

# Low soil temperature inhibits the effect of high nutrient supply on photosynthetic response to elevated carbon dioxide concentration in white birch seedlings

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**Summary** To investigate the interactive effects of soil temperature ( $T_{\text{soil}}$ ) and nutrient availability on the response of photosynthesis to elevated atmospheric carbon dioxide concentration ( $[\text{CO}_2]$ ), white birch (*Betula papyrifera* Marsh.) seedlings were exposed to ambient ( $360 \mu\text{mol mol}^{-1}$ ) or elevated ( $720 \mu\text{mol mol}^{-1}$ )  $[\text{CO}_2]$ , three  $T_{\text{soil}}$  (5, 15 and 25 °C initially, increased to 7, 17 and 27 °C, respectively, 1 month later) and three nutrient regimes (4/1.8/3.3, 80/35/66 and 160/70/132  $\text{mg l}^{-1}$  N/P/K) for 3 months in environment-controlled greenhouses. Elevated  $[\text{CO}_2]$  increased net photosynthetic rate ( $A_n$ ), instantaneous water-use efficiency (IWUE), internal to ambient carbon dioxide concentration ratio ( $C_i/C_a$ ), triose phosphate utilization (TPU) and photosynthetic linear electron transport to carboxylation ( $J_c$ ), and it decreased actual photochemical efficiency of photosystem II ( $\Delta F/F_m'$ ), the fraction of total linear electron transport partitioned to oxygenation ( $J_o/J_T$ ) and leaf N concentration. The low  $T_{\text{soil}}$  suppressed  $A_n$ , transpiration rate ( $E$ ), TPU,  $\Delta F/F_m'$  and  $J_c$ , but it increased  $J_o/J_T$ . The low nutrient treatment reduced  $A_n$ , IWUE, maximum carboxylation rate of Rubisco, light-saturated electron transport rate, TPU,  $\Delta F/F_m'$ ,  $J_c$  and leaf N concentration, but increased  $C_i/C_a$ . There were two-factor interactions for  $C_i/C_a$ , TPU and leaf N concentration, and a significant effect of  $\text{CO}_2 \times T_{\text{soil}} \times$  nutrient regime on  $A_n$ , IWUE and  $J_c$ . The stimulations of  $A_n$  and IWUE by elevated  $[\text{CO}_2]$  were limited to seedlings grown under the intermediate and high nutrient regimes at the intermediate and high  $T_{\text{soil}}$ . For  $J_c$ , the  $[\text{CO}_2]$  effect was significant only at intermediate  $T_{\text{soil}}$  + high nutrient availability. No significant  $[\text{CO}_2]$  effects were observed under the low  $T_{\text{soil}}$  at any nutrient level. Our results support this study's hypothesis that low  $T_{\text{soil}}$  would reduce the positive effect of high nutrient supply on the response of  $A_n$  to elevated  $[\text{CO}_2]$ .

**Keywords:** *Betula papyrifera* Marsh., boreal forest,  $\text{CO}_2$  enrichment,  $\text{CO}_2$ - $T_{\text{soil}}$ -nutrient interaction, gas exchange, global environmental change.

## Introduction

The photosynthetic and growth responses of  $\text{C}_3$  plants to elevated carbon dioxide concentration ( $[\text{CO}_2]$ ) show considerable diversity, ranging from highly positive to neutral and, in rare cases, even negative (Poorter 1993, Gunderson and Wullschleger 1994, Miglietta et al. 1996, Zhang and Dang 2007). Such variability in response complicates the prediction of ecosystem changes as  $\text{CO}_2$  continues to accumulate in the earth's atmosphere. Plant responses to elevated  $[\text{CO}_2]$  are modified by growing conditions (Miglietta et al. 1996, Midgley et al. 1999, Olszyk et al. 2003, Zhang and Dang 2006, Zhang et al. 2006, Cao et al. 2007, Zhang and Dang 2007). For instance, elevated  $[\text{CO}_2]$  increases photosynthesis (Davey et al. 1999, Eguchi et al. 2004) and growth (Baxter et al. 1997, Oren et al. 2001) in nutrient-rich but not in nutrient-poor soils. Other environmental factors that are known to influence the responses of  $\text{C}_3$  plants to elevated  $[\text{CO}_2]$  include soil moisture (Mishra et al. 1999, Robredo et al. 2007), light (Zebian and Reekie 1998, Marfo and Dang 2009) and air temperature (Allen et al. 1990, Pessarakli 2005). However, multiple factors often interact in natural ecosystems to affect plants, and the interactive effects may be of greater value than the main effects in predicting plant responses to elevated atmospheric  $[\text{CO}_2]$ .

Soil temperature ( $T_{\text{soil}}$ ) is an important environmental factor controlling the growth of northern forests (Bonan and Shugart 1989, Bonan 1992). There is great heterogeneity in  $T_{\text{soil}}$  among different sites within the boreal forest, ranging from near zero over permafrost to 35 °C on south-facing slopes and newly burnt sites (Bonan and Shugart 1989, Zasada et al. 1997). Low  $T_{\text{soil}}$  reduces root growth and nutrient uptake (Chapin 1974, Tachibana 1982, Pastor et al. 1987, Pritchard et al. 1990, Paré et al. 1993, Peng and Dang 2003). Plants growing in cold soils may experience feedback inhibition and photoinhibition of photosynthesis because of reduced sink strength (Bagnall et al. 1988, Lambers et al. 2008). Furthermore, low shoot water potentials associated with increased soil water viscosity and decreased root permeability

at low  $T_{\text{soil}}$  have been implicated in stomatal closure and stomatal limitation of carbon assimilation (Benzioni and Dunstone 1988, Dang and Cheng 2004). In spite of its negative effects on the physiology and overall growth of C<sub>3</sub> plants, low  $T_{\text{soil}}$  has been surprisingly neglected in most studies examining the impact of rising atmospheric [CO<sub>2</sub>] on boreal forest trees.

