Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO2] in white birch seedlings

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Summary To study the effects of elevated CO_2 concentration ([CO_2]) on relationships between nitrogen (N) nutrition and foliar gas exchange parameters, white birch (*Betula papyrifera* Marsh.) seedlings were exposed to one of five N-supply regimes (10, 80, 150, 220, 290 mg N l^{-1}) in either ambient [CO_2] (360 µmol mol^{-1}) or elevated [CO_2] (720 µmol mol^{-1}) in environment-controlled greenhouses. Foliar gas exchange and chlorophyll fluorescence were measured after 60 and 80 days of treatment. Photosynthesis showed a substantial down-regulation (up to 57%) in response to elevated [CO_2] and the magnitude of the down-regulation generally decreased exponentially with increasing leaf N concentration. When measured at the growth [CO_2], elevated [CO_2] increased the overall rate of photosynthesis (P_n) and instantaneous water-use efficiency (IWUE) by up to 69 and 236%, respectively, but decreased transpiration (E) and stomatal conductance (g_s) in all N treatments. However, the degree of stimulation of photosynthesis by elevated [CO_2] decreased as photosynthetic down-regulation increased from 60 days to 80 days of treatment. Elevated [CO_2] significantly increased total photosynthetic electron transport in all N treatments at 60 days of treatment, but the effect was insignificant after 80 days of treatment. Both P_n and IWUE generally increased with increasing leaf N concentration except at very high leaf N concentrations, where both P_n and IWUE declined. The relationships of P_n and IWUE with leaf N concentration were modeled with both a linear regression and a second-order polynomial function. Elevated [CO_2] significantly and substantially increased the slope of the linear regression for IWUE, but had no significant effect on the slope for P_n. The optimal leaf N concentration for P_n and IWUE derived from the polynomial function did not differ between the CO_2 treatments when leaf N was expressed on a leaf area basis. However, the mass-based optimal leaf N concentration for P_n was much lower in seedlings in elevated [CO_2] than in ambient [CO_2] (31.88 versus 37.00 mg g^{-1}). Elevated [CO_2] generally decreased mass-based leaf N concentration but had no significant effect on area-based leaf N concentration; however, maximum N concentration per unit leaf area was greater in elevated [CO_2] than in ambient [CO_2] (1.913 versus 1.547 g N m^{-2}).

Introduction

Carbon fixation is critical to the productivity of forest ecosystems. Detailed information on how increases in atmospheric carbon dioxide concentration ([CO_2]) will influence photosynthesis is critical for understanding how climate change will affect the structure, functioning and productivity of forest ecosystems.

The response of photosynthesis to increased [CO_2] often varies with other physiological and environmental variables (Kellomäki and Wang 1997, Laitinen et al. 2000, Murray et al. 2000, Ying 2002, Lewis et al. 2004, Zhang and Dang 2005, 2006). Nitrogen (N) is a key factor regulating photosynthetic responses to elevated [CO_2] (Lewis et al. 2004) because it is a major component of Rubisco and other photosynthetic enzymes and structures (Bond et al. 1999, Ripullone et al. 2003). Zhang and Dang (2006) and Zhang et al. (2006) have reported that elevated concentrations of N and CO_2 have synergistic effects on photosynthesis and biomass production of white birch seedlings, and many other studies have reported positive effects of increased N supply on the stimulation of photosynthesis by elevated [CO_2] (Roberntz and Stockfors 1998, Rogers et al. 1998, Murray et al. 2000). Low N supply, on the other hand, can decrease or even eliminate the positive effect of elevated [CO_2] on photosynthesis (Tissue and Oechel 1987, Tissue et al. 1993, El Kohen and Mousseau 1994, Thomas et al. 1994, Silvola and Alholm 1995, Curtis 1996, Nakano et al. 1997, Murray et al. 2000). It is believed that, when N supply is low, photosynthetic down-regulation in response to elevated [CO_2] occurs because an inadequate N supply restricts the development of new sinks and exacerbates the source–sink imbalance that tends to develop in response to elevated [CO_2] (Stitt and Krapp 1999, Isopp et al. 2000). In this study, we tested the hypothesis that the magnitude of photosynthetic down-regulation decreases as N supply increases and does not occur when

N becomes non-limiting.

