Effects of photoperiod, soil moisture and soil temperature on the northward migration potential of jack pine under the scenario of doubled atmospheric CO₂ concentration

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Abstract

Climate envelope models predict that boreal trees can potentially migrate northward by as much as 10° by 2100 as a consequence of the predicted climate change associated with the doubling of atmospheric CO_2 concentration, which might expose them to a new set of environmental conditions. The ability of the migrating trees to acclimate to the new set of environmental conditions may be critical for the actual scope and success of their migration or seed transfer. The changes in photoperiod, soil temperature and soil moisture associated with the migration will likely affect tree's ecophysiological traits. Furthermore, the combined effects of several environmental factors may be substantially different from the total of their individual effects. In this study, I investigated the interactive effects of (1) photoperiod and soil temperature and (2) photoperiod and soil moisture on the ecophysiological responses of jack pine (*Pinus banksiana* Lamb.) seedlings to elevated $[CO_2]$.

In the first set of experiments, jack pine seedlings were exposed to two $[CO_2]$ (400 and 950 µmol mol^{-1}), two soil temperatures (soil temperature at seed origin and $5^{°}$ C warmer) and three photoperiod regimes (photoperiod at seed origin, 5° and 10° north of the seed origin). It was found that the photoperiod regime associated with a $10^{°}$ northward migration advanced the timing of budburst by 10 days under the doubled $[CO_2]$ and current soil temperature at the seed origin. Also the photoperiod regimes at higher latitudes prolonged the process of bud setting. Photoperiod regimes at latitudes higher than the seed origin increased seedling height growth but did not have significant impact on seedling biomass. The elevated $[CO_2]$ increased the total leaf area per seedling, but reduced shoot to root ratio. Elevated $[CO_2]$ also increased the photosynthetic rate and photosynthetic water use efficiency (*WUE*). The maximum rate of carboxylation (V_{cmax}) and triose phosphate utilization (*TPU*) were affected by interactions involving CO_2 and photoperiod but no meaningful pattern could be discerned. Both CO_2 elevation and soil warming reduced the cold hardiness of jack pine seedlings as indicated by the injury index tested at -15 and -30º C testing temperatures. The lack of photoperiod effects on cold hardiness suggests that jack pine may be plastic enough to acclimate to the new photoperiod regime associated with climate change induced

northward migration. However, advanced budburst associated with long distance migration (e.g. 10° north) will likely expose the species to late-spring frost damage.

In the second set of experiments, seedlings were exposed to two $[CO_2]$ (400 and 950 µmol mol^{-1}), two soil moistures (60–70% and 30–40% of field capacity) and three photoperiod regimes (photoperiod at seed origin, 5° and 10° north of the seed origin). The results suggest that the responses of jack pine to climate change will become complicated under the interactive effects of the longer growing season photoperiod and faster rate of change in day length at higher latitudes, and soil moisture stress under elevated $[CO_2]$. Longer photoperiods at higher latitudes advanced budburst at both high and low soil moisture regime, which will likely increase the risk of late spring frosts damage prior to and during budburst. Longer summer photoperiods with northward migration increased the WUE under elevated $[CO_2]$ and low soil moisture regime. However, the significant 2- and 3-way interactions suggest that drought and longer photoperiods associated with northward migration will limit the positive effects of elevated $[CO_2]$ on growth and physiological processes in the species. Hydraulic conductivity in jack pine seedlings was significantly increased under elevated $[CO_2]$ while it was reduced at low soil moisture regime. The interactions of $[CO_2]$ and photoperiod had significant effects on the stem xylem vulnerability to cavitation. Tendency to embolize was significantly greater in the seedlings grown under elevated $[CO_2]$ with the photoperiod regime 10° north of the seed origin compared to those grown under ambient $[CO_2]$ with photoperiod 10° north of the seed origin and elevated $[CO_2]$ at the photoperiod regime of the seed origin. This result suggests that 10° northward migration under elevated CO_2 will affect the hydraulic behavior of the species and make it vulnerable to xylem cavitation.

