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Nutrient supply and belowground interaction alter responses to CO_2 elevation in black spruce and white spruce

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ABSTRACT

The carbon dioxide concentration ($[CO_2]$) in the atmosphere has been increasing since the start of the industrialization. The impact of increasing $[CO_2]$ on the physiology and growth of plants and on the composition, dynamics and productivity of plant communities have been studied extensively in the past few decades. However, the responses to CO_2 elevation can be altered by other environmental and biological factors. Such interactions, particularly those involving multiple factors, are complex and not well understood. In this study, we investigated the interactive effects of CO_2 elevation, nutrient availability and root interaction between plants on the total seedling photosynthesis, growth, and biomass allocation of two boreal conifer tree species, black spruce and white spruce. Seedlings were exposed to two $[CO_2]$ (380 vs. 720 $\mu mol\ mol^{-1}$) and two nutrient levels (low vs. high) with or without root interaction. We found that each of the three treatments influenced the effects of others and the two species also responded differently to those interactions. Root interactions reduced seedling growth only when nutrient supply was high. White spruce was more sensitive root interaction than black spruce while black spruce was more sensitive to the negative impact of low nutrient supply than white spruce. Surprisingly, CO_2 elevation and its interactions did not significantly affect seedling growth. The CO_2 elevation significantly increased total seedling photosynthesis while root interaction reduced it and the reduction was greater under the elevated CO_2. The photosynthetic response to CO_2 elevation was more sensitive to nutrient availability in white spruce than in black spruce. Seedling biomass was more sensitive to the negative impact of root interaction and low nutrient supply in black spruce than white spruce. Root interaction reduced biomass allocation to leaf and the impact was reduced by low nutrient. Biomass allocation to leaf was more sensitive to low nutrient supply in black spruce than white spruce. CO_2 elevation reduced but root interaction increased root/leaf mass ratio, partially offsetting each other when both treatments were applied. The root/leaf ratio of white spruce was more sensitive to CO_2 elevation while that of black spruce was more sensitive to root interaction and nutrient supply. Low nutrient supply and root interaction both had positive effect on root/leaf ratio and their effects were confounded when both were applied simultaneously. Therefore, their effects were observed only when one of them was absent.

1. Introduction

Climate change resulting from increasing carbon dioxide concentration ($[CO_2]$) in the atmosphere can affect the physiology, growth, morphology and geographic distribution of plants (Cao et al., 2007, 2008; Danyagri and Dang, 2014a, 2014b, Inoue et al., 2019, 2020; Lukac et al., 2010). For example, CO_2 elevation enhances the photosynthesis and growth of C_3 plants (Kubiske et al., 1998; Murray et al., 2000; Liu et al., 2002; Bigras and Bertrand, 2006; Zhang and Dang, 2007; Marfo and Dang, 2009; Wang and Taub, 2010; Newaz et al., 2016). CO_2 elevation can also increase biomass allocation to roots, particularly when nutrient supply is limited (Tingey et al., 2000; Liu et al., 2002; Zhang et al., 2006; Cao et al., 2008; Wang and Taub, 2010; Newaz et al., 2016). However, the effects of CO_2 elevation can be altered by other environmental or biological factors (Ambebe et al., 2010, 2013, Danyagri and Dang, 2013, 2014a, 2014b, Inoue et al., 2019, 2020, Li et al., 2013, 2015; Lukac et al., 2010; Marfo and Dang, 2009; Newaz et al., 2016, 2017; Oren et al., 2001; Tedla et al., 2019, 2020a, 2020b, Zhang and Dang, 2005, 2006, 2007; Zhang and Dang, 2013), for example, low nutrient supply reduces the positive effects of CO_2 elevation (Brooker, 2006; Danyagri and Dang, 2014b; Lukac et al., 2010; Oren et al., 2001; Zhang and Dang, 2006). CO_2 elevation can also

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increase plant demand for nutrients (Oren et al., 2001; Lukac et al., 2010; Li et al., 2013, 2015) and increased nutrient supply may be necessary to sustain the stimulation of CO_2 elevation to growth (Danyagri and Dang, 2014b; Li et al., 2013, 2015; Lukac et al., 2010; Proe et al., 2000). On the other hand, CO_2 elevation may partially ameliorate the limitation of low nutrient on photosynthesis and growth because increased [CO_2] increase the nutrient use efficiency (Danyagri and Dang, 2014b; Li et al., 2013, 2015; Warren et al., 2005). Furthermore, increased biomass allocation to roots under [CO_2] improves the exploration of the soil for nutrients and water (Pendall et al., 2004; Lambers and Oliveira, 2019).

The effects of CO_2 elevation vary with plant species (Joel et al., 2001; Zhang and Dang, 2005, 2007) and thus CO_2 elevations will likely alter interplant relationships within and between plant species within the same plant community (Poorter, 1998; Friend et al., 2000; Brooker, 2006), consequently their relative competitiveness and the species richness and composition of the plant community (Poorter, 1998; Brooker, 2006; Körner, 2006; Wang, 2007; Langley and Megonigal, 2010). Plant interactions are an important driver for the dynamics and species composition of a plant community (Drake and Leadley, 1991; Brooker, 2006; Lambers and Oliveira, 2019). The responses of plants grown in isolation can be quite different from the responses of plants grown together and interacting with other plants (Navas, 1998). This is because plants can modify the physical, chemical, and biological environment for other plants (Poorter and Pèrez-Soba, 2001; Wang, 2007; Lambers and Oliveira, 2019). Root interactions can affect the resource availability and other aspects of the soil environment (Arnone and Kestenholz, 1997; Casper and Jackson, 1997; Lambers and Oliveira, 2019). CO_2 elevations can have greater impact on roots than on aboveground organs (Bassirirad, 2000; Pendall et al., 2004; Ambebe and Dang, 2009, 2010; Ambebe et al., 2013; Inoue et al., 2020). However, the effects of root interactions on the response to CO_2 elevation have received little attention in the research community.

The climate change arising from increasing atmospheric CO_2 is expected to be the most prominent in the boreal region (Bonan and Shugart, 1989; Bonan et al., 1992; Sellers et al., 1997; IPCC, 2019). Soils in the region are generally deficient in nutrients, particularly nitrogen, due to the slow decomposition of organic matters (Dioumaeva et al., 2002), which is a major reason for the slow growth of boreal trees (Jarvis and Linder, 2000). There are also large variations in soil nutrient regimes across different sites in the region (Dioumaeva et al., 2002) and climate change will likely alter the decomposition rate of organic matters and soil nutrient regime (Dioumaeva et al., 2002). Such changes will affect not only the growth of individual species but interactions among individuals and among species, and the dynamics and composition of forest stands. However, there is a lack of information on such interactions in the literature. The objective of this study was to investigate the interactive effects of [CO_2], root interactions and soil nutrient supply on the photosynthesis, biomass allocation and growth of black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.).

