Effects of [CO$_2$] and nitrogen on morphological and biomass traits of white birch (*Betula papyrifera*) seedlings

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Abstract

To investigate the interactive effects of CO$_2$ concentration ([CO$_2$]) and nitrogen supply on the growth and biomass of boreal trees, white birch seedlings (*Betula papyrifera*) were grown under ambient (360 µmol mol^{-1}) and elevated [CO$_2$] (720 µmol mol^{-1}) with five nitrogen supply regimes (10, 80, 150, 220, and 290 µmol mol^{-1}) in greenhouses. After 90 days of treatment, seedling height, root-collar diameter, biomass of different organs, leaf N concentration, and specific leaf area (SLA) were measured. Significant interactive effects of [CO$_2$] and N supply were found on height, root-collar diameter, leaf biomass, stem biomass and total biomass, stem mass ratio (SMR), and root mass ratio (RMR), but not on root mass, leaf mass ratio (LMR), leaf to root ratio (LRR), or leaf N concentration. The CO$_2$ elevation generally increased all the growth and biomass parameters and the increases were generally greater at higher levels of N supply or higher leaf N concentration. However, the CO$_2$ elevation significantly reduced SLA (13.4%) and mass-based leaf N concentration but did not affect area-based leaf N concentration. Increases in N supply generally increased the growth and biomass parameters, but the relationships were generally curvilinear. Based on a second order polynomial model, the optimal leaf N concentration was 1.33 g m^{-2} for height growth under ambient [CO$_2$] and 1.52 g m^{-2} under doubled [CO$_2$]; 1.48 g m^{-2} for diameter under ambient [CO$_2$] and 1.64 g m^{-2} under doubled [CO$_2$]; 1.29 g m^{-2} for stem biomass under ambient [CO$_2$] and 1.43 g m^{-2} under doubled [CO$_2$]. The general trend is that the optimal leaf N was higher at doubled than ambient [CO$_2$]. However, [CO$_2$] did not affect the optimal leaf N for leaf and total biomass. The CO$_2$ elevation significantly increased RMR and SMR but decreased LMR and LRR. LMR increased and RMR decreased with the increasing N supply. SMR increased with increase N supply up to 80 µmol mol^{-1} and then leveled off (under elevated [CO$_2$]) or stated to decline (under ambient [CO$_2$]) with further increases in N supply. The results suggest that the CO$_2$ elevation increased biomass accumulation, particularly stem biomass and at higher N supply. The results also suggest that while modest N fertilization will increase seedling growth and biomass accumulation, excessive application of N may not stimulate further growth or even result in growth decline.
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1. Introduction

Human activities are causing a steady rise in CO$_2$ concentration ([CO$_2$]) in the atmosphere (IPCC, 1995, 2001). [CO$_2$] elevation can lead to changes in physiological and growth activities of plants, and consequently, changes in the biosphere (Eichelmann et al., 2004). Considerable attention has been devoted to plant physiological and growth responses to elevated [CO$_2$] (Rey and Jarvis, 1998; Roberntz and Stockfors, 1998; Rogers and Humphries, 2000; Jach and Ceulemans, 2000; Zhang and Dang, 2005; Karnosky et al., 2005; King et al., 2005; Cao et al., 2007; Kubiske et al., 2007). The net photosynthetic rate of trees generally increases in response to [CO$_2$] elevation if there are no other environmental factors limiting (Rey and Jarvis, 1998; Long et al., 2004; Karnosky et al., 2005). However, because the growth and photosynthesis of plants are influenced by many environmental factors, such as light, temperature, nutrition, and [CO$_2$], their responses to elevated [CO$_2$] are complex and depend on many physiological and environmental variables (Seppo and Wang, 1997; Karnosky et al., 2005; King et al., 2005). So plant responses should be studied not only under single factor treatments such as CO$_2$, but also under multifactor treatments.

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Nitrogen (N) is one of the key resources likely to regulate plant response to climate change (Lewis et al., 2004). N is often the primary factor limiting plant growth under current climatic conditions (Bormann and Likens, 1967; Vitousek and Howarth, 1991). Although high leaf [N] is associated with high rates of photosynthesis and high tree productivity, excessive amount of N reduces tree productivity (Manter et al., 2005). Declines in tree's growth response to elevated $[CO_2]$ with decreasing N availability has been demonstrated in trees grown in both growth chambers and the field (Stitt and Krapp, 1999; Harmens et al., 2000; Oren et al., 2001; Poorter and Pérez-Soba, 2001). However, since nearly all previous studies are conducted under two N supply regimes (Kellomäki and Wang, 1997b; Davey et al., 1999), it is difficult to evaluate the effect of elevated $[CO_2]$ on the growth–nitrogen relationship. This study investigated the responses of morphological and biomass traits in white birch (Betula papyrifera Marsh.) seedlings to elevated $[CO_2]$ under five different N levels. We examined the differences in the relationships between these traits and N supply under the current ambient and doubled $[CO_2]$.

