

Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings

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Summary To investigate the interactive effects of atmospheric carbon dioxide concentration ($[\text{CO}_2]$) and nutrition on photosynthesis and its acclimation to elevated $[\text{CO}_2]$, a two-way factorial experiment was carried out with two nutritional regimes (high- and low-nitrogen (N), phosphorus (P) and potassium (K)) and two CO_2 concentrations (360 and 720 ppm) with white birch seedlings (*Betula papyrifera* Marsh.) grown for four months in environment-controlled greenhouses. Elevated $[\text{CO}_2]$ enhanced maximal carboxylation rate (V_{cmax}), photosynthetically active radiation-saturated electron transport rate (J_{max}), actual photochemical efficiency of photosystem II (PSII) in the light ($\Delta F/F_m'$) and photosynthetic linear electron transport to carboxylation (J_c) after 2.5 months of treatment, and it increased net photosynthetic rate (A_n), photosynthetic water-use efficiency (WUE), photosynthetic nitrogen-use efficiency (NUE) and photosynthetic phosphorus-use efficiency (PUE) after 2.5 and 3.5 months of treatment, but it reduced stomatal conductance (g_s), transpiration rate (E) and the fraction of total photosynthetic linear electron transport partitioned to oxygenation (J_o/J_T) after 2.5 and 3.5 months of treatment. Low nutrient availability decreased A_n , WUE, V_{cmax} , J_{max} , triose phosphate utilization (TPU), $(F_m' - F)/F_m'$ and J_c , but increased J_o/J_T and NUE. Generally, V_{cmax} was more sensitive to nutrient availability than J_{max} . There were significant interactive effects of $[\text{CO}_2]$ and nutrition over time, e.g., the positive effects of high nutrition on A_n , V_{cmax} , J_{max} , $\Delta F/F_m'$ and J_c were significantly greater in elevated $[\text{CO}_2]$ than in ambient $[\text{CO}_2]$. In contrast, the interactive effect of $[\text{CO}_2]$ and nutrition on NUE was significant after 2.5 months of treatment, but not after 3.5 months. High nutrient availability generally increased PUE after 3.5 months of treatment. There was evidence for photosynthetic up-regulation in response to elevated $[\text{CO}_2]$, particularly in seedlings receiving high nutrition. Photosynthetic depression in response to low nutrient availability was attributed to biochemical limitation (or increased mesophyll resistance) rather than stomatal limitation. Elevated $[\text{CO}_2]$ reduced leaf N concentration, particularly in seedlings receiving low nutrition, but had no significant effect on leaf P or K concentration. High nutrient availability generally increased area-based leaf N, P and K concentrations, but had negligible effects on K after 2.5 months of treatment.

Keywords: boreal trees, chlorophyll fluorescence, $[\text{CO}_2]$ elevation, electron transport and partitioning, in situ gas exchange, in vivo carboxylation activity, nutrient availability.

Introduction

Increases in atmospheric carbon dioxide concentration ($[\text{CO}_2]$) can have profound effects on photosynthesis and dry mass production of plants (Morison 1985, Drake et al. 1997, Ward and Strain 1999, Loewe et al. 2000). However, the response is generally affected by other environmental factors, such as mineral nutrient supply, and studies on $\text{CO}_2 \times$ nutrient interactions have yielded conflicting results (Saxe et al. 1998, Curtis et al. 2000, Nowak et al. 2004). Most studies have shown that nutrient limitation reduces the responses of photosynthesis and growth to elevated $[\text{CO}_2]$ (Saxe et al. 1998). However, based on a model analysis of published data, Lloyd and Farquhar (1996) suggest that growth enhancement in response to elevated $[\text{CO}_2]$ increases with increasing nitrogen (N) limitation in some slow-growing plant species. Several researchers have found that the responses to elevated $[\text{CO}_2]$ are more accentuated in pot-grown plants or when nutrient availability is low (Arp 1991, Long and Drake 1991, Tissue et al. 1993, Teskey 1995, Johnson et al. 1995). Some researchers consider that the measured photosynthetic responses to elevated $[\text{CO}_2]$ are a reflection of CO_2 effects on plant nutrient conditions rather than a direct effect of $[\text{CO}_2]$ on photosynthesis (Arp 1991, Long and Drake 1991, Teskey 1995, Saxe et al. 1998). Studies on *Pinus taeda* L. and *Pinus sylvestris* L. have demonstrated that the relative enhancement of photosynthesis by elevated $[\text{CO}_2]$ is similar at high and low nutrient availabilities (Murthy et al. 1996, Kellomäki and Wang 1997). One explanation for these controversial findings is that the response may be species specific. A detailed understanding of nutrient effects on the magnitude of CO_2 stimulation of photosynthesis in different species is critical for predicting the physiological and growth performance of plants growing under different site conditions in response to climate changes associated with increasing atmospheric $[\text{CO}_2]$ (Saxe et al. 1998).

Photosynthesis can acclimate to the growth $[\text{CO}_2]$. How-

ever, the degree of acclimation is generally greater when N supply is low and can disappear when N supply is adequate (Drake et al. 1997, Stitt and Krapp 1999, Isopp et al. 2000). Photosynthetic acclimation is considered to be related to the source–sink relationship for carbohydrates, which in turn is influenced by the mineral nutrient regime (Saxe et al. 1998). By manipulating the source–sink balance of perennial ryegrass, Rogers et al. (1998) demonstrated that photosynthetic acclimation to elevated $[\text{CO}_2]$ at low N was a result of the low N limitation to sink development rather than the direct effect of nitrogen supply on photosynthesis. Similarly, Stitt and Krapp (1999) and Hymus et al. (1999) argue that inadequate N supply will restrict the development of new sinks and thus exacerbate the source–sink imbalance in plants grown in elevated $[\text{CO}_2]$. This theory is supported by the findings of some Free Air Carbon Dioxide Enrichment (FACE) studies (Medlyn et al. 1999, Lüscher et al. 2000, Ainsworth and Long 2005), but not by others (von Caemmerer et al. 2001).