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Abbreviations

A_n – Net photosynthetic rate (μmol m^{-2}S^{-1})

g_s – Stomatal conductance (mmol m^{-2}S^{-1})

J_{max} – Maximum rate of photosynthetic electron transport (μmol m^{-2}S^{-1})

LMR – Leaf mass ratio

M – Total biomass (gm)

PLA – Projected leaf area (cm^2)

R_d – day respiration (μmol m^{-2}S^{-1})

RGR$_D$ – Relative diameter growth (%)

RGR$_H$ – Relative height growth (%)

RGR$_V$ – Relative volume growth (%)

RLA – Root length to leaf area ratio (cm/cm^2)

RMR – Root mass ratio

SLA – Specific leaf area (cm^2/gm)

SMR – Stem mass ratio

SRA – Specific root surface area (cm^2/gm)

SRL – Specific root length (cm/gm)

SRR – Shoot to root ratio

TPU – Triose phosphate utilization rate (μmol m^{-2}S^{-1})

V_{cmax} – Maximum rate of carboxylation (μmol m^{-2}S^{-1})

WUE – Photosynthetic water use efficiency (mmol mol^{-1})

Acknowledgements

Chapter 1: General Introduction

Global atmospheric CO_2 is predicted to increase to as high as 950 μmol mol^{-1} by the end of the 21st century (Cao & Caldeira, 2010; IPCC, 2001; Luthi *et al.*, 2008). The atmosphere-ocean general circulation models (AOGCMs) predict that air temperature and precipitation will change following the increase in atmospheric [CO_2]. It is predicted that by the end of this century average global air temperature will increase by 1.8 - 4.0° C (Bigras & Bertrand, 2006; IPCC, 2007). An increase in global average temperature by 1.5 to 2.5° C may bring major changes in ecosystem structure and functions and shifts in species' geographical ranges and will expose approximately 20 to 30% of the plant and animal species (assessed so far) to increased risk of extinction (IPCC, 2007). High latitude ecosystems including boreal forest are predicted to warm substantially over the 21st century following anthropogenic climate change (IPCC, 2007; Pachauri & Reisinger, 2007). The mean annual temperature increase in the boreal region is likely to be as high as 5–7° C, which is higher than the global average increase (Christensen *et al.*, 2007; Eskelin *et al.*, 2011; Heimann & Reichstein, 2008; Montzka *et al.*, 2011). Since the extent of climate change is likely to be severe in the boreal region, impacts of climate change on boreal plants would also be greater (Barnett *et al.*, 2005; Juday *et al.*, 2005; Serreze *et al.*, 2000).

Global warming will accelerate land surface drying and increase the frequency and severity of droughts (Christensen *et al.*, 2007). It will alter the amount, intensity, frequency and type of precipitation. Due to a rise in temperature, precipitation falling as rain rather than snow is likely to increase, especially in the autumn and spring over lands in middle and high latitudes of the Northern Hemisphere, diminishing water resources in the summer when water is most needed (IPCC, 2007). The increase in atmospheric CO_2 and temperature and changes in the pattern of precipitation might alter the morpho-physiological processes in plants and thus affect their

25 growth and survival (Volder *et al.*, 2007). Warming temperatures may also be associated with

26 changes in ecosystem water balance, leading to a higher vapor pressure deficit and increased

27 evaporative demand and transpiration rate, which will affect the plant physiological processes

28 and thus plant growth, productivity and survival (Domec *et al.*, 2009; Meinzer, 2002b).

29   Light, temperature, soil moisture and atmospheric carbon dioxide concentration ($[CO_2]$)

30 are primary environmental factors that regulate the growth and geographic distribution of plants.

31 Light is the most crucial environmental factor that provides plants with a source of energy as

32 well as informational signals that control their growth and development and enable them to

33 occupy an ecological niche in space and time (Lambers *et al.*, 2008). Greater light availability

34 induces changes in foliage physiology (Tang *et al.*, 1999) and increases light-saturated rate of net

35 photosynthesis and dark respiration rate, as well as the photosynthetic light compensation point

36 (Loach, 1967; Pothier & Prevost, 2002). Photoperiod regulates the ratio of active and inactive

37 forms of phytochrome accumulated in plants, which controls some phenological and

38 physiological events in plants (Thomas & Vince-Prue, 1997). Summer and fall phenophases in

39 boreal trees like height growth cessation, bud set, leaf senescence and leaf drop are

40 predominantly controlled by photoperiod (Fracheboud *et al.*, 2009; Soolanayakanahally *et al.*,

41 2013). The length of photoperiod also affects the flowering response of long-day and short-day

42 plants (Lambers *et al.*, 2008). Affecting the chemical composition and thus physiological

43 processes in plants, the length of photoperiod acts as an important signal for acclimation to low

44 temperatures associated with the change of seasons (Lambers *et al.*, 2008). The ability to develop

45 a high degree of cold hardiness is critical for plants to survive severe winters (Li & Sakai, 1978;

46 Weiser, 1970). The decreases in photoperiod trigger the development of cold hardiness by

47 synthesizing membrane lipids with less saturated fatty acids (Lambers *et al.*, 2008).