2. Materials and methods

2.1. Materials and experimental design

The experiment was conducted in the Lakehead University greenhouses. White birch seeds (B. papyrifera Marsh.) were sown in horticultural trays filled with a mixture of peat moss and vermiculite (1:1, v/v). The germination completed within 10 days. After 6 weeks of growth in the tray under ambient $[CO_2]$, 180 seedlings of relatively uniform size were transplanted into PVC containers (21 cm height × 20.8 cm in top diameter) on April 26, 2005. The growing medium was a mixture of peat moss and vermiculite (1:1, v/v).

The experiment was comprised of two CO_2 treatments (ambient (360 µmol mol^{-1}) versus doubled (720 µmol mol^{-1})) and five nitrogen levels (10, 80, 150, 220, and 290 mg N L^{-1}). The phosphorus and potassium concentrations were 60 and 150 mg N L^{-1}, respectively, for all the treatments. The seedlings were fertilized once a week from April to June and twice a week afterwards. The experiment was a split-plot design where the CO_2 treatments were applied to main plots (greenhouses) and nitrogen treatments were applied to sub-plots (randomly located blocks within each greenhouse). The CO_2 treatments were applied independently to each of four environment-controlled greenhouses (two replications per treatment), whereas all the nitrogen treatments were applied to randomly located blocks within each greenhouse. The physical dimensions, layout, and environmental sensors and controllers are identical among the four greenhouses and the environmental conditions of all the greenhouses were monitored and controlled simultaneously by the same computer. There were nine seedlings in each treatment combination. The locations of the seedlings were randomized within each greenhouse. The seedlings were spaced far enough from each other to avoid mutual shading.

The $[CO_2]$ elevation was achieved using Argus CO_2 generators (Argus, Vancouver, BC, Canada). For all the treatments, day/night temperatures were set at 25–26/16–17 °C from April to June and 28–30/18–20 °C thereafter, with a 16-h photoperiod (on cloudy days, and early in the mornings and late in the evenings, natural light was supplemented with artificial light from high-pressure sodium lamps). The volumetric moisture content of the growing medium was maintained around 30% as measured with a HH2 Moisture Meter (Delta-T Devices, Cambridge, UK). Environmental conditions were monitored and controlled with an Argus control system (Argus, Vancouver, Canada). The experiment lasted for 90 days.

2.2. Measurements of growth and biomass

Five seedlings were selected randomly from each treatment for the measurement of total height and root-collar diameter. Five mature leaves were selected from each seedling and the area for each leaf was measured using a Win Folia system (Regent Instrument Inc., Canada). The seedlings were then harvested and oven-dried at 70 °C for 48 h. The dry mass of foliage, roots, and stems were measured separately on an electronic balance. The leaves for the leaf area measurement were measured separately from other leaves on the seedling.

2.3. Leaf nitrogen assay

The total nitrogen concentration of the sample leaves for area measurements was determined using the dry combustion method with a CNS-2000 (LECO Corporation, USA).

2.4. Data analysis

The data were analyzed using analysis of variance (ANOVA) with SAS statistics package (SAS Institute, Cary, NC). The ANOVA assumptions were tested before the analysis was conducted. The comparison of means was conducted using the LSD where ANOVA showed a significant effect. The relationships between growth and biomass parameters and leaf nitrogen concentration were modeled using a second order polynomial function and linear regressions.

