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Elevated CO_2 alters N-growth relationship in spruce and causes unequal increases in N, P and K demands

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ABSTRACT

CO_2 elevation stimulates plant growth, which in turn demands more nutrients to sustain. Since the increase of demand for nitrogen (N), phosphorus (P) and potassium (K) may be in different proportions, the optimal N–P–K ratios at elevated [CO_2] are likely different from those at the ambient [CO_2]. This study investigated the effects of various N supply levels with constant and variable (constant P and K concentrations) N–P–K ratios under ambient and elevated [CO_2] on black spruce (*Picea mariana* Mill. BSP) seedlings. One-year-old seedlings were exposed to two [CO_2] (370 vs. 720 μmol mol^{-1}), two nutrient ratio regimes (constant vs. variable N/P/K ratios) and six N concentrations (10, 80, 150, 220, 290 and 360 μmol mol^{-1}) in four environmentally controlled greenhouses for 3.5 months. Growth response to N varied with [CO_2] and N/P/K ratios: under the elevated [CO_2], height growth increased with increasing N supply when P and K concentrations were kept constant across different N levels, but it only increased when increasing N from 10 to 150 μmol mol^{-1}, and started to decline with further increase in N supply when N/P/K ratios were kept constant at different N levels; at the ambient [CO_2], height growth was greatest at 150 μmol mol^{-1} N and was generally greater at 220–360 than at 10 and 80 μmol mol^{-1} N in both nutrient ratio treatments. The foliage to root ratio, shoot mass ratio and total biomass generally increased with increasing N supply but root mass ratio decreased. The smallest specific leaf area occurred at the lowest N supply when N/P/K ratios were kept constant but at 220 μmol mol^{-1} N when P and K concentrations were kept constant across different N supplies. The results of leaf nutrient concentrations suggest that the elevated [CO_2] increased demand for N, P and K and the increase for N was greater than P and K, altering the relationship between growth and nitrogen supply. Under the elevated [CO_2], high N supplies resulted in growth suppression by critical toxicity content only in the constant N/P/K ratios treatment but low N supplies led to growth suppression by critical deficiency content in both nutrient ratio treatments. At the ambient [CO_2], in contrast, N/P/K ratio treatments did not affect growth suppression by critical deficiency or critical toxicity content. Because elevated CO_2 causes unequal increases in N, P and K demands, N–P–K ratios should be considered when modeling plant growth responses to elevated CO_2.

1. Introduction

The atmospheric carbon dioxide concentration in the lower atmosphere has been increasing since the beginning of the industrialization and will continue to rise for the rest of the century (IPCC, 2007). Increases in [CO_2] generally promote plant photosynthesis (Tognetti and Johnson, 1999; Taiz and Zeiger, 2002; Long et al., 2004; Ainsworth and Rogers, 2007) and growth (Israel et al., 1990; Saxe et al., 1998; Gavito et al., 2001; Phillips et al., 2009; Cole et al., 2010). Plants require more nutrients to sustain the stimulated growth (Taiz and Zeiger, 2002; Yazaki et al., 2004; Tissue and Lewis, 2010). Without sufficient nutrient supply, especially nitrogen (N), phosphorus (P) and potassium (K), the three most frequently limiting elements in natural soils (Brady and Weil, 2002; Lambers et al., 2008), the increase of growth will diminish (Brown and Higginbotham, 1986). Therefore, a good understanding of the relationship between CO_2 stimulation of growth and nutrient supply is essential for predicting the future trend of plant growth.

Many studies have been primarily focused on the effects of N supply with constant concentrations of K and P (Ingestad, 1979; Brown and Higginbotham, 1986; Griffin et al., 1993; Gavito et al., 2001; Cao et al., 2007, 2008), with only a few maintaining constant N/K and N/P ratios (Ingestad, 1979; Zhang et al., 2006; Ambebe et al., 2010). The purpose of maintaining constant N–P–K ratios

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(CNR) is to avoid N, P and K interactions that can potentially affect plant nutrient uptake and physiological functions (Newbery et al., 1995; Gusewell, 2005). For instance, limited N supply increases plant capacity to absorb N but suppresses the absorption of non-limiting nutrient elements, such as P and sulfur (Chapin III, 1991) and K (Timmer, 1991). On the other hand, high N supply induces K deficiency (van den Driessche and Ponsford, 1995) and high K supply negatively affects N and P uptake (Egilla and Davies, 1995). High N:K ratios reduce plant growth (Barbosa et al., 2000). Plant growth is generally more sensitive to N deficiency than to P (Saidana et al., 2009). These complications, however, can be avoided by maintaining N, P and K ratios constant (Ripullone et al., 2003; Zhang and Dang, 2006).

The adequate concentrations of N, P and K are critical to the physiology and growth of plants (Landis, 1989; van den Driessche, 1991; Marschner, 1995). Plant growth is dramatically reduced when concentrations of nutrient elements fall below the critical deficiency content or above the critical toxicity content (Timmer, 1991; Marschner, 1995; Brady and Weil, 2002). Within the range between the critical deficiency content and critical toxicity content, increasing nutrient supply generally results in greater plant growth, but the response patterns vary with elements (Timmer, 1991; Marschner, 1995). For example, increasing N supply produces gradual increases in plant growth, whereas increasing P and K results in a more dramatic growth increase within the lower range of availability (Epstein, 1972; Timmer, 1991; Marschner, 1995; Epstein and Bloom, 2005). Thus, the appropriate ratios among nutrient elements change with nutrient concentrations. To avoid growth suppressions, the concentration of each nutrient element needs to be maintained between the critical deficiency content and the critical toxicity content (Marschner, 1995; Brady and Weil, 2002) and the supply ratios among different nutrient elements should be adjusted accordingly.

