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Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO_2 and temperature

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Summary Biochemical models of photosynthesis suggest that rising temperatures will increase rates of net carbon dioxide assimilation and enhance plant responses to increasing atmospheric concentrations of CO_2. We tested this hypothesis by evaluating acclimation and ontogenetic drift in net photosynthesis in seedlings of five boreal tree species grown at 370 and 580 μmol mol^{-1} CO_2 in combination with day/night temperatures of 18/12, 21/15, 24/18, 27/21, and 30/24 °C. Leaf-area-based rates of net photosynthesis increased between 13 and 36% among species in plants grown and measured in elevated CO_2 compared to ambient CO_2. These CO_2-induced increases in net photosynthesis were greater for slower-growing *Picea mariana* (Mill.) B.S.P., *Pinus banksiana* Lamb., and *Larix laricina* (Du Roi) K. Koch than for faster-growing *Populus tremuloides* Michx. and *Betula papyrifera* Marsh., paralleling longer-term growth differences between CO_2 treatments. Measures at common CO_2 concentrations revealed that net photosynthesis was down-regulated in plants grown at elevated CO_2. *In situ* leaf gas exchange rates varied minimally across temperature treatments and, contrary to predictions, increasing growth temperatures did not enhance the response of net photosynthesis to elevated CO_2 in four of the five species. Overall, the species exhibited declines in specific leaf area and leaf nitrogen concentration, and increases in total nonstructural carbohydrates in response to CO_2 enrichment. Consequently, the elevated CO_2 treatment enhanced rates of net photosynthesis much more when expressed on a leaf area basis (25%) than when expressed on a leaf mass basis (10%). In all species, rates of leaf net CO_2 exchange exhibited modest declines with increasing plant size through ontogeny. Among the conifers, enhancements of photosynthetic rates in elevated CO_2 were sustained through time across a wide range of plant sizes. In contrast, for *Populus tremuloides* and *B. papyrifera*, mass-based photosynthetic rates did not differ between CO_2 treatments. Overall, net photosynthetic rates were highly correlated with relative growth rate as it varied among species and treatment combinations through time. We conclude that interspecific variation may be a more important determinant of photosynthetic response to CO_2 than temperature.

Keywords: Betula papyrifera, carbon fixation, elevated carbon dioxide, Larix laricina, ontogenetic drift, ontogeny, Picea mariana, Pinus banksiana, Populus tremuloides.

Introduction

Atmospheric concentrations of carbon dioxide (CO_2) are rising and are predicted to double within the next century (Houghton 1997). Increased concentrations of CO_2 and other greenhouse gases may result in climate warming. Interspecific variation in response of net photosynthetic CO_2 exchange to temperature and CO_2 concentration may play an important role in determining productivity and net carbon balance of plant communities in a changed global climate.

Photosynthetic carbon fixation in plants is limited by CO_2 and responds directly to increased concentrations of CO_2 and elevated temperatures (Bowes 1991). The accepted biochemical model of photosynthesis in C_3 plants predicts that CO_2 enhancement of net photosynthesis should increase at elevated temperatures (Long 1991, Kirschbaum 1994), because photorespiratory losses of CO_2 increase with increasing temperature and photorespiration is inhibited by elevated CO_2 concentrations (Bowes 1991, Long 1991).

Short-term physiological response models, however, frequently fail to predict actual responses of net photosynthesis of plants grown and compared across different environments. For example, plants grown at different temperatures and CO_2 concentrations exhibited responses in leaf net CO_2 exchange across the temperature treatments that were the opposite of model predictions (Ziska and Bunce 1994). This discrepancy between modeled and observed responses may result, in large part, from acclimation of net photosynthetic rates to atmospheric CO_2 concentration (Ceulemans and Mousseau 1994, Gunderson and Wullschleger 1994) and temperature (Berry and Björkman 1980). Likewise, expected increases in growth enhancements in response to CO_2 enrichment at elevated temperatures are not observed in some studies, and responses vary among species (e.g., Idso et al. 1987, Rawson 1992). Understanding acclimation of photosynthesis to CO_2 and temperature and potential interspecific variation is critical in predicting plant response to environmental change.

Acclimation of net photosynthetic rates to CO_2 enrichment may be linked to changes in leaf chemistry and structure and feedbacks governed by whole-plant growth dynamics. A common suite of responses to CO_2 enrichment is a reduction in the amount or activity of Rubisco (Bowes 1991), reduced leaf nitrogen (N) and specific leaf area (SLA, leaf area per unit leaf

mass) (Curtis 1996), which, collectively, should lead to decreased rates of net photosynthesis (Reich et al. 1997). Acclimation of net photosynthetic rates to CO_2 enrichment may also occur in concert with a limitation of carbon sinks for growth or storage (Arp 1991, Thomas and Strain 1991, Ziska et al. 1995). Plants grown in elevated CO_2 typically exhibit increased nonstructural carbohydrate concentrations (Poorter et al. 1997) that are hypothesized to result in a feedback inhibition of net photosynthetic rates in leaves (Stitt 1991). Among species, differences in relative growth rate may be associated with differences in growth and perhaps net photosynthetic responses to CO_2 enrichment. Faster-growing species are hypothesized to exhibit a larger growth response to CO_2 enrichment than slower-growing species, but the evidence is highly variable (Poorter 1993, Poorter et al. 1996) and species differences vary with plant age and size (Tjoelker 1997).

Phenotypic traits, including photosynthetic rates, may vary during plant growth and development, a phenomenon termed ontogenetic drift (Evans 1972). Ontogenetic drift complicates interpretation of treatment effects on traits at a common age when plants differ in size as a result of treatment effects on plant growth rate (Coleman et al. 1993, 1994). As plants increase in size, relative growth rates, and to a lesser extent rates of leaf net CO_2 exchange, decline (e.g., Poorter and Pothmann 1992). Thus, larger plants produced in a CO_2-enriched environment may exhibit lower net photosynthetic rates than smaller plants grown at the ambient CO_2 concentration when measured at a common CO_2 concentration, regardless of whether CO_2 acclimation of photosynthesis occurred. Comparisons of plants at both common sizes and times reveal the extent to which ontogenetic drift influences traits in their growth environment.

We tested the hypotheses that: (1) CO_2 effects on net photosynthetic rates are enhanced at elevated growth temperatures; (2) as a result of comparatively less acclimation in net photosynthesis to CO_2 enrichment, faster-growing tree species exhibit larger proportional responses in net photosynthesis to elevated CO_2 than slower-growing species; and (3) CO_2 effects on net photosynthesis vary with ontogeny. We chose five dominant tree species of the boreal forest in North America and examined interspecific variation in seedling response to increased CO_2 concentrations and temperatures of the growth environment during a three-month study. Growth enhancements of seedlings in response to CO_2 enrichment were initially greater among intrinsically fast-growing *Populus tremuloides* Michx. and *Betula papyrifera* Marsh. than slower-growing conifers, *Larix laricina* (Du Roi) K. Koch, *Pinus banksiana* Lamb., and *Picea mariana* (Mill.) B.S.P. (Tjoelker 1997). However, growth increases in response to CO_2 enrichment eroded through time for the broad-leaved species, whereas the conifers exhibited sustained increases in growth. Here, we explore the extent to which growth responses to elevated temperature and CO_2 enrichment are related to net photosynthetic rates and its variation with relative growth rate and ontogeny.