3. Results

3.1. Growth

The $[CO_2]$ and nitrogen treatments had significant interactive effects on the seedling height, root-collar diameter (Fig. 1), and the size (area) of mature leaves (Fig. 2a), but not on the specific leaf area (Fig. 2b). Under the ambient $[CO_2]$ (360 µmol mol^{-1}), seedling height was significantly smaller at the 10 µmol mol^{-1} N supply than at higher N supplies, but there were no significant differences in seedling height among other N supply levels (Fig. 1a). Under doubled $[CO_2]$ (720 µmol mol^{-1}), the seedlings were significantly shorter at 10 than at 80 µmol mol^{-1} N, and at 80 than at 150, 220, and
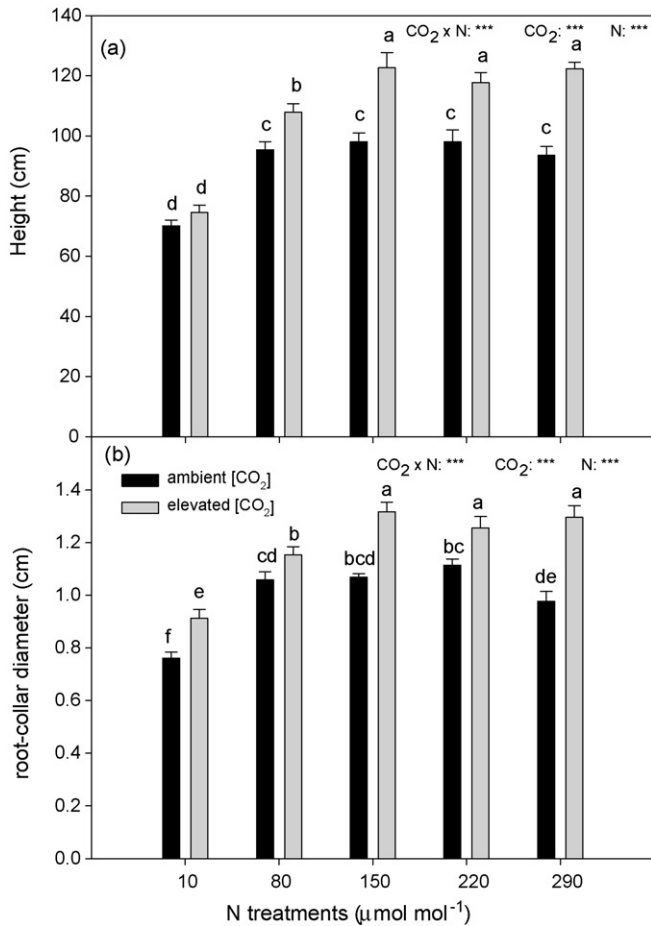
Fig. 1. Height (a) and root-collar diameter (b) of birch seedlings grown at five different levels of N supply and two [CO_2] for 90 days (means ± S.E., $n = 10$). Bars with different letters are significantly different (LSD, $P < 0.05$). (ns) $P \geq 0.05$; [*]$P < 0.05$; [**]$P < 0.01$; [***]$P < 0.001$.
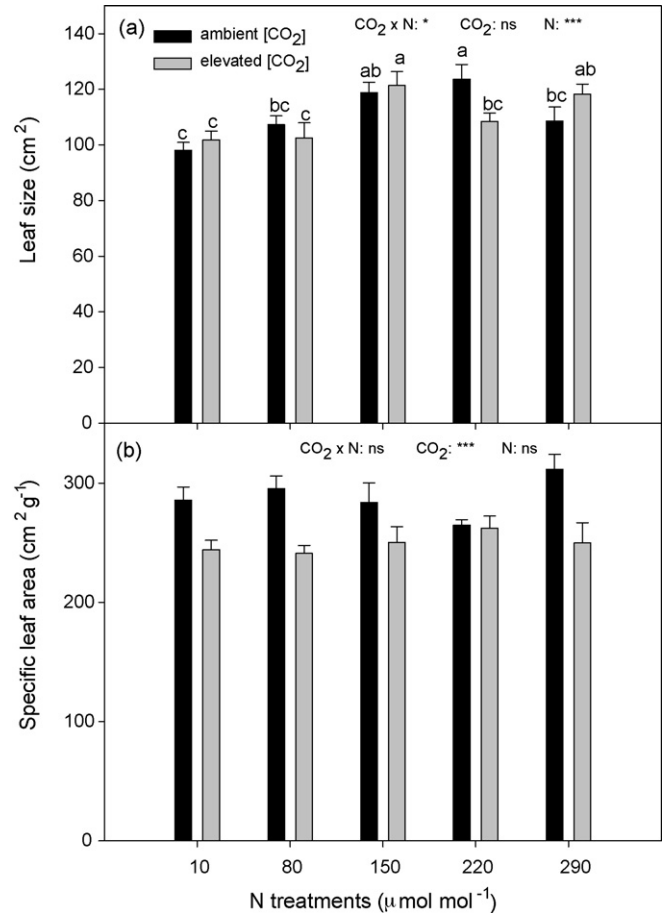

Fig. 2. Leaf size (a) and specific leaf area (b) of birch seedlings grown at five different levels of N supply and two [CO_2] for 90 days (means ± S.E., $n = 10$). Bars with different letters are significantly different (LSD, $P < 0.05$). (ns) $P \geq 0.05$; [*]$P < 0.05$; [**]$P < 0.01$; [***]$P < 0.001$.

290 μmol mol^{-1} N, but there were no significant differences in seedling height among 150, 200, and 290 μmol mol^{-1} N treatments (Fig. 1a). The [CO_2] elevation produced significantly taller seedlings only under 80 μmol mol^{-1} or higher N supplies, but not under the 10 μmol mol^{-1} N treatment (Fig. 1a). On average, the seedlings in the elevated [CO_2] were 20% taller than those in the ambient CO_2 treatment.