Optimal nutrient ratios may also be altered by elevated [CO_2]. Under elevated [CO_2], plants generally need more nutrients to support growth as it was mentioned here above, but the demand of increase may vary with elements. If the increase is not proportional, the optimal ratios of different nutrient elements will be changed. Research has indicated that the increase in demand is greater for N than P and K (Epstein, 1972; Timmer, 1991; Marschner, 1995; Epstein and Bloom, 2005) and plant growth is generally less sensitive to high P and K than to high N (Epstein, 1972; Timmer, 1991; Marschner, 1995; Epstein and Bloom, 2005). Consequently, increasing N, P and K to the same extent (i.e. maintain N/P/K ratio constant) in response to the higher N demand under elevated [CO_2] may result in P and K exceeding their critical toxicity content (Timmer, 1991; Marschner, 1995).

The interactive effects of [CO_2] and nutrient supply on plant physiology and growth have been studied extensively, but little consideration has been paid to nutrient ratios (Brown and Higginbotham, 1986; Johnsen, 1993a; Roberntz and Stockfors, 1998; Gavito et al., 2001; Cao et al., 2008; Ambebe et al., 2010). Elevated [CO_2] and high N supplies increase plant biomass production (Brown and Higginbotham, 1986; Griffin et al., 1993; Johnsen, 1993a; Elkohen and Mousseau, 1994; Zhang and Dang, 2006). Low P supply reduces photosynthetic rates under both ambient and elevated [CO_2] (Campbell and Sage, 2006) and deficiency in P reduces the enhancement of photosynthesis by CO_2 elevation (Tissue and Lewis, 2010). CO_2 elevation increases leaf and total biomass of spruce seedlings grown in high N supply, but the positive effect of CO_2 elevation on root biomass only occurs when N supply is low (Brown and Higginbotham, 1986).

To predict plant growth, modelers often establish relationships between nutrient supply and plant growth (McMurtrie, 1991; Dewar and McMurtrie, 1996). Since N is a critical constituent of amino acids, enzymes, nucleic acids, chlorophylls, and hormones in plants (Rook, 1991; Marschner, 1995; Taiz and Zeiger, 2002) and is required in greater quantities than any other mineral elements (Marschner, 1995; Nicodemus et al., 2008), the N level in soil is often used as a key factor in modeling plant growth responses to climate change (McMurtrie, 1991; Dewar and McMurtrie, 1996).The N concentration of plants, especially in leaves, is also used as a major driving factor in models (Hirose, 1988; Verkroost and Wassen, 2005; McMurtrie et al., 2008). Because of the interactions among nutrient elements and the effect of [CO_2] on optimal nutrient ratios, the effects of N on plant response to CO_2 elevation will likely vary with P and K supply. However, it is unclear how plants will respond to N under different P and K supplies under the ambient and elevated [CO_2].

In this study, the growth response of black spruce seedlings was examined at six levels of nitrogen supply and two nutrient ratios (constant N–P–K ratios and constant P and K concentrations (thus variable N–P–K ratios)) and two levels of [CO_2] (ambient 370 and elevated 720 μmol mol^{-1}). Since CO_2 elevation and high N supply can have a synergistic effect (Wang et al., 1995; Zhang et al., 2006), which may result in increased nutrient demands with altered N–P–K ratios, we hypothesize that under the elevated [CO_2], increasing N would increase seedling growth, particularly, at high N levels, to a greater extent in the constant P–K concentrations than constant N–P–K ratios due to the possible growth suppression by the toxic effects of P and K at high N levels in constant N–P–K ratios treatment, while low N supply would suppress growth at both the ambient and elevated [CO_2] due to N deficiency regardless of nutrient ratio treatment. We further hypothesize that changes in nutrient ratios would affect the relationship between growth and N supply.

2. Materials and methods

2.1. Plant materials

One-year-old black spruce seedlings were obtained from Hill's Tree Seedling Nursery in Thunder Bay, Ontario. The seedlings were relatively uniform in size at the beginning of the experiment (height = 22.8 ± 0.16 cm, root collar diameter = 2.05 ± 0.02 mm). The seedlings were potted (13 cm height, 12 cm diameter) with a mixture of peat moss and vermiculite (1:1, v/v).