Materials and methods

Plant material

We obtained seeds of quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), tamarack (*Larix laricina*), black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*) from northern Minnesota. The seeds were sown in 2.7-dm^3 pots (10.2 cm diameter, 33 cm height, PVC) filled with a 4:1 (v/v) mix of pure silica sand and a medium comprising equal proportions of loam, peat, and sand. Seeds were germinated under 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, Canada). After the seedlings emerged, each pot was watered to excess twice each day, once with a modified Hoagland's nutrient solution and once with deionized water. One or several plants of each species were grown separately in 10 to 15 pots in each growth chamber. The pots were periodically repositioned to prevent shading and to randomize any effect of position across all pots within a chamber.

Carbon dioxide and temperature treatments

Four weeks after sowing, when the seedlings were about 18 days old, we began the CO_2 and temperature treatments. Because of a limited number of chambers, the CO_2 (ambient = 370 μmol mol^{-1} and elevated = 580 μmol mol^{-1}) and temperature (day/night temperatures of 18/12, 21/15, 24/18, 27/21, and 30/24 °C) treatments were applied as a complete factorial across two time periods using a set of eight identical growth chambers (Conviron E15). The 21/15 and 27/21 °C treatments and both CO_2 treatments were applied in combination, each in two chambers in the first period; the 18/12, 24/18 and 30/24 °C treatments were applied at both CO_2 concentrations, each in one chamber in the subsequent period. Treatments were randomly assigned to chambers and lasted 91 days in each study period.

Day/night relative humidities were set at 60/65% in the 18/12 °C treatment, 65/70% in the three intermediate temperature treatments, and 70/75% at 30/24 °C to minimize vapor pressure gradients among temperature treatments (range 0.82 to 1.27 kPa). In each chamber, lighting provided by two 1000 W metal halide and two 1000 W sodium high-intensity-discharge lamps supplied a maximum of about 1200 μmol m^{-2} s^{-1} (PPFD) irradiance at plant height. Because of the varying light requirements of the species, the 16-h photoperiod was divided into two 5-h periods of one-half irradiance (532 ± 72 μmol m^{-2} s^{-1}, PPFD) at the beginning and end of the photoperiod with the middle 6-h period at full irradiance (1200 ± 98 μmol m^{-2} s^{-1}, PPFD), totaling 45 mol m^{-2} day^{-1}, representative of integrated irradiance on a clear sunny day. Light, temperature and humidity were monitored regularly to verify treatment conditions. A more detailed description of methods is provided by Tjoelker (1997).

Gas exchange

Light-saturated net photosynthetic rates and leaf stomatal conductance to water vapor (g_s) were determined throughout the

three-month study. Rates of CO_2 exchange were measured with infrared gas analyzers (LCA-3, Analytical Development Co. Ltd., Hoddesdon, U.K.), operated in an open configuration. Plants were typically measured the day preceding each of four to seven common harvest dates for which all five temperature treatments could be compared. For the first three measurement dates for the conifers, rates were determined on intact shoots bearing only cotyledons. As the conifer seedlings grew, rates were subsequently measured on shoots bearing primary or secondary needles. For *Populus* and *Betula*, we measured individual, fully expanded leaves at comparable unshaded canopy positions. The measurements took place in each controlled-environment chamber at light-saturated irradiances (> 750 μmol m^{-2} s^{-1}, PPFD) and at CO_2 concentrations and temperatures at which the plants were grown. Typically, measures of four plants were obtained for each species and treatment combination on each date.

Leaf and needle areas (one-sided, projected) of fresh material were measured with a video-image analysis system (AgVision, Decagon Devices, Inc., Pullman, WA). Leaves were oven-dried (65 °C) and dry masses determined. Photosynthetic rates were calculated on a leaf area (A_{area}, μmol m^{-2} s^{-1}) and mass basis (A_{mass}, nmol g^{-1} s^{-1}). Leaf temperature was estimated by energy balance equations.

To examine whether rates of net photosynthesis acclimated to CO_2 treatment, we determined responses of light-saturated rates of net photosynthesis to a short-term change in CO_2 concentration. After 13 weeks of treatment, we selected *Betula*, *Larix*, and *Picea* plants grown at 18/12, 24/18, and 30/24 °C and both CO_2 treatments. Both A_{area} and A_{mass} were determined at the temperature at which the plants were grown at both ambient and elevated CO_2 concentration (350 and 560 μmol mol^{-1} CO_2, respectively) by switching the concentration of CO_2 in the cuvette during measurement. Leaves or needle-bearing shoots of four plants per species in all six treatments were measured in this manner at irradiances exceeding photosynthetic light saturation (> 750 μmol m^{-2} s^{-1}).

We also examined the response of A_{area} to calculated internal CO_2 concentrations (C_i) for *Populus* and *Betula* plants from a common temperature treatment (27/21 °C) after 56 and 87 days of CO_2 exposure, respectively. For each species, measures of net CO_2 exchange were conducted at irradiances exceeding light saturation (> 760 μmol m^{-2} s^{-1}) and at CO_2 concentrations ranging from 0 to 1400 μmol mol^{-1} for each of three leaves from each temperature and CO_2 treatment combination. The response of net A_{area} to C_i was analyzed by a rectangular hyperbola (Ellsworth et al. 1995) of the form:

$$A_{area} = A_{sat}\left\{1 - \left(1 - \frac{R_{day}}{A_{sat}}\right)^{(1 - C_i/\Gamma)}\right\},$$

where A_{sat} is the PPFD- and CO_2-saturated rate of net CO_2 assimilation, R_{day} is leaf respiration in the light, and Γ is the CO_2 compensation point for net photosynthesis. For each species, statistical comparisons of the fitted response curves were made as described by Potvin et al. (1990) by fitting the complete dataset to the model, and by fitting the combined data for all leaves within each treatment combination individually to the model. Estimates of the maximum velocity of carboxylation ($V_{c,max}$, μmol m^{-2} s^{-1}) were obtained by fitting a biochemical model of photosynthesis to leaf data where C_i < 250 μmol mol^{-1} and the assimilation of CO_2 is assumed to be limited solely by the amount, activity, and kinetic properties of Rubisco (Harley et al. 1992). The relative stomatal limitation to photosynthesis was calculated by the graphical method (Farquhar and Sharkey 1982).

Total nonstructural carbohydrates and leaf nitrogen

Total nonstructural carbohydrate (TNC) concentrations of leaves and roots of harvested plants were determined 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. Following addition of anthrone, soluble sugars were determined spectrophotometrically at 625 nm. Starch was gelled and converted to glucose with amyloglucosidase (Sigma, St. Louis, MO). Glucose concentrations were determined by assaying with glucose oxidase. The sample was mixed with peroxidase-glucose oxidase-*o*-dianisidine dihydrochloride and absorbance measured at 450 nm after a 30-min incubation at 25 °C. Soluble sugars and starch concentrations are presented as glucose equivalents (mg glucose g^{-1} dry mass). Composite plant samples were collected from each of two chamber replications of the 21/15 and 27/21 °C treatment combinations after 34 and 61 days of treatment. Nitrogen concentrations of dried and ground leaf and needle tissues were measured with a CHN element analyzer (Perkin Elmer Corp., Norwalk, CT) for independent composite samples of mature leaves of three plants of each species grown at 24/18 °C and at both CO_2 concentrations.