Most studies have focused on the interactions between N and $[\text{CO}_2]$. However, different nutrient elements can interact with each other in affecting plant functions (Kozłowski et al. 1991, Newbery 1994, Gusewell 2005). For instance, heavy N fertilization can induce potassium (K) deficiency (van den Driessche and Ponsford 1995). It has been shown that foliage protein content can decline as foliage N increases as a result of other nutrient limitations, such as phosphorus (P), K or sulphur (S) (Brooks 1986, Nakaji et al. 2001, Warren and Adams 2002). Because gene activity and the synthesis of photosynthetic enzymes are influenced by N:P ratios (Stitt 1996, Nakaji et al. 2001), a curvilinear relationship between photosynthesis and foliar N concentration is often observed if P and K are not adjusted for N content in the fertilizer formulation (e.g., Mänter et al. 2005), whereas a linear relationship is found when P and K are adjusted based on the amount of supplied N (Ripullone et al. 2003). It is thus important to keep the ratios of N to other key nutrient elements constant to avoid confounding effects. Furthermore, nutrient-rich sites generally have higher concentrations of all three key elements (N, P and K) than poor sites. In this study, we examined the physiological responses of white birch seedlings to elevated $[\text{CO}_2]$ at low and high supplies of N, P and K where the ratios of N to P and K were kept constant between treatments.

White birch (*Betula papyrifera* Marsh.) is a pioneer boreal tree species with a high rate of juvenile growth and a relatively high nutrient demand (Burns and Honkala 1990, Peterson et al. 1997). We hypothesized that, under low nutrient conditions, birch seedling growth is limited by nutrients, but not by carbon acquisition, and that increasing atmospheric $[\text{CO}_2]$ would increase the nutrient-use efficiency for photosynthesis and thus permit re-allocation of some foliar nutrients to increase growth, resulting in photosynthetic down-regulation. Because white birch has an indeterminate growth pattern, we further hypothesized that, under high nutrient conditions, growth of birch seedlings is limited by carbon, and thus increasing $[\text{CO}_2]$ would stimulate growth and sink strength and, consequently, photosynthetic capacity would not down-regulate.

Materials and methods

Plant materials

In mid-February 2004, white birch seeds were germinated in the Lakehead University greenhouse in either ambient or 2× ambient $[\text{CO}_2]$. The seedlings were transplanted to large containers (21 cm in height, 20.8 cm in top diameter) when they were about 3 cm high on February 27, 2004. The growing medium was a (1:1; v/v) mixture of peat moss and vermiculite. The seedlings were relatively uniform in size at the beginning of the experiment.

Experiment design

The treatments consisted of two CO_2 concentrations (ambient (360 ppm) and 2× ambient (720 ppm)) and two nutrient availabilities (low and high). The nutrient treatments were nested within the greenhouse. The experiment was conducted in four environment-controlled greenhouses, two with ambient $[\text{CO}_2]$ and two with 2× ambient $[\text{CO}_2]$, resulting in two replications for each nutrient × CO_2 treatment combination. Each replication of a treatment combination had 10 seedlings of uniform size (80 seedlings in total). All seedlings were fertilized twice weekly: seedlings in the high nutrient treatment received a solution containing 100:44:83 ppm N,P,K, respectively, and seedlings in the low nutrient treatment received a solution containing 10:4.4:8.3 ppm N,P,K, respectively. Other environmental conditions for all the treatments were: day/night temperatures 25–26/16–17 °C from March to May and 28–30/18–20 °C starting in June; and a 16-h photoperiod (natural light was supplemented by high-pressure sodium lamps on cloudy days, early mornings and late evenings). All environmental conditions were monitored and controlled with an Argus control system (Vancouver, Canada). Volumetric water content of the growing medium was maintained around 50% as measured with a HH2 moisture meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U.K.). The seedlings were watered up to twice daily during the summer to maintain the soil water content at 50%. The experiment lasted about 3.5 months (from mid-February to early June 2004).

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

Foliage gas exchange was measured with a PP-Systems CIRAS-1 open gas exchange system (Hitchin, Hertfordshire, U.K.). The environmental conditions in the Parkinson broad-leaf chamber were controlled automatically. Photosynthesis (A) was measured sequentially at 50, 120, 180, 350, 500, 700 and 1000 $\mu\text{mol mol}^{-1} [\text{CO}_2]$ to establish $A-C_i$ curves. Other environmental conditions for the measurements were 26 °C air temperature, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) and 50% relative humidity (RH). The measurements were taken on the 5th mature leaf from the apex of three randomly selected seedlings from each treatment combination. All measurements were made between 0900–1130 h with the seedlings in their original positions and treatment conditions. The gas exchange parameters were expressed on a one-sided leaf area basis. The measurements were made 2.5 and

3.5 months after treatment initiation.

Net photosynthetic rate (A_n), stomatal conductance (g_s) and transpiration rate (E) were calculated according to Farquhar et al. (1980), von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991) and Harley et al. (1992). The *in vivo* maximal carboxylation rate (V_{cmax}), PAR-saturated electron transport rate (J_{max}) and the triose phosphate utilization (TPU) were calculated from the $A-C_i$ curve with the Photosyn Assistant software (Dundee Scientific, Scotland, U.K.). Wullschlegel (1993) suggests that TPU should be considered when there is an obvious saturation in the $A-C_i$ curve, which was the case in our study. The parameters for Rubisco kinetics (K_c , K_o and τ) and their temperature dependencies were taken from Bernacchi et al. 2001 and 2003. The relative limitation of the mesophyll (mesophyll resistance (R_m)/total resistance (R_{total})) was calculated as described by Berry and Downton (1982).

Chlorophyll fluorescence was measured with an FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments, Norfolk, U.K.). The probe was integrated in the leaf chamber of the gas exchange system and the control software for the two systems was integrated to allow simultaneous measurements of gas exchange and chlorophyll fluorescence. The following variables were obtained: chlorophyll fluorescence intensity (F); maximal chlorophyll fluorescence in light (F_m'); and the actual photochemical efficiency of photosystem II (PSII) in light ($(F_m' - F)/F_m'$ or $\Delta F/F_m'$) (Bilger and Björkman 1990). We obtained F_m' by illuminating the foliage with a pulse of strong light ($\sim 14,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 800 ms.