2.2. Experiment design

The experiment was carried out in four independent environment-controlled greenhouses with identical design and dimensions in the Forest Ecology Complex at the Thunder Bay Campus of Lakehead University. The treatments were two CO_2 concentrations (370 vs. 720 μmol mol^{-1}), two nutrient ratio regimes (constant vs. variable nutrient ratios) and six nitrogen concentrations (10, 80, 150, 220, 290 and 360 μmol mol^{-1}N). The 150 μmol mol^{-1} N supply with an N–P–K ratio of 5/2/5 is considered as the optimal nutrient regime for growing tree seedlings in controlled environments (Morrison, 1974; Ingestad, 1979; Landis, 1989; Ingestad and Agren, 1992; Zhang and Dang, 2007). One tenth of the optimal N supply concentration is considered a very low N supply level (Zhang et al., 2006). One tenth of the optimal N supply concentration is considered a very low N supply level (Zhang et al., 2006). In the constant nutrient ratio treatment, the concentrations of P and K varied with N levels to maintain constant N/P/K ratios at 5/2/5. In the variable nutrient ratio treatment, however, N levels were identical to those in the CNR treatment, but P and K concentrations were held constant across all N levels at 60 μmol mol^{-1} P and 150 μmol mol^{-1} K. Each [CO_2] treatment had two independent replicates (greenhouses), with the factorial combinations of nutrient ratio and N levels nested within the [CO_2] treatment. There were four seedlings in each treatment combination. The day/night air temperatures were controlled at 25–26/16–17 °C and the photoperiod was set at 16 h (natural daylength was extended using high-pressure sodium lamps when natural day length was less than 16 h) in all the greenhouses. All the experiment conditions (temperature, RH, [CO_2] and light) were monitored and controlled using a computerized Argus control system (Argus Control Systems Ltd., Vancouver, BC, Canada). All the seedlings were fertilized twice a week and watered once every 2 days to maintain water content above 30% (by volume) (Bergeron et al., 2004) in the growing medium. The growing medium water content was measured using an HH2 Moisture Meter attached to ML2X ThetaProbe (Delta-T Devices, Cambridge, UK). When the water content fell close to 30%, an extra watering was applied. The experiment lasted 3.5 months.

2.3. Measurements

Growth responses were measured on three seedlings in each treatment combination (only three seedlings were available in some combinations because of physical damages or mortality). Height and root collar diameter were measured at the beginning and the end of the experiment for determining increments and relative growth rates. The initial and end stem volume (V) was calculated from height (H) and RCD (D) using the following equation (van den Driessche, 1992):

$$V = (\pi D^2/4)H/3 \tag{1}$$

Relative growth rates were determined by dividing increments by the corresponding initial measurements.

At the end of the experiment, the seedlings were harvested and separated into foliage, stem and branches, and roots. The roots were scanned to determine the total root length using WinRHIZO (Regent Instruments Inc., Quebec City, Quebec, Canada), and the current year needles on the main stem were scanned using WinSEEDLE (Regent Instruments Inc., Quebec city, Quebec, Canada) to determine projected leaf area. The samples were then oven-dried at 70 °C for 48 h, to determine the dry mass. Then the foliage-root ratio (foliage mass/root mass), shoot mass ratio (shoot mass/total seedling mass), and root mass ratio (root mass/total seedling mass) were calculated. The samples for leaf area determination were weighed separately. Specific leaf area and specific root length (root length/root mass) were calculated from the projected leaf area, total root length and the corresponding dry mass.

2.4. Foliar nitrogen, phosphorus and potassium assay

The dried current year needles from terminal shoot were used for nutrient assays. Leaf N was determined using the dry combustion method on a CNS-2000 (LECO Corp., St. Joseph, MI, USA). The dried needles (about 0.5 g) were weighed directly on the ceramic crucible and recorded on the microprocessor. The crucible was placed into the autoloader rack for the analysis using the CNS-2000. Phosphorus and potassium concentrations were determined using the nitric/hydrochloric acid digestion method on an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Varian Canada Inc., Mississauga, ON, Canada). A 0.5 g dry sample was digested in 6 mL of nitric acid and 2 mL of hydrochloric acid for 8 h at 90 °C in a block digestor. Distilled water was added to the acid to dilute the mixture to 100 mL. The test tubes were shaken end-over-end to have a well-mixed solution, which was then filtered to remove particles. The P and K concentrations in the clear filtrate were determined on the inductively coupled plasma atomic emission spectrometer (ICP-AES). The mass-based nutrient concentrations were converted to leaf area-based using the specific leaf area.

2.5. Statistical analysis

The experiment was a split-plot design with CO_2 concentrations as main plots and combinations of nutrient ratio and N supply as subplots (the combinations were randomly allocated within each [CO_2]). The data were examined graphically for the normality of distribution (probability plots for residuals) and the homogeneity of variance (scatter plots). Since both of the two assumptions for Analysis of variance (ANOVA) were met, all subsequent analyses were done on the original data. When ANOVA showed a significant effect ($P < 0.05$) for a variable with more than two levels or an interaction, Least Significant Difference (LSD) multiple comparisons were conducted. All the analyses were conducted using the GenStat statistics package, 12th Edition (VSN international Ltd., Hemel Hempstead, UK, 2009).

3. Results

3.1. Height and root collar diameter increment and relative growth rates

The responses of height increment and relative growth rate to N supply were significantly influenced by [CO_2] and nutrient ratios (Table 1). Since the response patterns were similar, only the relative growth rate was presented. Under the elevated [CO_2], height growth increased with increasing N supply when P and K concentrations were kept constant across different N levels, but when N/P/K ratios were kept constant at different N levels it increased only with increasing N from 10 to 150 μmol mol^{-1} and started to decline with further increase in N supply; at the ambient [CO_2], height growth was greatest at 150 μmol mol^{-1} N and was generally greater at 220–360 than at 10 and 80 μmol mol^{-1} N in both nutrient ratio treatments (Fig. 1A). The CO_2 elevation increased height growth in all nutrient treatments, but the magnitude of increase was greater when the N/P/K ratios were kept constant across different nitrogen supplies than when the P and K concentrations were kept constant (Fig. 1A).