48   Soil temperature may have greater impact on boreal tree growth compared to air

49 temperature especially between late spring and early summer (Lupi *et al.*, 2012; Wu *et al.*, 2012)

50 since it usually lags behind air temperature in spring (Pregitzer *et al.*, 2000a) as well as during

51 diurnal temperature fluctuations (Bliss, 1956). Soil temperature can effectively modify

52 morphological and physiological traits in plants (Cai & Dang, 2002; Dang & Cheng, 2004) and

53 influence biomass allocation, organ development, stomatal conductance and nutrient and water

54 uptake in plants (Alvarez-Uria & Körner, 2007; Ambebe *et al.*, 2010; Frechette *et al.*, 2011;

55 Landhäusser *et al.*, 2001; Peng & Dang, 2003; Zhang & Dang, 2007). It significantly affects

56 shoot and root growth (Heninger & White, 1974; Lyford & Wilson, 1966). A high soil

57 temperature reduces photosynthetic rates and growth and a very high soil temperature may result

58 in higher seedling mortality (Ambebe *et al.*, 2010; Way & Sage, 2008b; Way & Sage, 2008a).

59 Increased soil temperature following global warming could reduce soil water content (Allison &

60 Treseder, 2008) and affect the movement of water through plants (Way & Oren, 2010). High

61 tensions in the xylem due to water stress may result in embolism if transpiration rates are not

62 efficiently controlled (Lambers *et al.*, 2008). When stomatal regulation could not limit the

63 transpiration, embolism occurs leading to shoot dieback and increased risk of tree mortality

64 (McDowell *et al.*, 2011; Tyree & Sperry, 1989). Low soil temperature can inhibit nutrient and

65 water uptake and limit shoot growth (Grossnickle, 2000; Peng & Dang, 2003). Low soil

66 temperature is considered one of the major constraints in the establishment of seedlings on boreal

67 reforestation sites (Stathers & Spittlehouse, 1990).

68   Water is the major medium for transporting metabolites through plant cell and plays a very

69 vital role in all physiological processes in plants. The availability of water strongly restricts

70 terrestrial plant production on a global scale. Physiological responses of trees to drought initially

3

71 start with reductions in net photosynthetic rate and stomatal conductance due to stomatal

72 limitations (Ambebe & Dang, 2009; de Miguel *et al.*, 2012; Mishra *et al.*, 1999) and eventually

73 hydraulic failure and xylem cavitation under severe droughts (McDowell *et al.*, 2011). Increased

74 frequency and severity of drought following global climate change may accelerate the

75 susceptibility of plants to embolism and limit the growth of plants (Lambers *et al.*, 2008).

76 Drought conditions inhibit root uptake of water due to high negative soil water potentials and

77 influence transpiration rates (Brodribb *et al.*, 2005; Wang *et al.*, 2003). When stomatal regulation

78 could not maintain water balance, embolism occurs and the ability of a species to withstand

79 embolism or to recover from it may significantly affect its ecological distribution (Tyree &

80 Sperry, 1989). Severe drought during growing season results in long-lasting growth reductions

81 and increase tree mortality (Bigler *et al.*, 2006; Waldboth & Oberhuber, 2009).

82   Atmospheric carbon-dioxide concentration [CO_2] is another important factor which

83 substantially influences plants physiological processes. Elevated [CO_2] usually promotes plant

84 growth and yield because of increased photosynthetic carbon acquisition and changes in carbon

85 allocation (Ainsworth & Long, 2005; Curtis & Wang, 1998; Eamus & Jarvis, 1989; Idso & Idso,

86 1994; Marfo & Dang, 2009; Norby *et al.*, 1999; Pregitzer *et al.*, 2000b). However, following an

87 initial enhancement of carbon assimilation, assimilation and growth decline (Brown, 1991), due

88 to modifications in several plant properties including phenology, morphology, carbon allocation

89 and photosynthesis (Ceulemans & Mousseau, 1994; Lee & Jarvis, 1995). Such photosynthetic

90 down-regulation takes place due to reduced chlorophyll concentration, reduced ribulose-1,5-

91 biphosphate carboxylase oxygenase (Rubisco) concentration, limitations in ribulose-1,5-

92 biphosphate (RuBP) and inorganic phosphate regeneration, decreased nitrogen concentration and

93 higher leaf mass/leaf area ratios (Tissue *et al.*, 1999). Elevated [CO_2] substantially increases the

94   water-use efficiency in boreal forests through partial closure of stomata during gas exchange and

95   directly or indirectly influence the water potential in plants, and thus the process of embolism

96   (Field *et al.*, 1995; Keenan *et al.*, 2013; Long *et al.*, 2004). Decreased stomatal conductance

97   indirectly stimulates photosynthesis in dry environments by reducing the rate of soil drying and