Based on Farquhar et al. (1980) and Genty et al. (1989), the simultaneously measured gas exchange and chlorophyll fluorescence are related as follows:

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

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

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

where J_T is the apparent rate of electron transport measured by chlorophyll fluorescence, PFD is the photosynthetic photon flux in the leaf chamber, V_c and V_o are the rates of carboxylation and oxygenation of Rubisco, respectively, calculated from the $A-C_i$ curve, A_n is net photosynthetic rate and R_d is daytime dark respiration.

The partitioning of total electron flow between carboxylation (J_c) and oxygenation (J_o) is determined as (Epron et al. 1995):

$$J_c = \frac{1}{3}(J_T + 8(A_n + R_d)) \quad (4)$$

$$J_o = \frac{2}{3}(J_T - 4(A_n + R_d)) \quad (5)$$

Leaf nutrient (N, P, K) assays

The 4th–6th fully expanded leaves from the apex were sampled from each seedling used for the gas exchange measurements. Total leaf N concentration (%) was assayed by the

Dumas method (McGill and Figueiredo 1993), and P and K concentrations were assayed as described by Kalra and Maynard (1998). The analyses were conducted in the Forest Soils Lab at Lakehead University. Nutrient concentrations were expressed on a leaf area basis.

Nitrogen- and P-use efficiencies (NUE and PUE, respectively) were obtained by dividing A_n at the growth [CO₂] by foliar nutrient concentration. The water-use efficiency of photosynthesis (WUE) was calculated by dividing A_n by E .

Statistical analysis

All data were examined graphically for normality of distribution (probability plots for residual analysis) and homogeneity of variance (scatter plots) with the software Data Desk (Version 6.01, 1996; Data Description, Ithaca, NY) before being subjected to analysis of variance (ANOVA). All the data except J_{max} in the first measurement satisfied the assumptions for ANOVA. The J_{max} data from the first measurement were log-transformed to meet these assumptions. Effects of nutrient availability, [CO₂] and their interaction were tested by two-way ANOVA. When the interaction between nutrient availability and [CO₂] was significant for a given parameter, a Scheffe's F test for post hoc pairwise comparisons was conducted.

Results

In situ gas exchange

Elevated [CO₂] and high nutrient availability significantly ($P < 0.001$) increased the light-saturated photosynthetic rate at the growth [CO₂] (A_n) by 41 and 59%, respectively, after 2.5 months of treatment (Figure 1), and by 43 and 93%, respectively, after 3.5 months of treatment. There was also a significant ($P < 0.01$) interaction between [CO₂] and nutrient availability on A_n after 3.5 months of treatment. The value of A_n in the elevated [CO₂] + high nutrient treatment was 173% higher than in the ambient [CO₂] + low nutrient treatment.

There were significant interactions between [CO₂] and nutrient availability on g_s and E at both measurement times ($P < 0.05$). The low nutrient treatment significantly reduced g_s and E in ambient [CO₂], but not in elevated [CO₂], and the elevated [CO₂] treatment significantly suppressed g_s and E in seedlings in the high nutrient treatment, but not in the low nutrient treatment (Figure 1). Overall, g_s and E were highest in the ambient [CO₂] and high nutrient treatments (Figure 1).

There were significant interactions between [CO₂] and nutrient availability on WUE after both 2.5 and 3.5 months of treatment ($P < 0.05$ and $P < 0.001$, respectively). The low nutrient treatment reduced WUE in elevated [CO₂], but not in ambient [CO₂], whereas elevated [CO₂] significantly increased WUE at both low and high nutrient availability, but the increase was much greater in the high nutrient treatment (Figure 1).