Black spruce and white spruce are two dominant conifer tree species in the boreal forest of North America and often form mixed-species stands on upland sites (Nienstaedt and Zasada, 1990; Sims et al., 1990; Viereck and Johnston, 1990). Black spruce is considered to be more plastic than white spruce because it can grow on a much wider range of sites from nutrient poor lowland sites (both mineral and peatland) to fertile upland sites (Nienstaedt and Zasada, 1990; Viereck and Johnston, 1990; Patterson et al., 1997). Species with greater plasticity generally respond better to increasing [CO_2] (Brown and Higginbotham, 1986; Midgley et al., 1999). On the other hand, black spruce generally grows slower and is less responsive to nutrient additions than white spruce (Patterson et al., 1997) and slow growing species are less responsive to CO_2 elevations than fast growing counterparts (Poorter, 1998; Tangley, 2001). Furthermore, fast growing species have high nutrient uptake kinetics and can deplete nutrient resources at a high nutrient site before slow growing species are able to extract enough nutrients (Jackson et al., 1990; Caldwell et al., 1996). However, species with high uptake kinetics may be disadvantaged when nutrient supply is low because of their high nutrient requirements (Jackson et al., 1990; Aerts and Chapin, 1999). Studies show that CO_2 elevations can increase nutrient use efficiency and partially mitigate the limitation of low nutrient supply to tree growth (Zhang and Dang, 2006; Zhang et al., 2006; Cao et al., 2007, 2008; Ambebe et al., 2009; Li et al., 2013, 2015). Apparently, the interactions between black spruce and white spruce under the interactive influence of CO_2 and nutrient conditions are extremely complicated. This study is aimed to obtain some insights on those relationships.

2. Materials and methods

2.1. Plant materials and experimental design

One-year old seedlings of black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) were obtained from the Boreal Tree Nursery (Thunder Bay, Ontario). We selected seedlings of relatively uniform size and shape for the experiment. The experiment was carried out in four environment-controlled greenhouses at Lakehead University Thunder Bay Campus.

There were four treatments: carbon dioxide concentration (380 vs. 720 μmol mol^{-1} [CO_2]), root interaction (with RI vs. without RI), nutrient (high N vs. low N) and species (black vs. white spruce). Each of the two [CO_2] was randomly assigned to two of the four greenhouses (2 replicates). Each greenhouse was divided into 8 blocks and each of the 4 RI-N combinations was randomly assigned to two of them as replicates (4 seedlings/species/replicate of each combination). The two species were nested within block. The CO_2 concentrations and other environmental conditions in each of the four greenhouses were monitored and controlled using an Argus Classic Control System (Argus, Vancouver, BC, Canada). CO_2 was elevated using GEN-2E CO_2 generators (Custom Automated Products Inc., Riverside, CA) and the CO_2 concentration in each of the greenhouses was allowed to fluctuate by ± 30 μmol mol^{-1} from the set point. Other environmental conditions were 55 ± 5% RH, 25 ± 2/15 ± 2 °C day/night temperature and 16 H photoperiod. The average PAR at the canopy was 660 μmol m^{-2}s^{-1} in the middle of sunny days. High pressure sodium lamps were used to extend the natural photoperiod to 16 H. The supplementary light was approximately 220 μmol m^{-2}s^{-1} at the canopy level.

Root interactions were achieved by growing the seedlings of both species in one large container (48 × 38 × 16 cm, 4 seedling per species, a total of 8 seedlings per container) and the seedlings of each species were evenly intermingled with those of the other species so that each seedling was adjacent to a different species. Each seedling in the no root interaction treatment was grown in an individual container (14.5 cm diameter, 15.5 cm height). The spatial distribution and spacing of seedlings within each block and average soil volume per seedling were comparable between the two root interaction treatments. The nutrient concentrations for the high nutrient treatment were 150, 60, 150, 80, 40, 60 mg/l of N, P, K, Ca, Mg and S, respectively and the concentrations were 10% of the above for the low nutrient treatment. The high nutrient formulation presents the optimal concentrations (Landis, 1989; Li et al., 2013) and the concentrations in the low nutrient treatment are within the deficiency range for the species under both ambient and elevated CO_2 (Li et al., 2013). The seedlings were fertilized once a week and watered between fertilizer applications.

2.2. Photosynthesis measurement

Photosynthesis was measured after 4-month treatment on 3 randomly selected seedlings from each replicate of each treatment combination (total of 192) using a PP-Systems CIRAS-1 open gas exchange system with a Parkinson conifer cuvette (PP-Systems, Amesbury, MA, USA). The environmental conditions in the leaf cuvette were controlled at 25 °C air temperature, 50% RH, 800 µmol m^{-2} s^{-1} PAR and the corresponding treatment [CO_2]. The whole tree photosynthetic rate (W_T, µmol CO_2 $tree^{-1}$ s^{-1}) was estimated as the product of leaf-level photosynthesis and total leaf area of the seedling according to Coleman et al. (1995).

2.3. Growth and biomass measurement

The height and root collar diameter (RCD) of all the seedlings were measured at the start and again at the end of the experiment. All the seedlings were harvested at the end of the experiment. The total projected leaf area (PLA) of the seedlings used for photosynthesis measurement was measured using a Regent WinSeedle system (Regent Instruments Inc., Quebec City, Quebec, Canada). Roots were washed and scanned with a Regents WinRhizo system (Regent Instruments Inc., Quebec City, Quebec, Canada) to determine total root length. All the specimens were then oven-dried at 70 °C for 48 h. The dry mass of foliage, roots and the remainder were weighed on an analytical balance. Specific leaf area was calculated as fresh leaf area per unit dry mass (cm^2 g^{-1}) and specific root length as fresh root length per unit dry mass (cm g^{-1}). Leaf mass ratio (LMR- ratio of leaf to total plant mass), root mass ratio (RMR, root mass per total seedling mass), root to shoot mass ratio (RSR- ratio of belowground to aboveground biomass) and root to leaf mass ratio (RLR) were used as indices of biomass allocation.

2.4. Statistical analysis

Analysis of variance (ANOVA) was conducted using GenStat version 12 (VSN International, Hemel Hempstead, UK). The ANOVA assumptions, i.e., the normality of distribution and homogeneity of variance, were checked using Shapiro-Wilk and Bartlett tests, respectively. Pairwise comparisons of means were conducted using Fisher's Least Significant Difference (LSD) when an interaction was significant ($P < 0.05$).

3. Results

3.1. Height and root collar diameter

The low nutrient supply reduced seedling height growth but the effect was not statistically significant when there was no root interaction; root interaction significantly reduced seedling height under high nutrient but not under low nutrient supply (Fig. 1a, Table 1). Root interaction reduced seedling height in both species and the reduction was greater in white spruce than black spruce; black spruce had greater height than white spruce and the difference was greater when was root interaction (Fig. 1b, Table 1). Low nutrient reduced root collar diameter and the effect was greater in black spruce than white spruce; black spruce had smaller root collar diameter than white spruce only when the nutrient supply was low (Fig. 1c, Table 1). [CO_2] or its interactions with other treatments had no significant effect on height or root collar diameter (Table 1).

