EFFECTS OF NUTRIENT SUPPLY, SOIL TEMPERATURE AND ROOT INTERACTIONS ON MORPHOLOGY, GROWTH, BIOMASS AND PHYSIOLOGICAL RESPONSES OF BLACK SPRUCE AND WHITE SPRUCE SEEDLINGS TO CO_2 ELEVATION

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Abstract

Changes in atmospheric CO_2 concentration ([CO_2]) and temperature may influence nutrient resources and how boreal species respond to their environment. The effects of increased [CO_2] on plants are generally positive but can be very complex because of interactions with soil temperature and nutrient availability on physiology, growth, root characteristics and biomass production. The issue is further complicated because species can respond differently to the interactive effects of these factors. Two experiments were conducted to determine how soil temperature, nutrient supply and inter-plant root interactions affect gas exchange, morphology, root traits and growth of black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings.

In the first experiment, the effects of [CO_2] (380 vs. 720 μmol mol^{-1}), nutrient supply (low vs. high) and inter-plant root interactions (no inter-plant root interaction vs. presence of inter-root interaction) were examined while in the second experiment, the nutrient treatment was substituted with soil temperature (20 vs 25 °C). One year old black spruce (Sb) and white spruce (Sw) seedlings were used in both experiments.

Elevated [CO_2] significantly increased both leaf level (P_n) and whole tree (W_T) photosynthetic rates but did not always increase height growth and biomass production. The CO_2 elevation also enhanced water and nutrient use efficiencies and the degree of enhancement was generally higher in seedlings grown with inter-plant root interaction and higher in black spruce than in white spruce. Soil temperature did not significantly influence water use efficiency but the 25 °C treatment significantly increased nitrogen and decreased phosphorus use efficiencies. Compared to the other factors, soil temperature had the least significant effect on most of the variables and did not influence photosynthetic response to CO_2 elevation. P_n, W_T, height growth, biomass production, specific root length, root length density were significantly greater in black spruce than in white spruce at the various treatment combinations. However, the slope of the W_T–biomass relationship, which may indicate W_T - biomass conversion, was greater in white spruce than in black spruce in the high nutrient. This suggests that species with greater W_T, may not necessarily be better at converting photosynthates into structural biomass.

The responses of seedlings grown without root interaction differed from those grown with root interaction in both direction and magnitude. Traits and responses associated with faster growth were generally increased when there was no inter-plant root interaction while traits such root mass density, which is associated with root longevity, increased with root interaction. This suggests differences in strategy between seedlings grown with and without root interaction

This study provides useful and information on responses of two of the most important boreal forest conifer species to interactions between CO_2, soil temperature and nutrient supply and how the responses are be modified by the roots of neighbouring plants. In modelling [CO_2], nutrient and soil temperature effects on black spruce and white spruce, the effects of belowground inter-plant interactions cannot be ignored as they significantly influence response variables.

Keywords: belowground interactions, boreal forest, competitive root interaction, gas exchange, resource use efficiency , specific root length, specific root area, whole tree photosynthetic rate.

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Abbreviations

A-C_i- Curve of assimilation rate versus intercellular CO_2 concentration

E- Transpirational water loss (mmol H_2O m^{-2} s^{-1})

HN- High nutrient treatment

IRI- presence of inter-plant root interactions

J_{max}- Maximal photosynthetic electron transport capacity (µmol m^{-2} s^{-1})

LMR- Leaf mass ratio

LN- low nutrient treatment

MRD: Mean root diameter (mm)

P_n- Net Photosynthetic rate (area based) at growth CO_2 concentration (µmol CO_2 m^{-2} s^{-1})

PNUE- Photosynthetic nitrogen use efficiency (µmol CO_2 g^{-1} N s^{-1})

PPUE- Photosynthetic phosphorus use efficiency (µmol CO_2 g^{-1} P s^{-1})

RBI- Root branching intensity (tips cm^{-1})

RCD- root collar diameter (cm)

RI- Inter-plant root interactions

RI_{No}-No inter-plant root interaction

RLD- Root length density (cm cm^{-3})

RLR- Root to leaf mass ratio

RM- Root mass (g)

RMR- Root mass ratio

RSA- Root surface area (cm^2)

RSR- Root to shoot mass ratio

RTD – Root tissue density (g cm^{-3})

SLA- Specific leaf area ($cm^2\ g^{-1}$)

SMR- Shoot mass ratio

SRA- Specific root surface area ($cm^2\ g^{-1}$)

SRL- Specific root length ($cm\ g^{-1}$)

SVR- Root surface area to volume ratio ($cm^2\ cm^{-3}$)

TPU- Triose phosphate utilisation ($\mu mol\ m^{-2}\ s^{-1}$)

TRL- Total root length (cm)

TRV- Total root volume (cm^3)

V_{cmax}- Maximum carboxylation capacity of Rubisco ($\mu mol\ m^{-2}\ s^{-1}$)

W_T- Whole tree photosynthetic rate ($\mu mol\ CO_2\ tree^{-1}\ s^{-1}$)

WUE- Photosynthetic water use efficiency ($\mu mol\ CO_2\ mmol\ H_2O^{-1}$)

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Dedication

To the Teacher who wakes up thinking about his/her students success

To the Professor whose sacrifices make all the difference in today and future leaders

To the Known and the Unknown whose financial support trains people like me.

CHAPTER 1: Research Introduction

The boreal forest occupies about 11% of the earth's terrestrial surface (Bonan and Shugart 1989, Saugier and Roy 2001) and plays an important role in the global carbon cycle (Baldocchi et al. 2000, Boisvenue and Running 2006). Globally, the region contains about 300 Pg of carbon, the equivalent of 50% of the carbon in the atmosphere (Cao and Woodward 1998, Gower et al. 2001). The region is large with high soil carbon sink and high net ecosystem exchange and is highly sensitive to changes in environmental variables (Gower et al. 2001). Hence, small changes in the region can have strong feedbacks on the climate, through changes in hydrologic cycle, albedo and carbon storage (Bonan et al. 1992, Barnett et al. 2005). Small changes in climate variables such as increase in carbon dioxide concentration ([CO_2]) can also significantly alter the boreal forest (Juday et al. 2005, Luthi et al. 2008). The ecological characteristics of the boreal biome also make it more vulnerable to warming and other global changes than other ecological regions (Chapin et al. 2006, Olsson 2009). The boreal forest is therefore both affected by and contributes to climate change (Juday et al. 2005).

Global atmospheric [CO_2] is predicted to increase from pre-industrial level of less than 300 to as high as 950 μmol mol^{-1} by the end of the 21st century (Wigley 1983, IPCC 2001, Luthi et al. 2008, Cao and Caldeira 2010). Warmer climates are predicted to result from the increased CO_2 and the average warming effect in the boreal region is likely to exceed the global mean (Serreze et al. 2000, Christensen et al. 2007, Heimann and Reichstein 2008, Montzka et al. 2011). For example, climate models predict that mean annual temperature in Canada's boreal region may rise by about 5-7 ^{o}C, higher than the 1.4-5.8 ^{o}C predicted global average (IPCC 2001, Christensen et al. 2007, Eskelin et al. 2011). The predicted increase in [CO_2] and temperature may affect growth and survival of plants by altering their physiology, phenology and competitive ability (Amthor 1995, Colombo et al. 1998, Volder et al. 2007) and the effects would be greater in the boreal region (Sellers et al. 1997, Serreze et al. 2000, Barnett et al. 2005, Juday et al. 2005)

Both short term and some long term, controlled environment and field studies indicate that CO_2 elevation increase photosynthesis, which may lead to greater biomass production and plant growth (Ainsworth and Long 2005, Ainsworth and Rogers 2007, Dang et al. 2008, Kirschbaum 2011, Barton et al. 2012). CO_2 elevation through its influence on stomatal conductance may reduce transpirational water loss leading to increase water use efficiency (Leuzinger and Körner 2007, Lambers et al. 2008, Barton et al. 2012). Elevated CO_2 may also decrease leaf nutrient content per unit leaf area and increase the efficiency of Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) in fixing carbon, thereby increasing nutrient use efficiency (Drake et al. 1997, Davey et al. 1999). The increased atmospheric [CO_2] may also act as driver to influence succession and stand diversity (Bolker et al. 1995, Wayne and Bazzaz 1995, Reich et al. 2001, Barnett et al. 2005, De Graaff et al. 2011) because of the differential physiological effect of CO_2 on different species (Poorter 1998, Poorter and Navas 2003, Dawes et al. 2011a). The effects of increased [CO_2] on plant growth is mostly positive. However, these potential effects are dependent on other factors, and in the boreal forest, nutrient availability and soil temperature are the key (Linder and Murray 1998, Jarvis and Linder 2000, Murray et al. 2000, Oren et al. 2001, Reich et al. 2006).

With the projected increase in air temperature in the boreal region, soil temperature will also increase because of the strong positive correlation and inter-dependency of the two (Zheng et al. 1993, Lupi et al. 2012). The impact of soil temperature on boreal tree growth may be higher than that of air temperature, especially between late spring and early summer (Jurik et al. 1988, Schwarz et al. 1997, Lupi et al. 2012, Wu et al. 2012). Soil temperature can modify plant morphology, physiology and traits such as organ development and biomass allocation, stomatal conductance, transpiration, nutrient and CO_2 uptake (Delucia 1986, Camm and Harper 1991, Delucia et al. 1992, Cai and Dang 2002, Domisch et al. 2002, Dang and Cheng 2004). Soil temperature is known to influence photosynthesis, growth and biomass production of boreal trees (Delucia 1986, Zhang and Dang 2005, Zhang and Dang 2007, Ambebe et al. 2010, Frechette et al. 2011). Soil temperatures below a species specific threshold value restrict nutrient availability and reduces the positive effects of CO_2 elevation on growth (Delucia 1986, Alvarez-Uria and Körner 2007, Ambebe et al. 2010) while very high soil temperatures may result in decline in photosynthesis and growth or increased mortality (Peng and Dang 2003, Way and Sage 2008a, Way and Sage 2008b). Soil warming is likely to affect nutrient availability in the soil through the stimulation of organic matter decomposition and mineralization of soil nutrients (Jarvis and Linder 2000, Lukac et al. 2010).

Nutrient availability influences plant response to increased [CO_2] (Bazzaz 1990, Bazzaz and Mcconnaughay 1992, Booker and Maier 2001, Zhang and Dang 2006, Lukac et al. 2010). Also, plant demand for nutrients may increase with increase in [CO_2] and temperature as growth increases with CO_2 (Gavito et al. 2001, Oren et al. 2001, Lambers et al. 2008, Lukac et al. 2010). The effects of CO_2 on plant growth and productivity may therefore be limited by nutrient availability on some sites (Oren et al. 2001, Zhang et al. 2006, Lukac et al. 2010). Most studies on plant nutrition modify the levels of nitrogen (N), because it is required in large quantities to support growth and most likely to limit carbon gain (Chapin et al. 1987, Lambers et al. 2008), leaving levels of other nutrient elements in the formulation intact. However, manipulating the level of only N may induce deficiency or toxicity in other nutrient elements (Proe et al. 2000, Reich et al. 2009) because of the stoichiometry between nutrient elements (Marschner 1995, Yuan and Chen 2009, Yuan et al. 2011). For example, photosynthesis is dependent on both leaf phosphorus ([P]) and [N] (Aerts and Chapin 1999, Ainsworth and Long 2005, Reich et al. 2009), so changing the concentration of only N may induce adverse P effects (Warren and Adams 2002). To avoid creating artificial nutrient deficiency or toxicity, it is essential to proportionally change the concentration of each nutrient element.

Altered temperature, nutrient and CO_2 regimes in the boreal forest may have direct impact on stand composition, physiology and inter-plant interactions (Brooker 2006, Brooker et al. 2008) and their interactive effects may be more important than the individual effects. While many studies have examined the individual or bi-factor effects of soil temperature, nutrient availability and CO_2 on boreal tree growth (Delucia and Smith 1987, Peng and Dang 2003, Way and Sage 2008a, Way and Sage 2008b), studies on the interactive effects of these factors on co-occurring boreal trees grown as individuals and in mixture is lacking. However, it is well known that plant-plant interactions are important mechanisms by which environmental drivers such as [CO_2], soil temperature and nutrient affect plant growth and productivity (Bazzaz and Mcconnaughay 1992, Brooker 2006, Engel et al. 2009, Dawes et al. 2011a). Differences in species responses to [CO_2], nutrient and soil temperature may lead to changes in relative species competitiveness, stand composition and distribution of species (Saxe et al. 2001, Brooker 2006).

This research focusses on the effects of nutrient supply and soil temperature on leaf level net and whole tree photosynthesis, seedling growth, biomass and allocation patterns, and root characteristics of black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca*

[Moench] Voss.) grown with and without inter-plant root interaction at ambient and elevated [CO_2]. The focus was on belowground interactions because root competition for nutrients can reduce leaf area, transpiration rate and growth (Jensen et al. 2011) more than above ground competition (Wilson 1988, Casper and Jackson 1997). Moreover, inter-plant root interactions can play an important role in determining competitive outcomes among individuals within communities (Arnone and Kestenholz 1997). Black spruce and white spruce were selected for the study because they co-occur, are dominant and economically important conifer species in the boreal forest (Bannan 1940, Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Patterson et al. 1997, Chen and Popadiouk 2002).

Photosynthesis, which serves as the basis of plant growth and biomass production, increases with increase CO_2 concentration because C_3 species, like black spruce and white spruce, are not saturated at current ambient [CO_2] (Amthor 1995, Ainsworth and Long 2005, Ainsworth and Rogers 2007, West-Eberhard et al. 2011). Increased [CO_2] with proportional increase in nutrient supply may enhance photosynthesis and growth (Oren et al. 2001, Reich et al. 2006, Lukac et al. 2010). It was hypothesized that increasing nutrient supply would enhance the positive effects of CO_2 elevation on photosynthetic rate, growth and biomass production and elevated [CO_2] would reduce the degree of low nutrient effect on the above responses. This is because in the short term, elevated [CO_2] may decrease the amount of Rubisco required to fix a given amount of carbon to partially compensate for low nutrient (Drake et al. 1997, Davey et al. 1999, Lambers et al. 2008). Also, increased biomass allocation to roots under elevated [CO_2] may allow for greater nutrient uptake (Rogers et al. 1994, Poorter and Nagel 2000, Pendall et al. 2004, Lambers et al. 2008).

Black spruce is able to grow on both cold peatlands and warm uplands while white spruce grows well mainly on warmer upland sites (Chapin 1983, Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Patterson et al. 1997, Wirth et al. 2008). Moreover, while black spruce response to temperature is mostly positive, that of white spruce is variable (Lloyd et al. 2005). This may indicate greater thermal plasticity in black spruce and species with greater plasticity respond better to CO_2 elevation (Midgley et al. 1999, Lloyd et al. 2005, Matesanz et al. 2010, Nicotra et al. 2010). Hence, it is hypothesized that CO_2 elevation and soil warming would enhance the growth, biomass and resource acquisition root traits in black spruce to a greater degree than in white spruce.

Plant roots communicate with each other and modify their response in response to abiotic factors and neighbouring roots (Friend et al. 2000, Cahill et al. 2010, Brassard et al. 2011). Moreover, different species respond differently to $[CO_2]$ and other treatments (Dawes et al. 2011a, Dawes et al. 2011b). Hence, the responses of isolated plants without inter-plant root interaction could differ from responses of plants with inter-plant root interaction in response $[CO_2]$, nutrient supply and soil warming, with greater use efficiency in plants grown with inter-plant root interactions.

The specific research objectives were to determine

i. how nutrient supply and inter-plant root interaction affect the photosynthetic response and nutrient use efficiency of black spruce and white spruce to CO_2 elevation (Chapter 2)

ii. the effects of nutrient supply on response of whole tree photosynthesis, biomass and morphology of the two species to CO_2 and root interactions (Chapter 3)

iii. the effects of nutrient availability, CO_2 and inter-plant root interaction on root traits of black spruce and white spruce (Chapter 4)

iv. how soil warming, inter-plant root interaction and [CO_2] affects growth, morphology and root characteristics of black spruce and white spruce seedlings (Chapter 5) and

v. how soil temperature and root interaction modify photosynthetic responses of black spruce and white spruce to CO_2 elevation (Chapter 6).

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CHAPTER 2: Nutrient effects on the physiological responses of black spruce and white spruce to CO_2 elevation with and without belowground interactions

Introduction

The atmospheric carbon dioxide concentration ([CO_2]) has been increasing since the start of industrial revolution in the mid-19th century (IPCC 2001, IPCC 2007, Conway and Tans 2011). The current [CO_2] of 380 μmol mol^{-1} (Conway and Tans 2011) may reach 730 to 1020 μmol mol^{-1} by 2100 (IPCC 2007). Increased [CO_2] may affect plant-plant interactions (Friend et al. 2000, Brooker 2006), species richness, composition and productivity of ecosystems (Brooker 2006, Körner 2006, Wang 2007, Langley and Megonigal 2010). At the individual plant level, increased [CO_2] generally enhances leaf level net photosynthesis (P_n), lowers stomatal conductance and transpiration rate (E), and increases water and nutrient use efficiencies (Ainsworth and Long 2005, Cao et al. 2007, Huang et al. 2007, Ambebe et al. 2010), at least in the short term. The effects of increased [CO_2] on plant growth and physiological processes may however, be influenced by other environmental factors such as nutrient availability (Ainsworth and Long 2005, Cao et al. 2007, Huang et al. 2007, Ambebe et al. 2010, Lukac et al. 2010).

The increase in atmospheric [CO_2] is expected to have profound impact on the boreal forest (IPCC 2007) and may change the region's species composition and the competitive ability and resource use of individual species (Stewart et al. 1998). Two congeneric conifer species in the region that may be impacted greatly by the predicted changes are black spruce (*Picea mariana* (Mill). B.S.P) and white spruce (*Picea glauca* (Moench) Voss) (Wang et al. 2006, Messaoud and Chen 2011). The two species are the most widely distributed conifers in the North American boreal forest (Nienstaedt and Zasada 1990, Sims et al. 1990, Viereck and Johnston 1990, Haavisto and Jeglum 1995). Black spruce grows on sites ranging from dry sands, gravels and shallow soils on bedrock, nutrient rich upland mineral soils to nutrient deficient lowland sites (Haavisto and Jeglum 1995). In Ontario's boreal forest, for example, 54% of black spruce stands are on upland mineral soils and 46% on peatlands (Haavisto and Jeglum 1995). On the other hand, white spruce grows best on moist upland sites and on well aerated, well drained sites (Nienstaedt and Zasada 1990). On moist upland mineral soils, the two species can co-occur with or without other species such as jack pine (*Pinus banksiana* Lamb.) (Nienstaedt and Zasada 1990, Sims et al. 1990, Haavisto and Jeglum 1995). The high economic values of the two species and the wide distribution of the species make them excellent materials for research in the context of climate change.

Nutrient availability can affect photosynthetic and transpiration rates and water use efficiency (Zhang et al. 2006, Cao et al. 2007, Ambebe et al. 2010). Nutrient availability also influences plants response to increased [CO_2] (Bazzaz 1990, Bazzaz and Mcconnaughay 1992, Booker and Maier 2001, Zhang and Dang 2006, Lukac et al. 2010). For example, low nutrient supply reduces photosynthetic gains from increased [CO_2] (Bazzaz 1990, Poorter 1998, Tangley 2001). Therefore, if any increase in boreal forest productivity due to increased [CO_2] is to be maintained, it may be necessary to increase soil nutrient supply (Lukac et al. 2010), especially on nutrient poor sites. Most studies on plant nutrition modify the levels of nitrogen (N), because it is required in large quantities to support growth and most likely to limit growth (Chapin et al. 1987), leaving the levels of other nutrient elements intact. However, deficiency and toxicity of other nutrient elements have significant effect on plants (Aerts and Chapin 1999, Lambers et al. 2008, Reich et al. 2009). For instance, photosynthesis rate varies with leaf phosphorus concentration ([P]) (Aerts and Chapin 1999, Ainsworth and Long 2005, Lambers et al. 2008, Reich et al. 2009) and P supply influences N effects on photosynthesis (Warren and Adams 2002, Warren et al. 2005). Leaf potassium concentration ([K]) affects photosynthesis through regulation of stomatal aperture (Pallardy 2008). Hence, an imbalance in nutrient supply may decrease photosynthetic rate and the beneficial effects of other treatments (Pallardy 2008). Moreover, it is not only N that limits growth of boreal trees; P and K deficiencies have been observed in black spruce (Wells 1994, Teng et al. 2003) and in white spruce (Phu and Gagnon 1975). Maintaining proper ratios of N, P and K in nutrient treatments may be key to avoid nutrient imbalance.

Inter-specific plant interactions are important in regulating species diversity and stand composition in plant communities (Brooker 2006). Previous studies indicate that there are substantial inter-specific variations in growth responses to rising [CO_2] (Bazzaz and Mcconnaughay 1992, Ainsworth and Long 2005, Brooker 2006, Engel et al. 2009, Lukac et al. 2010). The differences in responses may change species interactions in the future when atmospheric [CO_2] increases, leading to changes in relative species competitiveness, species composition of forest stands and distribution of species (Saxe et al. 2001, Brooker 2006). How will black spruce and white spruce growing on nutrient poor and nutrient rich conditions respond to CO_2 elevation and how does inter-plant root interaction modify the response

Black spruce may have lower nutrient requirement than white spruce (Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Patterson et al. 1997). Black spruce can grow on both rich upland sites and nutrient-poor peatlands while white spruce grows well only on fertile upland sites. Black spruce therefore appears physiologically more plastic than white spruce (Patterson et al. 1997). Species with greater plasticity tend to respond better to increasing [CO_2] (Brown and

Higginbotham 1986, Midgley et al. 1999, Matesanz et al. 2010), implying black spruce may benefit more from increased [CO_2]. Counteracting that, however, is the fact that black spruce has relatively slower growth rate than white spruce and species with inherent slow growth rate may benefit less from increased [CO_2] (Poorter 1998, Tangley 2001). Moreover, on fertile sites, fast growing species have high nutrient uptake kinetics (Jackson et al. 1990) and are able to deplete nutrient rich patches before slow growing species with low uptake kinetics are able to extract enough nutrients (Jackson et al. 1990, Caldwell et al. 1996, Lambers et al. 2008). Plants with high uptake kinetics may, on the other hand, may be at disadvantage where nutrient supply is low because of greater energy requirements (Jackson et al. 1990, Bassirirad 2000). As such, on nutrient poor sites, species adapted to low soil fertility with lower uptake kinetics may out-compete species adapted to high nutrient supply (Jackson et al. 1990, Aerts and Chapin 1999, Bassirirad 2000). Therefore, it is hypothesized that white spruce may benefit more than black spruce from the increased [CO_2] at high nutrient supply whereas CO_2 elevation may be more beneficial to black spruce under low nutrient supply conditions.

If the two species have different nutrient requirements, resource utilisation may be enhanced with the presence of inter-plant root interactions, particularly if they preferentially extract and use different forms of nutrient. As such, the relative enhancement in photosynthesis and nutrient use efficiencies due to increased [CO_2] could be higher in treatments with than without inter-plant root interactions.

It was hypothesized that increasing nutrient supply would increase the beneficial effects of CO_2 elevation on photosynthesis and that the CO_2 elevation would reduce the degree of low nutrient limitation on photosynthesis. This is because increased [CO_2] can partially compensate for low nutrient supply in the short term, by decreasing the amount of Rubisco required to fix a given amount of carbon (Pearcy and Björkman 1983, Drake et al. 1997, Davey et al. 1999, Lambers et al. 2008). Furthermore, increases in carbohydrate production resulting from elevated [CO_2] can promote the development of larger root system, leading to greater exploration for soil nutrients (Rogers et al. 1994, Pendall et al. 2004, Lambers et al. 2008).

In this chapter, I investigate the effects of nutrient availability on the physiological responses (gas exchange, nutrient relations) of black spruce and white spruce exposed to CO_2 elevation with and without inter-plant root interactions. Belowground competition for nutrients can reduce leaf area, number of leaves, transpiration rate and growth (Jensen et al. 2011) more than above ground competition (Wilson 1988, Carswell et al. 2000), hence, the focus.

Materials and Methods

Plant Materials

One-year old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings were obtained from a commercial tree seedling nursery for the study. Seedlings of relatively uniform size (height and diameter) were selected for the study. The growing medium used was a mixture of premium grade vermiculite and peat moss (50:50, v/v).

Experimental Design and Growth Conditions

The experiment was conducted at Lakehead University's Thunder Bay Campus. The treatments consisted of two CO_2 and nutrient (N) levels, two levels of inter-plant root interactions (RI) and two species (S) in a split-split-split plot design (App. 2.1 and 2.2). The [CO_2] was treated as the whole plot, with root interaction as the sub-plot, and nutrient supply the sub-sub plot. The nutrient treatment was further split into sub-sub-sub plots. The position of each species was completely randomised within the nutrient treatment. There were two CO_2 levels (ambient, 380 and elevated, 720 μmol mol^{-1}) with two independent replications each and two levels of RI (no inter-plant root interaction-RI_{No} vs. inter-plant root interaction-IRI) within each CO_2 treatment. The CO_2 elevation was achieved using electronic ignition CO_2 burners (model GEN-2E, Custom Automated Products, Inc, Riverside, CA). The [CO_2] in each of the four greenhouses was monitored and automatically controlled using Argus CO_2 sensors and control system (Argus, Vancouver, BC, Canada). In the no inter-plant root interaction (RI_{No}) treatment, a single seedling was grown in an individual container, while with the presence of inter-plant root interaction (IRI), 8 seedlings of each species were grown in a large container and were spaced at about the diameter of the container in the no root interaction treatment. The soil volume available per seedling was approximately the same for seedlings in the IRI and RI_{No} treatments. The high nutrient treatment composed of 150, 60, 150, 80, 40, 60 mg/l N, P, K, Ca, Mg and S, respectively, and was based on Landis (1989) recommendation for container-grown conifer seedlings during the rapid growth phase (App. 2.3). The low nutrient treatment was 10% of the high nutrient treatment concentrations. Fertiliser application was done once a week. Eight seedlings of each species per treatment combination were planted, bringing the total to 256 seedlings. See App. 2.1 for schematic presentation of design.

Environmental conditions in the greenhouses were controlled using an Argus control system as follows: relative humidity of 55 ± 5%, day and night air temperatures of 25 ± 2 °C and 15 ± 2 °C respectively. The seedlings received natural light (averaged 660 μmol m^{-2}s^{-}1) which was supplemented on cloudy days and early evenings with high pressure sodium vapour lamps up to a 17 hour photoperiod. The plants were maintained in a well-watered condition.

Gas exchange measurements

Foliar gas exchange was measured 4 months into the treatments. Three seedlings per treatment combination (total of 96) were randomly selected for the measurements. Gas exchange was measured on current year needles using a Ciras-1 open gas exchange system (PP-Systems, Amesbury, MA, USA) with a Parkinson conifer leaf cuvette. Environmental conditions in the cuvette were controlled automatically as follows: temperature 25 °C, relative humidity 50%. Saturating photosynthetic active radiation of 800 μmol m^{-2} s^{-1} was maintained during the measurements. The light was supplied from the cuvette's built-in tungsten lamp. A-C_i curves (net assimilation rate, A, vs inter-cellular CO_2 concentration, Ci) were measured sequentially at 50, 150, 250, 360, 500, 720, 900 and 1200 μmol mol^{-1} [CO_2]. Each series of A-Ci curve took approximately 20-30 minutes to complete. Net photosynthetic rate (P_n, μmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) at growth [CO_2] were estimated from the response curves. Photosynthetic water use efficiency (WUE, μmol CO_2 mmol H_2O^{-1}) was calculated as: WUE = P_n/E, where P_n and E were the rates at the corresponding growth [CO_2]. Maximal carboxylation capacity of Rubisco (V_{cmax}), maximal photosynthetic electron transport capacity (J_{max}) and triose phosphate utilisation (TPU) were estimated using the A-C_i models and curve-fitting utility developed by Sharkey et al. (2007). All the parameters were expressed on a projected leaf area basis. Projected leaf area was determined using the Regent WinSeedle system (Regent Instruments, Québec City, QC, Canada).

Foliar nutrient analyses

Following the gas exchange measurements, the three seedlings used for the measurements were harvested to determine specific leaf area and foliar nutrient concentration. The needles were oven-dried at 70 °C for 48 hours. Needle total nitrogen (N) concentration was determined using the dry combustion method on a LECO CNS-2000 analyser (LECO, St. Joseph's Michigan USA). Acid digestion method and the ICP-AES technique were used to determine foliar phosphorus (P) and potassium (K) concentrations. Mass-based nutrient contents were converted to area based concentrations. Photosynthetic nitrogen (PNUE) and phosphorus (PPUE) use efficiencies were calculated as P_n/[N] and P_n/[P] respectively).

Statistical analysis

The data were analysed using the analysis of general analysis of variance (ANOVA) in GenStat version 12 (VSN International, Hemel Hempstead, UK) with CO_2xRIxNxS as the treatment structure and Block/CO_2/RI/N/S as the block structure. The data met the assumptions for both normality and homogeneity (Shapiro Wilk and Bartlett tests), hence, no data transformation was required. Post-hoc tests were conducted using Fisher's Least Significant Difference (LSD) approach when ANOVA showed a significant ($P \leq 0.05$) interaction. When main effects and interactions are significant, only the significant interaction of the highest order was discussed. Relationships of P_n with J_{max}, V_{cmax} and TPU were modelled using simple linear regression for different combinations of species, CO_2 and nutrient supply. Differences between the slopes were compared with t-test based on (Zar 2010) as:

$$T_{stat} = \frac{(\beta_1 - \beta_2)}{(S_1 - S_2)}$$

where β_1 and β_2 are beta coefficients (slopes of two regressions) and S_1 and S_2 are the respective standard errors.

Results

Gas exchange

Carbon dioxide concentration ([CO_2]) and root interaction had significant interaction on net photosynthetic rate (P_n, Table 2.1). At the elevated [CO_2], the presence of root interaction significantly increased P_n with the reverse occurring at ambient [CO_2] (Fig. 2.1). Overall, the highest P_n rate was observed in seedlings grown with root interaction at elevated [CO_2] while the presence of root interaction at ambient [CO_2] significantly reduced P_n (Fig. 2.1). Nutrient supply also significantly affected seedlings P_n (Table 1). On average, P_n of seedlings grown in the high nutrient treatment were 17% higher than that of seedlings grown in the low nutrient treatment. P_n was 22% greater in black spruce.

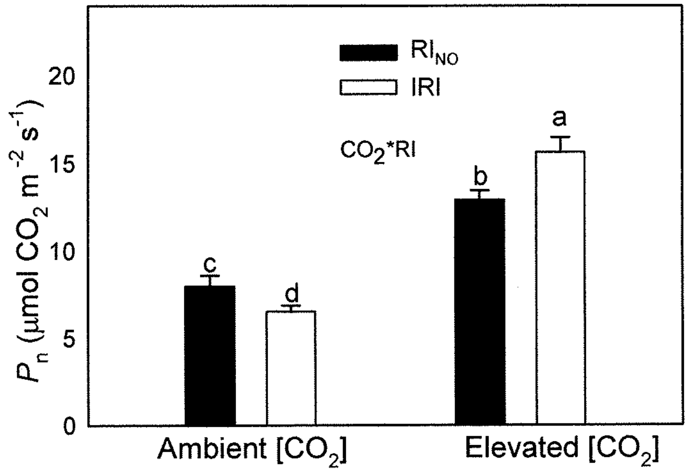

Fig. 2.1: Effects of [CO_2], inter-plant root interaction (RI) on photosynthetic rate (P_n) of black spruce and white spruce seedlings after months of treatment (n=24). The seedlings were exposed to two [CO_2] with or without inter-plant root interactions at low and high nutrient. In all figures, RI_{No} indicate no inter-plant root interaction while IRI indicates presence of inter-plant root interaction. Bars with the same letter or letters do not differ significantly ($P \leq 0.05$).

The response of transpiration rate (*E*) to root interaction differed significantly between the two species (Table 2.1). The presence of inter-plant root interaction significantly reduced *E* in white spruce (Fig. 2.2a). Neither [CO_2] nor nutrient supply significantly affected *E* (Table 2.1).

[CO_2], root interaction and species had significant interactive effects on photosynthetic water use efficiency (WUE, Table 2.1). At the ambient [CO_2], there was no statistical difference in WUE between any treatment combination (Fig. 2.2b). The CO_2 elevation significantly increased WUE in both species irrespective of the RI treatment (Fig. 2.2b). However, the level of WUE enhancement by CO_2 elevation was greater in black spruce grown with than without root interaction (Fig. 2.2b).

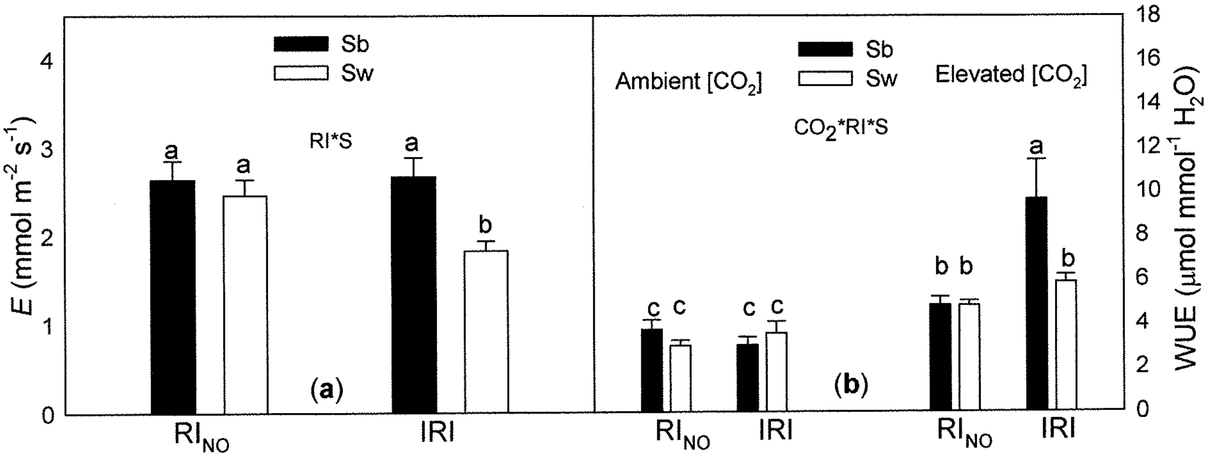

Fig. 2.2: Effects of [CO_2], species (S) and root interaction (RI) on transpiration rate-*E* (*a*) and water use efficiency-WUE (*b*) of black spruce and white spruce seedlings after 4 months of treatment. In Fig. ***a***, *n=24* while in ***b***, *n= 12.*

Table 2.1: *P* values from ANOVA on the effects of [CO_2], inter-plant root interaction (RI), nutrient supply (N) and species (S) on physiological characteristics of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were exposed to two CO_2 concentrations (380 and 720 μmol mol^{-1}) with and without inter-plant root interaction at two nutrient levels. Other abbreviations are defined in the text.

Factor	P_n	*E*	WUE	J_{max}	V_{cmax}	J_{max}/V_{cmax}	TPU
CO_2	**0.019**	0.894	**0.022**	0.572	0.771	0.167	0.405
RI	0.995	0.235	**0.014**	0.339	0.593	0.894	0.333
CO_2*RI	**0.047**	0.469	**0.012**	0.272	0.670	0.608	0.131
N	**0.033**	0.358	0.370	0.283	**0.047**	0.116	**0.037**
CO_2*N	0.290	0.177	0.296	0.190	0.497	0.364	0.733
RI*N	0.076	0.242	0.358	0.301	0.547	0.171	0.084
CO_2*RI*N	0.737	0.548	0.781	0.138	0.557	0.348	0.674
S	**<.001**	**0.003**	0.052	**0.009**	**0.019**	0.125	**0.007**
CO_2*S	0.110	0.137	0.080	0.271	0.149	0.347	0.930
RI*S	0.208	**0.047**	0.220	0.356	**0.020**	0.231	**0.014**
N*S	0.128	0.629	0.238	0.835	0.989	0.152	0.916
CO_2*RI*S	0.403	0.055	**0.015**	0.679	0.132	0.092	0.863
CO_2*N*S	0.243	0.140	0.709	0.387	0.573	**0.041**	0.453
RI*N*S	0.601	0.368	0.685	0.792	0.871	0.870	0.775
CO_2*RI*N*S	0.334	0.211	0.097	0.100	**0.027**	0.139	0.747

In-vivo biochemical activities of Rubisco

The maximum photosynthetic electron transport capacity (J_{max}) in black spruce was 17% higher than in white spruce (Table 2.1). No other factor had significant effect on J_{max} (Table 2.1).

There was a significant four-way interaction on maximum carboxylation capacity of Rubisco (V_{cmax}). At the ambient [CO_2], black spruce grown in the high nutrient treatment without root interaction had the highest V_{cmax} while white spruce in the low nutrient and with root interaction had the lowest V_{cmax} (Fig. 2.3a). The CO_2 elevation significantly increased V_{cmax} in white spruce grown in the high nutrient treatment with no root interaction and in black spruce grown in the high nutrient treatment with root interaction (Fig. 2.3a). The CO_2 elevation however, reduced V_{cmax} in black spruce grown in the high nutrient treatment without plant-plant root interaction (Fig. 2.3a).

