

# Effects of soil temperature and elevated atmospheric CO<sub>2</sub> concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings

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**Summary** One-year-old jack pine (*Pinus banksiana* Lamb.) and current-year white birch (*Betula papyrifera* Marsh.) seedlings were grown in ambient (360 ppm) or twice ambient (720 ppm) atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]) and at three soil temperatures ( $T_{\text{soil}} = 7, 17$  and  $27$  °C initially, increased to 10, 20 and 30 °C two months later, respectively) in a greenhouse for 4 months. In situ foliar gas exchange, in vivo carboxylation characteristics and chlorophyll fluorescence were measured after 2.5 and 4 months of treatment. Low  $T_{\text{soil}}$  suppressed net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) in jack pine in both CO<sub>2</sub> treatments and  $g_s$  and  $E$  in white birch in ambient [CO<sub>2</sub>], but enhanced instantaneous water-use efficiency (IWUE) in both species after 2.5 months of treatment. Treatment effects on  $g_s$  and  $E$  remained significant throughout the 4-month study. Low  $T_{\text{soil}}$  reduced maximal carboxylation rate ( $V_{\text{cmax}}$ ) and PAR-saturated electron transport rate ( $J_{\text{max}}$ ) in jack pine in elevated [CO<sub>2</sub>] after 2.5 months of treatment, but not after 4 months of treatment. Low  $T_{\text{soil}}$  increased actual photochemical efficiency of photosystem II (PSII) in the light ( $\Delta F/F_m'$ ) in jack pine, but decreased  $\Delta F/F_m'$  in white birch after 4 months of treatment. In response to low  $T_{\text{soil}}$ , photosynthetic linear electron transport to carboxylation ( $J_c$ ) decreased in jack pine after 2.5 months and in white birch after 4 months of treatment. Low  $T_{\text{soil}}$  increased the ratio of the photosynthetic linear electron transport to oxygenation ( $J_o$ ) to the total photosynthetic linear electron transport rate through PSII ( $J_o/J_T$ ) in both species after 2.5 months of treatment, but the effects became statistically insignificant in white birch after 4 months of treatment. High  $T_{\text{soil}}$  decreased foliar N concentration in white birch. Elevated [CO<sub>2</sub>] increased  $P_n$ , IWUE and  $J_c$  but decreased  $J_o/J_T$  in both species at both measurement times except  $J_c$  in white birch after 2.5 months of treatment. Elevated [CO<sub>2</sub>] also decreased  $g_s$  and  $E$  in white birch at high  $T_{\text{soil}}$ ,  $V_{\text{cmax}}$  in both species and triose phosphate utilization in white birch at low  $T_{\text{soil}}$  after 4 months of treatment, and  $\Delta F/F_m'$  in white birch after 2.5 months of treatment. Elevated [CO<sub>2</sub>] also increased foliar N concentration in both species. Low  $T_{\text{soil}}$  caused no permanent damage to PSII in either species, but jack pine responded and acclimated to low  $T_{\text{soil}}$

more quickly than white birch. Photosynthetic down-regulation and a decrease in photosynthetic electron transport to photorespiration occurred in both species in response to elevated [CO<sub>2</sub>].

**Keywords:** photosynthetic down-regulation, photosynthetic electron transport and allocation, water-use efficiency.

## Introduction

Photosynthetic responses to elevated atmospheric carbon dioxide concentration ([CO<sub>2</sub>]) can vary with other environmental conditions, e.g., photosynthetic down-regulation is greater when a plant is nutrient stressed (Rogers et al. 1998, Saxe et al. 1998, Davey et al. 1999, Liozon et al. 2000). Soil temperature is an important environmental factor affecting growth, carbon allocation and the distribution of plants (Cai and Dang 2002, Peng and Dang 2003). Schwarz et al. (1997) reported that photosynthesis is more sensitive to soil temperature than to air temperature. There are many studies on plant responses to increases in [CO<sub>2</sub>] and to increases in both [CO<sub>2</sub>] and air temperature, but there is a lack of information on the interactive effects of soil temperature and elevated [CO<sub>2</sub>] (Ward et al. 1999).

Soil temperature directly influences enzyme activities in roots, thereby affecting the absorption of mineral nutrients (Pastor et al. 1987, Paré et al. 1993), water transport (Long and Woodward 1998, Wan et al. 1999) and other metabolic processes. Low soil temperatures can inhibit photosynthesis (Vapaavuori et al. 1992, Folk et al. 1995, Cai and Dang 2002, Dang and Cheng 2004), the growth of shoots and leaves (Landhäusser and Lieffers 1998, Peng and Dang 2003) and roots (Folk et al. 1995, Domisch et al. 2001, Peng and Dang 2003). Because of low water absorption by roots, trees may experience water stress at low soil temperatures (Wan et al. 1999, Cai and Dang 2002, Dang and Cheng 2004). Low soil temperature can also decrease photosynthesis by reducing stomatal conductance ( $g_s$ ) to CO<sub>2</sub>. All these factors can influence the acclimation of plants to elevated [CO<sub>2</sub>] (Gavito et al. 2001). For

example, Huxman et al. (1998) found that  $g_s$  is less responsive to elevated  $[\text{CO}_2]$  under drought conditions.