Data analysis

The experimental treatments were arranged as a complete factorial combination of five species, two CO_2 concentrations, and, across two periods, five temperature regimes. A subset of the leaf gas exchange data was used to compare treatment effects on comparably mature foliage. For the conifers, mature secondary needles were sampled at each of four measurement periods at 52, 66, 80, and 90 days of treatment, except for *Pinus* for which measures were obtained for the first three dates. For the broad-leaved species, gas exchange data included four common measurement dates at 17, 25, 33 and 41 days of treatment. Means of each of the measurement dates were calculated and used in an analysis of variance of treatment effects for each species separately as follows. In the ANOVA, date (2 or 3 df) was considered a random effect and both CO_2 (1 df) and temperature (4 df) were considered fixed effects. The main effect of temperature treatment and the CO_2 by temperature interaction effect were partitioned into single-degree-of-freedom contrasts for both linear and quadratic terms to examine responses to temperature. In the full model, *F*-tests of treatment mean squared error against residual mean squared error (27 df) were used to examine the main effects of CO_2 and

temperature treatments, their potential interaction, and the extent that responses to temperature treatment were explained by a linear or quadratic model.

To examine ontogenetic changes in net CO_2 exchange rates, mean A_{mass} and A_{area} were regressed against mean ln-transformed plant mass for each species and treatment combination. Tests for homogeneity of slopes among treatment combinations were conducted by analysis of covariance with ln-transformed plant mass as the covariate. A significant treatment by plant size interaction indicates that differences between CO_2 or temperature treatments varied for small and large plants. A common slope and differences among intercepts indicates that treatment differences were maintained across the range of plant sizes. All analyses were conducted with statistical analysis software (JMP Version 3.15, SAS Institute Inc., Cary, NC).

Results

Net photosynthesis

Mean rates of light-saturated net photosynthesis on a leaf area or mass basis (Figure 1, Table 1) were generally higher in plants grown and measured at 580 μmol mol^{-1} CO_2 than in plants grown and measured at 370 μmol mol^{-1} CO_2. The main effects of growth temperature and CO_2 environment on net photosynthesis were independent except for A_{mass} of *Picea* and *Pinus* (Table 1). The response of net photosynthesis to growth temperature varied among species (Figure 1). For *Populus*, A_{area} increased with increasing temperature, whereas it decreased with increasing temperature in *Betula* and *Picea* (Table 1, Figure 1). There was no consistent response of A_{area} to temperature in *Larix* or *Pinus*. In the elevated CO_2 treatments, A_{mass} was depressed at the highest temperature in *Picea*, but was otherwise unaffected by temperature in any species. For *Pinus*, CO_2 enrichment reduced A_{mass} at the lowest growth temperature, but increased A_{mass} at the higher temperatures.

The hypothesis that the CO_2-induced enhancement of net photosynthetic rate increases with increasing growth temperature was not supported. Proportional responses of A_{area} and A_{mass} of plants grown and measured at 580 versus 370 μmol mol^{-1} CO_2 were generally comparable or exhibited declining trends with increasing growth temperature (Figure 2). Only for *Pinus* did proportional enhancement of rates of leaf net CO_2 exchange in response to CO_2 enrichment increase at higher temperatures compared to the lowest temperature treatment in agreement with model predictions (e.g., Long 1991).

The magnitude and direction of CO_2 effects on net photosynthesis depended on whether rates were expressed on a leaf area or leaf mass basis. For each species, CO_2-induced enhancement of net photosynthesis was lower for A_{mass} than for A_{area} (Table 2). Among the five species, elevated CO_2 increased mean A_{area} by 13% in *Populus* and 36% in *Picea*. On a mass basis, CO_2 treatment did not stimulate net photosynthesis in *Populus* or *Betula*, but increased A_{mass} by about 20% in the conifers.

The difference in the responses of A_{mass} and A_{area} to CO_2 enrichment was largely a result of decreased SLA (m^2 kg^{-1}) in plants grown in 580 compared to 370 μmol mol^{-1} CO_2 (Table 3). Except for *Pinus*, each species exhibited significantly lower SLA in the elevated CO_2 treatment than in the ambient CO_2 treatment. Furthermore, proportional reductions in SLA in response to CO_2 enrichment were greater for the broad-leaved species than for the needle-leaved species. Only *Larix*

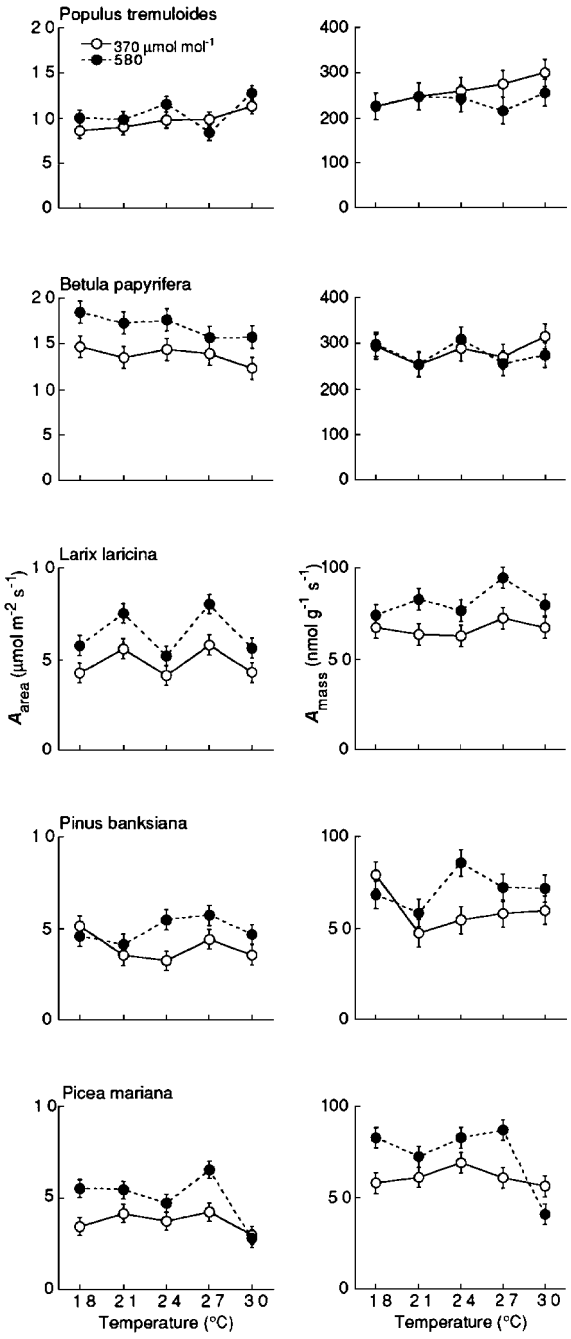

Figure 1. Area- and mass-based light-saturated rates of net photosynthesis of five boreal tree species grown and measured in ambient (○, 370 μmol mol^{-1}) and elevated (●, 580 μmol mol^{-1}) concentrations of CO_2 and five temperature treatments (light-period temperatures shown in this and subsequent Figures). Shown are mean values (± SE of treatment effect in ANOVA) between 17 and 41 days of treatment for *Populus* and *Betula* and 52 and 90 days for the conifers (n = 4 dates, except *Pinus*, n = 3 dates).

Table 1. Analysis of variance probabilities ($P > F$) of main effects of CO_2, temperature, and $CO_2 \times$ temperature interaction on gas exchange traits of five boreal species grown and measured in ambient (370 μmol mol^{-1}) and elevated (580 μmol mol^{-1}) CO_2 and five temperature treatments ranging from 18/12 to 30/24 °C.