Photosynthetic rate (P_n) is generally closely correlated to foliar N concentration. This relationship is often used to model the net productivity of the forest canopy (Aber et al. 1996, Kull and Jarvis 1996, Dang et al. 1997) and the primary productivity of forest ecosystems (Aber and Federer 1992, Sellers et al. 1992, Goodale et al. 1998, Zak et al. 2003, Pan et al. 2004). The impact of increases in atmospheric [CO_2] on this relationship will, therefore, have implications for the accuracy and reliability of these models in predicting forest productivity under future climate conditions. However, there is a paucity of data on the impact of elevated [CO_2] on the P_n–N relationship (Kellomäki and Wang 1997, Nakano et al. 1997, Peterson et al. 1999, Ellsworth et al. 2004). Most studies of P_n–N interactions have been restricted to only two N-supply regimes (high versus low, Rogers et al. 1998, Laitinen et al. 2000, Murray et al. 2000, Zhang and Dang 2006, Zhang et al. 2006), thus precluding assessment of the effects of elevated [CO_2] on the the P_n–N relationship. In this study, we investigated how atmospheric [CO2] (ambient or twice ambient) affected the photosynthetic response of white birch (*Betula papyrifera* Marsh.) seedlings to five N-supply regimes. There are two general forms of the P_n–N relationship: linear and curvilinear. Because elevated [CO_2] generally increases photosynthetic N-use efficiency (Davey et al. 1999, Peterson et al. 1999, Zhang and Dang 2006), we hypothesized that elevated [CO_2] will reduce the optimal leaf N concentration for photosynthesis when the P_n–N relationship is curvilinear and increase the slope when the relationship is linear.

Materials and methods

Materials and experimental design

The experiment was conducted in the Lakehead University greenhouses. Seeds of white birch were sown in horticultural trays filled with a 1:1 (v/v) mixture of peat moss and vermiculite. Germination (in ambient [CO_2]) was complete within 10 days. After six weeks, 180 seedlings of uniform size were selected and transplanted to PVC containers (21 cm height × 20.8 cm top diameter) filled with 1:1 (v/v) peat moss:vermiculite. The trees were acclimated in the new pots for one week before the treatments began.

The treatments comprised two CO_2 concentrations (360 (ambient) and 720 (elevated) $\mu mol\ mol^{-1}$) and five N-supply regimes (10, 80, 150, 220 and 290 mg l^{-1}). For all treatments, the phosphorus and potassium concentrations were 60 and 150 mg l^{-1}, respectively. No other mineral nutrients were provided because the irrigation water contains other nutrient elements in sufficient amounts. The seedlings were fertilized with nutrient solution once a week from April to June and twice a week thereafter. The experiment was a split-plot design where the CO_2 treatments were the main plots and N treatments were the subplots. The CO_2 treatments were applied in four environment-controlled greenhouses (two replications per treatment), whereas the N treatments were replicated in each greenhouse. There were nine seedlings in each treatment combination. The locations of the seedlings were randomized within each greenhouse. The seedlings were spaced far enough apart to avoid mutual shading.

The elevated [CO_2] was achieved with Argus CO_2 generators (Argus, Vancouver, BC, Canada). For all treatments, day/night temperatures were set at 25–26/16–17 °C from April to June and at 28–30/18–20 °C thereafter, and the photoperiod was set at 16 hours (natural light was supplemented with high-pressure sodium lamps on cloudy days, and early in the mornings and late in the evenings). The volumetric water content of the growing medium was maintained around 30% as measured with a HH2 moisture meter (Delta-T Devices, Cambridge, U.K.). Environmental conditions were monitored and controlled with an Argus environment control system.

Simultaneous measurements of gas exchange and chlorophyll fluorescence

Leaf gas exchange was measured after 60 and 80 days of treatment with a CIRAS-1 open gas exchange system (PP-Systems, Hitchin, Hertfordshire, U.K.). All measurements were made between 0900 and 1200 h on the 5th to 8th mature leaf from the apex. Five seedlings were selected randomly from each treatment combination for measurement. All measurements were made at both ambient and elevated [CO_2] with the seedlings in their original locations and treatment conditions. The environmental conditions in the leaf chamber (26 °C air temperature, 800 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux (PPF) and 50% RH) were controlled automatically by the system. The measurement of P_n at 360 $\mu mol\ mol^{-1}$ [CO_2] (P_{n360}) was used to calculate the down-regulation of photosynthesis (difference in P_{n360} between ambient and elevated [CO_2] divided by P_{n360} in the ambient [CO_2] treatment).

Simultaneously with the gas exchange measurements, chlorophyll fluorescence was measured with an FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments, Norfolk, U.K.). The chlorophyll fluorescence probe, leaf cuvette and control software for the two systems were integrated to allow simultaneous measurement of gas exchange and chlorophyll fluorescence. The fluorescence of light-adapted foliage was measured before (F_o') and after (F_m') illuminating the foliage with a pulse of strong light (14,000 $\mu mol\ m^{-2}\ s^{-1}$) for 800 ms. The apparent rate of electron transport (J_T) was calculated as $(F_m' - F_o')/0.5F_m'0.84I$, where I is photosynthetic photon flux (Farquhar et al. 1980, Genty et al. 1989).