The boreal forest covers 11% of the earth's terrestrial surface (Bonan and Shugart 1989) and contains around 800 Pg carbon (Apps et al. 1993), indicating that the physiology of boreal forests may have large impacts on the global carbon cycle (Peng and Apps 1998). Because variation is so great in soil temperature in the boreal forest, ranging from near zero over permafrost to 35 °C on south-facing slopes and newly burned sites (Bonan and Shugart 1989), a better understanding of the interactions between the effects of soil temperature and  $[\text{CO}_2]$  on trees may provide critical insights on the potential responses of the boreal forests to climate change associated with increasing atmospheric  $[\text{CO}_2]$ . The objective of this study was to investigate the interactive effects of soil temperature and elevated  $[\text{CO}_2]$  on the photosynthetic functions of a broadleaf (white birch, *Betula papyrifera* Marsh.) and a coniferous boreal tree species (jack pine, *Pinus banksiana* Lamb.).

## Materials and methods

### Plant materials

One-year-old seedlings of jack pine were obtained from Hills Tree Nursery in Thunder Bay, Ontario. White birch seedlings were grown from seed in greenhouses at Lakehead University, Thunder Bay, ON, Canada. In March 2003, all seedlings were transplanted to large containers (13.5-cm tall, 11-cm top diameter) filled with a 1:1 (v/v) mixture of peat moss and vermiculite.

### Experiment design

The experiment was conducted in greenhouses at Lakehead University and comprised two  $\text{CO}_2$  treatments (ambient (360 ppm) and twice ambient (elevated) (720 ppm)) and three soil temperature treatments (7, 17 and 27 °C at the beginning of the experiment in March, increased to 10, 20 and 30 °C in late June, respectively). The experimental design was a split-plot where  $\text{CO}_2$  treatments were the main plots and soil temperature treatments were the sub-plots. Soil temperature was regulated by circulating temperature-controlled water between the containers inside each thermostatically controlled box in which eight rows of 14 containers were installed. The boxes were insulated so that the soil temperature was independent of the air temperature in the greenhouse (a more detailed description of the system is given by Cheng et al. 2000). The  $\text{CO}_2$  treatments were applied simultaneously in separate greenhouses which were identical in design and layout. The elevated  $[\text{CO}_2]$  was achieved using Argus  $\text{CO}_2$  generators (Argus, Vancouver, BC, Canada). For all of the treatments, day/night temperatures were set at 25–26/16–17 °C from March to May and 28–30/18–20 °C starting in June, with a 16-h photoperiod (on cloudy days, and early in the mornings and late in the evenings, natural light was supplemented with artificial light from high-pressure sodium lamps). Environmental conditions were monitored and controlled with an Argus control system (Argus).

The growing medium was maintained at around 50% of saturated water content as measured with an HH2 Moisture Meter (Delta-T Devices, Cambridge, U.K.). Seedlings were watered up to twice a day during the summer to maintain a high soil water content. Seedlings were fertilized weekly with a solution of 100 ppm N, 35 ppm P and 66 ppm K. The temperature of the irrigation water and fertilizer solution was adjusted to match the soil temperature of the particular treatment.

### Simultaneous measurements of in situ gas exchange and chlorophyll fluorescence

Three seedlings per species were selected randomly from each treatment combination for gas exchange measurements. Measurements were made with a PP-Systems CIRAS-1 open gas exchange system (Hitchin, Hertfordshire, U.K.) between 0900 and 1130 h on the 5th mature leaf counting from the apex for white birch and on the fully developed needles near the shoot tip of jack pine, with the seedlings in their original positions and maintained under the respective treatment conditions. Environmental conditions in both the broadleaf and coniferous Parkinson leaf chambers were controlled automatically. Environmental conditions for measuring the  $A-C_i$  (photosynthetic rate–intercellular  $[\text{CO}_2]$ ) curves were 26 °C air temperature, 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation (PAR) and 50% RH. The in vivo maximal carboxylation rate ( $V_{\text{cmax}}$ ), PAR-saturated electron transport rate ( $J_{\text{max}}$ ), triose phosphate utilization (TPU) and other relevant parameters were calculated from the  $A-C_i$  curve according to Farquhar et al. (1980), von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991) and Harley et al. (1992). Wullschlegel (1993) suggests that TPU should be considered when there is obvious saturation in the  $A-C_i$  curve, as was the case in our study. The  $A-C_i$  curves were fit using Photosyn Assistant software (Dundee Scientific, Scotland, U.K.) to estimate  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and TPU. We used the parameters for the kinetics of Rubisco, i.e.,  $K_c$ ,  $K_o$  and  $\tau$ , and their temperature dependencies reported by Harley et al. (1992) and Wullschlegel (1993). Gas exchange parameters were expressed on a projected area basis. Projected leaf area for jack pine was measured with the Regent WinSeedle system (Regent Instruments, Québec City, QC, Canada).

Chlorophyll fluorescence was measured with an FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments, Norfolk, U.K.). The probe and control software for the two systems was integrated in the leaf chambers of the gas exchange system to allow simultaneous measurements of gas exchange and chlorophyll fluorescence. The following variables were obtained: fluorescence intensity at any time,  $F$ ; minimal fluorescence after dark adaptation,  $F_0$ ; maximal fluorescence after dark adaptation,  $F_m$ ; minimal fluorescence in light,  $F_0'$ ; maximal fluorescence in light,  $F_m'$ ; variable fluorescence after dark adaptation,  $F_v = F_m - F_0$ ; variable fluorescence in light,  $F_v' = F_m' - F_0'$ ; maximal photochemical efficiency of PSII (dark adapted leaf),  $F_v/F_m$ ; and actual photochemical efficiency of PSII in light ( $(F_m' - F)/F_m'$  or  $\Delta F/F_m'$ ), which is the efficiency under the degree of reaction center closure (Bilger

and Björkman 1990). Measurements of  $F_m$  and  $F_m'$  were obtained by illuminating the foliage with a pulse of strong light (around 14,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 800 ms. We measured  $F_v/F_m$  at predawn independently from the gas exchange measurement, whereas  $\Delta F/F_m'$  was measured simultaneously with each gas exchange measurement. Both gas exchange and chlorophyll fluorescence were measured 2.5 months after the initiation of the treatments and again at the end of the 4-month study.