The intermediate N treatments (80, 150, and 220 μmol mol^{-1} N) resulted in the highest diameter growth while the lowest N treatment (10 μmol mol^{-1} N) resulted in the smallest diameter growth under the ambient [CO_2] treatment, while the diameter was significantly smaller at 10 than at 290 μmol mol^{-1} N (Fig. 1b). Under the elevated [CO_2], however, the response pattern of root-collar diameter was similar to that of height: the diameter was significantly smaller at 10 than under 80 μmol mol^{-1} N, at 80 μmol mol^{-1} N than at higher N levels while there were not significant differences in root-collar diameter among the other three N levels (150, 220, and 290 μmol mol^{-1} N, Fig. 1b). The root-collar diameter was significantly larger under the elevated than the ambient [CO_2] in all N treatments (Fig. 1b). On average, the root-collar diameter in seedlings grown under the elevated [CO_2] was 19% larger than that of seedlings in the ambient CO_2 treatment.

There were significant interactions between [CO_2] and N on leaf size (area per leaf). However, there were no general trends evident (Fig. 2a). The CO_2 elevation significantly reduced the specific leaf area (by 13%), but the N treatments had no significant influence on this parameter (Fig. 2b).

3.2. Biomass

There were significant interactive effects of [CO_2] and N treatments on leaf mass, stem mass and total biomass, but not on root mass (Fig. 3). Under the ambient [CO_2], the leaf, stem, and total biomass were significantly lower at 10 μmol mol^{-1} N than at higher N treatments and there were no significant differences among the other N treatments (Figure 3). Under the doubled [CO_2], the leaf, stem, and total biomass were significantly lower at 10 than at 80 μmol mol^{-1} N, lower at 80 