In vivo Rubisco activity

Both elevated [CO₂] and high nutrient availability increased

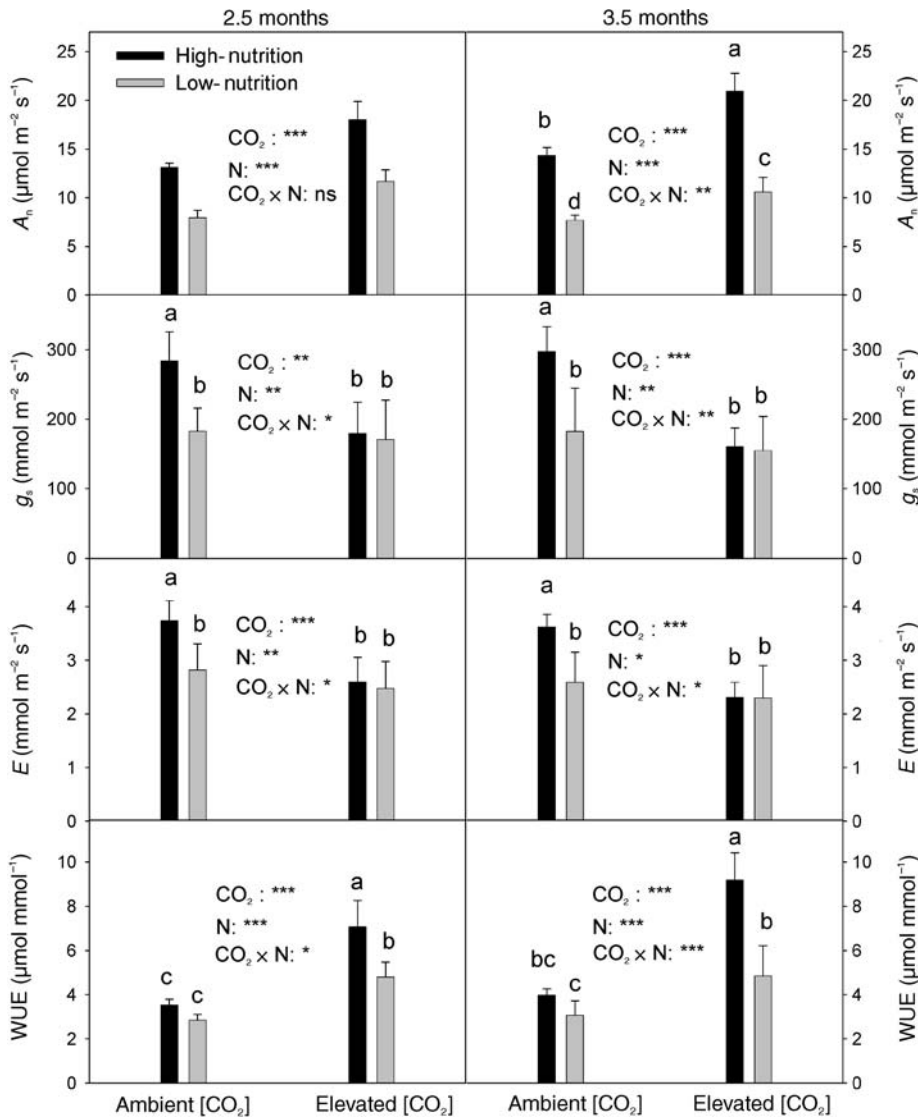


Figure 1. Effects of carbon dioxide concentration ($[CO_2]$; 360 versus 720 ppm) and nutrients (high versus low nitrogen (N), phosphorus (P) and potassium (K)) on net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration rate (E) and water-use efficiency (WUE) (mean \pm SEM, $n = 6$) in 1-year-old white birch seedlings. Measurements were taken at the growth $[CO_2]$ after 2.5 and 3.5 months of treatment. Significance values (*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; + = $P < 0.10$; and ns = $P > 0.10$) are based on ANOVA. Means sharing the same letter or letters do not differ significantly according to Scheffe's F test for post hoc pairwise comparisons conducted when the interaction between $[CO_2]$ and nutrient was significant for a given parameter. Because greenhouse had no significant effects on any of the variables ($P > 0.05$), data for each nutrient \times CO_2 combination were pooled.

V_{cmax} after 2.5 months of treatment ($P < 0.05$ and $P < 0.001$, respectively; Figure 2). There was a significant ($P < 0.10$) interaction between $[CO_2]$ and nutrient availability on V_{cmax} after 3.5 months of treatment: although the high nutrient treatment significantly increased V_{cmax} in both ambient and elevated $[CO_2]$, elevated $[CO_2]$ significantly increased V_{cmax} only in the high nutrient treatment (Figure 2).

The low nutrient treatment significantly reduced J_{max} , whereas elevated $[CO_2]$ had no significant effect on J_{max} after 2.5 months of treatment (Figure 2). After 3.5 months of treatment, there was a marginally significant ($P = 0.05$) interaction between $[CO_2]$ and nutrient availability on J_{max} : the low nutrient treatment significantly reduced J_{max} in both ambient and elevated $[CO_2]$, whereas elevated $[CO_2]$ significantly increased J_{max} only in the high nutrient treatment (Figure 2). Among the treatment combinations, J_{max} was highest in the elevated $[CO_2]$ + high nutrient treatment (Figure 2).

Low nutrient availability reduced TPU ($P < 0.001$) at both 2.5 and 3.5 months after initiation of treatments, but elevated

$[CO_2]$ had no significant effect on TPU (Figure 2).

There were significant interactions between the $[CO_2]$ and nutrient treatments on C_i at both 2.5 and 3.5 months after the start of treatments ($P < 0.05$ and $P < 0.001$, respectively; Figure 2). Elevated $[CO_2]$ generally increased C_i , significantly, but the increase was greater in the low nutrient treatment than in the high nutrient treatment (Figure 2). The low nutrient treatment increased C_i , but the effect was significant only in elevated $[CO_2]$ (Figure 2).

PSII efficiency and electron transport partitioning between carboxylation and oxygenation

The ratio $(F_m' - F)/F_m'$ was enhanced by high nutrient availability ($P < 0.001$), but was not significantly influenced by $[CO_2]$ ($P > 0.10$) after 2.5 months of treatment (Figure 3). After 3.5 months of treatment, however, nutrient and $[CO_2]$ interacted in affecting $(F_m' - F)/F_m'$ ($P < 0.05$): the low nutrient treatment significantly decreased $(F_m' - F)/F_m'$, but the effect was greater in elevated $[CO_2]$ than in ambient $[CO_2]$. Elevated

