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Ecophysiological responses of black spruce (*Picea mariana*) seedlings to nutrient supply, N-P-K ratio and photoperiod at current and elevated CO₂ concentration

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**Ecophysiological responses of black spruce (*Picea mariana*)
Seedlings to Nutrient Supply, N-P-K ratio and Photoperiod at
Current and Elevated CO₂ Concentration**

By

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ABSTRACT

The relationship between plant growth and nitrogen supply is frequently used in growth models to predict growth performance under global climate change scenarios. However, the relationship may be changed under elevated $[\text{CO}_2]$. Furthermore, plant responses to nitrogen supply may be modified by N-P-K ratio. Researchers using climate envelope models predict that boreal tree species will migrate northward during progress of the global warming associated with increasing atmospheric CO_2 and changes in precipitation. However, changes in photoperiod and nutrient supply associated with the northward migration to higher latitudes may influence survival and growth of the migrated plants. In this thesis, I investigated the ecophysiological responses of black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings to interactions between N supply and N-P-K ratios and between N supply and photoperiod under ambient and elevated $[\text{CO}_2]$.

In the first investigation, black spruce seedlings were exposed to six N levels (10, 80, 220, 290, 360 $\mu\text{mol mol}^{-1}$ N) with two scenarios of P and K supply (constant N/P/K ratio (CNR) and constant P and K concentration (VNR)) under two $[\text{CO}_2]$ (370 and 720 $\mu\text{mol mol}^{-1}$ $[\text{CO}_2]$) for 3.5 months. Under the elevated $[\text{CO}_2]$, the high N supplies with the CNR treatment resulted in critical toxicity content (CTC) growth suppression, but with the VNR treatment they resulted in growth promotion. Low N supplies, however, induced critical deficiency content (CDC) growth suppression in both the CNR and VNR. At the ambient $[\text{CO}_2]$, in contrast, the seedling growth was suppressed by CTC at high N supplies and CDC at low N supplies with both the VNR and CNR treatments. These trends were particularly obvious in height increment (Hi) and net photosynthetic rate (Pn). Compared to the intermediate N supply, under the elevated $[\text{CO}_2]$,

at the highest N supply, the CNR reduced H_i and P_n by 30.90% and 37.60%, respectively, but at the same N supply level, the VNR increased H_i and P_n by 30.96% and 13.24%, respectively; however, at the lowest N supply, the reduction of H_i and P_n were 87.81% and 63.70%, respectively (no significant difference between the CNR and VNR). At the ambient $[CO_2]$, also compared to intermediate N supply, at both the lowest and highest N supply levels, either the CNR or VNR both negatively affected H_i and P_n . The decrease in H_i and P_n compared to that at the lowest N supply were 229.41% and 53.70% (no significant difference between the CNR and VNR); the reduction in H_i at the highest N supply was 42.77% in the CNR and 24.03% in the VNR. The reduction in P_n at highest N supply level was 37.59% (no significant difference between the CNR and VNR). The trends also suggest that the seedlings require higher nutrient supply to support growth under elevated $[CO_2]$ and the increased nutrient demand appeared to be primarily for N. The preferential increases in N demand suggest an increase in N/P and N/K ratios under elevated $[CO_2]$. The relationship between growth and nitrogen supply was changed by elevated $[CO_2]$ and adjusted by N-P-K ratio.

In the second investigation, black spruce seedlings were exposed to two photoperiods (one at seed origin (PS) vs. one corresponding to a 10° northward migration (PNM)) and two nutrient supplies (30 vs. 300 $\mu\text{mol mol}^{-1}$ N) at two $[CO_2]$ (ambient 370 and elevated 720 $\mu\text{mol mol}^{-1}$ $[CO_2]$). The seedlings' growth and cold hardiness were examined after 4 months in the first growth season and their carryover effect was examined after a 1.5 month treatment in the second growth season. Between the two growing seasons, the seedlings were cold stored in -2 to -4 $^\circ\text{C}$ for 3 months. It was found that the greatest seedling height, root collar diameter and biomass were under the PNM, high N supply and elevated $[CO_2]$. The CO_2 elevation did not significantly affect seedling cold hardiness. Cold hardiness was the least under the low nutrient supply and the

PNM. The PNM treatment resulted in delayed bud set and earlier bud burst. The low N supply suppressed the seedlings' cold hardiness and all the seedlings that received the low N supply treatment died after cold storage. The elevated [CO₂] promoted the seedling growth but had no effect on terminal bud set and burst. The photoperiod associated with northward migration together with the low nutrient supply hindered black spruce seedlings from developing enough cold hardiness, and the photoperiod triggered delayed and advanced terminal bud set and burst.

The elevated [CO₂] increased demand for nutrient supply requiring more portion of N than P and K in the N-P-K ratio, which altered the relationship between black spruce growth and nitrogen supply. The CNR suppressed growth at higher N supply levels under the elevated CO₂ condition. The photoperiod changes associated with the northward migration led poor cold hardiness development. The low nutrient supply enhanced the negative photoperiod effect, which led to higher mortality. The predicted black spruce northward migration may fail because of the poor cold resistance ability.

Additional Keywords: climate envelope, photoperiodism, plant migration, critical deficiency content, critical toxicity content, cold hardiness, cold resistance, bud set, bud break.

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Chapter 1 General Introduction

The responses of black spruce (*Picea mariana* Mill. BSP) to the predicted global climate changes represent a key component of the boreal forest ecosystem responses because black spruce is an ecologically important and widely distributed tree species in the boreal forest. Some models predict that the average global air temperature will increase 1.8 and 4.0 °C by the end of this century (Bigras and Bertrand 2006, IPCC 2007), and the boreal forest will likely experience more temperature changes. Generally, CO₂ elevation and higher temperature increases photosynthesis (Roberntz and Stockfors 1998, Tognetti and Johnson 1999, Long et al. 2004, Nowak 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). However, predicting how black spruce may respond to the climate changes can be a challenge because of interactions among environmental factors. The interactions can result in conflicting results (Saxe et al. 1998, Curtis et al. 2000, Nowak et al. 2004). The atmosphere-ocean general circulation models (AOGCMs) predict that air temperature and precipitation will change along with the increasing [CO₂]. In response to such changes, to acclimate the elevated [CO₂], one of the responses is that the relationship between plant growth and nitrogen supply may change and the change may be modified new N-P-K ratio, and another one of the responses of many boreal tree species including black spruce will likely be northward migration to higher latitudes to meet the predicted temperature and water regime (McKenney et al. 2007, McKenney et al. 2011) which have been adapted by the species at their seed origin locations after hundreds and thousands of years.

Migrating to higher latitudes to avoid the stresses of the predicted changes in temperature and precipitation, black spruce will encounter different nutrient availability and photoperiod. Nitrogen supply and photoperiod are closely related to plant physiological activities and growth. Although there may be large amount of nutrients in the soils (Aerts et al. 2007, Mazzoleni et al. 2007), the amount of available nitrogen may be still low at high latitudes (CSIRO 2009), because of low soil temperatures and subsequent slow rates of organic decomposition and mineralization (Couteaux et al. 1995, CSIRO 2009). Photoperiod is the daily duration of light and varies with day of the year and latitude (Jackson 2009), but it does not change with climate. Thus, northward migration would mean that the species will grow in a different photoperiod regime that is characterized by longer daytime in the summer and shorter in the winter. Therefore, a good understanding of the effects of the nutrient availability and photoperiod is essential for predicting the potential northward migration.

Interactions among nutrient elements can affect plant responses to nitrogen supply. Nitrogen is a critical constituent of amino acids, enzymes, nucleic acids, chlorophylls, and hormones in plants (Rook 1991, Marschner 1995a, Taiz and Zeiger 2002) and is required in greater quantities than any other mineral element (Marschner 1995a, Nicodemus et al. 2008a). N supply is often used as a key factor in modeling plant growth responses (Verkroost and Wassen 2005, Coll et al. 2011, Nendel et al. 2011). However, the interactions among nutrient elements (Saxe et al. 1998, Curtis et al. 2000, Nowak et al. 2004) may result in different plant responses to nitrogen supply when changes in N supply are not synchronized with changes in other nutrients. However, there is a paucity of information describing the interactive effects of nutrient ratios and N supply on boreal trees under elevated [CO₂].

The changes in photoperiod associated with northward migration will lead to different combinations of photoperiod, nutrient and thermal regimes as compared to those at the original location of plant species, and these changes may alter the phenology and physiology of plants. Any physiological and /or phenological misalignment with the natural environment, particularly seasons, can have significant implications on the survival and growth of the species at the new location. Native plant species have adapted to the photoperiod and other environmental conditions in their habitats for hundreds and thousands of years, and formed their photoperiodisms, which control their phenological events and physiological processes (Thomas and Vince-Prue 1997). The photoperiod at higher latitudes is longer in the summer but shorter in the winter as compared to that at the original location of a species (Astronomical Applications Department 2009). Therefore, plants may advance or delay their phenological events and growth processes after migrating northward. For instance, plants may delay their bud set and development of cold hardiness in the fall, and thus increase the risk being damaged by early frosts. For species at their seed origin locations, bud break generally is controlled by temperature not photoperiod. An accumulation of temperatures above a threshold (usually 5°C) is used to predict the date of bud break and loss of cold hardiness (Colombo 1998). Korner and Basler (2010) pointed out that bud break in spring is controlled by winter chilling, photoperiod and temperature. Bud break of species like beech (*Fagus*) does not occur when photoperiod does not meet its critical long daylength even exceptionally high temperatures are applied (Korner and Basler 2010). When a migrated species grows under a changed photoperiod, its gene-based photoperiodism may still trigger bud break by the adapted daylength whose occur time now is advanced comparing to its seed origin location, so bud break may advance and the species will risk of spring frost damage. Whether and how changes in photoperiod will affect black spruce is

largely unknown but the information is pivotal for predicting the future distribution and growth of the species under the scenario of global climate change. The relatively shorter daylength and duration of cold hardening period and low nitrogen availability associated with the northward migration may also affect cold hardiness development and growth performance in the next growing season (Miller and Timmer 1997).

In this study I exposed black spruce seedlings to different combinations of [CO₂], nutrient supply and photoperiod, and examined their ecophysiological responses. The objectives of this study were to examine growth and photosynthetic responses of black spruce seedlings to nitrogen supply with different NPK ratio (Chapter 2 and 3), and to investigate the effects of photoperiod and nitrogen supply on growth and cold hardiness development (Chapter 4) at ambient and elevated [CO₂]. I have tested the following hypotheses: (1) under the elevated [CO₂], the increase N with the constant P and K concentration (VNR) would increase growth and photosynthesis, and the proportional increases of N, P and K (CNR) would suppress growth and photosynthesis at high N levels; (2) the low N supply would suppress growth and photosynthesis at both the ambient and elevated [CO₂]; (3) the nutrient ratios (CNR and VNR) would adjust the relationship between growth and N supply under elevated [CO₂]; (4) the photoperiod changes associated with northward migration would increase growth but constrain cold hardiness development; (5) the low nutrient supply would hinder cold hardening.

Chapter 2 Effects of nitrogen supply with constant and variable N-P-K ratios on growth response to CO₂ elevation

Introduction

The atmospheric carbon dioxide concentration in the lower atmosphere has been increasing since the beginning of industrialization and will continue to rise for the rest of the century (IPCC 2007). The increase of the atmospheric [CO₂] generally promotes 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 higher rate of 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 2002a, Lambers et al. 2008), the increase of growth will diminish (Brown and Higginbotham 1986). Therefore, a good understanding of the relationship between CO₂ stimulation of growth and nutrient supply is essential for predicting the future trend of plant growth under the warming climate.

Many studies have been primarily focused on the effects of N supply with constant concentrations of K and P (Ingestad 1979a, Brown and Higginbotham 1986, Griffin et al. 1993, Gavito et al. 2001, Cao et al. 2007, Cao et al. 2008), but only a few maintained constant N/K or N/P ratios (Ingestad 1979a, Zhang et al. 2006a, Ambebe et al. 2010). When N supply is varied, the purpose of maintaining constant N-P-K ratios (CNR) is to avoid N, P and K interactions that can potentially affect plant nutrient uptake and physiological functions (Newbery et al. 1995a, 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 1991a) and K (Timmer 1991). On the other hand, high N supply induces K deficiency (van den Driessche and Ponsford 1995a) and high K supply negatively affects N and P uptake (Egilla and Davies 1995). High N:K ratios reduce plant growth (Barbosa et al. 2000a). Plant growth is generally more sensitive to N deficiency than to P (Saidana et al. 2009a). These complications, however, can be avoided through constant ratios of N, P and K (Ripullone et al. 2003a, Zhang and Dang 2006a).

The concentrations of N, P and K are critical to the physiology and growth of plants (Landis 1989a, van den Driessche 1991a, Marschner 1995a). Plant growth is suppressed when concentrations of nutrient elements fall below the critical deficiency content (CDC) or above the critical toxicity content (CTC). (Timmer 1991, Marschner 1995a, Brady and Weil 2002a). Between CDC and CTC, increasing nutrient supply generally results in greater plant growth, but the response patterns vary with elements (Timmer 1991, Marschner 1995a). For example, increasing N supply produces gradual plant growth until CTC, whereas increasing P and K results in a more dramatic growth increase at lower range of availability (Epstein 1972a, Timmer 1991, Marschner 1995a, Epstein and Bloom 2005a). Therefore, the concentration of each element in plants needs to be maintained between CDC and below CTC to avoid growth suppressions (Marschner 1995a, Brady and Weil 2002a).

The optimal nutrient ratio for plant growth may be altered under elevated $[\text{CO}_2]$. Under elevated $[\text{CO}_2]$, plants generally need more nutrients to support growth, but the increased amounts of different elements may not be increased by the same proportion. Consequently, the optimal ratio of different nutrient elements may change under elevated $[\text{CO}_2]$. Research has indicated that the increase in demand is greater for N than for P and K (Epstein 1972a, Timmer

1991, Marschner 1995a, Epstein and Bloom 2005a) due to the relative insensitivity of plant growth to high P and K (Epstein 1972a, Timmer 1991, Marschner 1995a, Epstein and Bloom 2005a). Consequently, constant N/P/K ratios in meeting higher N demand under elevated [CO₂] may result in P and K exceeding their CTC (Timmer 1991, Marschner 1995a).

The interactive effects of [CO₂] and nutrient supply on plant physiology and growth have been studied extensively, but with little consideration of nutrient ratios (Brown and Higginbotham 1986, Johnsen 1993, Poorter 1993, Roberntz and Stockfors 1998, Gavito et al. 2001, Zhang and Dang 2006a, Cao et al. 2008, Ambebe et al. 2010). Elevated [CO₂] and high N supply increase plant biomass accumulation (Brown and Higginbotham 1986, Griffin et al. 1993, Johnsen 1993, Elkohen and Mousseau 1994, Zhang and Dang 2006a). Low P supply reduces photosynthetic rates under both ambient and elevated CO₂ conditions (Campbell and Sage 2006) and deficiency in P reduces the enhancement of CO₂ elevation on photosynthesis (Tissue and Lewis 2010). CO₂ elevations increase leaf and total biomass of spruce seedlings grown in high N supply, but the positive effect of CO₂ elevation on root biomass only occurs when N supply is low (Brown and Higginbotham 1986).

To predict plant growth, modelers need to 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 1995a, Taiz and Zeiger 2002) and is required in greater quantities than any other mineral element (Marschner 1995a, Nicodemus et al. 2008a), 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 factor in models (Hirose 1988, Verkroost and Wassen 2005, McMurtrie et al. 2008).

Because of the interactions among nutrient elements and the effect of [CO₂] on optimal nutrient ratios, N effect on plant response to CO₂ elevation likely varies with P and K supply. However, it is unclear how plants will respond to N under different P and K supplies and under the ambient and elevated [CO₂].

Black spruce (*Picea mariana* Mill. BSP) is a widely distributed tree species in the boreal forest and it grows at locations with various nutrient supplies. It is predicted that significant changes will occur in the boreal forest under the climate changes associated with increasing atmospheric CO₂ concentration (Bigras and Bertrand 2006, IPCC 2007) and nutrient availability will change following the climate changes. Although black spruce grows on various soil types with a wide range of N content from deficiency to sufficiency levels (Viereck and Johnston 1990), a recommended N supply is commonly used in controlled experiments (Morrison 1974, Ingestad 1979a, Landis 1989a, Ingestad and Agren 1992, Zhang and Dang. 2007), and an N-P-K ratio of 5/2/5 with 150 μmol mol⁻¹ N is generally used for growing black spruce seedlings in greenhouse conditions (Ingestad 1979a, Landis 1989a). When N supply is compared in experiment, one tenth of the N supply concentration is considered low N supply (Zhang et al. 2006a).

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 (CNR) and constant P and K concentrations (thus variable N-P-K ratios, VNR)) and two levels of [CO₂] (ambient 370 and elevated 720 μmol mol⁻¹). Since CO₂ elevation and high N supply can have a synergistic effect (Wang et al. 1995, Zhang et al. 2006a), which may result in increased nutrient demands with an altered N-P-K ratio, I hypothesized that under the elevated [CO₂], the increase in N with the VNR would increase seedling growth, particularly, at high N levels, more than the CNR due to

the possible growth suppression by the toxic effects of P and K at high N levels. On the other hand, the low N supply would suppress the seedling growth at both the ambient and elevated [CO₂] due to N deficiency regardless of nutrient ratios (VNR or CNR). Moreover, nutrient ratio would affect the relationship between growth and N supply.

Materials and methods

Plant materials

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

Experimental design

The experiment was carried out in four environmentally controlled identical design greenhouses in the Forest Ecology Complex at the Thunder Bay Campus of Lakehead University. The treatments were two CO₂ concentrations (370 vs. 720 μmol mol⁻¹), two nutrient ratios (constant vs. variable nutrient ratios) and six nitrogen concentrations (10, 80, 150, 220, 290 and 360 μmol mol⁻¹N). A recommended optimal N supply is commonly used in controlled experiments (Morrison 1974, Ingestad 1979a, Landis 1989a, Ingestad and Agren 1992, Zhang and Dang. 2007), and the N-P-K ratio of 5/2/5 with 150 μmol mol⁻¹ N is generally recommended (Ingestad 1979a, Landis 1989a). Other nutrients beside N, P and K were calcium (Ca), magnesium (Mg) and sulfur (S), and their ratios were 2.6/1.3/2. These elements were supplied

from Calcium Nitrate ($\text{Ca}(\text{NO}_3)_2$ 15.5% N, 18% Ca), Ammonium Nitrate (NH_4NO_3 , 17% $\text{NH}_4\text{-N}$, 17% $\text{NO}_3\text{-N}$), Microfine Super Phosphate (46% P_2O_5), Muriate of Potash (62% K_2O), Epson salt (9.8% Mg, 12.9% S), and Micromax micronutrient (12% S). When low N supply is designed to apply in experiment, one tenth of the N supply concentration is often considered as the lowest N supply level (Zhang et al. 2006a). In the constant nutrient ratio (CNR) treatment, the concentrations of P and K varied with N levels to maintain a constant N/P/K ratio at 5/2/5. In the variable nutrient ratio (VNR) treatment, however, N levels were identical to those in the CNR treatment, but P and K concentrations were held constant across all N levels ($60 \mu\text{mol mol}^{-1}$ for P and $150 \mu\text{mol mol}^{-1}$ for K). Each $[\text{CO}_2]$ treatment had two independent replicates (greenhouses), with the factorial combinations of nutrient ratio and N levels nested within the $[\text{CO}_2]$ treatment. Each treatment combination had 4 seedlings. The day/night air temperatures were controlled at 25-26/16-17 °C and the photoperiod at 16 hours (natural daylength was extended using high-pressure sodium lamps when natural daylength is less than 16 hours) for all the greenhouses. All the experiment conditions (temperature, $[\text{CO}_2]$ 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 two days to maintain water content above 30% (by volume) in the growing medium. The growing medium water content was measured using an HH2 Moisture Meter attached to ML2X ThetaProbe (Delta-T Devices, Cambridge, U.K.). When the water content fell close to 30% (Bergeron et al. 2004), an extra watering was applied. The experiment lasted 3.5 months.