The ratio of J_{max}/V_{cmax} was significantly affected by [CO_2], nutrient supply and species interactions (Table 2.1). At the ambient [CO_2], there was no significant difference in the ratio (Fig. 2.3b). The CO_2 elevation significantly increased the ratio in white spruce in the low nutrient treatment and in black spruce in the high nutrient treatment (Fig. 2.3b).

The response of triose phosphate utilization (TPU) to root interaction differed significantly between the two species (Table 2.1). The TPU of white spruce was significantly reduced with root interaction (Fig. 2.3c). The TPU of black spruce did not differ with root interaction (Fig. 2.3c). The high nutrient supply increased TPU by 17% while [CO_2] had no significant effect on TPU (Table 2.1).

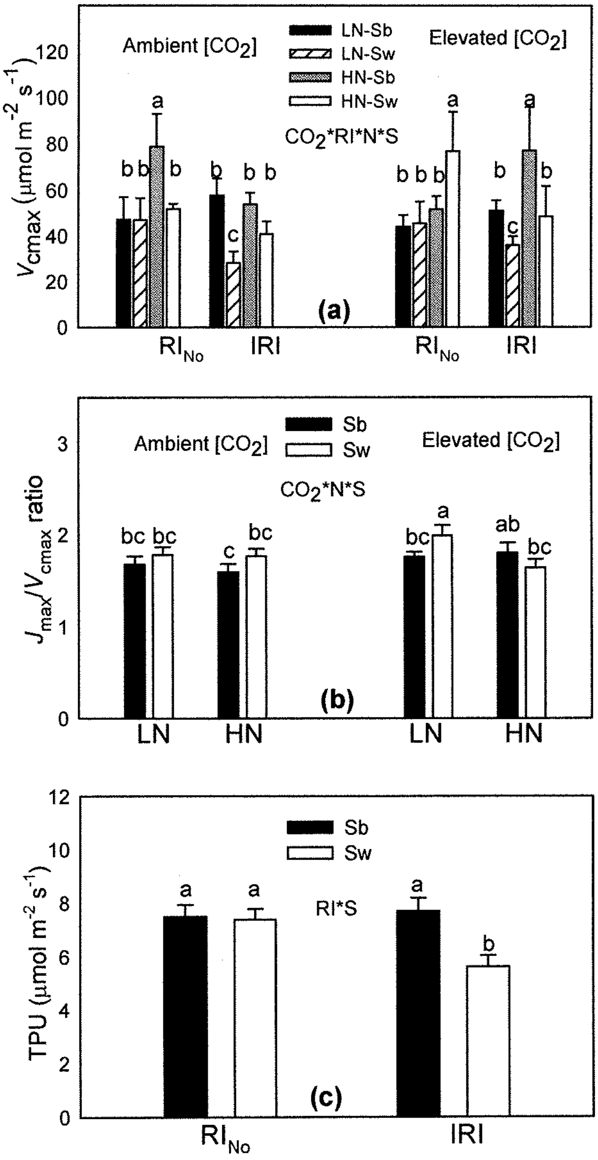

Fig. 2.3: Effects of [CO_2], nutrient supply (N), species (S) and root interaction (RI) on Rubisco's maximum carboxylation rate-V_{cmax} (*a*), ratio of maximum electron transport capacity-J_{max} to V_{cmax} (*b*) and triose phosphate utilisation-TPU (*c*) of black spruce and white spruce seedlings after 4 months of treatment. In Fig. ***a***, *n=6* while in ***b***, *n=12.* In Fig. ***c***, *n=24.*

There were generally positive linear relationship between J_{max} and P_n ($r^2 = 0.534\text{-}0.943$, Figs. 2.4a, 2.4b, 2.4d), with the exception of white spruce grown in elevated [CO_2] under the high nutrient treatment ($r^2 = 0.159$, $P = 0.199$, Fig. 2.4c). However, not all the individual slopes were ($P \leq 0.05$) significantly different from each other as indicated by the results of *t*-tests (App. 2.4). For example, the slope for black spruce grown in the high nutrient treatment under ambient [CO_2] was not statistically different from those of any other regressions at ambient [CO_2] but it was significantly different from that for white spruce grown in low nutrient and elevated [CO_2].

V_{cmax} and P_n were highly correlated in all treatment combinations ($r^2 = 0.768\text{-}0.951$, $P \leq 0.05$, Figs. 2.4e-2.4h). The slopes of all the regressions were significantly different from each other with the exception of black spruce in the high nutrient and elevated CO_2 treatment which was not significantly different from that of black spruce in the ambient [CO_2] with either high or low nutrient supply (App. 2.4).

The relationship between P_n and TPU were also highly significant ($r^2 = 0.617 - 0.900$, $P \leq 0.05$, Figs. 2.4i-2.4l). The slopes of all the regressions were statistically different from each other except that the CO_2 treatment significantly affected the slope for white spruce in the high nutrient treatment and black spruce in the low nutrient treatment differently (App. 2.4)

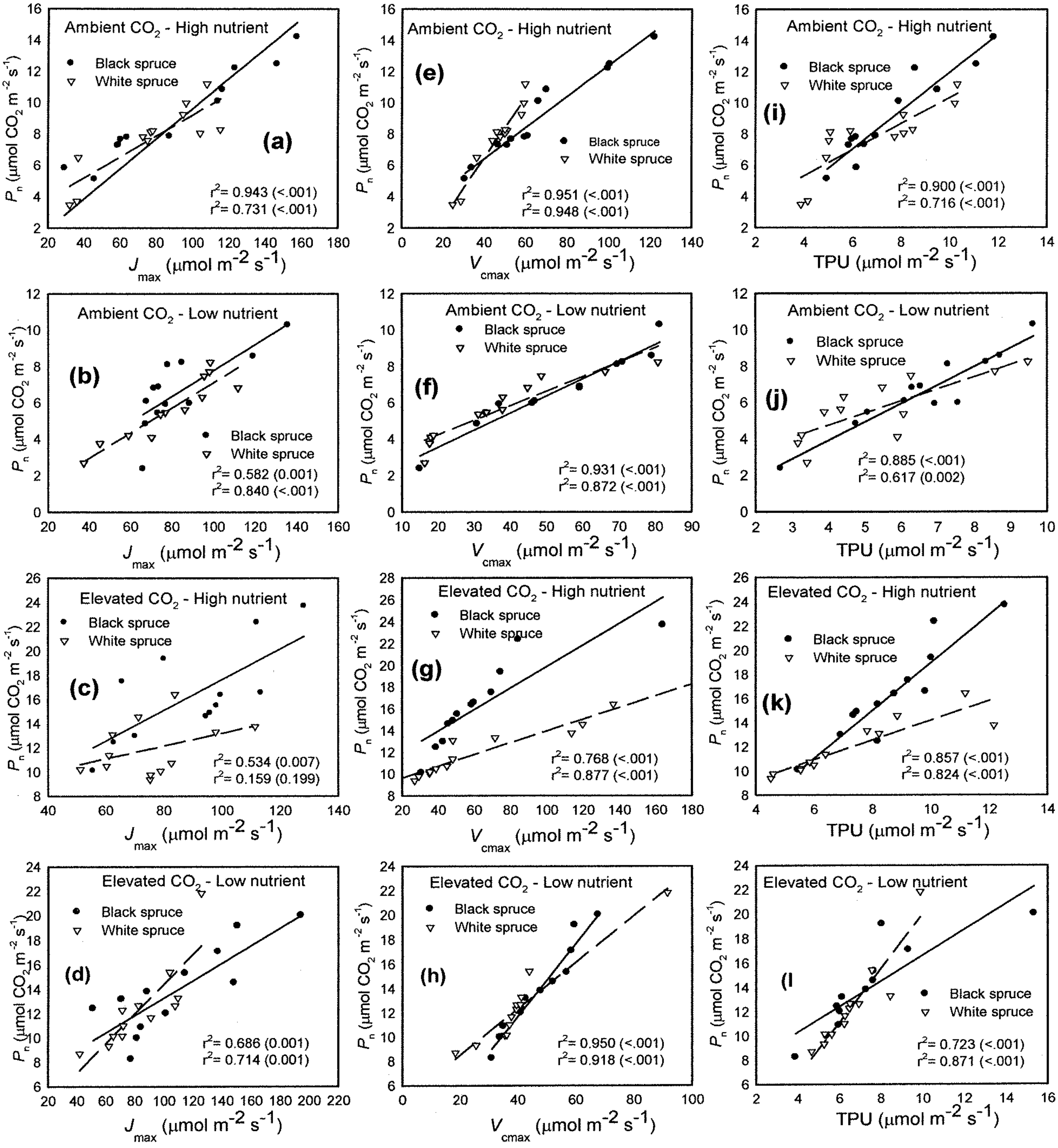

Fig. 2.4: Relationship between maximum photosynthetic electron transport capacity-J_{max} (left panel), maximum carboxylation rate-V_{cmax} (middle panel), triose phosphate utilisation-TPU (right panel) and net photosynthetic rate-P_n, of black spruce (straight line with filled circles) and white spruce (broken lines with open triangles) seedlings. The seedlings were exposed to two [CO$_2$] and two nutrient levels with or without inter-plant root interaction for 4 months.

Foliar nutrient concentration

[CO_2], root interaction and species had significant interaction on foliar [N] (Table 2.2). At the ambient [CO_2], white spruce grown without root interaction had significantly higher [N] than in the other treatments (Figs. 2.5a). The CO_2 elevation marginally reduced [N] in white spruce grown without root interaction but marginally increased it in white spruce grown with root interaction. There was however, no significant difference in [N] at elevated CO_2 among the treatment combinations (Figs. 2.5a). The high nutrient treatment increased [N] by 30%.

There was a significant three-way [CO_2] x RI x S interaction on foliar phosphorus concentration (Table 2.2). The response at ambient CO_2 followed the same trend as that of [N] (Fig. 2.5b). The CO_2 elevation significantly decreased [P] in white spruce grown without root interaction and in black spruce grown with root interaction (Fig. 2.5b).

Foliar potassium concentration ([K]) was significantly affected by RI x N (Table 2.2). The low nutrient treatment significantly reduced [K] when the two species grew together but had no significant effect on [K] when they grew separately (Fig. 2.5c). [K] was 21% higher in black spruce than in white spruce while [CO_2] had no significant effect on [K] (Table 2, Fig. 2.5c).

Foliar N/P ratio was significantly influenced by the interactions among the four factors (Table 2.2). The CO_2 elevation significantly increased the ratio when the seedlings were grown individually except in white spruce in the low nutrient (Fig. 2.5d). With root interaction however, the CO_2 elevation significantly increased the N/P ratio only in black spruce in the high nutrient treatment and in white spruce in the low nutrient treatment (Fig. 2.5d). At the ambient [CO_2], the presence of root interaction significantly increased N/P ratio in all treatment combinations except in individually grown white spruce in the low nutrient treatment (Fig. 2.5d). Net photosynthetic rate positively and more closely related with N/P ratio (*$R^2 = 0.308$, $P \leq 0.001$*, Fig. 2.6d) than with the concentration of individual nutrient elements (Figs. 2.6a-2.6c).

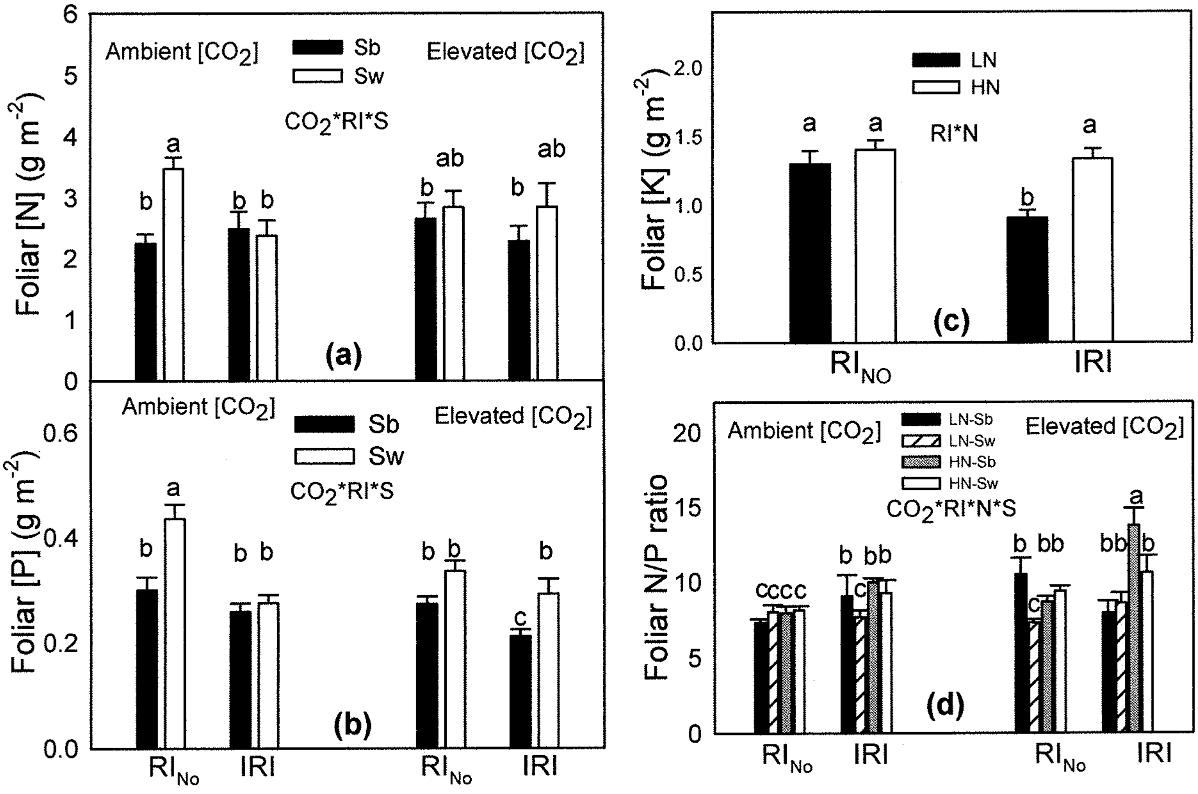

Fig. 2.5: Effects of [CO2], nutrient supply (N), species (S) and root interaction (RI) on foliar nitrogen concentration-[N] (*a*), phosphorus concentration-[P] (*b*), potassium concentration-[K] (*c*) and [N] to [P] ratio (*d*) of black spruce and white spruce seedlings after 4 months of treatment. In Figs. *a* and *b*, *n=12* while in *c*, *n=24*. In Fig. *d*, *n=6*.

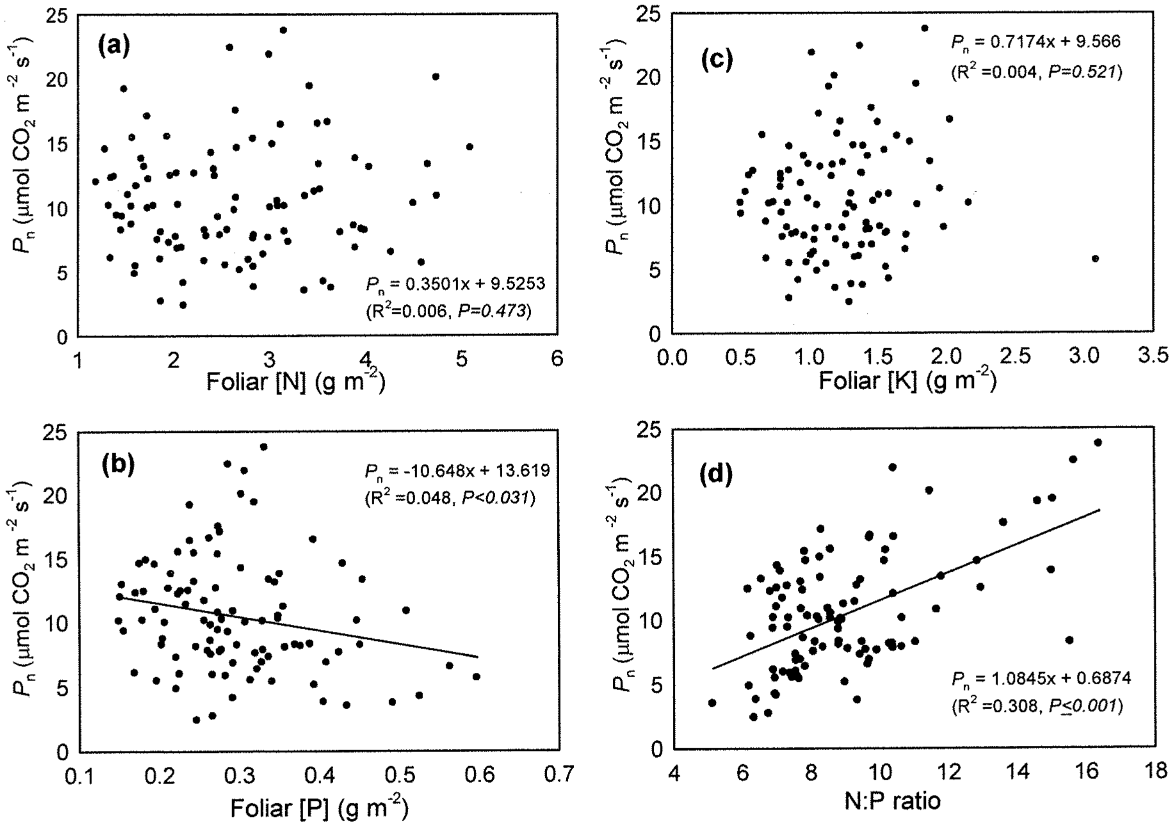

Fig. 2.6: Relationship between foliar nitrogen-[N], phosphorus-[P], potassium-[K], NP ratio and net photosynthetic rate-P_n, of black spruce and white spruce seedlings. The seedlings were exposed to two [CO_2] and two nutrient levels with and without inter-plant root interaction.

Table 2.2: *P* values from ANOVA on the effects of [CO_2], inter-plant root interaction (RI), nutrient supply (N) and species (S) on foliar nutrient concentration and nutrient use efficiency of black spruce and white spruce seedlings after 4 months of treatment.

Factor	**[N]**	**[P]**	**[K]**	**N/P ratio**	**PNUE**	**PPUE**
CO_2	0.968	0.363	0.295	**0.017**	**0.011**	**0.001**
RI	0.236	0.147	0.213	0.066	0.062	**0.009**
CO_2*RI	0.579	0.545	0.743	0.868	0.058	**0.015**
N	**0.005**	**0.023**	**0.009**	0.105	0.099	0.995
CO_2*N	0.274	0.892	0.362	0.422	0.080	0.408
RI*N	0.136	0.878	**0.043**	0.161	**0.043**	0.374
CO_2*RI*N	0.346	0.957	0.207	0.347	0.213	0.899
S	**0.007**	**<.001**	**0.001**	**0.022**	**<.001**	**<.001**
CO_2*S	0.595	0.842	0.159	0.153	0.115	**0.001**
RI*S	0.161	**0.049**	0.582	0.262	0.726	0.208
N*S	0.140	0.241	0.523	0.924	0.169	**0.006**
CO_2*RI*S	**0.013**	**0.010**	0.770	0.261	**0.052**	**0.041**
CO_2*N*S	0.186	0.114	0.810	0.994	0.174	**0.013**
RI*N*S	0.946	0.083	0.256	**0.017**	0.436	**0.041**
CO_2*RI*N*S	0.077	0.639	0.079	**0.001**	0.215	**0.014**

Photosynthetic nutrient use efficiency

[CO_2], root interaction and species had significant interactive effects on photosynthetic nitrogen use efficiency (PNUE, Table 2.2). While PNUE was significantly higher under elevated [CO_2] in all treatment combinations, the elevated CO_2 stimulation was greater with root interaction and significantly greater in black spruce with root interaction than in white spruce (Fig. 2.7a). At the ambient [CO_2], PNUE was significantly lower in individually grown white spruce than in the treatment other treatment combinations (Fig 2.7a). PNUE also differed significantly with RI x N interaction (Table 2.2). PNUE was significantly enhanced in the low nutrient treatment when the seedlings were grown together (Fig. 2.7b).

Photosynthetic phosphorus use efficiency (PPUE) was significantly affected by interactions among the four factors (Table 2.2). At the ambient [CO_2], there was no significant treatment difference, although white spruce appeared to have lower PPUE in all treatment combinations (Fig. 2.7c). At the elevated [CO_2] however, seedlings that were grown with root interaction had significantly greater PPUE than those grown individually with the exception of white spruce in the low nutrient treatment (Fig. 2.7c).

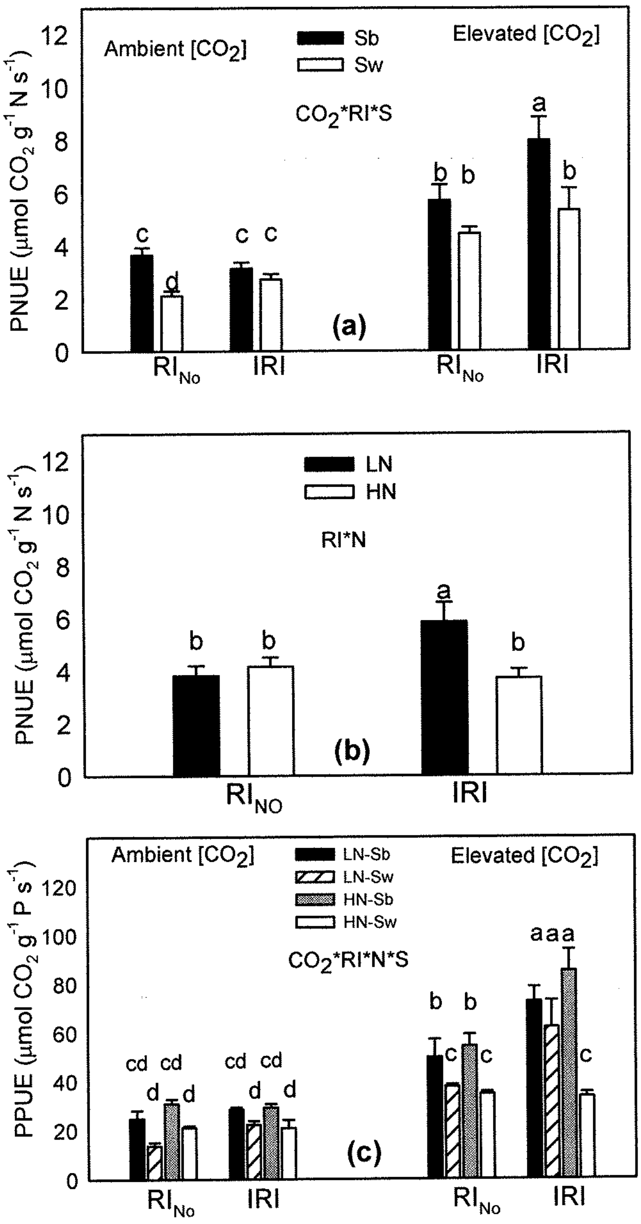

Fig. 2.7: Effects of [CO$_2$], nutrient supply (N), species (S) and root interaction (RI) on photosynthetic nitrogen use efficiency-PNUE (***a***, ***b***) and phosphorus use efficiency-PPUE (***c***) of black spruce and white spruce seedlings after 4 months of treatment. In Fig. ***a***, *n=12* while in ***b***, *n=24*. In Fig. ***c***, *n=6*.

Discussion

The data do fully not support the hypotheses that increasing nutrient supply would increase the beneficial effects of CO_2 elevation on photosynthesis and that the CO_2 elevation would reduce the degree of low nutrient limitation on photosynthesis. The greater photosynthetic enhancement of seedlings grown in elevated [CO_2] was independent of nutrient supply. The absence of significant CO_2 x Nutrient interaction might have been caused by the amount and rate of nutrient supplied. Landis (1989) recommends that for slow growing woody species such as spruce, the level of [N] needs to be increased to compensate for higher demand. However, in this study, the general nutrient treatment recommended for conifers by Landis (1989) for seedling rapid growth phase was used without adjustments for the specific species. Moreover, gradual but exponential nutrient supply may favour black spruce while white spruce responds well to high and constant nutrient feeding (Burgess 1990). However, the same rate of fertiliser was applied to all the seedlings, and that might have minimised the positive effects of high nutrient treatment thereby influencing interaction with other factors.

The results indicate that that nutrient availability did not alter photosynthetic responsiveness to [CO_2]. While the effect of elevated [CO_2] on photosynthesis is generally higher under enhanced nutrient conditions (Tissue et al. 1993, Murray et al. 2000, Oren et al. 2001, Springer et al. 2005), that was not the case in this present study. However, this result is consistent with some reports in the literature. Midgley et al. (1999) studied four congeneric *Leucadendron* species and observed no significant CO_2 x Nutrient interactions on photosynthetic rate, though each factor independently enhanced P_n. Norby and O'Neill (1991) reported that CO_2 stimulation on photosynthesis did not vary with nitrogen supply in yellow poplar (*Liriodendron tulipifera*) seedlings. In another study, Norby and O'Neill (1989) reported that CO_2 elevation increased both photosynthetic rate and growth in white oak irrespective of the nutrient treatment. In a free-air CO_2 enrichment experiment, Lee et al. (2011) found that the CO_2 enhancement of photosynthetic rate in 13 grassland species was independent of nitrogen supply. The result of this current study indicates that the limitations posed by low nutrient supply do not completely eliminate the beneficial effects of CO_2 elevation on photosynthesis.

The CO_2 elevation resulted in an 88% P_n enhancement across nutrient treatments. The 93% enhancement of P_n in black spruce by elevated [CO_2] is higher than the 33% enhancement reported by Johnsen (1993) for well watered well fertilised black spruce seedlings. However, the 83% elevated CO_2 induced P_n enhancement in white spruce observed in this study is lower than the 223% enhancement reported by Dang et al. (2008). The differences in magnitude of enhancement might be due to variations in experimental protocol and genetics. Johnsen's (1993) experiment was conducted in growth chambers while this study was carried out in greenhouses. Dang et al. (2008) used seedlings from 11 provenances across Ontario and did not have CO_2 replication while this study used only one provenance seed source with two CO_2 replications.

The hypothesis that CO_2 elevation would stimulate photosynthesis and nutrient use efficiencies to a greater extent in plants grown with inter-plant root interaction than in isolated individuals without inter-plant no root interaction is supported. The photosynthetic rates of both black spruce and white spruce were enhanced more by CO_2 elevation when the two species were grown together than in isolation. Furthermore, the effect of the root interaction treatment varied with CO_2 treatment. For example, growth without root interaction resulted in higher P_n than with root interaction under ambient [CO_2] but the trend was reversed under elevated [CO_2]. Navas (1998) analysed data from twenty studies and observed that 60% of the plant modified their response to

CO_2 elevation when grown in mixture as compared to growing individually. This indicates that P_n of black spruce and white spruce grown in mixture cannot be estimated from P_n of isolated individuals without inter-plant root interaction. For modelling the effects of climate change on black spruce and white spruce, these need to be recognised.

It has been reported elsewhere (Wang 2007) that plants are generally more stimulated by elevated [CO_2] when grown in monocultures or in isolation than in mixture. In contrast, however, Reekie and Bazzaz (1989) reported that while CO_2 elevation had no effect on the performance of *Ceeropia obtusifolia, Myrioearpa longipes, Piper auritum,* and *Triehospermum mexicanum* grown as individuals, their biomass were significantly enhanced under mixture. Similarly, Groninger (1996) observed greater photosynthesis enhancement by elevated [CO_2] in red maple (*Acer rubrum*) only when grown in mixture with loblolly pine but not in monoculture. In multi-species study, Navas et al. (1999) also reported that, CO_2 elevation stimulated greater biomass production in *Lotus pedunculatus* grown in mixture than in isolation or monoculture. Mixtures can out-yield monocultures or plants in isolation when the positive interactions of facilitation override the competitive interactions (Forrester et al. 2006). The higher P_n of seedlings grown with root interaction at elevated [CO_2] might be due to greater resource complementarity under CO_2 elevation which ultimately result in increased use efficiency and responsiveness to CO_2 (Teughels et al. 1995, Reich et al. 2004, Wang 2007). In fact, water and nutrient use efficiencies were generally higher at elevated [CO_2] when the two species were grown together with root interaction.

The expectation that white spruce would benefit more from higher nutrient supply and CO_2 elevation is not supported by the data. There was no significant CO_2 x N x S effect on net photosynthetic rate, indicating that photosynthetic rate of the two species responded similarly to

CO_2 and nutrient supply. Across all treatments, P_n was significantly higher in black spruce than in white spruce though [N] and [P] were on the average greater in white spruce. Plants with high foliar nitrogen concentration generally have high P_n rate (Aerts and Chapin 1999, Murray et al. 2000, Lambers et al. 2008, Pallardy 2008). Joel et al. (2001) observed a similar response in a field microcosm study and attributed the findings to timing of water and nutrient treatment. In this study however, the mechanisms for this observation might be related the low tissue potassium concentration ([K]) in white spruce and the reduced electron transport capacity (J_{max}) and sink strength (TPU) of the species and dependency of P_n on N/P ratio rather than [N] or [P]. First, K serves as an osmoticum for the flow of assimilates from source to sink (Marschner 1995). Plants with adequate [K] have enhanced RuBP carboxylase activity, increased synthesis of ATP, improved transport of assimilates from source to sink and ultimately increased photosynthetic rate (Peoples and Koch 1979, Marschner 1995). A decline in [K] may limit the utilization of triose-phosphate and ultimately photosynthesis (Nygren and Hari 1992). Secondly, both J_{max} and TPU were generally higher in black spruce than in white spruce (Fig. 2.3c) and both correlated well with P_n, hence, any reduction in either may limit P_n. The photosynthetic rate of white spruce appeared to be limited by both electron transport and sink strength, possibly through limitations imposed by low tissue K, rather just foliar [N]. Midgley et al. (1999) studied photosynthetic responses of *Leucadendron* species and concluded that the mechanism which controls photosynthetic response to CO_2 elevation is not primarily tissue nitrogen concentration but possibly plasticity of the species and efficiency in using the N especially under CO_2 elevated. Clearly, black spruce was more efficient in N utilisation at elevated [CO_2] as evident by the higher PNUE in black spruce grown with root interaction.

Finally, P_n related better with N/P ratio than either [N] or [P] and N/P ratio was generally in black spruce than in white spruce. Nutrient concentration ratios in plants can be more important than the concentration of individual nutrient elements (Lubbers and Boyce 2004) and photosynthesis in conifers is generally influenced by N/P ratio rather than individual nutrient concentrations (Reich and Schoettle 1988, Lubbers and Boyce 2004). Notwithstanding the balanced nutrient fertiliser formulation supplied, in this study, photosynthetic rate was more closely related to N/P ratio than individual nutrient element. In spruce seedlings, an N/P ratio of 10 is considered adequate (Linder 1995). However, the average N/P ratio in this study for both species were less than 10, and significantly lower in white spruce (black spruce's 9.4 vs. 8.6 in white spruce). The implication is that both species might have been nitrogen stressed even in the high nutrient treatment. However, because white spruce has higher demand for nutrients (Nienstaedt and Zasada 1990, Viereck and Johnston 1990), any nutrient stress would have been magnified in the species more than in black spruce (Patterson et al. 1997) hence, contributing to the lower P_n in white spruce. Differential response of the two species in nutrient use efficiencies may be key determinant of relative competitive ability of species, especially under limited nutrient conditions (Poorter et al. 1990).

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CHAPTER 3: Effects of nutrient supply and root interactions on growth, biomass and whole plant photosynthesis of black spruce and white spruce to CO_2 elevation

Introduction

Changes in global climatic conditions, particularly increased atmospheric carbon dioxide concentration ($[CO_2]$) have direct and indirect effects on plant growth, morphology and physiology (Lambers et al. 2008, Lukac et al. 2010). CO_2 elevation generally enhances photosynthetic CO_2 assimilation at the leaf and whole plant level (Kubiske et al. 1998, Murray et al. 2000, Liu et al. 2002, Wang and Taub 2010) and stimulates greater biomass production (Johnson et al. 1993, Bigras and Bertrand 2006, Zhang and Dang 2007, Marfo and Dang 2009). Depending on prevailing environmental conditions, CO_2 elevation may also influence biomass allocation patterns (Liu et al. 2002, Lambers et al. 2008, Wang and Taub 2010). For example, biomass allocation to root tends to be higher in elevated than ambient $[CO_2]$, particularly under limited nutrient conditions (Fitter et al. 1995, Tingey et al. 2000).

The effects of CO_2 elevation on plant growth may be species specific (Poorter 1998, Joel et al. 2001), hence, elevated $[CO_2]$ can affect intra and interspecific interactions (Poorter 1998, Friend et al. 2000, Brooker 2006), species richness and composition of a plant community (Poorter 1998, Brooker 2006, Körner 2006, Wang 2007, Langley and Megonigal 2010). However, the stimulatory effects of increased $[CO_2]$ on physiology and growth is influenced by other factors including nutrient availability (Oren et al. 2001, Lukac et al. 2010). Complementarily, the nutrient requirement of trees is influenced by atmospheric $[CO_2]$ with nutrient demand increasing at elevated $[CO_2]$ (Oren et al. 2001, Lukac et al. 2010). It is therefore, essential to consider the effects of CO_2 elevation on plant growth, biomass and whole plant photosynthetic rate in the context of nutrient supply.

Nutrient availability affects plant response to CO_2 elevation, with low nutrient supply decreasing the positive effects of CO_2 elevation (Bazzaz 1990, Bazzaz and Mcconnaughay 1992, Oren et al. 2001, Brooker 2006, Zhang and Dang 2006, Lukac et al. 2010). Therefore, to maintain or maximise the positive effects of CO_2 elevation on plant growth, nutrient supply needs to be adequate especially on nutrient poor sites and be in the right proportions (Proe et al. 2000, Lukac et al. 2010). In most studies on plant nutrition, the concentration of one nutrient element, mainly nitrogen (N), which is required in large quantities by plants, is modified leaving levels of other elements intact (Chapin et al. 1987). However, manipulating the concentration of only [N] may induce deficiency or toxicity in other nutrient elements (Proe et al. 2000, Reich et al. 2009) because of interactions between nutrient elements (Marschner 1995, Yuan and Chen 2009). For instance, photosynthesis is dependent on leaf phosphorus concentration (Aerts 1999, Ainsworth and Long 2005, Reich et al. 2009) and changing only N may induce adverse P effect (Warren and Adams 2002). Root biomass may also increase only when potassium concentration ([K]) is sufficient (Dighton et al. 1993, Reich et al. 2009). P and K deficiencies have been observed in black spruce (Wells 1994, Teng et al. 2003, Reich et al. 2009) and in white spruce (Phu and Gagnon 1975), indicating that their levels need to be considered in nutrient formulation for the species. Nutrient elements may interact with each other to modify their individual effects on plant growth and functionality (Dighton et al. 1993, Güswell 2005). For instance, the application of phosphorus may enhance nitrogen uptake (Graciano et al. 2006). Nitrogen supply has also been reported to significantly increase K and C: K ratio and the uptake of P and K (Newbery et al. 1995). Therefore, changing only the concentration of a single nutrient element, such as N, may incorrectly induce deficiency or toxicity in other nutrient elements. To avoid this, it is necessary to proportionally change the concentration of each nutrient element.

Plant-plant interactions are important in controlling environmental drivers which affect stand composition in plant communities (Drake and Leadley 1991, Brooker 2006). Plant responses to CO_2 elevation at the individual level without interaction from other plants differ from that of the community level where inter-plant interactions occur (Navas 1998). This is because response at the community level is determined by both the intrinsic response of the individual species and general characteristics of the species assemblage (Poorter and Pèrez-Soba 2001, Wang 2007). Root interactions are the specific focus of this study because belowground interactions can be stronger than above ground interactions and involve more resources than aboveground (Arnone and Kestenholz 1997, Casper and Jackson 1997). Moreover, belowground interactions have received less attention than aboveground interactions, though the relative effects of increased CO_2 elevation on roots may be greater than on aboveground organs (Bassirirad 2000, Pendall et al. 2004).

Black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) dominate the coniferous boreal forest of North America (Nienstaedt and Zasada 1990, Sims et al. 1990, Viereck and Johnston 1990) and can co-exist on upland sites. Of all forest biomes, climate changes associated with elevated atmospheric [CO_2] are expected to have the greatest impact on the boreal forest (Bonan and Shugart 1989, Bonan et al. 1992, Sellers et al. 1997). Because of slow organic matter decomposition rate in the boreal forest, most soils in the region are nutrient deficient (Dioumaeva et al. 2002), particularly nitrogen and tree growth in the region is often nutrient limited (Jarvis and Linder 2000). A good understanding of the response of boreal trees to increased [CO_2] under varied nutrient conditions is required to properly predict the responses of the boreal forest to climate change.

The objective of the study was to investigate growth, biomass and whole tree photosynthetic responses of congeneric black spruce and white spruce seedlings grown with and without plant-plant root interactions to the interactions between CO_2 elevation and nutrient availability. It was designed to address how nutrient supply and root interaction modify these responses to CO_2 elevation and whether high canopy photosynthetic rate translates into greater biomass production.