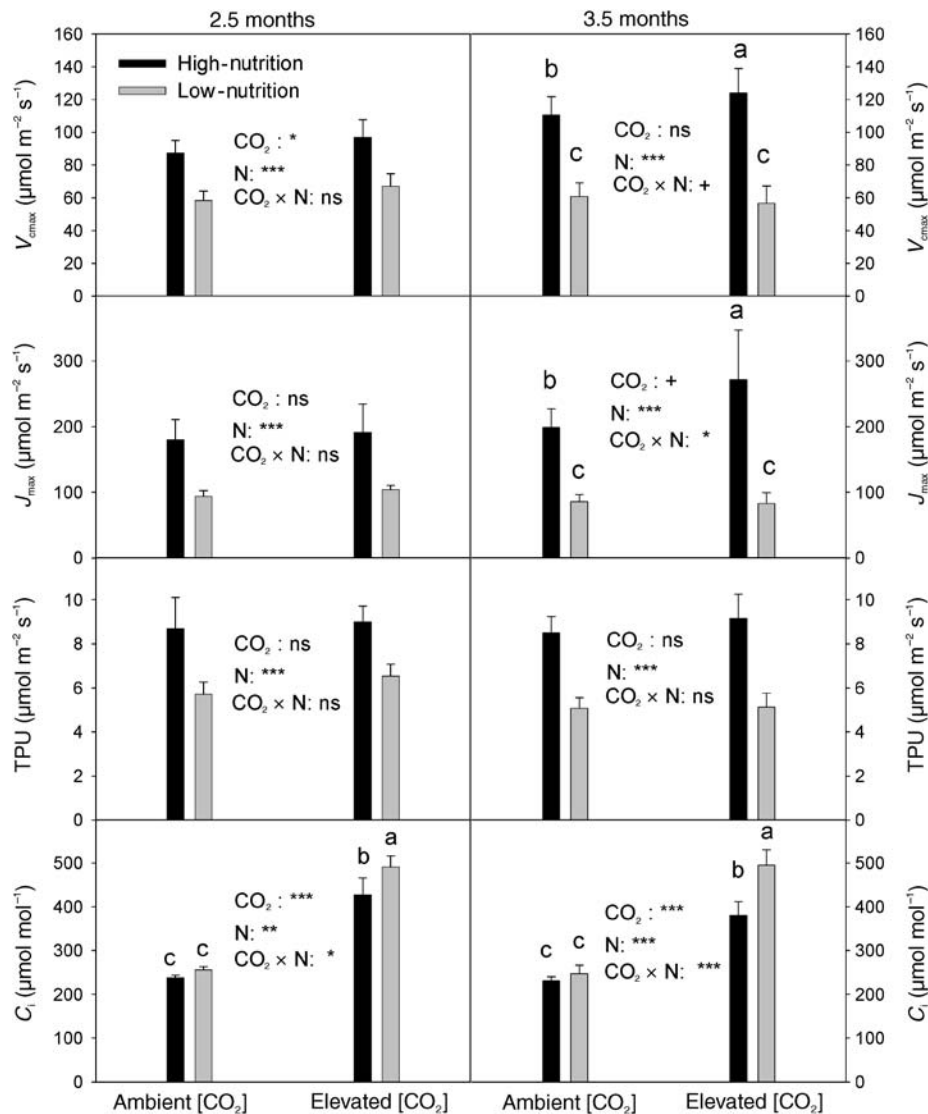


Figure 2. Effects of carbon dioxide concentration ([CO₂]; 360 versus 720 ppm) and nutrients (high versus low nitrogen (N), phosphorus (P) and potassium (K)) on in vivo maximal carboxylation rate (V_{max}), photosynthetically active radiation-saturated electron transport rate (J_{max}), triose phosphate utilization (TPU) and C_i (mean \pm SEM, $n = 6$) in 1-year-old white birch seedlings. Measurements were taken after 2.5 and 3.5 months of treatment. Significance values (*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; + = $P < 0.10$; and ns = $P > 0.10$) are based on ANOVA. Means sharing the same letter or letters do not differ significantly according to Scheffé's F test for post hoc pairwise comparisons conducted when the interaction between [CO₂] and nutrient availability was significant for a given parameter. Because greenhouse had no significant effects on any of the variables ($P > 0.05$), data for each nutrient \times CO₂ combination were pooled.

[CO₂] significantly decreased $(F_m' - F)/F_m'$ under conditions of low nutrient availability (Figure 3).

Both elevated [CO₂] and high nutrient availability increased J_c after 2.5 and 3.5 months of treatment ($P < 0.001$; Figure 3). After 3.5 months of treatment, there was also a significant interaction between nutrient availability and [CO₂]: the high nutrient treatment increased J_c in both ambient and elevated [CO₂], but the effect was greater in elevated [CO₂]; elevated [CO₂] increased J_c in the high nutrient treatment, but not in the low nutrient treatment (Figure 3).

The nutrient and [CO₂] treatments interacted with each other in affecting J_o ($P < 0.01$) after 2.5 months of treatment (Figure 3): the high nutrient treatment enhanced J_o in both ambient and elevated [CO₂], but the effect was greater in ambient [CO₂] than in elevated [CO₂]; elevated [CO₂] suppressed J_o at high nutrient availability, but not at low nutrient availability. As the treatments progressed to 3.5 months, the interaction between nutrient availability and [CO₂] became insignificant whereas the main effects remained significant, i.e., both ele-

vated [CO₂] and low nutrient availability decreased J_o ($P < 0.001$; Figure 3).

Elevated [CO₂] reduced J_o/J_T ($P < 0.001$), but nutrient availability had no significant ($P > 0.10$) effect on J_o/J_T , after 2.5 months of treatments (Figure 3). After 3.5 months of treatment, however, the high nutrient treatment significantly ($P < 0.001$) decreased J_o/J_T , and the suppression of J_o/J_T by elevated [CO₂] remained significant (Figure 3).

Foliar nutrient concentrations and photosynthetic nutrient-use efficiency

There was a significant ($P < 0.01$) interactive effect of [CO₂] and nutrient availability on total leaf N concentration after 2.5 and 3.5 months of treatments: the high nutrient treatment increased leaf N and the increase was greater in elevated [CO₂] than in ambient [CO₂]. At 2.5 months, elevated [CO₂] decreased leaf N in the low nutrient treatment (Figure 4), but no effect was detected at 3.5 months after treatment (Figure 4).

