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Acclimation of respiration to temperature and CO_2 in seedlings of boreal tree species in relation to plant size and relative growth rate

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Abstract

The role of acclimation of dark respiration to temperature and CO_2 concentration and its relationship to growth are critical in determining plant response to predicted global change. We explored temperature acclimation of respiration in seedlings of tree species of the North American boreal forest. *Populus tremuloides, Betula papyrifera, Larix laricina, Pinus banksiana,* and *Picea mariana* plants were grown from seed in controlled-environments at current and elevated concentrations of CO_2 (370 and 580 μmol mol^{-1}) in combination with three temperature treatments of 18/12, 24/18, and 30/24 °C (light/dark period). Specific respiration rates of roots and shoots acclimated to temperature, damping increases in rates across growth-temperature environments compared to short-term temperature responses. Compared at a standard temperature, root and shoot respiration rates were, on average, 40% lower in plants grown at the highest compared to lowest growth temperature. Broad-leaved species had a lower degree of temperature acclimation of respiration than did the conifers. Among species and treatment combinations, rates of respiration were linearly related to size and relative growth rate, and relationships were comparable among growth environments. Specific respiration rates and whole-plant respiratory CO_2 efflux as a proportion of daily net CO_2 uptake increased at higher growth temperatures, but were minimally affected by CO_2 concentration. Whole-plant specific respiration rates were two to three times higher in broad-leaved than coniferous species. However, compared to faster-growing broad-leaved species, slower-growing conifers lost a larger proportion of net daily CO_2 uptake as respiratory CO_2 efflux, especially in roots. Interspecific variation in acclimation responses of dark respiration to temperature is more important than acclimation of respiration to CO_2 enrichment in modifying tree seedling growth responses to projected increases in CO_2 concentration and temperature.

Keywords: allometry, elevated carbon dioxide, maintenance respiration, ontogeny, relative growth rate, temperature acclimation

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Introduction

Dark respiration in plants may consume over 50% of the net CO_2 fixed by photosynthesis (Farrar 1985). Respiratory CO_2 efflux is a key physiological process influencing the carbon balance of individual plants and ecosystems (Field *et al.* 1992; Ryan *et al.* 1995). While the effects of rising atmospheric concentrations of CO_2 and temperature increases on photosynthesis have received considerable attention, much less is known about the response of dark respiration. Understanding how temperature and CO_2 concentration alter respiration and its relationship to plant growth is fundamental to predicting the response of plants to environmental change.

Rates of dark respiration typically increase exponentially in response to short-term increases in ambient temperature. However, longer term responses to temperature may differ from predictions based on short-term response functions, since respiration acclimates to

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thermal environment (Stocker 1935; Scholander & Kanwisher 1959; Rook 1969; Collier & Cummins 1990) and species differ in this regard (Larigauderie & Körner 1995; Arnone & Körner 1997). When measured at a standard temperature, plants acclimated to low temperature have higher rates of respiration than plants acclimated to higher temperatures.

Rates of leaf dark respiration may be directly and reversibly inhibited by increased concentrations of CO_2 (Bunce 1994, 1995; Wullschleger *et al.* 1994; Ziska & Bunce 1994; Griffin *et al.* 1996). In addition to direct effects, respiration rates may be altered through a longer-term acclimation response to CO_2 enrichment (Bunce 1994; Wullschleger *et al.* 1994). Indirect effects of CO_2 concentration on respiratory processes and its relationship to growth are likely mediated by changes in tissue nitrogen and carbohydrate contents and construction costs (Poorter *et al.* 1992; Curtis 1996; Poorter *et al.* 1997; Wullschleger *et al.* 1997).

Respiratory responses to temperature and CO_2 enrichment have been examined in terms of growth and maintenance components (Lambers *et al.* 1983). It is generally assumed that temperature and CO_2 concentration should primarily affect maintenance respiration (Szaniawski & Kielkiewicz 1982; Amthor 1991; Ryan 1991). Growth temperature has been shown to alter the effect of CO_2 enrichment on plant respiration, reducing maintenance respiration at lower and not higher growth temperatures in one study (Ziska & Bunce 1993). Given a paucity of data, the potential interactive effects of CO_2 concentration and temperature on respiration warrant further consideration.

Elevated CO_2 concentration increased leaf maintenance respiration in *Gossypium hirsutum* (Thomas *et al.* 1993) and *Glycine max* (Thomas & Griffin 1994), and was attributed to increased starch accumulation. In contrast, reduced maintenance respiration in leaves of *Glycine max* in an elevated CO_2 concentration was attributed to a short-term inhibitory effect (Bunce 1995). Elevated CO_2 concentration reduced both growth and maintenance respiration in leaves of *Quercus alba* (Wullschleger & Norby 1992) and *Liriodendron tulipifera* (Wullschleger *et al.* 1992). In another study, neither growth nor maintenance respiration of woody stems of *Quercus alba* were affected by elevated CO_2 concentration, but respiration correlated with rates of stem growth (Wullschleger *et al.* 1995). In contrast, maintenance respiration of woody stems of *Pinus ponderosa* increased in trees grown in an elevated CO_2 concentration (Carey *et al.* 1996). Neither root growth nor maintenance respiration of roots of *Phaseolus vulgaris* were affected by soil CO_2 enrichment (Bouma *et al.* 1997). Together these studies indicate that responses differ among species, studies, and likely among plant tissues. Consequently, the responses of entire root, shoot, or plant respiration to CO_2 enrichment may differ from those of component tissues.

Since specific respiration rates vary with growth rate, it is useful to examine longer-term responses of respiration to growth environment in terms of the relationship between respiration and plant growth (Farrar & Williams 1991). In this regard, comparing rates of respiration at common plant sizes and relative growth rates (RGR) provides insight into the effect of growth environment on respiratory CO_2 efflux in the context of whole-plant growth. Furthermore, when contrasting growth environments result in different growth rates and, hence, diverging plant sizes through time, comparisons of plants at a common mass aids in determining the extent that plant traits are influenced by size or age differences, a phenomenon termed ontogenetic drift (Coleman *et al.* 1994).

The objective of this study was to examine the relationships between respiration and root and shoot growth in contrasting CO_2 concentrations and thermal environments among seedlings of five boreal tree species. As part of a larger study, plants of each species were grown from seed in controlled environments and specific rates of root and shoot dark respiration and RGR were determined in a series of destructive harvests (Tjoelker *et al.* 1998b). Here, we compare respiration rates at common plant masses and RGRs to test the hypothesis that differences in respiratory CO_2 efflux from plants result from contrasting size or growth rate differences among growth environments. We also test the hypothesis that CO_2 concentration or temperature acclimation of respiration alters the relationship between RGR and respiration. Finally, we examine the effects of CO_2 concentration and temperature acclimation of dark respiration rates on whole-plant respiratory CO_2 efflux in contrasting growth environments.