3.2. Whole seedling photosynthesis and specific leaf area

The CO_2 × RI × S 3-way interaction significantly affected whole tree photosynthesis (Table 1). The CO_2 elevation generally increased W_T in both species and the effect was greater on black spruce than on white spruce (not significant when there was no root interaction, Fig. 2a). Root interactions significantly reduced W_T in both species and under both [CO_2] (Fig. 2a). Black spruce had higher W_T than white spruce and the difference was greater under elevated [CO_2] (not significant in the combination of no-root interaction and ambient [CO_2], Fig. 2a).

The CO_2 × N × S interactions also significantly affected W_T (Table 1). The CO_2 elevation generally increased W_T but the effect on white spruce was not significant under the high nutrient condition (Fig. 2b). Low nutrient supply significantly reduced W_T and the effect was generally greater in black spruce than in white spruce (Fig. 2b). Black spruce had greater W_T than white spruce but the effect was not statistically significant under high nutrient and ambient [CO_2] (Fig. 2b).

The CO_2 × RI × S interaction significantly affected the specific leaf area (SLA, Table 1) and the response pattern was almost identical to that of photosynthesis (Figs. 1a & 2c). The CO_2 elevation significantly increased SLA only in white spruce with root interaction but it significantly altered the effects of root interaction and species: Under the ambient [CO_2], root interaction reduced SLA in both species and black spruce had higher SLA than white spruce under both root interaction treatments; under the elevated [CO_2], however, root interaction significantly reduced SLA only in black spruce and black spruce had significantly greater SLA than white spruce only when was no root interaction (Fig. 2c).

The interaction of RI × N × S also significantly affected SLA (Table 1). The root interaction generally reduced SLA but the effect was not significant for white spruce under the low nutrient treatment (Fig. 2d). The low nutrient treatment generally reduced SLA but the effect was not statistically significant in white spruce with root interaction (Fig. 2d). Black spruce generally had greater SLA than white spruce but the difference was not statistically significant under low nutrient with root interaction (Fig. 2d).

3.3. Biomass and biomass allocation

The RI × N × S interactions significantly affected seedling biomass (Table 1). Root interaction generally reduced seedling biomass, but the effect was statistically significant only in black spruce under the high nutrient treatment (Fig. 3). Low nutrient significantly reduced seedling biomass only in black spruce with no root interaction (Fig. 3). Black spruce generally had greater total biomass than white spruce but the effect was statistically significant only in the high nutrient supply with no root interaction (Fig. 3). [CO_2] or its interactions with other treatments had no significant effect on seedling biomass (Table 1).

The CO_2 × RI × S interaction significantly affected the leaf mass ratio (LMR) (Table 1). Root interaction significantly reduced LMR in both CO_2 treatments and the two species responded similarly (Fig. 4a). The CO_2 elevation decreased LMR only in black spruce with root interaction (Fig. 4a). Black spruce generally had greater LMR than white spruce but the difference was not statistically significant under the elevated [CO_2] with root interaction (Fig. 4a).

Root interaction and nutrient supply interactively affected LMR (Table 1): root interaction significantly reduced LMR under high nutrient but not under low nutrient condition, and the low nutrient reduced LMR only when there was no root interaction (Fig. 4b). Additionally, the two species responded differently: the low nutrient reduced LMR in black spruce but not in white spruce and black spruce had a greater LMR than white spruce only when the nutrient supply was high (Fig. 4c).

The [CO_2] × RI × S interaction significantly affected the root mass ratio (RMR) and root to shoot ratio (RSR, Table 1). The response patterns of the two parameters were similar: the CO_2 elevation increased RMR and RSR in white spruce in absence of root interaction but decreased them when there was root interaction (Fig. 4d & g); root interaction increase RMR and RSR under the ambient [CO_2] in both species but had no significant effect on them under the elevated [CO_2] (Fig. 4d & g).

Root interaction and nutrient supply also interactively affected RMR and RSR (Table 1): Root interaction significantly increased RMR and RSR under high nutrient but have no significant effect under low nutrient; The low nutrient significantly increased RMR and RSR in absence

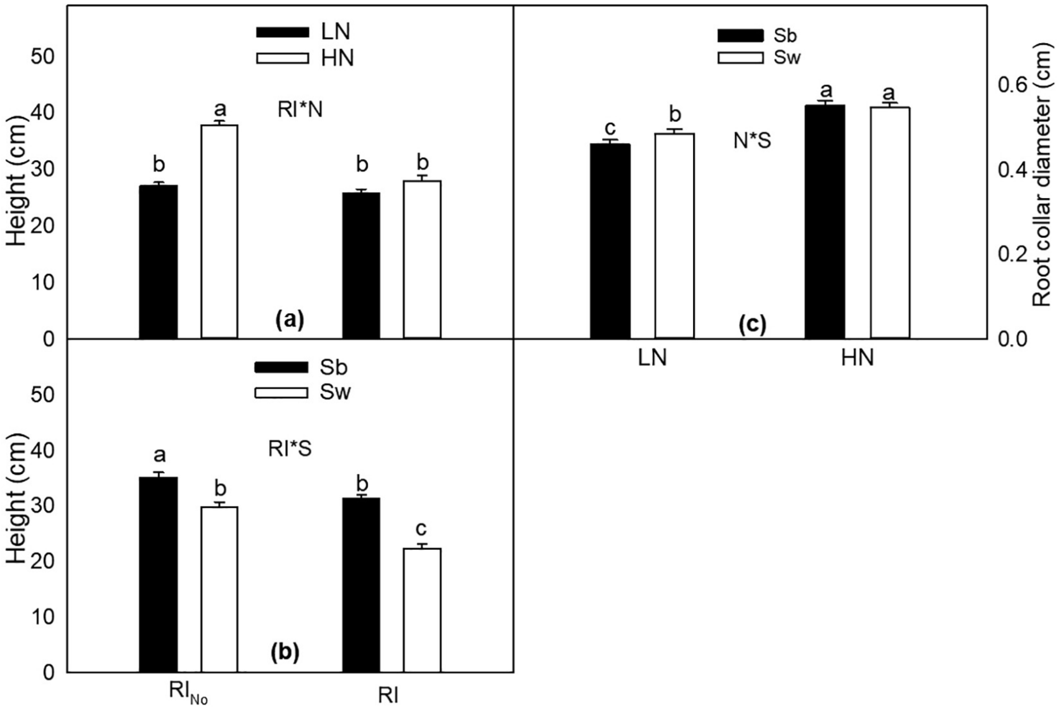

Fig. 1. Effects of interactions between root interaction (RI) and nutrient supply (N, (a)) and between RI and species (S, (b)) on seedling height, and the interactive effect of N and S on root collar diameter (*c*) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. Bars (mean + SEM) with different letters are significantly different from each other ($P \le 0.05$).

of root interactions, but did not significantly affect them when there was root interaction (Fig. 4e & h).