μmol mol^{-1} N than at higher N levels and there were no significant differences amount 150, 220, and 290 μmol mol^{-1} N levels (Fig. 3). The trends for the root biomass were similar to the above, but the interactions between CO_2 and N were not statistically significant ($P > 0.05$, Fig. 3c). However, the main effects of CO_2 and N were both highly significant ($P < 0.001$). The elevated [CO_2] resulted in significantly higher
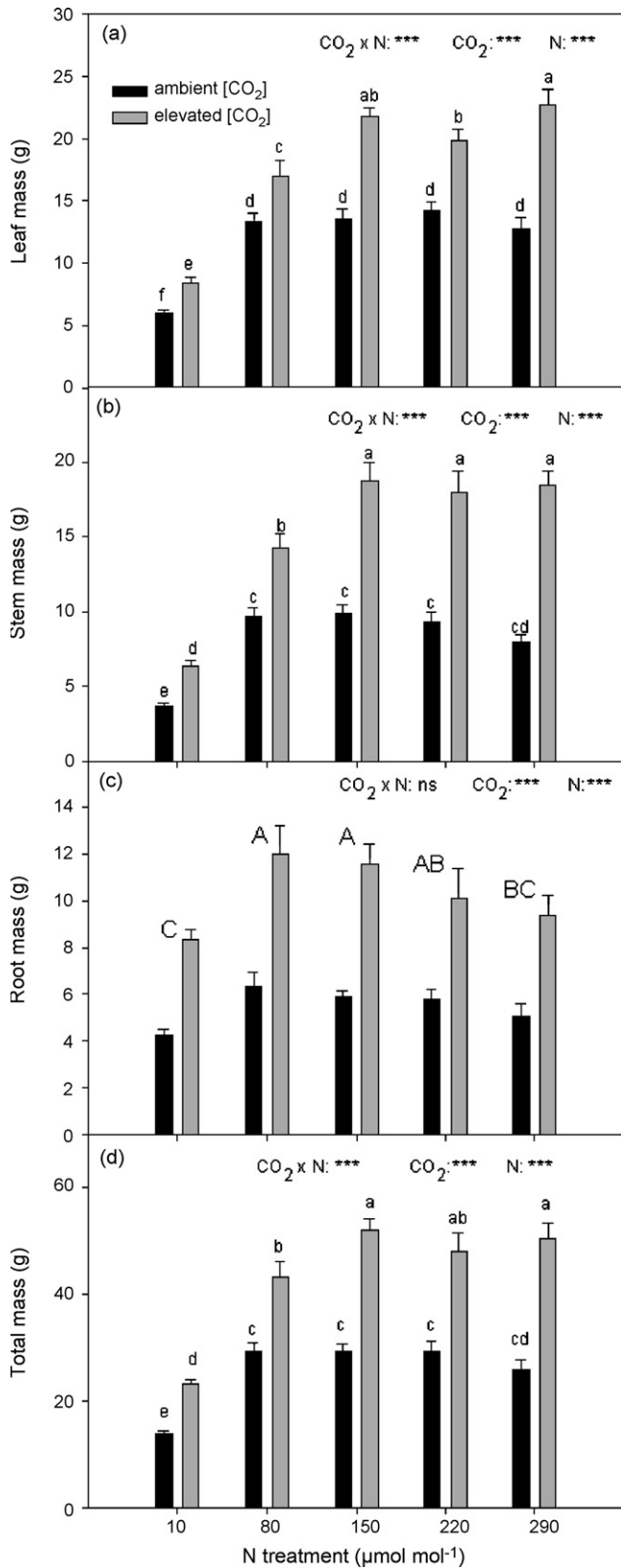
Fig. 3. Leaf mass (a), stem mass (b), root mass (c), and total mass (d) of birch seedlings grown at five different levels of N supply and two [CO_2] for 90 days (means ± S.E., $n = 10$). Bars with different letters are significantly different (LSD, $P < 0.05$). Lower case letters are for interactions and upper case letters are for nitrogen effects. (ns) $P \geq 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
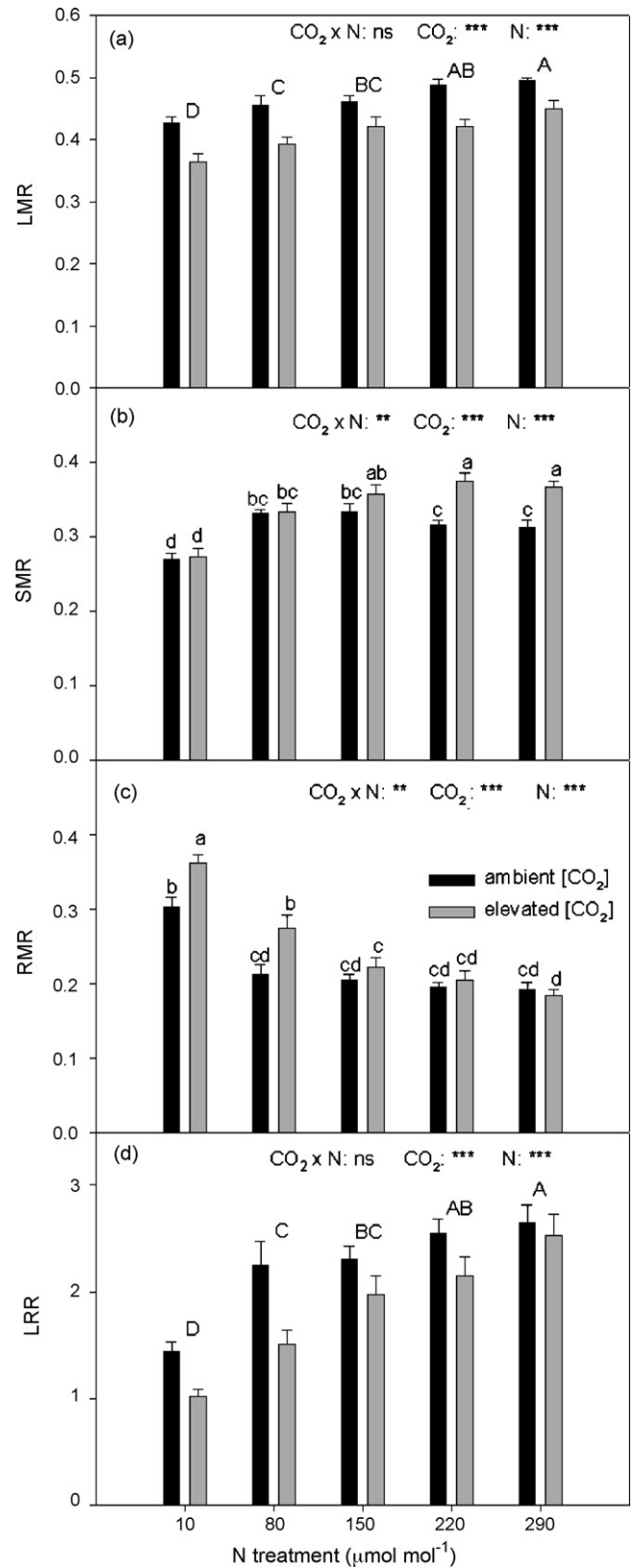
Fig. 4. Leaf mass ration (LMR), stem mass ratio (SMR), root mass ratio (RMR), and leaf to root ratio (LRR) of birch seedlings grown at five different levels of N supply and two [CO_2] for 90 days (means ± S.E., $n = 10$). Bars with different letters are significantly different (LSD, $P < 0.05$). Lower case letters are for interactions and upper case letters are for nitrogen effects. (ns) $P \geq 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
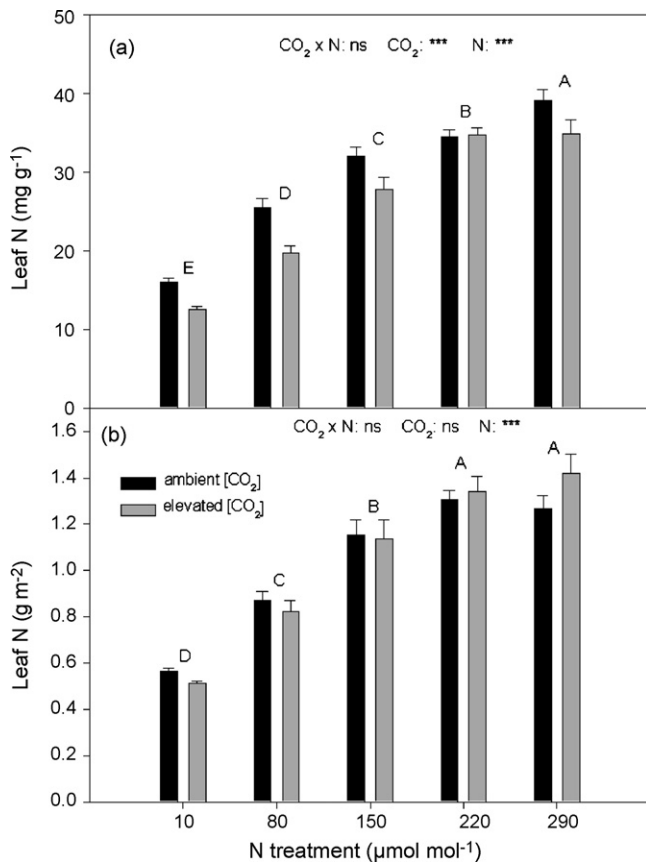
Fig. 5. Leaf N per unit mass (a) and area (b) of birch seedlings grown at five different levels of N supply and two [CO_2] for 90 days (means ± S.E., $n = 10$). Bars with different letters are significantly different (LSD, $P < 0.05$). The upper case letters are for nitrogen effects. (ns) $P \geq 0.05$; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

