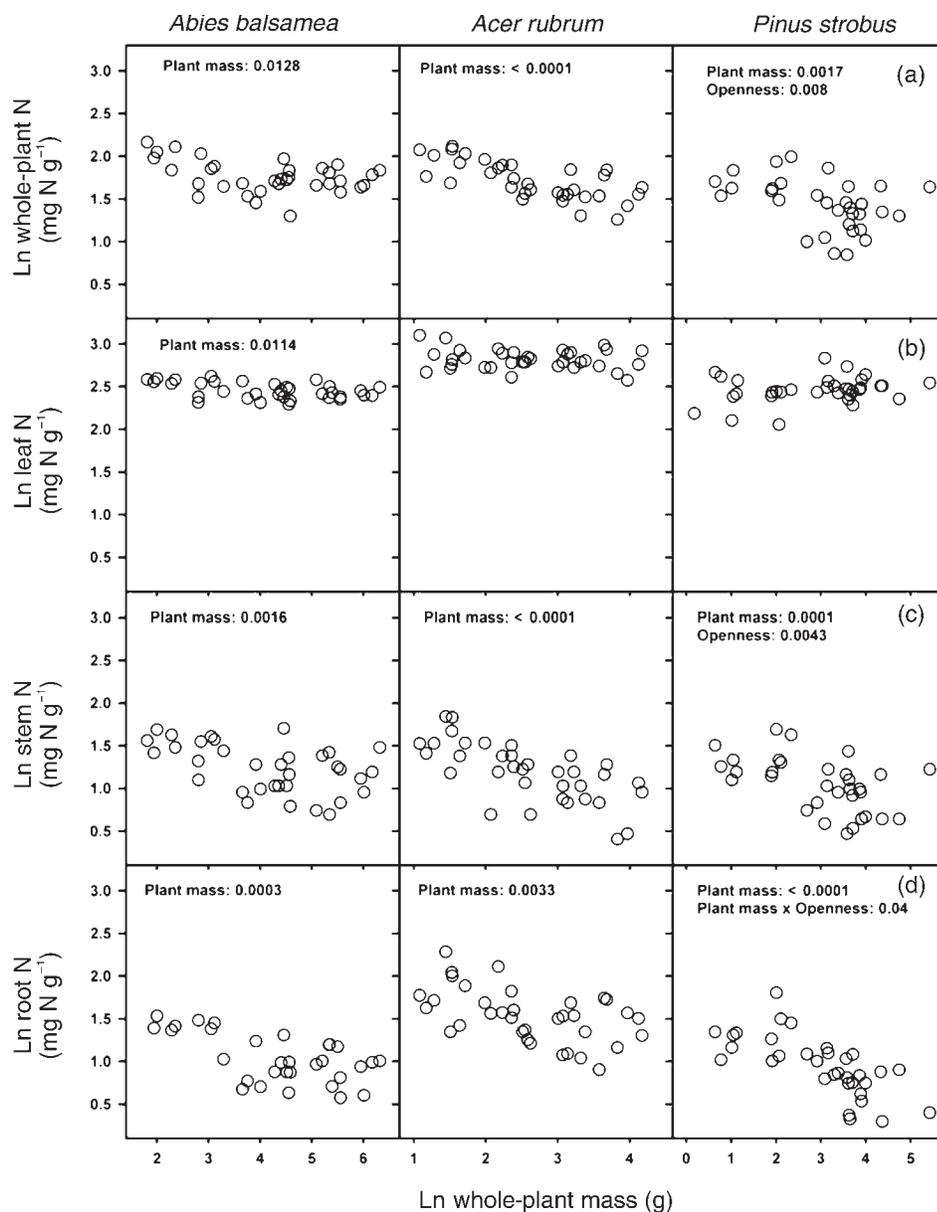


Figure 3. Changes in nitrogen concentration ( $\text{mg N g}^{-1}$ ) for whole plant (Panel a), leaf (Panel b), stem (Panel c) and root (Panel d) as a function of whole-plant mass (g) of naturally grown saplings in forest understories ranging in light from 1 to 13% canopy openness. The model included whole-plant mass (g), canopy openness (%), and two-way interaction. Only  $P$  values less than 0.05 are shown. Values represent measurements on single individuals.

creased with increases in whole-plant size. Similar changes were found for RMF and SMF in *Pinus* and for RMF in *Abies* (Figure 5, Panel a; Table 1). Irradiance did not affect biomass distribution in *Abies*, whereas *Acer* and *Pinus* individuals had a greater fraction of biomass in roots and a lower fraction in stems (and also leaves for *Acer*), with increasing irradiance (data not shown; Table 1). Thus, with respect to H.3, the fractional distribution of biomass to foliage decreased with size and increased with light as hypothesized, but in only one of the three species studied.