In this study, we examined the interactive effects of  $T_{\text{soil}}$  and nutrient availability on the response of net photosynthesis ( $A_n$ ) in white birch (*Betula papyrifera* Marsh.) to elevated [CO<sub>2</sub>]. White birch is a pioneer boreal tree species with a rapid rate of initial growth and a high nutrient demand (Burns and Honkala 1990, Zhang and Dang 2006). There are strong relationships between leaf nutrient concentrations and photosynthetic performance (Grassi et al. 2002, Bown et al. 2007). Nitrogen and phosphorus supply to leaves affect Rubisco activity (Fredeen et al. 1990, Jacob and Lawlor 1991, Warren and Adams 2004). Furthermore, Brooks (1986) has demonstrated significant reductions in the regeneration of the carboxylation substrate, RuBP, with declining leaf phosphorus levels. Plants that are provided with favorable nutrient conditions in cold soils may suffer from low nutrient stress because of the decrease in root uptake capacity (Setter and Greenway 1988). Thus, we hypothesized that low  $T_{\text{soil}}$  would reduce the positive effect of high nutrient supply on the response of  $A_n$  to elevated [CO<sub>2</sub>].

## Materials and methods

### Plant materials

Seeds of white birch were sown in germination trays in a greenhouse at Lakehead University. The growing medium was a 1:1 (v/v) mixture of peat moss/vermiculite. The greenhouse was maintained at 26/16 °C (day/night temperature), and the natural photoperiod was extended to 15 h by high-pressure sodium lamps. The growing medium was watered twice a day with normal tap water using a spray bottle. After 8 weeks, seedlings were selected for uniformity and transferred to plastic pots (13.5 cm tall and 11/9.5 cm top/bottom diameter) containing the same composition of growing medium described above. The pots were a built-in component of the  $T_{\text{soil}}$  control system that is described in the following section.

### Experimental design

The treatments consisted of two [CO<sub>2</sub>] (360 and 720 μmol mol<sup>-1</sup>), three  $T_{\text{soil}}$  (5, 15 and 25 °C initially, increased to 7, 17 and 27 °C, respectively, 1 month later) and three nutrient regimes (4/1.8/3.3, 80/35/66 and 160/70/132 mg l<sup>-1</sup> N/P/K). The CO<sub>2</sub> treatments correspond to approximately 'present' and 'year 2100' atmospheric CO<sub>2</sub> levels (Watson et al. 1990, Long et al. 2004, Zhou and Shangquan 2009). The  $T_{\text{soil}}$  treatments are in line with  $T_{\text{soil}}$  conditions at different sites within the ecological range of white birch in the boreal forest. The leaf nutrient concentrations for the intermediate and high

nutrient treatments are comparable to those in white birch trees that are naturally growing on nutrient-rich sites while the values for the low nutrient treatment are lower than the lowest levels in the field (Kopinga and van den Burg 1995, Zhang et al. 2006).

The experiment was a split-split plot design in which the [CO<sub>2</sub>] treatments were the main plots,  $T_{\text{soil}}$  were the sub-plots and nutrient regimes were the sub-sub-plots. Two separate environment-controlled greenhouses were maintained at 360 μmol mol<sup>-1</sup> and two at 720 μmol mol<sup>-1</sup>, representing two replications per CO<sub>2</sub> treatment. The elevated [CO<sub>2</sub>] was supplied by Argus CO<sub>2</sub> generators (Argus, Vancouver, BC, Canada). Three  $T_{\text{soil}}$  control boxes (one per  $T_{\text{soil}}$  treatment) were placed on separate benches in each greenhouse. The target  $T_{\text{soil}}$  was achieved by circulating temperature-controlled water between the pots fixed to the bottom of the  $T_{\text{soil}}$  control box. Each pot had a drainage hole drilled through the bottom of the box. For a detailed description of the  $T_{\text{soil}}$  control system, see Cheng et al. (2000). There were 10 randomly assigned seedlings in each of the three nutrient regimes within each  $T_{\text{soil}}$  control box. The nutrient treatments were applied once a week. Treatments started on 1 January 2008.

During the experiment, the four greenhouses were subjected to 26/16 °C (day/night air temperature) and a 16-h photoperiod (the natural light was supplemented with high-pressure sodium lamps on cloudy days, early mornings and late evenings). All the environmental conditions were monitored and controlled with an Argus environmental control system. The seedlings were watered regularly to keep the growing medium moist.

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

Two seedlings were randomly chosen from each greenhouse and  $T_{\text{soil}} \times$  nutrient treatment for gas exchange measurements after 3 months of treatments. The measurements were made on the fifth youngest fully developed leaf with a PP-Systems CIRAS-1 open gas exchange system (Hitchin, Hertfordshire, UK). The response of photosynthesis ( $A$ ) to intercellular [CO<sub>2</sub>] ( $C_i$ ) was measured over a range of eight external CO<sub>2</sub> partial pressures ( $C_a$ ) from ~50 to 1100 μmol mol<sup>-1</sup>. The environmental conditions in the leaf chamber were 26 °C air temperature, 800 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density and 50% relative humidity. The  $A/C_i$  curves were fitted using Photosyn Assistant software (Dundee Scientific, Scotland, UK) and analyzed with a biochemically based model (Farquhar et al. 1980, Harley et al. 1992) to determine the maximum carboxylation rate ( $V_{\text{cmax}}$ ), light-saturated electron transport rate ( $J_{\text{max}}$ ) and triose phosphate utilization (TPU) using the kinetic parameters of Wullschleger (1993).  $A_n$ , stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) were obtained from  $A/C_i$  curves where  $C_a$  equaled 360 μmol mol<sup>-1</sup> for ambient and 720 μmol mol<sup>-1</sup> for CO<sub>2</sub>-enriched leaves. All gas exchange parameters were calculated according to Farquhar et al. (1980).