Materials and methods

Plant material

Seeds of quaking aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.), tamarack (*Larix laricina* [Du Roi] K. Koch), black spruce (*Picea mariana* [Mill.] B.S.P.) and jack pine (*Pinus banksiana* Lamb.) were sown into 2.7 dm^3 pots (10.2 cm diameter, 33 cm height, PVC) filled with a 4 : 1 ratio (v/v) mixture of pure silica sand to a medium of equal proportions of loam, peat, and sand (by volume). Species are hereafter referenced by their generic names. The seeds germinated in uniform conditions of 370 µmol mol^{-1} CO_2, 20 °C, 80% RH, and a 16-h photoperiod at 577 µmol m^{-2} s^{-1} (PPFD) in controlled-environment chambers (Conviron E15, Controlled Environments, Inc., Winnipeg, Manitoba, Canada). Each

pot was watered to excess twice each day, once with a modified Hoagland's nutrient solution and once with deionized water. Plants of each species were grown separately in 10–15 pots in each growth chamber.

Carbon dioxide and temperature treatment

We selected CO_2 treatments of approximate current and future ($\approx 1.5 \times$ ambient) mean atmospheric concentrations and three light/dark period temperatures of 18/12, 24/18, and 30/24 °C. The CO_2 and temperature treatments were applied as a complete factorial in a set of six identical growth chambers (Conviron E15). Treatments began about 18 days after germination and lasted 91 days. The CO_2 concentrations averaged 370 and 580 μmol mol^{-1} in the ambient and elevated treatments.

Relative humidities were set at 60/65% (light/dark period) in the 18/12 °C treatment, 65/70% at 24/18 °C, and 70/75% at 30/24 °C to partially offset the increased vapor pressure deficits at higher temperatures (0.82–1.27 kPa). In each chamber, lighting consisted of metal halide and sodium high-intensity-discharge lamps, providing a maximum of about 1200 μmol $m^{-2}s^{-1}$ (PPFD) irradiance at plant height. Light, temperature and humidity were measured regularly to verify treatment conditions. A more detailed description of methods is provided elsewhere (Tjoelker *et al.* 1998b).

Dark respiration and growth

Plants were harvested at intervals of about 10 days, beginning at 7 days and ending at 91 days of CO_2 and temperature treatment in order to determine RGR (Tjoelker *et al.* 1998b). We measured rates of dark respiration of harvested plants at each of five to seven harvests (depending upon species) beginning at 19 days of treatment when plants were large enough to measure. Rates of net CO_2 efflux from intact root (but free of sand medium) and shoot systems were separately measured on harvested plants at their respective dark-period temperatures of 12, 18, and 24 °C in a temperature-controlled and darkened growth chamber. Plants were collected at the end of the dark period and measures completed within a 5-h period. After washing away the sand medium, intact plants were kept hydrated in the dark at their respective dark-period temperatures prior to measurement. The root was severed from the shoot, and the intact root and shoot placed in separate cuvettes for measurement of net CO_2 efflux. For several early harvests, we combined the roots or shoots of several plants to provide an adequate amount of sample for measurement. For larger plants, single plants or representative subsamples of shoots (7–10 cm length) and roots were used. We chose a functional approach to analysis of respiration (Poorter & Pothmann 1992) and plant growth data (Hunt 1982). The number of plants sampled at any single harvest was thus small and pooled sampling was required for respiration measures at the early harvests. Therefore, variation within treatments in respiration rates at individual harvests were either unavailable or not shown. At the final harvest mean coefficients of variation were 16% and 13%, respectively, for root and shoot specific respiration rates for the species and treatment combinations.

Rates of net CO_2 exchange were measured using infrared gas analysers and cuvettes (LCA-3 and PLC-C, Analytical Development Co. Ltd, Hoddesdon, UK), operated in an open configuration. Columns of magnesium perchlorate removed water vapour from the analyser air stream. We measured rates of CO_2 exchange at both CO_2 treatment concentrations (370 and 580 μmol mol^{-1}) in order to test for direct effects of CO_2 concentration on rates of dark respiration. For each plant sample the CO_2 concentration was switched in the cuvette during the measurement. Rates of dark respiration did not respond to a short-term (i.e. minutes) change in measurement CO_2 concentration (Tjoelker *et al.* 1999). Thus, we show rates determined at the CO_2 concentrations at which the plants were grown. Roots and shoots were oven dried (65 °C) and dry mass determined. Whole-plant respiration rates were calculated by summing root and shoot rates weighted by the proportion of dry mass in root and shoot tissues. To compare rates of respiration at a standard temperature, rates of both roots and shoots were adjusted to 18 °C using multipliers (Q_{10}) for each temperature interval obtained from measured temperature response curves of shoots of each species and treatment combination separately (Tjoelker *et al.* 1999).

Respiration-relative growth rate relationships

In the growth and maintenance model of respiration (Thornley 1970), growth respiration is proportional to the growth rate of a plant and maintenance respiration is proportional to plant dry mass. The relationship may be expressed in a linear form as:

$$R_s = g\,(\mathrm{RGR}) + m,$$

where R_s is the integrated daily rate of dark respiration (mmol CO_2 g^{-1} day^{-1}), RGR is the relative growth rate (g g^{-1} day^{-1}), the slope g is the growth coefficient (mmol CO_2 g^{-1} plant dry mass produced), and intercept m is the rate of maintenance respiration (mmol CO_2 g^{-1} plant dry mass day^{-1}). Rates of plant dark respiration were summed over 24 h with the assumption that shoots and roots respired throughout the light and dark periods.

Rates were adjusted for the 6 °C difference in the light and dark period temperatures as described above.

Data analysis

The experimental treatments were arranged as a complete factorial combination of five species, two CO_2 concentrations and three temperatures. Unless otherwise indicated, analyses were conducted separately for each species. Given the linear relationship between respiration rate and the log-transformed dry mass of roots and shoots, analysis of covariance was used to examine treatment effects on rates of dark respiration for roots and shoots of a common mass. In the same manner, rates were compared at mean root or shoot RGR, adjusted for the covariate relationship. In the full linear model, CO_2 (1 d.f.) and temperature (2 d.f.) and the interaction were considered fixed effects and ln-transformed root or shoot dry mass or RGR was included as the covariate. We present responses at the mean of the independent variable, corrected for the covariate regression relationship.