The increment and relative growth rate of root collar diameter and the relative growth rate of stem volume showed similar patterns in response to treatments (Table 1) and thus we only present the relative growth rate data for diameter. The responses to N varied with [CO_2] and nutrient ratios (Table 1): At the ambient [CO_2], the slowest growth occurred at 10 and 80 μmol mol^{-1} N levels, and highest at 150 μmol mol^{-1} N. Under the elevated [CO_2], however, the growth increased with increasing N from 10 to 80 μmol mol^{-1} N when P and K concentrations were kept constant but from 10 to 150 μmol mol^{-1} N when N/P/K ratios remained constant, and no further significant increases with further increases in N supply (Fig. 1B). The CO_2 elevation significantly increased diameter and stem volume growth in all nutrient treatments (Fig. 1B).

3.2. Biomass and biomass ratios

Although the foliage to root ratio and root mass ratio were significantly affected by the interaction between CO_2 and N supply (Table 1), the patterns of their responses to N were similar in the two CO_2 treatments: the foliage/root ratio generally increased (Fig. 1C) while the root mass ratio decreased (data not presented) with increasing N supply. N effects on total biomass varied with [CO_2] (Table 1) and the response patterns were similar to those of diameter and stem volume (Fig. 1B and D). The CO_2 elevation increased the total biomass at all but the lowest N supply (Fig. 1D). Total biomass was also significantly greater in treatments with constant P and K concentrations (9.63 ± 0.49 g) than in those with constant N/P/K ratios (8.19 ± 0.46 g, Table 1).

Table 1
P values for the treatment effect of CO_2 concentration (C), nutrient ratio (NR), nitrogen supply (N) and their interactions on growth increments (H_i – height and RCD_i – root collar diameter), relative growth rates (RGR_h – height, RGR_d – root collar diameter, and RGR_v – volume), biomass (TB – total biomass), mass ratios (FRR – foliage to root ratio, RMR – root mass ratio), specific leaf area (SLA), specific root length (SRL), mass-based nutrient concentrations (N_m – N, P_m – P, and K_m – K), and leaf area based nutrient concentration (N_m – N, P_m – P, and K_m – K) in black spruce seedlings.

Growth response	Treatment effects						
	C	NR	N	C × NR	C × N	NR × N	C × NR × N
H_i	**0.034**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
RCD_i	**0.038**	**0.005**	**<0.001**	0.413	**<0.001**	0.542	0.063
RGR_h	**0.052**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
RGR_d	**0.027**	**0.041**	**<0.001**	0.583	**<0.001**	0.204	**0.005**
RGR_v	**0.035**	**<0.001**	**<0.001**	0.097	**<0.001**	0.719	**0.015**
FRR	0.601	0.413	**<0.001**	0.389	**0.006**	0.593	0.706
RMR	0.691	0.239	**<0.001**	0.322	**0.002**	0.842	0.538
TB	0.158	**0.040**	**<0.001**	0.34	**0.006**	0.15	0.796
SLA	**0.031**	0.205	0.333	0.864	0.922	**0.024**	0.316
SRL	0.531	0.249	**0.007**	0.489	0.203	0.572	0.890
N_m	0.162	**0.046**	**<0.001**	0.425	0.708	0.577	0.655
P_m	0.476	**<0.001**	0.575	0.567	0.633	**<0.001**	0.823
K_m	0.078	0.208	**0.001**	0.055	**<0.001**	**<0.001**	0.094
N_a	0.166	**0.05**	**<0.001**	0.368	**0.023**	**0.053**	0.869
P_a	0.079	0.132	**<0.001**	0.645	**0.004**	0.239	0.914
K_a	0.299	0.16	**<0.001**	0.556	0.284	**0.042**	0.403

Bold values indicate $p \leq 0.05$.

3.3. Specific leaf area and specific root length

The specific leaf area was significantly higher at the ambient $[CO_2]$ ($7.62 \pm 0.08\ m^2\ kg^{-1}$) than at the elevated $[CO_2]$ ($6.79 \pm 0.08\ m^2\ kg^{-1}$) (Table 1). The effect of nutrient ratio on specific leaf area varied with the level of N: it was significantly higher when P and K concentrations were kept constant than with constant N/P/K ratios at 10 $\mu mol\ mol^{-1}$N, but the trend was the opposite at 220 $\mu mol\ mol^{-1}$ and higher N levels, while there was no significant difference between the two nutrient ratio regimes at 80 and 150 $\mu mol\ mol^{-1}$ N (Fig. 1E). The specific root length was significantly lower at 150 and 220 $\mu mol\ mol^{-1}$N that at other N levels (Table 1 and Fig. 1F).

3.4. N, P and K concentrations

Mass-based N concentration increased with increasing N supply (Fig. 2A) and were greater when P and K were kept constant than when N/P/K ratios were kept constant across different N levels (19.8 vs. 18.9 $mg\ g^{-1}$, Table 1). The response of leaf area-based N to N supply varied with $[CO_2]$ and nutrient ratio treatment (Table 1). In general, leaf area based N concentration increased with increasing N supply up to 150 $\mu mol\ mol^{-1}$, but the slope of increase was steeper under the elevated than ambient $[CO_2]$ and the CO_2 elevation resulted in higher foliar N concentration at all but the lowest N level (Fig. 2D). The nutrient ratio did not significantly affect foliar N concentration except at the highest N where foliar N concentration was significantly lower in treatments with constant P and K concentrations (Fig. 2D).