Based on Farquhar et al. (1980) and Genty et al. (1989), the

simultaneously measured gas exchange and chlorophyll fluorescence are related as:

$$J_T = (F_m' - F)/F_m'(\text{PPF})(0.84)0.5 \quad (1)$$

$$J_T = 4V_c + 4V_o \quad (2)$$

$$P_n = V_c - 0.5V_o - R_d \quad (3)$$

where  $J_T$  is apparent rate of electron transport measured by

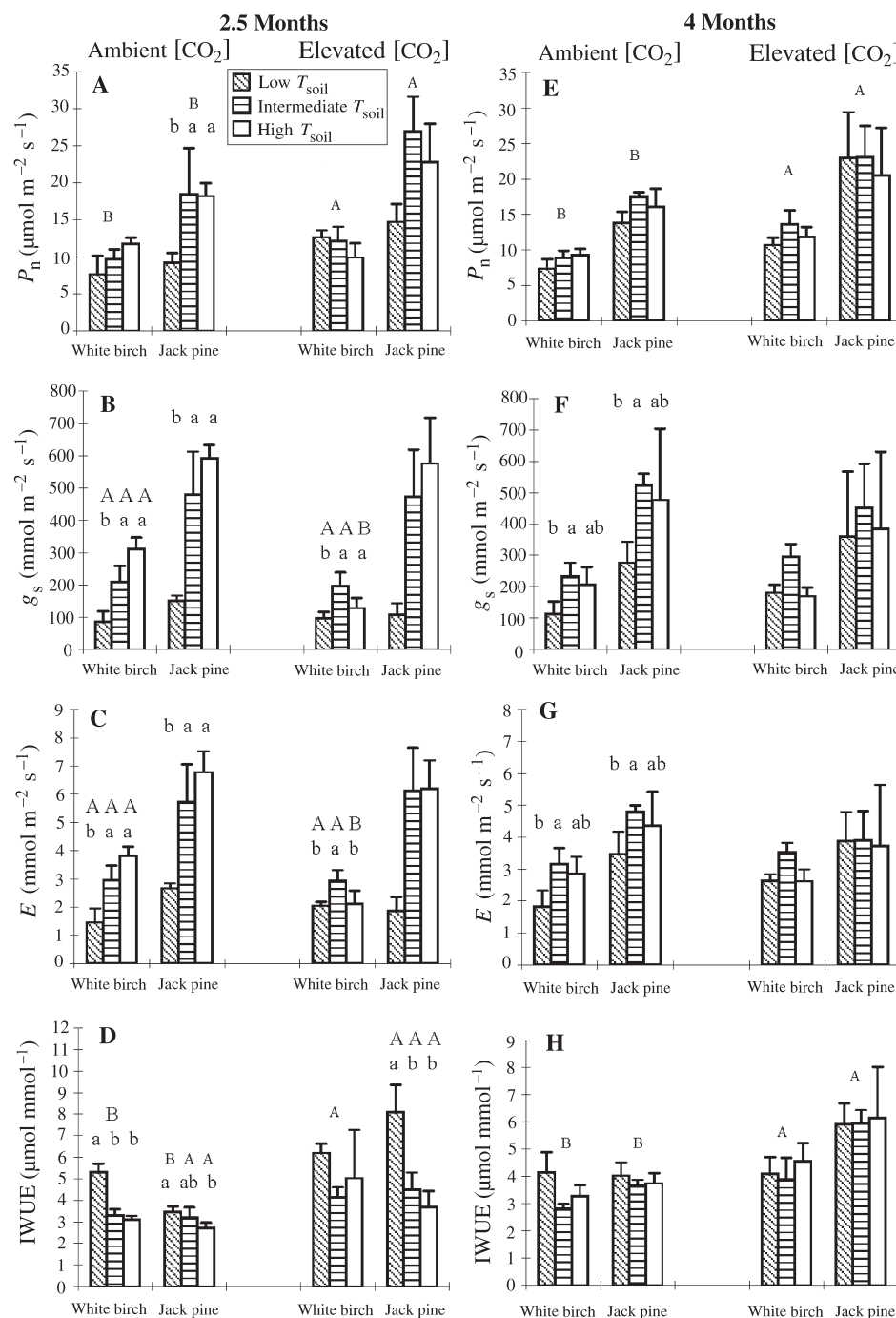


Figure 1. Effects of soil temperature and [CO<sub>2</sub>] on net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ) and instantaneous water-use efficiency (IWUE) (mean  $\pm$  SE,  $n = 3$ ). One-year-old jack pine and current-year white birch were subjected to ambient and twice ambient (elevated) [CO<sub>2</sub>] and three soil temperatures for 4 months. Measurements were taken 2.5 and 4 months after the start of the treatments. Lower-case letters and capital letters represent the effects of soil temperature and [CO<sub>2</sub>], respectively, based on Duncan's multiple range test. Means with different letters are significantly different from each other ( $P < 0.05$ ). For clarity, when there were no significant ( $P > 0.05$ ) interactions between soil temperature and [CO<sub>2</sub>] for a given parameter, the lower-case letters are shown only on the side of the ambient [CO<sub>2</sub>] treatment, but represent the soil temperature effects on trees grown in both ambient and elevated [CO<sub>2</sub>], and one capital letter (A or B) is used to represent the [CO<sub>2</sub>] effects on trees grown in the three soil temperatures. When the interaction between soil temperature and [CO<sub>2</sub>] was significant ( $P < 0.05$ ), the soil temperature and [CO<sub>2</sub>] effects are labeled separately for each treatment combination, and the absence of labels indicates no significant effects ( $P > 0.05$ ).