Chlorophyll fluorescence was measured with a portable FMS-2 pulse-amplitude modulated fluorometer (Hansatech

Table 1. *P*-values of ANOVA for the effects of [CO<sub>2</sub>], soil temperature ( $T_{\text{soil}}$ ), nutrient regime (N) and their interactions on net photosynthesis ( $A_n$ ), transpiration rate ( $E$ ), instantaneous water-use efficiency (IWUE), internal to ambient CO<sub>2</sub> concentration ratio ( $C_i/C_a$ ), maximum carboxylation rate ( $V_{\text{cmax}}$ ), light-saturated electron transport rate ( $J_{\text{max}}$ ), triose phosphate utilization (TPU), potential photochemical efficiency of photosystem II ( $F_v/F_m$ ), actual photochemical efficiency of photosystem II ( $\Delta F/F_m'$ ), photosynthetic linear electron transport to carboxylation ( $J_c$ ), the fraction of total photosynthetic linear electron transport partitioned to oxygenation ( $J_o/J_T$ ) and mass-based leaf nitrogen concentration ( $[N]_{\text{mass}}$ ) of white birch. Seedlings were subjected to two [CO<sub>2</sub>] (360 and 720  $\mu\text{mol mol}^{-1}$ ), three  $T_{\text{soil}}$  (5, 15 and 25 °C initially, increased to 7, 17 and 27 °C, respectively, 1 month later) and three N (4/1.8/3.3, 80/35/66 and 160/70/132  $\text{mg l}^{-1}$  N/P/K) regimes for 3 months.

Source	CO <sub>2</sub>	$T_{\text{soil}}$	N	CO <sub>2</sub> × $T_{\text{soil}}$	CO <sub>2</sub> × N	$T_{\text{soil}}$ × N	CO <sub>2</sub> × $T_{\text{soil}}$ × N
$A_n$	0.0658	0.0238	0.0257	0.4273	0.0869	0.0104	0.0753
$E$	0.7098	0.0145	0.6550	0.3876	0.2281	0.5021	0.5103
IWUE	0.2397	0.2019	0.0941	0.2253	0.0949	0.2102	0.0444
$C_i/C_a$	0.1814	0.6069	0.1068	0.2882	0.0618	0.5597	0.1682
$V_{\text{cmax}}$	0.1530	0.1278	0.1019	0.5983	0.5048	0.8702	0.3579
$J_{\text{max}}$	0.5462	0.1433	0.0550	0.3875	0.2274	0.6504	0.2206
TPU	0.1772	0.0826	0.0234	0.0312	0.0957	0.0229	0.4930
$F_v/F_m$	0.3596	0.3510	0.1176	0.3085	0.1161	0.2868	0.8324
$\Delta F/F_m'$	0.0449	0.0458	0.0277	0.9045	0.3792	0.4518	0.1459
$J_c$	0.2487	0.0363	0.0046	0.7744	0.0399	0.1886	0.0567
$J_o/J_T$	0.0439	0.0525	0.1952	0.9502	0.8145	0.1697	0.6175
$[N]_{\text{mass}}$	0.0998	0.2614	0.0715	0.0015	0.5342	0.4548	0.6500

Instruments, Norfolk, UK). Maximum ( $F_m'$ ) and steady-state ( $F_s$ ) fluorescence of light-adapted leaves were measured simultaneously with each gas exchange measurement with the chlorophyll fluorescence probe integrated into the leaf chamber of the CIRAS-1, whereas maximum ( $F_m$ ) and minimum ( $F_o$ ) fluorescence yields of dark-adapted leaves were independently sampled from the gas exchange measurements after dark adapting the leaves for 1 h in leaf clips. These measured variables were used to determine actual ( $\Delta F/F_m' = (F_m' - F_s)/F_m'$ ) and potential ( $F_v/F_m = (F_m - F_o)/F_m$ ) photochemical efficiency of photosystem II (PSII).

The rate of total electron transport through PSII ( $J_T$ ) and the partitioning of electrons between carboxylation ( $J_c$ ) and oxygenation ( $J_o$ ) were calculated according to Farquhar et al. (1980), Genty (1989) and Epron et al. (1995).

#### Leaf nitrogen assay

Following the gas exchange and chlorophyll fluorescence measurements, leaves were harvested and dried to constant mass at 70 °C. Total leaf nitrogen (N) concentration was determined by the dry combustion method using a LECO CNS-2000 analyzer (LECO Corporation, St. Joseph, MI, USA).

#### Statistical analysis

Data were analyzed with Data Desk 6.01 (Data Description 1996). All the data were examined graphically for normality and homogeneity of variance using probability plots and scatter plots, respectively. The above tests showed that all the data satisfied the assumptions for analysis of variance (ANOVA). The effects of [CO<sub>2</sub>],  $T_{\text{soil}}$ , nutrient regime and their interactions were then tested using a three-factor, split-split plot ANOVA. Differences were considered marginally significant at  $P \leq 0.10$  and significant at  $P \leq 0.05$ . When the effect of an interaction or a treatment involving more than two levels was

significant ( $P \leq 0.10$ ) for a given parameter, Scheffe's *F* test for post hoc pair-wise comparisons was conducted.

## Results

### *In situ* gas exchange

There was a significant main effect of  $T_{\text{soil}}$  and nutrient supply on  $A_n$ . In addition, the effects of [CO<sub>2</sub>] and [CO<sub>2</sub>] ×  $T_{\text{soil}}$  × nutrient supply on this parameter were marginally significant (Table 1).  $A_n$  significantly increased from the low to the high nutrient regime but only at the intermediate and high, not at the low  $T_{\text{soil}}$  where no significant nutrient effects were observed (Figure 1A). Furthermore, there were no significant differences between the intermediate and high nutrient regimes at intermediate and high  $T_{\text{soil}}$  (Figure 1A). The low  $T_{\text{soil}}$  significantly reduced  $A_n$  only at the intermediate and high but not at the low nutrient regime (Figure 1A). The differences in  $A_n$  between the intermediate and high  $T_{\text{soil}}$  were not statistically significant (Figure 1A). The [CO<sub>2</sub>] elevation significantly increased  $A_n$  under the intermediate and high  $T_{\text{soil}}$  at the intermediate and high but not the low nutrient level (Figure 1A). However,  $A_n$  was unaffected by [CO<sub>2</sub>] at low  $T_{\text{soil}}$  (Figure 1A).

No significant effects of [CO<sub>2</sub>] or nutrient supply alone or in combination were observed on  $g_s$  (data not shown) and  $E$  (Table 1). In contrast, there was a significant main effect of  $T_{\text{soil}}$  on both parameters, but no  $T_{\text{soil}}$ -related interaction (Table 1).  $g_s$  and  $E$  differed among all the three  $T_{\text{soil}}$  treatments and the responses were lowest in the low and highest in the intermediate  $T_{\text{soil}}$  (Figure 1B).