Measurements

Three seedlings were randomly chosen from the 4 seedlings in each treatment combination for measuring seedling responses (only 3 seedlings were available in some combinations due to damage or mortality). Height and root collar diameter were measured at the beginning and the end of the experiment for determining net growth 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):

$$V = (\pi D^2 / 4) H / 3 \quad (1)$$

The relative growth rates of height (RGR_h), RCD (RGR_d), and volume (RGR_v) were determined by dividing growth 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 needles on 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 hours, to determine the dry mass. Then the foliage-root ratio ($FRR = \text{foliage mass} / \text{root mass}$), shoot mass ratio ($SMR = \text{shoot mass} / \text{total seedling mass}$), and root mass ratio ($RMR = \text{root mass} / \text{total seedling mass}$) were calculated. The samples for leaf area determination were weighed separately. Specific leaf area (SLA) and specific root length ($SRL = \text{root length} / \text{root mass}$) were calculated from the projected leaf area, total root length and the corresponding dry mass.

Foliar nitrogen, phosphorus and potassium assay

The dried current 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 (P) and potassium (K) 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 hours at 90°C in a block digester. 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 (N_m , P_m and K_m) were converted to leaf area-based ones (N_a , P_a and K_a) using the specific leaf area.

Statistical analysis

The experiment was a split-plot design with CO₂ concentrations as main plots and combinations of nutrient ratios and N concentrations as subplots. 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).

Results

Height and RCD increment, and relative growth rates

Both height and RCD increments were generally increased by elevated $[\text{CO}_2]$, VNR, and the increase of N level, although the magnitude of change varied with treatment combinations (significant two- and three-way interactions for height and only $\text{C} \times \text{N}$ for RCD) (Table 2.1; Figures 2.1A and 2.1B). The largest height and RCD increments under the ambient $[\text{CO}_2]$ were observed at the intermediate N supply ($150 \mu\text{mol mol}^{-1}\text{N}$), whereas the largest growth increments under the elevated $[\text{CO}_2]$, were recorded at the highest N supply. The height growth difference between VNR and CNR treatments tended to occur at high N levels and more under the elevated $[\text{CO}_2]$ (Figure 2.1A).

The relative growth rates of height (RGR_h), RCD (RGR_d) and stem volume (RGR_v) were all significantly affected by $\text{C} \times \text{N}$ and the three-way interactions (Figures 2.1 C, 2.1D, and 2.1E; Table 2.1). Similar to height increment, the difference of RGR_h between VNR and CNR treatments tended to occur at high N levels and more under the elevated $[\text{CO}_2]$ (Figure 2.1C). Comparatively, RGR_d and RGR_v only appeared to be higher in VNR than CNR treatment at two low N levels under elevated $[\text{CO}_2]$ (Figure 2.1 D and 2.1E).

Table 2. 1. P values for the treatment effect of CO₂ 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 (B – total biomass), ratios (FRR – foliage to root ratio, SMR – shoot mass 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_a – N, P_a – P, and K_a – 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
SMR	0.691	0.239	<0.001	0.322	0.002	0.842	0.538
RMR	0.691	0.239	<0.001	0.322	0.002	0.842	0.538
B	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

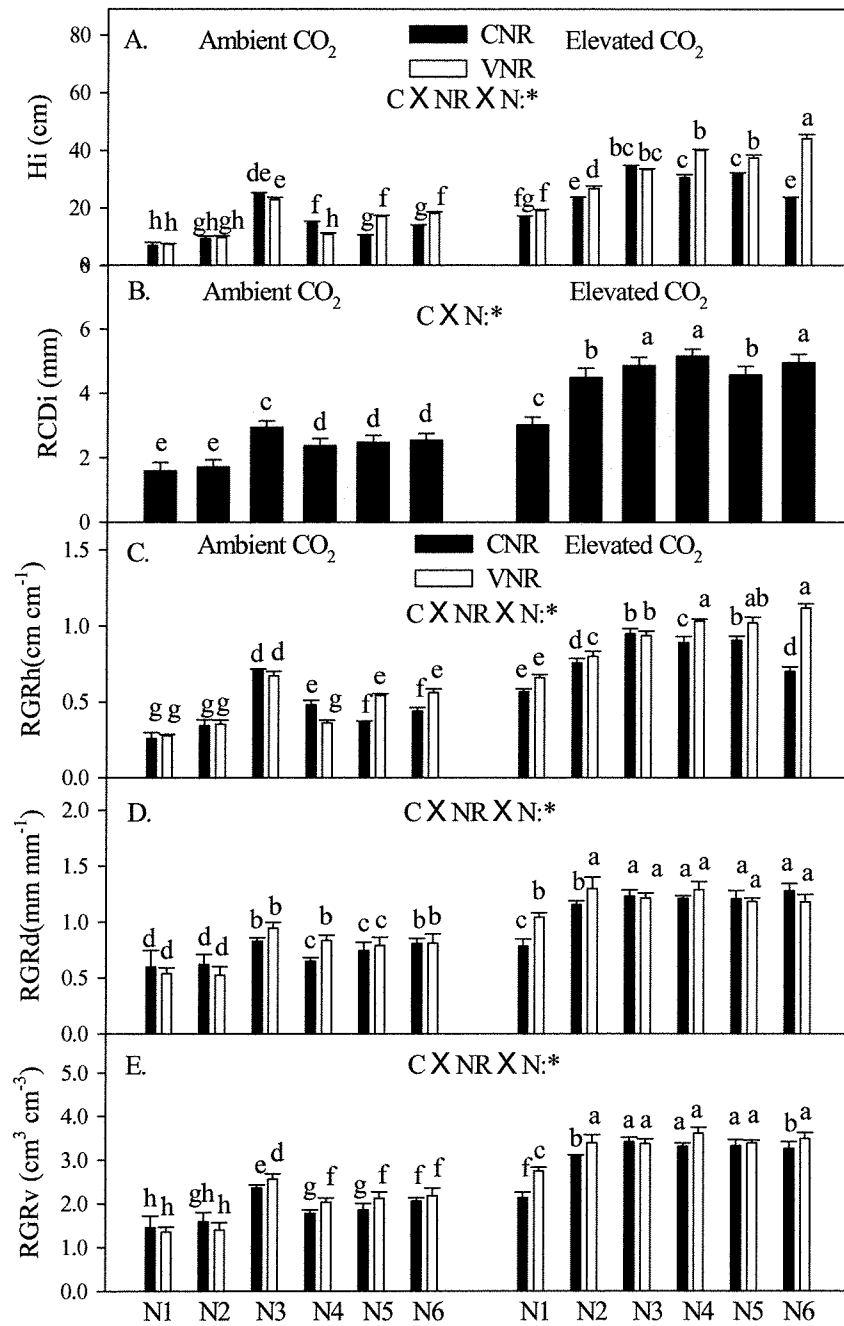


Figure 2. 1 Mean (+ SEM) height increment (H_i), root collar diameter increment (RCD_i), height relative growth rate (RGR_h), RCD relative growth rate and volume relative growth rate (RGR_v) of black spruce seedlings grown under two [CO_2] (ambient [CO_2] = $370 \mu mol mol^{-1}$; elevated [CO_2] = $720 \mu mol mol^{-1}$), 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 $60 \mu mol mol^{-1}$ and K at $150 \mu mol mol^{-1}$ and

variable N), and 6 N concentrations (10, 80, 150, 220, 290 and $360 \mu mol mol^{-1}$). Significant effects ($P \leq 0.05$) are indicated by *. Means with different letters were significantly different from each other ($P \leq 0.05$).

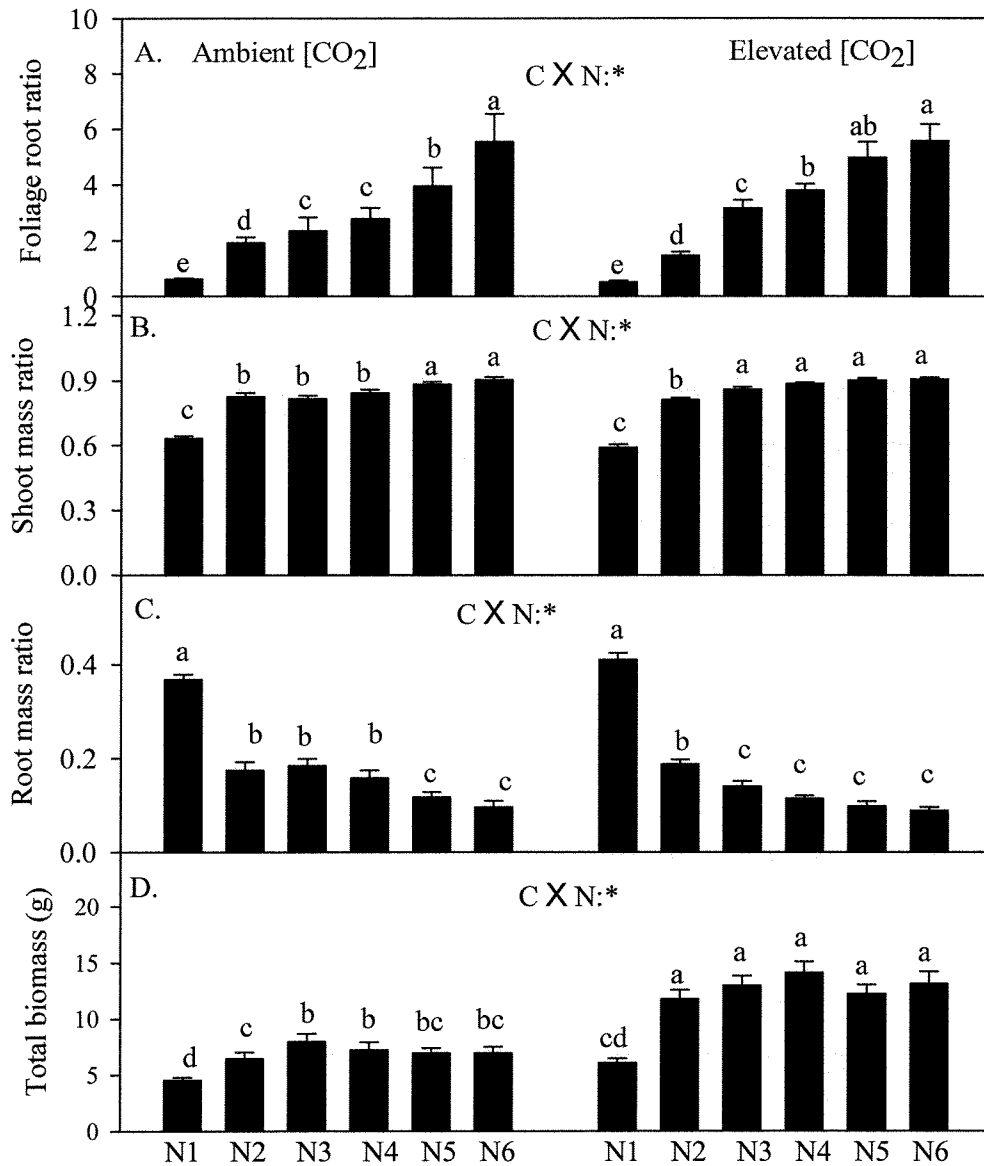


Figure 2. 2 Mean (+ SEM) foliage to root ratio, shoot mass ratio, root mass ratio and total biomass in black spruce seedlings (see Figure 2.1 and text for treatment descriptions). Significant effects ($P \leq 0.05$) are indicated by *. Means with different letters were significantly different from each other ($P \leq 0.05$).

Biomass and biomass ratios

At both $[\text{CO}_2]$, foliage to root ratio (FRR), shoot mass ratio (SMR) and total biomass (B) generally increased with the increase of N concentration (Figure 2.2 A, 2.2 B and 2.2 D), whereas root mass ratio (RMR) tended to decrease with N levels (Figure 2.2 C). The magnitude of change, however, was greater under the elevated $[\text{CO}_2]$ (significant $C \times N$ interaction, see Table 2.1). Total biomass was also significantly greater in the VNR than in the CNR treatment (Table 2.1) (treatment means were 9.63 ± 0.49 (g) for VNR, and 8.79 ± 0.46 (g) for CNR, respectively).

Specific leaf area (SLA) and specific root length (SRL)

SLA was significantly affected by CO_2 concentration, with treatment means of 7.62 ± 0.08 ($\text{m}^2 \text{kg}^{-1}$) and 6.79 ± 0.08 ($\text{m}^2 \text{kg}^{-1}$) for ambient and elevated $[\text{CO}_2]$, respectively (Table 2.1). The VNR treatment resulted in significantly lower SLA when N was at or above $220 \mu\text{mol mol}^{-1}\text{N}$, but higher SLA when N was at $10 \mu\text{mol mol}^{-1}\text{N}$ (significant $\text{NR} \times \text{N}$ interaction, see Table 2.1). The difference, however, disappeared at 80 and $150 \mu\text{mol mol}^{-1}\text{N}$ (Figure 2.3 A). Comparatively, SRL only differed with N levels and was significantly reduced at two intermediate N levels (150 and $220 \mu\text{mol mol}^{-1}\text{N}$) from that at the other N levels (Table 2.1; Figure 2.3 B)

N, P, K concentrations

Both mass-based and leaf area-based leaf N concentrations (N_m and N_a) increased with N supply and were greater with VNR (N_m : mean= 19.8 ± 0.64 (mg g^{-1}) for VNR, and 18.9 ± 0.62 (mg g^{-1}) for CNR; N_a : mean= 59.60 ± 5.30 (mg g^{-1}) for VNR, and 51.14 ± 3.62 (mg g^{-1}) for CNR, respectively) (Table 2.1; Figures 2.4A, 2.5A, and 2.5B). The significant difference of N_a between

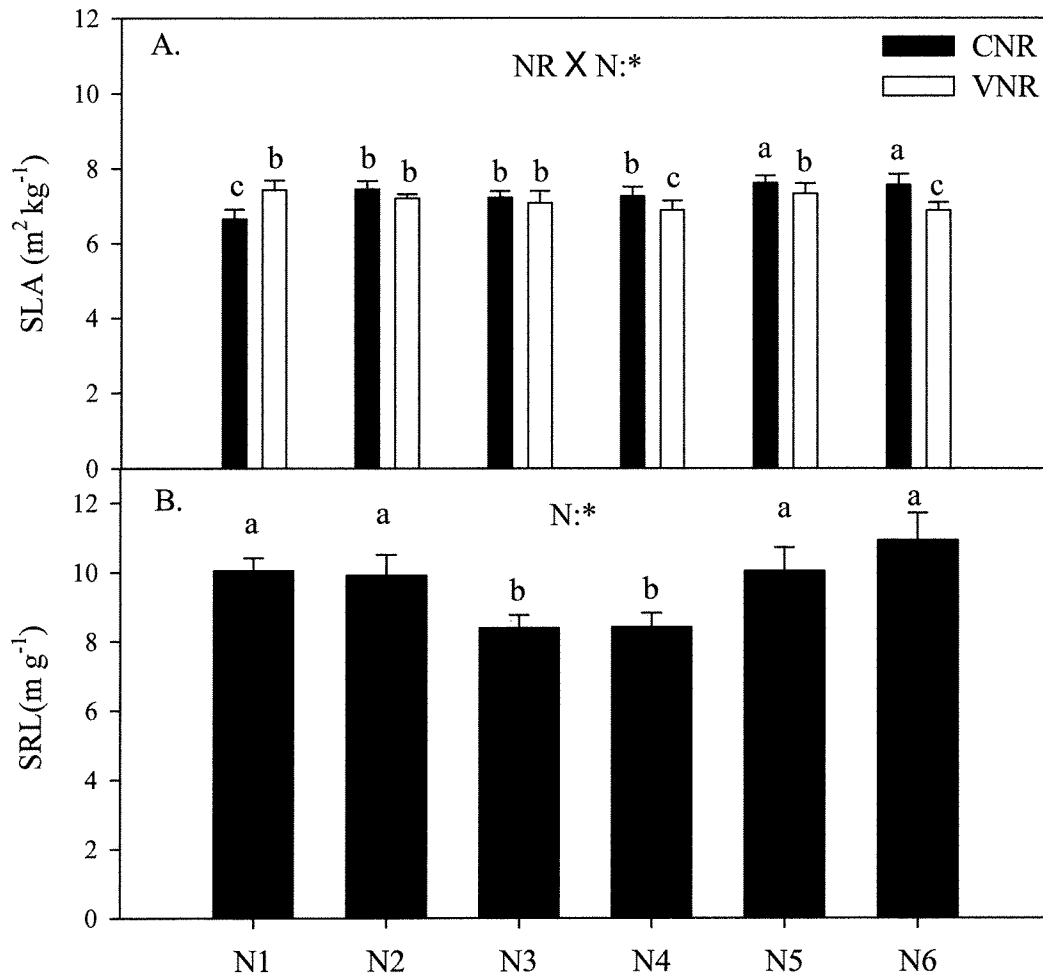


Figure 2. 3 Mean (+ SEM) specific leaf area (SLA) and specific root length (SRL) in black spruce seedlings (see Figure 2.1 and text for treatment descriptions). Significant effects ($P \leq 0.05$) are indicated by *. Means with different letters were significantly different from each other ($P \leq 0.05$)

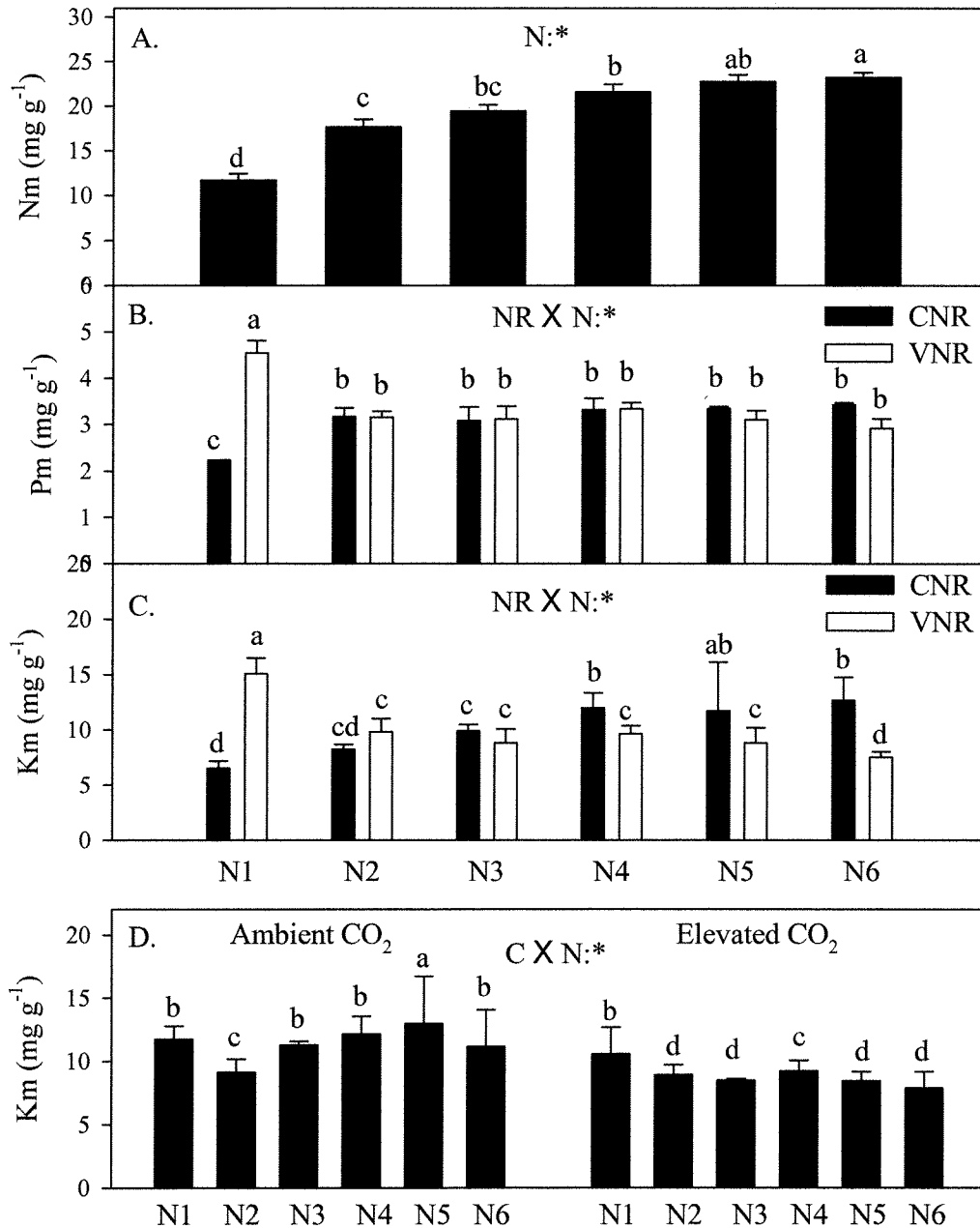


Figure 2. 4 Mean (+ SEM) mass based leaf nutrient concentrations of nitrogen (N_m), phosphorus (P_m) and potassium (K_m) in black spruce seedlings (see Figure 2.1 and text for treatment descriptions). Significant effects ($P \leq 0.05$) are indicated by *. Means with different letters were significantly different from each other ($P \leq 0.05$)