To evaluate the relative contributions to whole-plant  $R_d$  of variations in biomass distribution and  $R_d$  resulting from plant size and irradiance, we calculated the fraction of total plant  $R_d$  in each of three tissues: leaves, stems and roots. Overall, the stem fraction of total plant  $R_d$  was lower for *Acer* individuals compared with the conifer species (Figure 5, Panel b). For

both *Acer* and *Pinus* species, the root and stem  $R_d$  fractions of whole-plant  $R_d$  decreased and increased, respectively, with plant size. This pattern largely resulted from size-related shifts in biomass distribution away from roots and leaves and towards stems (Figure 5, Panel a). For *Acer*, high  $R_d$  of leaves resulted in a high foliar fraction of total  $R_d$  at all plant sizes. Only for *Pinus* did we find significant responses of leaf and root fractions of whole-plant  $R_d$  across light environments (data not shown).

## Discussion

### Acclimation of $R_d$ to low-light environment

Overall, our data do not support the hypothesis that species reduce tissue or whole-plant carbon losses when growing in in-

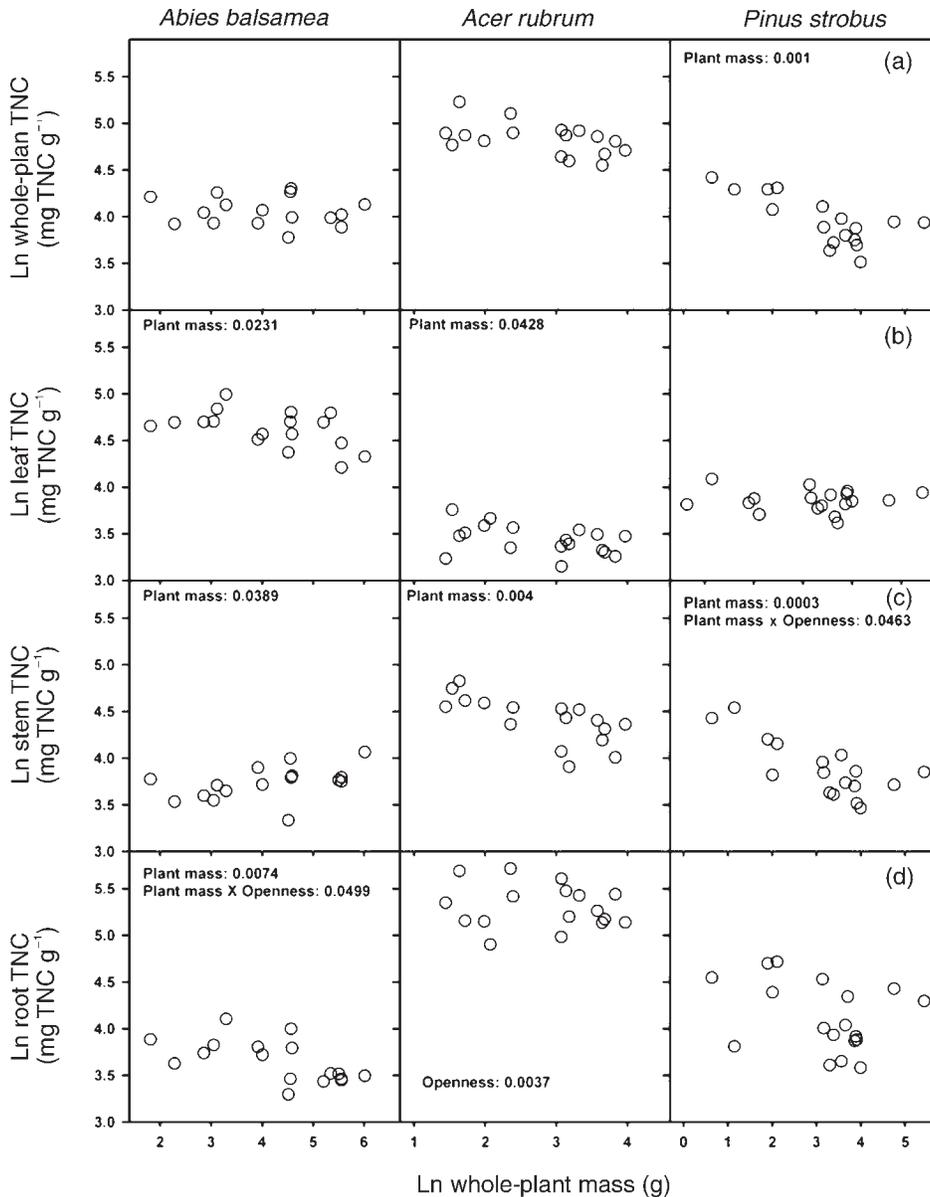


Figure 4. Changes in total nonstructural carbohydrate concentration ( $\text{mg TNC g}^{-1}$ ) for whole plant (Panel a), leaf (Panel b), stem (Panel c) and root (Panel d) as a function of whole-plant mass (g) of naturally grown saplings in forest understories ranging in light from 1 to 13% canopy openness. The model included whole-plant mass (g), canopy openness (%), and two-way interaction. Only  $P$  values less than 0.05 are shown. Values represent measurements on single individuals.

creasingly shaded environments (Givnish 1988, Walters and Reich 1999, Lusk and Reich 2000). We found no significant reductions in tissue  $R_d$  across the range of light environments studied ( $\sim 1$  to  $\sim 13\%$  of full sun; Figure 2). Comparable published data on the effects of variation in plant size and light environment on  $R_d$  are scarce. Studies on  $< 1$ -year-old seedlings grown indoors at 5 to 25% of full sun (Reich et al. 1998b) or at 15 to 75% of full sun (Walters et al. 1993a) showed that, at a common plant mass, whole-plant and tissue  $R_d$  were higher when seedlings were grown in high light. Although acclimation in mass-based  $R_d$  across a wide range of light gradients has been shown to occur (e.g., Chazdon and Kaufmann 1993, Ellsworth and Reich 1993, Lusk and Reich 2000), our data indicate that this phenomenon is not universal (Read and Hill 1985, Walters et al. 1993a, Kitajima 1994, Reich et al. 1998a). Perhaps the limited range of light environments in our study,