The nutrient-species interaction significantly affected RMR and RSR (Table 1). The low nutrient supply increased both RMR and RSR in both species, but the effect on the RMR of white spruce was statistically not significant; Black spruce had significantly lower RMR than white spruce under high nutrient but there was no significant difference between the two species under low nutrient (Fig. 4f & i).

The [CO_2] × RI × S interaction significantly affected root/leaf mass ratio (RLR, Table 1). The CO_2 elevation significantly reduced RLR in white spruce when there was root interaction but increased it in absence of root interaction while it had no significant impact on black spruce (Fig. 4j). Root interaction generally increased RLR but the effect was not statistically significant in white spruce under elevated [CO_2] (Fig. 4j). The two species responded differently to the interaction of [CO_2] and root interaction: black spruce had significantly smaller RLR than white spruce under the ambient when there was root interaction and under elevated [CO_2] in absence of root interaction (Fig. 4j).

Root interaction and species significantly affected the response of RLR to nutrient supply and vice versa (Table 1). Low nutrient supply significantly increased RLR in absence of root interaction while it had no significant effect when there was root interaction; root interaction significantly increased RLR under high nutrient but had no significant effect under low nutrient supply (Fig. 4k). Low nutrient supply significantly increased RLR in both species but the effect was greater in black spruce than in white spruce; black spruce had lower RLR under high nutrient and there was no significant difference between the two species under low nutrient (Fig. 4l).

Table 1
ANOVA P-values for the effects of [CO_2], root interaction (RI), nutrient supply (N), species (S) and their interactions on height and root collar diameter (RCD), whole tree photosynthesis (W_T), total biomass, leaf mass ratio (LMR), root mass ratio (RMR), root to shoot mass ratio (RSR), root to leaf mass ratio (RLR) and specific leaf area (SLA) of black spruce and white spruce seedlings. The seedlings were exposed to two [CO_2] (380 and 720 μmol mol^{-1}), two root interactions (with vs. without root interaction) and two nutrient supplies (high, HN vs. low, LN). Significant effects ($P \le 0.05$) are bold-faced.

Variation source	Height	RCD	W_T	Biomass	LMR	RMR	RSR	RLR	SLA
CO_2	0.103	0.156	0.216	0.067	0.530	0.166	0.187	0.832	0.794
RI	**0.002**	**0.013**	0.096	**0.002**	**< 0.001**	0.071	0.063	**0.018**	**0.009**
CO_2*RI	0.879	0.260	0.952	0.146	0.907	0.298	0.282	0.254	0.795
N	**< 0.001**	**< 0.001**	**0.012**	**0.001**	**< 0.001**	0.164	0.151	**0.016**	**< 0.001**
CO_2*N	0.513	0.909	0.910	0.657	0.169	0.263	0.351	0.238	0.366
RI*N	**< 0.001**	0.060	0.096	**0.013**	**0.002**	**0.010**	**0.011**	**0.039**	**0.009**
CO_2*RI*N	0.987	0.219	0.813	0.236	0.907	0.278	0.555	0.417	0.595
S	**< 0.001**	0.178	**< 0.001**	**< 0.001**	**< 0.001**	0.274	0.134	**< 0.001**	**< 0.001**
CO_2*S	0.177	0.533	**< 0.001**	0.119	0.978	0.569	0.498	0.357	0.883
RI*S	**0.002**	0.455	0.408	**0.008**	**0.005**	**< 0.001**	**< 0.001**	0.346	**< 0.001**
N*S	0.169	**0.049**	**0.002**	**0.038**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
CO_2*RI*S	0.967	0.410	**0.012**	0.492	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
CO_2*N*S	0.967	0.639	**0.012**	0.831	0.313	0.176	0.165	0.426	0.755
RI*N*S	0.333	0.082	0.723	**0.010**	0.132	0.651	0.346	0.374	**0.042**
CO_2*RI*N*S	0.059	0.414	0.117	0.611	0.576	0.989	0.856	0.661	0.110

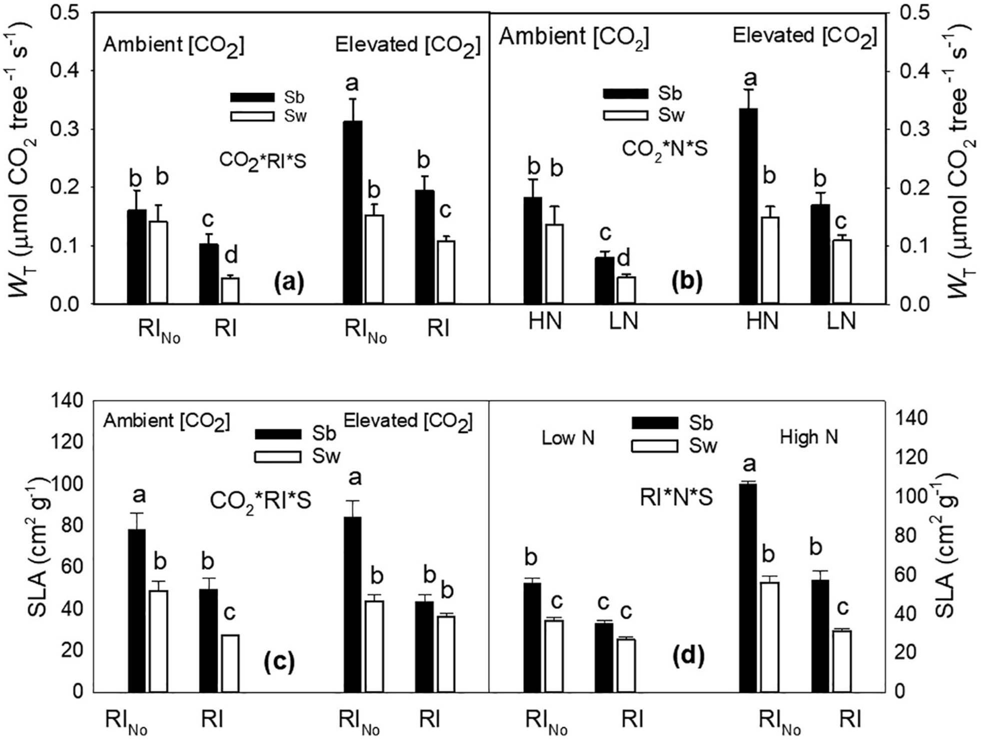

Fig. 2. Effects of [CO$_2$], nutrient supply (N), species (S) and root interaction (RI) on whole tree photosynthesis (a, b) and specific leaf area (SLA) of black spruce (Sb) and white spruce (Sw) seedlings. See Fig. 1 for more explanations.

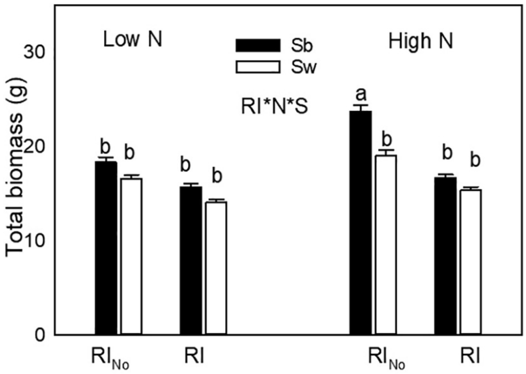

Fig. 3. Interactive effects of root interaction (RI), nutrient supply (N), and species (S) on the total seedling biomass of black spruce (Sb) and white spruce (Sw). See Fig. 1 for further explanations.