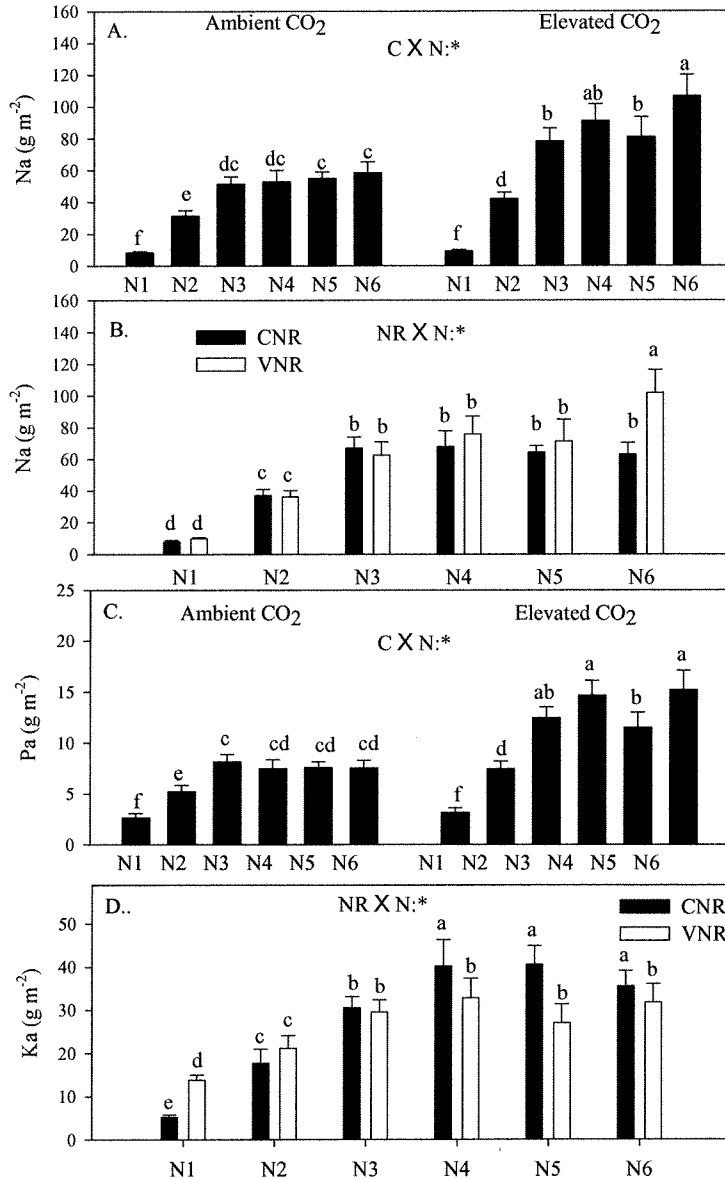


Figure 2. 5 Mean (+ SEM) leaf area based leaf nutrient concentrations of nitrogen (Na), phosphorus (Pa) and potassium (Ka) in black spruce seedlings (see Figure 2.1 and text for treatment descriptions). Significant effects ($P \leq 0.05$) are indicated by *. Means with different letters were significantly different from each other ($P \leq 0.05$).

CNR and VNR, however, only occurred at the highest N (significant NR x N interaction, see Table 2.1) (Figure 2.5A), while the increase of N_a with N supply was stronger under elevated $[CO_2]$ (significant C x N interaction) (Figure 2.5B).

The mass-based P concentration (P_m) only differed by nutrient ratio (significant NR) and the difference occurred only at the lowest N level (significant NR x N) (Table 2.1; Figure 2.4 B). Comparatively, P_a increased with the increasing N until $150 \mu\text{mol mol}^{-1}$ N in both $[CO_2]$. With further increase of N, P_a largely stayed constant under the ambient $[CO_2]$, but varied under elevated $[CO_2]$ (became significantly lower at $290 \mu\text{mol mol}^{-1}$ N) (Figure 2.5 C).

Both mass-based and area-based leaf K concentrations (K_m and K_a) differed among N supply treatment (significant N effect, see Table 2.1). The general pattern, however, was different; K_a increased with the increasing N at low range of N supply (Figure 2.5D), while the highest average K_m was observed at lowest N with VNR (Figure 2.4C). The change of K_m with the increasing N also varied with $[CO_2]$ (significant interactions of C x N and NR x N, see Table 2.1, Figures 2.4 C and 2.4D). Between the two nutrient ratios, both K_m and K_a in VNR appeared to be lower at the three high N levels, but higher at the lowest N level (significant NR x N interaction, see Table 2.1). No significant differences between VNR and CNR were observed at 80 and $150 \mu\text{mol mol}^{-1}$ N.

Discussions

The data support the hypotheses that the combination of elevated $[CO_2]$ and VNR at high N supply promotes black spruce seedling growth, whereas a combination of elevated $[CO_2]$ and CNR at high N supply suppresses growth. As suggested by others, elevated $[CO_2]$ increased

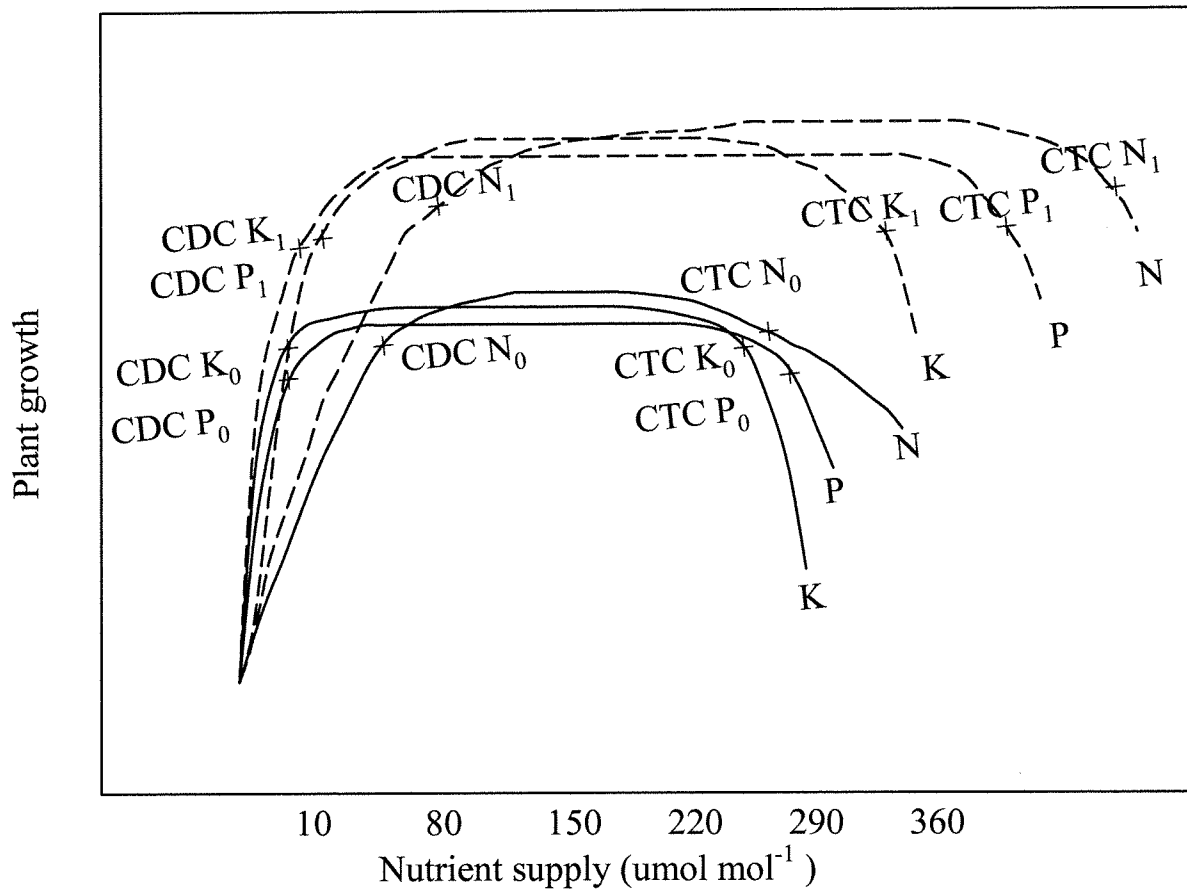


Figure 2. 6 Schematic representation of growth response of black spruce seedlings to nutrient supply under ambient (drawn based on the theory proposed by Marschner (1995)) and elevated $[\text{CO}_2]$ (proposed by the authors) concentrations. Solid lines are the response curves in ambient $[\text{CO}_2]$ and the dashed lines in elevated $[\text{CO}_2]$. “+” indicates the start of critical deficiency content (CDC) and critical toxicity content (CTC) and represents 90% of the maximum growth. 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 $[\text{CO}_2]$, respectively. The subscript “0” indicates the values for the ambient $[\text{CO}_2]$ and the “1” for the elevated $[\text{CO}_2]$.

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 with CNR 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 (Figure 2.6). Comparatively, height growth appeared to be more sensitive to the toxic effects of P and K than RCD or volume growth, as seen from the significant VNR-CNR difference in height growth when N supply $\geq 220 \mu\text{mol mol}^{-1}$ at both ambient and elevated $[\text{CO}_2]$. The growth of black spruce seedlings was also suppressed at low N supply, regardless of CO_2 concentrations or nutrient ratios, probably 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 elevation and this should be considered in growth prediction models. Although N supply has a strong relationship with growth (Rook 1991, Marschner 1995a, Taiz and Zeiger 2002, Nicodemus et al. 2008a) and plays a critical role in plant growth (Rook 1991, Marschner 1995a, Taiz and Zeiger 2002), the significant growth difference between two nutrient ratios at same N supply level in the experiment suggest that errors can occur in growth predictions if nutrient ratios are ignored. Since N supply is the primary element most commonly used by modelers (McMurtrie 1991, Dewar and McMurtrie 1996) for predicting plant growth, N-P-K ratio is then suggested to be considered in those prediction models. Because there are currently little data on the effects of nutrient ratios at various levels of nutrient supply, more research in this area is necessary.

The results of this study suggest that the biomass allocation in black spruce seedlings was primarily affected by N levels. As found by others, low N supply results in a larger RMR (Stulen

and Hertog 1993) and a smaller FRR (Stulen and Hertog 1993, Stitt and Krapp 1999), even though this relationship varied with CO₂ concentration. Pokorny et al. (2010) suggest that CO₂ elevation increases shoot growth and low N supply decreases shoot elongation growth. In this experiment, greater growth allocation to shoots by elevated [CO₂] occurred only at intermediate levels of N supply (150 and 220 μmol mol⁻¹ N), consistent with the observations that CO₂ elevation generally does not influence biomass allocations (Bosac et al. 1995, Curtis and Wang 1998, Riikonen et al. 2004, Zhang et al. 2006a, Ambebe and Dang 2010). Compared to N supply, the effect of nutrient ratio on biomass allocation was probably too small to be detected, as found by Ambebe et al. (2009) on white birch seedlings.

It is worthwhile to note the interactive effect of nutrient ratio and N supply on SLA. The VNR treatment resulted in higher SLA at the lowest N supply (10 μmol mol⁻¹N), but lower SLA at highest N supply (360 μmol mol⁻¹N), while the CNR treatment had an opposite effect on SLA. This phenomenon may result from more P and K uptake and less N uptake at the lowest N supply in the VNR treatment and less P and K uptake and more N uptake at high N supply in the CNR treatment. My results did not indicate positive relationships between SLA and N_m (Figures 2.1C, 2.1D, 2.1E, and 2.3A), as suggested by Reich et al. (1997) from two global data sets of 280 plant species or between SLA and relative growth rates as indicated by Poorter and Remkes (1990). The SLA did not change significantly as N supply increased, while N_m increased with increasing N supply. These differences could be the results of different scenarios of nutrient ratio used in this study compared to those by others.

The variation of specific root length (SRL) with N in black spruce seedlings may be caused by different scenarios of nutrient demands and uptakes (Ostonen et al. 2007). The low N supply could stimulate root growth and therefore increase RMR and SRL (Eissenstat 1992),

while high N levels and low root mass density may result in high nutrient demand and more growth of fine roots (Ryser and Lambers 1995). The lower SRL at intermediate levels of N supply may indicate a more balanced nutrient demand and uptake.

Based on Marschner's (1995) theory on the relationship between growth and nutrient supply, and the data collected in the experiment, a schematic representation (Figure 2.6) was drawn to describe the relationship between N, P and K supply and the growth of black spruce seedlings under both ambient and elevated CO₂ conditions. It is generally believed that elevated [CO₂] raises nutrient demands requiring proportionally more N and less P and K (Epstein 1972a, Timmer 1991, Marschner 1995a, Epstein and Bloom 2005a). The growth is suppressed when nutrient supply is below CDC or above CTC (Timmer 1991, Marschner 1995a, Brady and Weil 2002a). The CDC and CTC for black spruce seedlings were estimated from growth and leaf nutrient data of this experiment and determined from the nutrient supply points where 10 % growth reduction from the maximum occurred when nutrient supply changed (Timmer 1991). In Figure 2.6, the CDC of all 3 elements is below 80 $\mu\text{mol mol}^{-1}$ in both ambient and elevated CO₂ condition, while the CTC increase from about 220 in ambient CO₂ condition to over 290 in elevated CO₂ condition. As the CDC of N occurs before that of P and K (Figure 2.6), the black spruce seedlings in this experiment experienced N deficiency at low N supply in both CO₂ conditions. At ambient CO₂ condition black spruce seedlings may also suffer N and possible K CTC at high N supply levels.

Under elevated CO₂ condition, nutrient ratio effect became significant because the CO₂ elevation stimulated growth and increased demand for N to a greater extent than for P and K, thus moving the CTC of N to a higher point (CTCN₁) followed by CTCP₁ (P) and CTCK₁ (K). As in Figure 2.6, the CTCK₁ is below 360 $\mu\text{mol mol}^{-1}$ (which was the highest N and K supply

point applied in this experiment). The N supply is far below $CTCN_1$, so the highest N supply was still in sufficient supply range. Obviously, K supply in CNR treatment ($K = 360 \mu\text{mol mol}^{-1}$) exceeded the $CTCK_1$. The observed growth suppression in the CNR treatment but not in the VNR indicated that high P and K supply were the contributing factors (because both CNR and VNR had the same N supply levels). Comparatively, K supply likely played a more important role than P supply, because of the lower $CTCK_1$ than $CTCP_1$ and better match of leaf [K] with growth suppression. Therefore, under the elevated CO_2 condition, high N supply promoted growth in the VNR treatment because of the greater demand of seedlings for N while K and P supply were in the sufficient ranges. In the CNR treatment, however, growth was suppressed as K supply exceeded $CTCK_1$ (Figure 2.6).

Under the predicted climate change condition, whether nutrient ratio and N supply affect photosynthesis of black spruce seedlings is discussed in following Chapter 3.

Chapter 3 Photosynthetic responses to nitrogen supply with constant and variable N-P-K ratios under ambient and elevated [CO₂]

Introduction

Black spruce (*Picea mariana* Mill. BSP) is an economically and ecologically important and widely distributed tree species in the boreal forest. Global climate change models predict that the boreal forest will encounter significant increases of atmospheric temperature resulting from rising CO₂ concentration in the atmosphere (Bigras and Bertrand 2006, IPCC 2007). CO₂ concentration and temperature changes can have profound effects on plant photosynthesis (Harley and Sharkey 1991, Gunderson and Wullschleger 1994, Lloyd 1999, Ward and Strain 1999, Crous et al. 2008, Onoda et al. 2009). Generally, CO₂ elevation and higher temperatures increase photosynthesis (Roberntz and Stockfors 1998, Tognetti and Johnson 1999, Long et al. 2004, Nowak 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). However, predicting how trees will respond to climate change in terms of photosynthesis can be a challenge, because of the interactions among environmental factors that can lead to contradictory results (Saxe et al. 1998, Curtis et al. 2000, Nowak et al. 2004). Furthermore, the responses can vary with species.

Many studies have found that the CO₂ stimulation of photosynthesis cannot be sustained for a long term (Poorter 1998, Rogers and Ellsworth 2002, Norby and Iversen 2006, Norby et al. 2010b) because of photosynthetic acclimation or down-regulation (Johnsen 1993, Gunderson and Wullschleger 1994, Lewis et al. 2004, Kitaoa et al. 2005, Davey et al. 2006). However, the degree of acclimation is closely correlated to nutrient supply (Stitt and Krapp 1999, Isopp et al.

2000). Most studies have shown that the degree of CO₂ enhancement on photosynthesis declines over time due to limitation of nutrient supply (Gunderson and Wullschleger 1994, Saxe et al. 1998, Oren et al. 2001, Zhang and Dang 2006a, Reich et al. 2006a). Consequently, the stimulating effects of CO₂ elevation on growth are subdued on nutrient-limiting sites (Brown and Higginbotham 1986). The CO₂ enhancement effect on growth can be maintained or increased by increasing N supply (Oren et al. 2001, Lewis et al. 2004, Finzi et al. 2006, Zhang et al. 2006a).

The relationship between photosynthesis and nitrogen (N) has been studied extensively (Bowman and Conant 1994, Peterson et al. 1999, Cao et al. 2007, Crous et al. 2008). A close correlation exists between the CO₂ stimulation of photosynthesis and nutrient availability (Murthy et al. 1996, Kellomaki and Wang 1997b). The relationship can explain much of the variation in plant performance without considering the effects of phosphorus (P) and potassium (K) although P and K are also two important macronutrient elements to plant physiology and growth. N is a critical constituent of amino acids, enzymes, nucleic acids, chlorophylls, and hormones in plants (Rook 1991, Marschner 1995b, Taiz and Zeiger 2002) and is required for plant physiology and growth in greater quantities than any other mineral elements (Marschner 1995b, Nicodemus et al. 2008b). Nevertheless, other elements, such as P and K, can interact with N and affect plant physiology. For instance, low N supply increases plant capacity to absorb N but suppresses the absorption of non-limiting nutrient elements, such as P and sulfur (Chapin III 1991b) and K (Timmer 1991); high N supply induces K deficiency (van den Driessche and Ponsford 1995b); high N:K ratios reduce plant growth (Barbosa et al. 2000b), and high K supply negatively affects N and P uptake (Egilla and Davies 1995). However, plant growth is generally more sensitive to N deficiency than P deficiency (Saidana et al. 2009b). Because N:P ratio influences the synthesis of photosynthetic enzymes (Campbell and Sage 2006), a curvilinear

relationship between photosynthesis and foliar N concentration exists only when N and P are supplied with a certain ratio. Therefore, a certain N, P and K ratio can be maintained to avoid confounding effects (Ripullone et al. 2003b, Zhang and Dang 2006b).