We examined the relationships between respiration rates and RGR of roots, shoots, or whole-plants determined at growth conditions in destructive harvests throughout the 91 day study. For each species separately, the effects of CO_2 concentration and temperature treatment on the respiration-RGR relationships were tested with linear regression and analysis of covariance, using component RGR as the covariate. First, we tested for homogeneity of slopes among treatment combinations. Next, we used an analysis of covariance using same slopes to test differences in the intercept among the CO_2 and temperature treatments. To avoid extrapolation beyond the range of measured RGRs, we report adjusted least squares means of respiration rates of roots, shoots, and whole-plants to common RGRs across all growth environments for each species separately. Homogeneous slopes among the CO_2 and temperature treatments indicates that growth component of respiration did not differ across environments. Given homogeneous slopes among the growth environments, any differences in respiration rate across growth environments would result from differences in the intercept or maintenance component of respiration.

We estimated the proportional efflux of CO_2 in dark respiration as a percentage of integrated daily net CO_2 uptake. To this end, rates of respiration were summed over the light/dark period as described above. Net daily CO_2 uptake was calculated using mean rates of shoot photosynthesis (Tjoelker *et al.* 1998a) summed over the 16-h photoperiod with the assumption that rates were light-saturated throughout the photoperiod.

Results

Allometry of root and shoot respiration

Mean rates of entire root or shoot respiration (nmol s^{-1}) increased log-linearly with increasing mean root or shoot mass across harvests (Fig. 1). Compared at common sizes, rates of respiration measured at dark-period growth temperatures increased with increasing growth temperatures. In general, slopes of the allometric relationship between natural log transformed dry mass and respiration rates were less than 1.0, indicating that specific rates of respiration (nmol $g^{-1}s^{-1}$) declined with increasing size of roots or shoots. Slopes generally did not differ among temperature treatments with the exception of roots of *Larix* ($P = 0.004$) and shoots of *Betula* and *Pinus* ($P = 0.04$).

In contrast to the effects of temperature, CO_2 concentration generally did not alter the allometry of respiration of either roots or shoots. Rates of whole-plant respiration increased log-linearly with increasing plant mass in each of the five species (Fig. 2). In general, neither slopes nor intercepts differed between CO_2 treatments, indicating common size-dependent relationships. Between CO_2 treatments, slopes differed for *Picea* ($P = 0.05$, all other species, $P \geqslant 0.23$) and intercepts differed for *Larix* grown at 24/18 °C ($P = 0.04$), but not the other temperature treatments (data not shown). As a consequence, minimal declines in specific rates of respiration with CO_2 enrichment were largely a function of plant size in comparison to an acclimation response to CO_2 concentration.

Dark respiration at growth conditions

Mean specific rates of root and shoot dark respiration determined at growth conditions and at common sizes increased with higher growth temperature in each species ($P < 0.001$, Fig. 3). Although specific shoot respiration rates tended to be higher in *Larix* ($P = 0.08$) and *Pinus* ($P = 0.07$) grown in elevated compared to ambient CO_2 concentrations, rates generally neither varied between CO_2 concentrations ($P \geqslant 0.16$) nor were any CO_2–temperature interactions detected ($P \geqslant 0.62$, except for shoots of *Pinus*, $P = 0.11$). Therefore, the effect of thermal environment was predominately independent of growth CO_2 concentration. Accounting for dry mass differences, both root and shoot specific respiration rates in these species increased at higher growth temperatures and were largely unaffected by the CO_2 concentration of the growth environment. Across the 12 °C range in growth temperature, specific respiration rates nearly doubled in *Populus* and *Betula*. In the conifers, proportional

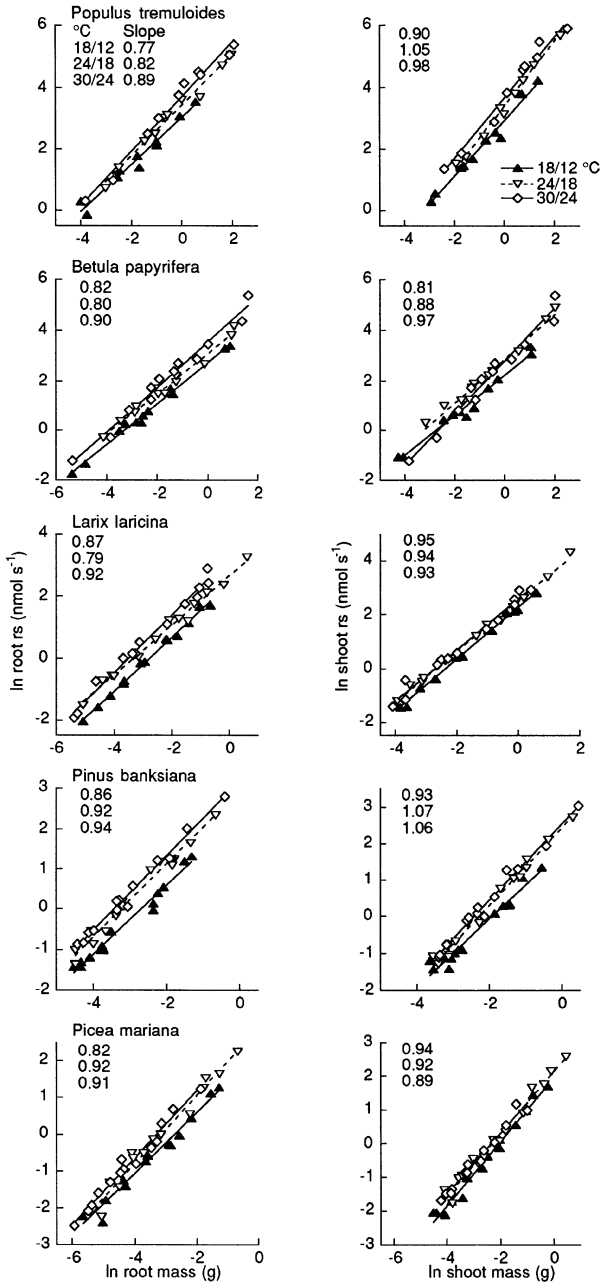

Fig. 1 Relationships between root or shoot size (ln dry mass, g) and dark respiration rates (nmol s^{-1}) in seedlings of five boreal tree species. Plants were grown at 18/12 (▲), 24/18 (▽), and 30/24 (◇) °C in combination with 370 and 580 µmol mol^{-1} CO_2 and measured at their respective dark-period temperatures. Symbols show data pooled across CO_2 treatments ($P \geq 0.19$ except *Larix* shoots, $P = 0.10$). Regression lines ($R^2 \geq 0.96$), and slope values for temperature treatments are shown for mean values from each of five to seven harvests. A slope less than 1.0 indicates a decline in specific respiration rate (nmol $g^{-1}s^{-1}$) with increasing size. Intercepts differed among growth temperature treatments ($P < 0.001$). Slopes differed among temperature treatments for *Betula* shoots, *Larix* roots, and *Pinus* shoots ($P < 0.05$).