4. Discussion

Our results demonstrate that the relative stimulation of CO$_2$ elevation to photosynthesis was greater under low nutrient supply than under high nutrient supply, that CO$_2$ elevation substantially reduced low nutrient limitation to photosynthesis, and that the degree of the responses was different between black spruce and white spruce. The stimulation of CO$_2$ elevation to the photosynthesis of black spruce and white spruce was 84% and 9%, respectively, under the high nutrient condition. Under the low nutrient, however, the stimulation was 114% and 133%, respectively, for black spruce and white spruce. Under the ambient CO$_2$, the low nutrient supply reduced photosynthesis by 56% and 66%, respectively, for black spruce and white spruce; Under the elevated CO$_2$, however, the corresponding reductions declined to 49% and 26%, respectively. Apparently, the CO$_2$ elevation was much more beneficial to white spruce than to black spruce under low nutrient conditions but the reverse was true under high nutrient conditions. The differences in the responses are most likely related to the relative sensitivity to and demand for nutrients: white spruce has higher nutrient demand and is more sensitive to changes in nutrient supply (Nienstaedt and Zasada, 1990; Patterson et al., 1997). Murray et al. (2000) have also found that the relative CO$_2$ stimulation of photosynthesis is greater under low than high nutrient conditions ((Murray et al., 2000). The large differences between the two species in responding to the CO$_2$-nutrient interaction suggest that increasing CO$_2$ in the atmosphere will likely influence the relative competitiveness of difference species, and thus the dynamics and species composition of forest stands. Our data further suggest that black spruce may become relatively more competitive on nutrient rich sites while white spruce may become relatively more competitive on sites with low nutrient availability. However, results on the interactive effects of nutrient supply and CO$_2$ elevation on tree growth are inconsistent in the literature.

Total photosynthesis and specific leaf area are generally positively correlated to the growth rate and biomass production of plants (Kirschbaum, 2011; Lambers and Oliveira, 2019). In fact, the correlation between growth rate and specific leaf area is generally so strong that specific leaf area is considered to be the best predictor of growth rate (Lambers and Oliveira, 2019). In this study, seedling photosynthesis and specific leaf area had almost identical patterns in their response to the interactions of CO$_2$ and nutrient. However, none of those responses were translated into a similar response in seedling growth or biomass. In fact, the growth and biomass were not significantly affected by the CO$_2$ elevation or its interaction with nutrient supply in the two species. The most likely reason for the lack of such a relationship in this study is that only a portion of the biomass and growth were formed under the treatment conditions while the foliage for the photosynthesis and specific leaf area measurement were entirely formed during the treatment. We used 1-year old seedlings which were grown under identical conditions in the first growing season. Thus, the treatment effects must have been diluted by the pre-treatment growth and biomass, making the parameters less sensitive to the treatments than they should have been. Additionally, black and white spruce are determinate

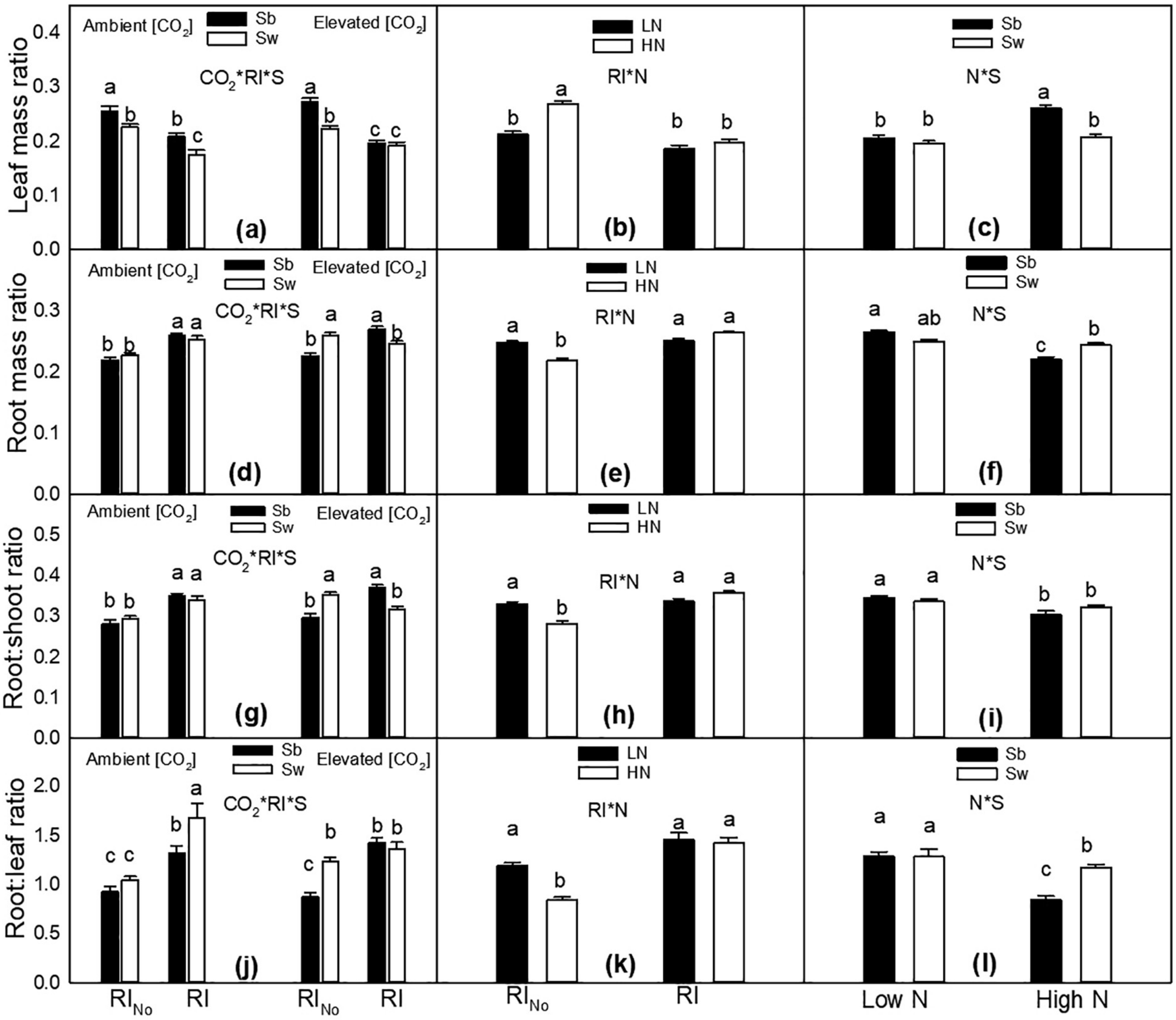

Fig. 4. Interactive effects of [CO_2], nutrient supply (N), species (S) and root interaction (RI) on leaf mass ratio (*a, b, c*), root mass ratio (*d, e, f*), root/shoot ratio (*g, h, i*) and root/leaf ratio (*j, k, l*) of black spruce (Sb) and white spruce (Sw) seedlings. Only significant ($P \leq 0.05$) interactions are shown. The specific significant interactions are noted in each figure. See Fig. 1 for further explanations.