Only nutrient regime, but not [CO<sub>2</sub>] or  $T_{\text{soil}}$ , had a significant main effect on IWUE (Table 1). However, the effect of [CO<sub>2</sub>] ×  $T_{\text{soil}}$  × nutrient regime was significant (Table 1). While IWUE was significantly higher in the high than low nutrient regime only at the low and intermediate  $T_{\text{soil}}$

in ambient [CO<sub>2</sub>], the nutrient effect in elevated [CO<sub>2</sub>] was significant only at the two higher but not at the low  $T_{\text{soil}}$  (Figure 1C). Furthermore, there were no significant differences between nutrient treatments at the high  $T_{\text{soil}}$  in ambient [CO<sub>2</sub>] (Figure 1C). The ranking of nutrient treatments for IWUE differed between [CO<sub>2</sub>] treatments: in ambient

[CO<sub>2</sub>], the intermediate nutrient regime was not significantly different from either the low or high nutrient levels at low  $T_{\text{soil}}$ , whereas there were no significant differences between the low and intermediate nutrient treatments at the intermediate  $T_{\text{soil}}$ ; in elevated [CO<sub>2</sub>], no significant differences were observed between the intermediate and high treatments at both the intermediate and high  $T_{\text{soil}}$  (Figure 1C). IWUE was not significantly affected by  $T_{\text{soil}}$  at any nutrient regime in ambient [CO<sub>2</sub>] (Figure 1C). In elevated [CO<sub>2</sub>], however, the low  $T_{\text{soil}}$  significantly suppressed IWUE only at the intermediate and high but not at the low nutrient regime where the effect of  $T_{\text{soil}}$  was insignificant (Figure 1C). In addition, no significant differences were detected between the intermediate and high  $T_{\text{soil}}$  in elevated [CO<sub>2</sub>] (Figure 1C). Elevated [CO<sub>2</sub>] significantly increased IWUE under the intermediate  $T_{\text{soil}}$  at the intermediate nutrient regime and under the high  $T_{\text{soil}}$  at the intermediate and high nutrient levels (Figure 1C). However, the effect of [CO<sub>2</sub>] on IWUE was generally insignificant under the low  $T_{\text{soil}}$  (Figure 1C).

There was no effect of  $T_{\text{soil}}$  alone or in combination on internal to ambient CO<sub>2</sub> concentration ratio ( $C_i/C_a$ ; Table 1). In contrast, the effect of [CO<sub>2</sub>] × nutrient regime was marginally significant (Table 1). Values of  $C_i/C_a$  were lowest in high nutrient regime + ambient [CO<sub>2</sub>] and highest in low nutrient regime + elevated [CO<sub>2</sub>]; however, the differences between the ambient and elevated [CO<sub>2</sub>] at the low nutrient regime were not statistically significant (Figure 1D).  $C_i/C_a$  increased from ambient to elevated [CO<sub>2</sub>] at the intermediate and high nutrient regimes (Figure 1D).

#### *In vivo Rubisco activity*

No significant individual or interactive effects of [CO<sub>2</sub>] or  $T_{\text{soil}}$  on  $V_{\text{cmax}}$  or  $J_{\text{max}}$  were found (Table 1). Nevertheless, the effect of nutrient was significant for  $J_{\text{max}}$  and marginally significant for  $V_{\text{cmax}}$  (Table 1).  $V_{\text{cmax}}$  and  $J_{\text{max}}$  were significantly higher at high than low nutrient regime, whereas there were no differences between the low and intermediate or the intermediate and high nutrient treatments (Figure 2A and B).

There was a significant effect of nutrient regime, and a marginal effect of  $T_{\text{soil}}$  and [CO<sub>2</sub>] × nutrient regime, on TPU (Table 1). TPU generally increased from the low to the