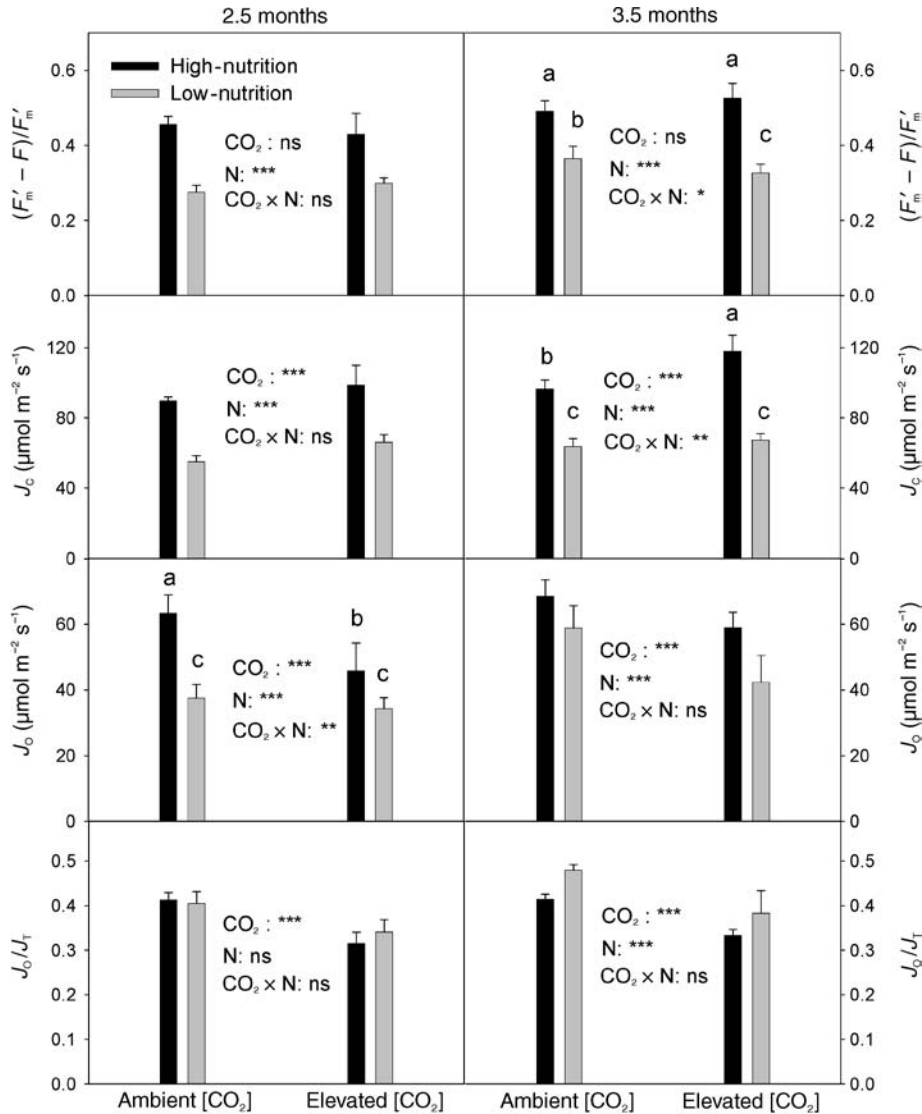


Figure 3. Effects of carbon dioxide concentration ($[CO_2]$; 360 versus 720 ppm) and nutrients (high versus low nitrogen (N), phosphorus (P) and potassium (K)) on actual photochemical efficiency of photosystem II in light ($(F_m' - F)/F_m'$), photosynthetic linear electron transport to carboxylation (J_c), photosynthetic linear electron transport to oxygenation (J_o) and J_o/J_T (mean \pm SEM, $n = 6$) in 1-year-old white birch seedlings. Measurements were taken after 2.5 and 3.5 months of treatment. Values of $(F_m' - F)/F_m'$ and J_T were derived from chlorophyll fluorescence measurements, and J_c and J_o were calculated from both chlorophyll fluorescence and gas exchange measurements. Significance values (*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; + = $P < 0.10$; and ns = $P > 0.10$) are based on ANOVA. Means sharing the same letter or letters do not differ significantly according to Scheffe's F test for post hoc pairwise comparisons conducted when the interaction between $[CO_2]$ and nutrient was significant for a given parameter. Because greenhouse had no significant effects on any of the variables ($P > 0.05$), data for each nutrient \times CO_2 combination were pooled.

Elevated $[CO_2]$ had no significant effect on leaf P concentration at either 2.5 or 3.5 months (Figure 4), but the high nutrition treatment increased leaf P at both measurement times (Figure 4). Neither $[CO_2]$ nor nutrient treatment had a significant ($P > 0.10$) effect on leaf K concentration after 2.5 months of treatment, but the high nutrition treatment had slight but statistically significant effects on leaf K after 3.5 months of treatment (Figure 4).

There were significant interactions ($P < 0.001$) between $[CO_2]$ and nutrition on photosynthetic NUE after 2.5 months of treatment: high nutrition availability decreased NUE in elevated $[CO_2]$, but not in ambient $[CO_2]$; elevated $[CO_2]$ increased NUE in both nutrient treatments but the enhancement was greater in the low nutrient treatment (Figure 4). At 3.5 months, the interaction became non-significant ($P > 0.10$), and both elevated $[CO_2]$ and low nutrient availability increased NUE ($P < 0.05$, Figure 4).

Elevated $[CO_2]$ increased PUE after 2.5 months of treatment, whereas the nutrient treatment had no effect. After

3.5 months of treatment, both elevated $[CO_2]$ and high nutrient availability increased PUE (Figure 4).

Discussion

High nutrient availability and elevated $[CO_2]$ enhanced birch seedling photosynthetic functions synergistically. Stimulation of photosynthesis by high nutrient availability was greater in elevated $[CO_2]$ than in ambient $[CO_2]$ and the $[CO_2]$ stimulation was greater at high nutrient availability than at low nutrient availability. The interactive effects of $[CO_2]$ and nutrient availability were significant for almost all photosynthetic parameters after 3.5 months of treatment. The high nutrient availability treatment increased A_n by 76% and the elevated $[CO_2]$ treatment increased A_n by 42%; however, A_n in the high nutrient + elevated $[CO_2]$ treatment was 173% higher than in the low nutrition + ambient $[CO_2]$ treatment.

Low A_n in the low nutrient treatment was a result of non-stomatal limitations. Both C_i (Figure 2) and R_m (Figure 5)