Black spruce is more plastic than white spruce because it can grow on more diverse site conditions including poor and fertile sites (Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Patterson et al. 1997). Species with greater plasticity respond better to increasing [CO_2] (Brown and Higginbotham 1986, Midgley et al. 1999), implying black spruce may benefit more from increased [CO_2]. However, black spruce has relatively slower growth rate and is less responsive to nutrient addition than white spruce (Patterson et al. 1997) and species with slower growth rate benefit less from increased [CO_2] (Poorter 1998, Tangley 2001). Fast growing species on fertile sites have high nutrient uptake kinetics that enable faster depletion of rich nutrient patches (Jackson et al. 1990) before slow growing species with low uptake kinetics are able to extract enough nutrients (Jackson et al. 1990, Caldwell et al. 1996). However, plants with high uptake kinetics may be disadvantaged when nutrient supply is low because of greater energy requirements (Jackson et al. 1990). Therefore, on nutrient poor sites, species adapted to low soil fertility with lower uptake kinetics may out-compete species adapted to high nutrient supply (Aerts and Chapin 1999). It was anticipated that white spruce will benefit more than black spruce from the increased [CO_2] at high nutrient supply whereas CO_2 elevation may be more beneficial to black spruce under low nutrient conditions. However, if the response of the two species to CO_2 elevation is more dependent on the species plasticity rather than inherent growth rate, then black spruce will benefit more than white spruce from the CO_2 elevation at the different nutrient treatments.

It was further predicted that whole tree photosynthetic rate, biomass and growth would be higher at elevated [CO_2] with increased nutrient supply and that CO_2 elevation may have partial ameliorating effect on the degree of low nutrient limitation on photosynthesis growth and biomass. This is because increased [CO_2] can partially compensate for low nutrient supply in the short term, by decreasing the amount of Rubisco required to fix a given amount of carbon (Pearcy and Björkman 1983, Warren et al. 2005). Furthermore, increased carbohydrate production as a result of elevated [CO_2] allow for the development of better root system, which leads to greater exploration for soil nutrients (Baker and Enoch 1983, Pendall et al. 2004, Lambers et al. 2008).

While high photosynthetic rates generally result in greater biomass production (Jackson et al. 1994, Drake et al. 1997, Quero et al. 2008, Kirschbaum 2011), there are instances where increased net photosynthetic rate do not lead to an increase in dry mass production (Drake and Leadley 1991, Norby et al. 1992, Olszyk et al. 2003). Growth or biomass production is controlled by carbon balance and biomass allocation (Lambers et al. 2008, Kirschbaum 2011) and whole tree photosynthetic rate is better related to growth than the rate per unit leaf area because it better reflects carbohydrate production. Relating whole tree photosynthetic rate (W_T) to total biomass production may provide a better picture of the overall effects of treatment on plant growth. The lack of a close relationship between W_T and total biomass may reflect variation in biomass allocation.

Materials and Methods

Plant Materials

One-year old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings were obtained from a local forestry nursery for the study. Seedlings of relatively uniform size (≈13 cm tall) were selected for treatments. The growing medium was a mixture of vermiculite and peat moss (50:50, v/v).

Experimental design and growth conditions

The experiment was conducted at Lakehead University's Thunder Bay Campus in four environmentally controlled greenhouses. A split-split-split plot experimental design with four factors was used. CO_2 concentration was treated as main plot, with inter-plant root interaction (RI) as the sub-plot, and nutrient supply (N) as the sub-sub plot, which was further split into the sub-sub-sub plot species treatment (App. 2.1 and 2.2). The position of seedlings of both species was completely randomised within the nutrient treatment. There were two CO_2 levels (380 and 720 μmol mol^{-1}, ambient and elevated respectively) with two replications. and two levels of inter-plant root interaction: no inter-plant root interaction (RI_{No}) and presence of inter-plant root interaction (IRI) within each CO_2 treatment, two nutrient levels and two species. Electronic ignition natural gas CO_2 generators (model GEN-2E, Custom Automated Products, Inc, Riverside, CA) were used to increase the CO_2 concentration. CO_2 levels in each of the four greenhouses were monitored and adjusted automatically with Argus CO_2 control system (Argus, Vancouver, BC, Canada). Seedlings of each species were either grown individually (one plant per pot) without inter-plant root interaction or combined in relatively larger pot where inter-plant root interaction occurred. Seedling distance was approximately the same for seedlings grown with and without inter-plant root interactions. The nutrient concentrations were: 150, 60, 150, 80, 40, 60 mg/l N, P, K, Ca, Mg, S, respectively, (high nutrient treatment, HN) and 10% of those in the high nutrient treatment (low nutrient treatment, LN). The high nutrient formulation was based on the recommendations of Landis (1989) for container tree seedlings during rapid growth phase. There were 8 seedlings per treatment combination, 256 in total. The seedlings were fertilised once a week. Seedlings were watered between fertiliser applications.

Relative humidity (55 ± 5%), day and night air temperature (25 ± 2 °C and 15 ± 2 °C respectively) in the greenhouses were controlled using an Argus control system. The seedlings received natural sunlight (PAR, approximately 660 μmol m^{-2}s^{-}1) and a 17-hour photoperiod. High pressure sodium vapour lamps were used to supplement the natural light on cloudy days, early mornings and late evenings and to extend the photoperiod. The supplementary light was approximately 220 μmol m^{-2}s^{-1} at the canopy level.

Measurements

Photosynthesis

Photosynthesis was measured 4 months into the treatment on 3 randomly selected seedlings per each treatment combination (*n = 96*). The measurement was carried out on current year needles using a PLC5 conifer cuvette attached to CIRAS-1 open gas exchange system (PP-Systems, Amesbury, MA, USA). A cuvette's temperature of 25 °C, RH of 50%, and PAR of 800 μmol m^{-2} s^{-1}were used for the measurements. Whole tree photosynthetic rate (W_T, μmol CO_2 tree^{-1} s^{-1}) was estimated as the product of net photosynthetic rate per unit leaf area at growth [CO_2] and total leaf area for each plant based on Coleman et al. (1995). Needle age was not considered in the determination of W_T because pre-treatment sub-sample photosynthetic measurement did not indicate significant differences in photosynthesis. Also, the seedlings developed new needles and branches on pre-existing shoots which made it impossible to place the PLC5 cuvette separately on the pre-treatment needles to measure their exact photosynthetic rate. Needle canopy position was not considered in the determination of W_T, because visible light at upper and lower canopy did not differ as measured with LI-190SA quantum sensor attached to LI-250A light meter (Licor Biosciences, Lincoln, USA).

Growth and biomass

Total height and root collar diameter (RCD) of all seedlings were measured before the commencement of treatment and immediately before the gas exchange measurement. Following the gas exchange measurements, all the seedlings were harvested for biomass analysis. The seedlings which were used for the gas exchange measurement were harvested separately from the remaining seedlings. The harvested seedlings were separated into aboveground parts (foliage and stem) and roots. The projected leaf area (PLA) of the 96 seedlings used for the gas exchange measurement were determined separately for current year and previous season needles using a Regent WinSeedle system (Regent Instruments Inc., Quebec City, Quebec, Canada). The PLA of the remaining foliage were also determined. The roots were washed free of growth media and scanned with Regents WinRhizo and Agfa Snapscan 600 (Agfa Graphics Corp, Branchburg, N.J., USA) to determine total root length. The materials were then oven-dried at 70 °C for 48 hours and weighed separately 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 mass) and root to leaf mass ratio (RLR) were used as indices of biomass allocation.

Statistical analysis

An analysis of variance (ANOVA) was carried out to quantity treatment effects on the response variables, with block/CO_2/RI/N/S as the block structure (GenStat version 12, VSN International, Hemel Hempstead, UK). Prior to the ANOVA, Shapiro Wilk and Bartelett tests were used to test the data's normality and homogeneity of variance, respectively. Pairwise comparisons of means were conducted using Fisher's least significant difference (LSD). The relationships between whole tree photosynthetic rate (W_T, x) and total tree biomass (y) were determined by simple linear regression as it provided the best fit.

Results

Height and root collar diameter growth

The response of height growth of the seedlings to nutrient supply differed significantly with presence of root interaction ($P<0.001$, Table 3.1). The high nutrient treatment significantly increased seedling height but only when there was no inter-plant root interaction (Fig. 3.1a). Although there was a significant species-RI interaction ($P\leq0.002$, Table 3.1), on height growth, the differences were in magnitude rather than direction of response. In general, black spruce was taller than white spruce and the seedlings were taller when grown without plant-plant root interaction (Fig. 3.1b). CO_2 had no significant effect on height growth ($P=0.103$, Table 3.1).

Diameter growth (RCD) of the two species responded differently to nutrient availability (*P≤0.049*, Table 3.1). RCD was greater in white spruce than in black spruce in the low nutrient treatment but there was no significant diameter difference in the high nutrient treatment (Fig. 3.1c). Both species responded positively to the increased nutrient supply but the magnitude of response was greater in black spruce than in white spruce (Fig. 3.1c)

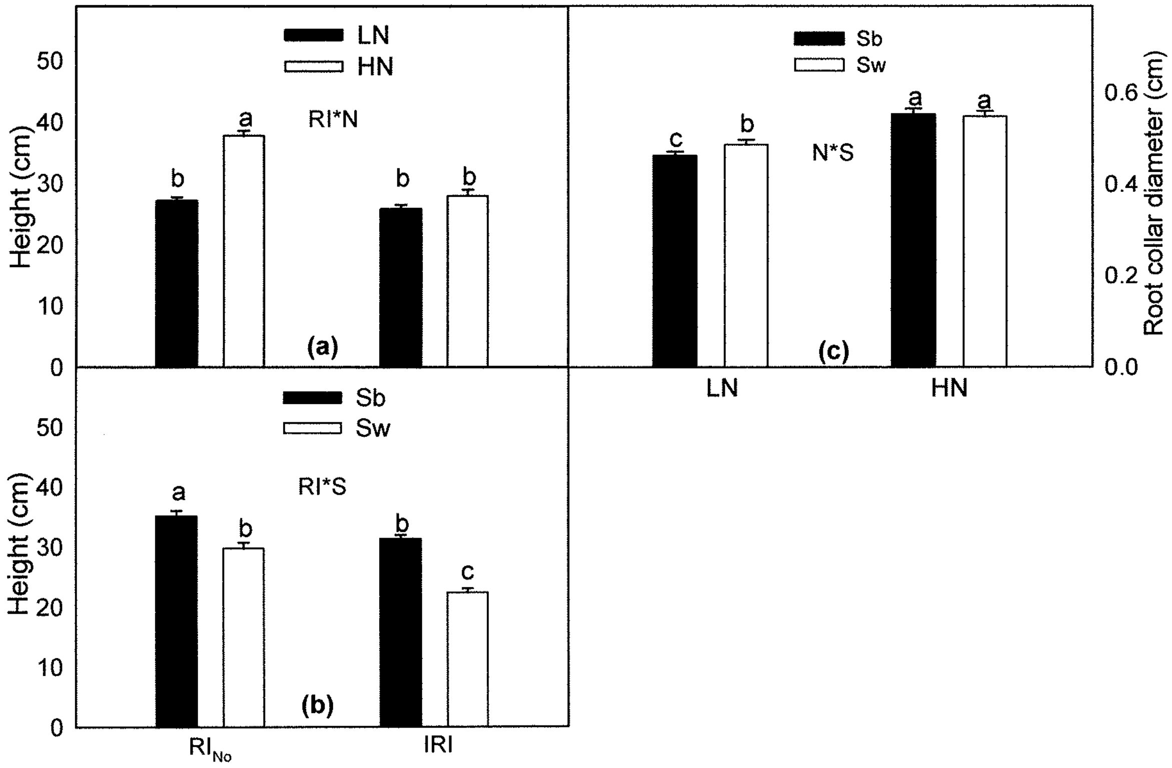

Fig. 3.1: Effects of [CO_2], nutrient supply (N), species (S) and inter-plant root interaction (RI) on height (*a*, *b*) and root collar diameter (*c*) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. In Fig. ***a***, ***b*** and ***c***, *n=64*. Each bar represents mean + standard error (SEM) and bars with different letters indicate significantly (*P≤0.05*) differently means.

Table 3.1: P values from ANOVA on the effects of $[CO_2]$, inter-plant root interaction (RI), nutrient supply (N) and species (S) on growth and biomass of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were exposed to two $[CO_2]$ (380 and 720 $\mu mol\ mol^{-1}$) with or without inter-plant root interactions at two nutrient levels (high, HN and low, LN). Significant effects (*$P \leq 0.05$*) are in bold.

Variation source	Height	RCD	Leaf area	W_T	Biomass	Leaf mass	Stem mass	Root mass
CO_2	0.103	0.156	0.061	0.216	0.067	0.127	0.100	**0.002**
RI	**0.002**	**0.013**	**0.010**	0.096	**0.002**	**0.002**	**<.001**	0.102
CO_2*RI	0.879	0.260	0.733	0.952	0.146	0.214	0.417	0.275
N	**<.001**	**<.001**	**<.001**	**0.012**	**0.001**	**<.001**	**0.011**	**<.001**
CO_2*N	0.513	0.909	0.485	0.910	0.657	0.511	0.552	0.114
RI*N	**<.001**	0.060	**0.006**	0.096	**0.013**	**0.003**	**0.033**	**0.024**
CO_2*RI*N	0.987	0.219	0.496	0.813	0.236	0.524	0.504	**0.005**
S	**<.001**	0.178	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*S	0.177	0.533	0.773	**<.001**	0.119	0.127	0.176	0.180
RI*S	**0.002**	0.455	**<.001**	0.408	**0.008**	**<.001**	**0.015**	**0.053**
N*S	0.169	**0.049**	**<.001**	**0.002**	**0.038**	**<.001**	0.300	0.068
CO_2*RI*S	0.967	0.410	**<.001**	**0.012**	0.492	**0.003**	0.964	0.097
CO_2*N*S	0.967	0.639	0.437	**0.012**	0.831	0.838	0.881	0.363
RI*N*S	0.333	0.082	**<.001**	0.723	**0.010**	**<.001**	0.079	0.169
CO_2*RI*N*S	0.059	0.414	0.415	0.117	0.611	0.822	0.525	0.697

Projected leaf area and whole plant photosynthesis

There was a significant (*$P<0.001$*) CO_2 x RI x S interaction on projected leaf area (PLA, Table 3.1). At the ambient [CO_2], the observed species differences were in magnitude rather than direction of response: PLA was significantly greater in black spruce than in white spruce and the magnitude of response was greater without inter-plant root interaction (Fig. 3.2a). The CO_2 elevation significantly increased PLA in black spruce grown without root interaction and in white spruce grown with root interaction (Fig. 3.2a). PLA of the two species also responded differently to root interaction and nutrient supply (*$P<0.001$*, Table 3.1). Within the low nutrient treatment, PLA was significantly greater in black spruce grown without root interaction than in the other treatment combinations (Fig. 3.2b). The high nutrient treatment increased PLA in all treatment combinations except in white spruce grown with root interaction (Fig. 3.2b). Generally, PLA was greater in black than in white spruce at all treatment levels except when the species were grown together in the low nutrient treatment (Fig. 3.2b).

Interactions among CO_2, RI and species significantly (*$P\leq0.012$*) affected whole plant photosynthesis (W_T, Table 3.1). At both [CO_2] levels, W_T was greater in seedlings grown without inter-plant root interaction than with root interaction (Fig. 3.2c). The CO_2 elevation significantly increased W_T in black spruce at both levels of root interaction but increased it in white spruce only with the presence of root interaction (Fig. 3.2c). At the ambient [CO_2], however, W_T was significantly higher in black spruce but only with root interaction (Fig. 3.2c). W_T was also significantly (*$P\leq0.012$*) affected by CO_2 x N x S interactions and the pattern of response was similar to the CO_2 x RI x S effects (Table 3.1). White spruce benefitted from the CO_2 elevation only in the low nutrient treatment while black spruce had significantly higher W_T in both nutrient treatments under elevated [CO_2] (Fig. 3.2d). W_T of black spruce was much higher than that of white spruce under elevated CO_2 and high nutrient supply as compared to the difference between the two species in the other treatment combinations (Fig. 3.2d).

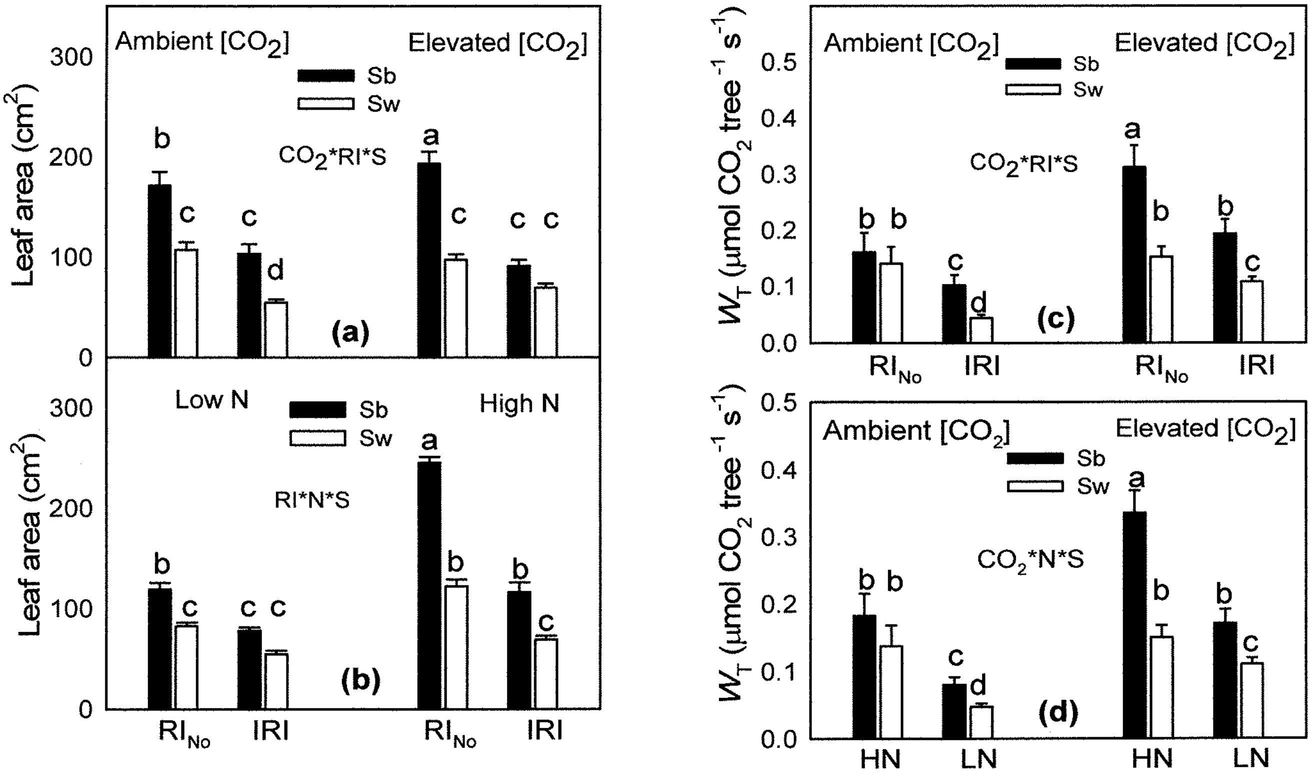

Fig. 3.2: Effects of [CO_2], nutrient supply (N), species (S) and inter-plant root interaction (RI) on projected leaf area (*a*, *b*) and whole tree photosynthesis(*c*, *d*) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. In Fig. ***a*** and ***b***, *n=32* while in ***c*** and ***d***, *n=12*.

Total biomass and organ mass

Total seedling biomass was significantly (*$P \leq 0.010$*) affected by interactions among RI x N x S (Table 3.1). Black spruce grown without root interaction in the high nutrient treatment had significantly greater biomass than the other treatment combinations (Fig. 3.3a). [CO_2] had no significant (*$P = 0.067$*) effect on total biomass (Table 3.1).

CO_2 x RI x S significantly (*$P \leq 0.003$*) affected leaf mass (Table 3.1). At the ambient [CO_2], treatment differences caused by root interaction were in magnitude and not direction of response: leaf mass was greater in seedlings grown without than with root interaction (Fig. 3.3b). Leaf mass was generally higher in black spruce than in white spruce (Fig. 3.3b). The CO_2 elevation enhanced leaf mass only in black spruce grown without inter-plant root interaction (Fig. 3.3b). Leaf mass was also significantly affected RI x N x S interactions (*$P \leq 0.001$*, Table 3.1). In the low nutrient treatment, leaf mass was significantly greater in black spruce grown without inter-plant root interaction than in the other treatments. The high nutrient treatment increased leaf mass in both species in the absence of root interaction but not with root interaction (Fig. 3.3c).

Stem mass was significantly (*$P \leq 0.033$*) affected by RI x N and RI x S interactions (Table 3.1). The high nutrient treatment increased stem mass only in seedlings grown without inter-plant root interaction (Fig. 3.3d). Moreover, stem mass of black spruce grown without root interaction was significantly (*$P \leq 0.015$*) higher than stem mass in the other treatment combinations (Fig. 3.3e). CO_2 had no significant effect on stem mass (Table 3.1).

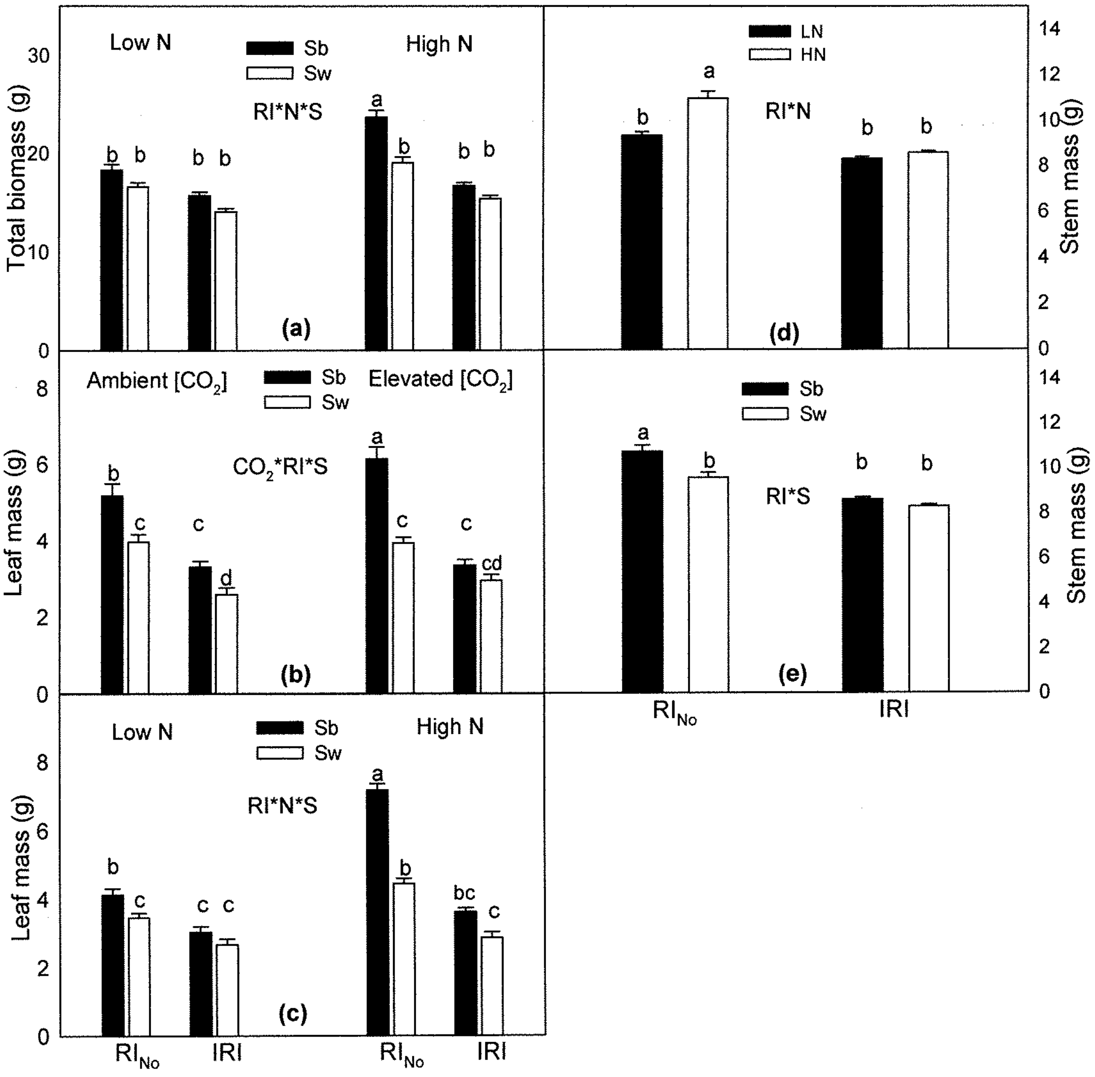

Fig. 3.3: Effects of [CO2], nutrient supply (N), plant-plant root interaction (RI) and species (S) on total biomass (*a*), leaf mass (*b*, *c*) and stem mass (*d*, *e*) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. In Figs. ***a*** ,***b*** and ***c***, *n=32*, while in Figs. ***d*** and ***e***, *n=64*.

Interactions among [CO_2], RI and N significantly (*$P \leq 0.005$*) influenced root mass (Table 3.1). At the ambient [CO_2], there was no significant difference among the treatments (Fig. 3.4a). The CO_2 elevation, however, significantly increased root mass in all treatment combinations except in seedlings grown in the low nutrient treatment with root interaction (Fig. 3.4a). Root mass of the two species also differed significantly with root interaction (Table 3.1). The presence of root interaction significantly reduced root mass in white spruce, and no other treatment difference occurred (Fig 3.4b).

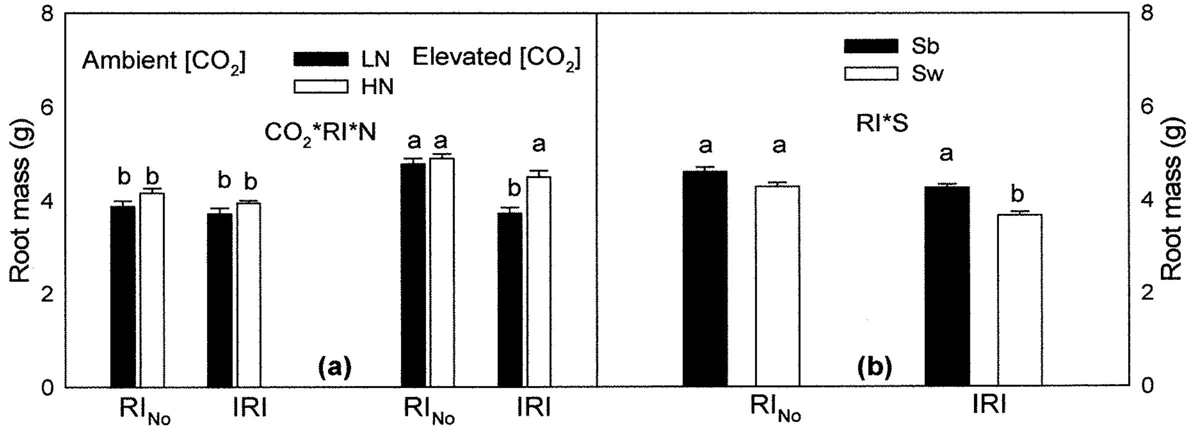

Fig. 3.4: Effects of [CO_2], nutrient supply (N), species (S) and plant-plant root interaction (RI) on root mass of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. In Fig. ***a***, *$n=32$*, while in ***b***, *$n=64$*.

Biomass allocation

Leaf mass ratio (LMR) was significantly (*$P<0.001$*) affected by CO_2 x RI x S interactions (Table 3.2). At both [CO_2], LMR was significantly higher in the absence of plant-plant root interaction (Fig. 3.5a). At the ambient [CO_2], it was the magnitude and not direction of response which varied between seedlings grown with and without root interaction: LMR was greater in black spruce than in white spruce and greater without root interaction than with root interaction (Fig.

3.5a). At the elevated CO_2,the presence of root interaction significantly reduced LMR in black spruce with no difference in the other treatment combinations. The high nutrient treatment significantly increased LMR in the absence of plant-plant root interaction ($P \leq 0.002$, Table 3.2, Fig. 3.5b). LMR of the two species differed with nutrient supply ($P < 0.001$, Table 3.2). The high nutrient treatment significantly increased LMR of black spruce with no significant difference in the other treatment combinations (Fig. 3.5c).

Root mass ratio (RMR) and root to shoot mass ratio (RSR) were significantly affected by CO_2 x RI x S interactions (Table 3.2). At the ambient [CO_2], there was no within RI significant species difference but both RMR and RSR were significantly greater with plant-plant root interaction (Figs. 3.5d and 3.5g). At the elevated [CO_2], root interaction had opposite effect on the species RMR and RSR. The CO_2 elevation increased RMR and RSR in white spruce in the absence of root interaction but decreased it in the presence of root interaction with no effect on RMR and RSR of black spruce (Figs. 3.5d and 3.5g).

Without root interaction, the high nutrient treatment decreased RMR and RSR and no other difference occurred (Fig. 3.5e and 3.5h). RMR and RSR of the two species responded differently to nutrient supply (Table 3.2). The nutrient treatment had opposite effect on the two species RMR and RSR. The low nutrient treatment decreased RMR and RSR in black spruce but not in white spruce while the high nutrient treatment increased RMR and RSR in black spruce but not in white spruce. RMR and RSR of white spruce did not change with nutrient supply (Fig. 3.5f and 3.5i)

The root to leaf mass ratio (RLR) of the two species responded differently to root interaction under the different [CO_2] ($P < 0.001$, Table 3.2). In the ambient [CO_2], there was no significant species difference in RLR when the seedlings were grown without root interaction, but with the presence of root interaction, RLR was significantly increased in both species with greater increase in white spruce (Fig. 3.5j). In the elevated [CO_2] however, RLR was significantly greater in white spruce when the species were grown without plant-plant root interaction but with root interaction, there was no significant species difference (Fig. 3.5j). Moreover, the elevated CO_2 decreased RLR in white spruce grown with root interaction (Fig. 3.5j). Root interaction significantly influenced the seedlings response to nutrient supply ($P \leq 0.039$, Table 3.2) and the trend was similar to the RI x N effects described previously for RMR (Figs. 3.5e, 3.5k). The two species also responded differently to nutrient supply ($P<0.001$, Table 3.2). The low nutrient treatment significantly lowered RLR and the reduction was greater in black spruce (Fig. 3.5l). The two species had similar RLR in the high nutrient treatment (Fig. 3.5l).

Table 3.2: *P* values from ANOVA on the effects of [CO_2], inter-plant root interaction (RI), nutrient supply (N) and species (S) on leaf (LMR) and root (RMR) mass ratios, root to shoot mass ratio (RSR), root to leaf mass ratio (RLR) and specific leaf area (SLA) of black spruce and white spruce seedlings after 4 months of treatment.

Variation source	LMR	RMR	RSR	RLR	SLA
CO_2	0.530	0.166	0.187	0.832	0.794
RI	**<.001**	0.071	0.063	**0.018**	**0.009**
CO_2*RI	0.907	0.298	0.282	0.254	0.795
N	**<.001**	0.164	0.151	**0.016**	**<.001**
CO_2*N	0.169	0.263	0.351	0.238	0.366
RI*N	**0.002**	**0.010**	**0.011**	**0.039**	**0.009**
CO_2*RI*N	0.907	0.278	0.555	0.417	0.595
S	**<.001**	0.274	0.134	**<.001**	**<.001**
CO_2*S	0.978	0.569	0.498	0.357	0.883
RI*S	**0.005**	**<.001**	**<.001**	0.346	**<.001**
N*S	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*RI*S	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*N*S	0.313	0.176	0.165	0.426	0.755
RI*N*S	0.132	0.651	0.346	0.374	**0.042**
CO_2*RI*N*S	0.576	0.989	0.856	0.661	0.110

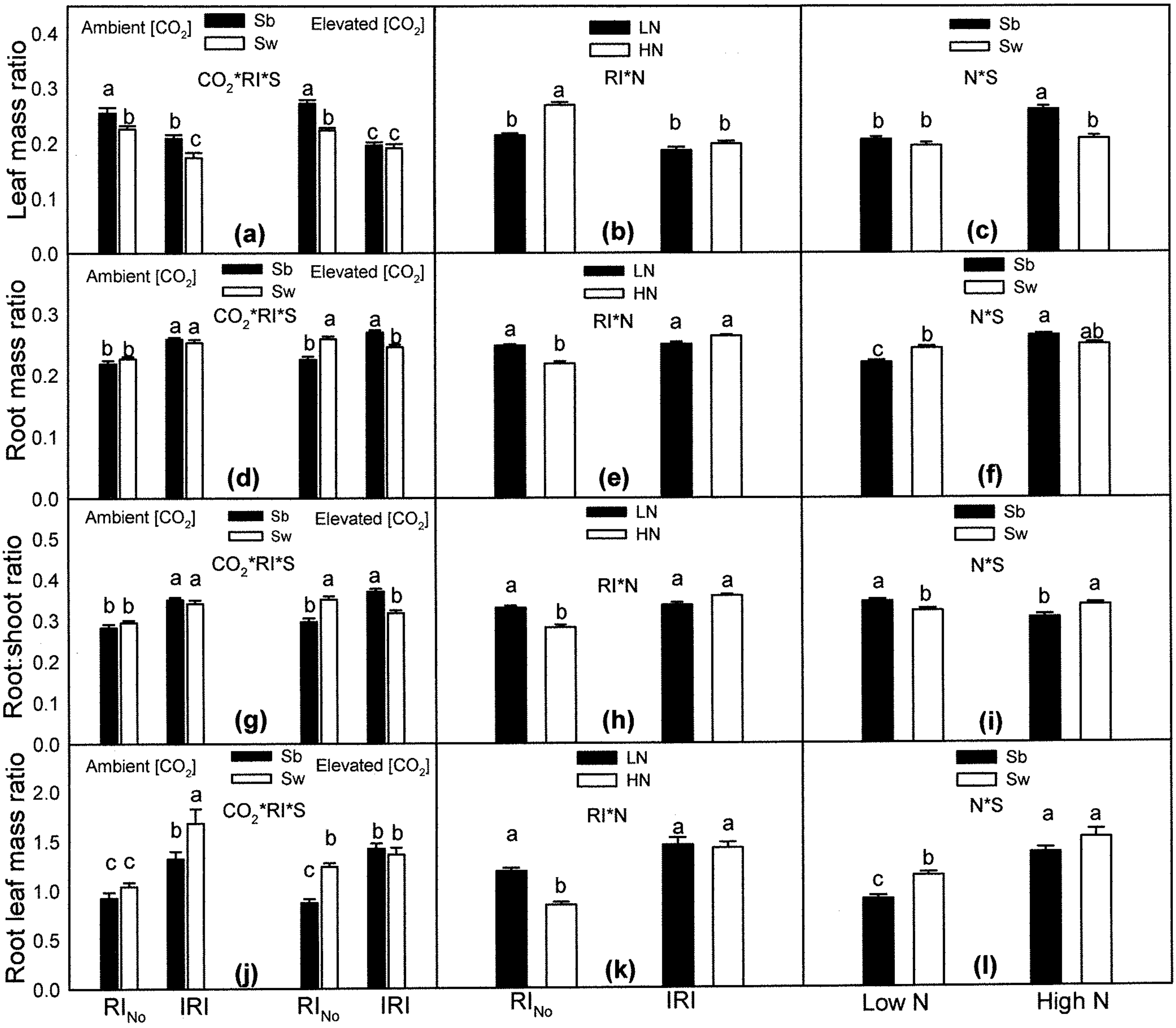

Fig. 3.5: Effects of [CO$_2$], nutrient supply (N), species (S) and plant-plant root interaction (RI) on leaf mass ratio (*a, b, c*), root mass ratio (*d, e, f*), root to shoot mass ratio (*g, h, i*) and root to leaf mass ratio (*j, k, l*) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. In Figs. on the left panel, *n=32*, while in the middle and right panels, *n=64*.

Specific leaf area

There was a significant (*$P<0.001$*) [CO$_2$], root interaction and species effect on specific leaf area (SLA, Table 3.2). In the ambient [CO$_2$], the species differences observed under the different RI was in magnitude and not in direction of response: SLA was significantly higher when there was no inter-plant root interaction (Fig. 3.6a). At the ambient [CO$_2$], SLA was significantly greater in black spruce than in white spruce (Fig. 3.6a). The CO$_2$ elevation significantly increased SLA in white spruce grown with root interaction but not in the other treatment combinations (Fig. 3.6a). Root interaction affected the two species SLA response to nutrient supply (*$P\leq0.042$*, Table 3.2, Fig. 3.6b). In the low nutrient treatment, SLA was significantly greater in black spruce grown without inter-plant root interaction than in the other treatment combinations (Fig. 3.6b). The high nutrient treatment however, significantly increased SLA in all treatment combinations except in white spruce grown with root interaction (Fig. 3.6b).