all at the dark end of the light availability gradient, either masked a pattern that could be seen across broader light gradients for the same species (e.g., Lusk and Reich 2000) or were beyond the lowest irradiance at which such acclimation can occur in our study species.

#### Specific $R_d$ , tissue $N$ , tissue $TNC$ and plant size

Our observations did not support the hypothesis that tissue  $R_d$  decreases with plant size. On the contrary, tissue  $R_d$  and consequently whole-plant  $R_d$  increased with increasing plant mass for all three species regardless of light environment. The hypothesis that  $R_d$  decreases with increasing plant size is based on both theory and empirical evidence showing a positive relationship between total  $R_d$  (i.e., maintenance plus growth respi-

Table 1. Summary of multiple regression analyses for the effects of whole-plant mass (g), canopy openness (%) and two-way interaction on: (a) leaf mass fraction; (b) stem mass fraction; and (c) root mass fraction; and on: (d) leaf mass-specific respiration rate ( $R_d$ ) fraction; (e) stem  $R_d$  fraction; and (f) root  $R_d$  fraction of whole-plant  $R_d$  of the tree species studied. Only  $P$  values less than 0.05 are shown.

Parameter	<i>Abies balsamea</i>		<i>Acer rubrum</i>		<i>Pinus strobus</i>	
	<i>F</i> ratio	<i>P</i>	<i>F</i> ratio	<i>P</i>	<i>F</i> ratio	<i>P</i>
<b>a. Leaf mass fraction (LMF)</b>						
Ln whole-plant mass			23.4	< 0.0001		
Canopy openness			14.9	0.0005		
Interaction	5.2	0.0301				
<b>b. Stem mass fraction (SMF)</b>						
Ln whole-plant mass			26.3	< 0.0001	18.6	0.0001
Canopy openness			23.1	< 0.0001	7.9	0.0084
Interaction	5.5	0.0257				
<b>c. Root mass fraction (RMF)</b>						
Ln whole-plant mass	8.2	0.0075	13.4	0.001	37.7	< 0.0001
Canopy openness			13.7	0.0008	8.9	0.0055
Interaction						
<b>d. Leaf <math>R_d</math> fraction of whole-plant <math>R_d</math></b>						
Ln whole-plant mass	5.4	0.0271				
Canopy openness						
Interaction						
<b>e. Stem <math>R_d</math> fraction of whole-plant <math>R_d</math></b>						
Ln whole-plant mass			33.2	< 0.0001	15.4	0.0004
Canopy openness			8.3	0.0077		
Interaction						
<b>f. Root <math>R_d</math> fraction of whole-plant <math>R_d</math></b>						
Ln whole-plant mass	11.1	0.0023	8.1	0.0077	50.5	< 0.0001
Canopy openness					7.6	0.0097
Interaction						