root biomass in all N treatments (Fig. 3c). The intermediate N levels (80 and 150 μmol mol^{-1}) resulted in highest root biomass and root biomass declined as N levels increased or decreased (Fig. 3c).

3.3. Mass ratios

The [CO_2] elevation significantly increased the leaf mass ratio (LMR) (Fig. 4a). The increases in N supply generally increased LMR but the differences between two adjacent levels of N were not always statistically significant (Fig. 4a).

There were significant interactive effects of N and CO_2 on the stem mass ratio (SMR) (Fig. 4b). The elevated [CO_2]

significantly increased SMR at 220 and 290 μmol mol^{-1} but not at lower N supplies (Fig. 4b). SMR was significantly lower at the lowest N supply (10 μmol mol^{-1}) than other N supplies under both [CO_2], but the differences between other N levels were less obvious and more complex (Fig. 4b).

There were also significant interactive effects of CO_2 and N on the root mass ratio (RMR). While the CO_2 elevation generally increased RMR, the difference was statistically significant only at the two lowest N levels (i.e., 10 and 80 μmol mol^{-1}, Fig. 4c). RMR generally declined with increasing N supply, but the decline was the most significant at 10, 80, and 150 μmol mol^{-1} (Fig. 4c).

The [CO_2] elevation significantly decreased the leaf/root mass ratio (LRR) (Fig. 4d). LRR generally increased with increasing N supply (Fig. 4d).

3.4. Leaf nitrogen concentration

Increasing N supply significantly increased both area- and mass-based leaf N concentrations (Fig. 5). The CO_2 elevation significantly reduced mass-based leaf N concentration, but not area-based leaf N (Fig. 5).

3.5. Relationships between growth/biomass parameters and leaf N concentration

Both linear regression and the second order polynomial model were highly significant ($P < 0.001$) for describing the relationships between growth/biomass parameters and leaf nitrogen concentration. However, the second order polynomial function performed better than linear regression as indicated by the much larger correlation coefficients for the model than for linear regressions (Table 1, Figs. 6 and 7). The correlation coefficients ranged from 0.64 to 0.84 for the second order polynomial model and from 0.58 to 0.76 for the linear regression.