98   therefore the water limitation of photosynthesis (Hungate *et al.*, 2002). By stimulating the root

99   growth (Curtis & Wang, 1998; Rogers *et al.*, 1994), elevated $[CO_2]$ enhances the availability of

100   water to plants. Elevated $[CO_2]$ can markedly influence the leaf morphology (Koch *et al.*, 1986;

101   Norby & O'Neill, 1989; Pettersson *et al.*, 1993) and the branching frequency of plants

102   (Samuelson & Seiler, 1993) and increase root biomass, root length, root branching and lateral

103   root production (Curtis & Wang, 1998; Day *et al.*, 1996; Janssens *et al.*, 1998; Rogers *et al.*,

104   1994; Tingey *et al.*, 2000). Elevated atmospheric $[CO_2]$ can affect the growth rhythm of trees by

105   altering the timing of bud burst and growth cessation and result in an increased cold hardiness in

106   early fall (Bigras & Bertrand, 2006; Ceulemans *et al.*, 1995).

107   However, the interactive effects of environmental factors on the growth and biomass

108   allocation in trees could be quite different from the total effects of individual factors (Curtis *et*

109   *al.*, 2000; Nowak *et al.*, 2004). Higher temperature with elevated $[CO_2]$ normally increases

110   photosynthetic capacity and accelerates plant growth (Ainsworth & Rogers, 2007; Cole *et al.*,

111   2010; Gavito *et al.*, 2001; Long *et al.*, 2004; Nowak *et al.*, 2004; Phillips *et al.*, 2009; Saxe *et al.*,

112   1998). But tree growth rate might not increase proportionally with increase in photosynthesis

113   because of other limiting factors (Hungate *et al.*, 2003; Luo *et al.*, 2004). An increase in

114   temperature alone would be beneficial for boreal plants (Briceño-Elizondo *et al.*, 2006;

115   Kellomäki & Wang, 1996), but interactions with other climate or site related factors can alter the

116   response (Lindner *et al.*, 2010). Elevated atmospheric temperature may modify water availability

117   to plants by changing precipitation and evaporative demand (Colombo *et al.*, 1998). Elevated

118   temperature increases evapotranspiration and therefore reduces soil moisture, which may limit

119   the distribution and growth of different tree species (Colombo *et al.*, 1998). The changes in the

120   amount and pattern of precipitation by global warming might affect plants' responses to

121   temperature increases (Curtis *et al.*, 2000; Nowak *et al.*, 2004). Higher temperatures extend

122   growing season and may increase photosynthesis, but if precipitation does not increase in the

123   winter season, detrimental effects are possible (Loustau *et al.*, 2005). Also, elevated [CO_2]

124   enhances leaf area, leaf dry weight and stem dry weight, whereas moisture stress significantly

125   reduces these plant traits (Mishra *et al.*, 1999). Therefore, under different sets of environmental

126   conditions plants' response could be different because of the conflicting impacts of the

127   interacting factors.

128   Conifers experienced extreme environmental conditions since their progression through

129   300 million years (Morse *et al.*, 2009) and fossil evidence indicates that forests have moved

130   across landscapes over millions of years in response to climate changes (Bunnell & Kremsater,

131   2012). There are evidences that responding to regional climate changes plant species are shifting

132   their ranges in altitude and latitude (Parmesan & Yohe, 2003; Walther *et al.*, 2002). Following

133   the predicted changes in environmental parameters many of the boreal tree species might migrate

134   further northward (McKenney *et al.*, 2007; McKenney *et al.*, 2011). But, under the rapid pace of

135   current climate change many species may not be able to follow the shift in their climate envelope

136   (Davis & Shaw, 2001). The spatial extent and success of such migration will depend on the

137   acclimating capability of the migrating species to the new photoperiod regimes in the sites where

138   they are migrating to along with changes in other environmental factors. The changes in

139   photoperiod associated with elevated [CO_2] and temperature driven migration may have adverse

140　effects, which may outweigh the positive effects of increased [CO_2] and temperature. For

141　example, migration associated changes in photoperiod may affect the timing of photoperiodically

142　controlled physiological and/or phenological events such as bud burst or bud setting and reduced

143　cold hardiness, all of which may expose plants to damages by adverse environmental conditions

144　and affect their growth and survival (Man *et al.*, 2009).