species and thus their potential height growth is pre-determined in the previous growing season, possibly limiting their response to treatments in this study. Dang and Lieffers (1989) report that the growth of black spruce is more closely related to the environmental conditions of the previous growing season than to those of the current growing season. Photosynthesis and specific leaf area, in contrast, were measured on leaves that were formed under during the treatment and thus their performance was better indicators of treatment effects. Furthermore, both CO_2 and nutrient supply had opposite effects on leaf and roots, as indicated by the opposite responses of leaf mass ratio and root mass ratio. The opposite effects may have offset each other, diminishing the treatment effects on total biomass. The results on the effects of CO_2 elevation on growth are inconsistent in the literature. Some find that there is a lack of CO_2 effects on growth and biomass in both controlled environment (El Kohen et al., 1992; Petterson and Mcdonald, 1992; Olszyk et al., 1998, 2003; Kilpeläinen et al., 2005; Zhang et al., 2006; Bloor et al., 2008; Inaunen et al., 2012; Inoue et al., 2019; Tedla et al., 2019) and field conditions (Körner et al., 2005) while others have observed that CO_2 elevations increase tree growth and/or biomass production (Callaway et al., 1994; Tissue et al., 1997; Zhang and Dang, 2007; Marfo and Dang, 2009; Li et al., 2013).

The most significant finding of this study is that root interaction influenced seedlings' response to CO_2 elevation and the effects varied with species. The interaction of CO_2, root interaction and species significantly affected nearly all the physiological and biomass allocation parameters. For example, in absence of root interactions, the CO_2 elevation increased the photosynthesis of black and white spruce by 94% and 8%, respectively. In the presence of root interaction, the corresponding stimulation was 90% and 146%, respectively for black and white spruce. The CO_2 elevation significantly reduced the biomass allocation to leaf in black spruce only when root interaction was present. The CO_2 elevation significantly increased the root mass ratio of white spruce in absence of root interaction but decreased it when root interaction was present. Roots can affect neighbouring plants by various means, such as modifying the biological and chemical environment, exudation of allolopathic substance, and modifying resource availability ((Jolliffe, 1997; Brassard et al., 2011; Jensen et al., 2011; Lambers and Oliveira, 2019). The significant interactions that we observed in this study have multiple implications, including the interpretation of research findings and comparisons between different studies. Plants are typically grown in individual containers in controlled environment studies and thus any potential impact of root interactions are precluded. Our finding adds an additional factor to consider when attempting to apply lab findings to field situations. Container limitation to root growth and resource availability is commonly used to explain differences in results between field and greenhouse studies. Our results suggest that root interactions may be another reason for the differences.

There are some limitations in this study and therefore cautions should be exercised when the results are applied beyond the inference sphere of the study. Firstly, the whole tree photosynthesis in this study was as the product of the photosynthetic rate of current year foliage and the total leaf area of the seedling. However, the seedlings contained foliage formed in the current year and the previous year. Foliage of different ages tend to have different rates of photosynthesis. Furthermore, the previous foliage in all the trees were formed under the same pre-treatment environmental conditions and should have responded differently to the treatments than the current foliage. The reported photosynthesis was only a rough estimate. Secondly, the experiment was carried out under the scenario of an even-mixture of black and white spruce. The influence of root interaction may be different when the mixing of the species is uneven because the interactions between individuals of the same species may be different from those between different species. The most important contribution of this study is that it demonstrates that belowground interaction and its interaction with nutrient supply can exert a significant impact on trees' responses to the elevation of CO_2 in the atmosphere and the interactions vary with species. We hope that this study will inspire more research in this area.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aerts, R., Chapin, F.S., 1999. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. In: Adv. Ecol. Res. Ed. A.H. Fitter. Academic Press, pp. 1–67.

Ambebe, T.F., Danyagri, G., Dang, Q.L., 2013. Low soil temperature inhibits the stimulatory effect of elevated [CO2] on height and biomass accumulation of white birch seedlings grown under three non-limiting phosphorus conditions. Nordic J. Botany 31, 239–246.

Ambebe, T.F., Dang, Q.L., Li, J.L., 2010. Low soil temperature inhibits the effect of high nutrient supply on photosynthetic response to elevated carbon dioxide concentration in white birch seedlings. Tree Physiol. 30, 234–243.

Ambebe, T.F., Dang, Q.L., 2010. Low moisture availability reduces the positive effect of increased soil temperature on biomass production of white birch (Betula papyrifera Marsh.) seedlings in ambient and elevated carbon dioxide concentration. Nordic J. Botany 28, 104–111.

Ambebe, T.F., Dang, Q.L., Marfo, J., 2009. Low soil temperature reduces the positive effects of high nutrient supply on the growth and biomass of white birch (Betula papyrifera Marsh.) seedlings in ambient and elevated carbon dioxide concentrations. Botany 87, 905–912.

Ambebe, T.F., Dang, Q.L., 2009. Low moisture availability inhibits the enhancing effect of increased soil temperature on net photosynthesis of white birch (Betula papyrifera Marsh.) seedlings under ambient and elevated carbon dioxide concentration. Tree Physiol. 29, 1341–1348.

Arnone, J.A., Kestenholz, C., 1997. Root competition and elevated CO_2: effects on seedling growth in *Linum usitatissimum* populations and *Linum-Silene cretica* mixtures. Funct. Ecol. 11, 209–214.

Bassirirad, H., 2000. Kinetics of nutrient uptake by roots: responses to global change. New Phytol. 147, 155–169.

Bigras, F.J., Bertrand, A., 2006. Responses of *Picea mariana* to elevated CO_2 concentration during growth, cold hardening and dehardening: phenology, cold tolerance, photosynthesis and growth. Tree Physiol. 26, 875–888.

Bloor, J.M.G., Barthes, L., Leadley, P.W., 2008. Effects of elevated CO_2 and N on tree-grass interactions: an experimental test using *Fraxinus excelsior* and *Dactylis glomerata*. Funct. Ecol. 22, 537–546.

Bonan, G.B., Pollard, D., Thompson, S.L., 1992. Effects of boreal forest vegetation on global climate. Nature 359, 716–718.

Bonan, G.B., Shugart, H.H., 1989. Environmental factors and ecological processes in boreal forests. Annu. Rev. Ecol. Syst. 20, 1–28.

Brassard, B.W., Chen, H.Y.H., Bergeron, Y., Parè, D., 2011. Differences in fine root productivity between mixed- and single-species stands. Funct. Ecol. 25, 238–246.