The mass-based P concentration was significant lower when N/P/K ratios were kept constant than when P and K concentrations were kept constant only at the lowest N level (Table 1 and Fig. 2B). Leaf area based P concentration increased with increasing N up to 150 $\mu mol\ mol^{-1}$ N in both $[CO_2]$ and was significantly higher under the elevated than ambient $[CO_2]$ in all but the lowest N level (Fig. 2E).

Mass-based and leaf area-based leaf K concentrations responded to N supply differently in different nutrient ratio treatments (Table 1). The most noteworthy result was that at the lowest N level the foliar K concentrations were significantly lower in the constant nutrient ratio treatment while the trend was the opposite when N supply was greater than 150 $\mu mol\ mol^{-1}$ (Fig. 2C and F). Leaf area based K concentration generally increased with increasing N supply, particularly in the constant nutrient ratio treatment (Fig. 2F). The CO_2 elevation significantly reduced mass based foliar K concentration at all but the lowest N level but had no significant effect on leaf area based K concentration (Table 1 and Fig. 2C and F).

4. Discussion

Our data support the hypotheses that the combination of elevated $[CO_2]$ and constant P and K concentrations at high N supply promotes black spruce seedling growth and that a combination of elevated $[CO_2]$ and constant N/P/K ratios at high N supply suppresses growth. As suggested by others, elevated $[CO_2]$ increases growth (Israel et al., 1990; Saxe et al., 1998; Gavito et al., 2001; Phillips et al., 2009; Cole et al., 2010) and therefore demands for nutrients (Taiz and Zeiger, 2002; Yazaki et al., 2004). The growth reduction under the constant N/P/K ratios at high N supply likely resulted from the toxic effects of P and K as the tolerance level of plants to P and K is much lower than that to N (Fig. 3). Height growth appeared to be more sensitive to the toxic effects of P and K than root collar diameter or volume growth, as seen from the differences in height growth between the two nutrient ratio treatments when N supply was at and above220 $\mu mol\ mol^{-1}$ at both ambient and elevated $[CO_2]$. The growth of black spruce seedlings was also suppressed at low N supply, regardless of CO_2 concentrations or nutrient ratios, possibly due to N deficiency. This is consistent with the theory of Marschner (1995) that deficiency or toxicity of a single or more elements (N, P and K) can suppress growth.

The results of the experiment suggest that nutrient ratio is an important factor affecting plant growth and growth response to CO_2 elevations and should be considered in growth prediction models. Although N supply has a strong relationship with growth (Rook, 1991; Marschner, 1995; Taiz and Zeiger, 2002; Nicodemus et al., 2008) and plays a critical role in plant growth (Rook, 1991; Marschner, 1995; Taiz and Zeiger, 2002), the significant growth difference between the two nutrient ratio regimes at same N supply level in this study suggests that the consideration of both nutrient levels and ratios should enhance the precision of growth predictions. Since N supply is the primary element most commonly used by modellers (McMurtrie, 1991; Dewar and McMurtrie, 1996) for predicting plant growth, N–P–K ratios should be considered in those prediction models. However, it should be pointed out that