			Temperature (T)[1]		$CO_2 \times T$	
Species	Trait[2]	CO_2	Linear	Quadratic	Linear	Quadratic
Populus	A_{area}	0.15	0.01[3]	0.22	0.58	0.54
	A_{mass}	0.21	0.15	0.89	0.28	0.97
	g_s	0.0003	0.01	0.14	0.91	0.40
Betula	A_{area}	0.0003	0.04	0.75	0.63	0.71
	A_{mass}	0.74	0.94	0.38	0.38	0.48
	g_s	0.08	0.40	0.0009	0.12	0.73
Larix	A_{area}	< 0.0001	0.85	0.06	0.98	0.84
	A_{mass}	0.0005	0.26	0.63	0.60	0.34
	g_s	0.02	0.03	0.57	0.72	0.27
Pinus	A_{area}	0.01	0.83	0.82	0.11	0.08
	A_{mass}	0.03	0.81	0.31	0.16	0.04
	g_s	0.24	0.02	0.61	0.44	0.59
Picea	A_{area}	0.0002	0.02	0.005	0.12	0.49
	A_{mass}	0.002	0.007	0.001	0.02	0.14
	g_s	0.001	0.97	0.009	0.82	0.84

[1] For temperature treatments, orthogonal linear and quadratic contrasts are shown.
[2] Light-saturated rates of carbon assimilation expressed on a leaf area (A_{area}, μmol m^{-2} s^{-1}) and leaf mass (A_{mass}, nmol g^{-1} s^{-1}) basis, g_s (mmol m^{-2} s^{-1}) is leaf stomatal conductance to water vapor.
[3] $P \leq 0.05$ are underlined.

exhibited differences in SLA among the temperature treatments and an interaction effect of $CO_2 \times$ temperature (Table 3).

Stomatal conductance and water-use efficiency

The effects of growth temperature and CO_2 environment on stomatal conductance to water vapor (g_s) were statistically independent in each species ($P \geq 0.12$, Table 1). Growth of plants in elevated CO_2 resulted in declines in mean g_s for all species ($P \leq 0.08$, Table 1, Figure 3) except *Pinus*. Proportional declines in g_s in plants grown in elevated CO_2 compared to ambient CO_2 were about 25% for *Populus*, *Larix*, and *Picea*, and about 10% for *Betula* and *Pinus*. Stomatal conductance increased linearly with increasing growth temperature for *Populus*, *Larix*, and *Pinus* ($P \leq 0.03$). For *Betula* and *Picea*, g_s was highest among the intermediate temperatures (quadratic effect, $P \leq 0.009$). As a result of an increase in A_{area} and simultaneous decrease in g_s in plants grown at 580 compared to 370 μmol mol^{-1} CO_2, instantaneous water-use efficiency increased from 40 to 80% among the five species grown in elevated CO_2 ($P \leq 0.002$, data not shown).

Ontogeny and acclimation of net photosynthesis to CO_2

The CO_2-induced enhancement of net photosynthetic rates was generally stable throughout the three-month period, although species differed in overall rates and proportional enhancement (Figure 4). To illustrate the predominant CO_2 treatment effect, both A_{mass} and A_{area} are shown in relation to ln-transformed plant mass, each averaged across the five temperature treatments, in Figure 4. For the three conifers, CO_2 enhancement of A_{mass} or A_{area} was maintained regardless of plant size, with the possible exception of newly germinated seedlings at 17 days of treatment. The broad-leaved species exhibited no difference in A_{mass} between the CO_2 treatments at any plant size, suggesting that acclimation to CO_2 treatment had already occurred by Day 17. For *Betula*, however, A_{area} was consistently higher in elevated CO_2 than in ambient CO_2 for leaves of similar age in both small and large plants.

Ontogenetic changes in area- and mass-based expressions of leaf photosynthetic rates were evident, although changes were relatively small in some species. In general, rates of net CO_2 exchange declined with increasing plant size during the three-month study. We found no evidence to support the hypothesis that CO_2-induced enhancements in A_{area} or A_{mass} decline with increasing plant size. Tests of homogeneity of slopes between the two CO_2 treatments for each species and temperature treatment combination failed to distinguish separate slopes for either A_{area} or A_{mass} regressed against ln-transformed plant mass ($P \geq 0.31$). Likewise, separate slopes analysis of differences in A_{area} or A_{mass} with increasing plant size among the temperature treatments also failed to reject the null hypothesis of equal slopes ($P > 0.05$) for each species except *Larix*. Therefore, differences in net photosynthetic rates between CO_2 treatments or among temperature treatments did not vary for small and large plants, suggesting a common response of net photosynthesis to CO_2 or temperature, regardless of plant size.

To examine the degree of acclimation in photosynthetic rates to CO_2 treatment, we measured A_{mass} and A_{area} of three species at two common measurement CO_2 concentrations (350 and 560 μmol mol^{-1}) after 13 weeks of exposure (Figure 5).

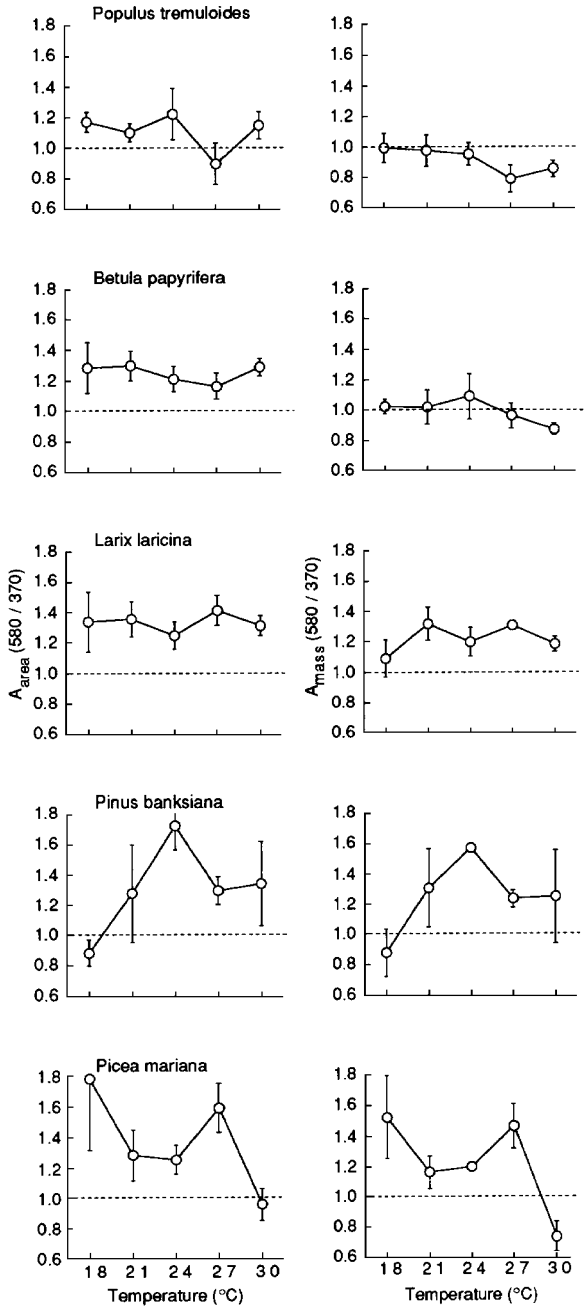

Figure 2. Effect of growth temperature on the response of area- (A_{area}, μmol m^{-2} s^{-1}) and mass-based (A_{mass}, nmol g^{-1} s^{-1}) net photosynthesis of leaves to CO_2 enrichment. Mean (± SE) proportional change in rates (rate at 580 divided by rate at 370 μmol mol^{-1} CO_2 growth environment) of plants grown and measured in elevated (580 μmol mol^{-1}) and ambient (370 μmol mol^{-1}) CO_2 are shown (see Figure 1 legend for sampling details).