Brooker, R.W., 2006. Plant-plant interactions and environmental change. New Phytol. 171, 271–284.

Brown, K., Higginbotham, K.O., 1986. Effects of carbon dioxide enrichment and nitrogen supply on growth of boreal tree seedlings. Tree Physiol. 2, 223–232.

Caldwell, M.M., Manwaring, J.H., Durham, S.L., 1996. Species interactions at the level of fine roots in the field: influence of soil nutrient heterogeneity and plant size. Oecologia 106, 440–447.

Callaway, R.M., DeLucia, E.H., Thomas, E.M., Schlesinger, W.H., 1994. Compensatory responses of CO_2 exchange and biomass allocation and their effects on the relative growth rate of ponderosa pine in different CO2 and temperature regimes. Oecologia 98, 159–166.

Cao, B., Dang, Q.L., Yu, X.G., Zhang, S.R., 2008. Effects of [CO2] and nitrogen on morphological and biomass traits of white birch (Betula papyrifera) seedlings. For. Ecol. Manage. 254, 217–224.

Cao, B., Dang, Q.L., Zhang, S.R., 2007. Relationship between photosynthesis and leaf nitrogen concentration under ambient and elevated [CO2] in white birch (Betula papyrifera) seedlings. Tree Physiol. 27, 891–899.

Casper, B.B., Jackson, R.B., 1997. Plant competition underground. Annu. Rev. Ecol. Syst. 28, 545–570.

Coleman, M.D., Isebrands, J.G., Dickson, R.E., Karnosky, D.F., 1995. Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. Tree Physiol. 15, 585–592.

Dang, Q.L., Lieffers, V.J., 1989. Climate and annual ring growth of black spruce in some Alberta pleatlands. Can. J. Bot. 19, 924–929.

Danyagri, G., Dang, Q.L., 2014a. Effects of elevated [CO2] and soil temperature on photosynthetic responses of mountain maple (Acer spicatum L.) seedlings to light. Environ. Exp. Bot. 107, 64–70.

Danyagri, G., Dang, Q.L., 2014b. Soil temperature and phosphorus supply interactively affect physiological responses of white birch to CO2 elevation. Am. J. Plant Sci. 5, 219–229. https://doi.org/10.4236/ajps.2014.52029.

Danyagri, G., Dang, Q.L., 2013. Effects of elevated [CO2] and low soil moisture on the physiological responses of mountain maple (Acer spicatum L.) seedling to light. PLoS One 8 (10), e76586. https://doi.org/10.1371/journal.pone.0076586.

Dioumaeva, I., Trumbore, S., Schuur, E.A.G., Goulden, M.L., Litvak, M., Hirsch, A.I., 2002. Decomposition of peat from upland boreal forest: Temperature dependence and sources of respired carbon. J. Geophys. Res. 107, 8222.

Drake, B.G., Leadley, P.W., 1991. Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO_2. Plant Cell Environ. 14, 853–860.

El Kohen, A., Rouhier, H., Mousseau, M., 1992. Changes in dry weight and nitrogen partitioning induced by elevated CO_2 depend on soil nutrient availability in sweet chestnut (*Castanea sativa* Mill). Ann. For. Sci. 49, 83–90.

Friend, A.L., Jifon, J.L., Berrang, P.C., Seiler, J.R., Mobley, J.A., 2000. Elevated atmospheric CO_2 and species mixture alter N acquisition of trees in stand microcosms. Can. J. For. Res. 30, 827–836.

Inoue, S., Dang, Q.L., Man, R., Tedla, B., 2019. Northward migration of trembling aspen will increase growth but reduce resistance to drought-induced cavitation in the xylem. Botany 97 (11), 627–638. https://doi.org/10.1139/cjb-2019-0099.

Inoue, S., Dang, Q.L., Man, R., Tedla, B., 2020. Photoperiod and CO_2 elevation influence morphological and physiological responses to drought in trembling aspen: implications to climate change-induced migration. Tree Physiol. https://doi.org/10.1093/treephys/tpaa044.

IPCC, 2019. Climate change and land: An IPCC Special Report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. https://www.ipcc.ch/site/assets/uploads/2019/08/Fullreport-1.pdf.

Jackson, R.B., Manwaring, J.H., Caldwell, M.M., 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature 344, 58–60.

Jarvis, P., Linder, S., 2000. Constraints to growth of boreal forests. Nature 405, 904–905.

Jensen, A.M., Löf, M., Gardiner, E.S., 2011. Effects of above- and below-ground competition from shrubs on photosynthesis, transpiration and growth in *Quercus robur* L. seedlings. Environ. Exp. Bot. 71, 367–375.

Joel, G., Chapin, F.S., Chiariello, N.R., Thayer, S.S., Field, C.B., 2001. Species-specific responses of plant communities to altered carbon and nutrient availability. Global Change Biol. 7, 435–450.

Jolliffe, P.A., 1997. Are mixed populations of plant species more productive than pure stands? Oikos 80, 595–602.

Kilpeläinen, A., Peltola, H., Ryyppö, A., Kellomäki, S., 2005. Scots pine responses to elevated temperature and carbon dioxide concentration: growth and wood properties. Tree Physiol. 25, 75–83.

Kirschbaum, M.U.F., 2011. Does enhanced photosynthesis enhance growth? Lessons learned from CO_2 enrichment studies. Plant Physiol. 155, 117–124.

Kubiske, M.E., Pregitzer, K.S., Zak, D.R., Mikan, C.J., 1998. Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO_2 and soil N availability. New Phytol. 140, 251–260.

Lambers, H., Oliveira, R.S., 2019. Plant Physiological Ecology. 3rd ed. Springer, New York. 736 p, ISBN-13: 978-3030296384.

Landis, T.D., 1989. Mineral nutrients and fertilization. In: Landis, T.D., Tinus, R.W., MacDonald, S.E., Barnet, J.P. (Eds.), The Container Tree Nursery Manual, Agric. Handbook 674. Washington D.C, USA, pp. 1–67.

Langley, J.A., Megonigal, J.P., 2010. Ecosystem response to elevated CO_2 levels limited by nitrogen-induced plant species shift. Nature 466, 96–99.

Li, J.L., Dang, Q.L., Man, R.Z., 2015. Photoperiod and nitrogen supply limit the scope of northward migration and seed transfer of black spruce in a future climate associated with doubled atmospheric CO_2 concentration. Am. J. Plant Sci. 6 (1), 189–200.

Li, J.L., Dang, Q.L., Man, R.Z., Marfo, J., 2013. Elevated CO_2 alters N- growth relationship in spruce and causes unequal increases in N, P and K demands. For. Ecol. Manage. 298, 19–26.

Liu, S.-R., Barton, C., Lee, H., Jarvis, P., Durrant, D., 2002. Long-term response of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) to CO_2 enrichment and nitrogen supply. I. Growth, biomass allocation and physiology. Plant Biosyst. 136, 189–198.