Leaf nitrogen assay

After 80 days of treatment (July 22, 2005), five mature leaves (including the leaf sampled for gas exchange measurement) were sampled from each seedling used for the gas exchange and chlorophyll fluorescence measurements. The leaves from the different treatments were selected in the same way to facilitate direct comparisons. The area of each leaf was measured with a WinFolia system (Regent Instruments Inc., Quebec City, QC, Canada). The leaves were subsequently oven-dried at 70 °C for 48 h and weighed. Leaf total N concentration was

determined by the dry combustion method with a CNS-2000 (LECO Corp., St. Joseph, MI, USA). Mass-based leaf N concentration was converted to area-based leaf N concentration by multiplying by the leaf area:mass ratio (i.e., specific leaf area).

Data analysis

Treatment effects were evaluated by analysis of variance (ANOVA) after examining the data graphically to confirm the normality of distribution (probability plots for residual analysis) and the homogeneity of variance (scatter plots). Multiple comparisons of means were conducted by the LSD method to identify variables that were significantly different from each other when ANOVA showed a significant effect. Where an interaction was significant, all the means involved in the interaction were compared. Regression analyses were conducted to examine relationships between leaf N concentration and relevant physiological variables.

Results

After 60 days of treatment, P_{n360} showed down-regulation in response to elevated [CO$_2$] in all seedlings except those in the highest N treatment, but the down-regulation was statistically significant only for seedlings in the lowest (10 mg N l^{-1}) and intermediate (150 mg N l^{-1}) N-supply regimes (20% and 31%, respectively, Figure 1A1). However, the photosynthetic down-regulation became statistically significant in all N regimes after 80 days of treatment (Figure 1B1).

The photosynthetic rate measured at the corresponding growth [CO$_2$] (P_n) was significantly higher in seedlings grown in elevated [CO$_2$] than in ambient [CO$_2$] after both 60 and 80 days of treatment, with the exception of seedlings in the lowest N treatment after 80 days where the difference was not statistically significant (Figures 1A2 and 1B2). However, the differences between CO$_2$ treatments were smaller after 80 days of treatment than 20 days earlier. At both measurement times, the magnitude of photosynthetic down-regulation generally corresponded with the magnitude of CO$_2$ stimulation of photosynthesis. For example, on Day 60, the percentage CO$_2$ stimulation was smallest at 150 and 10 mg N l^{-1} (31 and 50%, respectively), whereas photosynthetic down-regulation was 20 (second highest) and 31% (highest), respectively. After 80 days of treatment, the CO$_2$ stimulation of P_n was 14% (lowest) and 23% (second lowest) at 10 and 150 mg N l^{-1}, whereas the corresponding photosynthetic down-regulation was 57% (highest) and 34% (second highest). After 60 days of treatment, P_n was highest in seedlings in the intermediate N-supply regime (150 mg N l^{-1}) in both CO$_2$ treatments, and there were no significant differences between other N treatments (Figure 1A2). After 80 days of treatment, however, the interactions between [CO$_2$] and N regime became significant: in general P_n was higher with 150, 220 and 290 mg N l^{-1} with elevated [CO$_2$] than in the other treatment combinations, and P_n was lowest in seedlings in the lowest N-supply regime (Figure 1B2).

There were significant interactions between [CO$_2$] and N

treatment on stomatal conductance (g_s) after 60 days of treatment: g_s was highest in seedlings in the 10 mg N l^{-1} + ambient [CO$_2$] treatment and second highest in the 150 mg N l^{-1} + ambient [CO$_2$] treatment, whereas differences between other treatment combinations were small (Figure 1A3). Elevated [CO$_2$] generally decreased g_s, but the difference was not statistically significant in the 80 and 220 mg N l^{-1} regimes. Although the interactions remained significant after 80 days of treatment, the differences between the two [CO$_2$] treatments increased and became statistically significant for all nutrient treatments. At Day 80, g_s decreased significantly in response to elevated [CO$_2$] in all N-supply regimes (Figure 1B3). Nitrogen supply had no significant effect on g_s when [CO$_2$] was elevated, although there were significant variations in g_s of seedlings in the N-supply regimes in the ambient [CO$_2$] treatment (Figure 1B3). The responses of transpiration to [CO$_2$] and N supply were similar to those of g_s (Figures 1A3 to 1B4).

Elevated [CO$_2$] significantly and substantially increased instantaneous water-use efficiency (IWUE) in both measurement periods (Figures 1A5 and 1B5). The net increase was as high as 236% on Day 60 and 198% on Day 80. The nutrient effect on IWUE was fairly small after 60 days of treatment. The IWUE was significantly greater in seedlings in the 150, 220 and 290 mg N l^{-1} treatments than in the 10 and 80 mg N l^{-1} treatments, whereas there was no significant difference between IWUE of seedlings in the 10 and 80 mg N l^{-1} treatments (Figure 1A5). There were significant interactions between CO$_2$ and N treatments after 80 days of treatment, but there was little change in the ranking of IWUE among treatment combinations compared with Day 60. On Day 80, IWUE was highest in seedlings in the 150, 220 and 290 mg N l^{-1} supply regimes with elevated [CO$_2$] and lowest in the 10 mg N l^{-1} + ambient [CO$_2$] treatment (Figure 1B5).