Betula, *Larix*, and to a lesser extent *Picea*, exhibited down-regulation; i.e., both A_{mass} and A_{area} measured at common CO_2 concentrations were lower in plants grown at 580 μmol mol^{-1} CO_2 than in plants grown at 370 μmol mol^{-1} CO_2. At the same time, the plants exhibited a typical positive response to short-term increases in CO_2 concentration, increasing on average by

Table 2. Interspecific variation in proportional change in net photosynthetic rates in response to CO_2 enrichment. Mean responses (across five temperature treatments) of area- (A_{area}) and mass-based (A_{mass}) rates of photosynthesis to CO_2 concentration are shown as the ratio of rates determined at growth CO_2 concentrations of 580 and 370 μmol mol^{-1} (A580/A370). A value of 1.0 indicates no difference in rates between CO_2 treatments.

	A580/A370				Instantaneous
	Entire experiment[1]		Common time interval[2]		CO_2 response[3]
Species	A_{area}	A_{mass}	A_{area}	A_{mass}	
Populus	1.13	0.93	1.11	0.91	1.36
Betula	1.31	1.05	1.25	0.99	1.47
Larix	1.33	1.19	1.33	1.16	1.41
Pinus	1.26	1.21	1.24	1.18	–[4]
Picea	1.36	1.22	1.33	1.20	1.57
Mean	1.28	1.12	1.25	1.09	1.45

[1] Common sample dates (n = 4 to 7) range from 17 to 90 days of treatment across species.
[2] Common sample dates (n = 4) range from 17 to 48 days of treatment.
[3] Instantaneous CO_2 response = proportional change in rate measured at 560 compared to 350 μmol mol^{-1} CO_2.
[4] No data available.

45% (Table 2). Plants grown and measured at day/night temperature regimes of 18/12 and 30/24 °C showed similar responses (data not shown).

To explore the metabolic basis of photosynthetic acclimation, we determined A_{area}–C_i response curves for the two broad-leaved species. Down-regulation of photosynthesis in *Betula* and *Populus* plants grown in the elevated CO_2 treatment was evident in reduced CO_2-saturated rates of photosynthesis and $V_{c,max}$ (Figure 6). Estimates of $V_{c,max}$ were reduced by 29% in *Populus* and 19% in *Betula* for plants grown in 580 compared to 370 μmol mol^{-1} CO_2. Likewise, estimates of A_{sat} were reduced by 28% in *Populus* and 44% in *Betula*. In response to CO_2 enrichment, relative stomatal limitation to photosynthesis declined from 0.21 to 0.14 in *Populus* and from 0.30 to 0.27 in *Betula*, indicating that stomatal limitations to photosynthesis at elevated CO_2 were less important than biochemical limitations. As a consequence of down-regulation, A_{area} at a common measurement CO_2 concentration of 370 or 580 μmol mol^{-1} was reduced by about 30% in both *Betula* and *Populus* plants grown in elevated CO_2 compared to ambient CO_2.

Total nonstructural carbohydrates and leaf nitrogen

Leaf and root starch concentrations varied in parallel among the five species (Figure 7). Overall, *Populus* and *Betula* had higher starch concentrations than the conifers. Temperature and CO_2 treatments altered leaf and root starch concentrations and their effects were statistically independent (Figure 7). Across all species, growth in elevated CO_2 increased starch concentrations in leaves (P = 0.03) and roots (P = 0.07). For *Populus* and *Betula*, the CO_2-induced increases in leaf starch tended to be larger than the increases in root starch and this pattern was similar for total nonstructural carbohydrates

Table 3. Specific leaf area ($m^2\ kg^{-1}$) of leaves of five boreal tree species grown in 370 and 580 μmol mol^{-1} CO_2 and five temperature treatments.[1]

		Growth temperature (°C)						$P > F$[2]		
Species	CO_2	18/12	21/15	24/18	27/21	30/24	SE	CO_2	T	$CO_2 \times T$
Populus	370	26.5	28.3	28.1	28.4	26.3	2.1	0.002	0.34*q*	0.49*l*
	580	23.4	24.3	21.4	24.7	19.7				
Betula	370	21.8	19.6	20.0	20.2	25.9	2.1	0.002	0.18*q*	0.19*q*
	580	17.1	15.0	18.4	16.4	17.5				
Larix	370	15.7	11.7	15.2	13.3	15.7	0.5	< 0.001	< 0.001*q*	0.02*q*
	580	13.0	11.5	14.6	12.0	14.2				
Pinus	370	15.6	13.8	17.0	13.3	16.6	0.8	0.34	0.23*q*	0.35*l*
	580	15.0	15.0	15.6	13.4	15.1				
Picea	370	17.0	15.1	18.7	15.0	19.0	1.0	0.006	0.58*l*	0.08*q*
	580	15.1	13.6	17.9	13.5	14.8				

[1] Mean values of leaves sampled at four measurement dates between 17 and 41 days of treatment for *Populus* and *Betula* and between 52 and 90 days of treatment for the conifers. The SE is the standard error for treatment effect in analysis of variance.

[2] Linear (*l*) or quadratic (*q*) contrast of temperature effect that exhibited the lowest *P* value in analysis of variance.

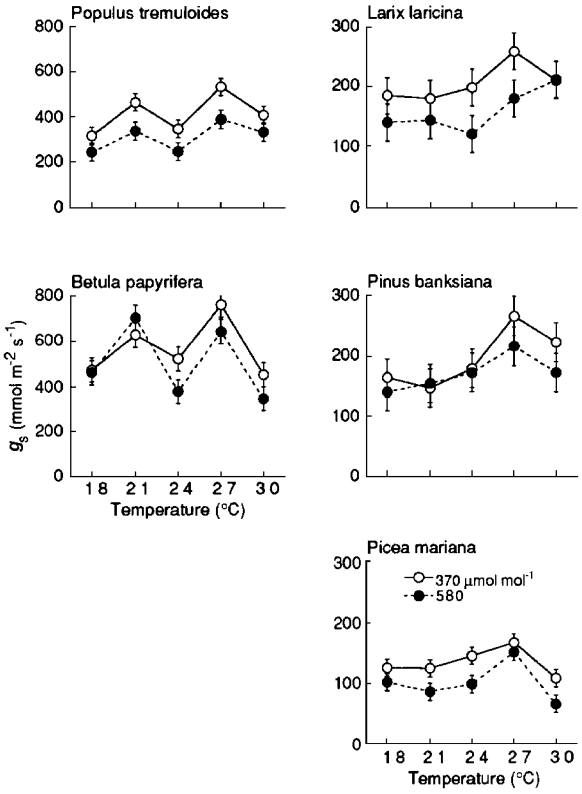

Figure 3. Response of leaf stomatal conductance (g_s, mmol $m^{-2}\ s^{-1}$) of five boreal tree species grown in ambient (○, 370 μmol mol^{-1}) and elevated (●, 580 μmol mol^{-1}) CO_2 in combination with five temperature treatments (°C). Mean values (± SE of treatment effect in ANOVA) are shown (see Figure 1 for sampling details).