145　　　Boreal forest is the most important forest type in Canada comprising of 74% of the total

146　forested land area (Forestry Canada, 1992). The climate of the boreal forest is characterized by

147　strong seasonal variation with short, moderately warm, moist summer and long, extremely cold,

148　dry winter (Larsen, 2013). Climate models suggest that the impact of global climate change

149　might be greatest in the southern boreal regions of North America (Reed & Desanker, 1992). An

150　increase of 3° C in annual mean temperature could decrease the extent of boreal forests by as

151　much as 37% (Peters, 1990). With continued climate changes tree populations become

152　progressively less well adapted to their environment and in order to survive, they either

153　acclimate to the changed environmental conditions in place or migrate to suitable locations

154　(Johnston *et al.*, 2009). At the pace of global climate change long-lived trees initially have to

155　acclimate to avoid extinction (Bradshaw & McNeilly, 1991).

156　　　Jack pine (*Pinus banksiana* Lamb.) is a conifer species of great ecological and economic

157　importance and in Canada it is the second most planted tree species after black spruce (Forestry

158　Canada, 1992; Fowells, 1965). In Ontario jack pine is a major species in forest management

159　comprising approximately 37% of the total annual softwood harvest volume (OMNR, 1991). The

160　species occurs extensively in the nutrient poor, droughty, sandy soils of Canadian boreal forests

161　(Cayford & McRae, 1983). The species is well adapted to frequent wild fires (Cayford & McRae,

162　1983) and regenerates in pure, even-aged, well-stocked stands from serotinous cones particularly

163 in burned dry sites of glaciofluvial sands and shallow bedrock soils (Carmean & Lenthall, 1989).

164 In the eastern part of its range, jack pine grows in a maritime climate but elsewhere it is found in

165 diverse climates characterized by short warm to cool summers, very cold winters and low

166 rainfall. The average January and July temperatures range from $-29°$ to $-4°$ C and from $13°$ to $22°$

167 C, respectively. Average annual maximum and minimum temperatures ranges from $29°$ to $38°$ C

168 and $-21°$ to $-46°$ C, respectively (Rudolf, 1965). Mean annual temperatures range between $-5°$ and

169 $4°$ C (Schoenike, 1976). The frost-free period averages from 50 to 173 days but is usually from

170 80 to 120 days. The average date of the last killing spring frost ranges from April 30 to about

171 July 1 and the average date of the first killing fall frost ranges from about August 10 to October

172 20 (Rudolf, 1965). Under forest conditions with adequate moisture jack pine seeds germinate

173 when air temperatures reach at $18°$ C (Rudolf, 1965). But, light availability significantly

174 influences the germination of the species (Rudolf, 1965).

175   Jack pine grows in a wide range of climatic conditions (Rudolf, 1965) with strong

176 fluctuations in most climatic variables. Usually, plants growing in fluctuating environmental

177 conditions develop strong physiological plasticity which allows them to cope with a wide range

178 of climatic conditions (Bradshaw & McNeilly, 1991) and under progressive environmental

179 change such plasticity allows a certain degree of adaptation without the need for genetic change

180 (Bradshaw & Hardwick, 1989).

181   Despite the high levels of genetic diversity, the distribution and productivity of jack pine in

182 Ontario are going to be affected by increases in atmospheric temperature (Colombo *et al.*, 2007).

183 Due to potential climate change induced northward migration the species might expose itself to a

184 different combination of soil temperature, moisture and photoperiod, which may alter its

185 morphological and physiological traits. Our knowledge on how the species will respond to the

186 combined effects of these environmental variables associated with northward migration is still

187 limited. Because of the great ecological and commercial values and sensitivity to climate

188 changes (Colombo *et al.*, 2007; Dang & Cheng, 2004; Rudolf, 1965), the species deserves

189 special attention in the context of responses to climate change. In this study I examined the

190 acclimatization capability of jack pine seedlings to elevated $[CO_2]$ and soil temperature and

191 altered soil moisture and photoperiod regimes. More specifically, I investigated (i) the interactive

192 effects of elevated soil temperature and $[CO_2]$ and changes in photoperiod associated with

193 northward migration on the morphological and physiological traits in jack pine and (ii) the

194 morpho-physiological plasticity and the vulnerability of the species to embolism under the

195 interactive effects of altered photoperiod, soil moisture and atmospheric $[CO_2]$. I hypothesize

196 that enhanced physiological activity and growth under northward migration associated longer

197 growing season photoperiod and elevated $[CO_2]$ will be coupled with reduced cold hardiness,

198 higher risks of late spring frost damage and increased vulnerability of the species to cavitation. A

199 clear understanding of the morphological and physiological responses of the species to changes

200 in photoperiods, soil temperature and soil moisture associated with migration or seed transfer

201 under elevated atmospheric $[CO_2]$ will enhance the managerial potentials to manage the species

202 at the pace of future climate changes.