Elevated [CO$_2$] significantly increased the apparent rate of electron transport (J_T) after 60 days of treatment but the effect disappeared after 80 days of treatment (Figures 2A and 2B). Although N supply had no significant effects on J_T after 60 days of treatment, J_T was significantly higher in seedlings in the 150, 220 and 290 mg N l^{-1} regimes than in the 10 and 80 mg N l^{-1} regimes on Day 80 (Figure 2B).

Elevated [CO$_2$] significantly increased photosynthetic N-use efficiency (PNUE) (Figure 3A). Increases in N supply generally had negative effects on PNUE but there was no significant difference between the 10 and 80 mg N l^{-1} regimes (Figure 3A).

Elevated [CO$_2$] significantly decreased mass-based leaf N concentration (N_m), but had no significant effect on area-based leaf N concentration (N_a) (Figures 3B and 3C). Both N_m and N_a increased with increasing N supply (Figures 3B and 3C).

The percent down-regulation of photosynthesis generally decreased as leaf N concentration increased (Figure 4). The linear model did not fit the data as well as the negative exponential function (Figure 4). The regression coefficient for the negative exponential function was 0.89 and 0.88 for N_a and N_m, respectively. The corresponding values for the linear model were 0.76 and 0.74.

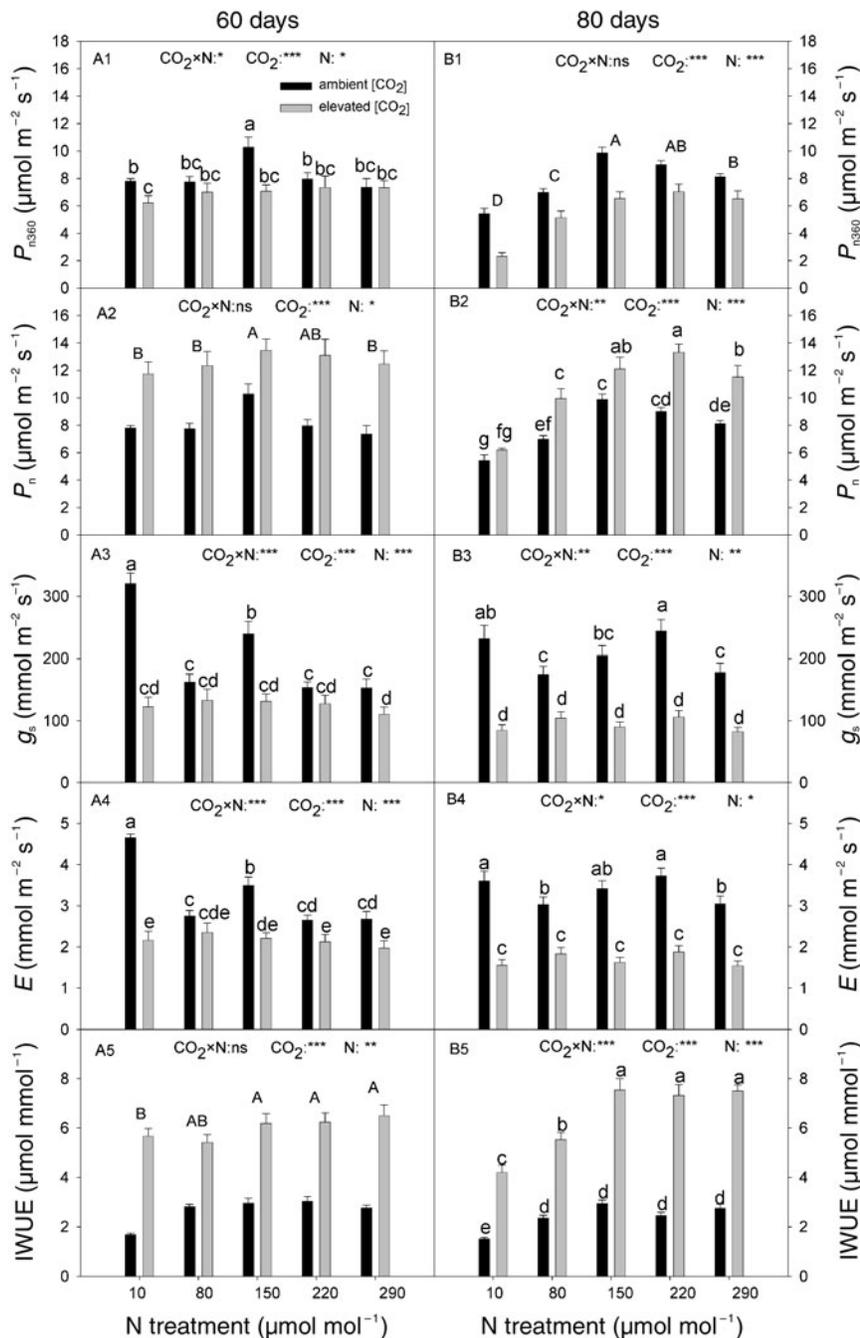The relationships between photosynthesis, IWUE and leaf