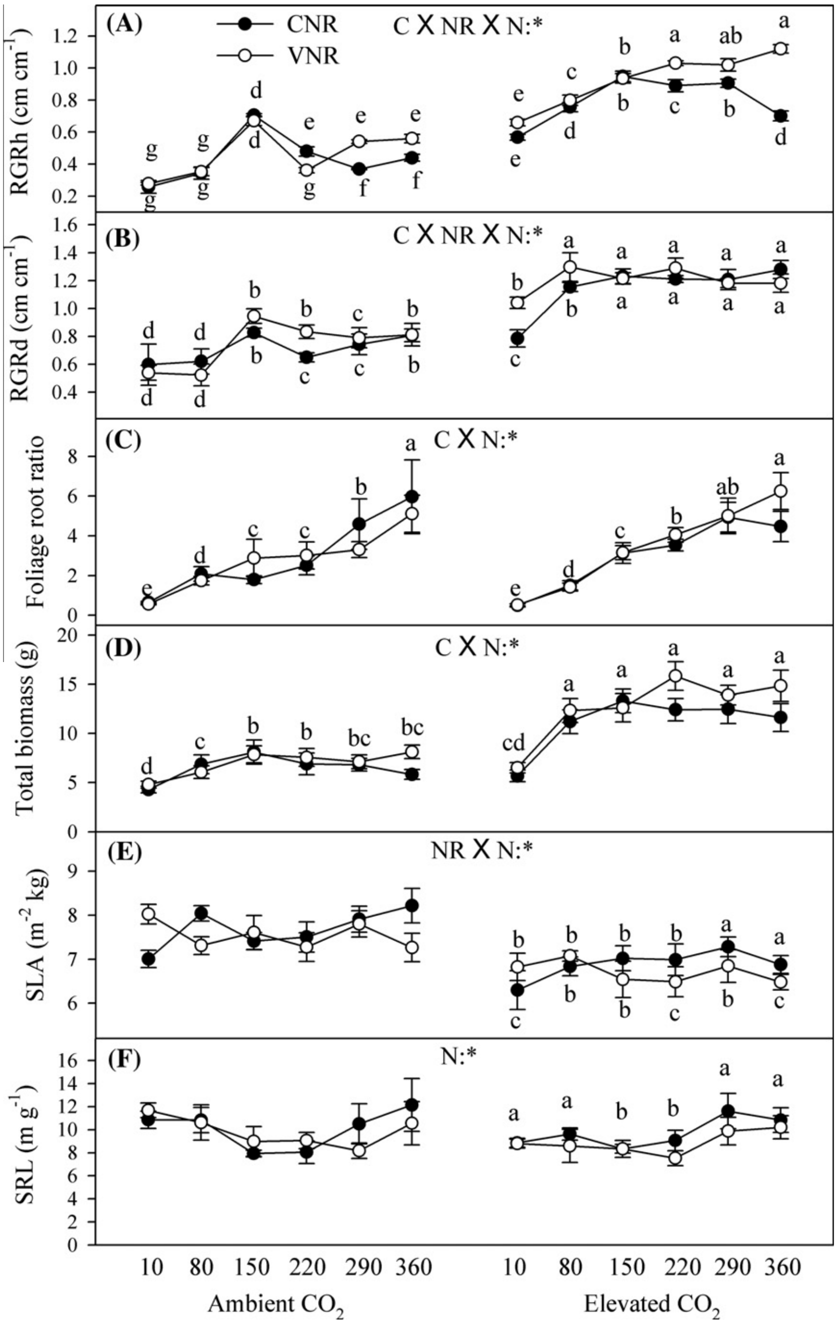

Fig. 1. Mean (±SEM) relative growth rate in height (RGR$_h$), foliage to root ratio, total biomass, specific leaf area (SLA) and specific root length (SRL) of black spruce seedlings grown under two [CO$_2$] (ambient [CO$_2$] = 370 μmol mol^{-1}; elevated [CO$_2$] = 720 μmol mol^{-1}), two nutrient ratios (CNR = constant N/P/K ratios at 5/2/5 and VNR = variable N/P/K ratios with constant concentrations of P at 60 μmol mol^{-1} and K at 150 μmol mol^{-1} and variable N), and 6 N concentrations (10, 80, 150, 220, 290 and 360 μmol mol^{-1}). Significant effects ($P \leqslant 0.05$) are indicated by *. Means with different letters were significantly different from each other ($P \leqslant 0.05$). Significant interactions between factors are indicated by symbol "X".

internal nutrient cycling may have a greater influence on nutrient uptake from the soil in conifers passing the seedling stage (Weatherall et al., 2006), therefore, cautions should be exercised when extrapolating the results from seedlings to large trees. Furthermore, the focus of this study was whether the N/P/K ratios affect black spruce growth as N availability changes. There are currently little data available on the effects of nutrient ratios at various levels of nutrient supply and we hope that this study will inspire more research in this area.

The results suggest that the biomass allocation in black spruce seedlings was primarily affected by N levels. As found by others, low N supplies result in a larger root mass ratio (Stulen and Hertog, 1993) and a smaller leaf to root ratio (Stulen and Hertog, 1993; Stitt and Krapp, 1999), although this relationship varied with CO$_2$ concentration. Pokorny et al. (2010) suggest that CO$_2$ elevation increases shoot growth and low N supply decreases it. In our experiment, the CO$_2$ elevation increased leaf to root ratio at 220 μmol mol^{-1} N, which is in contrast to the observation that CO$_2$ elevation generally does not influence biomass allocations (Bosac et al., 1995; Curtis and Wang, 1998; Riikonen et al., 2004; Zhang et al., 2006; Ambebe and Dang, 2010). However, the effect of N/P/K ratios on biomass allocation was probably too small to be detected, as found in white birch seedlings (Ambebe et al., 2009).