N-P-K ratio and concentration interactively affect plant growth. N effects are generally investigated with concentrations of P and K being maintained constant (Ingestad 1979a, Brown and Higginbotham 1986, Griffin et al. 1993, Gavito et al. 2001, Cao et al. 2007, Cao et al. 2008). There are only a few studies that maintain N/K and N/P ratios constant when N effects are investigated (Ingestad 1979b, Ripullone et al. 2003b, Zhang et al. 2006b, Ambebe et al. 2010). The reason to maintain N-P-K ratios constant while changing N supply is to avoid N, P and K interactions that can potentially affect plant nutrient uptake and physiological functions (Newbery et al. 1995b, Gusewell 2005). The concentrations of N, P and K as well as their ratios are critical to the physiology and growth of plants (Landis 1989b, van den Driessche 1991b, Marschner 1995b). The concentration of each element in plants needs to be maintained within a certain range to avoid going below or above the critical deficiency content (CDC) or critical toxicity content (CTC) (Marschner 1995b, Brady and Weil 2002b). When the concentrations of N, P and K in plant are below CDC or above CTC, plant growth is suppressed (Timmer 1991, Marschner 1995b, Brady and Weil 2002b). Between CDC and CTC, increasing nutrient supply generally results in greater plant growth, but the response patterns of plant growth differ with different elements (Timmer 1991, Marschner 1995b). For example, increasing N supply results in gradually ascending plant growth until CTC, whereas plant growth shows a dramatic increase when increasing P and K at low concentrations (above CDC), which then levels off until CTC (Epstein 1972b, Timmer 1991, Marschner 1995b, Epstein and Bloom 2005b).

A good understanding of how CO₂ elevation affects the photosynthesis–N relationship is a critical foundation for predicting plant growth trend under future climate conditions (Peterson et al. 1999, Reich et al. 2006b). Elevated [CO₂] and high N supply increase plant biomass production (Brown and Higginbotham 1986, Griffin et al. 1993, Johnsen 1993, Elkohen and Mousseau 1994, Zhang and Dang 2006a). Low P supply reduces photosynthetic rates under both ambient and elevated CO₂ conditions (Campbell and Sage 2006) and P deficiency reduces the enhancement of CO₂ elevation on photosynthesis (Tissue and Lewis 2010). The increase in demand for N is generally greater than that for P and K in response to CO₂ elevation (Epstein 1972a, Timmer 1991, Marschner 1995a, Epstein and Bloom 2005a), because plant growth is generally less sensitive to P and K (Epstein 1972a, Timmer 1991, Marschner 1995a, Epstein and Bloom 2005a). When high amounts of P and K are supplied, plants encounter a high risk of growth suppression from CTC restraint (Timmer 1991, Marschner 1995a). Since increasing N supply leads to high leaf [N], which is a result of increased N uptake (Kellomaki and Wang 1997a), and N plays a central role in photosynthesis, photosynthesis is generally closely correlated to N supply (Evans 1989). Modelers use N supply or leaf [N] as a key factor to predict plant photosynthesis and growth responses (Hirose 1988, McMurtrie 1991, Dewar and McMurtrie 1996, Verkroost and Wassen 2005, McMurtrie et al. 2008). Because of the interactions among N, P and K, nutrient ratios should be considered in investigation of nutrient effects on photosynthetic responses to CO₂ elevation or any other factors of climate change under different nutrient regimes. However, there is very little information in the literature on such effects of nutrient ratios.

Black spruce grows over a wide range of available N from deficiency to sufficiency (Viereck and Johnston 1990). However, the physiological responses of the species to CO₂

elevation are generally examined under optimal nutrient regimes (Morrison 1974, Ingestad 1979a, Landis 1989a, Ingestad and Agren 1992, Zhang and Dang. 2007). The objective of this study was to examine photosynthetic responses of black spruce seedlings to six levels of nitrogen supply under two different scenarios of nutrient ratios: constant N-P-K ratios (CNR) and constant P and K concentrations (thus variable N-P-K ratios, VNR), and two CO₂ concentrations (370 μmol mol⁻¹ and 720 μmol mol⁻¹). Since CO₂ elevation increases plant demand for nutrients, and the level of increase may vary with elements, possibly more N than P and K. When a constant N-P-K ratio (CNR) of a relatively greater P and K portion is applied, sufficient N supply may be associated with high P and K that exceed their critical toxicity content (CTC). Therefore, I hypothesize that nutrient ratios would influence photosynthetic responses of black spruce seedlings to the CO₂ elevation and the N supply, and that the CNR would reduce the seedlings' net photosynthetic rate ($P_{n\text{-growth}}$) at high N supplies, particularly under the elevated [CO₂].

Materials and methods

Plant materials

One-year-old black spruce (*Picea mariana* [Mill.] B.S.P.) seedlings were obtained from Hill's Greenhouse in Thunder Bay. The seedlings were relatively uniform in size at the beginning of the experiment (H=22.8±0.16cm, RCD=2.05±0.02cm). The seedlings were potted (13cm height, 12cm diameter) with a mixture of peat moss and vermiculite (1:1; v/v).

Experiment design

The experiment was carried out in four environment-controlled greenhouses in the Forest Ecology Complex at the Thunder Bay Campus of Lakehead University. The treatments consisted of two CO₂ concentrations (370 and 720 μmol mol⁻¹), two nutrient ratios (constant vs. variable nutrient ratios) and six nitrogen concentrations (10, 80, 150, 220, 290 and 360 μmol N mol⁻¹ solution). In the constant nutrient ratio (CNR) treatment, the concentrations of P and K varied with N levels to maintain N/P/K ratios constant at 5/2/5. In the variable nutrient ratio (VNR) treatment, N levels were identical to those in the CNR treatment, but P and K concentrations were constant across all the N levels (60 μmol mol⁻¹ P and 150 μmol mol⁻¹ K). Each of the CO₂ concentrations was replicated twice (greenhouses), with the factorial combinations of nutrient ratio and N levels nested within each CO₂ treatment. Each treatment combination had 4 seedlings. The day/night air temperatures were controlled at 25-26/16-17 °C and the photoperiod at 16 hours by supplementing natural light using high-pressure sodium lamps on shorter days for all the greenhouses. All the experiment conditions (temperature, [CO₂] 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 two days to maintain the water content of the growing medium above 30% (by volume). The growing medium water content was measured using an HH2 Moisture Meter attached to ML2X ThetaProbe (Delta-T Devices, Cambridge, U.K.). When the water content fell close to 30% (Bergeron et al. 2004), extra watering was applied. The experiment lasted 3.5 months.

Measurements of *in situ* gas exchange

Three seedlings were randomly chosen from each treatment combination. Photosynthetic responses to [CO₂] (*A/Ci* curves) were measured sequentially at 50, 150, 250, 370, 550, 720, 1000 and 1400 μmol mol⁻¹[CO₂] using a PP system Ciras-1 (PP System Inc. Amesbury, MA, USA). Net photosynthetic rate (*P_n*) was calculated (based on the data of the scanned projected leaf areas) according to Farquhar et al. (1980), von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991) and Harley et al. (1992). Other environment conditions in the leaf chamber were 25 °C air temperature, 800 μmol m⁻² s⁻¹ photosynthetically active radiation, and 50% relative humidity. The measurements were taken on the current year foliage on terminal shoots. All measurements were made between 0730-1130 h *in situ*. The *A/Ci* curves were analyzed to estimate *V_{cmax}* (maximum rate of carboxylation), *J_{max}* (light saturated rate of electron transport) and TPU (triose phosphate utilization) according to Sharkey et al. (2007).

Leaf mass and project leaf area

The foliage used for gas exchange measurement was then scanned to obtain projected leaf area using WinSEEDLE (Regent Instruments Inc., Quebec city, Quebec, Canada). The shoot for gas exchange was oven-dried at 75 °C for 48 hours and weighed on an analytical balance. The foliage mass and area were used to convert mass-based nutrient concentration to area-based values for calculating nutrient use efficiency.

Leaf nitrogen, phosphorus and potassium assay

The dry current year needles were then used for nutrient assays. Leaf [N] was determined using the dry combustion method with a CNS-2000 (LECO Corp., St. Joseph, MI, USA). The samples (about 0.5 g) were weighed directly on the ceramic crucible and recorded on the

microprocessor. The crucible was placed into an autoloader rack for analysis using the CNS-2000. Phosphorus (P) and potassium (K) 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 hours at 90°C in a block digester. Distilled water was added to the acid to dilute the solution 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).

Photosynthetic nutrient-use efficiency

Photosynthetic nitrogen-use efficiency (PNUE), phosphorus-use efficiency (PPUE) and potassium-use efficiency (PKUE) were calculated by dividing the net photosynthetic rate at the corresponding growth [CO₂] by leaf area-based N, P and K concentrations, respectively.

Statistical analysis

The data were examined graphically for the normality of distribution (probability plots for residuals) and homogeneity of variance (scatter plots). Since two assumptions for analysis of variance (ANOVA) were met, all subsequent analyses were done on the original data. When ANOVA (split-plot design) showed a significant effect ($P < 0.05$) for a variable with more than two levels or an interaction, the 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).

Results

Net photosynthetic rate (P_n)

The effect of nutrient ratio on net photosynthetic rate at growth $[CO_2]$ ($P_{n-growth}$) varied with $[CO_2]$ and N supply (significant 2- and 3-way interactions, see Table 3.1). Under the elevated $[CO_2]$, $P_{n-growth}$ increased with N supply from 10 to 150 $\mu\text{mol mol}^{-1}\text{N}$ in both nutrient ratios. At higher N levels, however, $P_{n-growth}$ remained high in the VNR treatment but started to decline in the CNR treatment (Figure 3.1A). At ambient $[CO_2]$, however, $P_{n-growth}$ generally increased with N from 10 to 150 $\mu\text{mol mol}^{-1}$ and decreased afterwards in both the VNR and CNR treatments. Between the two nutrient ratios, $P_{n-growth}$ generally did not differ at ambient $[CO_2]$, but was only significantly higher in VNR at high N levels of elevated $[CO_2]$ (Figure 3.1 A)

With increase of N supply, P_{n-370} reached its maximum at 80 $\mu\text{mol mol}^{-1}$ in the VNR treatment and at 150 $\mu\text{mol mol}^{-1}$ in the CNR treatment. At higher N supply, P_{n-370} remained high in the VNR but decreased in the CNR (significant NR, N, and NR \times N effects, see Table 3.1 and Figure 3.1 C). Between the two nutrient ratios, P_{n-370} was lower in the VNR at 10 $\mu\text{mol mol}^{-1}$ N but higher at 80, 290, and 360 $\mu\text{mol mol}^{-1}$ and much of the difference occurred under elevated $[CO_2]$ (significant NR and C \times NR, see Table 3.1 and Figure 3.1 B and C).

Table 3. 1 *P* values for the effects of CO₂ concentration (C), nutrient ratio (NR), nitrogen supply (N) and their interactions on net photosynthetic rate at growth [CO₂] (P_{n-growth}), net photosynthetic rate measured at a common 370 μmol mol⁻¹ [CO₂](P_{n-370}), maximum rate of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}), triose phosphate utilization (TPU), photosynthetic nitrogen-use efficiency (PUNE), phosphorus-use efficiency (PPUE) and potassium-use efficiency (PKUE) in black spruce seedlings grown under two [CO₂] (ambient [CO₂] = 370 μmol mol⁻¹ and elevated [CO₂] =720 μmol mol⁻¹), 6 N concentrations (10, 80, 150, 220, 290 and 360 μmol mol⁻¹), and 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) for 3.5 months.

Growth response	Treatment effects						
	C	NR	N	C×NR	C ×N	NR×N	C×NR×N
P _{n-growth}	0.022	0.001	<0.001	<0.001	0.003	<0.001	0.013
P _{n-370}	0.291	0.013	<0.001	0.002	0.256	0.011	0.208
V _{cmax}	0.021	0.084	<0.001	0.132	<0.001	0.027	0.566
J _{max}	0.05	0.626	<0.001	0.513	0.109	0.383	0.357
TPU	0.039	0.886	<0.001	0.93	0.103	0.341	0.293
PNUE	0.024	0.299	<0.001	0.028	0.476	0.159	0.324
PPUE	0.052	0.364	0.012	0.012	0.103	<0.001	0.003
PKUE	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

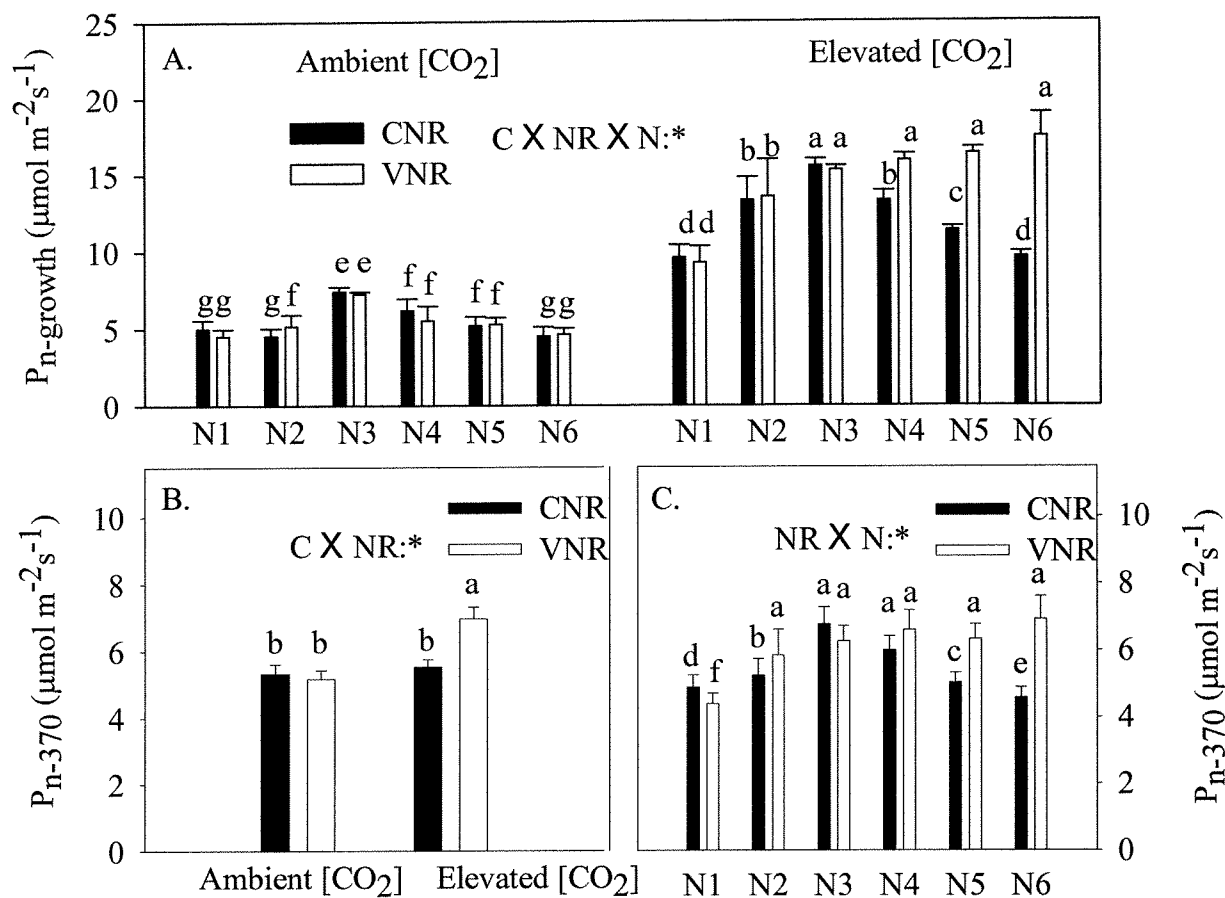


Figure 3. 1 Mean (+ SEM) net photosynthesis rate at growth [CO₂](P_n-growth) and common 370 μmol mol⁻¹ [CO₂] (P_n-370) in black spruce seedlings grown under two [CO₂] (ambient [CO₂] = 370 μmol mol⁻¹ and elevated [CO₂] = 720 μmol mol⁻¹), 6 N concentrations (10, 80, 150, 220, 290 and 360 μmol mol⁻¹), and 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). Significant difference is indicated by * (P ≤ 0.05). Means with different letters were significantly different from each other (P ≤ 0.05).

Biochemical parameters

The variation of maximum rate of carboxylation (V_{cmax}) with N supply differed with the $[\text{CO}_2]$ and the nutrient ratio (significant $\text{C} \times \text{N}$ and $\text{NR} \times \text{N}$ interactions, see Table 3.1 and Figures 3.2 A and 3.2 B). From N supply at 10 to 150 $\mu\text{mol mol}^{-1}$, V_{cmax} increased with increasing N in both CNR and VNR (Figure 3.2 B). At higher N, V_{cmax} largely remained high in the VNR but continuously declined in the CNR. Between the two $[\text{CO}_2]$, V_{cmax} was significantly higher in the elevated $[\text{CO}_2]$ at all N levels, except for 10 $\mu\text{mol mol}^{-1}\text{N}$ where no significant difference occurred (Figure 3.2 A). Between the CNR and VNR, higher V_{cmax} in the VNR treatment only occurred at high N supply levels (290 and 360 $\mu\text{mol mol}^{-1}\text{N}$) (Figure 3.2B).

Both the light saturated rate of electron transport (J_{max}) and triose phosphate utilization (TPU) were higher under the elevated $[\text{CO}_2]$ than the ambient $[\text{CO}_2]$, and increased with the N supply from 10 to 150 $\mu\text{mol mol}^{-1}$ but decreased at higher N (significant $\text{C} \times \text{N}$ effects, see Table 3.1, and Figures 3.2 C and 3.2 D). (Means of $J_{\text{max}} = 101.5 \pm 3.97$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for the elevated $[\text{CO}_2]$, 72.77 ± 2.56 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for the ambient $[\text{CO}_2]$; TPU = 8.14 ± 0.28 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for the elevated $[\text{CO}_2]$, 4.35 ± 0.18 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for the ambient $[\text{CO}_2]$).

Foliar nutrient use efficiency

The photosynthetic nitrogen-use efficiency (PNUE) generally decreased with the increasing N supply and was higher under the elevated $[\text{CO}_2]$ (significant N and C effect, see Table 3.1 and Figures 3.3 A and 3.3B). Between the two nutrient ratios, however, PNUE in VNR was lower under the ambient $[\text{CO}_2]$ but higher in the elevated $[\text{CO}_2]$ (significant $\text{C} \times \text{NR}$ effect, see Table 3.1).

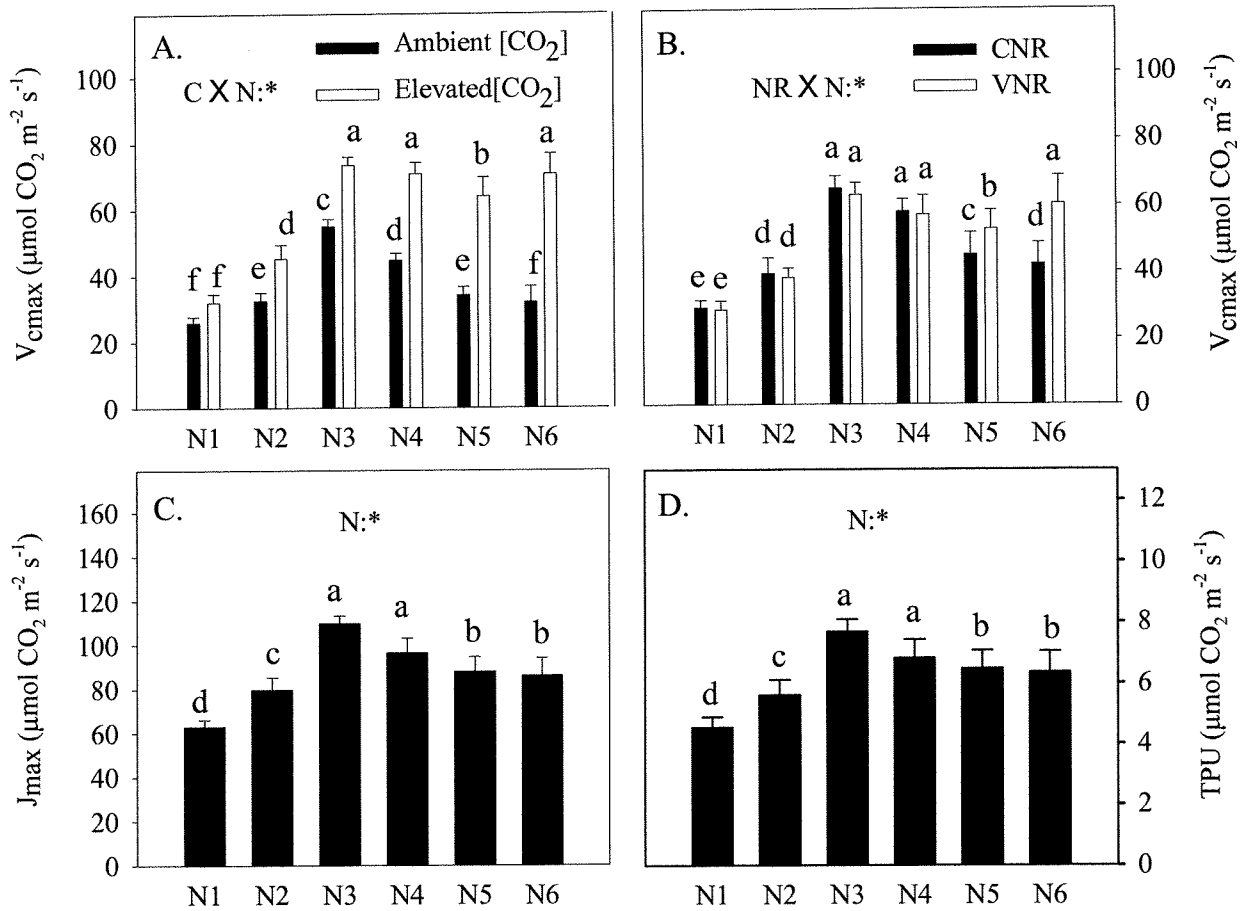


Figure 3. 2 Mean (+ SEM) maximum rate of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}), and triose phosphate utilization (TPU) in black spruce seedlings (see Figure 3.1 and text for treatment descriptions).