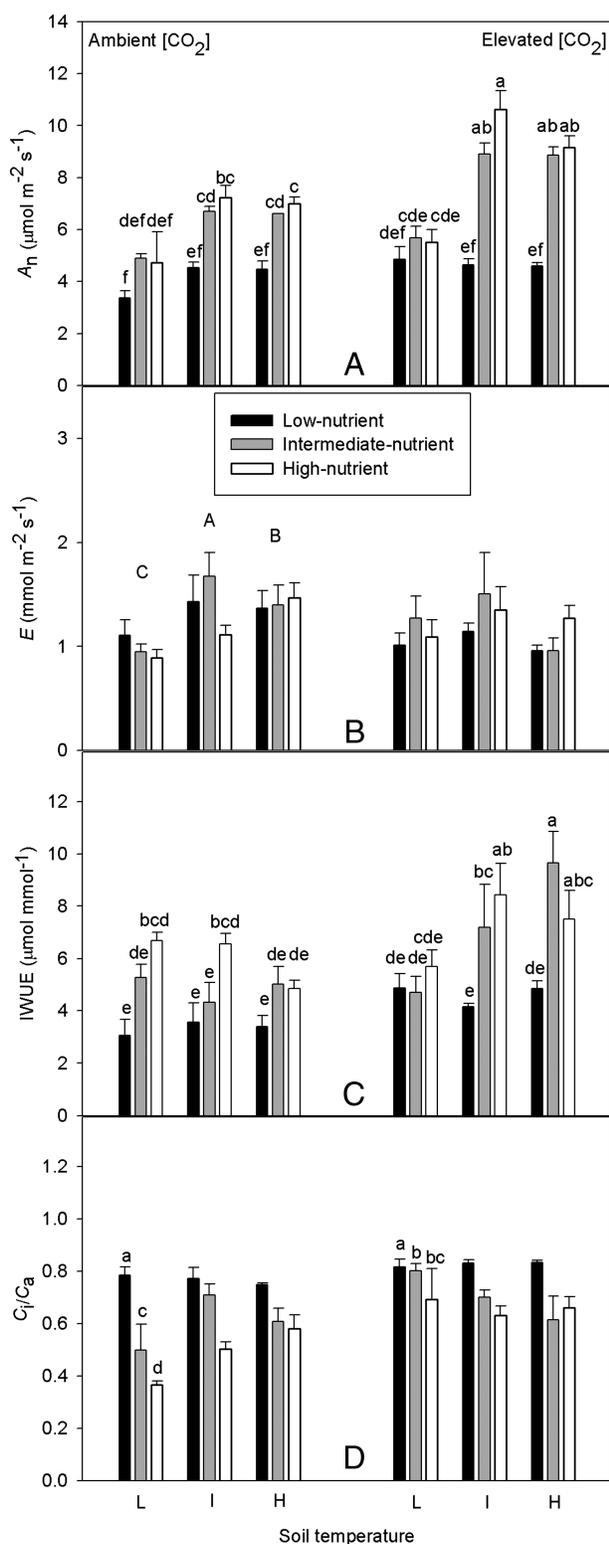


Figure 1. Effects of [CO<sub>2</sub>], soil temperature ( $T_{\text{soil}}$ ) and nutrient regime (N) on net photosynthesis ( $A_n$ ), transpiration rate ( $E$ ), instantaneous water-use efficiency (IWUE) and internal to ambient CO<sub>2</sub> concentration ratio ( $C_i/C_a$ ; mean  $\pm$  SE,  $n = 2$ ) in white birch. Seedlings were grown under two [CO<sub>2</sub>] (360 and 720  $\mu\text{mol mol}^{-1}$ ), three  $T_{\text{soil}}$  (5, 15 and 25 °C initially, increased to 7, 17 and 27 °C, respectively, 1 month later) and three N (4/1.8/3.3, 80/35/66 and 160/70/132  $\text{mg l}^{-1}$  N/P/K) regimes for 3 months. The significance levels (\*\* $P \leq 0.01$ , \* $P \leq 0.05$ , + $P \leq 0.1$ ) are based on ANOVA. In Figure 1A and C, the lowercase letters indicate CO<sub>2</sub> ×  $T_{\text{soil}}$  × N interactions. In Figure 1D, the letters indicate CO<sub>2</sub> × N interaction. The uppercase letters indicate  $T_{\text{soil}}$  effect. Means with different letters are significantly different from each other, according to Scheffe's  $F$  test. Note: when there was no CO<sub>2</sub>-related interaction for a given parameter, only the bars on the side of the ambient [CO<sub>2</sub>] were labeled. L, I and H represent the low, intermediate and high  $T_{\text{soil}}$ , respectively.

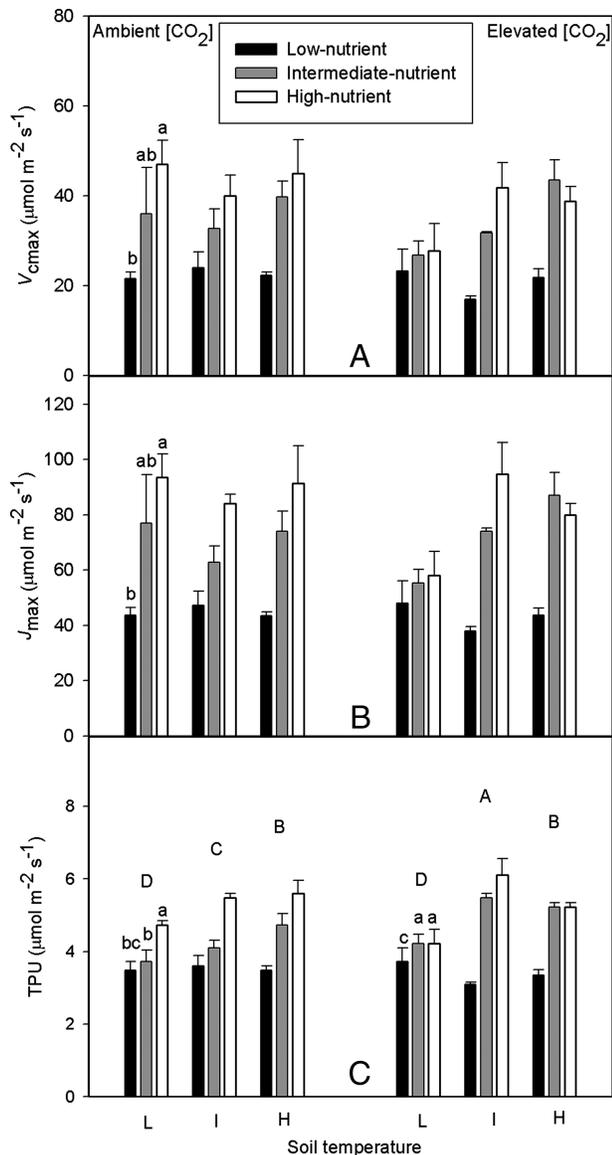


Figure 2. Effects of [CO<sub>2</sub>], soil temperature and nutrient regime on maximum carboxylation rate ( $V_{cmax}$ ), light-saturated electron transport rate ( $J_{max}$ ) and triose phosphate utilization (TPU; mean  $\pm$  SE,  $n = 2$ ). In Figure 2A and B, the letters indicate N effect. In Figure 2C, the lower- and uppercase letters indicate CO<sub>2</sub>  $\times$  N and CO<sub>2</sub>  $\times$  T<sub>soil</sub> interactions, respectively. See caption of Figure 1 for other explanations.

high nutrient regime in both [CO<sub>2</sub>] treatments (Figure 2C). However, the differences between the low and intermediate nutrient regimes in ambient [CO<sub>2</sub>] were not statistically significant (Figure 2C). Furthermore, no significant differences were observed between the intermediate and high nutrient regimes in elevated [CO<sub>2</sub>] (Figure 2C). The [CO<sub>2</sub>] elevation significantly increased TPU only at the intermediate but not at the low and high nutrient regimes (Figure 2C). Also, [CO<sub>2</sub>] and T<sub>soil</sub> had a significant interactive effect on TPU (Table 1). TPU was lowest in low T<sub>soil</sub> + elevated and highest in intermediate T<sub>soil</sub> + elevated [CO<sub>2</sub>], but the differences between low T<sub>soil</sub> + elevated [CO<sub>2</sub>] and low T<sub>soil</sub> + ambient [CO<sub>2</sub>] were

statistically insignificant (Figure 2C). TPU was significantly higher at the high than intermediate T<sub>soil</sub> in ambient [CO<sub>2</sub>] (Figure 2C). The [CO<sub>2</sub>] elevation significantly increased TPU only at the intermediate but not at the low and high T<sub>soil</sub> (Figure 2C).