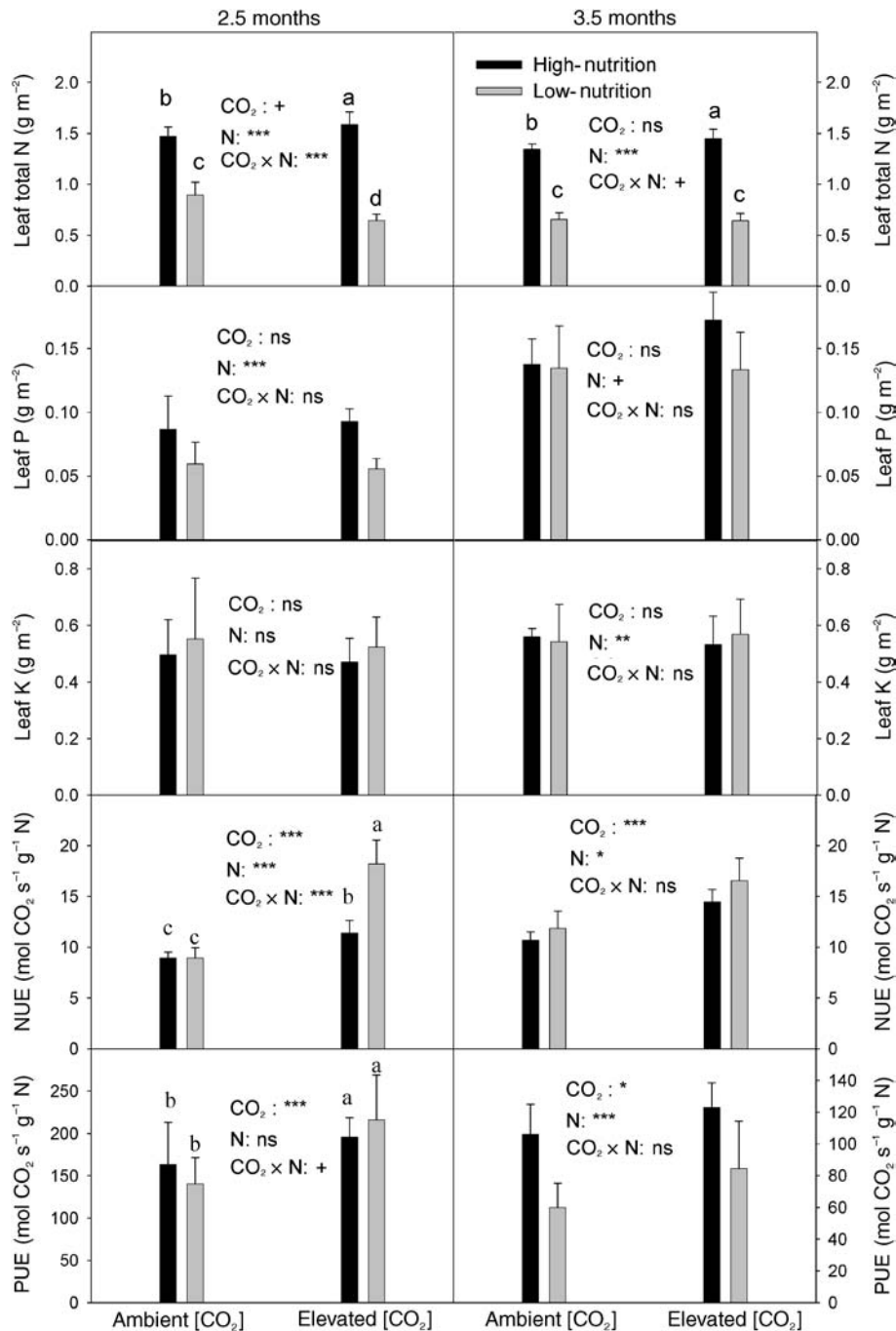


Figure 4. Effects of carbon dioxide concentration ([CO₂]; 360 versus 720 ppm) and nutrients (high versus low nitrogen (N), phosphorus (P) and potassium (K)) on total leaf N, P and K content, and photosynthetic N and P use efficiency (NUE and PUE, respectively; mean \pm SEM, $n = 6$) in 1-year-old white birch seedlings. Measurements were taken after 2.5 and 3.5 months of treatment. Significance values (***) = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; + = $P < 0.10$; and ns = $P > 0.10$ are based on ANOVA. Means sharing the same letter or letters do not differ significantly according to Scheffe's F test for post hoc pairwise comparisons conducted when the interaction between [CO₂] and nutrient was significant for a given parameter. Because greenhouse had no significant effects on any of the variables ($P > 0.05$), data for each nutrient \times CO₂ combination were pooled.

were higher in the low nutrient treatment than in the high nutrient treatment, whereas g_s was lower in the low nutrition treatment than in the high nutrition treatment only at ambient [CO₂], suggesting non-stomatal factors were primarily responsible for the low A_n at low nutrient availability (Farquhar and Sharkey 1982). This conclusion is consistent with the findings of Terry and Ulrich (1973) and Longstreth and Nobel (1980).

Our data did not support our first hypothesis that photosynthetic down-regulation occurs in response to elevated [CO₂] at low nutrient availability, but supported our second hypothesis

that the growth of white birch seedlings at high nutrient availability is carbon-limited and that photosynthesis is not down-regulated by elevated [CO₂]. We found that elevated [CO₂] resulted in up-regulation of photosynthesis in seedlings grown in the high nutrient treatment, which is in contrast to many published results (Saxe et al. 1998, Nowak et al. 2004, Riikonen et al. 2005), but in agreement with others (Ceulemans et al. 1997, Curtis et al. 2000). The results suggest that elevated [CO₂] in the presence of high nutrient availability substantially stimulated utilization of photosynthetic products. Root and leaf growth of the seedlings increased 47 and 35%, respectively, in

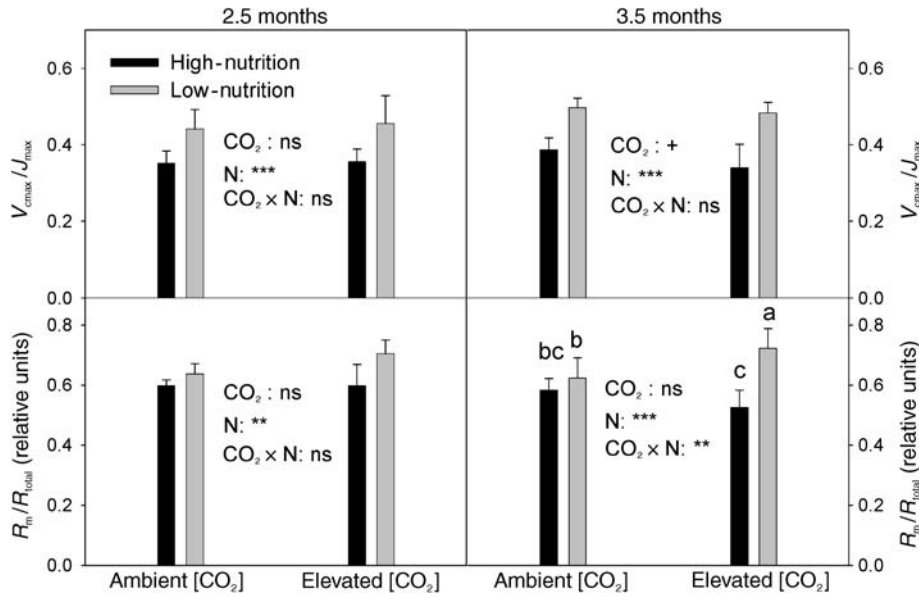


Figure 5. Effects of carbon dioxide concentration ($[CO_2]$; 360 versus 720 ppm) and nutrients (high versus low nitrogen (N), phosphorus (P) and potassium (K)) on maximal carboxylation rate (V_{cmax})/ photosynthetically active radiation-saturated electron transport rate (J_{max}) ratio and relative mesophyll limitation to photosynthesis (mesophyll resistance (R_m)/total resistance (R_{total})) (mean \pm SEM, $n = 3$) in 1-year-old white birch seedlings. Measurements were taken after 2.5 and 3.5 months of treatment. Significance values (***) = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; + = $P < 0.10$; and ns = $P > 0.10$ are based on ANOVA.