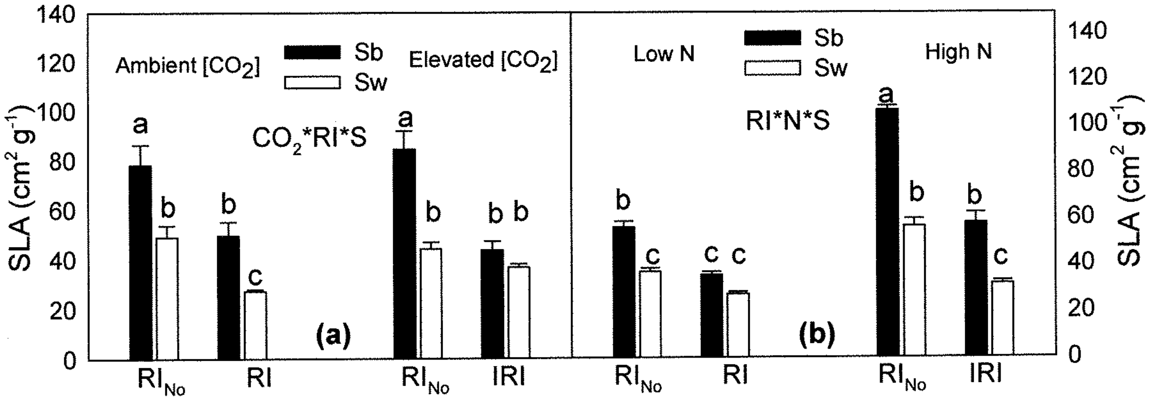

Fig. 3.6: Effects of [CO$_2$], nutrient supply (N), species (S) and plant-plant root interaction (RI) on specific leaf area of black spruce (Sb) and white spruce (Sw) seedlings (*$n=12$*) after 4 months of treatment.

Relationship between biomass and whole tree photosynthesis

Relationship between whole tree photosynthesis (W_T) and total biomass were explored for each species and subsequently for each treatment combination. W_T of the two species linearly and positively correlated with seedling biomass ($R^2 = 0.65$ and 0.67 for black spruce and white spruce, respectively) and the slope for each species, which may reflect conversion efficiency, significantly differed between the two species ($P \leq 0.001$, Fig. 3.7). The slope was significantly greater for white spruce than for black spruce indicating greater W_T to biomass conversion efficiency in white spruce (Fig. 3.7).

At the individual treatment combination level, the relationships were however, variable (Table 3.3, Fig. 3.8). In the ambient [CO_2] and in seedlings in the RI_{No} - high nutrient treatment, W_T positively related with biomass production in both species (Fig. 3.8a), while under elevated [CO_2], only the W_T of white spruce related with biomass (Fig. 3.8e). In the low nutrient- RI_{No} treatment however, W_T did not correlate with biomass at the ambient [CO_2] (Fig. 3.8b) while in the elevated [CO_2], the relationship was positive for black spruce (Fig. 3.8f) With the presence of root interaction, only W_T of white spruce correlated with biomass production in the high nutrient treatment under ambient [CO_2] (Fig. 3.8c), while in the elevated [CO_2], W_T of both species positively correlated with biomass (Fig. 3.8g). In seedlings grown with root interaction in the low nutrient treatment at ambient [CO_2], W_T correlated with biomass for both species while in elevated [CO_2], the relationship was positive only for black spruce (Table 3.3, Fig. 3.8h). Overall, white spruce had much lower W_T-biomass range than black spruce in all but the high nutrient-no root interaction treatment at ambient CO_2 (Fig. 3.8a)

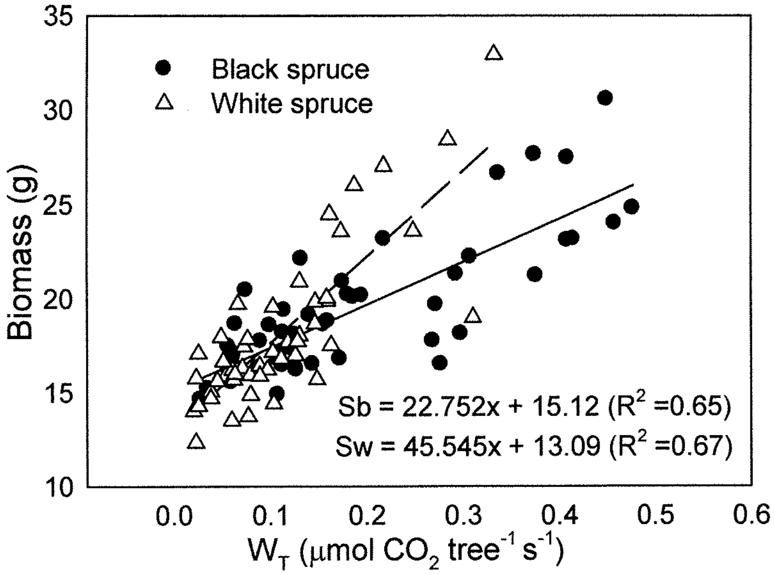

Fig. 3.7: Linear **r**elationship between whole tree photosynthetic rate-W_T and total seedling biomass for black spruce (circles) and white spruce (triangles) seedlings after 4 months of treatment.

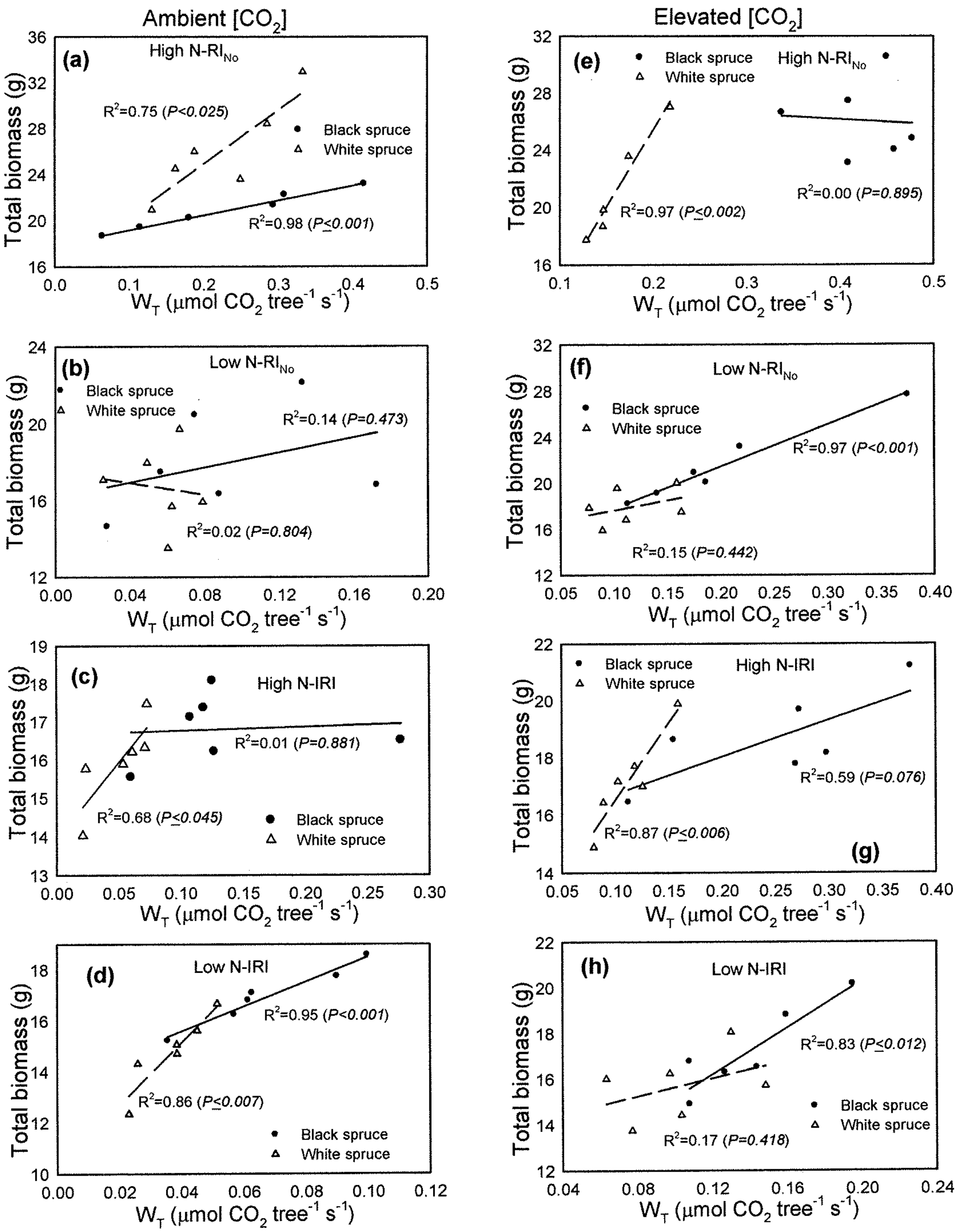

Fig. 3.8: Linear relationship between seedling total biomass and whole plant photosynthetic rate of black spruce (closed circles) and white spruce (open triangles) seedlings. Differences in slope were tested with t-test as [(slope$_1$-slope$_2$)/(r$_1$-error$_2$)]. The regression P-values are indicated in parentheses after each r^2 and full equations are in Table 3.3.

Table 3.3: Linear regression analysis of the relationship between total biomass and whole tree photosynthesis for black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment.

	Ambient CO_2				*Elevated CO_2*			
	a	***b***	***p***	***r^2***	***a***	***b***	***p***	***r^2***
No inter-plant root interaction								
Sb HN	17.87	12.82	**<0.001**	0.98	27.67	-3.79	0.895	0.00
Sw HN	15.53	46.86	**0.025**	0.75	108.33	3.76	**0.002**	0.97
Sb LN	16.17	19.50	0.473	0.14	14.15	36.50	**<0.001**	0.97
Sw LN	17.53	-15.35	0.804	0.01	15.94	17.21	0.442	0.15
Inter-plant root interaction								
Sb HN	16.67	0.97	0.881	0.01	15.43	12.96	0.076	0.59
Sw HN	13.94	39.93	**0.045**	0.68	11.15	53.61	**0.006**	0.87
Sb LN	13.63	48.92	**<0.001**	0.95	10.02	51.43	**0.012**	0.83
Sw LN	10.20	123.63	**0.007**	0.86	13.68	19.47	0.418	0.17

Discussion

Black spruce and white spruce seedlings grown at low and high nutrient regimes differed in their response to CO_2 concentration and the effects were modified by root interactions. Specifically, the two species height and diameter growth, leaf area, specific leaf area (SLA), whole tree photosynthesis (W_T) and total plant dry mass differed with resource availability and these were modified by root interaction. However, of the four primary response variables (height, diameter, W_T and total biomass), only the effects on W_T was significantly modified by [CO_2].

Elevated [CO_2] partially compensated for limitations imposed by low nutrient supply by increasing whole tree photosynthesis (W_T) in the low nutrient treatment. In white birch seedlings grown under low and high nutrient treatments, Zhang and Dang (2006) reported that, photosynthetic rate of seedlings grown in low nutrient treatment were significantly higher at elevated than ambient [CO_2], indicating elevated [CO_2] ameliorative effect on low nutrient supply. Norby and colleagues (1992) also observed that photosynthetic stimulation by [CO_2] elevation in yellow poplar was maintained despite significant reductions in foliar nutrient concentration. In Sikta spruce (*Picea sitchensis*), CO_2 elevation significantly increased photosynthetic rate at low nutrient condition by 19% in comparison with seedlings at ambient [CO_2] (Murray et al. 2000). The above examples and the current data on W_T indicate that CO_2 elevation may stimulate both leaf level photosynthesis and whole tree photosynthesis even when nutrients are low. However, on an absolute basis, the elevated [CO_2] stimulation of W_T was 20% greater under the high nutrient regime. Similarly, in both (Zhang and Dang 2006) and (Murray et al. 2000) studies, despite the increase in photosynthesis by elevated [CO_2] in the low nutrient treatments, absolute enhancements were much greater in high than low nutrient levels. I therefore postulate that low nutrient availability minimises the positive effects of CO_2 elevation but does not completely eliminate the positive effects and that CO_2 elevation may partially ameliorate the low nutrient limitation on photosynthesis.

The CO_2 elevation failed to stimulate significant height and diameter growth and biomass production in the two species. The initial expectation that growth and biomass would be enhanced by CO_2 elevation particularly with increased nutrient availability was not therefore supported by data for height, diameter and biomass. The absence of significant elevated CO_2 effect on either seedling height or total biomass might be due the fact that CO_2 elevation also failed to increase stem mass which accounted for over 80% of total biomass. The lack of CO_2 effect on stem mass, which ultimately influenced total biomass and height growth, might be due to greater maintenance respiration and carbon loss associated with woody stems under elevated [CO_2] (Edwards et al. 2002). Biomass component respiration rates were however, not measured in this study. Inaunen et al.(2012) reported that elevated [CO_2] failed to increase aboveground biomass and subsequently neutralised the positive effects of CO_2 elevation on growth. Körner et al. (2005) also observed in field grown trees that elevated [CO_2] failed to increase stem growth, stem biomass and subsequently affected overall biomass. In contrast to this study, some previous studies (Callaway et al. 1994, Tissue et al. 1997, Zhang and Dang 2007, Marfo and Dang 2009) reported greater tree growth or higher biomass production in response to CO_2 elevation. For example, Marfo and Dang (2009) studied the same two species and reported that CO_2 elevation significantly increased height, diameter and total biomass of the seedlings by 25, 33 and 75% respectively. The present findings are however, supported by some previous studies. Olszyk et al. (1998) studied Douglas-fir [(*Pseudotsuga menziesii* (Mirbel) Franco] and concluded that CO_2 elevation failed to stimulate height growth or biomass production. In white birch (*Betula papyrifera* Mash.) seedlings exposed to elevated [CO_2] for one growing season, Zhang et al.

(2006) observed no significant CO_2 elevation effect on either height or diameter growth. Bloor et al. (2008) also observed only a marginal enhancement of biomass and no height increase in *Fraxinus excelsior* exposed to elevated [CO_2]. Other studies also found no significant height difference in trees grown in elevated [CO_2] in comparison with plants grown in ambient [CO_2] (El Kohen et al. 1992, Pettersson and Mcdonald 1992, Kilpeläinen et al. 2005).

The CO_2 elevation increased whole tree photosynthetic rate but failed to increase biomass production. First of all, higher photosynthesis at elevated [CO_2] does not always translate into greater plant growth in a straightforward manner (Poorter et al. 1990, Luo et al. 1997, Körner et al. 2005, Körner 2006, Kirschbaum 2011, Inauen et al. 2012). This is because of adjustment in carbon allocation between leaves and other organs and or changes in specific leaf area (Poorter et al. 1990, Callaway et al. 1994, Lambers et al. 2008). A small increase in biomass allocation to non-photosynthetic organs, may significantly minimise growth, though photosynthesis may be high (Poorter et al. 1990, Callaway et al. 1994, Lambers et al. 2008). The changes in biomass allocation in response to CO_2, particularly between leaves and roots might have accounted for the lack of significant CO_2 effect on growth and biomass production despite the significant effect on whole plant photosynthesis. Secondary, biomass is accumulation of carbon over time while the photosynthetic data presented is per unit time. It is therefore an approximation of true whole tree photosynthetic rate but may not be the "true" whole tree photosynthetic rate. The increase in photosynthesis by elevated [CO_2] and the lack of biomass enhancement observed in this study supports some previous studies (Norby et al. 1992, Lee and Jarvis 1995, Kirschbaum 2011).

White spruce may be more efficient than black spruce in converting photosynthesis into biomass in the high nutrient treatment while the reverse may be true in the low nutrient treatment. Both

W_T and biomass were greater in black spruce and the range of the W_T-biomass relationship was greater in black spruce. However, the slope of the W_T-biomass relationship, which may indicate conversion efficiency, was either greater in white spruce or that of black spruce was not significant in the high nutrient treatment. In the low nutrient treatment however, the slope was either greater in black spruce or that of white spruce was not significant at all especially in elevated [CO_2]. Three conclusions can be drawn from this finding. First, a species with greater W_T or biomass may not necessarily have greater W_T- biomass conversion efficiency. Secondly, CO_2 elevation may increase W_T–biomass conversion efficiency in black spruce under nutrient poor conditions, but that of white spruce under nutrient high conditions may be independent of CO_2 concentration. Finally, the fact that black spruce had higher W_T and biomass but lower conversion efficiency with increased nutrient supply may indicate luxurious consumption in the species. The "conversion efficiency" reported is only an approximation and may not be the actual because whole tree photosynthesis was more or less an instantaneous measurement while total biomass production was carbon accumulated over time.

The hypothesis that white spruce would benefit more than black spruce from increased nutrient addition and CO_2 elevation is not supported. As previously discussed, CO_2 had no significant effect on either height growth or total biomass production. On the contrary, black spruce benefitted more from both increased nutrient supply and elevated [CO_2] in terms of W_T while the high nutrient treatment increased biomass production only in black spruce. Black spruce suppressed root biomass of white spruce when the two species were grown together with root interaction but not when they grew without root interaction (Fig. 3.4b). Black spruce also had greater leaf mass ratio than white spruce in the high nutrient treatment but similar LMR in the low nutrient treatment. In response to nutrient addition, black spruce that grew with root interaction increased its leaf area and specific leaf area while that of white spruce did not change. These outlined changes in black spruce in response to changes in resource supply may indicate greater plasticity in the species. Species with greater degree of physiological and morphological plasticity gain more from increase CO_2 elevation than less plastic species (Brown and Higginbotham 1986, Midgley et al. 1999) and that might explain why whole tree photosynthesis and total biomass production increased in black spruce more than in white spruce in response to resource supply. The fact that white spruce had lower W_T, height and biomass than black spruce, even in the high nutrient treatment, may suggests that the high nutrient supplied was not adequate for the species, confirming its relatively higher demand for nutrients than black spruce (Nienstaedt and Zasada 1990, Patterson et al. 1997). This is however, not certain because only two nutrient levels were used. The present results do not support Patterson et al. (1997) findings that white spruce more responsive to increase nutrient supply and hence has faster growth rate than black spruce.

Inter-plant root interactions can modify species response to resource supply. The presence of inter-plant root interaction reduced specific leaf area, whole tree photosynthetic rate, height and diameter growth and biomass production indicating competition. However, structural allocation to root increased with inter-plant root interaction. Plant mixtures may possess greater structural and functional versatility than isolated plants or monocultures in exploiting greater environmental resources (Jolliffe 1997, Brassard et al. 2011). However, under controlled conditions where heterogeneity in soil resources is minimal, that may not be the case, especially in short term studies. The observed reduction in W_T, growth and biomass production with the presence of root interaction might have been caused by the relatively lower leaf area, and reduced leaf biomass in seedlings grown with root interaction and suggest competition rather than facilitation. In a study involving *Quercus robur* L. (oak) seedlings, Jensen and colleagues (2011) reported that belowground competition for nutrients reduced leaf area, number of leaves, transpiration rate and ultimately plant biomass. It has also been established that belowground competition can reduce plant growth and productivity more than above ground competition (Wilson 1988, Casper and Jackson 1997). The amount of fertiliser supplied per seedling was the same, yet the root to shoot mass ratio increased with the presence of root interaction. Because changes in biomass allocation generally occurs under non-optimal nutrient conditions (Wilson 1988, Huante et al. 1995, Müller et al. 2000, Lambers et al. 2008), it is possible that the two species over competed for their own individual good and ended up depleting the resource faster than replenishing rates, especially at the low nutrient level.

Field application of this study requires some caution as the study was conducted under controlled conditions for one growing season. The whole tree photosynthesis reported in this study did not account for differences in needle age and needle canopy position, which may affect the true whole tree photosynthesis of the species. Aboveground plant-plant interactions were not considered because the amount of visible light in the upper canopy and at the root collar level did not differ. In cases where they differ, their effects cannot be ignored. Notwithstanding the limitations, this study is important in providing mechanistic understanding of how nutrient availability affects growth and whole tree photosynthesis of black spruce and white spruce under present and future CO_2 regimes and the vital role inter-plant root interactions play in modifying the response. Co-existing black spruce and white spruce may respond differently to nutrient supply in future climates when current CO_2 is doubled its present level. However, plant-plant root interactions will be a key in determining the degree of response. Species with higher photosynthetic rate and/or biomass production may not necessarily be the more efficient converter of photosynthesis to biomass. While CO_2 elevation may enhance whole tree photosynthesis under low nutrient conditions,, the positive effects are greater with increased nutrient supply.

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CHAPTER 4: Root interactions and nutrient supply modified root traits of black spruce and white spruce seedlings in response to CO_2 elevation

Introduction

As atmospheric carbon dioxide concentration ([CO_2]) increases from the current ~385μmol mol^{-1} (Conway and Tans 2011) to about 700 μmol mol^{-1} by the end of the 21st century (Christensen et al. 2007), the effects of [CO_2] on plant growth may increase. Studies in the past few decades (Crookshanks et al. 1998, Pregitzer et al. 2000, Ainsworth and Long 2005, Marfo and Dang 2009) have provided evidence that the potential effects of increased atmospheric [CO_2] on plant growth and biomass are generally positive. Roots generally benefit more from CO_2 elevation with relatively greater carbon allocation to roots than to above ground structures (Rogers et al. 1994, Tingey et al. 2000, Pendall et al. 2004). For instance, Tingey et al. (2000) reported that CO_2 elevation resulted in an average enhancement of 54% in fine root growth in conifer species. Fitter et al. (1995) also reported that more than half of the annual carbon produced by trees is allocated to roots under elevated [CO_2]. Using mini-rhizotrons, Pregitzer et al. (1995) observed that elevated [CO_2] consistently increased root production in *Populus* trees irrespective of the soil fertility level. Higher biomass allocation to roots under elevated [CO_2] may be necessary to increase nutrient uptake to match the greater plant growth under elevated [CO_2] and to reach functional equilibrium (Pendall et al. 2004).

Besides enhanced root biomass production, elevated [CO_2] also affects other important root traits such as specific root length (SRL), root diameter and total length, root length density (RLD), root branching intensity (RBI), specific root area (SRA) and number of root tips (Tingey et al. 2000).

These traits have a large impact on the functioning of the root system and carbon economy of the plant. For example, SRL characterises the economic aspect of a root system: the cost (root mass) of constructing a unit length of root (Eissenstat 1992, Ostonen et al. 2006, Ostonen et al. 2007). Root branching intensity (number of root tips per unit length) governs the exploration of the roots through the soil matrix thereby affecting nutrient acquisition (Fitter 1991). Elevated [CO_2] can increase (Pettersson and Mcdonald 1992), decrease (Larigauderie et al. 1994, King et al. 1997, Crookshanks et al. 1998) or cause no change (King et al. 1997) in SRL. The effects of increased [CO_2] on these root traits may however, be influenced by the fertility of the growing media (Gross et al. 1993, Norby 1994, Arnone and Kestenholz 1997).

Tree roots are vital to the acquisition of nutrients and water, anchorage into the soil and storage of assimilates produced from above ground (Atkinson 2000). Despite the importance of roots, particularly fine root (diameter < 2mm) traits, plant physiological ecologists have generally paid less attention to roots than to photosynthesis, leaf traits and above ground biomass (Crookshanks et al. 1998), probably because roots are more difficult to study. Furthermore, studies on root response to elevated [CO_2] and fertilisation tend to focus mostly on root biomass and turnover (Reich et al. 1998, Roumet et al. 2006, Comas and Eissenstat 2009). However, other root morphological characteristics, such as SRL and fine root diameter are important (Aerts et al. 1991, Aerts and Chapin 1999, Bonifas and Lindquist 2009) as they influence the competiveness of plants for nutrients and water, growth and long term survival of trees (Miller et al. 1991, Jones 1993, Casper and Jackson 1997). A detailed assessment of plant response to increasing [CO_2] and variations in nutrient resources requires that the responses of root systems and morphology be clearly understood (Norby 1994).

Plants adjust their root structure and physiology in response to nutrient availability and in the presence of neighbours (Arnone and Kestenholz 1997, Friend et al. 2000, Gersani et al. 2001, Cahill et al. 2010). For example, *Picea spp*, *Populus tremuloides* and *Abies balsamea* had lower horizontal and higher vertical fine root biomass heterogeneity in mixture than in monoculture (Brassard et al. 2011). Such adjustments may influence nutrient absorption capabilities which may ultimately affect stand species composition. Elevated [CO_2] as a resource can also alter competitive relationships within species and among species in communities (Bazzaz and Mcconnaughay 1992, Johnson et al. 1993, Navas 1998, Ainsworth and Long 2005, Lukac et al. 2010).

Black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) have an extensive distribution in North American coniferous boreal forest (Nienstaedt and Zasada 1990, Sims et al. 1990, Viereck and Johnston 1990, Gower et al. 2001). Black spruce can grow on low nutrient soils, while white spruce usually grows on richer upland sites (Larsen 1980). Black spruce regenerates on mineral substrates as well as on lowland sites with sphagnum seedbeds (Haavisto and Jeglum 1995, Chen and Popadiouk 2002). White spruce on the other hand, grows best on moist upland sites (Nienstaedt and Zasada 1990). Nutrient requirements of white spruce tend to be greater than that of black spruce (Nienstaedt and Zasada 1990). Notwithstanding the differences in nutrient requirements, both species co-occur on moist upland mineral soils (Nienstaedt and Zasada 1990, Sims et al. 1990, Wang and Macdonald 1993, Haavisto and Jeglum 1995).

Higher SRL and SRA are adaptations for greater nutrient absorption (Lõhmus et al. 1989, Lambers et al. 2008, Comas and Eissenstat 2009). By producing thinner and longer roots with high surface area, plants minimise the amount of investment in constructing roots for resource uptake (Eissenstat 1992, Leuschner et al. 2004). Hence, it was anticipated that the combination of high nutrient and elevated [CO_2] would confer on the seedlings traits such as high SRL, SRA, longer roots which are associated with faster growth rate, while at the low nutrient treatment, a high root mass density may be necessary. Furthermore, it was also hypothesised that the traits exhibited by seedlings grown with no root interaction would be altered with root interaction such that seedlings grown with no root interaction would have traits that confer faster growth rate while traits that confer longevity may be higher with root interaction.

The objective of this study was to assess the impact of nutrient availability root characteristics of black spruce and white spruce in response to CO_2 elevation in the presence and absence of plant-plant root interaction. Specifically, the following questions were asked: Do root traits of black spruce and white spruce respond differently to [CO_2] and nutrient supply? How are the effects of CO_2 elevation on the root characteristics modified by nutrient availability? Are the effects of [CO_2] and nutrient on the root morphology modified by inter-plant root interactions? To answer the above, black spruce and white spruce seedlings were grown at two nutrient treatments under ambient and elevated CO_2 concentrations.

Materials and methods

Plant materials

The plant materials were one-year old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings obtained from Hills Tree Nursery, Thunder

Bay, Ontario. At the beginning of the experiment, the seedlings were about 13 cm tall. Height and diameter of all seedlings were measured to ensure that only seedlings of relatively uniform size and form were used for treatment. The growing medium was a mixture of vermiculite and peat moss (50:50, v/v).

Experimental design and growth conditions

The experiment was conducted at Lakehead University's Thunder Bay campus in four similar-designed adjacent greenhouses. The experimental design was a split-split-split-plot with [CO_2] as the main plot, inter-plant root interaction (RI) as the sub-plot, and nutrient supply as the sub-sub plot. The position of seedlings of each species was completely randomized within each nutrient plot. There were two CO_2 levels (ambient, 380 and elevated, 720 μmol mol^{-1}) and each level had two independent replications. The elevated [CO_2] were achieved using natural gas CO_2 generators (model GEN-2E, Custom Automated Products, Inc, Riverside, CA). The CO_2 was evenly supplied to the plants through a network of perforated tubes and circulation fans above the benches. The levels of CO_2 in all the greenhouses were monitored and adjusted automatically with Argus CO_2 control system (Argus, Vancouver, BC, Canada).

Within each CO_2 treatment. there were two levels of root interaction, (no inter-plant root interaction, RI_{NO} vs. presence of inter-plant root interaction, IRI). In the treatment with no inter-plant root interaction (RI_{No}), seedlings of each species were individually (one plant per pot) such that roots of two plants did not physically interact. In the treatment with inter-plant root interaction (IRI), seedlings of the two species were grown in the same container to allow for inter-plant root communication. Each seedling was allocated an approximate soil volume of about 1769.38 cm^3. There were two nutrient levels: high and low. The formulation for the high nutrient (HN) treatment was 150, 60, 150, 80, 40, 60 mg/l nitrogen, phosphorus, potassium, calcium, magnesium and sulphur respectively and was based on Landis (1989) recommendations for growing container tree seedlings during rapid the growth phase. The low nutrient (LN) treatment was 10% of the high nutrient concentrations. Seedlings were manually fertilised once a week. For each treatment combination, there were eight seedlings of each species, (total of 256 seedlings).

Environmental conditions in all the greenhouses were monitored and adjusted automatically with an Argus control system. Relative humidity in all the greenhouses was 55 ± 5% and day/night air temperatures were 25 ± 2/15 ± 2 °C. Natural light was supplemented on cloudy days and evenings with high pressure sodium-vapour lamps up to a 17-hour photoperiod. Moisture content of the growing medium was about 30% in all treatment combinations as measured using an HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U. K.). The plants were well watered.

Measurement of root characteristics

After four months of treatment, all the plants were harvested and separated into shoot and root components. The roots were carefully washed with tap water to remove all growth media. They were evenly spread and scanned in grayscale at 300 dots per inch using AGFA SnapScan 600 (Agfa Graphics Corp, Branchburg, N.J., USA). A scan filter of 1.0 mm^2 was set such that only objects with area above the limit were scanned. WinRhizo software (Regent Instruments Inc, Quebec, Canada) was used to determine total root length (TRL), mean root diameter (MRD), total root volume (TRV), root surface area (RSA) and number of root tips. After the scanning, the roots were oven-dried at 70 °C for 48 hours to a constant mass and weighed on an analytical balance (precision of 0.001g) to determine dry mass. Root branching intensity (RBI) was calculated as the number of root tips per root length (tips cm^{-1}). Root tissue density (RMD) and root length density (RLD) were determined as root dry mass per unit root volume (g cm^{-3}) and the length of roots per unit volume of growth media (cm cm^{-3}) respectively. Specific root length (SRL) and specific root area (SRA) were determined as root length per unit dry mass (cm g^{-1}) and root surface area per unit root dry mass (cm^2 g^{-1}) respectively. Details of each measured trait and its key significance are summarised as Table 4.1.

Statistical analysis

The effects of CO_2, root interaction, nutrient supply and species on root traits were tested with the general ANOVA procedure in GenStat version 12 (VSN International, Hemel Hempstead, UK). CO_2, root interaction (RI), nutrient supply and species were considered as fixed factors while each greenhouse was treated as random factor. Shapiro Wilk (normality) and Bartlett (homogeneity) tests showed that the data conformed to ANOVA's normality and homogeneity of variance assumptions. Fisher's Least Significant Difference (LSD) was used to perform pair-wise means comparisons. Mean comparisons were made on the rule that significant interactions override main effects and higher order significant interactions override lower level interactions. Therefore, when both main effects and interactions are significant ($P \leq 0.05$), only the significant higher interactions are presented. In the graphical presentation of results, only means of significant factors/interactions are presented.

Table 4.1: Measured root traits and their key functional significance. The signs (+) and (-), respectively indicate positive or negative relationship between a trait and its function.

Trait	**Abbreviation**	**Key significance**	**Main reference**
Total root length	TRL (cm)	Nutrient uptake (+) Life span (-)	(Eissenstat 1992, Ryser 1998)
Mean root diameter	MRD (cm)	Nutrient uptake rate (-), water transport (+), soil penetration (+), life span (+)	(Eissenstat 1992, Ryser 1998, Roumet et al. 2006)
Root surface area	RSA (cm^2)	Potential nutrient and water absorption (+)	(Chapin et al. 1986)
Root mass	RM (g)	Root system size, amount of belowground assimilate	(Atkinson 2000)
Root tips	Tips	Nutrient absorption (+)	(Paula and Pausas 2011)
Branching intensity	RBI (tips cm^{-1})	Nutrient exploration (+) Efficient transport (+)	(Comas and Eissenstat 2009, Paula and Pausas 2011)
Root length density	RLD (cm cm^{-3})	Nutrient and water exploration (+)	(Atkinson 2000)
Root mass density	RMD (g cm^{-3})	Life span (+), defence (+)	(Ryser 1998)
Specific root length	SRL (cm g^{-1})	Nutrient and water acquisition (+)	(Eissenstat 1992, Ostonen et al. 2006)
Specific root area	SRA (cm^2 g^{-1})	Nutrient, water absorption (+)	(Lõhmus et al. 1989)

Results

The [CO_2], root interaction and species (*$P \leq 0.040$*) influenced total root length (TRL) of seedlings (Table 4.2). At the ambient [CO_2], both species had significantly longer roots when they grew without root interaction than with root interaction, and within each RI, TRL of black spruce was longer than TRL of white spruce (Fig. 4.1a). At the elevated [CO_2], black spruce still had significantly greater TRL but the RI effect became statistically non-significant (Fig. 4.1a).The CO_2 elevation significantly increased TRL of seedlings grown with root interaction (Fig. 4.1a). TRL was also significantly (*$P<0.001$*) affected by interaction among CO_2, nutrient and species on (Table 4.2). The CO_2 elevation significantly increased TRL only in white spruce grown without root interaction in the high nutrient treatment (Fig. 4.1b). TRL of black spruce did not change with nutrient supply.

Mean root diameter (MRD) was significantly (*$P<0.001$*) influenced by interactions among [CO_2], root interaction and species (Table 4.2). At the ambient [CO_2], MRD did not differ with any treatment (Fig 4.1c). However, CO_2 elevation significantly increased MRD in black spruce grown without root interaction but reduced it with root interaction and there were no species differences within each RI (Fig. 4.1c). The MRD of the two species responded differently to CO_2 and nutrient supply (*$P \leq 0.016$*, Table 4.2). At ambient [CO_2], the low nutrient treatment significantly increased MRD in black spruce (Fig. 4.1d). In contrast, the CO_2 elevation significantly increased MRD of black spruce at the high nutrient treatment but reduced it when nutrient supply was low. Generally, MRD of white spruce did not respond to CO_2 or nutrient treatment (Fig. 4.1d).

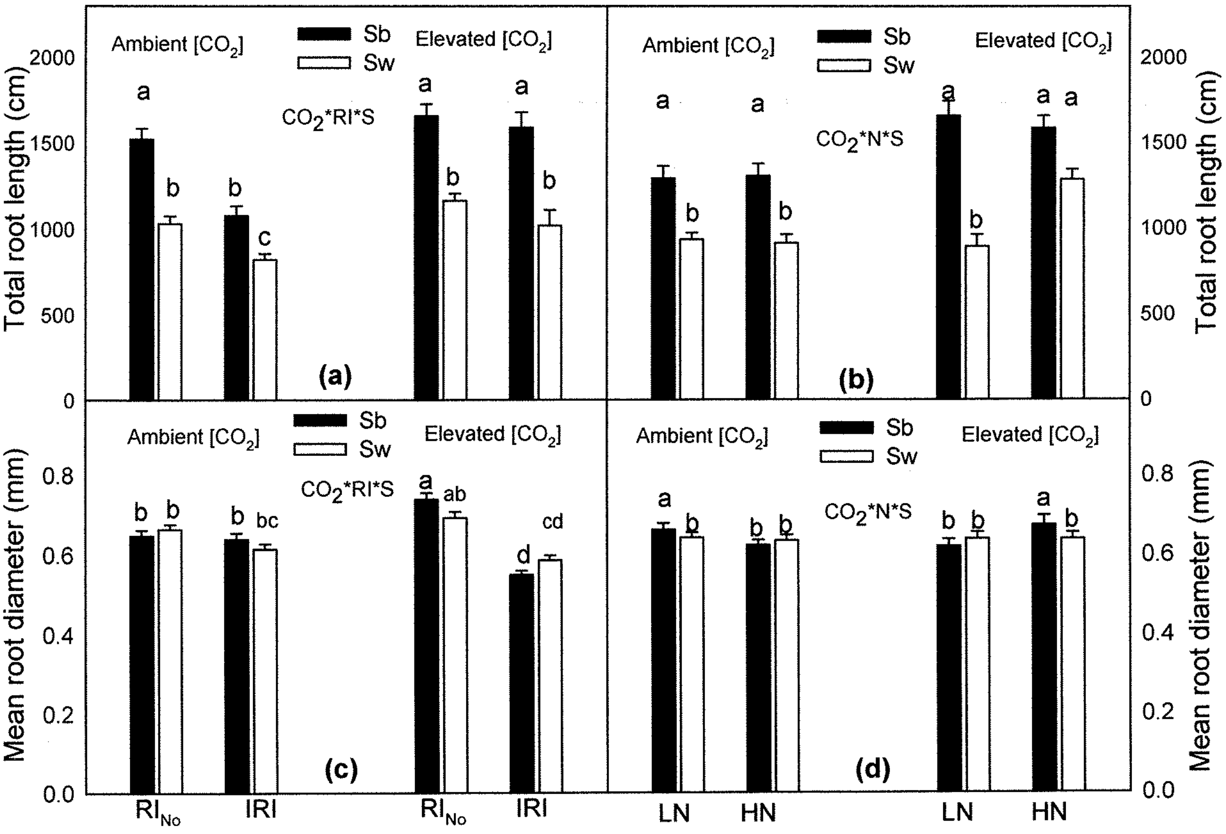

Fig. 4.1: Effects of [CO_2], nutrient supply (N), species (S) and inter-plant root interaction (RI) on total root length (*a*, *b*) and mean root diameter (*c* and *d*) of black spruce (Sb) and white spruce (Sw) seedlings after months of treatment (*n=32*). The seedlings were grown with (IRI) and without (RI_{NO}) inter-plant root interaction under two nutrient levels (HN-high nutrient, LN-low nutrient) at ambient and elevated [CO_2]. Each bar represents mean + SEM (standard error of the mean) and bars with different letters differ significantly ($P \leq 0.05$) from each other.