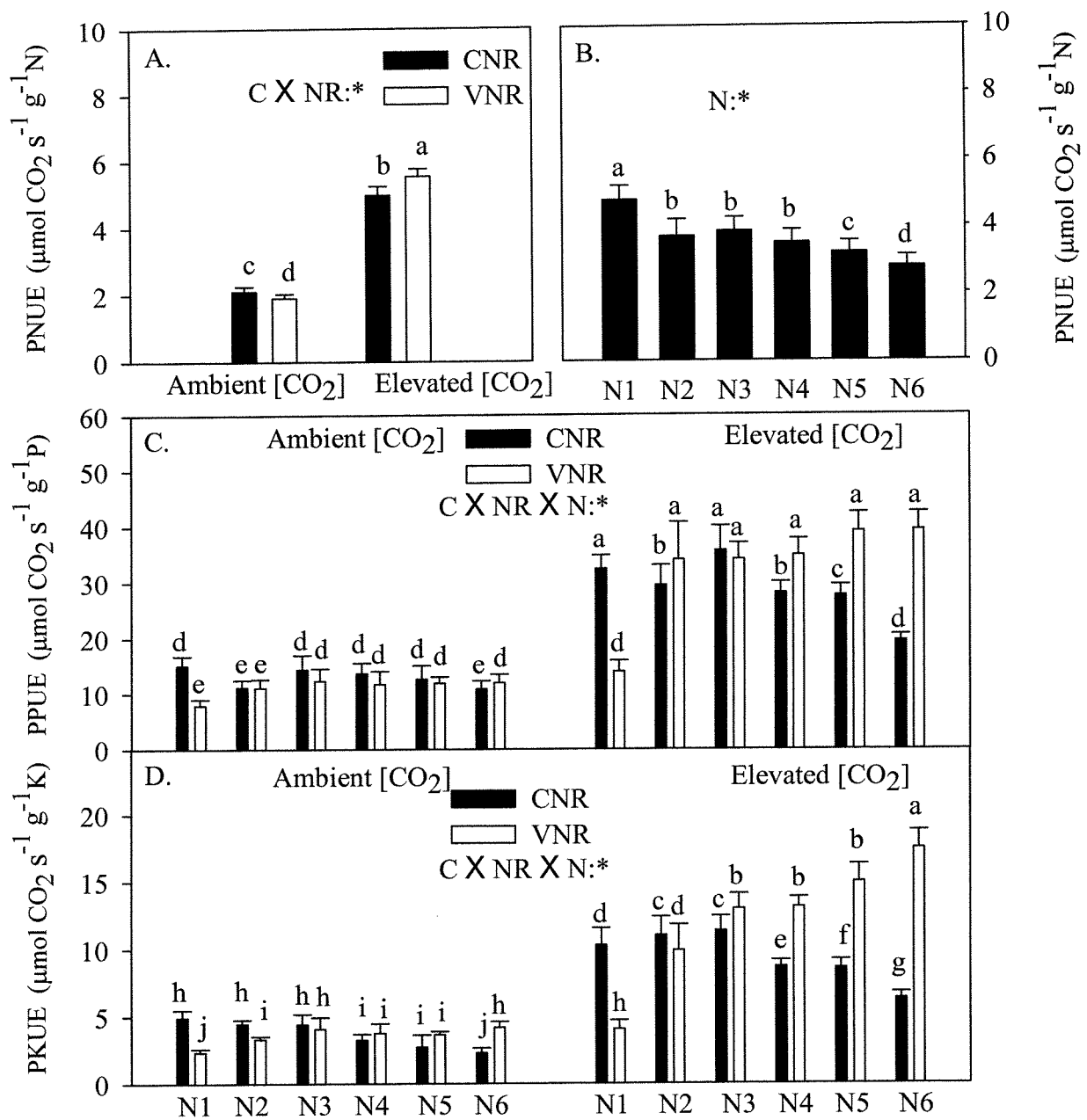


Figure 3.3 Mean (+ SEM) photosynthetic nitrogen-use efficiency (PUNE), phosphorus-use efficiency (PPUE), and potassium-use efficiency (PKUE) in black spruce seedlings (see Figure 3.1 and text for treatment descriptions).

The variation of photosynthetic phosphorus-use efficiency (PPUE) with nutrient ratio differed among N levels, which largely occurred under the elevated [CO₂] (significant C × NR × N interactions, see Table 3.1 and Figure 3.3 C). Under the elevated [CO₂], PPUE increased from 10 to 80 μmol mol⁻¹ in the VNR treatment and remained high but generally decreased in the CNR treatment within the range of N between 10 and 360 μmol mol⁻¹. Therefore, PPUE in the VNR treatment was lower at the low N and higher at the high N levels, compared to that in the CNR treatment. In addition, PPUE was generally greater under the elevated [CO₂] than the ambient [CO₂].

The treatment responses of photosynthetic potassium-use efficiency (PKUE) were similar to those of PPUE, except for the greater difference between nutrient ratios, especially under elevated [CO₂] (significant C, NR, N, and their interactions, see Table 3.1 and Figure 3.3D). In both the CO₂ concentrations, PKUE in the VNR was lower at the low N and higher at the high N, in comparison with that in the CNR.

Discussion

As found by others (Israel et al. 1990, Roberntz and Stockfors 1998, Gavito et al. 2001, Nowak et al. 2004, Ainsworth and Rogers 2007, Cole et al. 2010), the CO₂ elevation increased P_{n-growth}, but the magnitude of change depended on N supply and nutrient ratio (Table 3.1A and Figure 3.1A). The significant difference of P_{n-growth} between the two nutrient ratios at the high N confirms the hypothesis that the constant nutrient ratio (CNR) resulted in a reduction of the seedlings' net photosynthetic rate (P_{n-growth}) at the high N supply and the elevated [CO₂]. The fact that P_{n-growth} in the VNR and the elevated [CO₂] did not show any sign of suppression with the

increase of N at high levels suggests that the $P_{n\text{-growth}}$ suppression was possibly due to toxic levels of P or K. Comparatively, the high K is more likely the responsible factor because of its relatively lower CTC than P (Figure 2.6) and the higher leaf [K] associated with the CNR treatment at the high N.

At the ambient $[\text{CO}_2]$, however, $P_{n\text{-growth}}$ showed suppression at both low and high N levels and the nutrient ratio did not make a significant difference, indicating that the N supply may be the limiting factor. At the high N levels, the leaf [N] may have reached or surpassed the CTC of N (Figure 2.6) and overwhelmed the effects of the nutrient ratio and therefore other nutrients (P and K). Comparatively, the suppression of $P_{n\text{-growth}}$ at the low N supply may have resulted from CDC limitation of N (Epstein 1972a, Timmer 1991, Marschner 1995a, Epstein and Bloom 2005a).

The results in this study suggest that photosynthetic down-regulation did not occur in black spruce seedlings after 3.5 months of growth under elevated $[\text{CO}_2]$. Although under elevated $[\text{CO}_2]$, photosynthetic down-regulation is observed frequently at low N supply (Stitt and Krapp 1999, Ellsworth et al. 2004, Nowak et al. 2004), the $P_{n\text{-370}}$ in this experiment did not show any significant differences between the ambient and elevated $[\text{CO}_2]$, even at the low N levels. These results are consistent with the theory of Long et al. (2004) and findings by Wang et al. (1995) and Zhang and Dang (2006a), but contrary to the observations of others (Johnsen 1993, Gunderson and Wullschleger 1994, Lewis et al. 2004, Kitaoa et al. 2005, Davey et al. 2006, Watanabe et al. 2011).

The data in this study suggest that biochemical parameters of photosynthesis are interactively affected by $[\text{CO}_2]$, N-P-K ratio and N supply. The increases of V_{cmax} and light saturated rate of electron transport (J_{max}) by CO_2 elevation are consistent with the observations of

Darbah et al. (2010) on aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) seedlings, but different from those on Douglas-fir (*Pseudotsuga menziesii*) (Lewis et al. 2004), cottonwood (*Populus deltoides*) (Tissue and Lewis 2010), and hybrid larch (*Larix gmelinii* var. *japonica* × *Larix kaempferi*) (Watanabe et al. 2011) seedlings where V_{cmax} and J_{max} generally decrease with increasing $[CO_2]$, and on cottonwood (*Populus deltoides*) (Tissue and Lewis 2010).

Research has indicated that both J_{max} and triose phosphate utilization (TPU) are more related to P supply as inorganic phosphate (P_i) concentration in cytosol largely determines the exportation of triose phosphate to cytosol or storage in chloroplasts (Lambers et al. 2008). Therefore, low P supply generally reduces J_{max} (Wykoff et al. 1998) and TPU (Watanabe et al. 2011). In this study, neither J_{max} nor TPU was significantly affected by the nutrient ratio, possibly due to a large range of sufficient P concentration (Figure 2.6), as indicated by Warren and Adams (Warren and Adams 2002) that light-saturated photosynthesis is not affected by P supply when P concentration is adequate in plants. Both J_{max} and TPU increased with the increasing N, as reported by Maier et al. (2008) where N fertilization increased J_{max} in 25 year old loblolly pine (*Pinus taeda* L.). The decrease of J_{max} and TPU at low and high N levels is probably due to CDC and CTC suppression by N (Figure 2.6).

The nutrient ratios changed the relationship between photosynthesis and leaf [N] in this study. Ellsworth et al. (2004) pointed out that the effects of elevated $[CO_2]$ on V_{cmax} are largely through the changes in leaf [N] (Ellsworth et al. 2004). The results in this study indicated that the relationship between V_{cmax} and leaf [N] was affected by the nutrient ratio through different proportions of P and K. The high leaf [N] (N_m) at the high N supply levels resulted in greater V_{cmax} with the VNR treatment, but not in the CNR treatment, possibly due to the toxic effects of K as occurred to $P_{n-growth}$. The theory of Lewis et al (2004) states that elevated $[CO_2]$ decreases

leaf [N] and reduces net photosynthetic rates measured at a common [CO₂]. Cao et al (2007) report that the magnitude of photosynthetic acclimation in white birch (*Betula papyrifera* Marsh.) in response to CO₂ elevation decreases with increases in leaf N concentration (Cao et al. 2007). Zhang and Dang (2006a) find that no photosynthetic down-regulation occurs in white birch seedlings in response to CO₂ elevation at various levels of N supply when N/P/K ratios are maintained constant while varying N supply. In this experiment, the leaf [N] increased with the increasing N, but this increasing trend of leaf [N] did not show corresponding increases of net photosynthetic rate at the high N supply levels under the elevated [CO₂], rather it was adjusted by the nutrient ratio changes where by the CNR decreased P_{n-growth} and the VNR increased P_{n-growth}. The nutrient ratio effect also observed on significant lower P_{n-370} in the CNR than the VNR treatment at the high N and the elevated [CO₂] conditions, which was likely due to the toxic level of K associated with increasing N uptake in the CNR of high P and K proportions. Leaf [K] changes caused by nutrient ratio treatments showed a corresponding trend to P_{n-growth}, indicating that photosynthetic responses were not only correlated to leaf [N], but leaf [K] in the experiment, endorsing the statement that the relationship between photosynthesis and leaf [N] is influenced by nutrient ratio (Cao et al. 2007).

The nutrient ratio modified the responses of photosynthetic nutrient use efficiency of N, P and K (PNUE, PPUE and PKUE, respectively) to [CO₂] and N supply. CO₂ elevation in this study generally resulted in greater PNUE, PPUE and PKUE. While PNUE was greater under elevated [CO₂], PNUE decreased as the N supply increased in this study. The enhancing effect of CO₂ elevation on PNUE agrees with findings of Onoda et al. (Onoda et al. 2009), and the negative relationship between PNUE and leaf [N] is consistent with results of Ripullone et al. (Ripullone et al. 2003a). Deficiency of P and K can result in PNUE reductions (Watanabe et al.

2011). However, that the VNR resulted in greater PNUE under the elevated $[\text{CO}_2]$ but smaller PNUE at the ambient $[\text{CO}_2]$ indicates the effect of nutrient ratio can be altered under different CO_2 conditions. Hidake and Kitayama (Hidaka and Kitayama 2009) showed that PPUE increases with decreasing P availability; the data of PPUE (P availability only increased with increases of N supply in the CNR treatment) agreed with this statement. However, with the same P availability, PPUE decreased when the N supply at lowest level, indicating limited N supply constrained effect of P supply on PPUE. The data of PKUE showed a similar trend as PPUE. However, the PKUE significantly increased under elevated $[\text{CO}_2]$ with increases of N supply in the VNR treatment (K availability remained equal across all N supply levels), indicating an enhanced effect of increasing N supply on PKUE; the reverse trend occurred with PKUE in the CNR treatment (K availability increased with increases of N supply), indicating the high K availability decreased the PKUE. Therefore, these results suggest that nutrient ratio adjusts the relationship between photosynthesis and N supply.

Under the predicted climate change condition, to avoid the stresses of predicted temperature and precipitation, black spruce may migrate northward. Because of inevitable photoperiod and nutrient availability changes, whether the photoperiod and nutrient supply affect its survival and growth in migrated locations is discussed in following Chapter 4.

Chapter 4 Interactive effects of photoperiod and nutrient supply on the potential northward migration of black spruce

Introduction

Global warming has been progressing as the concentration of greenhouse gases in the atmosphere rises (Papadopol 2000, Shafer et al. 2001). The average global temperature is predicted to increase between 1.8 and 4.0 °C by the end of this century (IPCC 2007). Northern America will be one of the regions where the global warming would be the most prominent (Hansen et al. 1996, Bronson et al. 2009). Because of this warming trend, the areas with optimal temperature and precipitation ranges for plant growth will likely shift to higher latitudes in Northern Hemisphere (Walther et al. 2002). The shifts in suitable temperature and precipitation ranges are the premises of predicted plant migrations (Houlder et al. 2000, Pearson and Dawson 2003). Migration may become an inevitable plant response to the climate changes (Pitelka 1997, De Frenne et al. 2011). Based on projections of temperature and precipitation across North America by using Atmosphere Ocean General Circulation Models, a 10 degree northward shift (about 1100 kilometers) for 130 North American tree climate envelopes is predicted to occur between 2071 and 2100 (McKenney et al. 2007, McKenney et al. 2011). However, optimal temperatures and water regimes may not guarantee the success of plant migration, because plants will also need to adjust to the photoperiod and nutrient changes in their predicted new locations.

The changes in photoperiod may affect the timing of photoperiodically controlled growth events of migrated plants and lead to untimely phenological responses, such as earlier bud burst or delayed bud setting and cold hardening. The responses may affect plant growth or even survival. Photoperiod is the relative lengths of day and night in a daily cycle of 24 hours. The

photoperiod is determined by the latitude of a location and not influenced by global climate changes. The photoperiod does not change from year to year and is a reliable indicator of the time of year (Jackson 2009). To a certain location, native plant species have adapted to the photoperiod in their habitats for hundreds and thousands of years, forming their photoperiodisms, rhythms of development, and phenology (Thomas and Vince-Prue 1997). Photoperiodisms control the performances through phytochrome perceives daylength (or nightlength).

Phytochrome is a plant pigment that changes forms according to the ratio of red to far-red light. The ratio of active and inactive form accumulated in the plant is controlled by photoperiod, and this ratio controls plant phenology and other physiological events (Thomas and Vince-Prue 1997, Lambers et al. 2008). At higher latitude the photoperiod will be longer in the summer but shorter in the winter. For instance, with a 10° northward migration the photoperiod will be 2.2 hours longer days in the summer and 1.98 hours shorter days in the winter on Solstice days (Astronomical Applications Department 2009). If the daylengths on August 1 and May 15 are the critical photoperiod for triggering bud set (Bigras and Hebert 1996, Bigras and Bertrand 2006) and bud break (Man et al. 2009, Frechette et al. 2011), the date for these critical photoperiods will come 19 days later and 21 days earlier, respectively, if plants migrate 10° degrees toward the north. Therefore, the migrated plants may start growth earlier and end growth later than they do at their original location. Earlier bud bursts will expose plants to the risk of frost damage in the spring (Man et al. 2009) while a delayed onset of cold hardening may expose the plants to frost damage late in the fall. Although longer photoperiod and longer growing season at higher latitudes can increase plant growth as measured by dry-matter production and leaf size (Hay 1990), the relatively shorter daylength during the winter and shorter duration of dormancy may affect cold hardiness development and performance in the next growing season.

The development of the terminal bud is indicative of the beginning of cold hardiness development (Bigras and Colombo 2001, Colombo and Templeton 2006). Thus, a better understanding of how photoperiod will interact with warmer temperature and CO₂ elevation in affecting plant physiology may be pivotal for predicting the future trend of plant migration and growth under global climate change conditions.

The growth of natural forests at high latitudes is limited by available nitrogen (N) (CSIRO 2009). Though ecosystems at high latitudes can store large amounts of nutrient in the soils, available N concentration is lower further north due to the low rate of decomposition and mineralization of organic materials at higher latitudes (Cousteaux et al. 1995). The predicted 2-4°C increase in mean summer temperature (based on the General Circulation Models, GCMs) does not necessarily translate into an corresponding increase in available N because of interactions among soil temperature, moisture, and low temperature adapted decomposing microorganisms (Robinson 2002). Therefore, black spruce, one of the key tree species in the boreal forests, may experience a shortage of available N when it migrates northward. Although low N supply generally helps induce cold hardening (Landis 1989a), a certain amount of N is required for the proper development of cold hardiness and other physiological functions (Miller and Timmer 1997). However, there are contradictory reports in the literature on the relationship between cold hardiness and N supply (Puertolas et al. 2005), varying from no relationship (Birchler et al. 2001, Floistad 2002), positive (Bigras et al. 1996, Rikala and Repo 1997), and to negative (Calme et al. 1993).

Black spruce (*Picea mariana* (Mill) B.S.P.) is very sensitive to photoperiod (D' Aoust and Hubac 1986) and has been found to have genetically adapted to the local photoperiod of its habitat (Thomas and Vince-Prue 1997). It can continue vegetative growth and does not initiate

terminal buds when exposed to long day treatment (Odlum and Colombo 1989). Furthermore, its terminal bud formation cannot be initiated by low air temperature without short daylength treatment, although it can be induced by a combination of low nitrogen supply and low temperature (Bigras et al. 1996). Similarly, long photoperiods prevent white spruce (*Picea glauca* (Moench) Voss) and Engelmann spruce (*P. engelmannii* Parry) from forming terminal buds (Arnott 1974). The growth of both black and white spruce is reported to be sensitive to long photoperiod (Watt and McGregor 1963) and short photoperiod induces their dormancy (Thomas and Vince-Prue 1997). Therefore, photoperiod changes by northward migration will affect black spruce growth. However, there is a paucity of information regarding the possible migration of black spruce in response to the global climate change. Although the premise of northward migration for boreal tree species has been documented scientifically (Pitelka 1997, Higgins et al. 2003, Pearson 2006, McKenney et al. 2007, McKenney et al. 2009, McKenney et al. 2011), the potential effects of changes in photoperiod and nutrient regime on the survival and growth of black spruce under elevated CO₂ condition are largely unknown (Bronson et al. 2009).