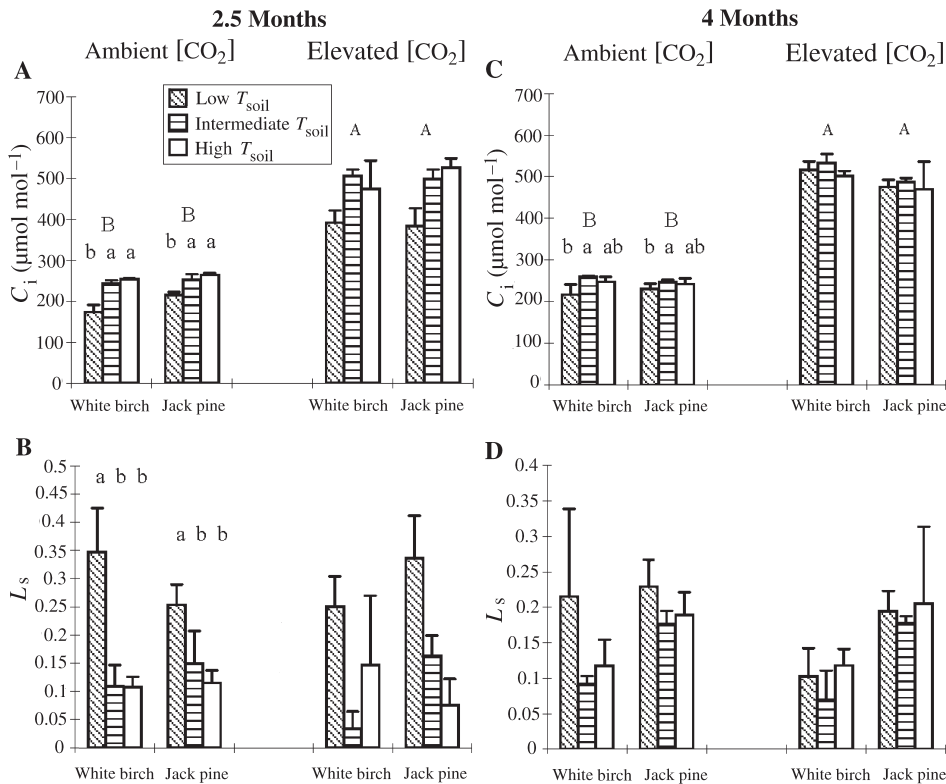


Figure 2. Effects of soil temperature and [CO<sub>2</sub>] on intercellular [CO<sub>2</sub>] (C<sub>i</sub>) and stomatal limitation to photosynthesis (L<sub>s</sub>) (mean ± SE, n = 3). We calculated L<sub>s</sub> as described by Berry and Downton (1982). One-year-old jack pine and current-year white birch were subjected to ambient and twice ambient (elevated) [CO<sub>2</sub>] and three soil temperatures for 4 months. Measurements were taken 2.5 and 4 months after the start of the treatments. For the interpretation of the letters indicating the results of the statistical analysis, see caption of Figure 1.

chlorophyll fluorescence and PPF is the photosynthetic photon flux in the leaf chamber, V<sub>c</sub> and V<sub>o</sub> are the rates of carboxylation and oxygenation of Rubisco, respectively (calculated from the A–C<sub>i</sub> curve), P<sub>n</sub> is the rate of net photosynthesis, and R<sub>d</sub> is daytime dark respiration.

The partitioning of total electron flow between carboxylation (J<sub>c</sub>) and oxygenation (J<sub>o</sub>) was determined as (Epron et al. 1995):

$$J_c = 1/3(J_T + 8(P_n + R_d)) \quad (4)$$

$$J_o = 2/3(J_T - 4(P_n + R_d)) \quad (5)$$

#### Foliar nitrogen

The total foliar nitrogen concentration was determined by Kjeldahl analysis at the end of the experiment. Measurements were made on the seedlings used for gas exchange measurements.

#### Statistical analysis

All data were examined graphically for the normality of distribution by probability plots and the homogeneity of variance by scatter plots before the analysis of variance (ANOVA) was carried out. Those data that did not follow a normal distribution were log-transformed and the transformed data satisfied the normal distribution assumption for ANOVA. The effects of species, soil temperature, CO<sub>2</sub> and their interactions were tested using the ANOVA procedure of the SAS statistical package (1996, SAS Institute, Cary, NC). When the ANOVA

showed a significant soil temperature effect, further comparisons among the three soil temperatures were conducted with Duncan's Multiple Range Test. When significant interactions between species and soil temperature or CO<sub>2</sub> were detected, further separate ANOVAs were conducted.