Table 4.2: ANOVA *P*-values on the effects of [CO_2], inter-plant root interaction (RI), nutrient supply (N) and species (S) on TRL, MRD, RSA, RM, number of tips, RBI, RLD, RMD, SRL and SRA of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were grown with (IRI) and without (RI_{NO}) inter-plant root interaction at two nutrient levels (HN-high nutrient, LN-low nutrient) under ambient and elevated [CO_2]. Abbreviations are defined in Table 4.1.

Source	**TRL**	**MRD**	**RSA**	**Tips**	**RBI**	**RMD**	**RLD**	**RM**	**SRL**	**SRA**
CO_2	0.105	0.956	0.196	0.069	0.175	0.565	0.105	**0.002**	0.406	0.537
RI	0.164	**0.014**	0.054	0.350	0.694	0.125	0.164	0.102	0.357	0.098
CO_2*RI	0.385	**0.029**	0.757	0.166	0.207	0.704	0.385	0.275	0.175	0.659
N	0.568	0.811	0.535	0.107	0.121	0.781	0.568	**<.001**	0.829	0.878
CO_2*N	0.549	0.083	0.365	0.360	0.893	0.288	0.549	0.114	0.520	0.382
RI*N	0.249	0.217	0.313	**0.005**	0.205	0.564	0.249	**0.024**	0.360	0.450
CO_2*RI*N	0.341	0.885	0.341	0.125	0.833	0.422	0.341	**0.005**	0.605	0.573
S	**<.001**	0.612	<.001	**<.001**	0.959	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*S	**0.035**	0.983	0.061	**0.044**	0.864	**0.034**	**0.035**	0.180	0.370	0.090
RI*S	0.294	0.236	**0.012**	0.060	**0.002**	0.213	0.294	**0.053**	0.087	**<.001**
N*S	**0.005**	0.410	**0.049**	**<.001**	0.454	**0.006**	**0.005**	0.068	0.085	0.066
CO_2*RI*S	**0.040**	**<.001**	0.782	**0.003**	0.988	0.477	**0.040**	0.097	0.167	0.544
CO_2*N*S	**0.001**	**0.016**	0.068	**0.006**	0.388	**<.001**	**0.001**	0.363	**<.001**	**<.001**
RI*N*S	0.631	0.220	0.292	0.251	**<.001**	**<.001**	0.631	0.169	0.869	0.205
CO_2*RI*N*S	0.627	0.916	0.932	**<.001**	**0.027**	**0.004**	0.627	0.697	0.345	0.626

Root surface area (RSA) of the two species varied with RI (*P≤0.012*) and nutrient supply (*P≤0.049*, Table 4.2). RSA of black spruce 48% higher than that of white spruce when grown with root interaction. RSA of black spruce was 41% higher than in white spruce when grown without root interaction (Fig. 4.2a). RSA of white spruce grown without root interaction did not differ from that of black spruce grown with root interaction (Fig. 4.2a). Both species had lower RSA when grown together than grown individually (Fig. 4.2a). RSA of black spruce did not differ with nutrient supply but the low nutrient treatment reduced RSA of white spruce (Fig. 4.2b). Black spruce had significantly greater RSA than white spruce in both nutrient treatments (Fig. 4.2b). [CO_2] had no significant (*P=0.196*) effect on RSA (Table 4.2).

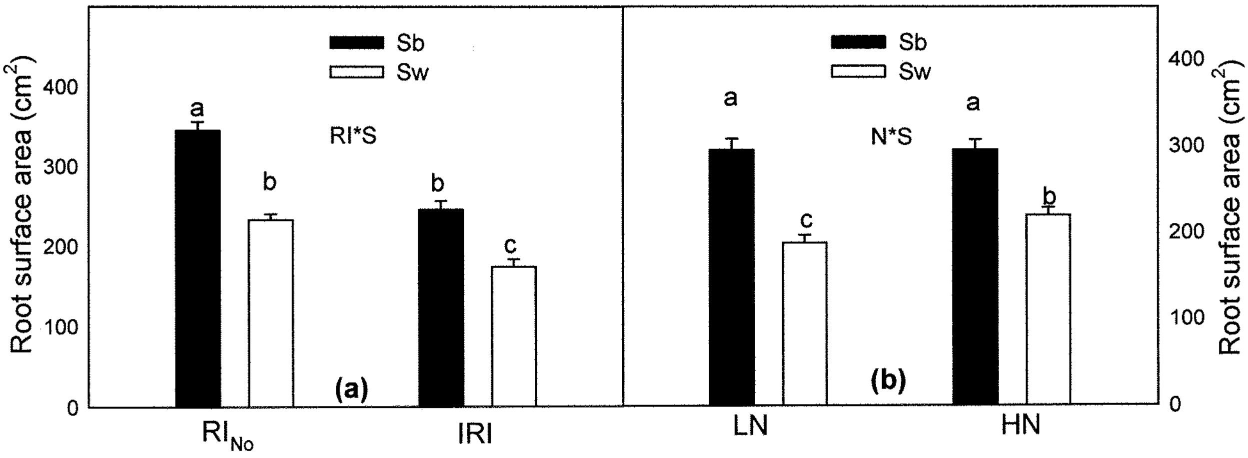

Fig. 4.2: Effects of [CO_2], nutrient supply (N), species (S) and root interaction (RI) on root surface area of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment (*n=64*).

There was a significant four-way (*P<0.001*) interaction on the number of root tips per seedling (Table 4.2). At the ambient [CO_2], black spruce grown without root interaction in the low nutrient treatment had the highest number of root tips (Fig. 4.3a). The low nutrient treatment significantly reduced the number of root tips in white spruce grown with root interaction at ambient CO_2 (Fig. 4.2a). The CO_2 elevation significantly increased the number of root tips in black spruce grown with root interaction at both the low and high nutrient levels and in white spruce with root interaction in the high nutrient treatment (Fig. 4.3a). However, the CO_2 elevation significantly decreased root tips in black spruce that grew without root interaction in the high nutrient treatment (Fig. 4.3a).

Seedling root branching intensity (RBI) was significantly ($P \leq 0.027$) affected by interactions among the four factors (Table 4.2). In the ambient [CO_2], seedlings grown with root interaction had lowered RBI in the low nutrient treatment and black spruce grown without root interaction in the high nutrient treatment had the lowest RBI (Fig. 4.3b). At the elevated [CO_2], RBI was significantly reduced in seedlings that grew without root interaction but was increased with root interaction in black spruce in the high nutrient treatment and white spruce in the low nutrient treatment (Fig. 4.3b).

Root tissue density (RTD) was significantly ($P \leq 0.004$) affected by interactions among the four factors (Table 4.2). At the ambient [CO_2] and within the RI_{No} treatment, RTD was higher in white spruce supplied with the high nutrient while no other difference occurred in the other treatments (Fig. 4.3c). The trend was similar for seedlings in the IRI treatment at ambient [CO_2] but the mean RTD in the IRI treatment was higher than in the RI_{No} treatment (Fig. 4.3c). The CO_2 elevation increased RTD in white spruce supplied with the low nutrient treatment at both levels of RI but reduced it in white spruce supplied with high nutrient in the IRI treatment (Fig. 4.3c). Generally, RTD was lower in black spruce than in white spruce particularly when there was no root interaction. Moreover, CO_2 and nutrient supply did not affect RTD of black spruce while white spruce responded to changes in both (Fig. 4.3c).

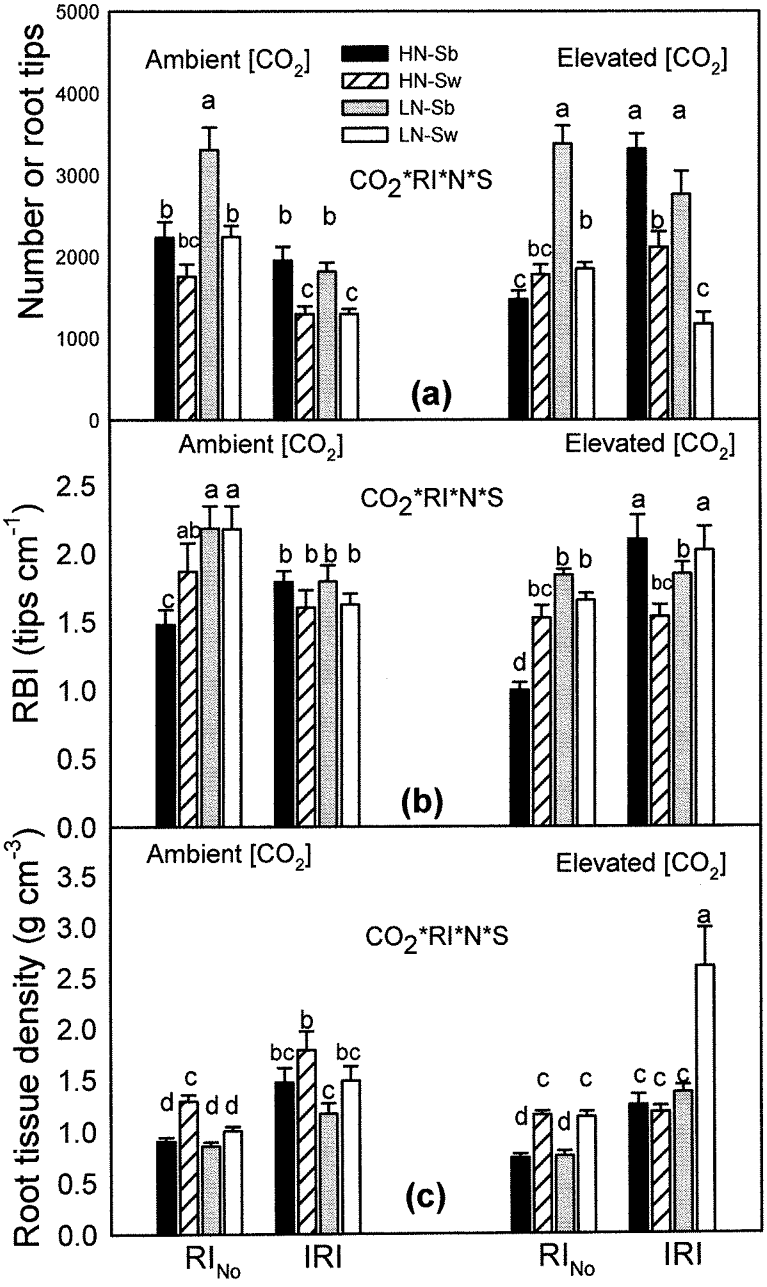

Fig. 4.3: Effects of $[CO_2]$, nutrient supply (N), species (S) and root interaction (RI) on number of root tips (*a*), root branching intensity (*b*) and root tissue density (*c*) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment (*n=16*).

Root length density (RLD) of the two species responded differently to [CO_2] and root interaction (*$P \leq 0.040$*, Table 4.2). At the ambient [CO_2], RLD was significantly higher when there was no root interaction but the difference disappeared at elevated [CO_2] (Fig. 4.4a). At both [CO_2] and RI treatments, black spruce had higher RLD than white spruce but the difference was smaller at ambient [CO_2] (Fig. 4.4a). Furthermore, CO_2 elevation significantly increased RLD of both species when root interaction was present but had no significant effect on RLD when there was no root interaction (Fig. 4.4a). The CO_2 treatment significantly (*$P < 0.001$*) modified the species RLD response to nutrient supply (Table 4.2). In the ambient [CO_2], RLD was significantly higher in black spruce at both nutrient levels and the nutrient treatment did not affect RLD of either species (Fig. 4.4b). However, the CO_2 elevation significantly increased RLD of both species at the high nutrient treatment but only that of black spruce at the low nutrient level (Fig. 4.4b).

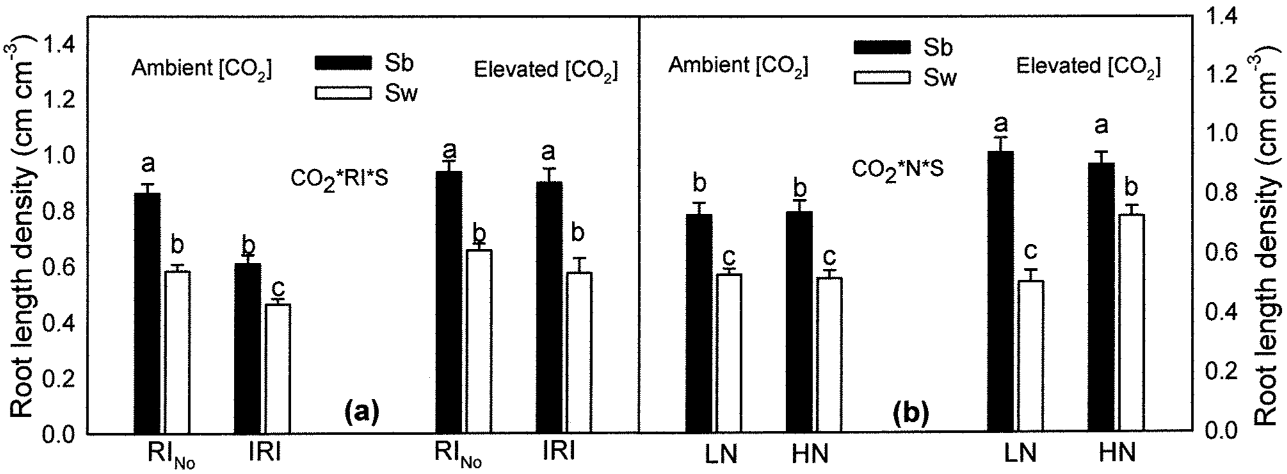

Fig. 4.4: Effects of [CO_2], nutrient supply (N), species (S) and root interaction (RI) on root length density of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment (*$n=32$*).

Root mass was significantly (*$P \leq 0.005$*) affected by interactions among [CO_2], root interaction and nutrient supply (Table 4.2). The CO_2 elevation significantly increased root mass in all but seedlings grown in the high nutrient with root interaction (Fig.4.5a). Seedlings in the ambient [CO_2] did not respond to nutrient or root interaction treatment (Fig. 4.5a). The RI x S interaction on root mass was marginally (*$P=0.053$*) significant. The presence of root interaction significantly reduced root mass in white spruce but had no significant effect on root mass when there was no root interaction (Fig. 4.5b).

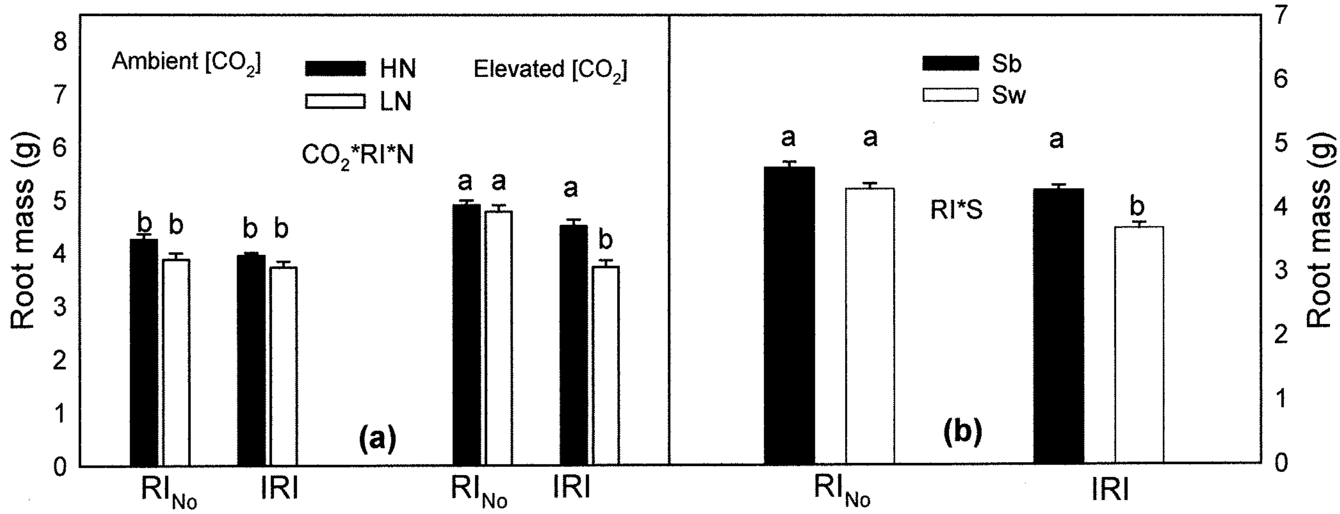

Fig. 4.5: Effects of [CO_2], nutrient supply (N), species (S) and root interaction (RI) on root mass of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment (*$n=64$*).

CO_2, nutrient supply and species had significant (*$P<0.001$*) interaction on specific root length (SRL) and specific root area (SRA, Table 4.2). The [CO_2] and nutrient supply did not influence black spruce's SRL and SRA (Figs. 4.6a and 4.6b). Both SRL and SRA of white spruce showed opposite response to nutrient supply under the different [CO_2]: the low nutrient treatment resulted in significantly higher SRL and SRA at ambient [CO_2], but significantly lowered SRL and SRA under elevated [CO_2] (Figs. 4.6a and 4.6b). SRA of the two species responded differently to root interaction (*$P<0.001$*, Table 4.2). SRA of black spruce without root interaction was 32% higher than that of black spruce grown with root interaction while the SRA of white spruce did not vary with root interaction (Fig. 4.6c).

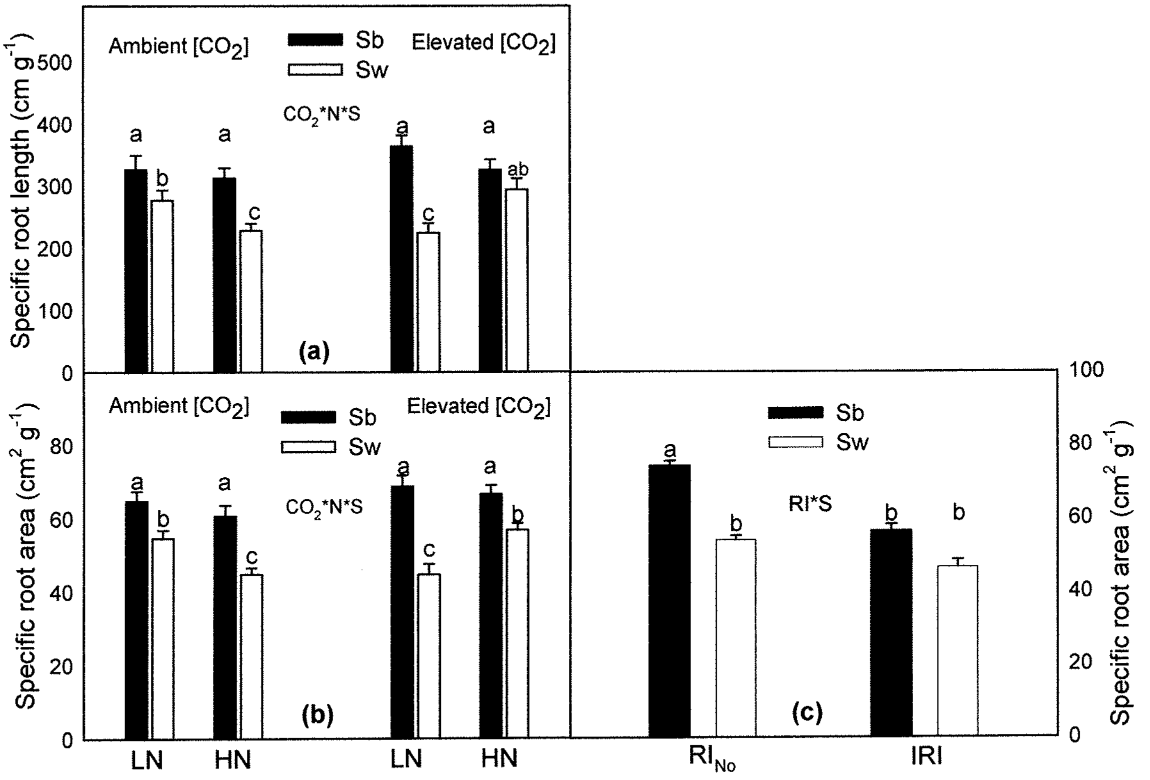

Fig. 4.6: Effects of [CO_2], nutrient supply (N), species (S) and root interaction (RI) on specific root length (*a*) and specific root surface area (***b***, ***c***) of black spruce (Sb) and white spruce (Sw) seedlings after 4 months of treatment. In Figs. ***a*** and ***b***, *$n=32$* while in ***c***, *$n=64$*.

Discussions

Tree roots are important for the acquisition and transport of nutrients and water, anchorage into the soil and the storage of assimilates produced from above ground (Atkinson 2000). Fine roots in particular serve as the principal pathway for water and nutrient absorption (Eissenstat 1992). At the same time, roots represent significant cost to a plant's overall carbon economy (Eissenstat 1992). Knowledge of root traits required for maximising nutrient absorption and/or maintenance in response to current and future [CO_2] at different nutrient regimes is required for successful management of trees. Roots traits of black spruce and white spruce seedlings differed in response to nutrient availability and the effects were modified by [CO_2]. Total root length (TRL), average fine root diameter (MRD), root surface area (RSA), root branching intensity (RBI), root length density (RLD), root mass density (RMD), specific root length (SRL), specific root area (SRA) and root mass differed between the two species in response [CO_2], root interactions, nutrient supply.

The hypothesis that high nutrient and elevated [CO_2] would confer on the seedlings traits such as high SRL, SRA, longer roots that are associated with faster growth rate with the low nutrient conferring traits needed for longevity is generally but not completely supported. However, this was species specific. Black spruce had higher photosynthetic rate, greater biomass and greater height growth than white spruce (Chapters 2 and 3 of this thesis). Black spruce also had longer roots with greater surface area and maintained a constantly higher SRL and SRA at both [CO_2] and this did not differ with nutrient treatment. Furthermore, root mass density, a trait associated with root maintenance and lifespan (Ryser 1998, Atkinson 2000, Comas et al. 2002, Holdaway et al. 2011) was generally lower in black spruce with increased nutrient supply. This indicates that black spruce traded-off lower tissue density, defence and longevity trait in favour of high specific root length for faster growth and greater biomass production (Chapin et al. 2011). In contrast to black spruce, white spruce had generally lower SRL and SRA but higher RMD and these varied with nutrient supply and CO_2 concentration, which also indicate some trade-off. These findings suggest that the trade-offs and the plants response to CO_2 and nutrient supply was species-specific. The data on black spruce indicate that specific root length and specific root surface area of the species are not influenced by nutrient availability. Forde and Lorenzo (2001) reported that nutrient effects on SRL is species-specific and that some species may not change their SRL in response to nutrient availability. Similarly, Hutchings and de Kroon (1994) observed that many species do not change their SRL as a function of nutrient supply. The apparent trade-off observed however, seem to be not so distinct probably because this was a controlled experiment lasting only one growing season.

So what was the underlying cause of the variation in SRL and SRA in white spruce in response to CO_2 and nutrient supply? Higher SRL and SRA are achieved by roots being longer in length (Lõhmus et al. 1989, Ostonen et al. 2007, Lambers et al. 2008, Paula and Pausas 2011), or having either smaller diameter or lower root mass density-RMD (Eissenstat 1991, Ryser and Lambers 1995) or their combinations. Though changes in root diameter have been reported as a possible cause of variation in SRL and SRA (Eissenstat 1991, Ryser and Lambers 1995), it is ruled out because diameter of white spruce did not change with nutrient-CO_2 treatment. Differences in white spruce's total root length and root mass density may better explain the findings. First, root length of white spruce in the high nutrient treatment increased at elevated [CO_2], probably due to increased photosynthetic rate and this provided the carbon needed for greater root length extension, hence, greater SRL and SRA. Secondly, RMD was lower in white spruce under elevated [CO_2] at the high nutrient treatment resulting in higher SRL. Overall, these findings support studies by Eissenstat (1991) that variation in SRL is caused by root diameter only when RMD is constant and when RMD varies, root diameter does not affect SRL.

Highly branched roots systems are more efficient in water transport and nutrient uptake than roots with low branching intensities (Fitter 1986, Paula and Pausas 2011). High RBI also confers greater competitive ability for soil moisture and nutrient on plants (Fitter 1986, Paula and Pausas 2011) by increasing the volume of soil explored per unit fine root biomass invested (Comas and Eissenstat 2004). However, when there is adequate soil moisture and nutrient avaialability, high RBI may not be well utilised (Tani et al. 2003). Black spruce has lesser demand for water and nutrients than white spruce (Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Patterson et al. 1997), hence, less need to invest in high RBI, especially when there is no competition for either resource. This might explain why RBI was generally lower in black spruce grown without inter-plant root interactions and in the high nutrient treatment but was either higher or the same as that of white spruce when grown with root interaction. Furthermore, each new extension of plant root comes at a cost (Eissenstat 1992, Yanai and Eissenstat 2002). To increase efficiency at lower construction cost, individually grown black spruce in the high nutrient treatment under elevated [CO_2] decreased the construction of new tips and subsequently reduced RBI. This was compensated for by increased root length density and a constant SRL. In the presence of plant-plant root interaction and the low nutrient treatment however, both species generally had similar RBI to increase resource exploration and exploitation.

Root traits exhibited by seedlings grown with plant-plant interactions differed from traits exhibited by individually seedlings. TRL, MRD, RSA, number of tips, RBI, RTD, SRA,SRL and root mass all varied with root interaction. These support the theory that plants alter their fine root characteristics in response to the presence of neighbouring plants (Gersani et al. 2001, Hodge 2009) and that roots integrate information about neighbours (Cahill et al. 2010). Responses of most of the traits such as TRL, RSA, SRA, RLD which are associated with high growth rate were reduced with plant-plant root interaction. In contrast, a trait like root mass density, which is associated with slow growth rate and greater longevity was generally higher with than without root interaction. The contrasting responses tend to indicate that in the presence of plant-plant root interaction, root competition was either perceived or was apparent and the plants' strategy was geared towards defence or probably became victims of tragedy of the commons (Gersani et al. 2001). In the case of the treatment without root-root interaction however, the strategy was geared towards faster growth. Interestingly, in the presence of root interaction, the CO_2 elevation had opposite effect on root length and root diameter, two key parameters upon which key parameters are derived. The CO_2 elevation increased root length but decreased root diameter. This is an important strategy in maximising nutrient absorption under elevated [CO_2], because absorption is increased with increased length and surface area and not increased diameter (De Kroon et al. 2012).

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CHAPTER 5: Effects of CO_2 and soil warming on growth, biomass and root traits of black spruce and white spruce seedlings grown with and without inter-plant root interaction

Introduction

Tree growth and biomass production are affected by various environmental factors and their interactions (Huang et al. 2007, Lambers et al. 2008, Lukac et al. 2010). In the boreal forest, soil temperature and nutrient availability are major factors influencing tree growth (Jarvis and Linder 2000). Air temperature is predicted to rise with the increase in atmospheric [CO_2 (IPCC 2001, Christensen et al. 2007). Air temperature positively correlates with soil temperature as both are determined by the energy balance at the ground surface (Zheng et al. 1993). Hence, increases in air temperature may result in increased soil temperature (Domisch et al. 2002) and the changes may affect tree growth (Ambebe et al. 2010). In conifers, particularly, spruce, the relative sensitivity to soil temperature may be greater than to air temperature (Jurik et al. 1988, Schwarz et al. 1997, Lupi et al. 2012, Wu et al. 2012). The effects of increased atmospheric [CO_2] and warming may also be higher in the boreal forest than in other ecological regions (Christensen et al. 2007).

Climate models predict that mean annual temperature in Canada's boreal region may increase by about 5-7 °C, higher than the 1.4–5.8 °C predicted global average (IPCC 2001, Christensen et al. 2007). There is evidence that the region is warming up faster than other regions on earth (Serreze et al. 2000). Increased atmospheric [CO_2] may interact with soil temperature to change species composition, competitive ability and resource use of plant communities (Stewart et al. 1998). These changes and interaction may compound the management of the boreal forest.

Soil temperature affects plant's morphology and physiological processes and characteristics, including organ development and biomass allocation, stomatal conductance, nutrient uptake (Lawrence and Oechel 1983, Camm and Harper 1991, Landhäusser et al. 1996, Stewart et al. 1998, Landhäusser et al. 2001, Cai and Dang 2002, Dang and Cheng 2004, Pregitzer and King 2005, Alvarez-Uria and Körner 2007). The limitations posed by low soil temperature on tree growth in the boreal forest is well known (Delucia and Smith 1987, Grossnickle 1987) For example, soil temperatures below 10 °C can limit shoot growth by inhibiting nutrient and water uptake (Bonan 1992, Grossnickle 2000, Peng and Dang 2003). Low soil temperatures also result in greater root biomass allocation (Clarkson et al. 1988, Tierney et al. 2001), thereby reducing potential photosynthetic productivity and growth. The effect of supra-optimal soil temperature in conifers such as black spruce is also well known (Peng and Dang 2003, Way and Sage 2008a, Way and Sage 2008b). For instance, under controlled conditions, photosynthetic rate in black spruce declines at a soil temperature of 30 °C (Way and Sage 2008a), while at 35 °C, mortality can reach 80% in black spruce seedlings (Peng and Dang 2003).

Despite our current knowledge on boreal trees, particularly spruce response to soil temperature, there remains a gap; how will CO_2 elevation affect spruce growth, biomass and fine root morphology in the context of predicted soil warming? What role does inter-plant root interaction play in modifying the response? Soil warming is very important for the survival and early development of newly planted seedlings (Grossnickle and Blake 1985). The successful management of the boreal forest requires a good understanding of how the effects of soil temperature on growth, morphology and root traits change with CO_2 elevation.

Black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) were studied in this research. The two species were selected because of their wide natural distribution in the boreal forest, economic importance, and their ability co-existence on some sites (Bannan 1940, Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Patterson et al. 1997). Both species have shallow root system (Bannan 1940, Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Haavisto and Jeglum 1995) with black spruce generally lacking a tap root (Viereck and Johnston 1990). Black spruce can grow on both peatlands where soil temperatures are low and on warmer upland sites while white spruce grows well on only warmer upland sites (Grossnickle and Blake 1985, Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Grossnickle 2000, Wolken et al. 2011). Soil temperatures between 19 and 21 °C are considered ideal for the growth of black spruce and white spruce seedlings (Heninger and White 1974, Tryon and Chapin 1983, Grossnickle and Blake 1985, Odlum and Ng 1995). In a controlled environment, a soil temperature of 15 °C is considered ideal for maximum root biomass production in both species (Peng and Dang 2003). Maximum total biomass production in black spruce and white spruce are achieved at respective soil temperatures of 16 and 14 °C under greenhouse conditions (Peng and Dang 2003).

This study was undertaken to examine the effects of interactions between [CO_2] and soil temperature on growth, biomass, morphology and root characteristics of black spruce and white spruce seedlings grown with and without inter-plant root interactions. Responses measured included seedling height and diameter, biomass and biomass allocation patterns, root length and diameter, number of root forks, branching intensity, specific root length, specific root surface area and the relationship between specific root length and root surface to volume ratio. Because black spruce can grow on both cold peatlands and warm uplands, it is considered to be more plastic than white spruce and more plastic plants respond better to treatment than less plastic species (Midgley et al. 1999, Pritchard et al. 1999, Hodge 2004). Hence, it was hypothesized that soil warming and elevated CO_2 would enhance the growth, biomass and resource acquisition root traits of black spruce to a greater degree than in white spruce.

Plant roots integrate information about roots of their kind and of other species and on available resources and modify their responses accordingly to optimise resource utilisation (Walker et al. 2003, Cahill et al. 2010, Armas and Pugnaire 2011). CO_2 may also differentially modify individual species response in plant mixture creating a community response that is different from those of individual species (Navas 1998, Navas et al. 1999). Hence, it was hypothesized that the responses of seedlings grown without inter-plant root interaction differ from those of seedlings grown with root interaction in response [CO_2] and soil warming.

Materials and methods

Plant materials

One-year old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings were obtained from a local commercial nursery (Hills Tree Nursery, Thunder Bay, Ontario) for the study. Diameter and height of the seedlings were measured before treatment, therefore, diameter and height data provided in subsequent sections of this chapter is growth increment. All seedlings selected for treatment were relatively uniform in size. The growing medium was a mixture of vermiculite and peat moss (50:50, v/v).

Experimental design and environmental conditions

The experimental design was split-split-split plot. [CO_2] was the whole plot, with soil temperature as the sub-plot, and inter-plant root interaction (RI) as the sub-sub plot. Equal number of seedlings of the two species were randomly position within each sub-sub treatment unit. Each fixed factor had two levels. The CO_2 levels were 380 and 720 μmol mol^{-1}, ambient and elevated respectively with two replications each. Electronic ignition natural gas CO_2 generators (model GEN-2E, Custom Automated Products, Inc, Riverside, CA) were used to generate CO_2. The CO_2 was distributed to the plants through a network of perforated tubes and circulation fans placed above the benches. The levels of CO_2 in the greenhouses were monitored and controlled automatically with Argus CO_2 Control System (Argus, Vancouver, BC, Canada). Within each [CO_2], there were two soil temperatures (20 and 25 °C).

Based on the soil temperature range considered ideal for the two species optimal growth (Heninger and White 1974, Tryon and Chapin 1983, Grossnickle and Blake 1985, Odlum and Ng 1995), the 20 °C (T_{20}) was chosen. The 5 °C additional warming (T_{25}) is projection based on

IPCC (2001) predictions for air temperature. Each soil temperature was controlled separately using a soil temperature control system consisting of a large leak-proof box (196 x 112 x 16 cm, L x W x D). The design is described in detail in Cheng et al. (2000). The soil temperature was controlled by circulating temperature-controlled water in the space between the planting containers. To ensure even temperature distribution within the system, each control unit was equipped with two circulatory pumps (model AC-2CP-MD, March Mfg. Inc., Glenview, Illinois, USA) to distribute the temperature-controlled water. The system was insulated to minimise heat exchange with the greenhouse air. Each day-time soil temperature was lowered by approximately 4-6 °C at night to cater for lower night temperatures taking into account the lag in soil temperature changes.

Each soil temperature was split into two areas and the RI treatment was randomly allocated to the two areas. Eight seedlings of each species per treatment combination were planted within each T-RI treatment. In the treatment without inter-plant root interaction (RI_{NO}), each seedling was grown in an individual pot. In the treatment where inter-plant root interaction occurred (IRI), seedlings of the two species were grown in the same container. A total of 256 seedlings were planted and there was no mortality.

The plants received natural light which was supplemented by high pressure sodium vapour lamps on cloudy days and early evenings. The supplementary light was approximately 220 μmol m-2s-1 at the canopy level. A 17-hour photoperiod during the experimental duration. Relative humidity (55 ± 5%) and air temperature (day, 25 ± 2 °C and night, 15 ± 2 °C) in the greenhouses were automatically controlled using an Argus control system. All the seedlings were fertilised once a week with nutrient solution containing 150, 60, 150, 80, 40, 60 mg/l N, P, K, Ca, Mg, S, respectively. Plants were watered between fertiliser applications as required based on physical observation and the use of HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U. K.).

Measurements and Harvest

Seedling height, and root collar diameter (RCD) were measured with ruler and a digital calliper, respectively, at the end of the treatment. After four months of treatment, all the 256 seedlings were harvested and separated into foliage, stem (including branches) and roots. The foliage were scanned with WinSeedle (Regent Instruments Inc, Quebec, Canada) to determine projected leaf area. The roots were carefully washed of all growth media and scanned at 300 dots per inch (DPI) using AGFA SnapScan 600 (Agfa Graphics Corp, Branchburg, N.J., USA). A scan filter of 1.0 mm^2 was set to allow only needles with area above the limit to be measured. WinRhizo software was used to determine total root length (TRL, cm), mean root diameter (MRD, mm), root volume (RV, cm^3), root surface area (RSA, cm^2) and number of root tips. The foliage, stem and roots were subsequently oven-dried at 70 °C for 48 hours and weighed on an analytical balance (precision of 0.001g) to determine dry mass. Shoot mass ratio (aboveground to total biomass, SMR), root to shoot mass ratio (RSR, ratio of belowground to above ground mass) were used as indices of biomass allocation. Root length to leaf area ratio (RLA) was used to estimate proportional water absorbing and transpirational surface. Specific root length (SRL, cm g^{-1}) and specific root surface area (SRA, cm^2 g^{-1}) were determined as root length per unit dry mass and root surface area per unit root dry mass respectively. Root branching intensity (RBI) was calculated as the number of root tips per root length (tips cm^{-1}). Surface area to volume ratio (SVR, cm^2 cm^{-3}) was also calculated.

Statistical analysis

Data were analysed using mixed model analysis of variance (ANOVA) procedure in GenStat version 12 (VSN International, Hemel Hempstead, UK). Before the ANOVA test, Shapiro Wilk and Bartelett tests were used to test the data's normality and homogeneity of variance respectively. CO_2, soil temperature, root interaction and species were treated as fixed factors in the analysis. Post-hoc mean comparisons were carried out with Fisher's least significant difference (LSD). The mean comparisons were made on the rule that significant interactions override main effects and higher order significant interactions override lower level interactions. Hence, when main effects and interactions are significant ($P \leq 0.05$), only the significant higher order interactions are presented. Relationship between SRL and SVR was established with simple linear regression. Differences in slopes were compared based on Zar (2010).