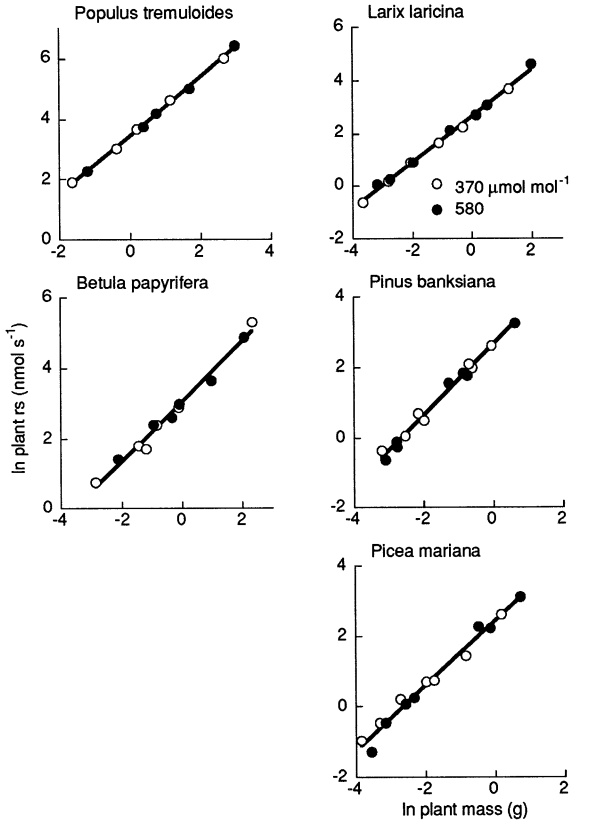

Fig. 2 Relationships between plant size (ln dry mass, g) and respiration rate (ln plant rs, nmol s^{-1}) in seedlings of five boreal tree species. Mean values of plants grown and measured at 370 (○) or 580 (●) µmol mol^{-1} CO_2 in the 24/18 °C temperature treatment are shown. Linear regression slopes were 0.97 for *Populus*, 0.85 for *Betula*, 0.88 for *Larix*, 1.00 for *Pinus*, and 0.92 for *Picea* ($R^2 \geq 0.98$). Between CO_2 treatments slopes differed statistically only for *Picea* ($P = 0.05$, all other species $P \geq 0.23$) and intercepts differed only for *Larix* ($P = 0.04$).

increases in rates, especially for shoots, averaged about 50%, lower than the response of broad-leaved species.

Acclimation to thermal environment

Respiration acclimated to thermal environment in all species. Increases in specific respiration rates across growth temperatures were less than that predicted based on short-term response functions (Fig. 3). Consequently, mean specific respiration rates adjusted to a standard temperature (18 °C) were lower in roots and shoots of plants of all species grown in warmer growth environments (Fig. 4). Compared at the mean dry masses among growth environments (Fig. 4), specific respiration rates of roots and shoots determined at 18 °C declined, often approximately linearly,

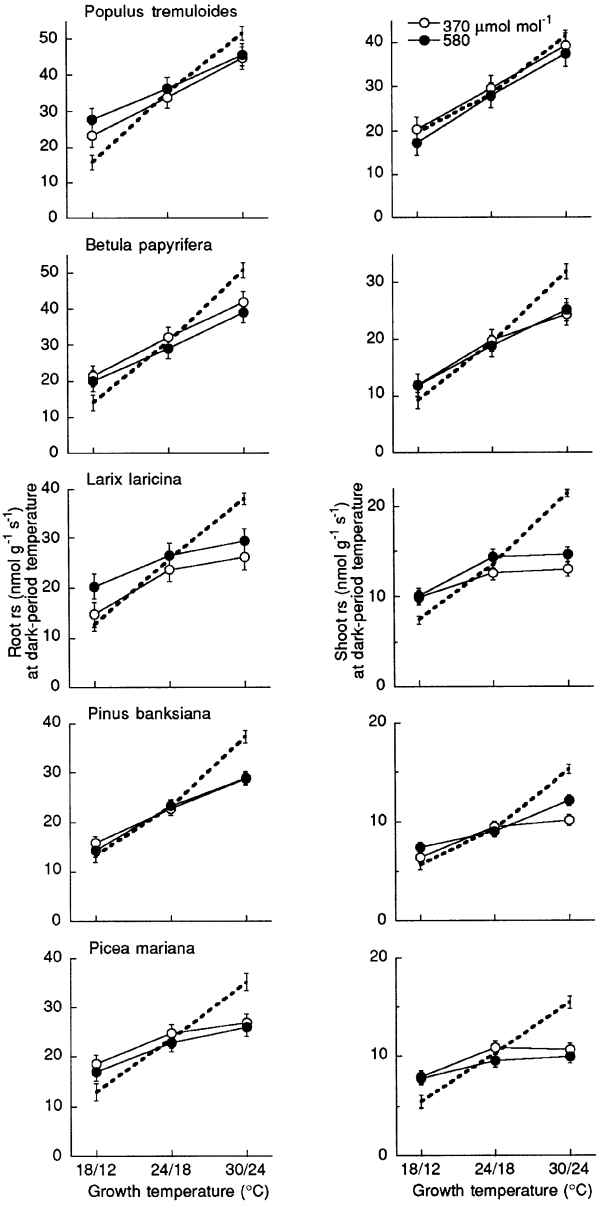

Fig. 3 Acclimation of specific dark respiration rates (nmol $g^{-1}s^{-1}$) of roots and shoots of five boreal tree species grown and measured at 370 (○) and 580 (●) µmol mol^{-1} CO_2 and three growth temperatures of 18/12, 24/18 and 30/24 °C (light/dark period). Shown are mean (± SE) responses at dark-period temperatures across five to seven harvests, corrected for the covariate regression relationship of dry mass and specific respiration rate for each species separately. Dotted lines connect predicted rates (± SE) of respiration at 12 and 24 °C in the absence of acclimation, based on short-term response functions. Predicted rates were projected from the mean rate at 18 °C (across both CO_2 treatments) using measured Q_{10} values for each 6 °C interval (Tjoelker *et al.* 1999).