The ability to develop a high degree of cold hardiness is critical for the species to survive the severe winter conditions in the boreal forest (Weiser 1970, Li and Sakai 1978). With a proper level of cold hardiness, black spruce can survive temperatures well below -40°C (Lamhamedi and Bernier 1994). Cold hardiness affects not only plant survival in the winter, but also plant growth in the next growth season, because the terminal bud formation, bud size and the timing of bud burst are closely related to cold hardiness (Colombo et al. 1995). Currently, boreal forests have been experiencing significant environmental changes as a result of increasing atmospheric CO₂ concentration and associated rises in temperature (Bigras and Bertrand 2006, IPCC 2007). Black spruce is predicted to migrate northward in response to the predicted changes in

temperature and precipitation (Malcolm et al. 2002, McKenney et al. 2007, Soja et al. 2007, McKenney et al. 2011). Whether black spruce can develop enough cold hardiness at the potential northward migration locations is a critical process to the black spruce survival and growth.

The overall objective of this study was to examine the interactive effects of photoperiod, nutrient supply and CO₂ concentration on growth and cold hardiness of black spruce seedlings grown under two levels of nitrogen supply (30 and 300 μmol mol⁻¹N), two photoperiods (one for seed origin (PS) and the other one for a location of 10 degrees further north (predicted northward migration - PNM)), and two [CO₂] (370 and 720 μmol mol⁻¹). The specific objectives of the study differed with experimental stages: effects of photoperiod and nutrient supply on growth and cold hardiness development in the first growing season and bud phenology and growth in the second growing season. Since black spruce will likely encounter changes in both photoperiod and available nutrient when migrating northward under the predicted scenario of climatic change, a good understanding of the effects of photoperiod and nutrient regime on the development of cold hardiness becomes critical. I hypothesized that the photoperiod change associated with northward migration would promote the growth and the low nutrient supply would have the opposite effect. Since the development of cold hardiness requires duration of certain photoperiod and adequate nutrient condition, I also hypothesized that the shortened period of time for cold hardiness development associated with northward migration and the low nutrient supply would reduce cold hardiness quality of black spruce seedlings.

Materials and methods

Plant materials

Black spruce (*Picea mariana* [Mill.] B.S.P.) seeds were collected from a 64 year-old black spruce stand near Huntsville, Ontario, Canada (45°26'49.54"N, 79°14'35.15"W) in 2009. The stand was composed of 70% black spruce, 20% balsam fir (*Abies balsamea* (L.) Mill.), and 10% larch (*Larix decidua* Mill.) in a lowland riparian zone near Lake Fish. About 5,000 (about 0.1pound) seeds were collected from 30 trees at least 50 m apart from each other. The seeds were germinated in the greenhouses of Lakehead University Thunder Bay Campus. Four hundred eighty germinants were chosen randomly and potted (15cm height, 13cm diameter) with a mixture of peat moss and vermiculite (1:1; v/v).

Experiment design

The treatments consisted of two CO₂ concentrations (370 vs. 720 μmol mol⁻¹), two photoperiods (PS for seed origin with 15 and 10 h for active growing and cold hardening stages, respectively, and PNM for 10 degrees north from the seed origin with 17 and 8 h for active growing and cold hardening stages, respectively), and two nutrient supply levels (30 vs. 300 μmol mol⁻¹N). The 15-hour photoperiod represents the actual median photoperiod during the growing season at the seed origin and the 17-hour for the corresponding median photoperiod 10 ° north from the seed origin. The 10 hours and 8 hours represent the median photoperiods during cold hardening phase, respectively, for PS and PNM locations. The concentrations of P and K were the same for both N treatments, 60 and 150 μmol mol⁻¹, respectively. The experiment was

carried out in four greenhouses with identical design features and control devices. Each of the two CO₂ levels was randomly assigned to two independent greenhouses.

All other environmental factors ([CO₂] and temperature) were automatically controlled and monitored using an Argus control system (Argus Control Systems Ltd, Vancouver, BC, Canada). The day/night temperatures were 25-26/16-17 °C for all the greenhouses. The same temperature regime was used for the two photoperiod treatments based on the assumption of the climate envelope shift that the predicted northward migration would have the same temperature and water regime as the current regime at the location of seed origin (McKenney et al. 2007, McKenzie et al. 2011). The photoperiod in all the greenhouses was set to long days and the short days were achieved through manual shading of the seedling blocks in early mornings and late evenings with the standard blackout used by tree nurseries. Each photoperiod treatment started with summer long days (15 hours for 75 days for PS and 17 hours for 95 days for PNM) followed by the corresponding short days (10 hours for 50 days for PS and 8 hours for 30 days for PNM). The calculation of daylength was based on the annual daylength curve and sum of accumulated temperature of the growing season for the experimental locations (Figure 4.1). The seedlings were fertilized once a week using liquid fertilizer at the previous described nutrient concentrations. The water content of the growing medium was maintained above 30% monitored with an HH2 Moisture Meter (Delta-T Devices, Cambridge, U.K.). The seedlings were watered every two days. When the volumetric water content of the growing medium declined to 30% (Bergeron et al. 2004), an extra watering was applied.

After 125 days treatment (Growing season I), the seedlings were randomly chosen for measuring seedling height, root collar diameter, terminal bud size, biomass, chlorophyll fluorescence and electrical conductivity. Among the seedlings, ten seedlings from each treatment

combination were used for chlorophyll fluorescence measurement, fifteen for electrical conductivity, and three for terminal bud size and biomass. Those samples used for biomass measurement were oven-dried at 75 °C for 48 hours (separately for shoots and roots) and their dry mass was determined on an analytical balance.

Another six seedlings were randomly chosen and placed in cold storage (temperature -2 to -4 °C, 3 months) for the next phase of the experiment (Growing season II). The second phase of the experiment was aimed to examine the carrying over effects of treatments applied in the first phase on the bud phenology and growth in the next growing season. These seedlings were exposed to growth conditions described previously again. The seedlings who received PS treatment were under short day photoperiod for first three weeks and then changed to long day photoperiod for the next six weeks; the seedlings who received PNM treatment were under short day photoperiod for one week and then changed to long day photoperiod for the next eight weeks (i.e., in 63 days experiment, PS treatment was divided to 21 days of short day photoperiod and 42 days of long day photoperiod, but PNM treatment 7 days of short and 56 days of long). The advance of two weeks long day photoperiod treatment on PNM reflects the photoperiod changes of the new growing season when black spruce grows at the 10 degree northward location compared to its seed origin (Figure 4.1).

Chlorophyll fluorescence measurement

At the end of Growing season I, 10 seedlings were selected from each treatment combination for determination of chlorophyll fluorescence on terminal shoots using a FMS 2, a pulse modulated chlorophyll fluorometer (Hansatech Instruments Ltd, King's Lynn, Norfolk, England). Chlorophyll fluorescence of initial (F_o) and maximum (F_m) were obtained after

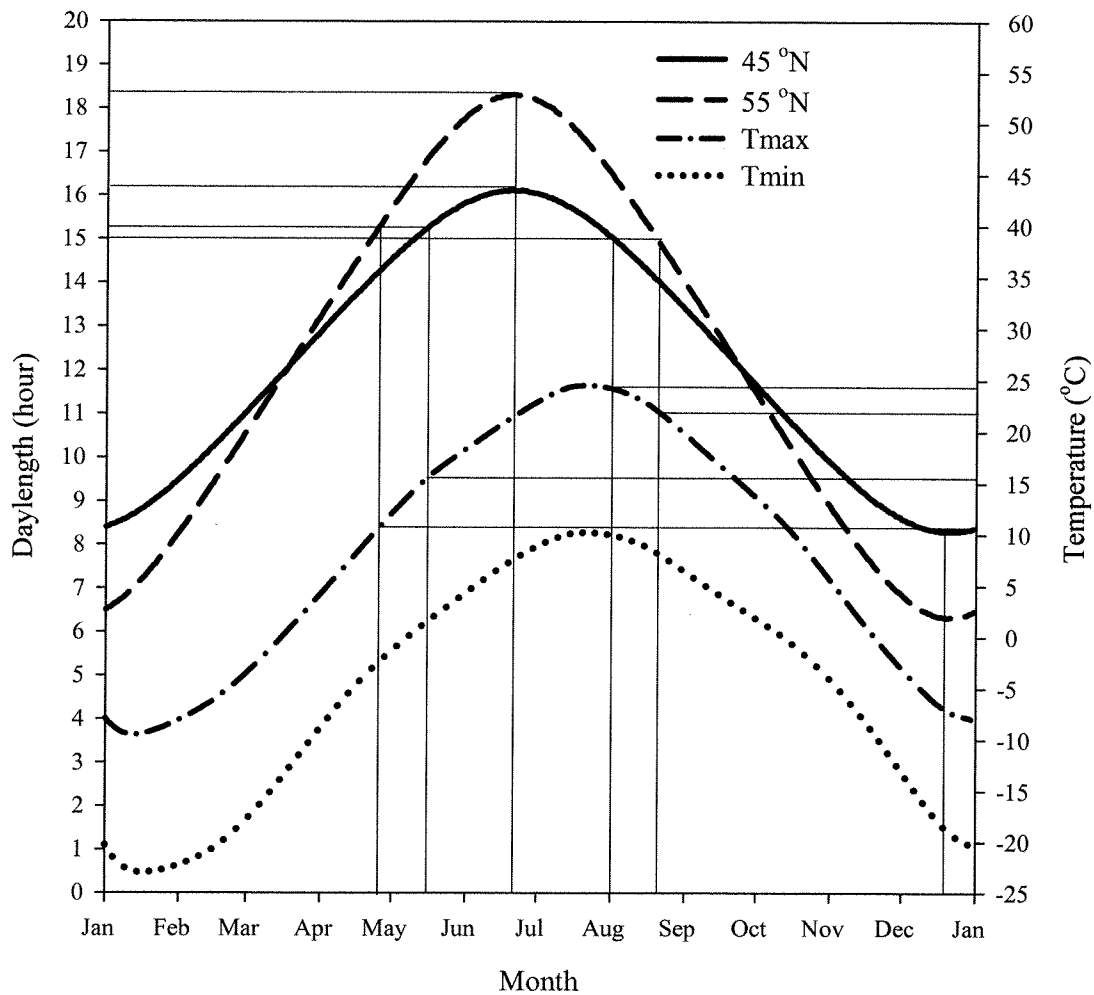


Figure 4. 1 Schematic diagram of daylength and air temperature at 45° (seed origin) and 55° N latitude (10° north from seed origin). T_{max} and T_{min} are monthly maximum and minimum air temperatures at the location of seed origin (Data source: U.S. Naval Observatory (Astronomical Applications Department, 2009) and WorldClim (Hijmans et al., 2005)).

30-minute dark adaptation. The variable chlorophyll fluorescence (F_v) was calculated as the difference between F_m and F_o , and maximum quantum efficiency of PSII photochemistry was calculated as the ratio, F_v / F_m .

Electrical Conductivity and index of injury

At the end of phrase I, fifteen terminal shoot tips were randomly collected from each treatment combination for cold hardiness assessment using the methods described by Colombo et al. (1984, 2002). A 2-3 cm long shoot tip was cut using scissors and rinsed with distilled water. Three shoot tips from each treatment combination were used for electrical conductivity measurement at each of the testing temperatures, non-frozen (control), -5, -15, -30 and -60 °C. The testing treatments were achieved using a programmable freezer (Foster Refrigeration Ltd, King's Lynn Norfolk, U.K.) by gradual decrease of temperature at 5 °C per hour. After one hour exposure to the testing temperatures, samples were gradually returned to room temperature at 5 °C per hour. Each shoot tip was then placed into a jar and immersed in 100 ml distilled water and incubated at room temperature (20-25 °C) for 24 hours. The solutions were measured for electrical conductivity using an electrical conductivity meter (Fisher Accumet AR 20, Fisher Scientific, Ottawa, Ontario, Canada). The electrical conductivity from the no freezing treatment was used as the control (ECC), for comparing the electrical conductivity of samples subjected to freezing temperatures (ECF). After the measurement of electric conductivity for ECC and ECF, all the solutions were placed in an oven at 80 °C for two hours and then cooled to the room temperature over night; the solutions were measured for electrical conductivity again as killed electrical conductivity (ECK).

The index of injury (I_i) is inversely related to cold hardiness and was calculated using the following equation [1] (Colombo et al. (1984, 2002):

$$I_i = \frac{\frac{ECF}{ECK} - \frac{ECC}{ECK}}{1 - \frac{ECC}{ECK}} \times 100 \quad [1]$$

where: I_i = index of injury (%)

ECC = electrical conductivity of control shoot tips,

ECF = electrical conductivity of the solution measured following freezing shoot tips at -5, -15, -30 and -60°C,

ECK = electrical conductivity of the solution measured after killing shoot tips at 80 °C.

Morphological and biomass measurements

Thirty seedlings from each treatment combination were measured for height and root collar diameter (RCD) after four months (125 days) growth (Growing season I). Three sample seedlings, randomly chosen from each treatment combination, were harvested and shoot and root dry mass was obtained on an analytical balance to determine root mass ratio (RMR = root mass/total seedling mass) and total biomass. The terminal bud size (bud length and width) of all seedlings was measured using a high-resolution digital imaging system, AMS-MV2 (Advanced Microscopy Group, Mill Creek, WA, USA, 2008) and Micron Imaging Software (USB2) 1.08 (Westover Scientific, Inc., Mill Creek, WA, USA, 2008). Height and RCD were also measured at the end of the second phase of the experiment to determine height and RCD increment and relative growth rate. The stem volume (V) at the end of Growing season I and Growing season II

was calculated from height (H) and RCD (D) using the following equation (van den Driessche 1992):

$$V = (\pi D^2/4) H/3 \quad [2]$$

The relative growth rates of height (RGR_h), RCD (RGR_d), and volume (RGR_v) were determined by dividing the growth increments with the seedling sizes at the end of Growing season I. The bud phenology was observed daily, and days to bud burst (DBB) and days to bud burst after long photoperiod (DBBL) were recorded. DBB was the number of days after cold storage (including the two weeks of spring short days) when terminal buds burst. DBBL was the number of days after spring short days when bud burst occurred (total of long days). Seedling mortality was calculated by dividing the number of dead seedlings by the total number of seedlings in a treatment combination.

Statistical analysis

The experiment was a split-plot design with CO₂ treatment as the main plots and factorial combinations of photoperiod and nutrient supply as the split plots. The data were examined graphically for normality (probability plots for residuals) and homogeneity of variance (scatter plots). Both assumptions for analysis of variance (ANOVA) were met. When ANOVA showed a significant effect ($P < 0.05$) for an interaction, Least Significant Difference (LSD) multiple comparisons were conducted. Since all the seedlings that received low nutrient treatment (30 $\mu\text{mol mol}^{-1}\text{N}$) died in the second phase of the experiment, the nutrient supply was removed from the analysis for the second phase data. All the analyses were conducted using the GenStat statistics package, 12th Edition (VSN international Ltd, Hemel Hempstead, UK, 2009).

Results

Growing season I

Height and RCD

Height and root collar diameter (RCD) were significantly affected by the interactions between photoperiod and nutrient supply and between CO₂ concentration and nutrient supply (Table 4.1). The CO₂ elevation and high N significantly increased height growth and the CO₂ elevation effect was greater in the high nutrient treatment (Figure 4.2 A). The height was smaller at the photoperiod of the seed origin than that of 10 degrees north, especially at high nutrient supply (Figure 4. 2 C). While the general treatment responses for RCD were similar to those of height, the effects of the [CO₂] and photoperiod on RCD were largely in the high nutrient treatment (insignificant C and P, but significant C x N and P x N, see Table 4.1 and Figures 4.2 B and 4.2D).

Bud size

Both bud length and bud width were longer and wider at the high nutrient level (significant N effect) and the effect largely occurred in the PS (significant P x N effect) (Table 4.1 and Figures 4.3A and 4.3B).

RMR and total biomass

Both root mass ratio (RMR) and total seedling biomass of the seedlings increased with nutrient level (significant N effect, see Table 4.1 and Figure 4.4). Among the eight treatment combinations, the difference between the two photoperiods in RMR was greater in the ambient

Table 4. 1 ANOVA P values for the effects of CO₂ concentration (C), photoperiod (P), nutrient supply (N) and their interactions on seedling growth including height (H), root collar diameter (RCD), bud length (BL), bud width (BW), root mass ratio (RMR), and total biomass (TM), index of injury (I_i) (at test temperatures of -5, -15, -30, and -60 °C), measurements, the initial (F₀), maximum (F_m), and variable (F_v) of chlorophyll fluorescence, and maximum quantum efficiency of PSII (F_v/F_m) of black spruce grown under two [CO₂] (370 and 720 μmol mol⁻¹), two photoperiods (one at the seed origin and one at a 10° northward migration), and two nutrient supply levels (30 and 300 μmol mol⁻¹ N) for 125 days.

Source of variation	Treatment effects						
	C	P	N	C×P	C×N	P×N	C×P×N
H	0.035	0.015	<0.001	0.564	0.002	0.005	0.659
RCD	0.115	0.062	<0.001	0.981	<0.001	<0.001	0.206
BL	0.097	0.049	<0.001	0.134	0.403	0.002	0.652
BW	0.37	0.137	0.002	0.173	0.268	0.002	0.383
RMR	0.239	0.012	<0.001	0.889	0.005	0.718	0.02
TM	0.527	0.114	<0.001	0.806	0.107	0.05	0.115
F ₀	0.032	0.21	<0.001	0.926	0.434	<0.001	0.916
F _m	0.045	0.187	0.871	0.568	0.195	0.013	0.581
F _v	0.052	0.182	0.001	0.511	0.152	0.05	0.508
F _v / F _m	0.097	0.275	<0.001	0.479	0.249	<0.001	0.851
I _i at -5°C	0.904	0.08	0.002	0.379	0.161	0.802	0.709
I _i at -15°C	0.072	0.043	<0.001	0.871	0.008	0.034	0.754
I _i at -30°C	0.057	<0.001	<0.001	0.038	0.246	0.022	0.946
I _i at -60°C	0.071	0.626	<0.001	0.867	0.494	0.128	0.124

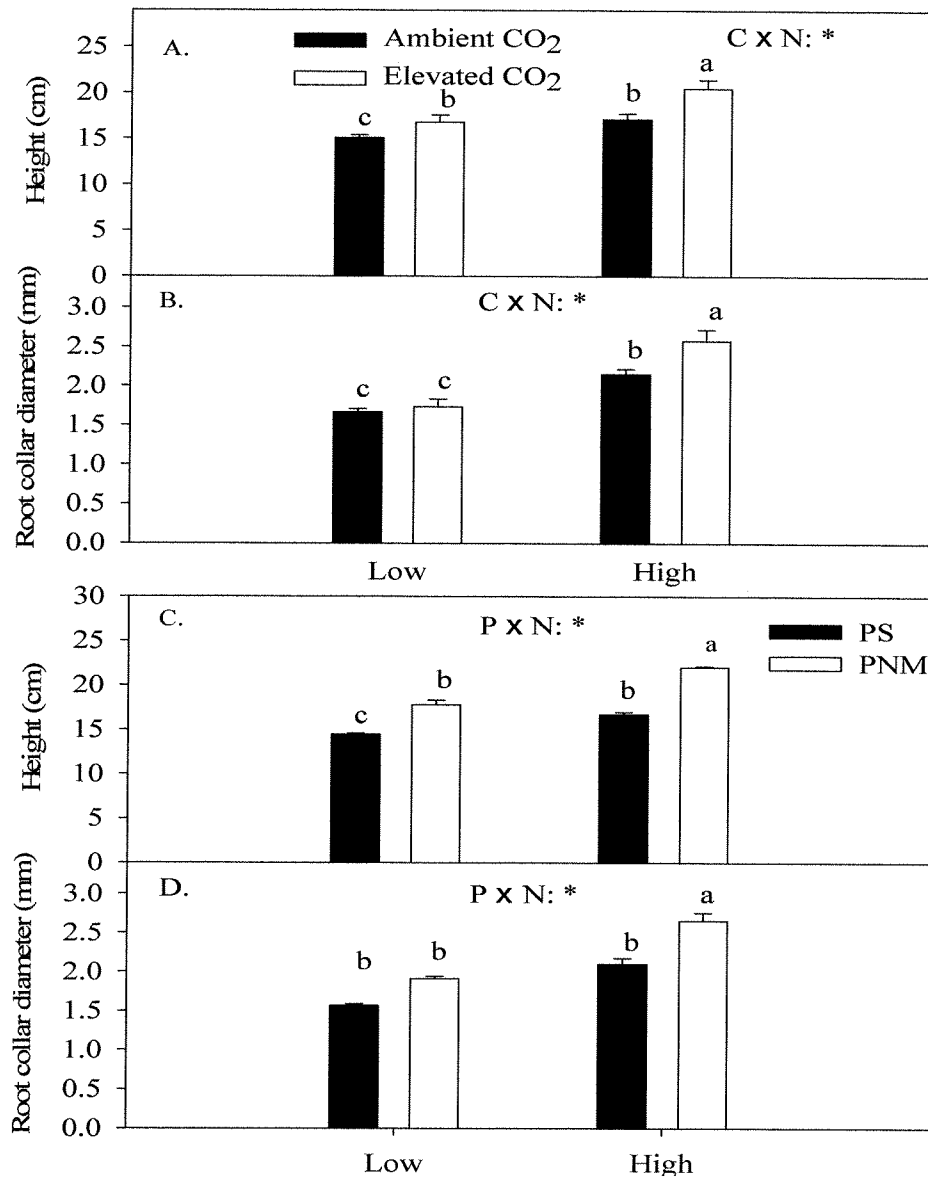


Figure 4. 2 Mean (+ SEM) height and root collar diameter of black spruce seedlings grown under two [CO₂] (C) levels (370 (ambient) vs. 720 (elevated) $\mu\text{mol mol}^{-1}$ [CO₂]), two photoperiods (P) (PS - photoperiod at the seed origin, PNM corresponding to a 10° northward migration), and two nutrient supply (N) levels (30 (Low) vs. 300 (High) $\mu\text{mol mol}^{-1}$ N) for 125 days. “*” indicates significance at $P \leq 0.05$. Means with different letters were significantly different from each other ($P \leq 0.05$).