Results

Height and diameter growth

There was a significant ($P \leq 0.042$) four-way interaction on height growth (Table 5.1). In the ambient CO_2, soil warming significantly increased height growth of white spruce grown without root interaction and in black spruce grown with root interaction (Fig. 5.1). White spruce grown with root interaction at ambient [CO_2] had the least height at both temperatures. In the elevated [CO_2], the T_{20} treatment significantly increased height of white spruce grown without root interaction and that of black spruce with root interactions (Fig. 5.1). At the 25 °C warming treatment, the CO_2 elevation increased height growth of black spruce grown without root interaction and that of white spruce grown with root interaction (Fig. 5.1).

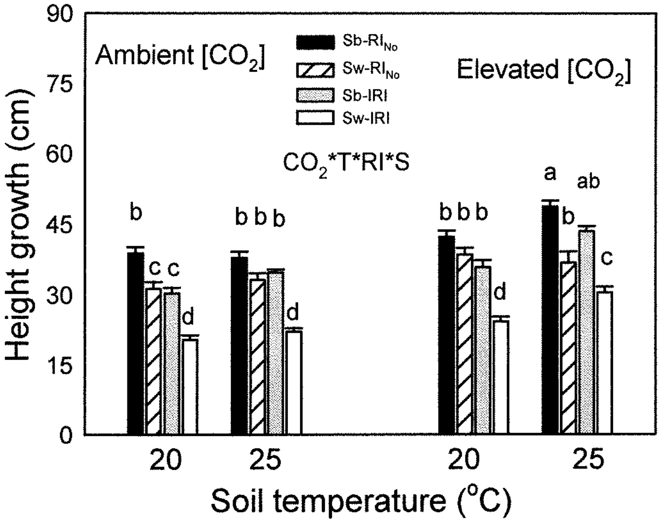

Fig. 5.1: Effects of [CO_2], soil temperature (T), species (S) and root interaction (RI) on height growth of black spruce and white spruce seedlings (*n=16*) after 4 months of treatment. The seedlings were exposed to two CO_2 concentrations (380 and 720 µmol mol^{-1}) with and without root interaction at 20 and 25 °C soil temperatures. Each bar represents mean + SEM and bars with different letters differ significantly ($P \leq 0.05$) from each other.

CO_2 ($P \leq 0.045$) and root interactions ($P < 0.001$) had significant but non-interactive effect on seedling diameter growth (RCD, Table 5.1). RCD of seedlings grown in elevated [CO_2] were 15% greater than at ambient (5.99 vs 5.12 mm respectively) and seedlings grown without plant-plant root interaction had a 26% greater RCD (6.20 vs 4.91 mm respectively, Table. 5.1). Soil warming did not significantly ($P=0.507$) influence RCD and neither did the two species ($P=0.191$) differ in their RCD (Table 5.1).

Table 5.1: *P* values from ANOVA on the effects of[CO_2], soil temperature (T), inter-plant root interaction (RI) and species (S) on growth, biomass and organ biomass of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were exposed to two [CO_2] (380 and 720 μmol mol^{-1}) with and without root interaction at 20 and 25 °C soil temperature. All abbreviations are defined in the text.

Treatment	Height	RCD	Biomass	Leaf mass	Stem mass	Root mass
CO_2	0.141	**0.045**	**0.039**	0.131	0.066	**0.042**
T	**0.007**	0.507	0.370	0.803	0.662	**0.007**
CO_2*T	**0.037**	0.208	0.365	0.326	0.651	0.053
RI	**<.001**	**<.001**	**<.001**	**<.001**	**0.001**	**0.039**
CO_2*RI	0.309	0.257	0.720	0.989	0.970	0.342
T*RI	**0.003**	0.850	0.785	0.108	0.720	0.293
CO_2*T*RI	0.252	0.854	0.648	0.953	0.757	0.437
S	**<.001**	0.191	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*S	0.441	0.292	**0.047**	**0.024**	0.237	**0.002**
T*S	0.058	0.474	0.618	0.397	0.507	0.075
RI*S	**<.001**	0.953	**0.051**	**0.001**	**0.047**	0.204
CO_2*T*S	0.055	0.364	0.600	0.175	0.991	0.483
CO_2*RI*S	0.667	0.337	0.377	0.063	0.340	0.165
T*RI*S	0.946	0.319	**0.019**	**<.001**	0.178	0.510
CO2*T*RI*S	**0.042**	0.340	0.954	0.285	0.679	0.994

Biomass and biomass allocation

The total biomass of the two species responded differently to CO_2 treatment (*P≤0.047*, Table 5.1). The CO_2 elevation significantly increased total biomass in black spruce but not in white spruce (Fig. 5.2a). Soil temperature, root interaction and species also had significant (*P≤0.019*) interactive effects on total biomass (Table 5.1). At 20 °C, biomass of black spruce grown without root interaction was significantly greater than that of white spruce grown at the same treatment ,but with root interaction there was no significant different in the two species biomass (Fig. 5.2b). The 25 °C warming treatment significantly increased biomass of black spruce grown with root interaction but not in white spruce at the same treatment (Fig. 5.2b). Moreover, total biomass of white spruce did not respond to soil warming (Fig. 5.2b).

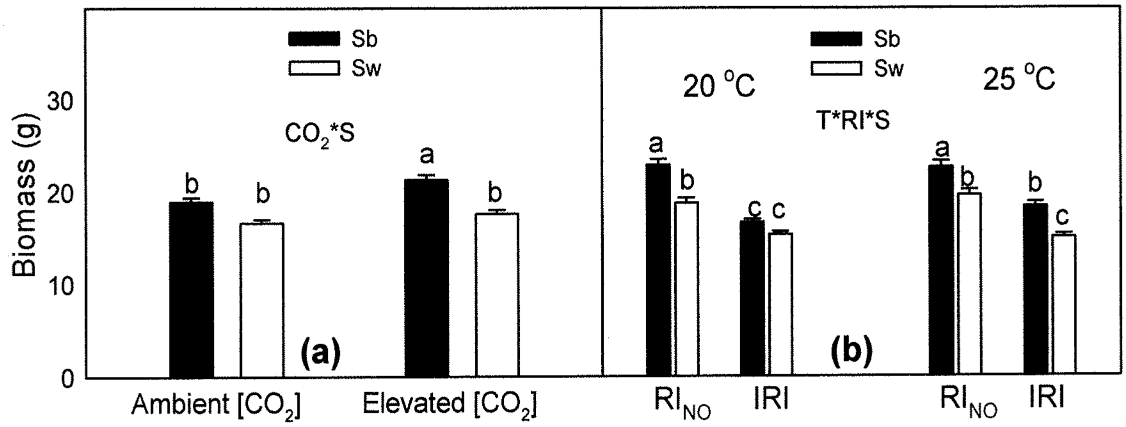

Fig. 5.2: Effects of $[CO_2]$, soil temperature (T), species (S) and root interaction (RI) on seedling total biomass of old black spruce and white spruce seedlings after 4 months of treatment. In Fig. ***a***, *n=64* while in ***b***, *n=32.*

Leaf mass of the two species responded differently to CO_2 treatment (*$P \leq 0.024$*, Table 5.1). Leaf mass of white spruce did not respond to CO_2 treatment but the CO_2 elevation significantly increased leaf mass in black spruce (Fig 5.3a). Furthermore, at ambient [CO_2], leaf mass of black spruce was 37% higher than that of white spruce and this difference increased to 48% under elevated [CO_2] (Fig. 5.3a). The leaf mass of black spruce grown in elevated [CO_2] was 16% higher than that of the species at ambient [CO_2]. There was also a significant (*$P<0.001$*) T*RI*S effect on leaf mass (Table 5.1). The response patterns were similar at T_{20} and T_{25} (Fig. 5.3b): black spruce had higher leaf mass than white spruce in both root interaction treatments and the magnitude of response was greater without root interaction (Fig. 5.3b). The 25 °C warming treatment significantly increased leaf mass of white spruce grown without root interaction and in black spruce grown with root interaction (Fig. 5.3b). Leaf mass of white spruce did not respond to the temperature treatment.

Both species had significantly (*$P \leq 0.047$*) greater stem mass when grown without root interaction than with root interaction (Fig. 5.3c). Moreover, the stem mass of black spruce grown without root interaction was significantly greater than stem mass of white spruce grown without root interaction (Table 5.1, Fig. 5.3c). In the presence of root interaction, the two species had statistically similar stem mass (Fig. 5.3c).

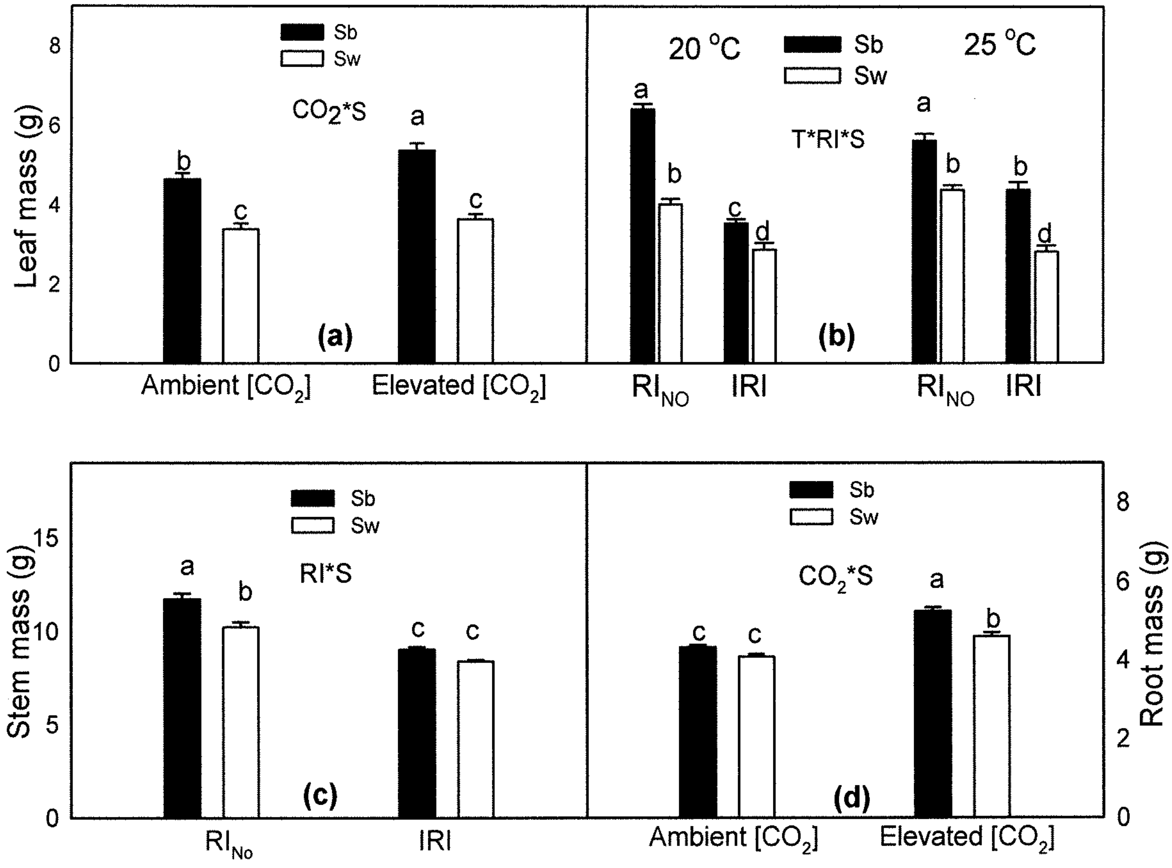

Fig. 5.3: Effects of [CO_2], soil temperature (T), species (S) and inter-plant root interaction (RI) on leaf (*a, b*), stem (*c*) and root mass (*d*) of black spruce and white spruce seedlings after 4 months of treatment. In Figs. ***a***, ***c*** and ***d***, *n=64* ,while in ***b***, *n=32*.

Root mass of the two species responded differently to CO_2 concentration (*$P \leq 0.002$*, Table 5.1). CO_2 elevation significantly increased root mass of both species but the magnitude of increase was greater in black spruce than in white spruce (Fig. 5.3d). At the ambient [CO_2], the two species had similar root mass (Fig. 5.3d). The 25 °C warming treatment significantly increased root mass by 8% relative to root mass of seedlings grown at 20 °C treatment

CO_2, root interaction and species had significant (*$P<0.001$*) effects on shoot mass ratio (SMR, Table 5.2). At the ambient [CO_2], SMR was greater in seedlings without root interaction than in seedlings with root interaction and there was no significant difference between the species (Fig. 5.4a). The CO_2 elevation significantly reduced SMR of white spruce grown without root interaction (Fig. 5.4a).

SMR of the two species responded differently to soil warming based on the presence or absence of root interactions (*$P\leq0.001$*, Table 5.2). At the T_{20}, there was no significant species difference in SMR, but root interaction reduced SMR (Fig. 5.4b). However, no such effect was detected in the 25 °C warming treatment (Fig. 5.4b). The 25 °C warming treatment significantly reduced SMR in white spruce grown without root interaction but significantly increased it in black spruce grown with root interaction (Fig. 5.4b).

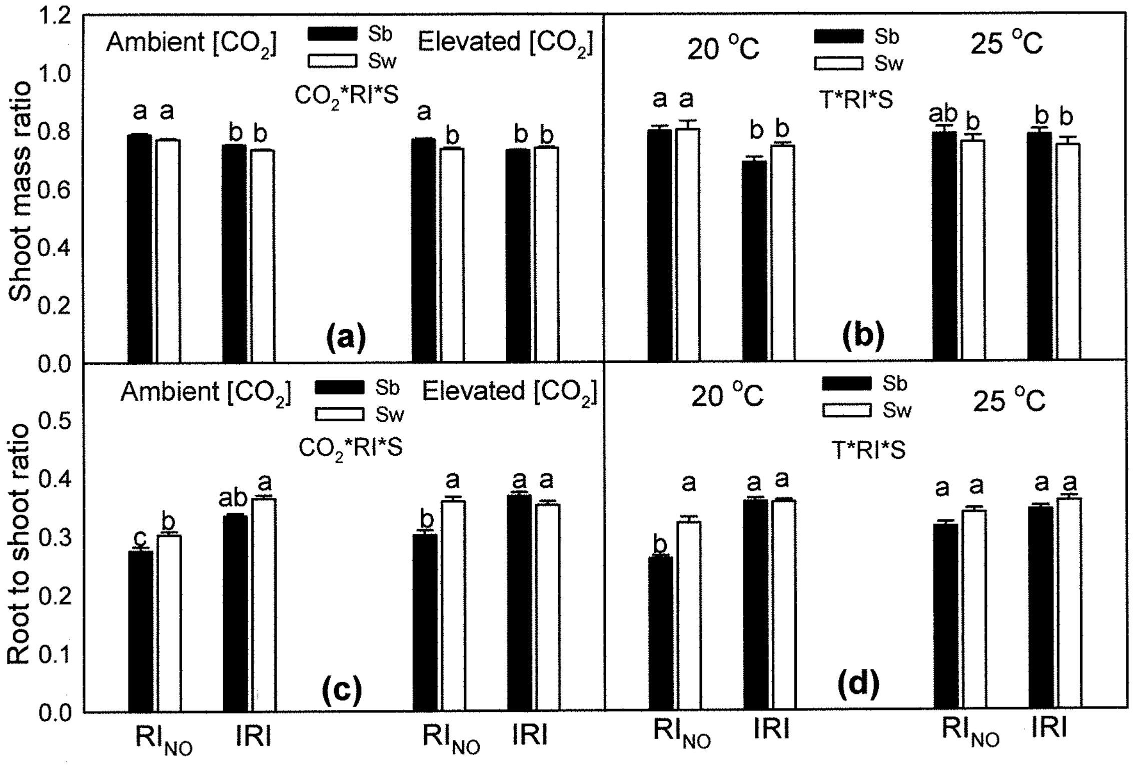

Fig. 5.4: Effects of [CO$_2$], soil temperature (T), species (S) and inter-plant root interaction (RI) on shoot mass ratio (*a, b*) and root to shoot mass ratio (*c, d*) of black spruce and white spruce seedlings after 4 months of treatment (*n=32*).

Root to shoot mass ratio (RSR) was significantly ($P<0.001$) affected by interactions among CO_2, root interactions and species (Table 5.2). At the ambient [CO_2], the presence of root interaction significantly increased RSR in both species but the difference was greater in white spruce than in black spruce (Fig. 5.4c). At the elevated CO_2 however, the effect of root interaction became insignificant for white spruce. The CO_2 elevation significantly increased RSR in both species when grown without root interaction (Fig. 5.4c). The soil warming treatment significantly ($P\leq0.001$) increased RSR black spruce grown without plant-plant root interaction (Table 5.2, Fig. 5.4d). No other significant treatment difference was detected (Fig. 5.4d).

Total root length, mean root diameter and root forks

The CO_2 elevation significantly ($P\leq0.035$) increased total root length (TRL) of seedling by 31% (1522 vs. 1162 cm). TRL of the two species also differed significantly ($P<0.001$): TRL of black spruce was 32% longer than that of white spruce (1529 vs 1156 cm). Neither soil temperature ($P=0.274$) nor inter-plant root interaction ($P=0.301$) affected TRL (Table 5.2).

Root interaction significantly ($P\leq0.039$) affected mean root diameter (MRD): the presence of root interaction significantly reduced MRD by 21% (0.6 vs 0.71 mm) and no other significant effect on MRD was observed.

The number of root forks was significantly influenced by CO_2 ($P\leq0.033$) and species ($P<0.001$). The CO_2 elevation increased number of root forks by 23% (10811 vs. 8797) and the number of forks in black spruce was 47% more than in white spruce (11657 vs. 7951).

Table 5.2: *P* values from ANOVA on the effects of [CO_2], soil temperature (T), plant-plant root interaction (RI) and species (S) on biomass ratios and root characteristics of black spruce and white spruce seedlings after 4 months of treatment.

Treatment	SMR	RSR	RLA	TRL	SRL	RSA	RBI
CO_2	0.080	0.075	0.238	**0.035**	**0.018**	**0.020**	0.176
T	0.301	0.311	0.871	0.274	0.679	0.772	0.741
CO_2*T	0.695	0.692	0.602	0.743	0.960	0.741	0.161
RI	**0.011**	**0.012**	**0.015**	0.301	0.691	0.467	0.130
CO_2*RI	0.197	0.205	0.289	0.118	0.100	0.410	0.475
T*RI	0.102	0.112	0.538	0.541	0.902	0.679	0.924
CO_2*T*RI	0.418	0.393	0.762	0.532	0.920	0.955	0.468
S	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*S	0.300	0.317	0.078	0.493	**<.001**	**0.001**	**0.010**
T*S	0.129	0.193	**0.011**	0.494	0.259	0.828	**0.006**
RI*S	**<.001**	**<.001**	**0.001**	0.081	**<.001**	**<.001**	**<.001**
CO_2*T*S	0.854	0.885	**0.020**	0.672	0.652	0.453	0.084
CO_2*RI*S	**<.001**	**<.001**	**<.001**	0.411	0.811	**0.031**	**0.007**
T*RI*S	**0.001**	**0.001**	**<.001**	0.820	0.422	0.171	**<.001**
CO_2*T*RI*S	0.923	0.989	**0.035**	0.460	0.087	0.261	0.181

Root length to leaf area ratio

There was a significant (*$P \leq 0.035$*) four-way interaction on ratio of seedling root length to leaf area (RLA, Table 5.2). At the ambient [CO_2], both species had response to soil temperature and warming had no significant effect on seedling RLA (Fig. 5.5). RLA was significantly higher in white spruce in the IRI treatment than at the other treatment combinations and no other difference occurred at ambient [CO_2] (Fig. 5.5). The CO_2 elevation significantly increased RLA in black spruce grown with root interaction at the 20 °C treatment but not at the 25 °C or in white spruce at either temperature (Fig. 5.5).

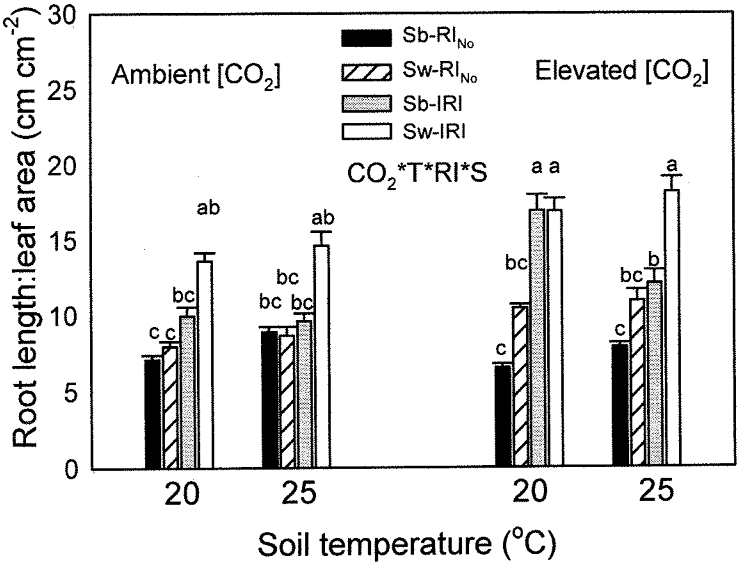

Fig. 5.5: Effects of [CO_2], soil temperature (T), species (S) and inter-plant root interaction (RI) on ratio of root length to leaf area of black spruce and white spruce seedlings after 4 months of treatment (*n= 16*).

Specific root length, specific root surface area and root branching intensity

Specific root length (SRL) of the two species responded differently (*$P<0.001$*) to CO_2 treatment (Table 5.2). The CO_2 elevation significantly increased SRL in white spruce but had no effect on SRL in black spruce (Fig. 5.6a). SRL of the two species also responded differently to root interactions (*$P<0.001$*, Table 5.2). SRL of white spruce significantly increased with root interaction while black spruce showed no significant response to root interaction (Fig. 5.6b). SRL of black spruce did not differ with either CO_2 or root interaction (Figs. 5.6a, b).

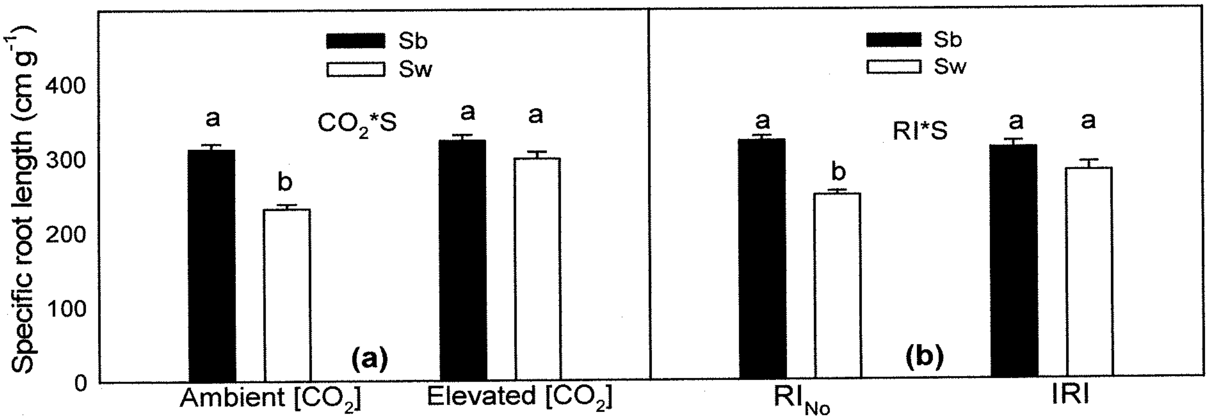

Fig. 5.6: Effects of [CO_2], species (S) and root interaction (RI) on specific root length of black spruce and white spruce seedlings after 4 months of treatment (*$n=64$*).

[CO_2] modified the effect of species and root interaction on specific root surface area (SRA) (*$P\leq0.031$*, Table 5.2). At the ambient [CO_2], SRA was significantly greater in black spruce that grew without root interaction and no other difference was observed (Fig. 5.7). The CO_2 elevation significantly increased SRA in both species when they were grown with root interaction (Fig. 5.7).

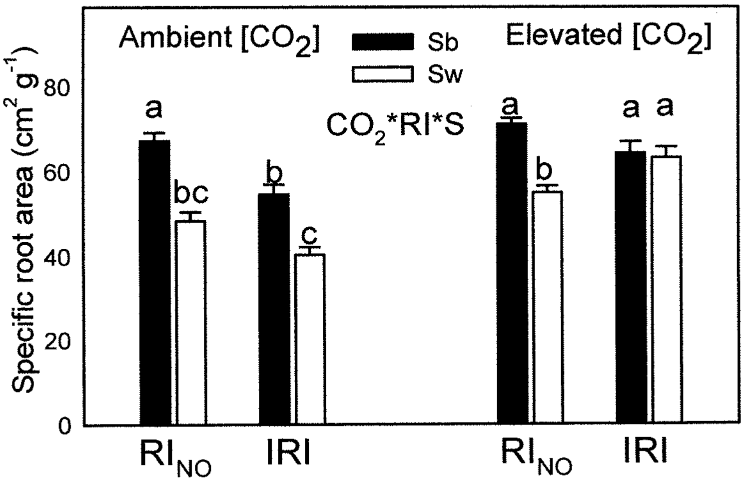

Fig. 5.7: Effects of [CO$_2$], species (S) and inter-plant root interaction (RI) on specific root surface area (*n=32*) of black spruce and white spruce seedlings after 4 months of treatment.

There was a significant (*P<0.001*) interaction among CO$_2$, root interaction and species on root branching intensity (RBI, Table 5.2). At the ambient [CO$_2$], there was no significant difference in any treatment combination (Fig. 5.8a). At elevated CO$_2$ however, RBI was significantly increased in black spruce that grew with root interaction but was reduced in black spruce grown without root interaction (Fig. 5.8a). RBI of the two species responded differently to soil warming and the effects were modified by root interactions (*P<0.001*, Table 5.2). At the 20 °C temperature treatment, the only significant difference was between black spruce grown with and without root interaction: RBI was significantly higher with root interaction than without root (Fig. 5.8b). At the 25 °C warming treatment, the only significant difference was between black spruce grown without root interaction and white spruce with root interactions: the white spruce had significantly greater RBI (Fig. 5.8b).

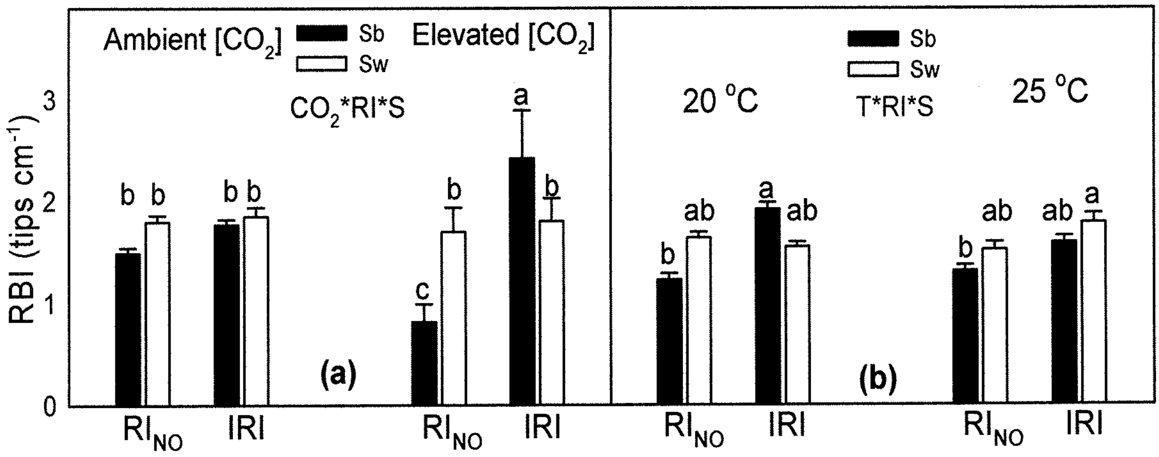

Fig. 5.8: Effects of [CO$_2$], soil temperature (T), species (S) and inter-plant root interaction (RI) on root branching intensity (RBI) of black spruce and white spruce seedlings after 4 months of treatment (*n=32*).

Relationship between specific root length and surface area: volume ratio

There was a strong positive linear relationship between seedling specific root length and root surface area to volume ratio among all treatment combinations (Fig. 5.9a-5.9h). The slopes of the relationships generally differed between the two species at the various treatment combinations, with two exceptions (Fig. 5.9a-5.9h). The slopes of the two species did not differ from each other in the combination of T_{25}-root interaction at ambient [CO$_2$] (Figs. 5.9e and 5.9g). The slopes in all the other treatment combinations were significantly higher in black spruce than in white spruce (Figs. 5.9b, c, d, f, h), except in the T_{20}-RI_{No} treatment at ambient [CO$_2$] which was reversed (Fig. 5.9a).

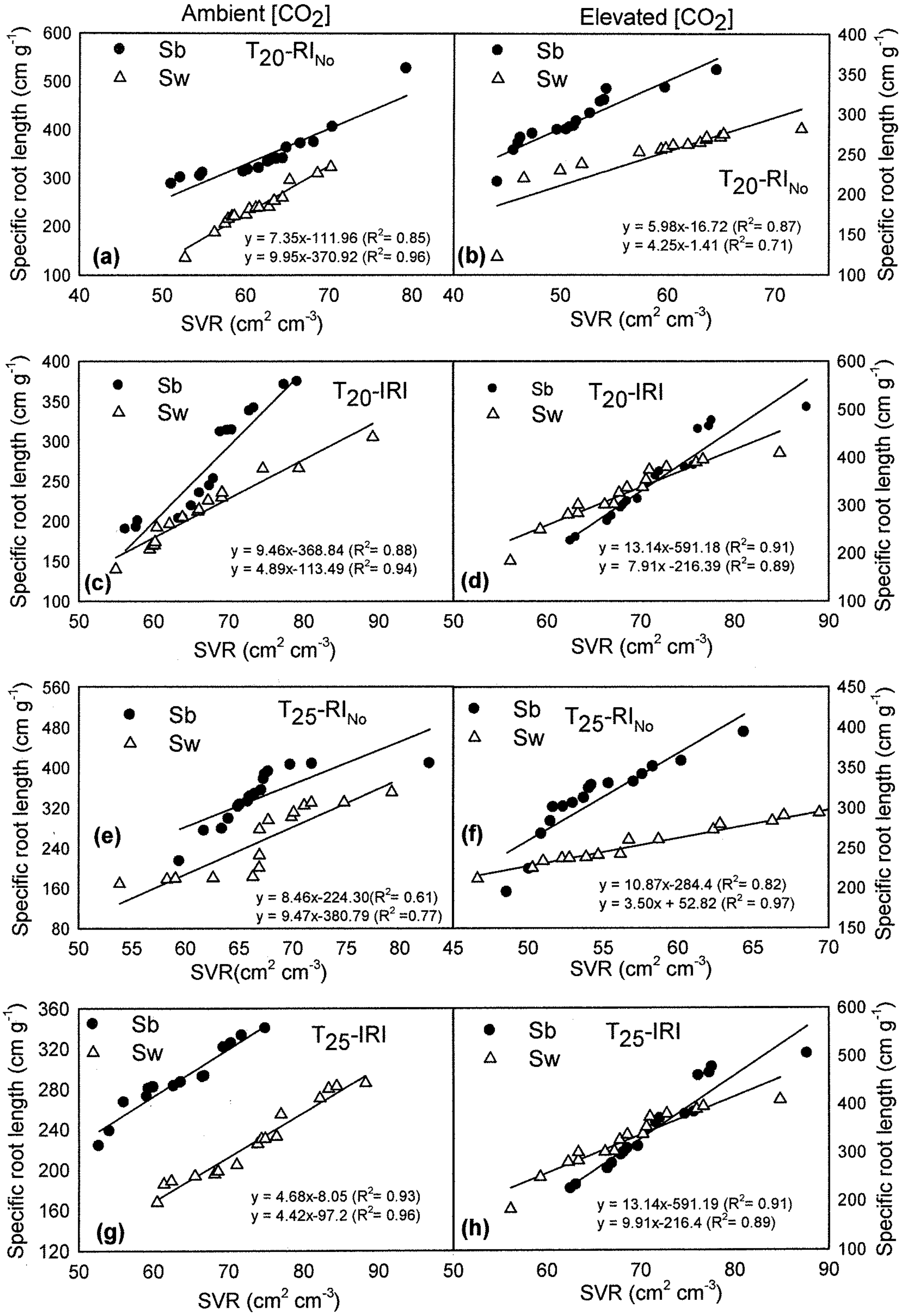

Fig. 5.9: Relationship between specific root length and root surface to volume ratio (SVR) of black spruce and white spruce seedlings.

Discussion

The effects of CO_2 elevation and soil warming on growth, biomass production and root morphology of black spruce and white spruce were generally additive and not interactive. With the exception of height growth and the ratio of root length to leaf area (RLA) where $[CO_2]$ and temperature effects were interactive, their effects on the other parameters were either not significant or additive. For instance, CO_2 elevation and the warming treatment influenced total biomass, leaf mass, shoot mass ratio (SMR), root to shoot ratio (RSR) root branching intensity (RBI) but the effects were non-interactive. It appears from this study and some previous studies that CO_2 and soil temperature effects on boreal trees are interactive only when soil temperature is limiting or below a species threshold. For example, in two previous studies (Zhang and Dang 2007, Ambebe et al. 2010) that have reported significant CO_2-soil temperature interactive effects, there were no significant treatment differences in temperatures between 17-27 °C, with differences occurring only at temperatures below 10 °C. In the physiological measurements (Chapter 6), the effects of CO_2 and soil temperature on gas exchange were also generally additive but not interactive. It is therefore reasonable to conclude that at soil temperatures above the species-specific threshold, the effects of both CO_2 and warming on growth, biomass and root characteristics of black spruce and white spruce, are additive and not interactive. The temperature threshold for both species is less than 15 °C (Cheng 2009).

The hypothesis that CO_2 elevation and soil warming would enhance the growth, biomass and resource acquisition root traits of black spruce to a greater degree than in white spruce is partly supported. Both the CO_2 elevation and soil warming (25 °C) increased height growth in both species (with root interaction) but the degree of enhancement was greater in black spruce than in white spruce. The CO_2 elevation also increased total biomass, leaf and root mass more in black spruce than in white spruce. Furthermore, total biomass, leaf mass were enhanced by the warming treatment to a greater extent in black spruce than in white spruce. As discussed previously, the effects of soil temperature and CO_2 were however, mostly non-interactive. CO_2 and soil temperature appeared to have opposite effect on the species root branching intensity (RBI). With the presence of root interaction, the CO_2 elevation significantly increased RBI in black spruce. However, with warming and root interaction, RBI was marginally decreased in black spruce but increased marginally in white spruce. The higher leaf mass, shoot mass ratio, number of root forks, total root length, specific root length and specific root surface area of black spruce contributed to greater photosynthetic rate in black spruce (Chapter 6), which led to the increased height growth and biomass production.

One key trait for successful nutrient exploration and uptake is the specific root length (Eissenstat 1991, Eissenstat 1992, Atkinson 2000, Pregitzer et al. 2002, Ostonen et al. 2007), with high SRL generally leading to increased nutrient exploration and uptake. However, to further increase root absorption capability, plants need to also maximise their surface to volume ratio (SVR) (Paula and Pausas 2011). Plants which are able to increase and coordinate both SRL and SVR maximise their root-soil interface to increase their root absorption potential for the same carbon investment (Larcher 2003, Paula and Pausas 2011). This relationship was positive for both species, with black spruce generally having greater slope especially at elevated [CO_2]. This may indicate greater nutrient absorption potential in black spruce than in white spruce. However, both foliar nitrogen and phosphorus concentrations were generally greater in white spruce than in black spruce, with potassium was greater in black spruce than in white spruce (Chapter 6). Hence, other factors and traits in addition to SRL and SVR determine actual nutrient absorption rate. The actual nutrient absorption potential of the two species was however, not measured.

Responses of seedlings grown with root interaction cannot be completely inferred from the results of plants that are individually grown. However, this is species and resource dependent. Height growth, total biomass, leaf and root mass were greater in seedlings grown without root interaction than with root interaction. On the contrary, specific root length, specific root surface area and root branching intensity were on average greater in seedlings grown with inter-plant root interaction. Biomass allocation to roots was also significantly greater when the seedlings were grown with root interaction than without root interaction. Further, the ratio of root length to leaf area was generally higher with than without root interactions. These findings support the hypothesis that plants alter their root growth in the presence of neighbouring plants Gersani et al. (2001). It also supports the finding that the response of plants grown in isolating differs from that of the same species in mixture (Navas 1998). Further, the results reflect differences in strategy between seedlings grown individually without inter-plant root interaction and seedlings with root interaction. When the different seedlings shared the growing medium with neighbours, the strategy was geared towards nutrient acquisition. However, for the seedlings grown without belowground neighbour, no perceived belowground competition was sensed, hence, the strategy was rapid shoot growth which resulted in greater height growth and biomass production. Plants allocate greater biomass to organs responsible for the acquisition of limited resources (Shipley and Meziane 2002). The greater relative biomass allocation to roots when the seedlings were grown together indicate that nutrient was more limited with the presence of inter-plant root interaction. The greater SRL in white spruce grown with root interaction in relation to white spruce grown in isolation may be a strategy to acquire more nutrient since high SRL increases nutrient uptake (Eissenstat 1991, Eissenstat 1992, Ryser and Lambers 1995, Ryser and Eek 2000). In most controlled experiments, seedlings are grown without plant-plant interaction and that may significantly affect interpretation of results. In modelling the response of black spruce and white spruce to future[CO_2] and soil warming, the effects of inter-plant root interactions need to be considered because they modify response variables in both direction and magnitude.