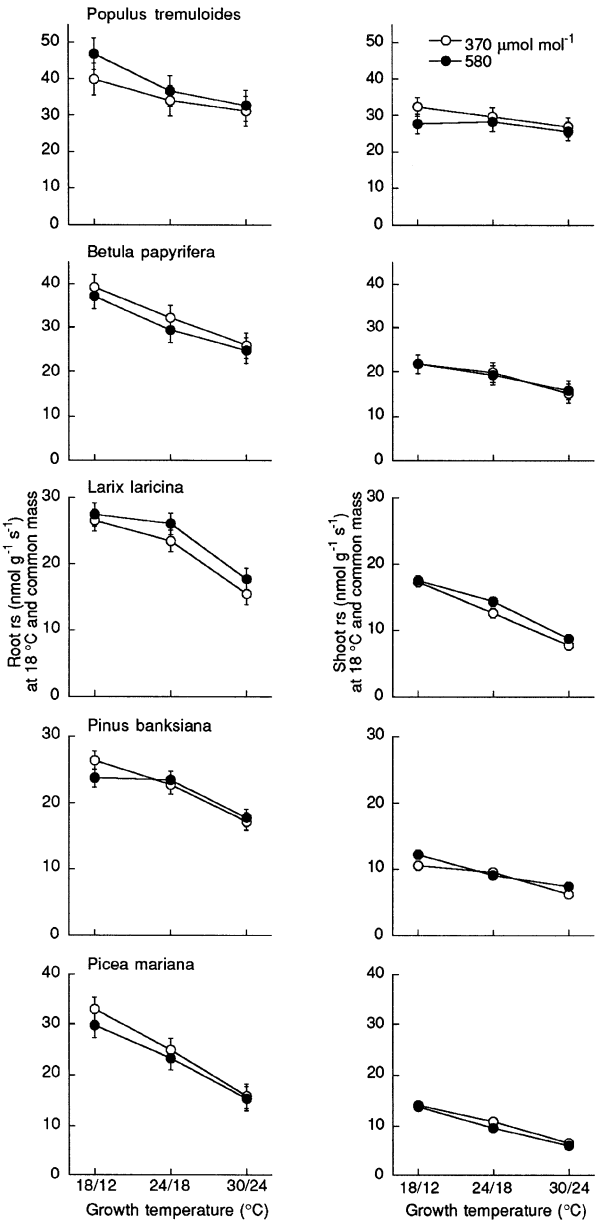

Fig. 4 Acclimation of specific dark respiration rates to thermal environment for five boreal tree species. Specific rates of respiration were adjusted to a standard temperature of 18 °C. Mean rates are shown at the mean ln-transformed root or shoot dry mass among growth environments for each species separately, corrected for the covariate regression relationship. Shown are least squares means (± SE) of five to seven harvests of plants grown and measured at 370 (○) and 580 (●) µmol mol^{-1} CO_2. In the absence of temperature acclimation, rates would be identical across growth temperatures. For each species, specific respiration rates differed among growth temperatures ($P \leq 0.05$), except for shoots of *Populus* ($P = 0.36$). Specific respiration rates generally did not differ between CO_2 treatments ($P \geq 0.25$), except for shoots of *Larix* ($P = 0.09$) and *Pinus* ($P = 0.13$).

Table 1 Interspecific variation in thermal acclimation of dark respiration among seedlings of five boreal tree species*

	Respiration ratio		
Species	Root	Shoot	Plant
Populus	0.74	0.87	0.82
Betula	0.66	0.71	0.69
Pinus	0.70	0.60	0.64
Larix	0.61	0.48	0.53
Picea	0.50	0.45	0.45

*Ratio of specific respiration rates at 18 °C for plants of a common size grown at 30/24 vs. 18/12 °C (see Fig. 4). A ratio of 1.0 would indicate no change in rates across thermal environments. Values indicate the proportion of specific respiration rates of plants acclimated to 30/24 compared to 18/12 °C. Species are ranked from lowest to highest degree of acclimation.

with increased growth temperature from 18/12 to 30/24 °C. The main effects of temperature and CO_2 concentration were statistically independent in each species ($P \geqslant 0.25$).

Species differed in their magnitude of acclimation to thermal environment, especially for shoots (Figs 3, 4). *Populus* and *Betula* had a lower degree of thermal acclimation than did the three conifers. Between the 18/12 and 30/24 °C growth environments, reductions in whole-plant rates of respiration ranged from 18% in *Populus* to 55% in *Picea* (Table 1). In contrast, growth CO_2 concentration had minimal effects on specific rates of respiration for plants compared at a common mass among growth environments. Only specific shoot respiration rates tended to be higher in *Larix* ($P = 0.09$) and *Pinus* ($P = 0.13$) grown in elevated compared to ambient CO_2 concentrations (Fig. 4).

Relationship between respiration and relative growth rate

As predicted by the two-component model of growth and maintenance respiration, specific rates of respiration in growth conditions were generally linear functions of RGR for each species in each of the six treatment combinations (Table 2). For each species, tests for homogeneity of slopes between CO_2 concentration treatments in each temperature treatment did not distinguish separate slopes for shoots, roots, or plants. Likewise, slopes did not differ among temperature treatments at each CO_2 concentration. Thus, the apparent growth component of respiration was not significantly altered by temperature or CO_2 concentration in these five species.

In contrast, the linear regression intercepts or apparent maintenance respiration rates differed among the growth environments for individual species. Same-slopes analysis of covariance was used to examine the relationship between specific respiration and RGR for roots, shoots, and whole plants of each species separately (Table 2). Overall, specific respiration rates at common RGRs among growth environments (for each species and plant component separately) increased with higher growth temperatures, and CO_2 concentration and temperature effects were independent (Table 2). The response patterns were comparable to those of the common mass comparisons (Fig. 3). However, several exceptions were noted. In response to CO_2 enrichment, specific root respiration of *Pinus* was 10% lower and both root and shoot respiration of *Picea* were about 15% lower compared to plants grown and measured at the ambient CO_2 concentration. In contrast, specific respiration rates of shoots of *Larix* were higher in the elevated than ambient CO_2 concentration ($P = 0.13$). Given that the slopes (i.e. growth respiration coefficients) of the respiration-RGR relationships did not differ between CO_2 treatments, apparent maintenance respiration was reduced in *Picea* grown in the elevated CO_2 concentration.