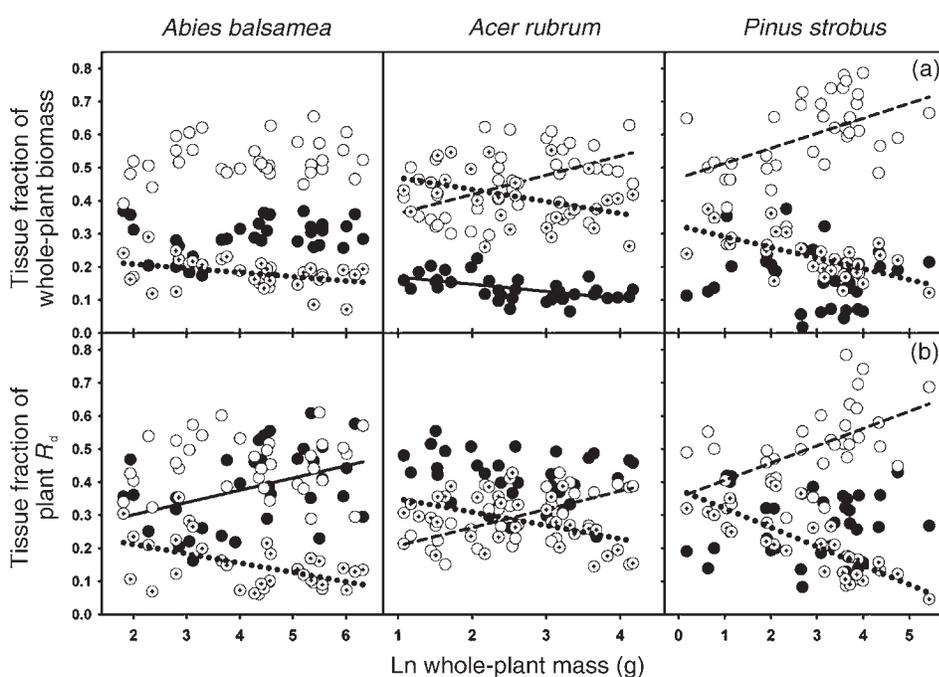


Figure 5. Changes in biomass distribution (Panel a) and tissue respiration distribution (Panel b) as a function of Ln whole-plant mass (g) of naturally grown saplings in forest understories ranging in light from 1 to 13% canopy openness. Values represent measurements on single individuals with solid (●), empty (○) and cross hair (⊕) symbols representing leaf, stem and root tissues, respectively. Regression lines (solid for leaves, short dash for stems and dotted for roots) illustrate significant variables at  $P < 0.05$  (see Table 1). Abbreviation:  $R_d$  = mass-specific respiration rate.

ration) and relative growth rates (Givnish 1988, Poorter et al. 1990, Walters et al. 1993b, Tjoelker et al. 1999b), as well as negative relationships of both components to plant size. As roots and shoots grow larger, the proportion of metabolically active meristems declines and the proportion of structural support tissue increases. For instance, as a first-year seedling increases in age and mass, its relative growth rate decreases with concomitant reductions in growth and maintenance  $R_d$  (e.g., Walters et al. 1993b, Reich et al. 1998a, 1998b, Tjoelker et al. 1999a). However, in our field study of older saplings, height growth in *Abies* and *Acer* was unrelated to plant mass, although *Pinus* height growth rate declined with plant mass (data not shown).

Before discussing these potentially conflicting results, we present two caveats. First, our respiration measurements were made late in the growing season when most growth had ceased for leaves and stems (and likely roots) of the studied species (Kozlowski and Ward 1957a, 1957b). Hence, we assumed that the measured  $R_d$  would largely correspond to maintenance rather than to growth respiration, at least for aboveground tissues. The second caveat is that wound responses could have been a source of variation in  $R_d$ , although studies on detached leaves and branches have provided no evidence of such wound effects (e.g., Mitchell et al. 1999). Moreover, wound responses would likely cause a similar directional change in measured values for all species under all environmental classes. Overall, the strength and consistency of the patterns we observed for all species in relation to plant size are difficult to explain as artifacts of our measurement protocol.