whether expressed on a leaf mass or area basis (Table 4). Comparison of changes in area-based TNC concentrations and leaf mass per area (1/SLA) between CO_2 concentrations suggested that increases in leaf mass per area (i.e., decreases in SLA) in response to CO_2 enrichment were only partly explained by increased TNC (Table 4). The low temperature treatments resulted in higher leaf starch concentrations in *Populus* and *Betula* compared with the high temperature treatments, whereas needle starch concentrations of the conifers differed minimally between temperature treatments (species × temperature effect, $P = 0.004$). Root starch concentrations increased in response to the low temperature treatments in *Pinus* and *Picea*, but were unaffected by the temperature treatments in *Populus* and *Betula*. In summary, both low temperatures and elevated CO_2 increased starch concentrations and this occurred primarily in leaves of broad-leaved species and roots of coniferous species. Overall, leaf N concentration on a mass basis declined in leaves grown in elevated CO_2 and the effect ($P = 0.0001$) tended to be greater in *Populus* and *Betula* than in the conifers (species × CO_2 effect, $P = 0.13$, Figure 8). Leaf N content on an area basis, however, did not vary between CO_2 treatments. Overall, N concentration was higher in the broad-leaved species than in the conifers, whereas N content on an area basis exhibited an opposite trend, because of differences in SLA among species. Despite declines in leaf N concentration, net photosynthetic rates on a leaf N basis increased in plants grown and measured in elevated CO_2 compared to plants grown and measured in ambient CO_2 (Figure 8).

Relationship between net photosynthesis and relative growth rate

To explore the extent to which differences in relative growth rates among species and treatments are correlated with differences in net photosynthetic rates, we examined variation in both instantaneous measures of relative growth rate (Tjoelker 1997) and mean A_{area} and A_{mass} (Figure 9). Overall, relative growth rate was positively correlated with mean A_{area} ($r = 0.72$) and A_{mass} ($r = 0.85$). The slopes differed between CO_2 treatments for A_{area} ($P = 0.0003$), but not for A_{mass}. An index of the whole-canopy rate of net CO_2 exchange (i.e., $A_{mass} \times$ LWR, where LWR is the leaf dry mass fraction of the plant), was also positively correlated with relative growth rate ($r = 0.87$). Thus,

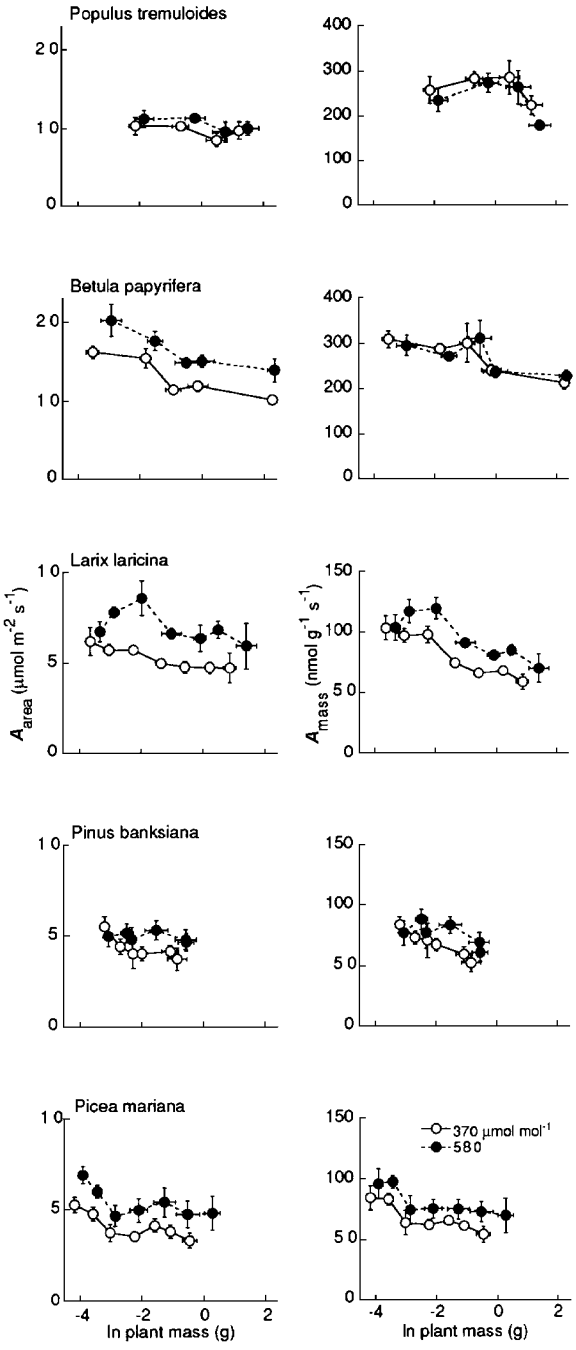

Figure 4. Ontogenetic change in light-saturated rates of leaf net photosynthesis on an area and mass basis for five boreal tree species grown in ambient (○, 370 μmol mol^{-1}) and elevated (●, 580 μmol mol^{-1}) CO_2 over a three-month period. Mean (± SE) rates of net photosynthesis under growth conditions are shown in relation to mean (± SE) plant mass (ln-transformed), calculated from the five temperature treatments to illustrate the predominant CO_2 treatment effect.

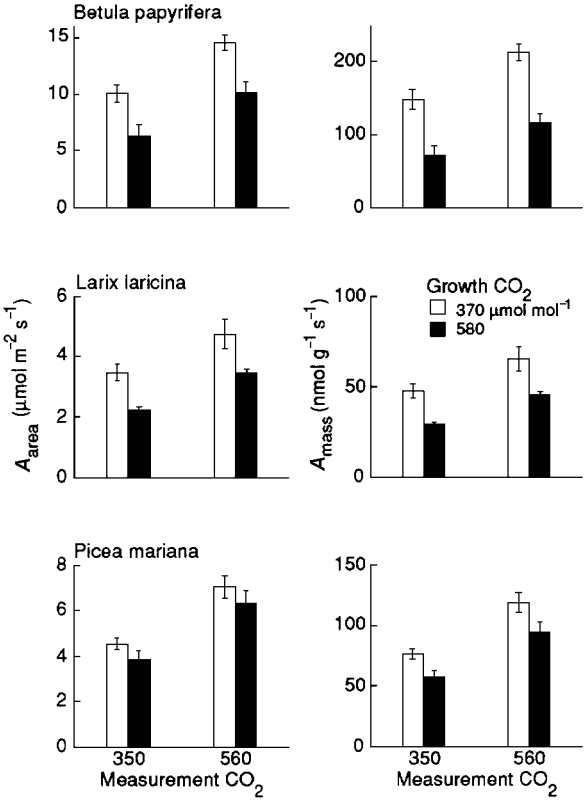

Figure 5. Acclimation of net leaf CO_2 exchange to growth CO_2 concentration. Mean rates of four plants (± SE, for *Larix* and *Picea*; ± 95% confidence intervals for *Betula* data obtained from response curves) were determined at measurement CO_2 concentrations of 350 and 560 μmol mol^{-1} after 13 weeks of growth in 370 and 580 μmol mol^{-1} CO_2 and 24/18 °C.

species, treatment and ontogenetic variation in net canopy photosynthetic rate were strongly associated with variation in relative growth rate, supporting the hypothesis that variation in gas exchange rates underpin species differences in relative growth rate across contrasting CO_2 and thermal environments.