9

Chapter 2: Morphological responses of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod

2.1 Introduction

Phenological sensitivity reflects the ability of a species to track climate change and indicates whether the species will persist or decline in response to climate changes (Cleland *et al.*, 2012). Phenological events are easily observable and most sensitive traits to climate change (Soolanayakanahally *et al.*, 2013). Plants respond to changes in growing conditions and altered availability of resources induced by climate change by making phenotypic and physiological adjustments, or by migrating to a new area having similar environmental conditions that they are adapted to, or by combinations of the above responses and eventually by genetically adapting to the new conditions (Aitken *et al.*, 2008). Historical evidence indicates that all the above mechanisms have occurred in plants' responses to climate change (Bunnell & Kremsater, 2012). During the process of migration and adaptation plants encounter multiple challenges. Since it is almost impossible to get exactly the same environmental conditions to migrate to, plants usually migrate to a relatively favorable site and phenotypic plasticity plays a critical role in the success of such a migration. Phenotypically plastic changes have a large influence on the immediate fate of the migrating plants via their effects on survival and reproduction (Johnston *et al.*, 2009).

Each environmental factor such as atmospheric carbon dioxide concentration [CO_2], temperature and photoperiod has its own impacts on the growth and biomass allocations in trees (Huang *et al.*, 2007; Lukac *et al.*, 2010). Increased atmospheric [CO_2] generally increases the photosynthetic capacity of plants leading to an increased carbon balance, growth and yield (Ainsworth & Long, 2005; Curtis & Wang, 1998; Idso & Idso, 1994; Marfo &

226   Dang, 2009; Norby *et al.*, 1999; Pregitzer *et al.*, 2000) because of increased photosynthetic

227   carbon acquisition and changes in biomass allocation (Eamus & Jarvis, 1989). Elevated [CO_2]

228   can markedly alter the structure and physiology of plants, such as increased leaf expansion and

229   cell wall extensibility and often cell turgor pressure, leading to increased leaf and root growth.

230   Increased atmospheric [CO_2] can increase total leaf area (Koch *et al.*, 1986), leaf mass (Norby &

231   O'Neill, 1989), leaf mass to area ratio (Pettersson *et al.*, 1993), and branching frequency

232   (Samuelson & Seiler, 1993). Elevated atmospheric [CO_2] enhances root growth (Curtis & Wang,

233   1998; Rogers *et al.*, 1994) and affects many important root traits (Tingey *et al.*, 2000). Root

234   biomass, root length, root branching and lateral root production are reported to increase in

235   response to elevated [CO_2] (Day *et al.*, 1996; Janssens *et al.*, 1998; Rogers *et al.*, 1994). Elevated

236   [CO_2] can affect the growth rhythm of trees by altering the timing of bud burst and growth

237   cessation and results in an increased seedling cold tolerance in early fall (Bigras & Bertrand,

238   2006; Ceulemans *et al.*, 1995). It can significantly advance the date of bud burst and increase

239   total foliage area as a result of increased number and length of shoots and increased individual

240   foliage area (Jach & Ceulemans, 1999). Early bud burst, however, may increase the risk of frost

241   damage from late spring frosts (Murray *et al.*, 1989).

242   Soil temperature influences the growth and physiology in plants, such as biomass

243   allocation, shoot and root growth, stomatal conductance and nutrient and water uptake (Alvarez-

244   Uria & Körner, 2007; Cai & Dang, 2002; Camm & Harper, 1991; Dang & Cheng, 2004;

245   Landhäusser *et al.*, 2001). The soil temperature in the boreal forest is often too low for the

246   optimal physiological activities and growth (Stathers & Spittlehouse, 1990). For example, low

247   soil temperature can inhibit nutrient and water uptake, limit shoot growth (Grossnickle, 2000;

248   Peng & Dang, 2003) and reduce photosynthetic productivity (Tierney *et al.*, 2001).Therefore, the

249   increase of soil temperature by climatic warming would be beneficial to boreal plants (Alvarez-

250   Uria & Körner, 2007; Ambebe *et al.*, 2010; Domisch *et al.*, 2002). Warmer soil temperatures

251   improve water uptake, root growth and root permeability (Häussling *et al.*, 1988).

252   Photoperiod regulates annual growth cycles in temperate and boreal trees and influences

253   their geographic distribution (Campbell & Sugano, 1975; Campbell, 1979; Weiser, 1970;

254   Hänninen, 2006). Plants are generally adapted to the photoperiod regime of their habitats over

255   hundreds and thousands of years (Thomas & Vince-Prue, 1997). Photoperiod regulates the

256   development of cold hardness in the fall and the timing of bud flush in the spring (Fracheboud *et*

257   *al.*, 2009; Soolanayakanahally *et al.*, 2013). However, photoperiod is not considered in climate

258   envelop models. If species migrate naturally or with human assistance based on the shift of their

259   climate envelopes, it is possible that the species may not be able to acclimate to the photoperiod

260   regimes at their new location.