Root length to leaf area ratio determines the balance between water and nutrient uptake capacity and their utilisation in transpiration and photosynthesis (Körner and Renhardt 1987, Fitter and Hay 2002). RLA also indicates the relative sizes of the organs responsible for above and belowground resource capture (Tani et al. 2003) and it increases with water and nutrient stress (Boot and Mensink 1990, Boot and Mensink 1991, Aref and El-Juhany 1999, Tani et al. 2003). The increase in the ratio with resource limitation is necessary to obtain more of the limiting resource to better support aboveground transpiration and photosynthetic activity. Though root to shoot ratio provide similar information as RLA does, RLA is more precise because water and nutrient absorption is based more on surface area (root length) than on mass (Eissenstat 1991, Tani et al. 2003). In this study, white spruce grown with root interaction maintained a higher RLA at both temperature and CO_2 treatments, an indication that in comparison with black spruce, nutrient was more limiting in white spruce when grown together with black spruce. The CO_2 elevation however, increased RLA in black spruce grown with root interaction in the 20 but not in the 25 °C temperature treatment. The species differences in the RLA further support the earlier claim that white spruce may have higher nutrient demand than black spruce and may further be an indicator of greater competitiveness in black spruce.

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CHAPTER 6: Soil warming did not alter photosynthetic responses of black spruce and white spruce seedlings to CO_2 elevation and plant-plant root interaction

Introduction

Soil temperature is a key factor that influences photosynthesis and tree growth in the boreal forest (Delucia 1986, Bonan and Van Cleve 1992, Bergh and Linder 1999, Dang and Cheng 2004, Ambebe et al. 2010, Frechette et al. 2011). Soil temperature also affects other plant's physiological traits such as stomatal conductance, transpiration rate, water and nutrient uptakes and resource use efficiencies (Lopushinsky and Max 1990, Schwarz et al. 1997, Freckleton and Watkinson 2001, Dang and Cheng 2004, Lukac et al. 2010). The impact of soil temperature on spruce photosynthetic rate is generally higher than air temperature effects (Schwarz et al. 1997, Lupi et al. 2012, Wu et al. 2012).

Temperatures are predicted to rise as atmospheric carbon dioxide concentration rises (IPCC 2001, Christensen et al. 2007). The effects of increased atmospheric [CO_2] and warming are likely to be higher in the boreal region (Christensen et al. 2007). While global temperatures are predicted to rise by 1.4–5.8 °C by the end of this century (IPCC 2001), models predict this may be as high as 5-7 °C in the Canada's boreal region (Christensen et al. 2007, Eskelin et al. 2011). Though the boreal forest is presently characterised by low soil temperatures (Domisch et al. 2002), there is evidence that the region has been warming up faster than other regions on earth (Serreze et al. 2000). Increased atmospheric [CO_2] coupled with warmer temperatures may change species composition, competitive ability and resource use (Stewart et al. 1998). In addition to the direct effects of CO_2 elevation and warming, climate change indirectly affects forest communities through plant-to-plant interactions for resources such as nutrients and moisture (Colombo 1998).

Black spruce (*Picea mariana* (Mill). B.S.P) and white spruce (*Picea glauca* (Moench) Voss) are widely distributed conifer species in the boreal forest (Nienstaedt and Zasada 1990, Sims et al. 1990, Viereck and Johnston 1990, Haavisto and Jeglum 1995, Way and Sage 2008a). The Arctic Climate Impact Assessment (ACIA) climate models suggest that the rapid warming of the boreal forest may not allow the growth of commercially valuable white spruce while the spread of black spruce in western Canada's boreal forest may be reduced on poor sites (Juday et al. 2005). On the other hand, the increased in air and soil temperature and [CO_2] can extend the growing season for the species and increase their photosynthetic rate and biomass production as long as there is enough nutrients and moisture (Strömgren and Linder 2002).

Black spruce grows on both cold peatlands and upland sites while white spruce grows only on upland sites (Grossnickle and Blake 1985, Nienstaedt and Zasada 1990, Viereck and Johnston 1990, Grossnickle 2000, Wolken et al. 2011). Root growth of both species increase rapidly at soil temperatures above 10 °C (Grossnickle 2000). Under field conditions, soil temperatures between 19 and 21 °C are considered optimal for the growth of black spruce and white spruce seedlings (Heninger and White 1974, Tryon and Chapin 1983, Grossnickle and Blake 1985, Odlum and Ng 1995). A soil temperature of 22 °C (black spruce) and 21 °C (white spruce) have been reported as the optimum for maximum photosynthetic rate of the two species (Dang and Cheng 2004). A soil temperature of 14.1 °C is the reported threshold for black spruce photosynthetic activity, below which photosynthesis rapidly declines or ceases (Cheng 2009).

Maximum nutrient uptake in spruce occurs at 20 °C soil temperature and higher temperatures can reduce root uptake of nitrate and ammonium (Gessler et al. 1998).

The effects of soil temperature (Chalupa and Fraser 1968, Cleve et al. 1990, Bergh and Linder 1999, Cai and Dang 2002, Peng and Dang 2003, Dang and Cheng 2004, Lahti et al. 2005, Zhang and Dang 2007, Way and Sage 2008a, Bronson et al. 2009, Cheng 2009) and CO_2 (Brown and Higginbotham 1986, Tjoelker et al. 1998, Murray et al. 2000, Bigras and Bertrand 2006, Zhang and Dang 2007, Dang et al. 2008, Marfo and Dang 2009) on spruce growth, biomass and photosynthetic rate are well studied. However, currently, only one study (Zhang and Dang 2007) has examined the interactions between CO_2 and soil temperature on boreal black spruce and white spruce. And at present, no published study has addressed the effects of plant-plant root interaction on physiological responses of the two species in response CO_2 and soil temperature, despite the fact that the species can form mixed-wood in nature. Belowground interactions are important because they account for significant changes in above ground tree response to the environment (Casper and Jackson 1997). This study attempts to address some of the knowledge gaps. Specifically, this study was aimed at evaluating the effects of [CO_2] and soil temperature on gas exchange and foliar nutrient status of black spruce and white spruce seedlings grown with and without inter-plant root interaction.

The seedlings were exposed to two CO_2 concentrations (380 and 720 μmol mol^{-1}) and two soil temperature regimes (20 and 25 °C) either without inter-plant root interactions or with root interactions. The 20 °C temperature mimics the current most suitable soil temperature for the species while the 5 °C warming increment was based on temperature projections for the boreal region (Christensen et al. 2007, Eskelin et al. 2011). It was hypothesized that CO_2 elevation and soil warming would collectively enhance photosynthetic rate in both species more than each factor alone. Black spruce is believed to be more plastic than white spruce in acclimating to soil temperature because it is able to grow on both peatlands and uplands. It was therefore expected that black spruce would benefit more from warming than white spruce. Nutrient demands of the two species differ (Nienstaedt and Zasada 1990, Viereck and Johnston 1990) and nutrient extraction and use capabilities are generally improved when roots of different species interact in non-exploitative manner (Richards et al. 2010). It was therefore hypothesized that the presence of root interactions would enhance seedling overall water and nutrient use efficiencies.

Materials and Methods

Plant Materials, Experimental Design and Growth Conditions

Container nursery stock of one year old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) seedlings were obtained from a local commercial tree nursery (Hill Tree Nursery, Thunder Bay). The study was conducted at Lakehead University's Thunder Bay Campus in four greenhouses with identical design. Seedlings selected for treatment were approximately of the same size and form. The growing medium was a 50:50 (volume/volume) mixture of peat moss and vermiculite.

The experimental design was split-split-split plot with CO_2 concentration as the main plot, soil temperature (T) as the sub-plot, and root interaction (RI) as the sub-sub plot, which was further split into two units. Position of seedlings of each species was completely randomised in RI treatment . There were two CO_2 levels (ambient, 380 and elevated, 720 μmol mol^{-1}) with two replications each, two soil temperature regimes (20 and 25 °C) within each CO_2 treatment, two levels of inter-plant root interactions (no root interaction RI_{NO} vs. inter-plant root interactions-IRI) and two species (black spruce and white spruce). Electronic ignition CO_2 burners (model GEN-2E, Custom Automated Products, Inc, Riverside, CA) were used to generate CO_2 in the elevated greenhouses. The level of CO_2 in each greenhouse was monitored and automatically adjusted using Argus CO_2 sensors and control system (Argus, Vancouver, BC, Canada).

Within each CO_2 level, there were two daytime soil temperatures (20 and 25 °C). Each day-time soil temperature was lowered by about 4-5 °C at night. .Because soil temperature change is slower than changes in air temperature, switching between day and night soil temperatures occurred an hour before greenhouse air temperature was changed. Each soil temperature was independently controlled using a soil temperature control system consisting of a large leak-proof box (196 x 112 x 16 cm, L x W x D). Temperature-controlled water was circulated in the space between the planting containers to achieve the target soil temperature. For uniform temperature distribution within the system, each control unit was equipped with two circulatory pumps (model AC-2CP-MD, March Mfg. Inc., Glenview, Illinois, USA) to distribute the temperature-controlled water. The system was insulated to minimise heat exchange with air.

For each CO_2-temperature treatment, eight seedlings of each species were grown in each of the root interaction treatments (RI). "No inter-plant root interaction (RI_{No})," indicates where each seedling was grown in an individual container, while with "the presence of inter-plant root interaction", (IRI), all the seedlings were grown in a large container and were spaced at about the diameter of the container in the no root interaction treatment. In the RI_{No} treatment, the seedlings were grown in individual containers with soil volume of about 1769.38 cm^3. In IRI the treatment, eight seedlings of each species were grown together in a large container to allow for inter-plant root interactions (16 per pot). Drain-through stainless steel threaded nipples with washers were used to fix the pots to the temperature box. Further details of the soil temperature protocol is in Cheng et al. (2000) for details on system construction). A total of 256 seedlings were planted.

Argus control system was used to control environmental conditions in the greenhouses as follows: relative humidity of 55 ± 5%, day and night air temperatures of 25 ± 2 °C and 15 ± 2 °C respectively. Plants received natural light averaged 660 μmol $m^{-2}s^{-1}$,which was supplemented on cloudy days and early evenings with high pressure sodium vapour lamps for 17 hour photoperiod. Fertiliser was applied weekly with nutrient solution of 150 mg N l^{-1} water, 60 mg P l^{-1}, 150 mg K l^{-1}, 80 mg Ca l^{-1}, 40 mg Mg l^{-1} and 60 mg S l^{-1}. Plants were watered between fertiliser applications.as required based on physical observation and probe measurements using HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U. K.).

Gas exchange measurements

Leaf gas exchange measurements were carried out 4 months into the treatment. Three seedlings per treatment combination (total of 96) were randomly selected for the measurements. The measurements were done on current year needles using Ciras-1 open gas exchange system (PP-Systems, Amesbury, MA, USA) with a Parkinson conifer leaf cuvette. A cuvette temperature (25 °C) and relative humidity (50%) were maintained during the measurements. Measurements were done at saturating photosynthetic active radiation (PAR) of 800 μmol m^{-2} s^{-1}. The light was supplied from the cuvette's built-in tungsten lamp. A-C_i curves were measured sequentially at 50, 150, 250, 380, 500, 720, 900 and 1200 μmol mol^{-1} [CO_2]. Net photosynthetic rate (P_n, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance to water vapour (g_s)

presented are from the corresponding growth [CO_2]. Photosynthetic water use efficiency (WUE, μmol CO_2 mmol H_2O^{-1}) was calculated as: WUE = P_n/E. The analysis of the A–Ci response curves involved the calculation of parameters that may potentially limit photosynthesis: J_{max}, the RuBP regeneration rate mediated by maximum electron transport rate; V_{cmax}, the maximum carboxylation capacity of ribulose-1,5-bisphosphate carboxylase/oxygenase, and TPU, the rate of triose phosphate utilization. TPU is a major indicator of inorganic phosphate availability in the Calvin cycle and photosynthate translocation from leaf to sinks. This was done using the curve-fitting utility proposed by (Sharkey et al. 2007). All the parameters were expressed on a projected leaf area basis. Leaf area was determined using the Regent WinSeedle system (Regent Instruments, Québec City, QC, Canada).

Foliar nutrient analyses

After the gas exchange measurements, the three seedlings used for the measurements were harvested to determine projected leaf area, specific leaf area (SLA) and foliar nutrient concentration. The same needles used for gas exchange measurements were used in the determination of SLA and nutrient concentration. The needles were oven-dried at 70 °C for 48 hours and weighed to determine SLA. Needle total nitrogen (N) concentration was determined using the dry combustion method with LECO CNS-2000 analyser (LECO, St. Joseph's Michigan USA). Foliar phosphorus (P) and potassium (K) concentrations were determined by the acid digestion method and the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) technique. Photosynthetic nitrogen and phosphorus use efficiencies (PNUE and PPUE) were respectively calculated as P_n/N and P_n/P.

Statistical analysis

The data were analysed using the mixed model analysis of variance (ANOVA) procedure in GenStat version 12 (VSN International, Hemel Hempstead, UK). Block/CO2/Tem/RI/S was used as the block structure. No data transformation was necessary as all data met normality (Shapiro Wilk) and homogeneity (Bartlett) assumptions. Post-hoc mean comparisons were carried out with Fisher's Least Significant Difference (LSD). Post-hoc tests were made based on the rule that significant interactions override main effects and higher order significant interactions override lower level interactions. Simple linear regression was used to model the relationship between net photosynthesis and foliar nutrient concentration.

Results

Net photosynthetic rate, transpiration rate and water use efficiency

Net photosynthetic rate (P_n) of seedlings at the corresponding growth [CO_2] was about 78% ($P \leq 0.047$) higher at elevated than at ambient [CO_2] [CO_2] (Table 6.1, Fig. 6.1). CO_2 elevation resulted in photosynthetic up-regulation (Fig. 6.1). P_n of seedlings grown at elevated but measured at ambient [CO_2] was 10% higher than P_n of seedlings grown and measured at ambient [CO_2] (Fig. 6.1). There was no interaction between [CO_2] and the other treatments. The two species P_n responded differently to soil temperature ($P<0.001$, Table 6.1). P_n of white spruce was significantly lower than of black spruce at the 20 °C treatment but the difference became insignificant at the 25 °C warming treatment (Fig. 6.2). Moreover, the P_n of black spruce did not differ with soil warming (Fig. 6.2).

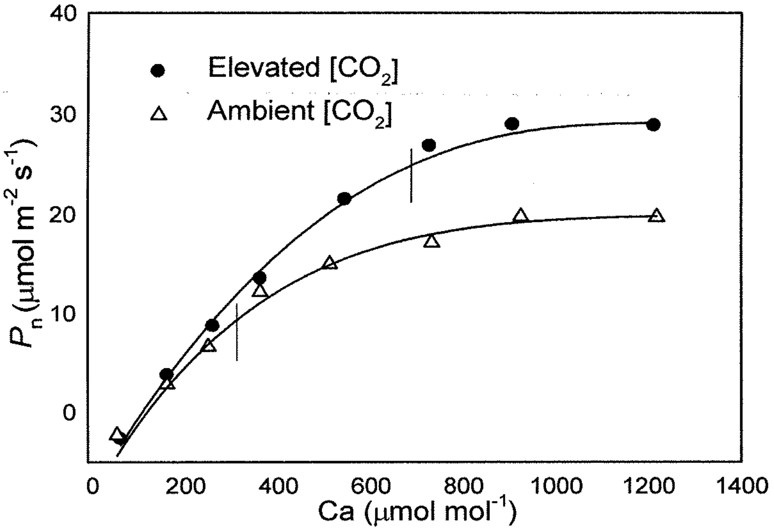

Fig. 6.1: Effects of CO_2 on net photosynthetic rate of black spruce and white seedlings after 4 months of treatment. Each point on the curve is the mean of 6 measurements.

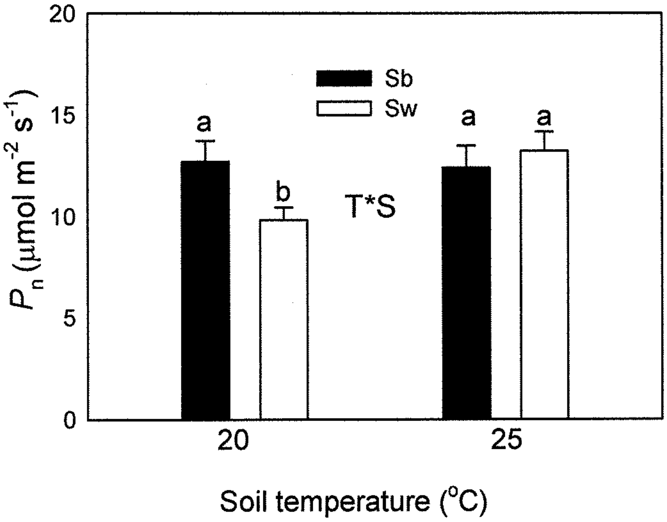

Fig. 6.2: Effects of soil temperature (T) and species (S) on net photosynthetic rate at growth [CO_2] -P_n of black spruce and white spruce seedlings after 4 months of treatment (*n=24*). The seedlings were exposed to two [CO_2], two temperatures at two levels of root interaction. Each bar represents mean + s.e.m and bars with different letters differ significantly (*$P \leq 0.05$*).

Table 6.1: *P* values from ANOVA on the effects of [CO_2], soil temperature (T), inter-plant root interaction (RI) and species (S) on physiological characteristics of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were exposed to two CO_2 concentrations (380 and 720 μmol mol^{-1}) with and without inter-plant root interactions at two soil temperatures.

Factor	P_n	E	**Gs**	**WUE**	J_{max}	V_{cmax}	**TPU**
CO_2	**0.047**	0.782	0.498	**0.050**	0.374	0.440	**0.023**
T	0.172	0.810	0.779	0.057	0.560	0.725	0.356
CO_2*T	0.329	0.437	0.328	0.067	0.889	0.934	0.959
RI	0.254	**0.015**	0.108	**0.014**	**0.011**	0.082	**0.010**
CO_2*RI	0.410	0.491	0.537	**0.010**	0.154	0.348	0.092
T*RI	0.810	0.556	0.901	0.613	0.153	0.321	0.131
CO_2*T*RI	0.600	0.376	0.760	0.166	0.754	0.955	0.544
S	0.059	**0.020**	0.129	0.446	0.390	0.473	0.458
CO_2*S	0.073	0.152	0.660	0.926	0.803	0.640	0.649
T*S	**<0.001**	0.226	0.748	0.458	**0.043**	0.346	**0.037**
RI*S	0.129	**0.006**	0.124	0.410	0.294	0.546	0.119
CO_2*T*S	0.298	0.120	0.216	0.530	0.860	0.358	0.708
CO_2*RI*S	0.266	0.203	0.941	0.623	0.509	0.367	0.619
T*RI*S	0.758	0.920	0.167	0.606	0.282	0.362	0.528
CO_2*T*RI*S	0.460	0.055	0.222	0.316	0.131	0.094	0.326

Transpiration rate (*E*) of the two species responded differently (*P≤0.006*) to root interactions (Table 6.1). The presence of root interactions significantly reduced transpiration rate in white spruce and no other significant difference occurred (Fig. 6.3a). None of the treatments had significant effect on stomatal conductance (G_S, Table 6.1).

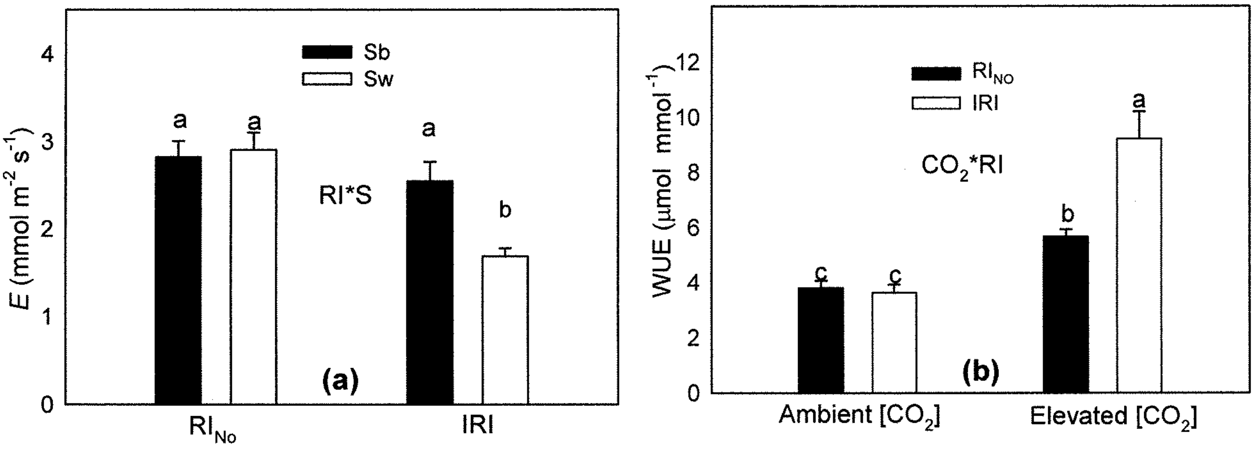

Fig. 6.3: Effects of [CO2], species (S) and root interaction (RI) on seedlings transpiration rate, *E* (***a***) and photosynthetic water use efficiency, WUE (***b***), of black spruce and white spruce seedlings after 4 months of treatment (*n=24*).

Photosynthetic water use efficiency (WUE) was significantly (*P≤0.010*) affected by interactions between CO_2 and root interactions (Table 6.1). The CO_2 elevation significantly enhanced WUE in all seedlings but the level of enhancement was significantly greater with root interaction that without root interaction (Fig. 6.3b). Root interaction significantly increased WUE under elevated but not at ambient [CO2] (Fig. 6.3b).

Electron transport, carboxylation capacity and triose-phosphate utilisation

The maximum photosynthetic electron transport capacity ($J_{\max}$) of the two species responded differently to soil temperature (*P≤0.043*, Table 6.1). Soil warming significantly increased $J_{\max}$ in white spruce but had no significant effect on black spruce. $J_{\max}$ was significantly lower in white spruce than in black spruce at the 20 °C treatment but the difference became insignificant at the 25 °C (Fig. 6.4a). The presence of root interactions significantly reduced $J_{\max}$ by 40% (*P≤0.011*, Table 6.1). [CO$_2$] had no significant (*P=0.374*) effect on $J_{\max}$ (Table 6.1). None of the treatments had significant effect on the maximum carboxylation capacity of (V_{cmax}, Table 6.1).

The CO$_2$ elevation significantly (*P≤0.023*) increased triose-phosphate utilisation (TPU) by 10% (Table 6.1). The presence of root interaction significantly reduced TPU by 40% (*P≤0.010*, Table 6.1). TPU of the two species responded differently to soil warming (*P≤0.037*, Table 6.1) and the pattern of response was similar to those of P_n and $J_{\max}$ (Fig. 6.4b).

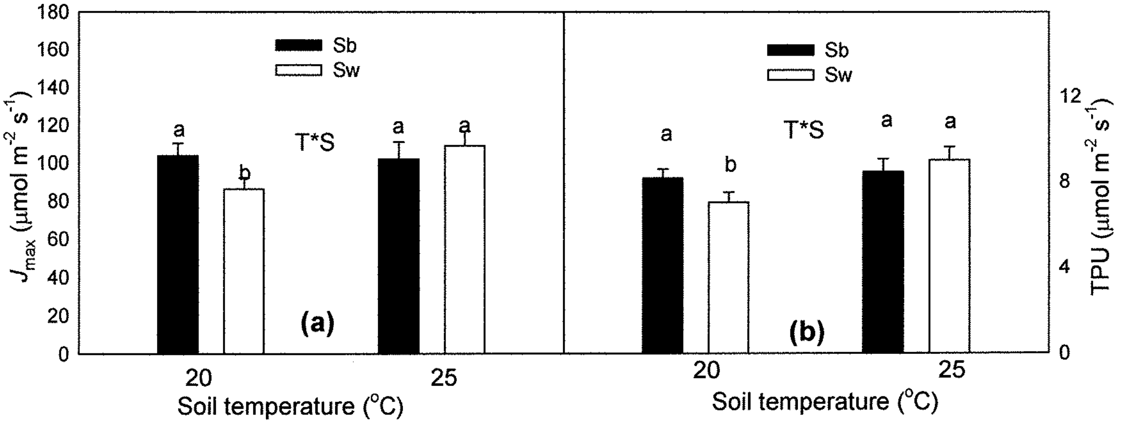

Fig. 6.4: Effects of soil temperature (T) and species (S) on seedlings maximum photosynthetic electron transport capacity-$J_{\max}$ (***a***), and triose phosphate utilisation-TPU (***b,***) of black spruce and white spruce seedlings after 4 months of treatment (*n=24*).

Specific leaf area

CO_2, RI and S had a significant (*$P \leq 0.012$*) three-way interactive effect on specific leaf area (SLA, Table 6.2). At ambient [CO_2], SLA was significantly higher in black spruce than in white spruce irrespective of the RI treatment but the mean SLA was greater in seedlings grown without root interaction (Fig. 6.5). At the elevated [CO_2] however, the presence of root interaction significantly reduced SLA of black spruce and the effect on white spruce became insignificant (Fig. 6.5). Furthermore, the significant difference in SLA between black spruce and white spruce with root interaction under the ambient [CO_2] became statistically insignificant at the elevated [CO_2] (Fig. 6.5). Soil temperature had no significant *($P=0.137$)* effect on SLA (Table 6.2).

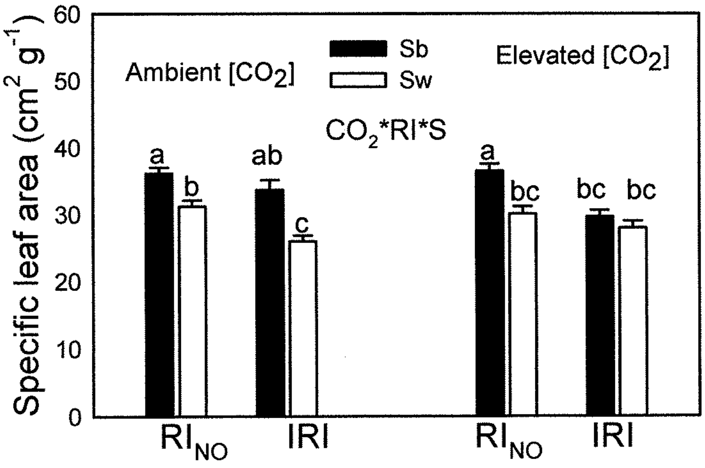

Fig. 6. 5: Effects of CO_2, root interactions (RI) and species (S) on specific leaf area (SLA) of black spruce and white spruce seedlings (*$n=12$*).

Table 6. 2: *P* values from ANOVA on the effects of [CO_2], soil temperature (T), inter-plant root interaction (RI) and species (S) on foliar nutrient concentration, nutrient use efficiency and specific leaf area (SLA) of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were exposed to two CO_2 concentrations (380 and 720 μmol mol^{-1}) with and without below-ground interactions at two soil temperature levels for 4 months.

Factor	**SLA**	**[N]**	**[P]**	**[K]**	**N/P**	**PNUE**	**PPUE**
CO_2	0.646	0.237	0.788	0.371	0.087	**0.004**	**0.025**
T	0.137	0.715	0.509	0.600	0.695	0.631	0.623
CO_2*T	0.948	0.450	0.316	0.174	0.905	0.515	**0.050**
RI	**0.007**	0.087	**0.046**	0.168	0.080	0.948	0.321
CO_2*RI	0.696	0.136	0.217	0.862	0.456	0.971	0.472
T*RI	0.868	0.143	0.819	0.349	0.070	0.338	0.684
CO_2*T*RI	0.209	0.806	0.648	0.626	0.490	0.694	0.386
S	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CO_2*S	0.133	0.300	**0.015**	0.516	**0.001**	**<.001**	**<.001**
T*S	0.075	0.766	0.402	0.784	0.745	**<.001**	**<.001**
RI*S	0.502	**0.025**	0.325	0.637	**<0.001**	0.063	0.063
CO_2*T*S	0.454	0.853	0.209	0.617	0.170	0.093	0.192
CO_2*RI*S	**0.012**	0.296	**0.046**	0.222	0.102	0.176	**0.003**
T*RI*S	0.944	0.398	0.440	0.296	0.435	0.279	**0.013**
CO_2*T*RI*S	0.999	0.796	0.967	0.824	0.146	0.974	**0.044**

Foliar Nutrient concentrations

Neither CO_2 ($P=0.237$) nor soil temperature ($P=0.715$) had significant effect on foliar nitrogen concentration ([N], Table 6.2). The two species differed in their foliar [N] in response to root interactions ($P\leq0.025$, Table 2). Foliar [N] was significantly higher in white spruce than in black spruce when there was no root interaction but there was no such difference when root interaction was significant (Fig. 6.6a). The presence of root interaction significantly reduced the foliar [N] of white spruce but root interaction did not affect [N] of black spruce (Fig. 6.6a).

Foliar phosphorus concentration ([P]) was affected by interactions among CO_2 x RI x S interactions ($P\leq0.046$, Table 6.2). At ambient [CO_2], [P] was significantly higher in white spruce grown without root interaction than in the other treatment combinations (Fig. 6.6b). At the elevated [CO_2], the presence of root interaction significantly increased foliar [P] in white spruce but there was no difference between [P] of white spruce grown with and without root interaction (Fig. 6.6b). There was no difference in black spruce foliar [P] at the various treatment combinations (Fig. 6.6b). Soil temperature had no significant ($P=0.509$) effect on foliar [P] (Table 2).

With the exception of species differences ($P<0.001$), no other factor significantly affected needle potassium concentration ([K], Table 6.2). Black spruce had 20% higher [K] than white spruce (Fig. 6.6c).

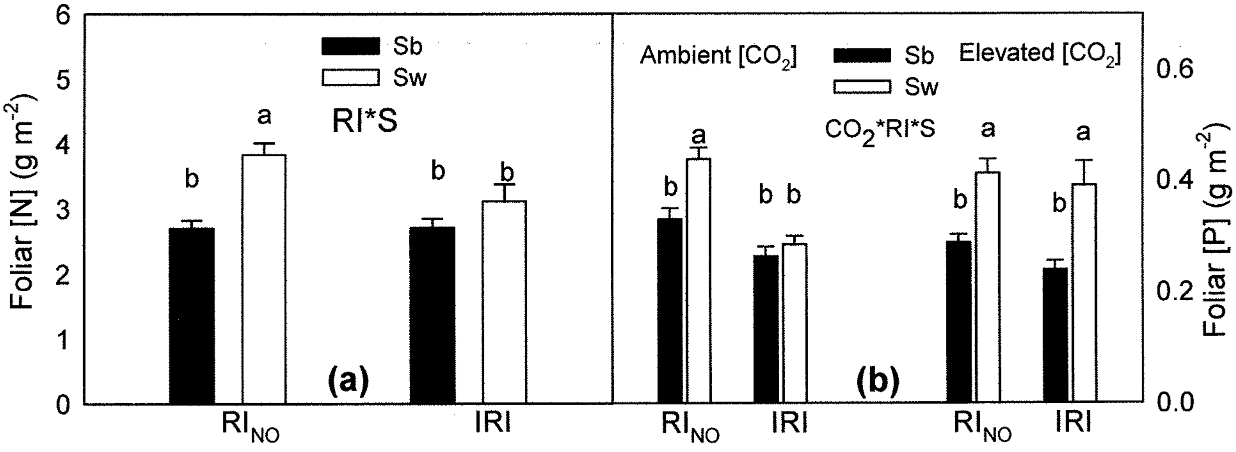

Fig. 6.6: Effects of [CO2], soil temperature (T), species (S) and inter-plant root interaction (RI) on foliar nitrogen concentration-[N] (*a*, *n=24*) and phosphorus concentration-[P] (***b***, *n=12*) of black spruce and white spruce seedlings after 4 months of treatment.

The two species responded differently to CO_2 concentration ($P \leq 0.001$) in their N/P ratios (Table 6.2). At ambient the [CO_2], there was no significant difference in the N/P ratio of two species (Fig. 6.7a). However, CO_2 elevation significantly increased the ratio in black spruce but not in white spruce (Fig. 6.7a). The N/P of the two species also responded differently to root interactions ($P<0.001$, Table 6.2). The two species had similar N/P ratio when they grew without root interaction (Fig. 6.7b). However, the presence of root interaction significantly increased N/P in black spruce (Fig. 6.7b).

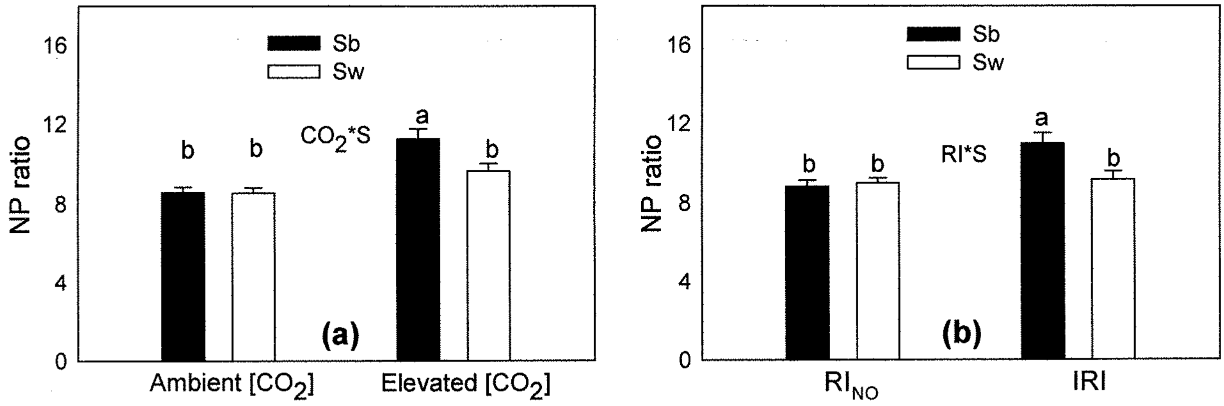

Fig. 6.7: Effects of [CO_2], species (S and) root interaction (RI) on nitrogen: phosphorus (N/P) ratio of black spruce and white spruce seedlings (*n=24*).

Relationship between nutrient concentration and photosynthesis

Net photosynthetic rate of the two species positively correlated with N, P, K concentrations and NP ratio (Figs. 6.8a-6.8d). The slopes of the P_n-nutrient relationships were all significant ($P<0.001$), and the co-efficient of determination ranged from 2 to 54% when data were analysed separately for each species (Figs. 6.8a-6.8d). The slopes were significantly higher in black spruce than in white spruce except the P_n-P relationship where white spruce had significantly higher slope (Fig. 6.8b). P_n in black spruce correlated better with N/P ratio than with either [N] or [P] while that of white spruce positively correlated better with [N] than with N/P ratio. P_n of both species positively correlated with [K], with R^2 of 36 and 60% respectively for black spruce and white spruce (Fig. 6.8c).

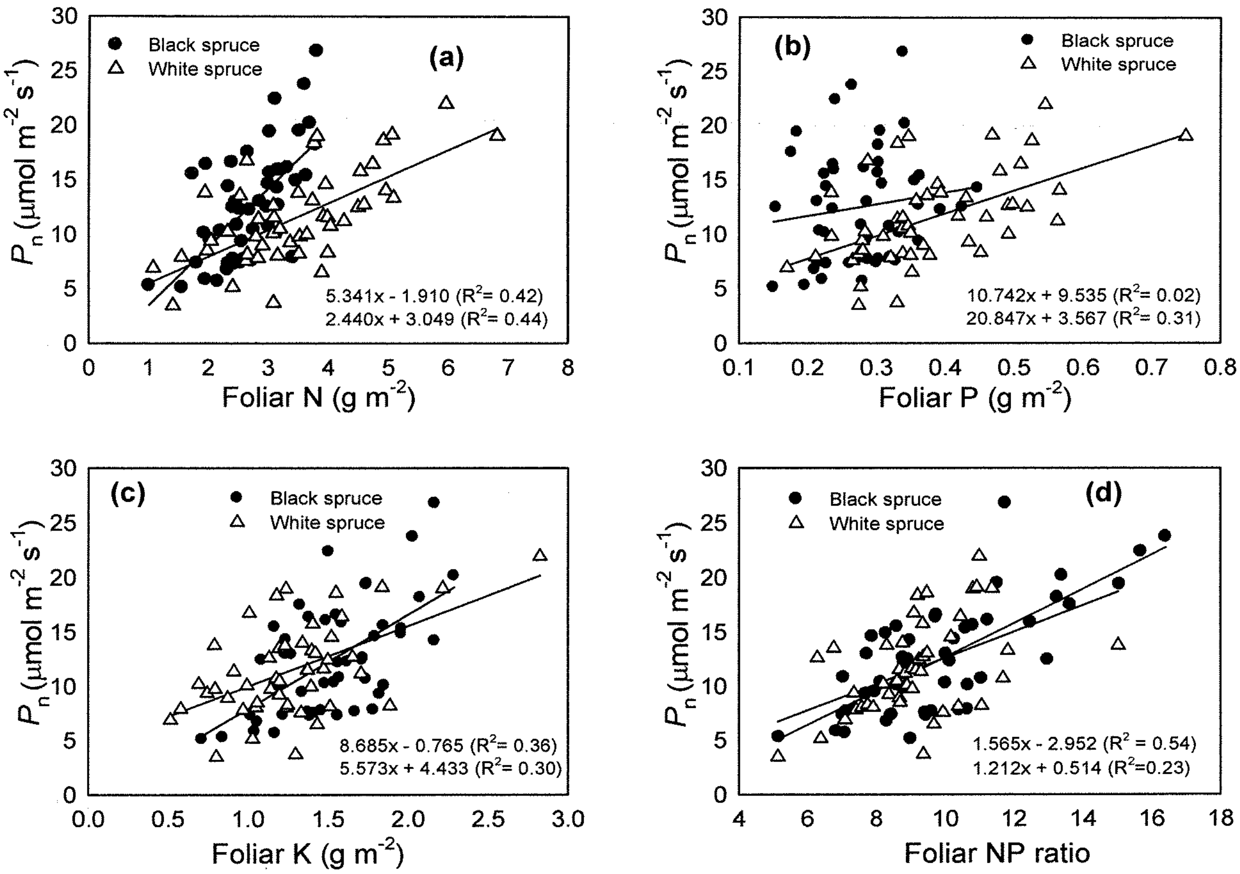

Fig. 6.8: Relationship between foliar nitrogen-[N], phosphorus-[P], potassium-[K], N/P ratio and net photosynthetic rate-P_n, of black spruce and white spruce seedlings after 4 months of treatment. The seedlings were exposed to two [CO$_2$] and two soil temperatures with and without plant-plant root interaction for 4 months.