4. Discussion

Both linear regression and second order polynomial performed well in describing the relationships between the growth/biomass parameters and foliage nitrogen concentration in white birch seedlings. However, the much higher correlation coefficients suggest that the second order polynomial is more appropriate. The relationship between physiological/growth

Table 1
Linear regressions for relationships between grown/biomass and leaf nitrogen concentration

Parameter	Elevated [CO_2]			Ambient [CO_2]		
	Intercept	Slope	Correlation coefficient	Intercept	Slope	Correlation coefficient
Height	66.34	40.79	0.76	64.23	25.98	0.58
Diameter	0.85	0.33	0.67	0.66	0.32	0.67
Leaf mass	7.34	10.10	0.66	3.32	8.37	0.70
Stem mass	5.01	9.68	0.65	2.72	5.22	0.58
Total biomass	22.30	20.09	0.59	10.17	14.89	0.61

All the regressions were statistically highly significant ($P < 0.001$).
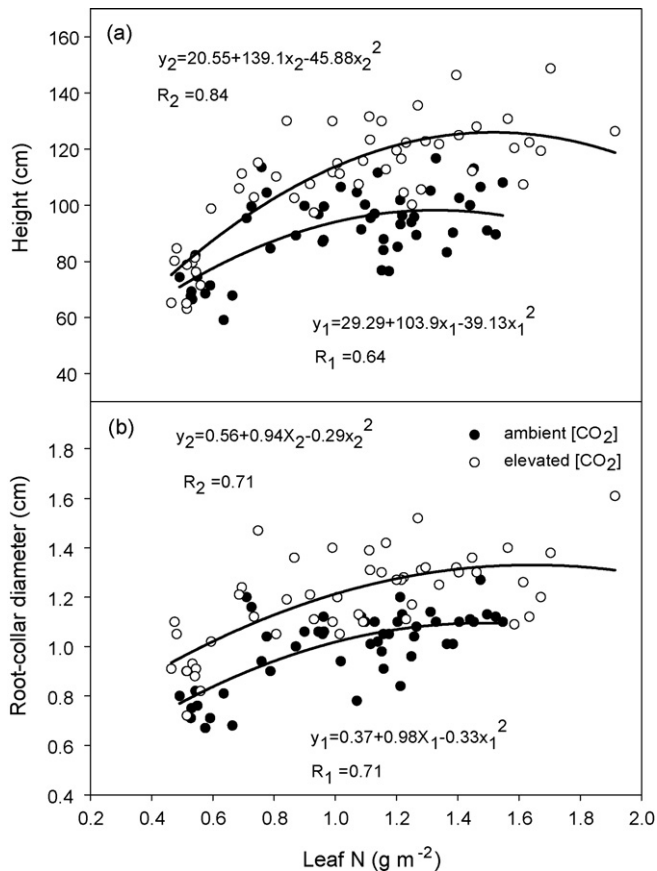
Fig. 6. Relationship of height and root-collar diameter with area-based leaf nitrogen concentration of birch seedlings grown at five different levels of N supply and two $[CO_2]$ for 90 days.
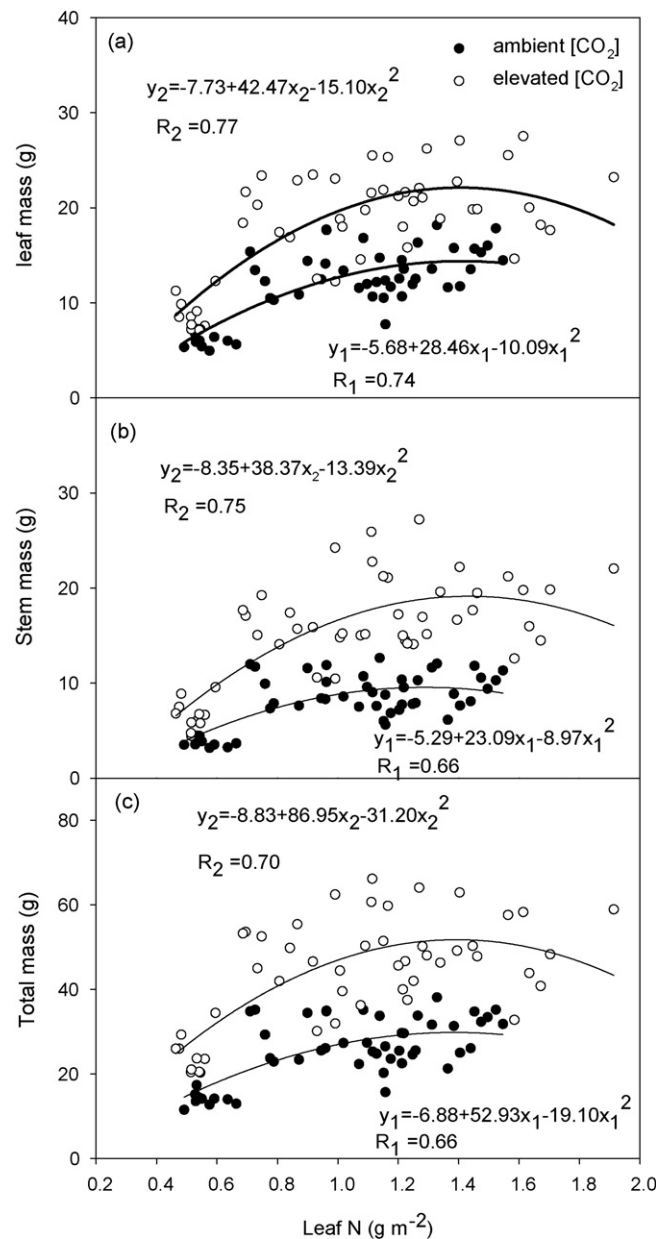