Figure 1. Effects of carbon dioxide concentration ([CO_2]) and nitrogen (N) supply on light-saturated rate of photosynthesis measured at 360 µmol mol^{-1} [CO_2] (P_{n360}) and at the corresponding growth [CO_2] (P_n), stomatal conductance (g_s), transpiration (E) and instantaneous water-use efficiency (IWUE) of birch seedlings after 60 (A1–A5) and 80 days (B1–B5) of treatment (mean ± SE, n = 10). The significance of main effects and interactions are indicated as: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Bars with different letters differ significantly according to the LSD test ($P < 0.05$). Lower-case letters represent the interactive effect of CO_2 and N, and capital letters represent the N effect.

N concentration were modeled using both a second-order polynomial function and a linear function, separately for each CO_2 treatment. In general, the two functions produced similar regression coefficients (R, Table 1). However, the polynomial function provided a better fit than the linear function for the relationship between P_n and N_m in elevated [CO_2] (Table 1).

For the linear function, we examined the effect of [CO_2] on the slope with the Student's t test (Steel and Torrie 1980). The slopes for the IWUE–N_a (Figure 5) and IWUE–N_m (Figure 6) relationships were both significantly greater ($P < 0.05$) in elevated [CO_2] than in ambient [CO_2]. However, there was no sig-

nificant difference ($P > 0.05$) between the two CO_2 treatments for the P_n–N_a or P_n–N_m regression (Figures 5 and 6).

For the polynomial function, we determined the optimal N_a or N_m for P_n and IWUE by setting the first derivative of the equation to zero and solving the equation for leaf N concentration. Elevated [CO_2] increased the optimal N_a for photosynthesis from 1.464 g N m^{-2} at ambient [CO_2] to 1.509 g N m^{-2} (Figure 5A), but decreased the optimal N_a from 37.0 to 31.9 mg g^{-1} (Figure 6A). The range of N_a in elevated [CO_2] was higher than in ambient [CO_2] (Figure 5) with a maximum value of 1.547 g N m^{-2} in ambient [CO_2] and 1.913 g N m^{-2} in elevated [CO_2], whereas the ranges for N_m were similar in the
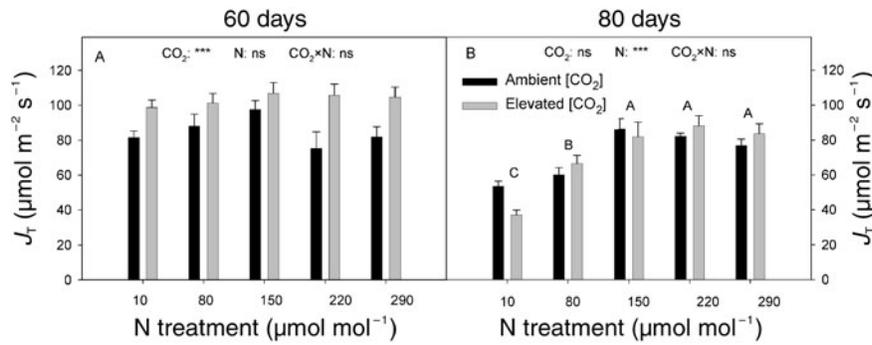
Figure 2. Effects of carbon dioxide concentration ([CO₂]) and nitrogen (N) supply on the apparent rate of electron transport (J_T) of birch seedlings after 60 (A) and 80 days (B) of treatment. Measurements were made at the corresponding growth [CO₂]. The significance of main effects and interactions are indicated as: ns, not significant; and ***, $P < 0.001$. Bars with different letters differ significantly according to the LSD test ($P < 0.05$).

two [CO₂] treatments (Figure 6). Elevated [CO₂] increased the optimal N_a for IWUE from 1.582 to 1.638 g N m^{-2} (Figure 5B) but did not affect the optimal N_m for IWUE (Figure 6B).