Although we were unable to identify the mechanism underlying the increase in  $R_d$  with increasing plant size, we considered and rejected the following possibilities. It has been shown that variation in tissue N concentration could help explain differences in  $R_d$  (Poorter et al. 1990) because  $R_d$  is often linearly related to tissue N concentration (Ryan 1995, Ryan et al. 1996, Reich et al. 1998a, 1998b). However, we found that tissue N concentration decreased with increasing plant mass (Figure 3) and thus, changes in tissue N concentration cannot explain the observed patterns. Additionally,  $R_d$  expressed per unit N for tissues and the whole plant were higher for larger plants than for smaller plants (data not shown) and relationships between plant mass and respiration rate per unit N have even steeper slopes than for mass-based  $R_d$ , because mass-based  $R_d$  increased with increasing plant mass whereas tissue N decreased. This implies that the costs associated with protein turnover are higher for larger plants than for smaller plants growing in these low-light environments, or that the effects of other factors that influence size-related  $R_d$  (such as the availability of substrate) were greater in the larger plants. There is evidence that the accumulation of TNC directly affects  $R_d$  (Tjoelker et al. 1999b) and, in shaded plants, substrate availability may regulate  $R_d$ . However, whole-plant and tissue TNC concentrations were either unrelated to or decreased with increasing plant size (Figure 4). In summary, neither tissue N nor TNC concentrations reconcile the increases in specific  $R_d$  with increasing plant size.

#### *Biomass distribution, $R_d$ and growth in deeply shaded understories*

Differences in biomass distribution and changes in the contributions of tissue fractions to whole-plant  $R_d$  could underlie the differential ability of species to grow in low-light environments. In *Pinus*, increasing plant size resulted in significant proportional increases in stem biomass at the expense of biomass distribution to needles or roots, or both (Figure 5). Stem  $R_d$  contributed a greater fraction of whole-plant  $R_d$  in larger *Pinus* plants than in smaller *Pinus* plants. It is known that successful recruitment of *Pinus* is poor in low-light environments (Canham et al. 1994) and beneath conspecific stands (Frelich and Reich 1995), despite the ability of *Pinus* seeds to germinate there. Our data suggest that the high cost of maintaining increases in biomass coupled with increasing stem biomass at the expense of photosynthetic tissue provide a mechanistic explanation of the failure of *Pinus* to grow in low-light environments or beneath conspecific trees.

The broad-leaved *Acer* showed a similar size-related response with respect to biomass distribution but a slightly different respiratory cost compared with the evergreen *Pinus*. In general, as *Acer* plants grew larger, the fraction of whole-plant biomass distributed to stems increased but with stems responsible for a smaller fraction of total  $R_d$  compared with large *Pinus* plants. With increasing plant size, *Abies* showed significant changes only in root biomass distribution and the root tissue fraction of total plant  $R_d$ .

#### Conclusions

We found no reduction in tissue or whole-plant  $R_d$  in plants growing in increasingly deeply shaded understories. Therefore, our data do not support the notion that plant species reduce whole-plant carbon losses when growing in low-light environments. However, the limited range of light environments in our study (e.g., compared with the study by Lusk and Reich (2000) with the same species) might have been beyond the lowest irradiance at which such reductions can occur.

Our results do not support the notion of a consistently negative relationship between  $R_d$  and plant mass because  $R_d$  was consistently higher in intraspecific analyses of larger plants in our study. Furthermore, our data do not support the finding of Tjoelker et al. (1999b)—based on very young seedlings grown under conditions conducive to rapid growth—that  $R_d$  is a predictable positive function of the combination of N and TNC concentrations in some cold-temperate tree species. However, when the respiration and N data for these three species were expressed on a per plant basis (not per unit mass), and combined with similar data for 40 other species including grasses, forbs and woody plants, there was a single strong relationship of  $R_d$  per plant and N per plant that fit aggregated data for plants of all sizes (Reich et al. 2006). This scaling relationship was observed among individuals within each of the three species in this study, although the slopes for these three were on the high end of the range of slopes among species. This suggests that constraints that operate at the largest ranges in plant

size and number of taxa that are often real and have implications for scaling across such large ranges do not apply identically to every case at narrower domains.

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