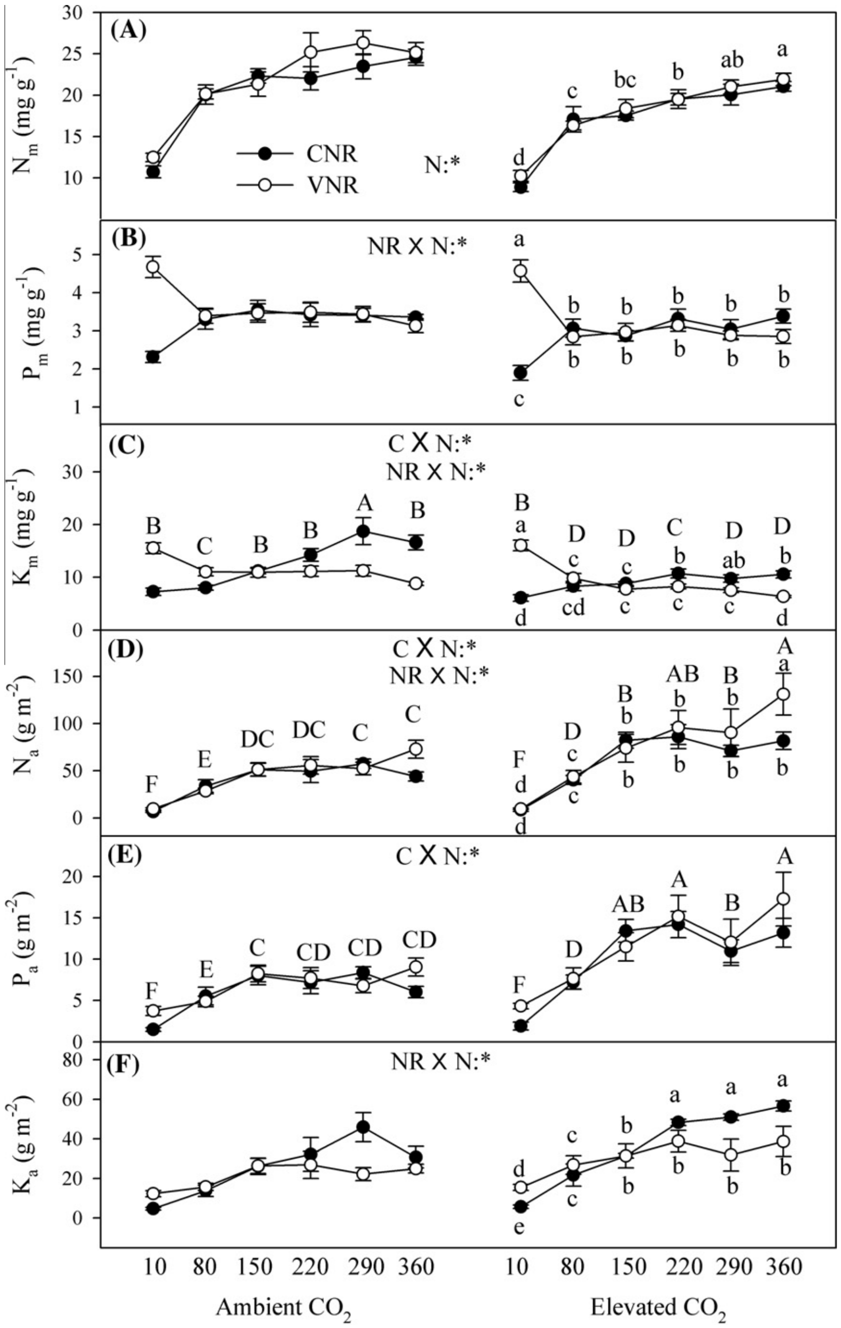

Fig. 2. Mean (±SEM) mass and area based leaf concentrations of nitrogen (N_m and N_a), phosphorus (P_m and P_a) and potassium (K_m and K_a) in black spruce seedlings (see Fig. 1 and text for treatment descriptions). Significant effects ($P \leqslant 0.05$) are indicated by *. Means with different uppercase or lowercase letters were significantly different from each other ($P \leqslant 0.05$). The uppercase letters indicate interactions between [CO_2] and N concentration; the other significant interaction differences are shown in lowercase letters.

Our results on leaf nitrogen concentration indicate that the CO_2 elevation generally increased area-based leaf [N] but had no significant effect on mass-based leaf [N]. The difference in response between area-based and mass-based measurements may be attributable to growth dilution on mass based measurements (Johnsen, 1993b). Some studies have found that that CO_2 elevations reduce both area-based and mass-based leaf [N] (Ellsworth et al., 2004) while others have reported that that CO_2 elevations reduce mass-based but not area-based leaf [N] (Curtis and Wang, 1998; Norby et al., 1999). Our data of increased leaf [N] in the elevated [CO_2] suggest that the CO_2 elevation may have stimulated nitrogen uptake. The fact that the stimulatory effect was detected in leaf-based but not in mass-based measurement may suggest that the CO_2 elevation probably resulted in elevated carbohydrate accumulation in the foliage. Increased carbohydrate accumulation in the foliage may lead to the feedback inhibition and eventually down regulation of photosynthesis (Lambers et al., 2008).

We have drawn a schematic representation (Fig. 3) to describe the relationship between N, P and K supply and the growth of black spruce seedlings under the ambient and elevated CO_2 conditions based on Marschner's theory (1995) on the relationship between growth and nutrient supply, and the data of this study. It is generally believed that elevated [CO_2] raises nutrient demands in greater proportion for N than for P and K (Epstein, 1972; Timmer, 1991; Marschner, 1995; Epstein and Bloom, 2005). The growth is zsuppressed when nutrient supply is below the critical deficiency