## Results

### Gas exchange

Low T<sub>soil</sub> significantly (P < 0.001) decreased P<sub>n</sub> in jack pine 2.5 months after initiation of the treatment (Figure 1A), but the effect became statistically insignificant by the end of the 4-month study (Figure 1E). There was no significant difference in P<sub>n</sub> between the intermediate and high T<sub>soil</sub> treatments. Elevated [CO<sub>2</sub>] significantly (P < 0.001) increased P<sub>n</sub> in both species at both measurement times.

Low T<sub>soil</sub> significantly reduced g<sub>s</sub> in both species after 2.5 months (P < 0.001 for jack pine, P < 0.01 for birch at ambient [CO<sub>2</sub>], P < 0.05 for birch at elevated [CO<sub>2</sub>]) and 4 months of treatment (P < 0.001 for both species), whereas there was no significant difference in g<sub>s</sub> between the intermediate and high T<sub>soil</sub> treatments (P > 0.05) (Figures 1B and 1F). Elevated [CO<sub>2</sub>] reduced g<sub>s</sub> in white birch in the high T<sub>soil</sub> treatment after 2.5 months of treatment (P < 0.01), but not in the other T<sub>soil</sub> treatments (P > 0.05; Figure 1B). Elevated [CO<sub>2</sub>] did not affect g<sub>s</sub> in jack pine (P > 0.05).

The responses of E to the T<sub>soil</sub> treatments and elevated [CO<sub>2</sub>] were similar to those of g<sub>s</sub>. Low T<sub>soil</sub> reduced E in both species at both 2.5 and 4 months after treatment (P < 0.01), and high

$T_{\text{soil}}$  reduced  $E$  of white birch in elevated [CO<sub>2</sub>] ( $P < 0.01$ ; Figures 1C and 1G). Elevated [CO<sub>2</sub>] decreased  $E$  of white birch at high  $T_{\text{soil}}$  ( $P < 0.05$ ).

In both species, IWUE was highest at low  $T_{\text{soil}}$  after 2.5 months of treatment ( $P < 0.05$ ), whereas there were no significant differences in IWUE between the intermediate and high  $T_{\text{soil}}$  treatments ( $P > 0.05$ ; Figure 1D). The low  $T_{\text{soil}}$  effect became nonsignificant after 4 months of treatment ( $P > 0.05$ ; Figure 1H).

Elevated [CO<sub>2</sub>] significantly ( $P < 0.05$ ) enhanced IWUE in white birch in all three  $T_{\text{soil}}$  treatments and IWUE in jack pine at low  $T_{\text{soil}}$  after 2.5 months of treatment (Figure 1D). After 4 months of treatment, enhancement of IWUE by elevated [CO<sub>2</sub>] was significant in all  $T_{\text{soil}}$  treatments and in both species ( $P < 0.05$  for white birch,  $P < 0.001$  for jack pine; Figure 1H).

Low  $T_{\text{soil}}$  significantly ( $P < 0.001$ ) reduced intercellular [CO<sub>2</sub>] ( $C_i$ ) in both species but there was no significant difference in  $C_i$  between the intermediate and high  $T_{\text{soil}}$  treatments at 2.5 months (Figure 2A). After 4 months of treatment,  $C_i$  was still significantly lower in the low  $T_{\text{soil}}$  treatment than in the in-

termediate  $T_{\text{soil}}$  treatment but the difference was nonsignificant for high  $T_{\text{soil}}$  (Figure 2C).

Low  $T_{\text{soil}}$  significantly ( $P < 0.001$ ) increased  $L_s$  in both species after 2.5 months of treatment (Figure 2B), but the effect became nonsignificant ( $P > 0.05$ ) after 4 months (Figure 2D).

#### *In vivo biochemical activities of Rubisco*

After 2.5 months of  $T_{\text{soil}}$  treatment,  $V_{\text{cmax}}$  of jack pine was significantly ( $P < 0.01$ ) lower at low  $T_{\text{soil}}$  than at intermediate  $T_{\text{soil}}$ , whereas there was no significant difference between the low and high or between the intermediate and high  $T_{\text{soil}}$  treatments, and there was also no significant  $T_{\text{soil}}$  effect on  $V_{\text{cmax}}$  of white birch after 2.5 months of treatment ( $P > 0.05$ ; Figure 3A). All of the low  $T_{\text{soil}}$  effects on  $V_{\text{cmax}}$  became nonsignificant after 4 months of treatment ( $P > 0.05$ ). Elevated [CO<sub>2</sub>] significantly ( $P < 0.05$ ) decreased  $V_{\text{cmax}}$  in both species after 4 months of treatment (Figure 3D).

After 2.5 months of treatment,  $J_{\text{max}}$  of jack pine was significantly ( $P < 0.05$ ) lower at low  $T_{\text{soil}}$  than at intermediate  $T_{\text{soil}}$ , whereas there was no significant difference between low  $T_{\text{soil}}$  and high  $T_{\text{soil}}$  or between intermediate  $T_{\text{soil}}$  and high  $T_{\text{soil}}$ , and