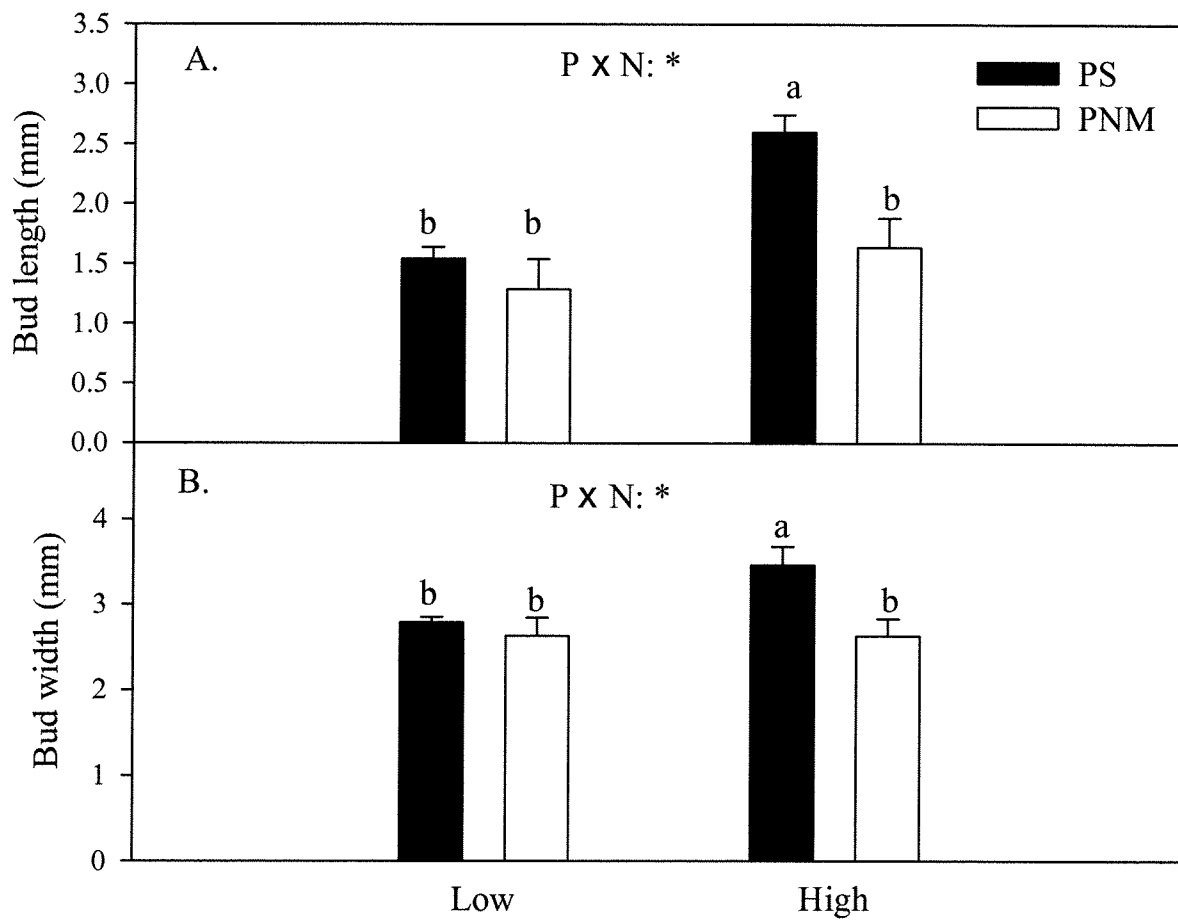


Figure 4. 3 Mean (+ SEM) bud length and width in black spruce seedlings. (see Figure 4.2 for details).

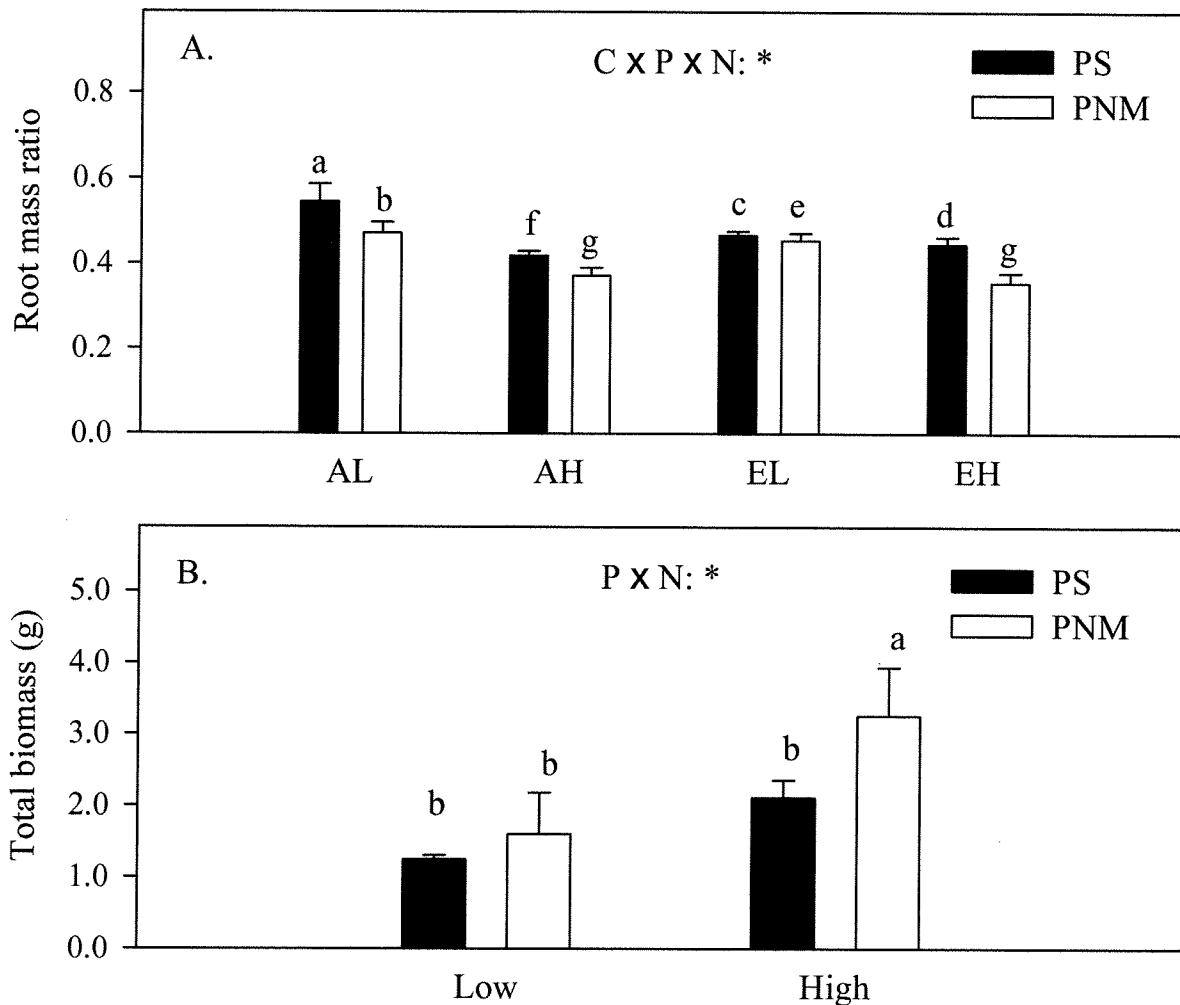


Figure 4. 4 Mean (+ SEM) root mass ratio and total biomass of black spruce seedlings.

(AL= ambient [CO₂] with low nutrient supply, AH= ambient [CO₂] with high nutrient supply, EL= elevated [CO₂] with low nutrient supply, and EH= elevated [CO₂] with high nutrient supply) (see Figure 4.2 for details).

[CO₂] with low nutrient supply, and the elevated [CO₂] with high nutrient supply than that in other treatment combinations (significant P, C x N, and C x N x P, see Table 4.1 and Figure 4.4A). Comparatively, the difference between the two photoperiods in total biomass was greater at high nutrient level (significant P x N, see Table 4.1 and Figure 4.4B).

Chlorophyll fluorescence

Initial (F_0), maximum (F_m), and variable (F_v) were greater at the elevated [CO₂] (significant C effect, see Table 4.1; at the ambient [CO₂], mean of $F_0 = 153 \pm 4.84$ bit, $F_m = 717 \pm 15.98$ bit, $F_v = 564 \pm 12.57$ bit, under the elevated [CO₂], mean of $F_0 = 161 \pm 3.64$ bit, $F_m = 813 \pm 11.32$ bit, $F_v = 652 \pm 9.89$, respectively). Among the four treatment combinations by nutrient supply and photoperiod, the difference between the two photoperiods occurred primarily at low nutrient supply in F_0 , F_m , and F_v , but no significant difference appeared at the high nutrient supply (Figures 4.5 A, 4.5 B and Figure 4.5 C). Inversely, maximum quantum efficiency of PSII photochemistry (F_v/F_m) was greater in the PNM treatment than the PS treatment at the high nutrient supply, but no significant difference appeared between the two photoperiod treatments at the low nutrient supply (significant P x N effect, see Table 4.1 and Figures 4.5 D). Between the two nutrient levels, F_0 was greater at low nutrient supply, while F_v and F_v/F_m were greater at high nutrient supply, and F_m had no significant difference between the two nutrient levels (significant N effect, see Table 4.1 and Figures 4.5 A, 4.5 C and 4.5 D).

Index of injury (Ii)

The index of injury in black spruce seedlings generally decreased with the increasing N

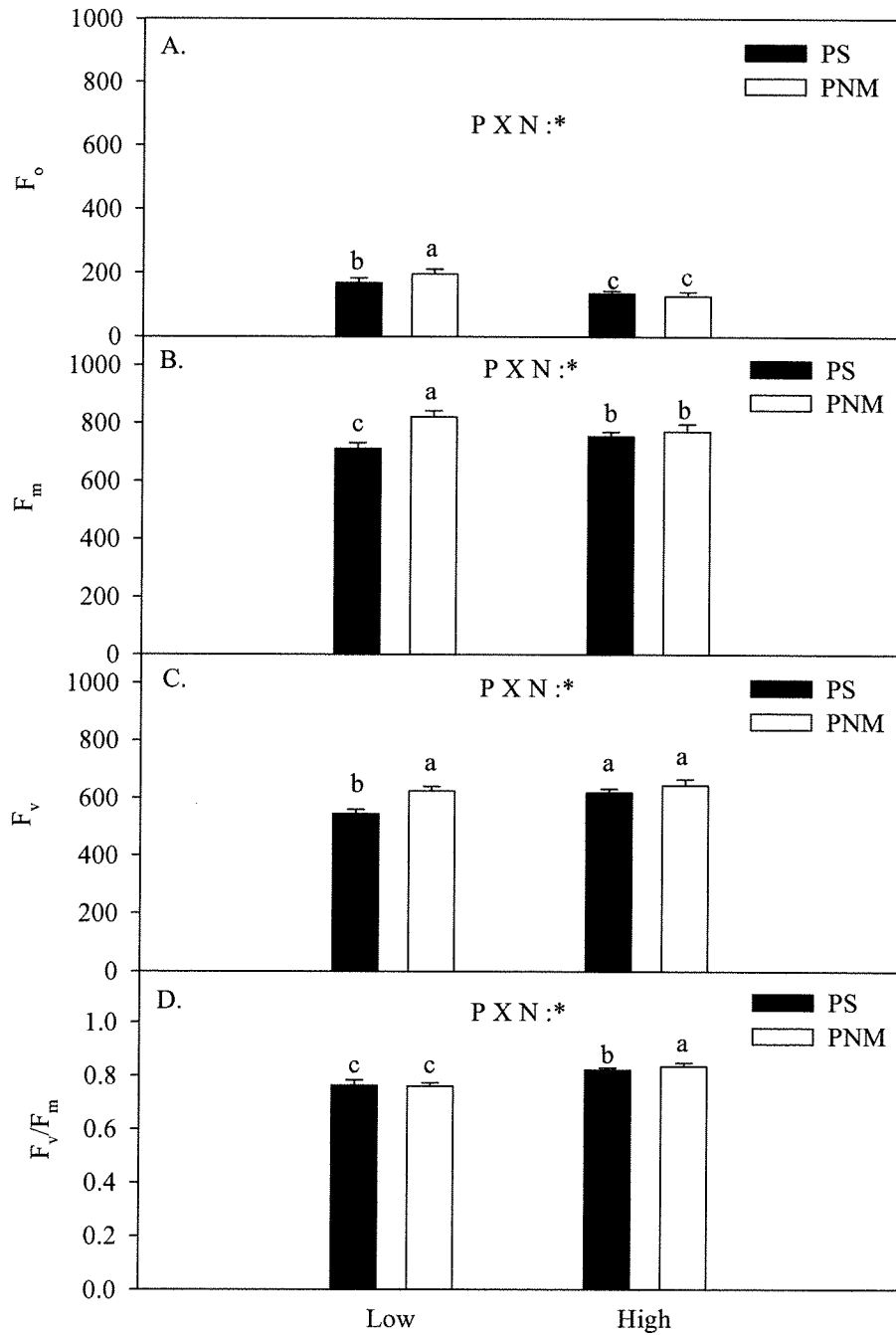


Figure 4. 5 Mean (+ SEM) of initial (F_0), maximum (F_m), variable (F_v) chlorophyll fluorescence and maximum quantum efficiency of PSII (F_v/F_m) of black spruce seedlings. Other explanations are as in figure 4.2

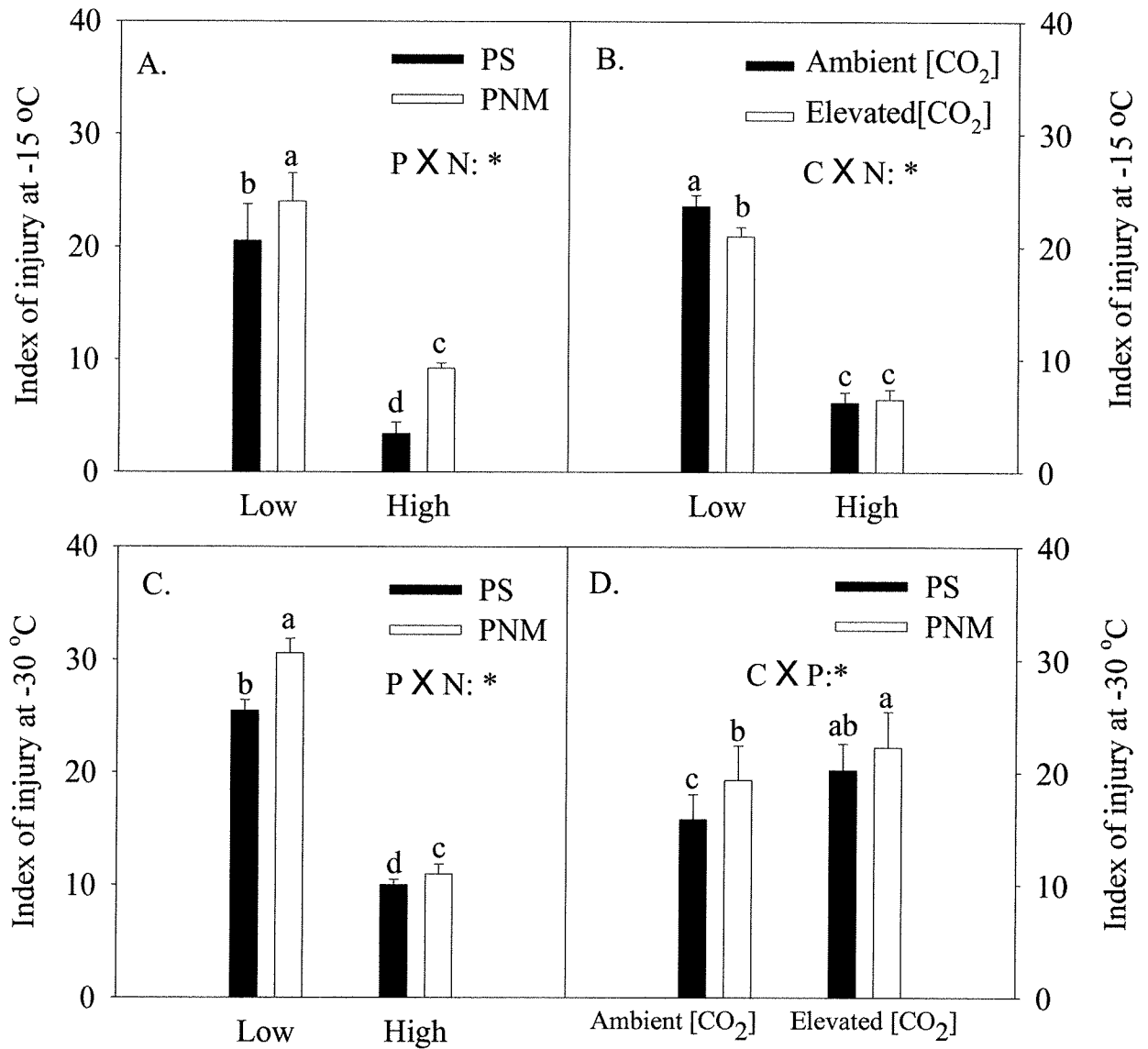


Figure 4. 6 Index of injury ($\bar{X} + \text{SEM}$) at -5, -15, -30 and -60 °C of black spruce seedlings.