What is the nature of the relationship between respiration and RGR across the five study species which differ widely in RGR? Across all species and harvests within each of the six treatment combinations, specific rates of respiration and RGR were positively correlated and the relationships were linear (Fig. 5). Tests of homogeneity of slopes failed to statistically distinguish separate slopes among the treatment combinations for roots ($P \geqslant 0.15$). For shoots, slopes differed among the three temperature treatments ($P = 0.003$); the slope of the relationship for plants grown at 30/24 °C was higher than the other growth temperatures. Slopes of the shoot respiration-RGR relationships did not differ between CO_2 concentrations at any growth temperature ($P \geqslant 0.17$). For whole-plant respiration, slopes did not differ between CO_2 concentrations at any growth temperature ($P \geqslant 0.37$), but showed an increasing trend with increasing growth temperatures ($P = 0.09$). By contrast, linear regression intercepts increased with higher growth temperatures markedly in roots ($P < 0.0001$) and less so in shoots ($P < 0.0001$). Intercepts tended to be lower in roots ($P = 0.07$) grown in an elevated compared to ambient CO_2 concentration but not in shoots ($P = 0.53$). Thus, for a given RGR, specific respiration rates increased at higher growth temperatures. The effects of growth temperature and CO_2 concentration on the intercepts were statistically independent in roots ($P = 0.76$), shoots ($P = 0.30$), and plants ($P = 0.17$). The relationship between specific respiration and RGR across these five species was affected by growth temperature;

Table 2 Mean specific rates of respiration (nmol $g^{-1}s^{-1}$) for seedlings of five boreal tree species grown at 370 and 580 µmol mol^{-1} CO_2 and three temperature treatments at the mean relative growth rate among growth environments within each species and plant part separately*

		Growth Environment								
		18/12		24/18		30/14°C		ANCOVA ($P > F$)†		
Species	Plant part	370	580	370	580	370	580	CO_2	T	$CO_2 \times T$
Populus	Root	31.2	32.4	32.9	32.5	40.6	41.9	0.77	0.005	0.95
	Shoot	20.5	17.4	29.6	27.9	39.0	37.3	0.36	***	0.96
	Plant	23.8	21.4	30.4	29.0	38.8	38.7	0.50	***	0.89
Betula	Root	23.1	22.6	32.9	28.2	39.0	38.2	0.34	***	0.65
	Shoot	12.8	13.1	19.9	18.2	23.4	24.7	0.98	***	0.76
	Plant	15.5	15.8	23.3	21.2	27.4	28.6	0.89	***	0.72
Larix	Root	18.6	17.1	22.1	21.9	26.2	30.1	0.63	***	0.31
	Shoot	10.5	10.3	12.1	13.3	13.2	15.2	0.13	***	0.39
	Plant	12.3	12.0	14.6	15.7	16.3	19.7	0.12	***	0.23
Pinus	Root	15.9	16.2	23.0	20.2	31.8	26.9	0.05	***	0.28
	Shoot	6.5	7.9	9.3	8.6	10.6	11.8	0.18	***	0.10
	Plant	9.4	10.8	13.1	12.1	16.6	16.2	0.99	***	0.25
Picea	Root	19.5	14.2	23.6	18.2	31.1	29.4	0.01	***	0.57
	Shoot	8.3	7.2	10.5	8.1	11.6	11.1	0.03	***	0.35
	Plant	11.1	9.2	13.7	10.5	15.3	15.3	0.03	***	0.22

*Rates were measured at the growth CO_2 concentrations and dark-period temperatures at which the plants were grown and rates were corrected for the covariate regression relationship.
†Relative growth rate of root, shoot, or plant included as the covariate for each species separately.
***$P < 0.0001$.

for a given RGR rates increased with higher growth temperatures, especially in roots. In contrast, CO_2 concentration had little effect on the respiration-RGR relationships.

Temperature effects on respiratory CO_2 losses in relation to net CO_2 uptake

Specific respiration rates were higher in roots than shoots and differed among species (see Fig. 3). Among species, specific rates of both roots and shoots ranked higher in *Populus* and *Betula* than the conifers. Despite both lower RGRs and specific rates of respiration in conifers than broad-leaved species, the proportion of net daily CO_2 uptake respired was larger in the conifers (Fig. 6). *Larix*, *Pinus*, and *Picea* respired between 50 and 70% of net daily CO_2 uptake compared to 20 and 45% for *Populus* and *Betula*. As a result of incomplete acclimation of specific respiration rates to temperature, respiratory losses of CO_2 as a proportion of daily net CO_2 uptake increased at higher growth temperatures in each species ($P < 0.0001$). Species differences appeared greatest in roots. In conifer roots respiratory losses of CO_2 as a proportion of daily net CO_2 uptake were roughly double that of the broad-leaved species.

Discussion

Temperature acclimation of dark respiration

Rates of respiration of plants in their growth environments increased with higher growth temperatures, but were minimally affected by CO_2 concentration. However, both roots and shoots had a large acclimation response to thermal environment. With increasing growth temperatures, acclimated rates were much lower than expected from instantaneous temperature-response models. Consequently, the magnitude of increase in rates of respiration across growth temperatures did not increase to the extent predicted by short-term temperature responses. Given a mean Q_{10} of 2.2 (12–24 °C), rates would have increased by a factor of 2.6 rather than 1.5–2.0 over the 12 °C range in growth temperatures if acclimation had not occurred.