#### Photochemical efficiency of PSII

No significant effects of [CO<sub>2</sub>], T<sub>soil</sub> or nutrient regime were observed on  $F_v/F_m$  (Table 1, Figure 3A). However,  $\Delta F/F_m'$  significantly responded to all three environmental factors, but not to their interactions (Table 1).  $\Delta F/F_m'$  declined with increased [CO<sub>2</sub>] (Figure 3B). Both low T<sub>soil</sub> and low nutrient regime significantly decreased  $\Delta F/F_m'$ , whereas there were no significant differences between the intermediate and high T<sub>soil</sub> or the intermediate and high nutrient regimes (Figure 3B).

$J_c$  was significantly affected by T<sub>soil</sub>, nutrient regime, [CO<sub>2</sub>]  $\times$  nutrient regime and [CO<sub>2</sub>]  $\times$  T<sub>soil</sub>  $\times$  nutrient regime (Table 1). However, the effect of the three-factor interaction was marginal. The low nutrient regime significantly reduced  $J_c$  at the intermediate and high but not at the low T<sub>soil</sub> where the effect of nutrient regime was insignificant (Figure 3C). However, there were no significant differences between the intermediate and high nutrient regimes at the intermediate and high T<sub>soil</sub> (Figure 3C). The low T<sub>soil</sub> significantly suppressed  $J_c$  only at the high nutrient regime in ambient [CO<sub>2</sub>] and at the intermediate and high nutrient regimes in elevated [CO<sub>2</sub>], but no significant differences were noted between the intermediate and high T<sub>soil</sub> in either ambient or elevated [CO<sub>2</sub>] (Figure 3C). The effect of T<sub>soil</sub> on  $J_c$  was not significant at the intermediate nutrient regime in ambient [CO<sub>2</sub>] and at the low nutrient regime in both ambient and elevated [CO<sub>2</sub>] (Figure 3C). The [CO<sub>2</sub>] elevation significantly increased  $J_c$  only in intermediate T<sub>soil</sub> + high nutrient regime (Figure 3C). No significant effect of [CO<sub>2</sub>] on  $J_c$  was detected in any other treatment.

There was no significant effect of nutrient alone or in combination on  $J_o/J_T$  (Table 1). In contrast, there was a significant main effect of both [CO<sub>2</sub>] and T<sub>soil</sub>:  $J_o/J_T$  significantly decreased from ambient to elevated [CO<sub>2</sub>] and low to high T<sub>soil</sub> (Table 1, Figure 3D). However, the differences in  $J_o/J_T$  between the intermediate T<sub>soil</sub> and either the low or high T<sub>soil</sub> were not statistically significant (Figure 3D).

#### Total leaf N concentration

There was a marginally significant main effect of [CO<sub>2</sub>] and nutrient regime and also a significant effect of [CO<sub>2</sub>]  $\times$  T<sub>soil</sub> on leaf N concentration (Table 1). Leaf N concentration was the highest in ambient [CO<sub>2</sub>] + low T<sub>soil</sub> and the lowest in elevated [CO<sub>2</sub>] + low T<sub>soil</sub> and elevated [CO<sub>2</sub>] + high T<sub>soil</sub> (Figure 4). There were no significant differences between the intermediate and high T<sub>soil</sub> in ambient [CO<sub>2</sub>] (Figure 4). Elevated [CO<sub>2</sub>] significantly decreased leaf N concentration at all T<sub>soil</sub> (Figure 4). Leaf N concentration was significantly higher at the high than low nutrient regime, whereas the dif-

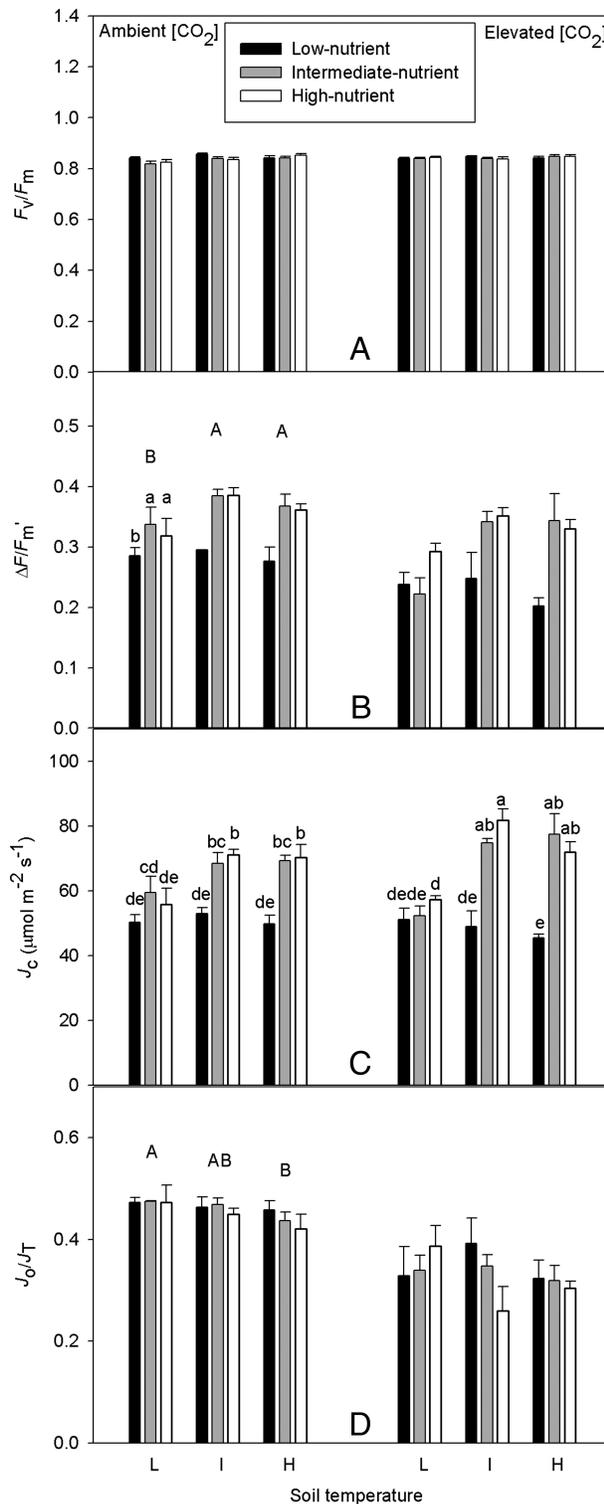


Figure 3. Effects of [CO<sub>2</sub>], soil temperature and nutrient regime on potential photochemical efficiency of photosystem II ( $F_v/F_m$ ), actual photochemical efficiency of photosystem II ( $\Delta F/F_m'$ ), photosynthetic linear electron transport to carboxylation ( $J_c$ ) and the fraction of total photosynthetic linear electron transport partitioned to oxygenation ( $J_o/J_T$ ; mean  $\pm$  SE,  $n = 2$ ). The absence of labels indicates no significant effects ( $P > 0.1$ ). See captions of Figures 1 and 2 for other explanations.