Other explanations are as in Figure 4.2.

supply at all freezing temperatures between -5 and -60°C (significant N effect, see Table 4.1 and Figures 4.6A, 4.6B, and 4.6C; mean index of injury at -5°C was 4.20 ± 0.34 for the low nutrient supply, 2.87 ± 0.20 for the high nutrient supply; mean index of injury at -60°C was 30.91 ± 0.81 for the low nutrient supply, 15.15 ± 0.89 for the high nutrient supply). At the freezing temperatures of -15 and -30°C, however, the PNM resulted in higher seedling injury than PS and the effect was greater at the high N supply for -15°C and at the low N supply for -30°C (significant P and P × N effects, see Table 2.1 and Figures 4.6A and 4.6C). Significant decrease of seedling injury at -15°C by [CO₂] elevation occurred only in low N supply and increase of seedling injury at -30°C by PNM only in ambient [CO₂] (significant C × N at -15°C and C × P at -30°C, see Table 2.1 and Figures 4.6B and 4.6D).

Growing season II

In the Growing season II, the growth variables of black spruce seedlings were not affected by [CO₂] levels, except on F_v/F_m ratio; were affected by photoperiod on height, RGR_h, RGR_d, RGR_v, DBB, mortality and F_v/F_m; were affected by interactions of [CO₂] and photoperiod on RGR_d and F_v/F_m (Table 4.2). None of the seedlings receiving low nutrient treatment ($30 \mu\text{mol mol}^{-1}\text{N}$) during the first year survived in the Growing season II of the experiment.

Height, RCD and relative growth rates

The PNM significantly increased total height (H) (mean of height was 30.47 ± 1.40 cm for the PNM, 26.49 ± 1.03 cm for the PS), but decreased relative growth rates in height (RGR_h) and volume (RGR_v) of black spruce seedlings (with the PNM, mean of RGR_h = 0.44 ± 0.01 cm cm⁻¹,

Table 4. 2 ANOVA *P* values for the effects of CO₂ (C) concentration, photoperiod (P), and their interactions of black spruce seedlings on height (H), RCD, height increment (H_i), RCD increment (RCD_i), relative growth rates of height (RGR_h), RCD (RGR_d) and volume (RGR_v), root mass ratio (RMR), total biomass (TM), total days to bud burst (DBB), total long days to bud burst (DBBL), mortality, measurements of initial (F₀), maximum (F_m), variable (F_v) chlorophyll fluorescence and maximum quantum efficiency of PSII photochemistry (F_v/F_m) after Growing season II.

Source of variation	Treatment effects		
	C	P	C × P
H	0.056	0.029	0.221
RCD	0.112	0.483	0.896
H _i	0.076	0.382	0.896
RCD _i	0.277	0.368	0.407
RGR _h	0.157	0.018	0.486
RGR _d	0.14	0.003	0.042
RGR _v	0.278	0.005	0.081
RMR	0.532	0.057	0.173
TM	0.393	0.124	0.777
DBB	0.595	0.01	0.594
DBBL	0.332	0.947	0.567
Mortality	0.795	0.012	0.423
F ₀	0.094	0.643	0.949
F _m	0.631	0.996	0.723
F _v	0.723	0.929	0.687
F _v /F _m	0.011	0.032	0.053

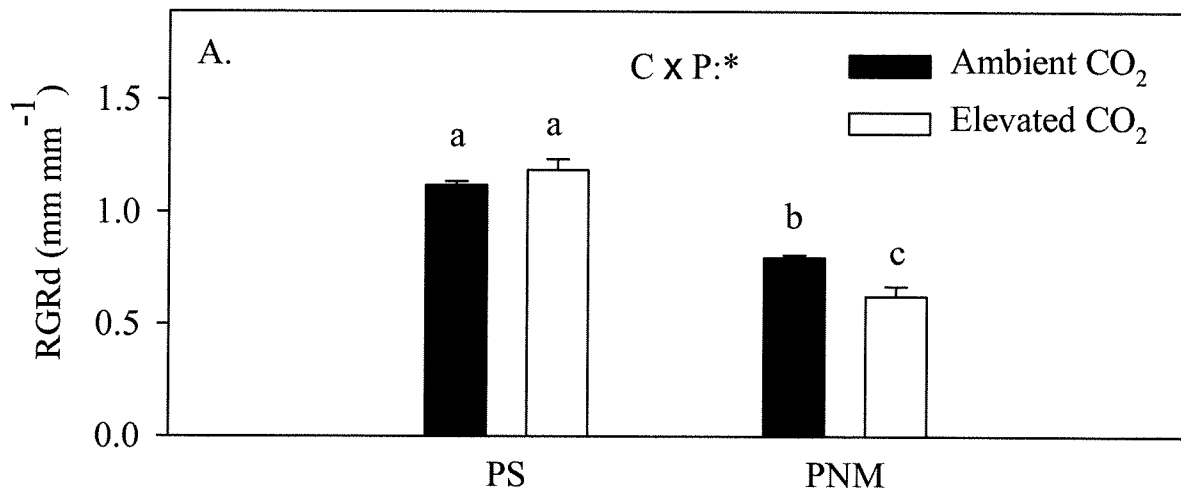


Figure 4. 7 Mean (+ SEM) of relative growth rate in root collar diameter (RGRd)I of black spruce seedlings. The seedlings from the first phase were cold stored for three months before the treatments were resumed in this second phase. The measurements were taken after Growing season II resumption of treatments. Other explanations are as in Figure 4.2.

$RGR_v=3.23\pm 0.15 \text{ cm}^3 \text{ cm}^{-3}$; with the PS, mean of $RGR_h = 0.59\pm 0.01 \text{ cm cm}^{-1}$, $RGR_v=6.39\pm 0.22 \text{ cm}^3 \text{ cm}^{-3}$, respectively.) (Table 4.2). The decrease of RGR_d by the PNM was greater under the elevated $[\text{CO}_2]$ (significant $C \times P$ effect, see Table 4.2 and Figure 4.7A). Photoperiod did not affect RCD, height increment (H_i), RCD increment (RCD_i), root mass ratio (RMR), or total biomass (TM) of black spruce seedlings in the second growing season.

DBB and mortality

Total days to bud burst (DBB) was significantly less and mortality was greater at the PNM (significant P effect, see Table 4.2; mean of DBB was 10.35 ± 1.5 days for the PNM, and 20.95 ± 0.68 days for PS; mean of mortality was 41.46 ± 4.81 percent for the PNM, 4.17 ± 0.41 percent for the PS, respectively). Photoperiod did not affect total long days to bud burst (DBBL) (Table 4.2).

Chlorophyll fluorescence

Among the chlorophyll fluorescence measurements on black spruce seedlings, only maximum quantum efficiency of PSII photochemistry (F_v/F_m) was significantly greater under the elevated $[\text{CO}_2]$ than the ambient $[\text{CO}_2]$ ($P=0.011$) and at the PNM than the PS photoperiod (significant C and P effects, see Table 4.2 and Figure 4.8). The significant photoperiod effect however primarily occurred under the elevated $[\text{CO}_2]$. Photoperiod did not affect initial (F_0), maximum (F_m), and variable fluorescence (F_v) (Table 4.2).

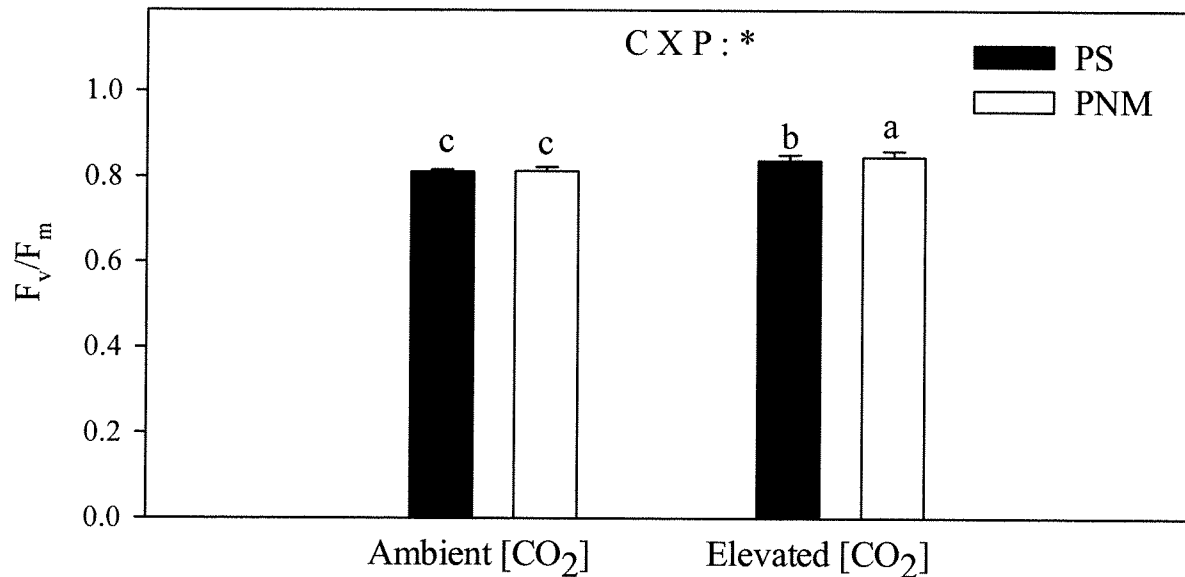


Figure 4. 8 Maximum quantum efficiency of PSII (F_v/F_m) ($\bar{X} + SEM$) of black spruce seedlings. Other explanations are as in Figure 4.2

Discussions

The data support the hypothesis that the elevated [CO₂], photoperiod associated with northward migration (PNM) and high nutrient supply increase the growth of black spruce seedlings. The enhanced effects varied with the time of seedling growth. In the first growing season, it supports the hypothesis that the elevated [CO₂], PNM photoperiod, and high nutrient supply enhance height and diameter growth and seedling total biomass. These are consistent with the findings indicated by many studies that CO₂ elevation enhances plant photosynthesis and promotes growth (Elkohen and Mousseau 1994, DeLucia and Thomas 2000, Yazaki et al. 2004,

Bigras and Bertrand 2006, Norby et al. 2010a). The data also suggest that the effects of the elevated $[\text{CO}_2]$ and PNM photoperiod were greater at high nutrient supply, similar to the synergetic relationship between $[\text{CO}_2]$ and nutrient supply shown by Wang et al. (1995) and Zhang et al. (2006a), as well as by the results in chapter 2 of this study. The enhanced growth by the PNM was probably due to longer daylength (15 h vs. 17 h) and more growing days (75 days vs 95 days). As black spruce is sensitive to photoperiod change (D' Aoust and Hubac 1986), it continues vegetative growth under long days without setting buds (Odlum and Colombo 1989). The greater total mass, height and diameter in the PNM treatment with the high N supply were probably a result of increased carbon allocation to aboveground growth, as indicated by the smaller root mass ratio (RMR). Comparing to high RMR indicates stresses of nutrient supply (Chapin III 1980) or drought (Guo et al. 2010), the low RMR represented promoted height and RCD growth and heavier biomass accumulation in this study. The lowest RMR was observed in the PNM under elevated $[\text{CO}_2]$, indicating that the effect of PNM was enhanced by the elevated $[\text{CO}_2]$.

The PNM-enhanced growth did not fully continue in the second growing season. Although seedlings with the PNM broke buds almost 10 days earlier than those in PS, due to triggering of earlier long photoperiod signal, the resultant greater number of growing days did not translate into greater seedling growth. Although the seedlings still had greater total height and maximum quantum efficiency (F_v/F_m) in the PNM, their relative growth rates in height, root collar diameter and volume were lower than in the PS. The relative growth rate in PNM in height, root collar diameter and volume declined 25.17%, 38.28% and 49.37% respectively, since relative growth rates are more affected by light intensity than by duration (Leopold and Kriedemann 1975). Northward migration to higher latitudes will lead to lower light intensity than

at the seed origin because sunlight angles are lower at high latitudes. Thus, the results imply that the species will likely have lower relative growth rates when black spruce migrates northward. However, the negative impact of lower RGR may be more than offset by the longer photoperiod and longer growing season associated with the northward migration. It should be pointed out that the effect of light intensity was not tested in this experiment, but should be studied in future efforts. In addition, the seedlings in the PNM not only had a lower relative rate of growth in the next season but also suffered a mortality rate of more than 40%, as compared to the mortality rate of less than 5% in the PS treatment.

The data support the hypothesis that the cold hardiness quality is reduced by the PNM and low nutrient supply but regardless to the $[CO_2]$. The size of the terminal bud was much smaller in the PNM treatment, indicating lower level of cold hardiness quality (Colombo and Templeton 2006). Although the process of cold hardening (indicated by terminal bud setting) is generally induced by both lower temperature and shorter photoperiod, some species, such as black spruce, are more sensitive to daylengths, black spruce can set buds at warmer temperatures with shorter daylength, grow more needle primordia, and consequently build up its cold hardiness (Colombo et al. 1989, Colombo et al. 2003). The index of injury tested at $-15^{\circ}C$ and $-30^{\circ}C$ shows that the PNM treatment resulted in more severe freezing injury than the PS treatment, indicating PNM suppressed the seedlings' cold hardiness development. According to Krause et al. (1984, 1989) and Bigras and Bertrand (2006), greater F_v/F_m and F_o indicate higher growth potential and chloroplast damage, both of which mean reduced cold hardiness. The low N supply resulted in a higher index of injury at all test freezing temperatures, indicating cold hardiness development demanded certain N supply. Although cold hardiness may be initiated by short photoperiods, low temperatures and low nutrient supply combinations (Landis 1989a), too

low in N supply may be not the adequate for development of cold hardening. This argument agrees with Miller and Timmer (1997), Bigras et al. (1996) and Rikala and Repo (1997).

The data suggest that nutrient supply is important to the growth of black spruce in terms of taking advantage of elevated [CO₂] and longer daylength when black spruce migrates northward under predicted climate change. The high nutrient supply also increases cold hardiness of buds, shown by the data of bud size and index of freezing injury, as suggested by Miller and Timmer (1997), Bigras et al. (1996) and Rikala and Repo (1997). That all the black seedlings receiving the low N treatment died after the cold storage, indicating the consequence of poor cold hardiness. The low nutrient supply and the PNM together probably were the cause of the death of seedlings after cold storage because of the poor cold hardiness quality.

Under the predicted scenario of climate change, trees likely have longer daylength and duration of growing season when black spruce migrates northward. However, black spruce may not be able to take advantage of this benefit due to poor bud formation and cold hardiness and therefore higher risk of frost damage and reduced potential for growth. The reduced cold hardiness may constrain that black spruce successfully migrate to predicted northward locations. These results suggest that black spruce northward migration needs to establish a new trade-off between growth and stress resistance to cope with photoperiod and nutrient availability changes from seed origin to northward locations.

Chapter 5 General Discussions

The global climate change imposes stresses to plants. There are two primary mechanisms of acclimation that plants use in response to stresses: tolerance and avoidance (Taiz and Zeiger 2002, Lambers et al. 2008). Plants can tolerate the stresses physiologically and morphologically, so that they can continue to exist in the changed environment. In contrast, the plants can avoid the stresses by migrating to a new location when the environment conditions at the current site become less favorable. Research has suggested that the environmental condition in the boreal forest region will become less and less favorable for the existence and growth of boreal plants (Soja et al. 2007). This thesis was set out to examine the plasticity of black spruce in acclimating to certain aspects of future climatic conditions and the ability of the species to cope with the new environmental conditions associated with a northward migration as predicted by climate envelope models.

To assess the acclimation under the stresses of global climate change, a relationship between growth or photosynthesis and nitrogen supply is commonly used as a tool and the relationship may be affected by different combinations with other mineral elements. Among mineral elements, nitrogen (N), phosphorus (P) and potassium (K) are three major macronutrients. Since plant amino acids, enzymes, nucleic acids, chlorophylls, and hormones use N as a critical constituent (Rook 1991, Marschner 1995a, Taiz and Zeiger 2002) and the quantity required is larger than any other mineral element (Marschner 1995a, Nicodemus et al. 2008a), the N availability is often used as a key factor to assess plant growth responses to climate change (McMurtrie 1991, Dewar and McMurtrie 1996). However, because of the effect of interactions

among nutrient elements, an N-P-K ratio effect should be taken into account in order to explain the relationship between growth and N supply precisely.

N-P-K ratio modified the responses of the black spruce seedlings to elevated $[\text{CO}_2]$ and N supply. Under the elevated $[\text{CO}_2]$, the seedlings required higher nutrient supply to support growth; the increased nutrient demand appeared to be primarily for N; consequently, the results indicated a corresponding increase in N / P and N / K ratios. The CNR treatment at high N supplies resulted in CTC related growth suppression, while the VNR treatment at the low N supply induced CDC related growth suppression. At the ambient $[\text{CO}_2]$, the seedling growth was suppressed by CTC at the high N supply and CDC at the low N supply with both the CNR and VNR. The CTC of N, P and K are not proportionally changed under the elevated $[\text{CO}_2]$. The data of growth, net photosynthetic rate and leaf [N], [P] and [K] suggest that the CTC of N is greater than the CTC of K. When N and K supply were kept proportionally (e.g., the CNR treatment in this experiment), the relatively high K concentration suppressed net photosynthetic rate (P_n) and height growth at the high N supply levels. Since more portion of N is needed in N-P-K ratio under predicted $[\text{CO}_2]$, N availability should be the primary factor that is taken into account for future sustainable forest management.

The relationship between photosynthesis and N supply is adjusted by nutrient ratio. Ellsworth et al.'s (2004) theory says that because there are strong correlations between leaf [N] and V_{cmax} maintaining leaf carboxylation capacity in elevated $[\text{CO}_2]$ depends on the maintenance of canopy N stocks (Ellsworth et al. 2004). The nutrient ratio and N supply significantly affected the V_{cmax} , indicating nutrient ratio effect cannot be ignored. J_{max} and TPU were significantly affected interactively by $[\text{CO}_2]$, nutrient ratio and N supply. They were significantly lower at the low and high N supply levels, suggesting some effects of nutrient ratio existed. The data of leaf

[N] and photosynthetic rate indicated a different relationship pattern, which against Lewis et al.'s (2004) theory of which elevated [CO₂] decreases leaf [N] and reduces net photosynthetic rates measured at a common [CO₂]. Photosynthetic nutrient-use efficiency suggests that P and K influence on N performance, indicating significant nutrient ratio effect.

Under the stress of the predicted climate change, boreal black spruce may migrate northward to avoid the stresses of predicted changing temperatures and precipitations. The migration may take place from west to east, low elevation to high elevation and south to north. If the migrating direction is from west to east at same latitudes, then photoperiods will not change, and even from low to high elevations, photoperiods of the two locations will not change much, so that the effects of photoperiod on plants will not be significant. However, if the migration is take place from south to north and passes over 10 ° latitudes, the changes of photoperiod and nutrient availability associated with northward migration may constrain the migration. Black spruce not only has adapted temperatures and precipitations at its seed origin locations for hundred and thousand years, but also has adapted its photoperiod changes and has formed its unique photoperiodism controlling its growth events. It is impossible that black spruce will form its photoperiodism to acclimate the sudden photoperiod changes caused by migration because photoperiodism is a gene controlled phenological response (Murneek 1948, Thomas 1991, Thomas and Vince-Prue 1997). The photoperiod changes associated with northward migration induced the late bud set and early bud burst that led to the poor cold hardiness. The low nutrient availability in the potential migration locations may also suppress cold hardiness development. In this study, the photoperiod of 10° northward migration location (PNM) with high nutrient supply resulted in 7.05 times more freezing index of injury than the photoperiod of seed origin location (PS) of black spruce seedlings (freezing index of injury tested at -15°C:

PNM=24.01±0.72; PS=3.40±0.29). The PNM resulted in greater seedling mortality (41.46±4.81 percent) than the PS's (4.17±0.41 percent). Although elevated [CO₂] and longer growing season at predicted northward locations promoted the seedling growth in growing season, but lack of enough cold hardiness put the seedlings on survival challenge. Black spruce cannot survival without obtaining sufficient cold hardiness quality.

In this study, two aspects of black spruce responding to the predicted global climate were briefly discussed above. To take advantage of elevated [CO₂], black spruce will require more N supply support its growth demand and proportionally less P and K supply. Therefore, a new optimal N-P-K ratio will form under future elevated [CO₂]. To avoid the predicted stresses of temperature and precipitation, black spruce may migrate northward but the migration will be challenged by acclimation of photoperiod and nutrient availability changes. Gene based black spruce photoperiodism cannot adjust to photoperiodic change in the short term, and the photoperiodism may lead poor cold hardiness development and delayed or advanced phenology. Consequently, survival of migrated black spruce will be challenged by the harsh winter and the spring frost.

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