Lukac, M., Calfapietra, C., Lagomarsino, A., Loreto, F., 2010. Global climate change and tree nutrition: effects of elevated CO_2 and temperature. Tree Physiol. 30, 1209–1220.

Marfo, J., Dang, Q.L., 2009. Interactive effects of carbon dioxide concentration and light on the morphological and biomass characteristics of black spruce and white spruce seedlings. Botany. 87, 67–77.

Midgley, G.F., Wand, S.J.E., Pammenter, N.W., 1999. Nutrient and genotypic effects on CO_2-responsiveness: photosynthetic regulation in *Leucadendron* species of a nutrient-poor environment. J. Exp. Bot. 50, 533–542.

Murray, M.B., Smith, R.I., Friend, A., Jarvis, P.G., 2000. Effect of elevated $[CO_2]$ and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (*Picea sitchensis*). Tree Physiol. 20, 421–434.

Navas, M.L., 1998. Individual species performance and response of multispecific communities to elevated CO_2: a review. Funct. Ecol. 12, 721–727.

Newaz, S., Dang, Q.L., Man, R.Z., 2017. Eco-physiological potential of jack pine for assisted northward migration: interactions among photoperiod, [CO2] and moisture stress. Nordic J. Botany. https://doi.org/10.1111/njb.01325.

Newaz, M.S., Dang, Q.-L., Man, R.Z., 2016. Morphological response of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod. Am. J. Plant Sci. 7, 879–893.

Nienstaedt, H., Zasada, J.C., 1990. Picea glauca (Moench) voss, white spruce. In: Burns, R. M., Honkala, B.H. (Eds.), Silvics of North America, vol. 1. Conifers, Agricultural Handbook 654. USDA Forest Service, Washinton D.C, pp. 204–226.

Olszyk, D., Wise, C., Vaness, E., Tingey, D., 1998. Elevated temperature but not elevated CO_2 affects long-term patterns of stem diameter and height of Douglas-fir seedlings. Can. J. For. Res. 28, 1046–1054.

Olszyk, D.M., Johnson, M.G., Tingey, D.T., Rygiewicz, P.T., Wise, C., VanEss, E., Benson, A., Storm, M.J., King, R., 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for 4 years. Can. J. For. Res. 33, 269–278.

Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schafer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G., Katul, G.G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411, 469–472.

Patterson, T.B., Guy, R.D., Dang, Q.L., 1997. Whole-plant nitrogen and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species. Oecologia 110, 160–168.

Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y., Megonigal, J.P., Olsrud, M., Ryan, M.G., Wan, S., 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytol. 162, 311–322.

Poorter, H., 1998. Do slow-growing species and nutrient-stressed plants respond relatively strongly to elevated CO_2? Global Change Biol. 4, 693–697.

Poorter, H., Pèrez-Soba, M., 2001. The growth response of plants to elevated CO_2 under non-optimal environmental conditions. Oecologia 129, 1–20.

Proe, M.F., Midwood, A.J., Craig, J., 2000. Use of stable isotopes to quantify nitrogen, potassium and magnesium dynamics in young Scots pine (*Pinus sylvestris*). New Phytol. 146, 461–469.

Sellers, P.J., Hall, F.G., Kelly, R.D., Black, A., Baldocchi, D., Berry, J., Ryan, M., Ranson, K.J., Crill, P.M., Lettenmaier, D.P., Margolis, H., Cihlar, J., Newcomer, J., Fitzjarrald, D., Jarvis, P.G., Gower, S.T., Halliwell, D., Williams, D., Goodison, B., Wickland, D.E., Guertin, F.E., 1997. BOREAS in 1997: Experiment overview, scientific results, and future directions. J. Geophys. Res. 102, 28731–28769.

Sims, R.A., Kershaw, H.M., Wickware, G.M., 1990. The autecology of major tree species in the north central region of Ontario. Forestry Canada, Ontario Region, COFRDA Report 3302, Sault Ste. Marie, Ontario. 126 p.

Tangley, L., 2001. High CO_2 levels may give fast-growing trees an edge. Science 292, 36–37.

Tedla, B., Dang, Q.L., Inoue, S., 2020a. Freeze-thaw events delay spring budburst and leaf expansion while longer photoperiods have opposite effect under different $[CO_2]$ in white birch: advance it under elevated but delay it under ambient $[CO_2]$. Environ. Exp. Bot. 173. https://doi.org/10.1016/j.envexpbot.2020.103982.

Tedla, B., Dang, Q.L., Inoue, S., 2019. White birch has limited phenotypic plasticity to take advantage of increased photoperiods at higher latitudes north of the seed origin. For. Ecol. Manage. 451 (2019), 117565. https://doi.org/10.1016/j.foreco.2019.117565.

Tedla, B., Dang, Q.L., Inoue, S., 2020b. CO_2 elevation and photoperiods north of seed origin change autumn and spring phenology as well as cold hardiness in boreal white birch. Frontiers Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00506.

Tingey, D.T., Phillips, D.L., Johnson, M.G., 2000. Elevated CO_2 and conifer roots: effects on growth, life span and turnover. New Phytol. 147, 87–103.

Tissue, D.T., Thomas, R.B., Strain, B.R., 1997. Atmospheric CO_2 enrichment increases growth and photosynthesis of *Pinus taeda*: a 4 year experiment in the field. Plant Cell Environ. 20, 1123–1134.

Viereck, L.A., Johnston, W.F., 1990. Picea mariana (Mill). B.S.P. In: Burns, R.M., Barbara, H.H. (Eds.), Silvics of North America, vol. 1. Conifers, Agricultural Handbook 654. USDA Forest Service, Washington D.C., pp. 227–237.

Wang, X., 2007. Effects of species richness and elevated carbon dioxide on biomass accumulation: a synthesis using meta-analysis. Oecologia 152, 595–605.

Wang, X., Taub, D., 2010. Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: a meta-analytical synthesis using pairwise techniques. Oecologia 163, 1–11.

Warren, C.R., McGrath, J.F., Adams, M.A., John, F.M., Mark, A.A., 2005. Differential effects of N, P and K on photosynthesis and partitioning of N in *Pinus pinaster* needles. Ann For Sci. 62, 1–8.

Zhang, S.R., Dang, Q.L., 2005. Effects of soil temperature and elevated CO2 concentration on gas exchange, in vitro carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. Tree Physiol. 25, 609–617.

Zhang, S., Dang, Q.L., 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. Tree Physiol. 26, 1457–1467.

Zhang, S., Dang, Q.L., 2007. Interactive effects of soil temperature and CO_2 on morphological and biomass traits in seedlings of four boreal tree species. For. Sci. 53, 453–460.

Zhang, S., Dang, Q.L., Yu, X., 2006. Nutrient and $[CO_2]$ elevation had synergistic effects on biomass production but not on biomass allocation of white birch seedlings. For. Ecol. Manage. 234, 238–244.

Zhang, S.R., Dang, Q.L., 2013. CO2 elevation improves photosynthetic performance in progressive warming environment in white birch seedlings. F1000Research (http://f1000research.com/articles/2-13/v1).