Discussion

Our data support the hypothesis that the magnitude of photosynthetic down-regulation in response to elevated [CO₂] decreases as N supply increases. The percentage down-regulation of photosynthesis decreased exponentially with increasing leaf N concentration, and the trend was similar whether the leaf N concentration was expressed on an area or mass basis. However, even at the highest leaf N concentration, photosynthetic down-regulation was above zero (about 20%). The highest leaf N concentration in this study was comparable to the highest value found in birch trees growing under natural environmental conditions (Perry and Hickman 2001), and our lowest leaf N concentration was lower than that found for seedlings in the low-N treatments in nearly all other published studies (Nakano et al 1997, Rogers et al. 1998, Peterson et al. 1999, Zhang and Dang 2006) but not as low as found in seedlings in the low-N treatment in the study of Tissue et al. 1993. Examination of the root system at the end of the experiment showed that root growth was not limited by the container. These results suggest that photosynthesis of white birch trees
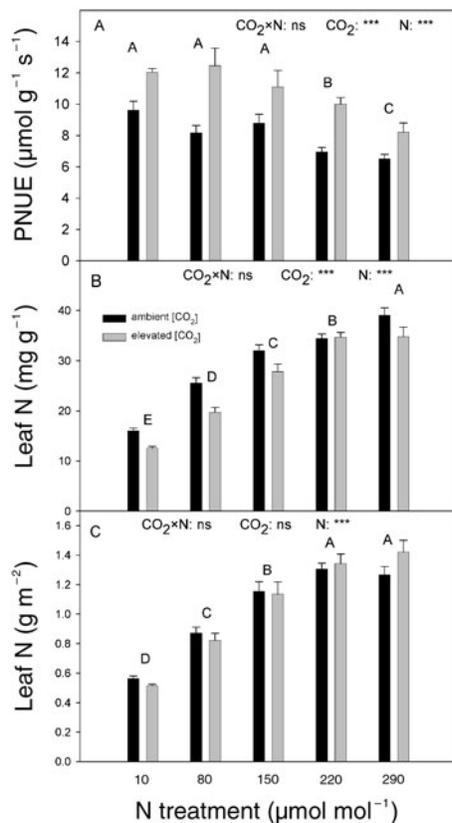

Figure 3. Effects of carbon dioxide concentration ([CO₂]) and nitrogen (N) supply on photosynthetic N-use efficiency (PNUE) and total leaf N concentration of birch seedlings after 80 days of treatments. The significance of main effects and interactions are indicated as: ns, not significant; and ***, $P < 0.001$. Bars with different letters differ significantly according to the LSD test ($P < 0.05$).
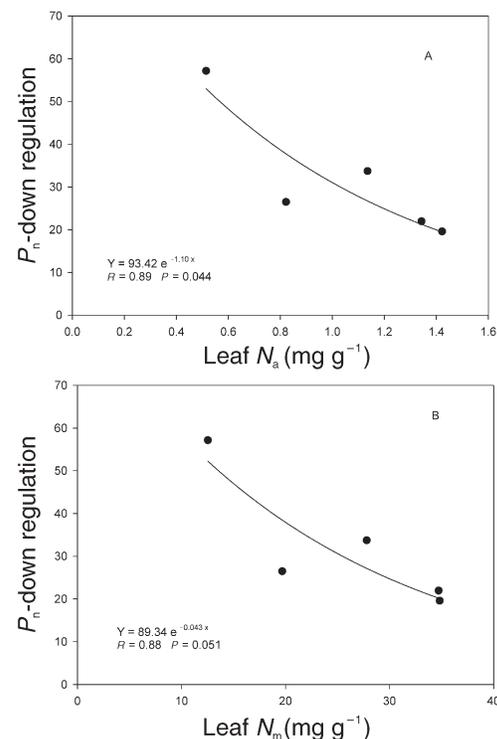

Figure 4. Relationship between percent down-regulation of photosynthetic rate (P_n) and area- (N_a) and mass-based (N_m) leaf nitrogen concentration.

Table 1. Regression analyses of P_n–nitrogen (N) and IWUE–N relationships. Abbreviations: P_n = light saturated rate of photosynthesis; IWUE = instantaneous water use efficiency; N_a = area-based leaf N concentration; and N_m = dry-mass-based leaf N concentration. For all analyses, $P <$ 0.0001.

Relationship	[CO$_2$]	a + bx			a + bx + cx^2			
		a	b	R	a	b	c	R
P_n–N_a	360	2.51	4.82	0.74	−0.42	13.07	−4.46	0.74
	720	3.65	5.70	0.80	−1.73	19.48	−6.46	0.74
IWUE–N_a	360	0.72	1.50	0.80	−0.21	3.94	−1.24	0.75
	720	2.26	3.55	0.76	0.27	9.22	−2.81	0.77
P_n–N_m	360	2.67	0.16	0.75	−1.57	0.56	−0.0076	0.71
	720	4.61	0.18	0.63	−5.24	1.14	−0.018	0.80
IWUE–N_m	360	0.73	0.052	0.79	−0.054	0.15	−0.0019	0.75
	720	2.14	0.15	0.81	0.41	0.36	−0.0042	0.80

will acclimate to increases in atmospheric [CO$_2$] even on nutrient-rich sites. However, even in the lowest N-supply regime, photosynthetic down-regulation was less than 60% and the photosynthetic rate of seedlings was higher in elevated [CO$_2$] than in ambient [CO$_2$] in all the N treatments, suggesting that white birch will likely benefit from increases in atmospheric [CO$_2$] across all sites, and the benefit will increase with increasing soil fertility.