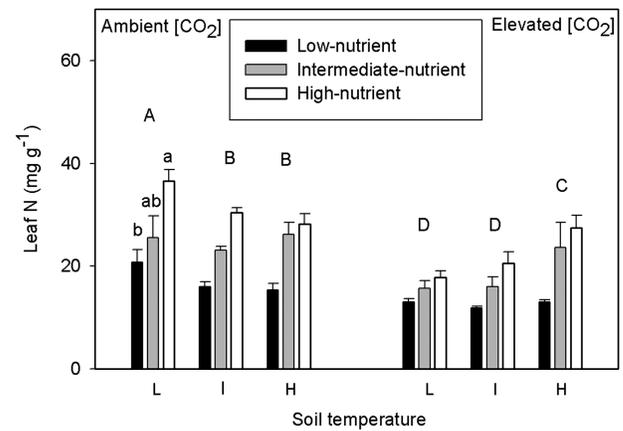


Figure 4. Effects of [CO<sub>2</sub>], soil temperature and nutrient regime on total leaf nitrogen concentration (mean  $\pm$  SE,  $n = 2$ ). See captions of Figures 1 and 2 for other explanations.

ferences between the intermediate nutrient treatment and either the low or high nutrient levels were not statistically significant (Figure 4).

## Discussion

Elevated [CO<sub>2</sub>] significantly increased  $A_n$  under the intermediate and high  $T_{\text{soil}}$  but only at the intermediate and high nutrient regimes, not at the low nutrient level. Similar responses of  $A_n$  to elevated [CO<sub>2</sub>] and nutrient availability have been obtained with seedlings of loblolly pine (*Pinus taeda* L., Tissue et al. 1993), perennial ryegrass (*Lolium perenne* L., Davey et al. 1999) and Japanese larch (*Larix kaempferi* Carr., Eguchi et al. 2004) grown under favorable  $T_{\text{soil}}$  conditions. In a previous study with white birch seedlings (Zhang and Dang 2006), the increase in  $A_n$  from ambient to elevated [CO<sub>2</sub>] at high nutrient regime was associated with an increase in  $V_{\text{cmax}}$ . The important role of  $V_{\text{cmax}}$  in the photosynthetic response of both coniferous and deciduous species to elevated [CO<sub>2</sub>] has been demonstrated (Tissue et al. 1997, Murray et al. 2000, Ainsworth et al. 2002). In the present study, however, elevated [CO<sub>2</sub>] did not increase  $V_{\text{cmax}}$  at any  $T_{\text{soil}}$  or nutrient regime, suggesting that the observed increases in  $A_n$  cannot be explained by higher  $V_{\text{cmax}}$ . The lack of positive response of  $V_{\text{cmax}}$  to elevated [CO<sub>2</sub>] could be possibly attributed to the decline in leaf N concentration under elevated [CO<sub>2</sub>] (Griffin and Seemann 1996, Midgley et al. 1999). Ellsworth et al. (2004) have reported strong correlations between the responses of leaf N concentration and  $V_{\text{cmax}}$  to [CO<sub>2</sub>] elevation. For 4-month-old potted white birch seedlings, Zhang and Dang (2006) have demonstrated that supplying optimal nutrient levels at least twice a week is crucial for maintaining higher leaf N concentrations and, consequently,  $V_{\text{cmax}}$  in elevated than ambient [CO<sub>2</sub>]. The high [CO<sub>2</sub>]-related decrease in leaf N concentration at the intermediate and high nutrient levels under the warmer  $T_{\text{soil}}$  conditions in this study was probably reflective of the low frequency of fertilizer applica-

tion. On the other hand, reduced plant sink strength at the low  $T_{\text{soil}}$  might have induced an accumulation of excess carbohydrates in the leaf under elevated  $[\text{CO}_2]$ , diluting N concentration (DeLucia 1986, Bagnall et al. 1988).

According to Farquhar et al. (1980) and Hymus et al. (2001), high atmospheric  $[\text{CO}_2]$  would stimulate electron flow to the photosynthetic carbon reduction cycle and competitively suppress electron allocation to the photorespiratory carbon oxidation pathway in plants growing under non-limiting nutrient conditions, leading to increased  $A_n$ . In accord with this hypothesis, the  $[\text{CO}_2]$  elevation significantly increased  $J_c$  but decreased  $J_o/J_T$  under intermediate  $T_{\text{soil}}$  + high nutrient supply. However, no significant effects of elevated  $[\text{CO}_2]$  on  $J_c$  were found in any other treatments, suggesting that other factors were more important than  $J_c$  for the observed increases in  $A_n$  under elevated  $[\text{CO}_2]$ .  $C_i$  significantly increased from ambient to elevated  $[\text{CO}_2]$  at each  $T_{\text{soil}}$  and nutrient level (data not shown). Our data support the finding of other researchers (e.g. Agrawal 1999, Midgley et al. 1999) that  $C_i$  is the decisive factor for the higher  $A_n$  in elevated  $[\text{CO}_2]$ .