261   Although the individual effects of elevated atmospheric [CO_2], warmer soil temperature

262   and prolonged photoperiod on plant growth are mostly positive, their combined effects may not

263   be equal to the sum of the individual effects (Curtis *et al.*, 2000; Nowak *et al.*, 2004). Soil

264   temperature and light regime influence the responses of plants to elevated [CO_2] (Allen *et al.*,

265   1990; Marfo & Dang, 2009; Mishra *et al.*, 1999; Robredo *et al.*, 2007; Zebian & Reekie, 1998).

266   However, elevated [CO_2] in combination with increased soil temperature has no significant

267   effect on the overall height growth of jack pine seedlings, but significantly increase the total

268   biomass (Cantin *et al.*, 1997). (Cantin *et al.*, 1997) report that the growth of jack pine seedlings

269   under elevated [CO_2] and temperature results in reduced leaf mass ratio as more biomass is

270   allocated to roots. The effects of individual environmental factors or bi-factor effects on boreal

271   tree growth are well documented (DeLucia & Smith, 1987; Fraser, 1962; Li, 2012; Peng &

272 Dang, 2003; Soolanayakanahally *et al.*, 2013; Way & Sage, 2008b; Way & Sage, 2008a), but the

273 understanding of interactive effects of multiple environmental variables such as [CO_2], soil

274 temperature and photoperiod, on boreal tree growth is still limited.

275 Jack pine (*Pinus banksiana* Lamb.) is a major tree species in the boreal forests of Canada

276 holding great ecological and commercial values and thus deserves special attention in the context

277 of climate change. Atmosphere Ocean General Circulation Models predict a 10 degree

278 (approximately 1000 kilometers) northward shifts in the climate envelopes of 130 North

279 American tree species between 2071 and 2100 (McKenney *et al.*, 2007; McKenney *et al.*, 2011).

280 Following the predicted shift in climate envelopes jack pine might need to migrate 10° northward

281 between 2071 and 2100 (Pearson & Dawson, 2003). In that case, the species will be exposed to a

282 different photoperiod regime, e.g. the photoperiods will be longer in the summer and shorter in

283 the winter with faster transition between seasons than the regimes that it has adapted to, which

284 might affect the phenological events of the species. But, the impacts of changes in photoperiod

285 regimes associated with migration or seed transfer of jack pine are not yet well documented.

286 Since the impacts of elevated atmospheric [CO_2] and warmer soil temperature on the growth of

287 jack pine are mostly positive (Cantin *et al.*, 1997; Dang & Cheng, 2004; Tjoelker *et al.*, 1998), I

288 hypothesize that elevated [CO_2] and soil temperature and prolonged photoperiods associated

289 with northward migration or seed transfer will enhance the growth performance of jack pine but

290 early budburst or delayed bud setting will increase the risks of frost damage. The overall

291 objective of this study was to assess the interactive effects of photoperiod, soil temperature and

292 CO_2 elevation on the morphological traits of jack pine in the context of northward migration. The

293 specific objectives were to: 1) assess the tri-factor effects on the phenological traits of jack pine

294 seedlings, 2) assess the impacts of elevated [CO_2], soil temperature and photoperiod on growth

295 performance and biomass allocation patterns and 3) investigate the spatial extent of possible

296 northward migration following the predicted shift in climate envelope.

297

2.2 MATERIALS AND METHODS

2.2.1 Plant materials

300   One-year old jack pine (*Pinus banksiana* Lamb.) seedlings were obtained from a

301 commercial tree seedling nursery. The seedlings were raised from seeds collected from

302 Kakabeka region ($48^o57^{'}$ N & $90^o44^{'}$ W) and appropriately cold hardened. A total of 608

303 seedlings of relatively uniform size (average height 13.05; average root collar diameter 0.21 cm)

304 were chilled and planted in plastic pots of 15cm in height and 13cm in diameter, filled up with a

305 mixture of premium grade vermiculite and peat moss (50:50, v/v).

2.2.2 Experimental design

307   The experiment was set on November 20, 2013 and continued until May 31, 2014

308 mimicking the actual day lengths and temperatures of a typical growing season for the period of

309 April 16 to October 31 (Table 1).

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Table 2.1: Biweekly mean day length and air and soil temperatures from April 16 to October 31. Air temperature is based on 10-year average (2004-2013). Soil temperatures are biweekly averages of soil temperatures at 5 and 20 cm depths in 2012 and 2013.