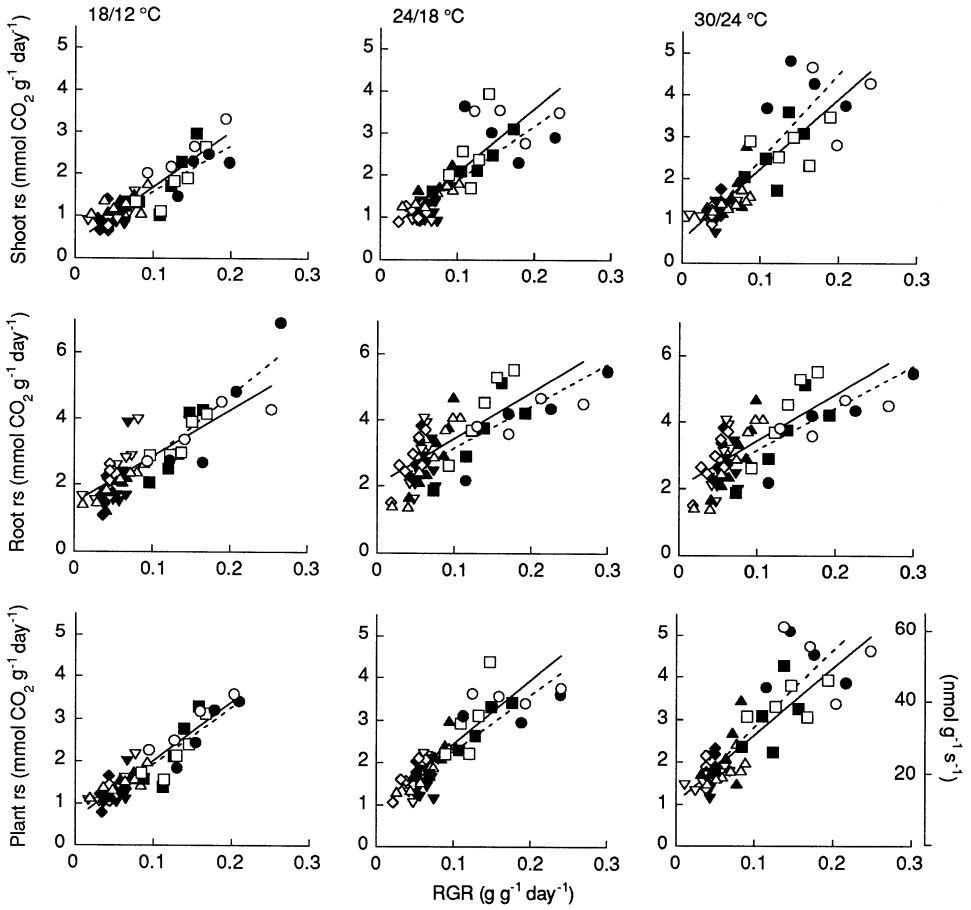

Fig. 5 Relationships between relative growth rate and dark respiration among seedlings of five boreal tree species grown in three thermal environments. Relationships did not differ between ambient (white symbols, 370 μmol mol^{-1}) and elevated (black symbols, 580 μmol mol^{-1}) CO_2 environments. Points are of individual species [*Populus tremuloides* (○), *Betula papyrifera* (□), *Larix laricina* (△), *Pinus banksiana* (◇), and *Picea mariana* (▽)] at either ambient or elevated CO_2 at a given harvest date. Specific respiration rates were determined seven times during the 91-day experiment. Individual linear regression R^2 ranged from 0.65 to 0.83 for shoots, 0.38–0.79 for roots, and 0.71–0.89 for plants (all $P \leq 0.0003$). Inset *y*-axis shows rates scaled to units of nmol g^{-1} s^{-1}.

Species differed in their degree of thermal acclimation. In a study of 19 alpine and lowland plant species, acclimation patterns to temperature ranged from full to no acclimation (Larigauderie & Körner 1995). We concur that predictions of respiration responses at the community level will be difficult given the wide variation in thermal acclimation among species. Among the five boreal tree species, temperature acclimation was larger among the slower-growing conifers than the faster-growing *Populus* and *Betula*. We explore the mechanistic basis of acclimation in dark respiration to growth temperature in a separate study that included measurements of short-term temperature responses, demonstrating an association of temperature acclimation with increases in leaf nitrogen concentration in colder environments (Tjoelker *et al.* 1999).

Plant size and CO_2 concentration effects on respiration

Our results demonstrated that specific rates of respiration in young plants decline with increasing plant dry mass in agreement with prior studies (Poorter & Pothmann 1992; Walters *et al.* 1993b). As roots and shoots grow larger, the proportion of metabolically active meristems declines while the proportion of structural support tissue, such as woody stems and coarse roots, increases. Likewise, RGR declines with increasing plant age and size. Since age, morphology, and RGR underlie the observed declines in specific rates of respiration with increasing plant dry mass, extrapolation of these findings to large, mature trees in the field should be considered with caution.

We found no evidence of a direct, short-term inhibition of dark respiration by an elevated concentration of CO_2

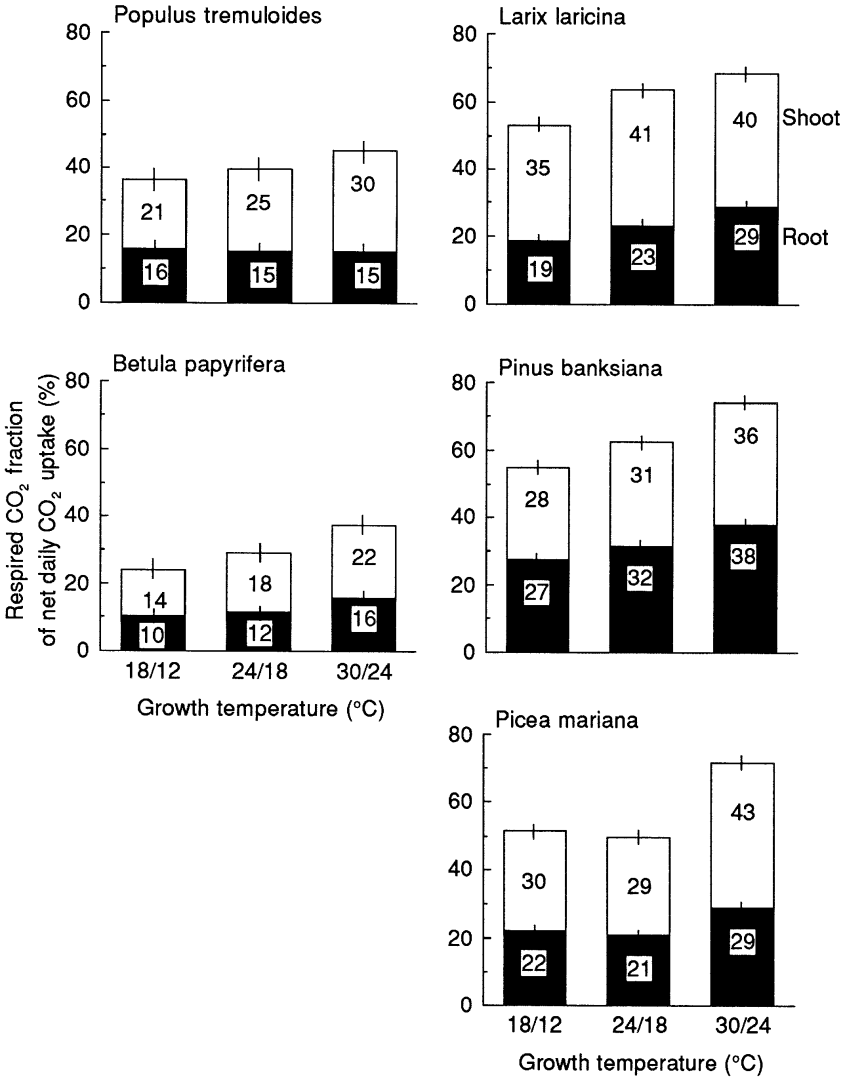

Fig. 6 Whole-plant respiratory CO_2 efflux of roots (■) and shoots (□) as a proportion of daily net photosynthetic CO_2 uptake of seedlings of five boreal tree species grown in three temperature treatments. Mean (± SE) response of plants at a common mass (0.230 g) were corrected for the covariate regression relationship with mass. Means did not differ between CO_2 treatments ($P = 0.25$), and therefore temperature effects are pooled across CO_2 treatments. Photosynthesis data were obtained from Tjoelker *et al.* (1998a).