Discussion

Our data do not support the hypothesis that a warmer environment will enhance the response of net photosynthesis to increased concentrations of CO_2. Although our findings do not preclude the likelihood that short-term (i.e., minutes to hours) responses of leaf net CO_2 exchange follow model predictions, over the longer term (i.e., days or weeks) this was not the case. Growth in different thermal and CO_2 environments resulted in partial acclimation of net photosynthetic rates to both of these factors in seedlings of all five tree species. Given that acclimation responses appear critical in determining plant response to environmental change, it is essential to incorporate this phenomenon into models of leaf-level responses scaled to larger spatial and temporal dimensions. This feature is commonly lacking in present models (e.g., Kirschbaum 1994).

Acclimation in photosynthesis to CO_2 environment

Relative enhancement in photosynthetic rate in response to an elevated growth CO_2 concentration varied among the five tree species, even though they all share the same C_3 photosynthetic biochemical pathway (Table 2). Our findings contradict the prediction that faster-growing species would exhibit greater proportional increases in rates of leaf net CO_2 exchange when

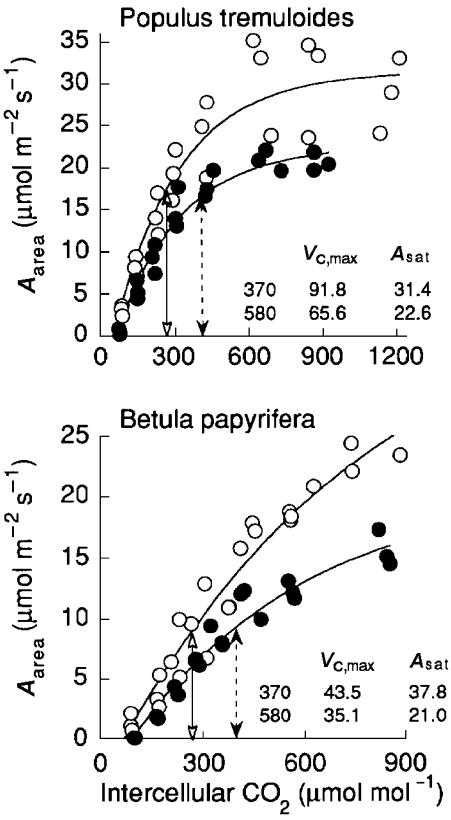

Figure 6. Acclimation of net photosynthesis to CO_2 treatment in seedlings of *Populus tremuloides* and *Betula papyrifera*. The A_{area} versus C_i (intercellular CO_2, μmol mol^{-1}) curves are shown for plants grown at ambient (○, 370 μmol mol^{-1}) and elevated (●, 580 μmol mol^{-1}) CO_2 at 27/21 °C. Three leaves in each treatment combination were measured at 27 °C after 56 days of treatment for *Populus* and after 87 days of treatment for *Betula*. Nonlinear regression lines are shown ($R^2 \geq 0.92$). Arrows indicate rates of net photosynthesis at growth CO_2 concentrations. Abbreviation: $V_{c,max}$(μmol m^{-2} s^{-1}) is the maximum velocity of carboxylation.

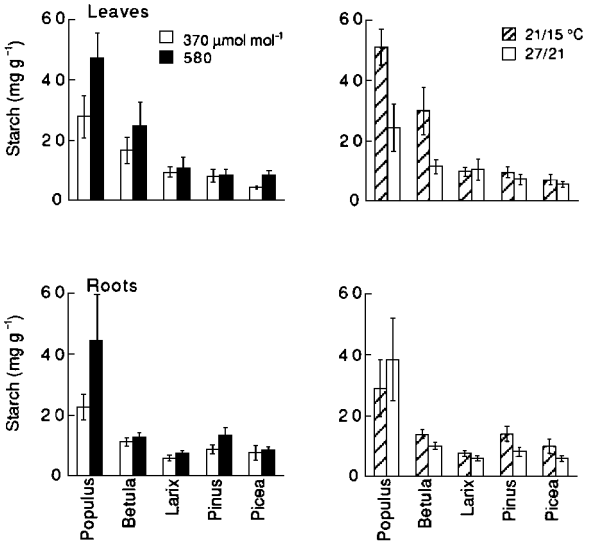

Figure 7. Starch concentration in leaves and roots of five boreal tree species grown in ambient (370 μmol mol^{-1}) and elevated (580 μmol mol^{-1}) CO_2 and 21/15 and 27/21 °C temperature treatments. Means (± SE) are shown based on two composite samples collected at 34 and 61 days of treatment.

grown and measured at 580 compared to 370 μmol mol^{-1} CO_2 than slower-growing species. Species differences appear to result from acclimation to CO_2 environment in terms of SLA, TNC, and leaf N concentration, factors that underlie leaf-based rates of net photosynthesis (Reich et al. 1997). Intrinsically slower-growing species did not exhibit greater acclimation of net photosynthesis and leaf structure to elevated CO_2 than faster-growing species as hypothesized. Likewise, a study of five slow-growing wild herbaceous species found no acclimation of net photosynthesis to CO_2 concentration when grown in elevated CO_2 (Stirling et al. 1997), contrary to predictions based on their minimal growth responses to CO_2 enrichment. Together, these findings suggest that slow-growing species are not intrinsically prone to exhibit greater acclimation in net photosynthesis to CO_2 than faster-growing species.

For individual species, differences in net photosynthetic rates between CO_2 environments were similar for plants compared at either common times or common plant sizes. Although photosynthetic rates declined with increasing plant size through time, the effect of CO_2 enrichment on net photosynthesis was relatively independent of plant size differences. Thus, the hypothesis that effects of CO_2 on net photosynthesis vary with ontogeny was not supported.

We found evidence of down-regulation of photosynthesis for plants grown in CO_2 enrichment in the four species measured. For *Populus* and *Betula*, down-regulation was associated with reduced $V_{c,max}$, reflecting a reduction in quantity or activity of Rubisco, or both, in plants grown in CO_2 enrichment, a common (Long et al. 1993, Gunderson and Wullschleger 1994), but not universal response (Harley et al. 1992). Com-

Table 4. Area- and mass-based concentrations of total nonstructural carbohydrates (TNC) in leaves[1] and associated leaf mass per unit area (1/SLA) of five boreal tree species grown in ambient (370 μmol mol^{-1}) and elevated (580 μmol mol^{-1}) CO_2.

	TNC (mg g^{-1})		TNC (g m^{-2})		1/SLA (g m^{-2})	
Species	370	580	370	580	370	580
Populus	132	176	6.0	10.0	47.8	55.6
Betula	54	65	2.9	4.5	53.2	67.1
Larix	45	50	3.4	4.2	75.1	81.8
Pinus	41	42	2.8	2.9	69.1	70.6
Picea	53	67	3.5	5.2	68.8	78.1
SE[2]		6.0		0.54		2.95
Species (S)		< 0.001		< 0.001		< 0.001
CO_2		0.02		0.01		0.05
S × CO_2		0.006		0.009		0.34

[1] Mean values are based on two composite samples collected at 34 and 61 days of treatment for plants grown at 27/21 and 21/15 °C.

[2] Standard error of the treatment effect and ($P > F$) in analysis of variance.