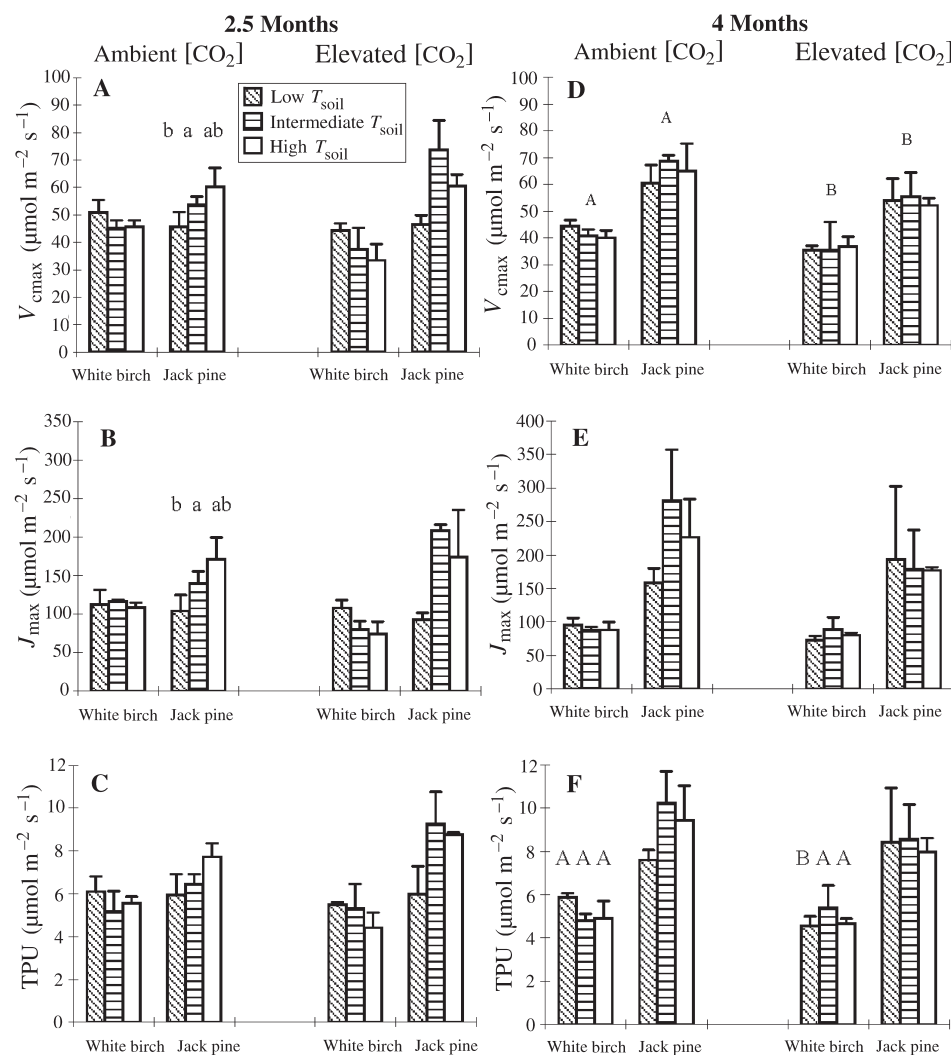


Figure 3. Effects of soil temperature and [CO<sub>2</sub>] on maximal carboxylation rate ( $V_{\text{cmax}}$ ), PAR-saturated electron transport rate ( $J_{\text{max}}$ ) and triose phosphate utilization (TPU) (mean  $\pm$  SE,  $n = 3$ ).

One-year-old jack pine and current-year white birch were subjected to ambient and twice ambient (elevated) [CO<sub>2</sub>] and three soil temperatures for 4 months. Measurements were taken 2.5 and 4 months after the start of the treatments. For the interpretation of the letters indicating the results of the statistical analysis, see caption of Figure 1.

there was also no significant  $T_{\text{soil}}$  effect on  $J_{\text{max}}$  of white birch ( $P > 0.05$ ; Figure 3B). The low  $T_{\text{soil}}$  effects on  $J_{\text{max}}$  became nonsignificant after 4 months of treatment ( $P > 0.05$ ). Elevated  $[\text{CO}_2]$  had no significant effect ( $P > 0.05$ ) on  $J_{\text{max}}$  in either species ( $P > 0.05$ ; Figures 3B and 3E). Elevated  $[\text{CO}_2]$  decreased TPU of white birch at low  $T_{\text{soil}}$  after 4 months of treatment ( $P < 0.05$ ; Figure 3F).

#### Photochemical efficiency of PSII

Neither  $T_{\text{soil}}$  nor elevated  $[\text{CO}_2]$  significantly affected  $F_v/F_m$  in either species ( $P > 0.05$ , data not presented). However,  $\Delta F/F_m'$  was significantly lower in the low  $T_{\text{soil}}$  treatment than in the other  $T_{\text{soil}}$  treatments in white birch, whereas it was significantly higher at low  $T_{\text{soil}}$  than at high or intermediate  $T_{\text{soil}}$  in jack pine after 4 months of treatment ( $P < 0.01$ ; Figure 4D). Elevated  $[\text{CO}_2]$  significantly ( $P < 0.05$ ) decreased  $\Delta F/F_m'$  in white birch after 2.5 months of treatment (Figure 3A), but the effect became nonsignificant after 4 months of treatment (Figure 4D).

#### Photosynthetic electron flow to carboxylation ( $J_c$ ) and oxygenation ( $J_o$ )

Low  $T_{\text{soil}}$  significantly reduced  $J_c$  in jack pine after 2.5 months of treatment ( $P < 0.01$ ), whereas there was no significant difference in  $J_c$  between the intermediate and high  $T_{\text{soil}}$  treatments ( $P > 0.05$ ; Figure 4B), and the low  $T_{\text{soil}}$  effect became nonsignificant after 4 months of treatment ( $P > 0.05$ ; Figure 4E). Low  $T_{\text{soil}}$  significantly decreased  $J_c$  of white birch after 4 months of treatment ( $P < 0.001$ ; Figure 4E). Elevated  $[\text{CO}_2]$  significantly increased  $J_c$  of jack pine at both measurement times ( $P < 0.05$  and  $P < 0.001$ , respectively) and that of white birch after 4 months of treatment ( $P < 0.001$ ; Figures 4B and 4E).