and relatively small long-term effects of CO_2 concentration in shoots and roots of seedlings of the five tree species. However, we interpret our findings with caution given that the magnitude of short or long-term inhibition of respiration rates between 370 and 580 μmol mol^{-1} CO_2 is likely small (less than 15–20% based on literature reports) and perhaps within the limits of resolution of our instruments and sample sizes. Furthermore, we measured rates of root respiration at CO_2 concentrations far lower than those typical of soil environments, which might be important if root specific respiration rates respond to short-term changes in CO_2 concentration (Qi *et al.* 1994; Burton *et al.* 1997). However, our finding of no effect of CO_2 concentration on root respiration between 370 and 580 μmol mol^{-1} CO_2 is in agreement with findings of Bouma *et al.* (1997), who found no effect of changes in soil CO_2 concentration on respiration rates in citrus and bean. Comparisons of our relative growth rate-respiration relationships of roots with those of hydroponically grown and measured plants (Poorter *et al.* 1991) were similar, evidence of the generality of the relationship that would perhaps not be the case if root respiration rates were significantly affected by the measurement CO_2 concentration.

At the whole-plant level, an often overlooked source of variation in rates of respiration is its size dependence or ontogenetic drift (Coleman *et al.* 1994). Thus, the size difference of plants grown in contrasting CO_2 environments, in part, influences the comparisons typically made at one or more common harvests in most studies. In other words, larger plants produced in a high CO_2 concentration may simply have lower specific respiration rates owing to their larger size, rather than a functional adjustment to CO_2 concentration. The distinction be-

tween ontogenetic drift and functional adjustment in interpreting variation in plant traits is often ignored (Coleman *et al.* 1994), and in the case of CO_2 enrichment studies may aid in interpreting the often contradictory results concerning long-term effects of CO_2 concentration on dark respiration in plants. An alternative and perhaps complementary approach involves analysis of respiration in terms of its relationship to growth.

Growth and maintenance respiration

Insight into the variation in respiration rates among species and growth environments may be gained by examining the underlying processes that drive respiratory CO_2 efflux. To this end the relationship of respiration rate to plant growth and maintenance is of concern in interpreting plant responses. Although inexact, respiration can be examined in terms of a growth component, associated with the construction costs of synthesizing new plant biomass (Lambers *et al.* 1983), and a maintenance component, the cost of maintaining existing plant biomass (Amthor 1984). The lack of suitable models to link growth and maintenance respiration to respiratory biochemistry remain a concern in interpreting these components.

Both within and especially among species, specific rates of root and shoot respiration increased with increasing RGR of these plant components (Fig. 5). Interspecific variation in rates of respiration were largely associated with species differences in RGR (Poorter *et al.* 1991; Reich *et al.* 1998). Overall, CO_2 concentration had little or no effect on respiration-RGR relationships at the three growth temperatures, in contrast to the dominant effect of thermal environment on maintenance, but not growth respiration. Our experiment did not examine whether CO_2 effects differed between stems and foliage, and thus does not preclude the possibility that stems and foliage responded independently and perhaps differently to short- and long-term effects of CO_2 concentration. Maintenance respiration rates were reduced only in *Picea* and in roots of *Pinus* in response to CO_2 enrichment. In contrast, rates of leaf net photosynthesis acclimated to CO_2 concentration in varying degrees in all five species (Tjoelker *et al.* 1998a). Regardless of tissue-specific differences in response of respiration to CO_2 enrichment, our findings suggest that compared to the effects of growth temperature, changes in growth CO_2 concentration from 370 to 580 μmol mol^{-1} had minimal effect on the relationship between respiration and growth of young tree seedlings.

Increased growth temperatures were associated with higher rates of maintenance respiration of shoots and roots in agreement with previous findings (Szaniawski & Kielkiewicz 1982; Ryan 1991). Thus, despite a degree of thermal acclimation, specific rates of respiration increased in the warmer growth environments. In addition, maintenance respiration was higher in roots than shoots. For the three conifers, roots had a larger increase in specific respiration rates across thermal environments. Given comparable increases in air and soil temperature in response to climate warming, root respiration may increase to a larger extent than shoot respiration.

Respiration and whole-plant carbon budget

To what extent does respiration influence the carbon budget of these plants? Total daily respiratory losses of CO_2 ranged from 20 to 70% of net daily photosynthesis among species and temperature treatments (Fig. 6). Despite their slower growth rates, respiratory losses in conifers were larger than the faster-growing *Betula* and *Populus*. Although data of this type are scarce, our findings concur with those of Poorter *et al.* (1990) and Walters *et al.* (1993a), in which proportional respiratory losses of carbon among herbaceous and woody species, respectively, decreased with increasing species RGR. The increases in whole-plant respiratory losses as a fraction of net daily CO_2 uptake across growth temperatures are likely muted by temperature acclimation of dark respiration rates. Despite increases in respiratory CO_2 efflux with increased growth temperatures, the greatest effect of higher temperatures was to increase growth. Species and growth temperature differences in proportional CO_2 losses of net daily CO_2 uptake were larger in roots than shoots. These findings may be explained, in part, by species differences in rates of photosynthesis and morphology. Faster-growing species had higher rates of photosynthesis (Tjoelker *et al.* 1998a) and a higher leaf area: plant dry mass ratio than the slower-growing conifers (Reich *et al.* 1998; Tjoelker *et al.* 1998b). In comparison, differences in root and shoot dry mass fraction among species were minor and, hence, relatively unimportant in explaining species differences in respiratory losses. Thus, despite their higher rates of root and shoot respiration, the broad-leaved species were able to fix more carbon per unit plant mass and use proportionally less in respiration than the conifers. Relatively high root respiration rates among slower-growing species are related to increased costs of nutrient acquisition (Poorter *et al.* 1991).

Conclusions

Compared to the effects of thermal environment, increasing concentrations of CO_2 from 370 to 580 μmol mol^{-1} had little effect on respiration rates and their relationship to growth in seedlings of five boreal tree species. Rates of respiration acclimated to thermal

environment and species differed in this regard. Acclimation resulted in reduced respiratory efflux of CO_2 at higher temperatures, and increased efflux at lower temperatures compared to what would occur based on instantaneous temperature-response models. Thus, increases in respiration at high growth temperatures and decreases in rates at low growth temperatures were muted as a result of thermal acclimation. Both within and across species, respiration rates were linearly related to RGR in the various CO_2 and thermal environments. Slower-growing conifers lost a larger proportion of net daily CO_2 in dark respiration in all environments. Increases in root maintenance respiration may be particularly important in response to climate warming.

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