Our observation of down-regulation of photosynthesis in the high N treatments contrasts with the finding of Zhang and Dang (2006) for the same species where no down-regulation occurred in response to a doubling of the ambient [CO$_2$] in a high-nutrient treatment. The two experiments were carried out in the same facility, and the foliar N concentrations are compa-
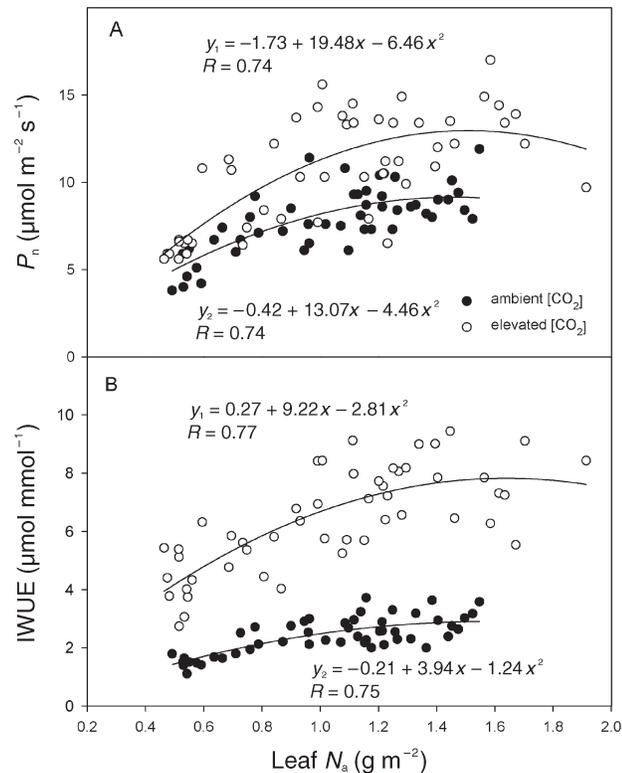

Figure 5. Relationships between light-saturated rate of photosynthesis at the growth carbon dioxide concentration (P_n), photosynthetic water-use efficiency (IWUE) and area-based leaf nitrogen concentration (N_a) in birch seedlings after 80 days of CO$_2$ and nitrogen treatments.
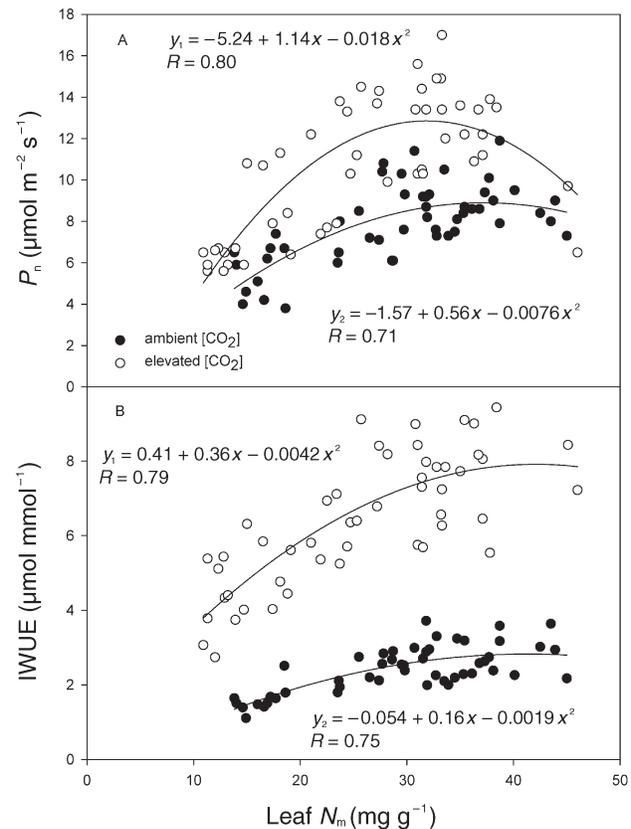

Figure 6. Relationship between light-saturated rate of photosynthesis at the growth carbon dioxide concentration (P_n), photosynthetic water-use efficiency (IWUE) and mass-based leaf nitrogen concentration (N_m) in birch seedlings after 80 days of CO$_2$ and nitrogen treatments.

rable between the two studies. Seedling age and treatment protocols (including environmental conditions, pot size and shape and growing medium) are also comparable between the two studies except for the nutrient ratios. Zhang and Dang (2006) kept the ratios of N to P and K constant between the low and high nutrient treatments, whereas we kept the concentrations of P and K constant in all the N treatments and so the ratios of N to P and K increased with increasing N in our study. A similar phenomenon has been observed in studies of Douglas-fir (*Pseudotsuga menziesii*) by Manter et al. (2005) and Ripullone et al. (2003). The ratios of N to other nutrient elements were kept constant among different nutrient treatments in the study by Ripullone et al. (2003), who found that the rate of photosynthesis increased linearly with increasing foliar N concentration. In contrast, Manter et al. (2005) kept the concentrations of other nutrient elements constant among treatments (thus varying ratios of N to other nutrient elements) and found a curvilinear relationship between photosynthesis and foliar N concentration. These results suggest that nutrient ratios influence the relationship between photosynthesis and foliar N concentration and the acclimation of photosynthesis to elevated [CO$_2$]. Several researchers (Brooks 1986, Nakaji et al. 2001, Warren and Adams 2002) have reported that foliage protein concentrations decline as foliage N concentration increases as a result of limitations by other nutrient elements, such as P, K or S. Stitt (1996) and Nakaji et al. (2001) report that N:P ratios influence both gene activity and synthesis of photosynthetic enzymes.