Nutrient use efficiencies

Photosynthetic nitrogen use efficiency (PNUE) of the two species responded differently to CO_2 (*$P<0.001$*, Table 6.2). Under ambient [CO_2], the two species had similar PNUE (Fig. 6.9a). However, CO_2 elevation significantly enhanced PNUE in black spruce but not in white spruce (Fig.6.9a). Furthermore, PNUE of white spruce did not show significant response to CO_2 elevation (Fig. 6.9a). PNUE of the two species also responded differently to soil warming (*$P<0.001$*, Table 6.2). The 20 °C temperature treatment significantly reduced PNUE in white spruce but not in black spruce and there was no other significant difference (Fig. 6.9b). Root interactions had no significant (*$P=0.948$*) effect on PNUE (Table 6.2).

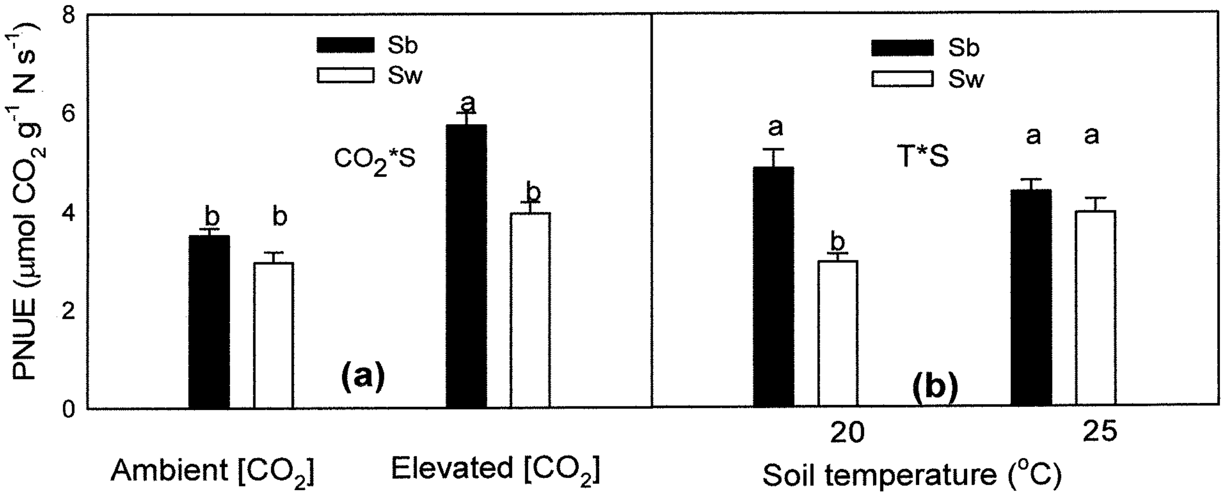

Fig 6.9: Effects of [CO_2], soil temperature (T), species (S) and root interaction (RI) on photosynthetic nitrogen use efficiency-PNUE of black spruce and white spruce seedlings after 4 months of treatment (*$n=24$*).

There was a significant (*P≤0.044*) four-way interactive effect on photosynthetic phosphorus use efficiency (PPUE, Table 6.2). At ambient [CO_2], PPUE was generally low and did not differ between treatments (Fig. 6.10). The CO_2 elevation significantly increased PPUE in black spruce but not in white spruce and soil warming significantly reduced PPUE in black spruce with root interaction (Fig. 6.10). Furthermore, white spruce PPUE did not differ with any treatment (Fig. 6.10).

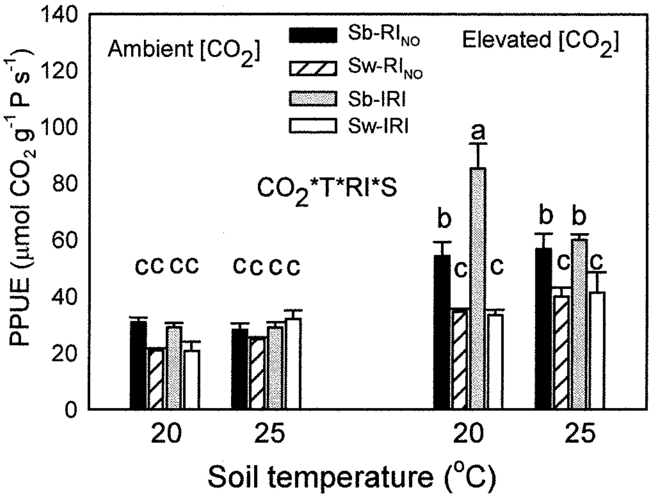

Fig. 6.10: Effects of [CO_2], soil temperature (T), species (S) and root interaction (RI) on photosynthetic phosphorus use efficiency-PPUE of black spruce and white spruce seedlings after 4 months of treatment (*n=6*).

Discussion

The 5 °C soil warming did not alter the responses of photosynthesis to the CO_2 elevation in black spruce and white spruce. The effects of CO_2 and soil temperature on net photosynthetic rate (P_n) in the two species were independent of each other, i.e., there was no significant interaction between them. The CO_2 elevation increased P_n by about 78% but independent of soil temperature. The initial hypothesis that CO_2 elevation and warming would synergistically enhance photosynthetic rate was therefore not supported. The current findings are inconsistent with the work of Zhang and Dang (2007) who reported significant interactions between CO_2 and soil temperature on growth and biomass responses of the same species. The conflicting findings might be attributed to the temperature control protocol and the temperature ranges used in the two studies. Zhang and Dang (2007) maintained a constant day and night soil temperature while in this study, nocturnal soil temperature was lowered by 4-5 °C to be at par with greenhouse air temperature. Such a protocol took into account the daily changes of soil temperature in the field. Reduced night soil temperatures can decrease carbon expenditure during the night thereby lowering sink strength during the day and ultimately reducing net photosynthetic rate through feedback inhibition (Turnbull et al. 2002). Small changes in night soil temperature can result in significant changes in plant respiration-photosynthetic relationship (Turnbull et al. 2002) thereby affecting plant responses to other factors, such as CO_2 concentration. Furthermore, in conifers, temperature changes may supersede CO_2 responses on photosynthesis, respiration and growth (Lewis et al. 2002, Luomala et al. 2003, Lewis et al. 2004).

Furthermore, Zhang and Dang (2007) used a much wider range of temperature (7-30 °C) than the current study (20-25 °C) and only the lowest soil temperature (7 °C) affected the photosynthetic response to the CO_2 elevation in their study. The lack of significant CO_2-temperature interaction in this study, however, agrees with the findings of Zhang and Dang (2005) in jack pine (*Pinus banksiana* Lamb.) and current-year white birch (*Betula papyrifera* Marsh.) seedlings. The lack of significant CO_2 and temperature interaction suggests that effects of the two factors on trees may be additive and not interactive unless temperature is below the species threshold and or possibly at supra-optimal levels.

The CO_2 elevation resulted in photosynthetic up-regulation. P_n measured at a common CO_2 and the corresponding growth [CO_2] were both higher in seedlings grown at the elevated than at ambient [CO_2]. The current finding of up-regulation however, is in contrast with some previous studies on the species. Johnsen and Seiler (1996), Tjoelker et al. (1999) and Bigras and Bertrand (2006) observed photosynthetic down-regulation in black spruce exposed to elevated [CO_2] and attributed the cause to decreased foliar [N] and increased carbohydrate accumulation in the needles leading to reduced sink strength. In another study, Dang et al. (2008) reported P_n down-regulation in white spruce that grew at 530 and 700 μmol mol^{-1} in relation to seedlings that grew at 360 μmol mol^{-1} and attributed the cause to reduced V_{cmax} and J_{max}. Generally, photosynthetic down-regulation is linked with reduced carboxylation capacity of Rubisco (V_{cmax}), reduced electronic transport capacity (J_{max}) and limited inorganic phosphate regeneration (TPU), reduced specific leaf area and decreased nitrogen [N] at elevated CO_2 (Tissue et al. 1999). However, in this study, the CO_2 elevation had no significant effect on V_{cmax}, J_{max} and only marginal effect on SLA. Consistent with the lack of down-regulation of P_n, foliar [N] also did not decrease with exposure to elevated CO_2 but rather the CO_2 elevation increased [P]. Moreover, the rate of triose phosphate utilization, a major indicator of inorganic phosphate availability in the Calvin cycle and determinant of photosynthetic source-sink activity was significantly higher in elevated than at ambient [CO_2], supporting the observed up-regulation. Photosynthetic up-regulation has been observed in other woody species. Reddy et al. (2010) studied *Gmelina arborea* Roxb for two seasons and observed up-regulation of P_n in the trees grown in elevated [CO_2]. Zotz et al. (2005) exposed *Fagus sylvatica, Quercus petraea, Carpinus betulus, Tilia platyphyllos, Acer campestre,* and *Prunus avium* to three years of CO_2 treatment and observed no photosynthetic down-regulation but rather signs of leaf level photosynthetic up-regulation. Similarly, Pushnik et al. (1995), Zhang and Dang (2006) and Spring and Thomas (2007) observed photosynthetic up-regulation in woody seedlings and saplings exposed to elevated [CO_2].

It must be stated that though up-regulation was observed in this four-month study, there is some evidence that in the long term, the positive effect of CO_2 elevation on P_n may be down-regulated in the treatment where inter-plant root interaction occurred. This claim is supported by the reduced [N] in white spruce grown with root interaction, the lowered SLA in black spruce grown with root interaction at elevated CO_2 and finally the reduced triose phosphate utilisation (sink strength) in seedlings having root interactions.

Black spruce was insensitive to soil warming compared to white spruce. Hence, the hypothesis that black spruce would benefit more from soil warming is not supported. Net photosynthetic rate, maximum electron transport rate (J_{max}), triose phosphate utilisation (TPU), N/P ratio and photosynthetic nitrogen use efficiency (PNUE) and all other measured parameters (except PPUE) in black spruce did not change with soil temperature. In white spruce, however, the 5 °C warming treatment enhanced the species P_n, J_{max}, TPU and PNUE. The fact that black spruce maintained a high P_n at both temperatures suggest that the species has wider temperature optimum range or greater physiological thermal plasticity which. Black spruce is known to be highly sensitive to high soil temperatures (Dang and Lieffers 1989, Dang and Cheng 2004, Juday et al. 2005, Way and Sage 2008b) with temperatures of 30 °C and above causing significant decline in photosynthetic rate or even death (Peng and Dang 2003, Way and Sage 2008a). However, the species may also have high thermal plasticity, which enables it to grow on wide range of sites ranging from cold peatlands to warm uplands including burnt sites (Van Cleve and Yarie 1986, Viereck and Johnston 1990, Peng and Dang 2003). Similar to this study, Way and Sage (2008b) reported that photosynthetic rate of black spruce grown and measured at different soil temperatures did not differ from each other and suggested thermal acclimation as the reason for the observation. The non-responsive of black spruce to the temperature treatment has been previously observed in the species (Bronson and Gower 2010). This finding has both ecological and economic implications. Ecologically, soil warming within the range used in this study may benefit white spruce (increasing both photosynthetic rate and nitrogen use efficiency), with no effect black spruce's. Indirect effects of warming on the species include improved drainage, increased nutrient cycling which may affect competing vegetation. Economically, deliberate practices such as ploughing, mounding aimed solely at increasing soil temperature for black spruce growth may only add to cost but no significant photosynthetic enhancement.

The photosynthetic rate of black spruce was more closely related with NP (r^2= 0.54,) than with either [N] (r^2 =0.44 or [P] (r^2 =0.02) while that of white spruce closely related with [N] (r^2 =0.44) than either NP (r^2 = 0.23) or [P] (r^2 =0.31). P_n rate of both species also positively correlated with [K]. It must be noted that the P_n-nutrient relationship was not explored for each treatment combination because the ANOVA did not indicate significant four-way interaction for either P_n or foliar nutrient concentration. Also worthy of noting is the fact that [P] was much higher in white spruce than in black spruce while [K] was higher in black spruce than in white spruce. These differences coupled with the high P_n-N correlation in white spruce and the P_n-NP correlation in black spruce may reflect genetic and environmental differences in nutrient absorption between the two species (Chapin et al. 1986). Plants adapted to infertile sites have greater absorption affinity for the highly mobile K while on the contrary, plants adapted to fertile sites have greater affinity for P (Chapin et al. 1986). This might explain why black spruce which can grow on infertile sites had greater [K] than white spruce while [P] was higher in white spruce, which is adapted to fertile site. The ability of black spruce to obtain proportional amount of N, P and K as reflected in this study may be the key to the success of species on upland sites where it grows with white spruce. The photosynthesis dependency of black spruce on NP ratio rather than just [N] or [P] has been reported in other conifers (Reich and Schoettle 1988, Lubbers and Boyce 2004). The higher responsiveness of white spruce to [N] than black spruce has been previously reported by Patterson et al. (1997).

The positive effect of CO_2 elevation on photosynthetic water use efficiency and phosphorus use efficiency were greater in seedlings with root interactions than in seedlings without root interactions. Furthermore, transpirational water loss of seedlings with root interaction was significantly lower than in seedlings without root interactions, particularly in white spruce. Plants with high WUE are able to maintain growth or photosynthesis even when stomata are partially closed due to water stress (Picon-Cochard et al. 2001). Therefore, root interaction between or within the two species, may enable the species to cope with moisture stress better than isolated stands of the individual species. However, because the effects of intra and inter-specific interactions were not separated in this study, it may be premature to conclude that the higher

WUE in seedlings grown with root interaction was due to inter or intra-specific differences. The higher WUE due to the presence of plant-plant interaction was largely due to reduction in transpiration loss, especially in white spruce rather than increased in photosynthetic rate. The high WUE of seedlings grown in elevated CO_2 has been previously observed in both species (Wang et al. 1995, Dang et al. 2008) while the relatively greater WUE enhancement of the seedlings grown with inter-plant interactions is previously reported (De Boeck et al. 2006, Richards et al. 2010).

Within the temperature range used in this study, soil warming did not influence the response of the species to changes in CO_2 concentrations. Moreover, soil warming did not influence nutrient status of the two species nor affect black spruce response to other treatments. This study clearly outlines the importance of inter-plant root interaction in photosynthetic responses of the two species to soil temperature and CO_2. Field application requires caution as results presented here are from short term controlled studies on young seedlings.

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CHAPTER 7: General Discussion and Conclusion

This research addressed the effects of nutrient supply and soil temperature on leaf level and whole tree photosynthesis, growth, morphology, biomass and root characteristics of black spruce and white spruce grown with and without inter-plant root interaction at current and future [CO_2]. Because tree roots are vital in resource acquisition and transport and storage of assimilates (Atkinson 2000), this study focussed on how changing root zone nutrient and temperature influence seedlings response to CO_2 elevation. Global climate change is occurring (Christensen et al. 2007, Eskelin et al. 2011) and different species may respond differently to the changes (Dawes et al. 2011) and the magnitude of response may differ with fertility levels (Oren et al. 2001, Lukac et al. 2010). While it is difficult to predict the exact outcome of interactions between CO_2, nutrient and temperature on boreal black spruce and white spruce from this study, due to future uncertainties, the results of this work provide mechanistic understanding and physiological basis for some of the changes expected.

CO_2 elevation may increase photosynthetic rate without affecting height growth or biomass production. The CO_2 elevation increased both leaf level (P_n) and whole tree (W_T) photosynthetic rates, similar to previous studies (Bigras and Bertrand 2006, Zhang and Dang 2006, Dang et al. 2008, Quero et al. 2008). However, the CO_2 elevation did not enhance biomass production and height growth in the CO2 x RI X N x S experiments, despite having singnificant positive effect on both P_n and W_T. The failure of CO_2 elevation to enhance stem mass, which accounted for over 80% of total biomass contributed to the absence of significant elevated CO_2 effect on seedling height and total biomass. Changes in biomass allocation between leaves and roots in response to

CO_2 also contributed to the lack of significant CO_2 effect on growth and biomass production despite the significant effect on whole plant photosynthesis. These results suggest that in studies involving CO_2, it is vital to assess the effects of the factor on both the physiological and growth responses because CO_2 elevation may differentially affect photosynthesis and growth.

White spruce was more efficient than black spruce in converting photosynthesis into biomass especially in the high nutrient treatment with the reverse also being true. Leaf level and whole tree photosynthesis and biomass production were greater in black spruce than in white spruce but the slope of W_T –biomass relationship, which may indicate conversion efficiency was greater in white spruce than in black spruce at the high nutrient treatment. The greater W_T-P_n "conversion efficiency" in white spruce was independent of inter-plant root interaction and CO_2 treatment, and this may indicate an inherent genetic ability. However, it was dependent on nutrient supply with greater conversion efficiency at high nutrient levels. Practices such as fertiliser application, site preparation that increase site fertility may help increase biomass productivity of white spruce more than in black spruce. Though black spruce had greater absolute W_T and biomass, the "conversion efficiency" at high nutrient supply was generally non-significant or lower than in white spruce while the slope at the low nutrient treatment was generally higher than in white spruce. This seems to suggest that luxurious nutrient utilisation occurred in black spruce supplied with the high nutrient dosage. I conclude that species with high photosynthetic rate and high biomass production may not necessary be the most efficient converter of photosynthate into structural carbon.

The effects of CO_2, nutrient supply, soil warming and inter-plant root interactions on physiology, above and belowground morphological traits differed between the two species. Of almost all the key parameters (height and radial growth, biomass, leaf area, leaf level and whole tree photosynthetic rate, nutrient use efficiency, specific leaf area and specific root length), the positive responses were generally greater in black spruce than in white spruce. Some of the parameters in black spruce did not change with treatment, and may suggest inherent genetic trait. For example, total root length, root length density, specific root length of black spruce were generally high regardless of [CO_2], soil temperature, nutrient supply or roots of other species. Black spruce had longer roots with high surface area and smaller diameter, which might have enabled it explore for more nutrient resources to take advantage of the increased CO_2 to increase growth. Moreover, SRL on black spruce did not depend on changes in soil temperature or nutrient supply but SRL in white spruce was affected by both factors.
Another difference between the two species was in their response to the different nutrient elements. Photosynthetic rate generally depended on N/P rather than on either [N] or [P]. And photosynthetic rate in black spruce was more sensitive to N/P ratio than to [N] or [P] while that of white spruce was more sensitive to N. The greater photosynthesis dependency on N/P ratio rather than just [N] or [P] has been reported in other conifers (Reich and Schoettle 1988, Lubbers and Boyce 2004). The differences in the nutrient affinity of the two species may contribute to their ability to co-exist, particularly on upland sites.

Soil temperature had less effect on photosynthesis, growth, biomass and root characteristics than the other treatments. In most of the responses, the effect of soil temperature was not significant compared to CO_2, RI or N effects. Moreover, soil warming did not alter photosynthetic responses of the species to CO_2 elevation indicating additive rather than interactive effect. Soil temperature in both controlled and field conditions has been known to affect gas exchange and plant growth (Grossnickle 2000, Zhang and Dang 2005, Zhang and Dang 2007, Melillo et al. 2011). In the boreal forest, where low soil temperatures limit tree growth (Grossnickle 2000, Jarvis and Linder 2000), soil warming is essential to increase plant growth. However, it is evident from this study and previous works of Zhang and Dang (2005) and Ambebe et al (2010) that the effect of soil temperature on CO_2 response is important if the warming raises the temperature from below to above a species-specific threshold. For instance in both Zhang and Dang (2005) and Ambebe et al. (2010), the effects of soil warming above the species threshold were generally not significant. On boreal sites with temperatures above the threshold for the studied species, soil warming treatments may not change photosynthesis, growth and biomass production response to CO_2 elevation. However, at temperatures below the species-specific threshold, CO_2 elevation and soil temperature may interact to influence response parameters. Moreover, at temperatures above the species threshold, nutrient availability and neighbour's identity may be more important in determining species response to CO_2 elevation than soil warming.

Finally, this thesis answers the question of whether responses of plants grown in mixture can be extrapolated from responses of individuals grown in isolation with no inter-plant root interaction. With the presence of inter-plant root interaction, root competition was apparent. In almost all the parameters measured from leaf level photosynthetic rate, whole tree photosynthesis, height growth and biomass production to root traits, the responses of seedlings grown with root interaction generally differed from responses of seedlings grown without root interaction. Leaf mass, leaf area, specific leaf area, height growth, total seedling biomass, total root length, specific root length were generally greater with no root interaction than with root interaction. The differences in response between seedlings grown with and without root interactions were in direction, magnitude of response or both. For example, most of the differences observed in leaf traits (Chapter 3) and root traits (Chapter 4) were in magnitude rather than direction of response, with seedlings grown with root interaction having lower responses than those without plant-plant root interaction. In some of the responses, however, the differences were in direction rather than magnitude of response. For instance, leaf level photosynthetic rate was higher in seedlings grown without root interaction at ambient [CO_2] but reversed at elevated [CO_2] (Chapter 2). Generally, root tissue density was greater when seedlings were grown together with the reverse when there was no inter-plant root interaction. These findings are generally in line with some previous studies (Bazzaz and Mcconnaughay 1992, Navas 1998, Navas et al. 1999, Friend et al. 2000, Falik et al. 2003, Brooker 2006, Wang 2007) that plant response at the individual level may differ from community level response. It must however, be noted that in most of the responses, species identity did not generally change. That is to say, the species which had greater response when grown individually generally had greater response when grown with root interaction. In most controlled experiments, individual plants are potted. While results from such studies are useful in many extent, they may not provide a complete picture of plant responses to resources because in field situations, plant roots may interact. In modelling photosynthetic and growth responses of black spruce and white spruce to soil nutrient supply, temperature and [CO_2], the effects of belowground plant interactions need to be considered.

There were some limitations associated with this study. The study was conducted under controlled conditions for one growing season. The plant materials used were seedlings and response of seedlings to CO_2 and nutrient supply may differ from that of matured plants (Lee and Jarvis 1995, Zhou et al. 2011). The whole tree photosynthetic rate reported in this study did not account for differences in needle age and needle canopy position, and these may affect the true whole tree photosynthesis (Zhou et al. 2011). Furthermore, this study did not separate the effects of inter-specific root interaction from intra-specific root interaction. Therefore, the differences revealed in this study may contain the confounding effects. Further research is warranted to separate these effects. Future research need to address the interactive effects of CO_2, soil temperature and nutrient supply on species responses at the individual, monoculture and mixture levels. Notwithstanding the limitations, this study provides mechanistic understanding of how soil temperature, inter-plant root interactions and nutrient supply affect black spruce and white spruce physiological, morphological, root traits and biomass response to CO_2 elevation.

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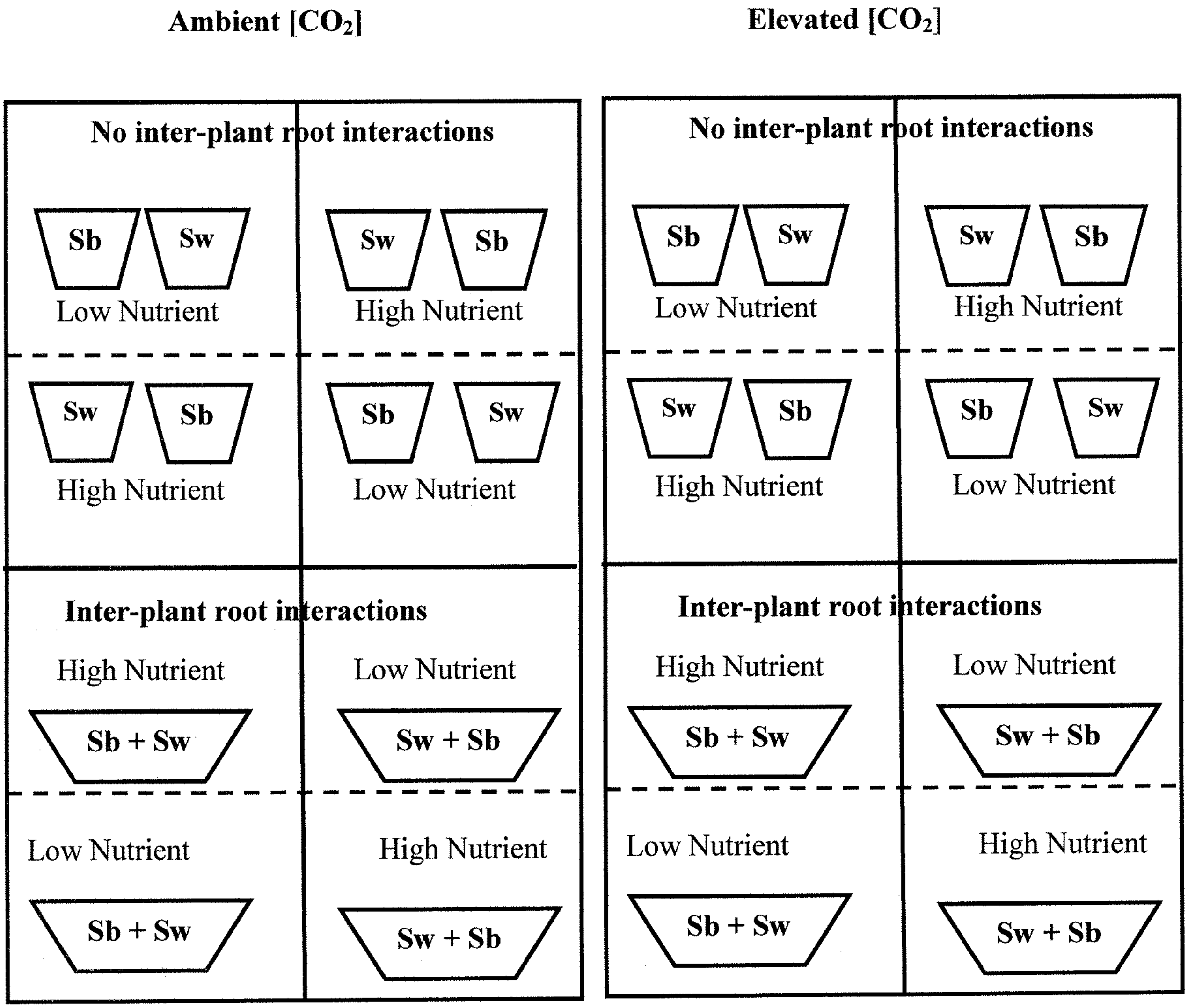
APPENDICES

Appendix 2.1: Schematic presentation of experiment 1: CO_2, inter-plant root interaction, nutrient supply and species



Appendix 2.2: Linear model and expected mean square table for experiment 1

$Y_{ijklmn} = \mu +$

$\beta_i + C_j + \omega_{ij} +$

$R_k + CR_{ik} + \omega CR_{ijk} +$

$N_l + CN_{il} + RN_{kl} + CRN_{ikl} + \omega CRN_{ijkl} +$

$S_m + CS_{im} + RS_{km} + NS_{lm} + CRS_{ikm} + CNS_{lkm} + RNS_{klm} + CRNS_{ijklm} + \omega CRNS_{ijklm}$

$\varepsilon_{(ijklm)n}$

$i = 1, 2; j = 1, 2; k = 1, 2; l = 1, 2; m = 1, 2; n = 1,2,3,\ldots 8$[1]

Y_{ijklmn} = response variable such as height growth, photosynthesis

μ = the grand mean

β_i = the i^th^ replicate effect (random)

C_j = the fixed effect of the j^th^ level of CO_2

ω_{ij} = the whole plot random effect of the j^th^ CO_2 within the i^th^ block. This is assumed to be identically and independently distributed as N (0, δ^2)

R_k = the fixed effect of the k^th^ level of plant-plant root interaction (RI)

CR_{jk} = the interactive effect of the j^th^ CO_2 at k^th^ RI

ωCR_{ijk} = the sub-plot random effect of the j^th^ CO_2 at k^th^ RI within the i^th^ block

N_l = the fixed effect of the l^th^ nutrient supply

CN_{jl} = the interactive effect of the j^th^ CO_2 at l^th^ nutrient supply

RN_{kl} = the effect of k^th^ RI at l^th^ nutrient supply

CRN_{jkl} = the effect of the j^th^ CO_2 at the k^th^ RI and l^th^ nutrient supply

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[1] 8 samples for growth and root data and 3 for gas exchange measurements

ωCRN_{ijkl} = the sub-sub plot random effect of the jth CO_2 at kth RI and lth nutrient supply within the ith block

S_m = the fixed effect of the mth species

CSj_m = the effect of jth CO_2 at mth species interaction

RS_{km} = the effect of kth RI at mth species interaction

NS_{lm} = the effect of lth nutrient supply at mth species interaction

$CRSj_{km}$ = the effect of the jth CO_2 at kth RI and mth species

CNS_{jlm} = the effect of the jth CO_2 at lth nutrient treatment on the mth species

RNS_{klm} = the effect of the kth RI at lth nutrient supply and mth species

$CRNS_{jklm}$ = the effect of the ith CO_2 treatment at the kth RI in lth nutrient supply on the mth species

$\omega CRNS_{ijklm}$ = the random effect of the jth CO_2 at kth RI and lth nutrient supply within the ith block on the mth species

$\varepsilon_{(ijklm)n}$ = the error term and is assumed to be identically and independently distributed as $N(0, \delta^2)$

EMS Table

		2	***2***	***2***	***2***	***2***	***8***	***EMS***	***Df***	***Test stat***
		R	**F**	**F**	**F**	**F**	**R**			
		i	**j**	**k**	**l**	**m**	**n**			
β_i	*A*	1	2	2	2	2	8	$\delta^2 + 64\delta^2\omega + 128\delta^2\beta$	1	*A/C*
C_j	*B*	1	0	2	2	2	8	$\delta^2 + 64\delta^2\omega + 64\Phi C$	1	*B/C*
$\omega_{(ij)}$	*C*	1	1	2	2	2	8	$\delta^2 + 64\delta^2\omega$	1	
R_k	*D*	1	2	0	2	2	8	$\delta^2 + +32\delta^2\omega CR + 64\Phi R$	1	*D/F*
CR_{ik}	*E*	1	0	0	2	2	8	$\delta^2 + +32\delta^2\omega CR + 32\Phi CR$	1	*E/F*
$\omega CR_{(i)jk}$	*F*	1	0	0	2	2	8	$\delta^2 + 32\delta^2\omega CR$	2	
N_l	*G*	1	2	2	0	2	8	$\delta^2 + 16\delta^2\omega CRN + 64\Phi N$	1	*G/K*
CN_{jl}	*H*	1	0	2	0	2	8	$\delta^2 + 16\delta^2\omega CRN + 32\Phi CN$	1	*H/K*
RN_{kl}	*I*	1	2	0	0	2	8	$\delta^2 + 16\delta^2\omega CRN + 32\Phi RN$	1	*I/K*
CRN_{jkl}	*J*	1	0	0	0	2	8	$\delta^2 + 16\delta^2\omega CRN + 16\Phi CRN$	1	*J/K*
$\omega CRN_{(i)jkl}$	*K*	1	0	0	0	2	8	$\delta^2 + 16\delta^2\omega CRN$	4	
S_m	*L*	1	2	2	2	0	8	$\delta^2 + 8\delta^2\omega CRNS + 64\Phi S$	1	*L/T*
CS_{jm}	*M*	1	0	2	2	0	8	$\delta^2 + 8\delta^2\omega CRNS + 32\Phi CS$	1	*M/T*
RS_{km}	*N*	1	2	0	2	0	8	$\delta^2 + 8\delta^2\omega CRNS + 32\Phi RS$	1	*N/T*
NS_{lm}	*O*	1	2	2	0	0	8	$\delta^2 + 8\delta^2\omega CRNS + 32\Phi NS$	1	*O/T*
CRS_{ikm}	*P*	1	0	0	2	0	8	$\delta^2 + 8\delta^2\omega CRNS + 16\Phi CRS$	1	*P/T*
CNS_{jlm}	*Q*	1	0	2	0	0	8	$\delta^2 + 8\delta^2\omega CRNS + 16\Phi CNS$	1	*Q/T*
RNS_{klm}	*R*	1	2	0	0	0	8	$\delta^2 + 8\delta^2\omega CRNS + 16\Phi RNS$	1	*R/T*
$CRNS_{jklm}$	*S*	1	0	0	0	0	8	$\delta^2 + 8\delta^2\omega CRNS + 8\Phi CRNS$	1	*S/T*
$\boldsymbol{\omega} CRNS_{(ijkl)m}$	*T*	1	1	1	1	0	8	$\delta^2 + 8\delta^2\omega CRNS$	8	
$\varepsilon_{(ijklm)n}$	*U*	1	1	1	1	1	1	δ^2	224	No test

Appendix 2.3: Fertilizer Chemical Formulation

	Nitrogen (N)	**Phosphorus (P)**	**Potassium (K)**	**Magnesium (Mg)**	**Calcium (Ca)**	**Sulphur (S)**
Target level (mg/l)	150	60	150	40	80	60
Amount in water	-	-	0.5	2.9	15	2
Amount in growth medium			6.2	4.3	3.8	-
Amount to add	150	60	143.3	32.8	31.2	58

Sources of fertiliser	***Amount required per treatment (mg/l)***
Calcium nitrate (19% Ca, 15.5% N)	322.15
Epsom salt (9.8% Mg, 12.9% S)	326.53
Microfine SuperPhosphate (20%P)	597.77
Micromax micronutrient with 12% S	132.50
Muriate of Potash (62% K_2O)	278.44
Ammonium nitrate (NH_4NO_3) 17% NH_4, 17% NO_3	294.12

Appendix 2.4: Probability values of 2-tailed studentized t-tests comparing the slopes of linear regressions for P_n on J_{max}, V_{cmax} and TPU for one year old black spruce (Sb) and white spruce (Sw) seedlings. The seedlings were exposed to two [CO_2] and two nutrient levels (HN, high nutrient and LN, low nutrient). The regression of P_n on J_{max} for white spruce in the high nutrient and elevated CO_2 treatment was not statistically significant (*P>0.05*) and thus was not included in the analysis (continued on next page)

		Ambient [CO_2]				**Elevated [CO_2]**		
P_n -J_{max}		HN-Sb	HN-Sw	LN-Sb	LN-Sw	HN-Sb	LN-Sb	LN-Sw
Ambient [CO_2]	HN-Sb	-						
	HN-Sw	0.968	-					
	LN-Sb	0.746	**0.050**	-				
	LN-Sw	0.522	0.433	0.862	-			
Elevated [CO_2]	HN-Sb	0.092	0.35	**0.013**	0.067	-		
	LN-Sb	0.654	0.102	**<.001**	0.768	**0.031**	-	
	LN-Sw	**0.017**	**0.<.001**	**<.001**	**0.005**	0.696	**<.001**	-

		Ambient $[CO_2]$				**Elevated $[CO_2]$**			
P_n -V_{cmax}		HN-Sb	HN-Sw	LN-Sb	LN-Sw	HN-Sb	HN-Sw	LN-Sb	LN-Sw
Ambient $[CO_2]$	HN-Sb								
	HN-Sw	**<.001**	-						
	LN-Sb	**<.001**	**<.001**	-					
	LN-Sw	**<.001**	**<.001**	**<.001**	-				
Elevated $[CO_2]$	HN-Sb	0.914	**<.001**	0.631	**0.003**	-			
	HN-Sw	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	-		
	LN-Sb	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	-	
	LN-Sw	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	-
P_n –TPU									
Ambient $[CO_2]$	HN-Sb	-							
	HN-Sw	**0.006**	-						
	LN-Sb	**<.001**	**0.006**	-					
	LN-Sw	**<.001**	**<.001**	**<.001**	-				
Elevated $[CO_2]$	HN-Sb	**<.001**	**<.001**	**<.001**	**<.001**	-			
	HN-Sw	**<.001**	0.623	**<.001**	**<.001**	**<.001**	-		
	LN-Sb	**0.036**	**<.001**	0.695	**<.001**	**<.001**	**0.021**	-	
	LN-Sw	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	-