Low  $T_{\text{soil}}$  significantly increased  $J_o/J_T$  of jack pine at both measurement times ( $P < 0.05$ ), whereas the effect of low  $T_{\text{soil}}$  on white birch was significant only at 2.5 months of measurement ( $P < 0.001$ ; Figures 4C and 4F). Elevated  $[\text{CO}_2]$  significantly suppressed  $J_o/J_T$  in both species at both measurement times ( $P < 0.05$ ; Figures 4C and 4F).

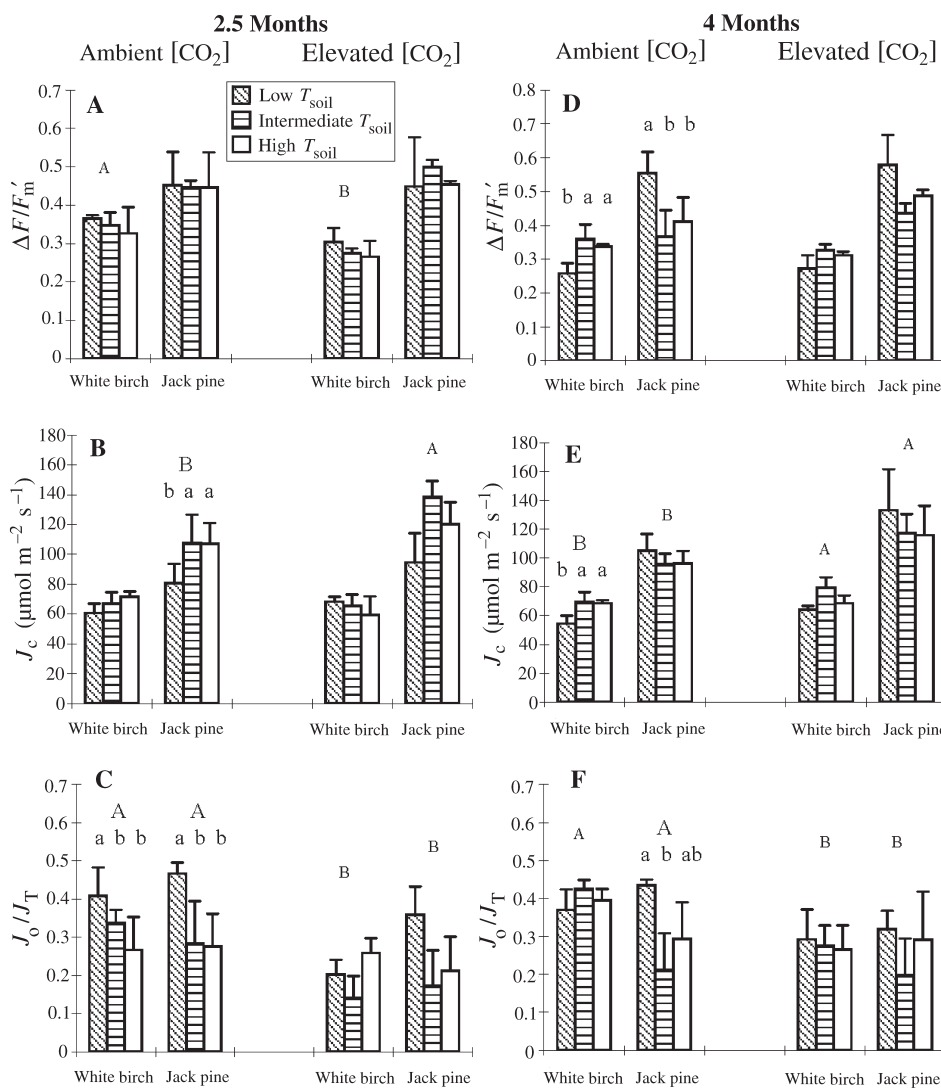


Figure 4. Effects of soil temperature and  $[\text{CO}_2]$  on actual photochemical efficiency of PSII ( $\Delta F/F_m'$ ), total electron flow between carboxylation ( $J_c$ ) and total photosynthetic linear electron transport rate through PSII ( $J_o/J_T$ ) (mean  $\pm$  SE,  $n = 3$ ). One-year-old jack pine and current-year white birch were subjected to ambient and twice ambient (elevated)  $[\text{CO}_2]$  and three soil temperatures for 4 months. Measurements were taken 2.5 and 4 months after the start of the treatments. For the interpretation of the letters indicating the results of the statistical analysis, see caption of Figure 1.

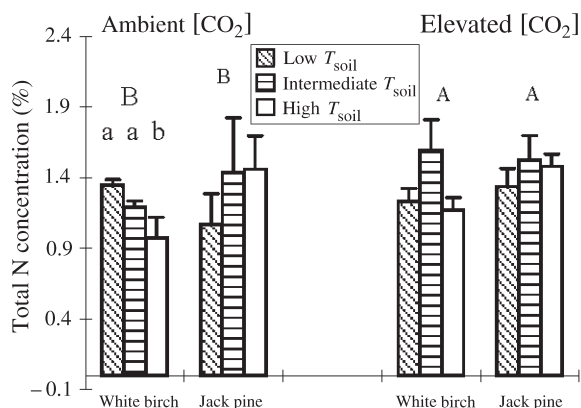


Figure 5. Effects of soil temperature and [CO<sub>2</sub>] on total foliar N concentration (mean  $\pm$  SE,  $n = 3$ ). One-year-old jack pine and current-year white birch were subjected to ambient and twice ambient (elevated) [CO<sub>2</sub>] and three soil temperatures for 4 months. Measurements were taken 2.5 and 4 months after the start of the treatments. For the interpretation of the letters indicating the results of the statistical analysis, see caption of Figure 1.