The simple linear and the second-order polynomial regression functions generally performed equally well in describing the relationships between P_n or IWUE and leaf N concentration, except for the relationship between P_n and N_m in elevated [CO$_2$], where the polynomial function gave a much better fit to the data. Both linear (Kellomäki and Wang 1997, Nakano et al. 1997, Peterson et al. 1999, Ripullone et al. 2003, Ellsworth et al. 2004) and curvilinear (Evans 1983, DeJong and Doyle 1985, Terashima and Evans 1988, Cheng and Fuchigami 2000, Manter et al. 2005) patterns are documented in the literature either when the leaf N concentration is expressed on a leaf area basis (Evans 1983, DeJong and Doyle 1985, Terashima and Evans 1988, Kellomäki and Wang 1997, Peterson et al. 1999, Cheng and Fuchigami 2000, Ripullone et al. 2003, Ellsworth et al. 2004) or on a leaf mass basis (Peterson et al. 1999, Manter et al. 2005). Theoretically, if the range of foliar N concentrations is wide enough, the relationship should be curvilinear and consist of four phases: (1) a deficiency phase where the rate of photosynthesis is limited by N and increases linearly with increasing foliar N; (2) an optimum phase where foliar N concentration is optimal for photosynthesis and the rate of photosynthesis peaks; (3) a phase of superfluous N uptake, in which additional N is stored in the leaf with no impact on the rate of photosynthesis; and (4) a toxicity phase, where further N uptake causes the rate of photosynthesis to decline (Landis et al. 1989, Salisbury and Ross 1992). Besides toxicity, other mechanisms can also cause a reduction in

photosynthetic rate when excess N is supplied, such as an increase in CO$_2$ transfer resistance between the intercellular spaces and the chloroplast (Evans 1983, Terashima and Evans 1988), a decline in total Rubisco content because gene activity and protein synthesis are influenced by high N:P ratios, low P concentrations and high carbohydrate concentrations (Stitt 1996, Nakaji et al. 2001) and a decline in activated Rubisco content associated with concomitant nutrient limitations (Cheng and Fuchigami 2000). We believe that the various response patterns reported by different authors reflect different portions of the response curve examined rather than differences in the form of the overall response function. In our study, most data fell in the linear portion of the response curve with only a few points falling on the curvilinear portion. Thus, the linear and curvilinear models performed similarly in fitting the data.

Our results do not support the hypothesis that elevated [CO$_2$] increases the slope when the P_n–N relationship is linear and reduces the optimal foliar N concentration for photosynthesis when the P_n–N relationship is curvilinear. Elevated [CO$_2$] had no significant effect on the slope of the P$_n$–N regression regardless of whether leaf N concentration was expressed on an area or a mass basis. We found that elevated [CO$_2$] decreased the optimal N_m for photosynthesis from 37.00 to 31.88 mg g^{-1}, a 14% reduction. However, close examination of Figure 6A shows that the P_n–N curve in elevated [CO$_2$] turned downward primarily because of points in the high N range, and there is no statistical tool available to test the significance of the difference in the optimal leaf-N for photosynthesis. Thus, there are insufficient data in the high N range to draw a definite conclusion about the effect of elevated [CO$_2$] on the optimal foliar N concentration for photosynthesis. We can conclude, however, that elevated [CO$_2$] moved the P_n–N curve upward with no significant impact on the general shape of the relationship.

Elevated [CO$_2$] significantly increased the slope of the IWUE to leaf N concentration relationship. This is probably the result of increased N allocation to the photosynthetic apparatus with increasing leaf N concentration. Because the basic structural needs for N must be satisfied first, the proportion of leaf N allocated to photosynthetic apparatus increases with increasing leaf N (Field and Mooney 1986, Evans 1989) until N uptake becomes superfluous. This explanation is consistent with the linear portion of the P_n–N relationship. Presumably, elevated [CO$_2$] (and the associated increase in intercellular [CO$_2$]) increases the photosynthetic-use efficiency of only that portion of N that is allocated to the photosynthetic apparatus. Consequently, the greater that portion is, the greater the increase in IWUE in response to increasing [CO$_2$], leading to a greater slope of the IWUE–N relationship.

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