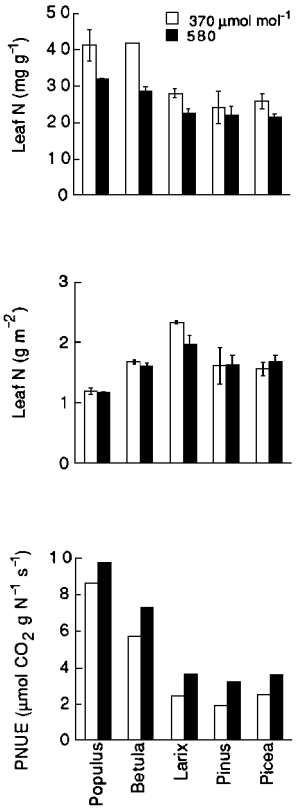

Figure 8. Leaf nitrogen concentration and content and photosynthetic nitrogen-use efficiency (PNUE) among seedlings of five boreal tree species grown in ambient (370 µmol mol^{-1}) and elevated (580 µmol mol^{-1}) CO_2. Means (± SE) are shown for plants grown at 24/18 °C and measured at 24 °C .

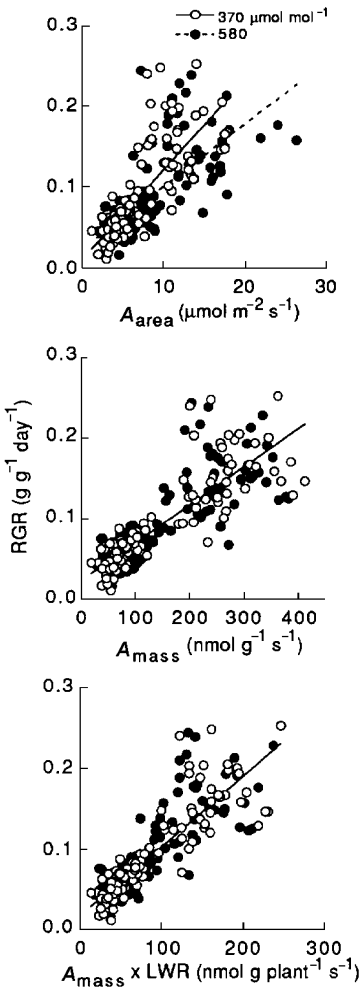

Figure 9. Relationship between net photosynthetic CO_2 exchange and instantaneous relative growth rate (RGR) among seedlings of five boreal tree species grown in 370 or 580 µmol mol^{-1} CO_2 at five temperature treatments (n = 150 for each CO_2 treatment). Each point represents an individual harvest of each species and temperature treatment combination.

paring the magnitude of short-term CO_2 enhancements of net photosynthetic rates with differences in rates between growth CO_2 concentrations during the study demonstrated that down-regulation of photosynthesis occurred in all species, and that species differed in degree of acclimation (see Table 2).

We found evidence of species-specific differences in adjustment of several leaf traits across growth environments. Changes in leaf chemistry and structure were noted in reduced N concentrations, increased TNC, and lower SLA in plants grown in the elevated CO_2 treatment, a common suite of responses to CO_2 enrichment (Curtis 1996, Roumet et al. 1996). Moreover, these responses were greater among broad-leaved species than among conifers. Increased TNC concentrations in leaves only partially accounted for the decreases in SLA in response to CO_2 enrichment, suggesting other changes in morphology occur in leaves grown at high CO_2 concentrations. Changes in SLA accounted for the differences in the response of leaf area- and mass-based rates of net photosynthesis to CO_2 and thus are important in interpreting species differences in response to increasing CO_2 concentrations. We also note that, among the species studied, *Pinus* exhibited the smallest changes in these leaf traits and leaf net photosynthesis responded most closely to model predictions of a greater CO_2 response at higher temperatures. Thus, interspecific variation in plasticity of leaf traits across growth environments appears critical in interpreting plant response to CO_2.

Despite declines in leaf N concentration in response to CO_2 enrichment, net photosynthesis expressed per unit leaf N (i.e., photosynthetic N-use efficiency) increased in plants grown and measured in elevated CO_2. This response is opposite to the typical decrease in photosynthetic N-use efficiency with declining leaf N either within or among species (Reich et al. 1991). An increased photosynthetic N-use efficiency in response to CO_2 enrichment may lead to increased nitrogen productivity of leaves (Garnier et al. 1995) and whole plants (Roumet et al. 1996), and may be a general response of plants to CO_2 enrichment (Luo et al. 1994). Although broad-leaved species had a higher photosynthetic N-use efficiency than conifers, proportional increases in photosynthetic N-use efficiency in response to CO_2 enrichment appeared larger among conifers than among broad-leaved species.

TNC and sink limitations

Species differed in starch and TNC concentrations and in their response to treatment combinations, suggesting that source–sink relationships play an important role in the response of net photosynthesis to CO_2 and thermal environment. Accumulation of carbohydrates often indicates a reduced sink strength for carbon at the whole-plant level (Farrar and Williams 1991, Stitt 1991). Consistent with this idea, *Populus* and *Betula* had higher foliar starch concentrations and a greater increase in starch and TNC concentration in response to CO_2 enrichment than the conifers. Because *Populus* and *Betula* have high leaf area ratios (Tjoelker 1997) and net photosynthetic rates, these species were perhaps more sink-limited than the conifer seedlings under the high light and nutrient conditions of this study, a response that would be magnified in elevated CO_2. Species differences in responses of net photosynthesis and TNC to growth CO_2 concentration are consistent with the hypothesis of a carbohydrate feed-back limitation to net photosynthesis in elevated CO_2 (Farrar and Williams 1991, Stitt 1991).

Populus and *Betula* also exhibited greater increases in leaf starch concentration in response to low temperatures than the conifers. The accumulation of starch in leaves of *Populus* and *Betula* grown at low temperatures may reflect a temperature limitation of carbon export (i.e., phloem-loading) from leaves or limited sink development at low temperatures, or both (Farrar and Williams 1991). Higher starch concentrations in leaves than in roots of plants grown at low temperatures tends to support the former hypothesis.

Net photosynthesis in relation to growth

In the species studied, net photosynthetic rates exhibited modest declines with increasing plant size compared with other growth traits (Tjoelker 1997). Furthermore, net photosynthetic rates for *Populus* and *Betula* exhibited larger declines than those for conifers over a common range of plant sizes. Thus, variation in ontogenetic drift affects the magnitude of species-specific differences in net photosynthetic rates and other traits at a common time.

Two- to threefold higher leaf area ratios in combination with higher net photosynthetic rates accounted for the greater relative growth rates of *Populus* and *Betula* compared to the conifers (Tjoelker 1997). Rates of leaf and canopy net CO_2 exchange were correlated with relative growth rates among species and treatment combinations, providing evidence that leaf-level rates of gas exchange are not independent of whole-plant growth dynamics. Thus, species differences in leaf-level photosynthetic acclimation to CO_2 are linked to growth dynamics at the whole-plant scale.

Conclusions

The response of net photosynthesis across elevated CO_2 and temperature environments failed to support model predictions based on the biochemistry of net photosynthesis (Long 1991, Kirschbaum 1994). Proportional increases in net photosynthetic rates in response to CO_2 enrichment were not enhanced at elevated growth temperatures. Adjustments in leaf structure and chemistry to the growth environment and associated alterations in net photosynthetic rates suggest an important role of acclimation in determining plant responses to changing environments. Furthermore, a greater plasticity of leaf traits, such as SLA, leaf N concentration, and TNC across growth environments among broad-leaved seedlings than among needle-leaved seedlings underpins tree species differences in photosynthetic acclimation and growth response to elevated CO_2 and temperature.

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