Means sharing the same letter or

letters do not differ significantly according to Scheffe's F test for post hoc pairwise comparisons conducted when the interaction between $[CO_2]$ and nutrient was significant for a given parameter. Because greenhouse had no significant effects on any of the variables ($P > 0.05$), data for each nutrient \times CO_2 combination were pooled.

response to elevated $[CO_2]$ at high nutrient availability (S. Zhang and Q.-L. Dang, unpublished data). White birch has an indeterminate pattern. Elevated $[CO_2]$ would be less likely to result in photosynthetic up-regulation in trees with determinate growth, particularly in the short term, because such trees are unable to take advantage of favorable current environmental conditions by increasing their leaf area. We speculate that photosynthetic up-regulation may occur in response to elevated $[CO_2]$ in young stands of trees with indeterminate growth patterns growing on sites with high nutrient availability, particularly following fires or fertilization. However, as the stand further develops to form a closed canopy, light becomes a limiting factor for photosynthesis and growth because of self-shading and mutual shading; consequently, photosynthetic up-regulation in response to elevated $[CO_2]$ is less likely to occur in stands with a closed canopy.

Our results support the theory that elevated $[CO_2]$ can lead to a rapid depletion of soil N by pot-grown plants unless the supply is replenished by increased supply (Pettersson and McDonald 1994). Our elevated $[CO_2]$ treatment significantly reduced the foliar N concentration of seedlings grown at low nutrient availability, but not those grown at high nutrient availability. Elevated $[CO_2]$ initially increased the V_{cmax} of seedlings grown at both low and high nutrient availabilities, but the stimulation at low nutrient availability disappeared over time, whereas the stimulation at high nutrient availability increased over time. Ellsworth et al. (2004) have reported strong correlations between the responses of leaf N concentration and V_{cmax} to $[CO_2]$ elevation in 16 species and concluded that maintaining leaf carboxylation capacity in elevated $[CO_2]$ depends on the maintenance of canopy N stocks, and that photosynthetic

adjustment is associated with leaf N depletion. However, the decrease in foliar N concentration in the low nutrient treatment did not result in photosynthetic down-regulation in our study, supporting the theory of Long et al. (2004) that Rubisco activity may increase in response to $[CO_2]$ elevation even though Rubisco content may decline.

Triose phosphate utilization, an indicator of the transport of photosynthetic products from chloroplasts to sinks, was not significantly affected by elevated $[CO_2]$. In conjunction with the lack of photosynthetic down-regulation, this result suggests that pot size did not constrain sink development in our white birch seedlings and that the source-sink balance was maintained in elevated $[CO_2]$. The biomass data indicate that elevated $[CO_2]$ did not significantly affect the shoot/root ratio of the seedlings (S. Zhang and Q.-L. Dang, unpublished data), despite the low nutrient supply (10:4.4:8.3 ppm N,P,K, respectively) in the low nutrient treatment. Our results do not support the theory that plants will down-regulate their photosynthetic capacity when grown in elevated $[CO_2]$ and poor soil nutrition (Sage and Sharkey 1989, Curtis and Wang 1998, Saxe et al. 1998). The results are, however, in agreement with the findings of most field experiments conducted under natural rooting conditions (Huxman et al. 1998, Lee et al. 2001, Rogers and Ellsworth 2002, Nowak et al. 2004). Kubiske et al. (2003) emphasized that trees rooted freely in the ground can exhibit photosynthetic acclimation to elevated $[CO_2]$. Long et al. (2004) suggested that photosynthetic down-regulation is not necessarily a characteristic response to elevated $[CO_2]$ in plants grown in chambers or pots.

Both nutrition and $[CO_2]$ affected the actual overall electron transport efficiency through PSII ($(F_m' - F)/F_m'$) and the elec-

tron partitioning between Rubisco carboxylation and oxygenation. The high nutrient treatment stimulated $(F_m' - F)/F_m'$ and the proportion of the total electron flow partitioned to carboxylation, but suppressed the electron partitioning to Rubisco oxygenation or photorespiration (J_o/J_T), resulting in a substantial increase in net photosynthesis. Elevated [CO₂] generally suppressed J_o/J_T in both nutrient treatments and decreased $(F_m' - F)/F_m'$ in the low nutrient treatment at the second measurement. The decrease in $(F_m' - F)/F_m'$ and increase in electron partitioning to photorespiration in response to low nutrient availability may be related to photoprotective mechanisms. Low carboxylation capacity at low nutrient availability could potentially produce excess amounts of excited electrons that would be detrimental to the photosynthetic systems, particularly PSII centers. Because the photorespiratory pathway is more efficient in the safe dissipation of excess energy (Berry and Björkman 1980), an increase in electron flow through the photorespiratory pathway would protect the photosynthetic systems. Yin et al. (2004) suggest that the operation of non-carboxylation energy-dissipation pathways is especially relevant under suboptimal conditions when abiotic stresses prevail. Under such conditions, non-assimilatory electron flow pathways, e.g., O₂-dependent pseudocyclic electron transport coupling with Mehler-ascorbate peroxidase reaction and photorespiration, may replace assimilatory electron flow to protect the photosystems from photodamage (Ort and Baker 2002). Additionally, white birch seedlings reduced the total electron transport capacity relative to carboxylation capacity in the low nutrient treatment (Figure 5).

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