#### Foliar nitrogen concentration

High  $T_{\text{soil}}$  resulted in significantly reduced foliar N concentration in white birch ( $P < 0.01$ ; Figure 5). Elevated [CO<sub>2</sub>] significantly increased leaf N concentration in both species ( $P < 0.05$ ; Figure 5).

#### Discussion

Both the reduction in mesophyll activities and the decline in stomatal conductance in response to the  $T_{\text{soil}}$  treatments contributed to the decline in photosynthesis in jack pine after 2.5 months of treatment, but the decline in  $g_s$  was the primary factor. Low  $T_{\text{soil}}$  also reduced  $P_n$ ,  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and  $J_c$  of jack pine, whereas it had no significant effect on these parameters in white birch, although it decreased  $g_s$  and  $E$  in both species. These results suggest that the reduction in  $P_n$  in jack pine was primarily caused by low  $T_{\text{soil}}$  limiting mesophyll activities, whereas the concurrent increases in stomatal limitation to photosynthesis ( $L_s$ ) and IWUE and decreases in  $C_i$  and  $P_n$  suggest that the decrease in  $g_s$  was the primary factor (Farquhar and Sharkey 1982). The reduction in  $g_s$  may be related to non-hydraulic signals that roots sense at low  $T_{\text{soil}}$  (Day et al. 1991) and to water stress resulting from low root permeability and high water viscosity at low  $T_{\text{soil}}$  (Kaufmann 1975).

The proportion of total electron transport through PSII to photorespiration increased in response to the low  $T_{\text{soil}}$  treatment in both species after 2.5 months of treatment and the effect remained significant after 4 months of treatment in jack pine. Low  $T_{\text{soil}}$  may cause feedback inhibition and photo-inhibition of photosynthesis because of reduced sink strength (Lambers et al. 1998). Increases in electron flow to photorespiration may minimize or prevent damage to the PSII. The corresponding increase in  $\Delta F/F_m'$  in jack pine after 4 months of low  $T_{\text{soil}}$  treatment supports this theory, as does the observation that the absence of a significant increase in  $J_o/J_T$  in white

birch at low  $T_{\text{soil}}$  was accompanied by a decrease in  $\Delta F/F_m'$ .

The findings that  $T_{\text{soil}}$  did not significantly affect predawn  $\Delta F/F_m$  of dark-adapted leaves and all the  $\Delta F/F_m$  values were within the normal range for non-stressed plants suggest that the low  $T_{\text{soil}}$  treatment did not cause permanent damage to PSII in the study species. Our results are consistent with data obtained for *Populus tremuloides* Michx. (Landhäusser and Liefers 1998, Wan et al. 1999) and Scots pine (Domisch et al. 2001).

Elevated [CO<sub>2</sub>] significantly decreased the allocation of electron transport to photorespiration ( $J_o/J_T$ ) in both species; i.e., it increased electron allocation to Rubisco carboxylation. This finding supports the theory that elevated [CO<sub>2</sub>] stimulates photosynthesis by promoting Rubisco carboxylation and suppressing photorespiration (Lambers et al. 1998). Elevated [CO<sub>2</sub>] stimulation of photosynthesis has been observed in this study and many other studies (Drake and Leadley 1991, von Caemmerer et al. 2001, Gunderson et al. 2002, Rogers and Ellsworth 2002, Naumburg et al. 2003).

Photosynthetic down-regulation occurred in both species after 4 months in elevated [CO<sub>2</sub>], as evidenced by decreases in  $V_{\text{cmax}}$ , which were unaffected by soil temperature. Idso and Kimball (1989), Arp (1991), Long and Drake (1991) and Teskey et al. (1995) suggest that photosynthetic down-regulation is primarily a phenomenon or artifact associated with studies on potted plants where root growth and thus sink demand for photosynthates are limited by the pot. However, our data do not support this conclusion. If pot limitation were involved, there should have been greater down-regulation of TPU (an indicator of photosynthate translocation from leaf to sinks) after 4 months of treatment than after 2.5 months of treatment because the pot should have become more limiting over time. Additionally, pot limitation and thus photosynthetic down-regulation should have occurred earlier at high  $T_{\text{soil}}$  because root growth rate increases with soil temperature (Peng and Dang 2003), but our data showed no such trend. Furthermore, if pot size were the limiting factor, the trees should have experienced more severe N shortage in elevated [CO<sub>2</sub>] than in ambient [CO<sub>2</sub>] because of the higher growth rate in elevated [CO<sub>2</sub>]. However, we found that elevated [CO<sub>2</sub>] had no effect on TPU except in white birch at low  $T_{\text{soil}}$  after 4 months of treatment, and the foliar N concentration was significantly higher in elevated [CO<sub>2</sub>] than ambient [CO<sub>2</sub>]. Additionally, several elevated [CO<sub>2</sub>] studies have shown that photosynthetic down-regulation can occur in the field (Li et al. 1999, Tissue et al. 1999, Jach and Ceulemans 2000, Laitinen et al. 2000).

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