Fig. 7. Relationship of leaf mass (a), stem mass (b), and total mass (c) with area-based leaf nitrogen concentration of birch seedlings grown at five different levels of N supply and two $[CO_2]$ for 90 days.

parameters and leaf nitrogen can be linear or curvilinear, depending on the range of leaf nitrogen and the ratios between nitrogen and other nutrient elements. Over a wide enough range, the relationship generally consists of an upward linear section, a plateau and a downward section (Landis et al., 1989; Salisbury and Ross, 1992). Both linear (Kellomäki and Wang, 1997b; Nakano et al., 1997; Peterson et al., 1999; Ripullone et al., 2003; Ellsworth et al., 2004) and curvilinear relationships (Evans, 1983; DeJong and Doyle, 1985; Terashima and Evans, 1988; Cheng and Fuchigami, 2000; Manter et al., 2005; Cao et al., 2007) are reported in the literature. We believe that the differences in the shape of the response curves in the literature reflect differences in the range of nitrogen used in each study and thus the portion of the response curve examined. Within the same range, the relationship tends to be linear when the ratios of nitrogen to other nutrient elements are kept constant among different N treatments (Ripullone et al., 2003) but curvilinear when the concentrations of other nutrient elements are constant and thus the ratios decline with increasing N levels (Manter et al., 2005; Cao et al., 2007). The latter is the case in this study. The curvilinear relationships found in this study are consistent with our previous findings on the relationships between physiological traits and leaf nitrogen concentration in the same species (Cao et al., 2007). Readers are referred to Cao et al. (2007) for a more comprehensive discussion on the mechanisms governing the shape of physiological response curves to nitrogen. Additionally,

both this and our earlier study (Cao et al., 2007) show that trees grown under different CO_2 concentrations followed different curves in regard to the above relationships. These results suggest that modelers need to be cautious when using nitrogen to scale physiological/growth parameters from leaf to tree/canopy or from tree to ecosystem levels under future climate conditions involving elevated $[CO_2]$. Nitrogen is commonly used for such up-scaling (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).

It was worthy to note that the CO_2 elevation reduced LMR but increased the SMR and RMR with increasing N supply. The SMR was increased primarily at high nitrogen levels (150, 220,

and 290 μmol mol^{-1}). At the same time, higher N supply significantly increased the LMR, while decreased the RMR irrespective of ambient or double [CO_2]. But for SMR, it was increased by nitrogen treatments under doubled [CO_2] and reduced under ambient [CO_2]. All these results suggest that the CO_2 elevation stimulated the seedlings to allocate more biomass to stems and roots rather than to leaves, and the biomass allocated to the root was reduced with high levels of [N] in soil.

Leaf [N] often increased with increasing soil N availability because of increased N uptake by plants (Kellomäki and Wang, 1997a,b; Hobbie et al., 2001). However, our data show that the CO_2 elevation significantly decreased the leaf N per unit mass ($p < 0.01$) by 11.2%. A possible accumulation of carbohydrates in leaves was the most likely reason for this effect. The accumulation of carbohydrates in the leaves under elevated CO_2 concentration is a universal phenomenon in all C_3 plants that have been studied (Drake et al., 1997). Such an accumulation would presumably dilute the N content per unit leaf mass but should not affect area-based N concentration. The significant decline in specific leaf area in response to the CO_2 elevation and the fact that the CO_2 elevation did not significantly affect the area-based leaf N concentration in this study can be considered as indirect evidence supporting the above argument. Roberntz and Stockfors (1998) have found that CO_2 elevations can result in a translocation of nitrogen from leaf to other organs of the tree, resulting in a decrease in leaf nitrogen concentration. However, we do not think that it is the case in this study because there was no significant change in the area-based leaf nitrogen concentration in response to the CO_2 treatments.

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