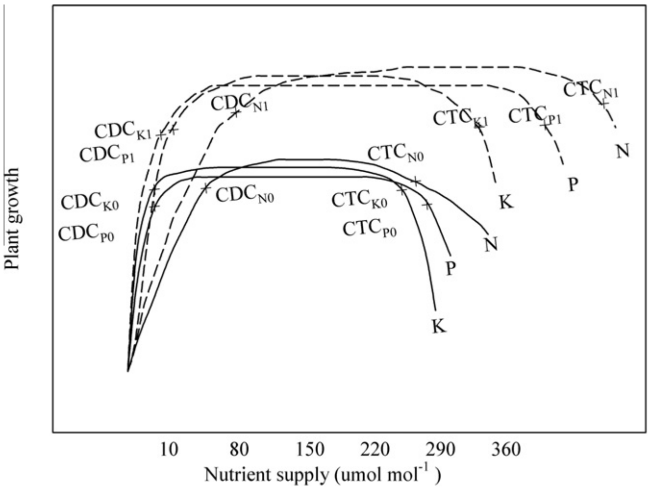

Fig. 3. Schematic representation of growth response of black spruce seedlings to nutrient supply under ambient (drawn based on the theory proposed by Marschner (1995)) and elevated [CO_2] (proposed by the authors) concentrations. Solid lines are the response curves in ambient [CO_2] and the dashed lines in elevated [CO_2]. " + " indicates the start of critical deficiency content (CDC) and critical toxicity content (CTC). CDC and CTC are defined as the low and high nutrient supply, respectively, at which the growth is at 90% of the maximum growth (Marschner, 1995). "N_0" and "N_1", "P_0" and "P_1", and "K_0" and "K_1" show the CDC and CTC points for nitrogen (N), phosphorus (P) and potassium (K) under ambient and elevated [CO_2], respectively. The subscript "$_0$" indicates the values for the ambient [CO_2] and the "$_1$" for the elevated [CO_2].

content or above the critical toxicity content (Timmer, 1991; Marschner, 1995; Brady and Weil, 2002). The critical deficiency content and critical toxicity content are nutrient supply points where a 10% growth reduction from the maximum growth is reached because of nutrient deficiency or toxicity (Timmer, 1991). The estimates of the critical deficiency content and the critical toxicity content for black spruce seedlings were presented in Fig. 3. The critical deficiency content values for N, P and K were all below 80 μmol mol^{-1} under both ambient and elevated CO_2 condition, while the critical toxicity content values for N, P and K were estimated to be above 220 μmol mol^{-1} in the ambient [CO_2] and 290 μmol mol^{-1} under the elevated [CO_2]. Since the critical deficiency content for N was higher than P and K (Fig. 3), the lowest N supply was below the critical deficiency content for N as indicated by the decreased growth while P and K were still in their sufficient supply range with the constant P–K concentrations treatment in both CO_2 conditions. At the ambient [CO_2] black spruce seedlings may have also suffered critical toxicity N content growth suppression and possibly critical toxicity K content at high N supply levels. It should be pointed out that the above calculations were based on the assumption that the combination of 150 μmol mol^{-1} N, 60 μmol mol^{-1} P and 150 μmol mol^{-1} K was the optimal nutrient regime for growing black spruce seedlings. The N/P/K ratios of 5/2/5 and P and K concentrations were derived from studies on several *Picea*, *Pinus* and *Betula* species (Morrison, 1974; Ingestad, 1979; Landis, 1989; Zhang and Dang, 2007). Those studies were all carried out in controlled environmental conditions. If the actual optimal nutrient regime differs for the genetic material used in this study, the specific threshold values may be different from those presented but the general trends of responses should hold.

The CO_2 elevation increased the critical toxicity content for N, P and K but the magnitude of the increase was greater for N than P and K (Fig. 3). Because of these differences, the nutrient ratio effect can be directly linked to critical toxicity content growth suppression. As seen in Fig. 3, the critical toxicity content for K is below 360 μmol mol^{-1} (which was the highest N and K supply point applied in this study). The N supply is far below the critical toxicity content, so the highest N supply was still in the sufficient supply range. Obviously, K supply in the constant N–P–K ratio treatment (K = 360 μmol mol^{-1}) exceeded the critical toxicity K content. The observed growth suppression in the constant nutrient ratios treatment but not in the constant P–K concentration treatment indicated that high P and K supply were the contributing factors for the decline in growth (because the N supply levels were the same in the two nutrient ratio treatments). Comparatively, K supply likely played a more important role than P supply, because of the lower critical toxicity content for K than for P. The relatively higher K supply in the constant nutrient ratio treatment with the high N supply levels suppressed the growth because the K supply had passed over the critical toxicity content under the elevated [CO_2]. Therefore, under the elevated [CO_2], high N supply promoted growth in the constant P–K concentrations treatment because of the greater demand of seedlings for N while K and P supply were in their sufficient ranges (Fig. 3).

In conclusion, nutrient ratio and N supply interactively affected the growth of black spruce seedlings. Under the elevated [CO_2], the seedlings needed more nutrient supply, primarily N, to support growth, resulting a demand for higher N/P and N/K ratios. The constant N/P/K ratio treatment at high N supply led to growth suppression caused by the critical toxicity K content K, while the growth suppression in the constant P–K concentration treatment at low N supply resulted from the critical deficiency content for N. At the ambient [CO_2], growth suppression was induced by the critical toxicity content for N at high N supply or the critical deficiency content for N at low N supply in both nutrient ratio treatment. The ratios of N, P and K significantly affected the relationship between plant growth and N supply and thus should be considered in plant growth models. However, we would like to point out that this study was conducted on small seedlings under controlled environmental conditions in the greenhouse and only for one growing season. While short-term studies on young trees can provide valuable insights on physiological acclimations to treatments, the inference sphere of such studies is limited. The environmental conditions in the field are much more variable and complicated, which will affect responses. Furthermore, adult trees may be less responsive than seedlings, particularly in the long term. Additionally, the nutrient availabilities applied in this experiment were anthropogenic. While the results can be referenced to environmentally controlled greenhouses and nurseries, cautions should be exercised when extrapolating the results to young trees in the field or adult trees.

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