The lack of positive response of  $A_n$  to elevated  $[\text{CO}_2]$  at the low nutrient regime reflects photosynthetic down-regulation (Evans 1989, Murray et al. 2000, Eguchi et al. 2004, Cao et al. 2007). To support this claim, we found a 39% decline in  $A_n$  from ambient to elevated  $[\text{CO}_2]$  at the low nutrient regime, when  $A_n$  of ambient and  $\text{CO}_2$ -enriched leaves were compared at a common  $C_a$  of  $360 \mu\text{mol mol}^{-1}$  (data not shown).  $A_n$  generally increases in response to elevated  $[\text{CO}_2]$  (Drake et al. 1997, Saxe et al. 1998, Zhang and Dang 2006, Huang et al. 2007). However, if the increased production of carbohydrate cannot be utilized due to nutrient limitation to growth, carbohydrates will accumulate in the leaf. Two mechanisms have been proposed whereby the accumulation of starch can directly inhibit  $A_n$  in elevated  $[\text{CO}_2]$ . First, high levels of carbohydrates may hinder  $\text{CO}_2$  diffusion from the intercellular airspaces to the stroma in chloroplasts (Makino 1994, Eguchi et al. 2004). Second, carbohydrate accumulation may induce a feedback inhibition of carbohydrate synthesis, with the result that  $A_n$  is inhibited because  $P_i$  is not regenerated rapidly enough (Lambers et al. 2008). Stitt (1991) has proposed another feedback mechanism in which carbohydrate loading indirectly causes a decrease in the levels of proteins and other components of the photosynthetic apparatus. Because elevated  $[\text{CO}_2]$  did not significantly reduce  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and TPU at any nutrient level in this study, high diffusion resistance in chloroplasts may be the main cause for the down-regulation of  $A_n$  in elevated  $[\text{CO}_2]$  + low nutrient supply.

The low  $T_{\text{soil}}$  suppressed  $A_n$  at the intermediate and high nutrient regimes, consistent with the works of King et al. (1999), Dang and Cheng (2004) and Zhang and Dang (2005). Furthermore, there were no significant differences in  $A_n$  between the ambient and elevated  $[\text{CO}_2]$  at this  $T_{\text{soil}}$ . The fact that low  $T_{\text{soil}}$  significantly decreased  $g_s$  and  $E$  but had no significant effects on  $V_{\text{cmax}}$  and  $J_{\text{max}}$  suggests that

the reduction in  $A_n$  was primarily caused by the decline in  $g_s$ . Zhang and Dang (2005) have found that  $g_s$  is the main limiting factor for  $A_n$  in white birch and jack pine seedlings growing under low  $T_{\text{soil}}$  conditions in ambient and elevated  $[\text{CO}_2]$ . The reduction in  $g_s$  may be ascribed to a decline in leaf water potential (Benzioni and Dunstone 1988, Dang and Cheng 2004). Low  $T_{\text{soil}}$  may reduce water supply to the shoot by increasing soil water viscosity and decreasing root permeability (Kaufmann 1977, Gurdarshan and Reynolds 1996, Richardson 2000, Öpik and Rolfe 2005). Alternatively, the decrease in  $g_s$  could be related to non-hydraulic signals that roots sense in cold soils (Day et al. 1991). There were no significant effects of  $T_{\text{soil}}$  on  $A_n$  at the low nutrient regime, indicating a stronger nutrient than  $T_{\text{soil}}$  effect on  $A_n$  at this nutrient level.

Lambers et al. (2008) have suggested that a decrease in  $A_n$  under low  $T_{\text{soil}}$  could be potentially associated with photoinhibition. However, this study's finding that low  $T_{\text{soil}}$  did not significantly affect  $F_v/F_m$  and all the values of  $F_v/F_m$  were within the normal range (0.75–0.85, Ball et al. 1994) for non-stressed plants points to the absence of photoinhibition. In other words, there was no loss in the yield of PSII photochemistry due to the low  $T_{\text{soil}}$ . Similar results have been obtained with jack pine (*Pinus banksiana* Lamb., Zhang and Dang 2005), trembling aspen (*Populus tremuloides* Michx., Landhäusser and Lieffers 1998) and Scots pine (*Pinus sylvestris* L., Domisch et al. 2001). The significant decrease in  $\Delta F/F_m'$  and increase in  $J_o/J_T$  under low  $T_{\text{soil}}$  were possibly related to photoprotective mechanisms. Photorespiration could prevent the photosynthetic apparatus from photodamage by consuming excessive assimilatory power, a prerequisite of which should be an increase in photorespiratory activity or, at least, no decrease (Zhang and Dang 2005, Zhang and Dang 2006, Lambers et al. 2008).

In conclusion, the  $[\text{CO}_2]$  elevation significantly increased  $A_n$  under the intermediate and high  $T_{\text{soil}}$  at the intermediate and high nutrient regimes but not at low nutrient availability. In contrast, no significant differences in  $A_n$  were observed between ambient and elevated  $[\text{CO}_2]$  at the low  $T_{\text{soil}}$ . These findings support our hypothesis that low  $T_{\text{soil}}$  reduces the positive effect of high nutrient availability on the response of  $A_n$  to elevated  $[\text{CO}_2]$ . Like atmospheric  $[\text{CO}_2]$ , soil fertility is predicted to increase in the future due to an increase in anthropogenic nitrogen deposition (Galloway et al. 2004, LeBauer and Treseder 2008). Although  $[\text{CO}_2]$  and nutrient availability are known to synergistically affect  $A_n$  of white birch growing under favorable  $T_{\text{soil}}$  conditions (Zhang and Dang 2006), the results of the present study reveal that elevated  $[\text{CO}_2]$  + high soil fertility may not be expected to increase  $A_n$  in trees growing in cold soils. In other words, the enhancement of photosynthesis by  $[\text{CO}_2]$  elevation depends on both nutrient availability and  $T_{\text{soil}}$ . The differences in response can have important implications on the productivity of different sites within the boreal forest, given the great variation in  $T_{\text{soil}}$  across the boreal landscape. However, because this short-term study with seedlings was conducted in a con-

trolled environment, the findings may not accurately reflect long-term acclimation of mature trees to field conditions (Curtis and Wang 1998, Pritchard et al. 1999, Bond 2000, Wigley and Schimel 2000). Therefore, the results should not be applied directly to trees growing in forests without validation. They, however, highlight a necessity to take  $T_{\text{soil}}$  into account when investigating the effect of nutrient availability on the response of  $A_n$  to high atmospheric [CO<sub>2</sub>]. This controlled-environment study should pave the way for further research to determine whether the different responses of  $A_n$  to [CO<sub>2</sub>] resulting from the various combinations of  $T_{\text{soil}}$  and nutrient availability are exhibited in the field.

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