Actual time period	Experiment time period	Mean day length (hours)			Mean air temperature (^{o}C) at seed origin		Soil temperature (^{o}C)
		P_s	P_{nm1}	P_{nm2}	Day	Night	
Apr. 16-30	Nov. 20-30	14.14	14.56	15.11	8	1	3
May 01-15	Dec. 01-15	14.91	15.50	16.29	10	4	7
May 16-31	Dec. 16-31	15.56	16.32	17.35	14	7	9
Jun. 01-15	Jan. 01-15	15.98	16.85	18.08	17	9	12
Jun. 16-30	Jan. 16-31	16.08	16.98	18.26	20	12	14
Jul. 01-15	Feb. 01-14	15.87	16.72	17.89	22	14	16
Jul. 16-31	Feb. 15-28	15.38	16.08	17.04	22	14	16
Aug. 01-15	Mar. 01-15	14.68	15.21	15.93	22	14	15
Aug. 16-31	Mar. 16-31	13.86	14.22	14.68	21	13	15
Sep. 01-15	Apr. 01-15	12.98	13.16	13.40	18	11	13
Sep. 16-30	Apr. 16-30	12.11	12.12	12.13	14	8	10
Oct. 01-15	May 01-15	11.24	11.08	10.87	12	5	8
Oct. 16-31	May 16-31	10.36	10.02	9.58	7	2	6

Note: P_s, P_{nm1} & P_{nm2} represent the seed origin, 5° north of seed origin & 10° north of seed origin, respectively.

The experiment was conducted in four green houses at Lakehead University's Thunder Bay campus. The treatments were consisted of two levels of [CO_2] (400 and 950 µmol mol^{-1}), two soil temperatures (the average soil temperature at seed origin (T_s) and 5 ^{o}C warmer (T_e)) and three photoperiod regimes (P_s, P_{nm1} and P_{nm2}, indicating photoperiods at seed origin and 5° and 10° north of the seed origin, respectively). The 10° north of the seed origin represents the predicted northward shift of the climate envelopes for boreal tree species (McKenney *et al.*, 2007; McKenney *et al.*, 2011) and the 5° north is the midpoint between the current and the predicted future location of the species. The soil temperature of seed origin was derived from the biweekly mean soil temperature of the years 2012 and 2013 at Kakabeka region. Soil temperatures at 5 cm and 20 cm depths were averaged. Based on the biweekly average, soil temperatures were adjusted periodically. Since [CO_2] was hard-to-randomize, the experiment was carried out following a split plot design, with [CO_2] as the whole plot, soil temperature as

330   the sub-plot and photoperiod as the sub-sub plot. Two levels of $[CO_2]$ were randomly assigned to

331   four independent greenhouses with two replicates of each. The two levels of soil temperature

332   were set up within each level of $[CO_2]$ and then three levels of photoperiod were nested within

333   each soil temperature.

2.2.3 Environmental controls

335   To elevate $[CO_2]$ in the greenhouses, electronic ignition natural gas CO_2 generators (model

336   GEN-2E, Custom Automated Products Inc, Riverside, CA) were used. Circulation fans were

337   used to make the air composition even throughout the greenhouse. The $[CO_2]$ in each greenhouse

338   was monitored and controlled automatically with an Argus Environment Control System (Argus,

339   Vancouver, BC, Canada). The soil temperatures were controlled using a soil temperature control

340   system consisting of a large leak-proof wooden box (196 cm long, 112 cm wide and 16 cm deep)

341   designed by (Cheng *et al.*, 2000). The seedling containers were mounted within the soil

342   temperature control box and temperature-controlled water was circulated in the space between

343   the containers to maintain the desired soil temperature. To ensure the even distribution of

344   temperature within the system, each control unit was equipped with circulatory pumps (model

345   AC-2CP-MD, March Mfg. Inc., Glenview, Illinois, USA). The system was insulated to minimize

346   heat exchange with the greenhouse air. Each day-time soil temperature was lowered by

347   approximately 4-6 $^{\circ}$C at night to cater for lower night temperature. The lengths of the

348   photoperiod for active growing and cold hardening phases for each of the three locations were

349   set periodically based on the actual biweekly average photoperiods during the growing season

350   and cold hardening phase at corresponding locations. Each photoperiod treatment was started

351   with summer long days followed by the corresponding short days. The length of natural

352   photoperiod was extended using high-pressure sodium lamps when natural day lengths were

16

353   shorter than the set photoperiods. Photoperiods shorter than the natural day length were achieved

354   through manual shading of the seedlings in the early mornings and late evenings following

355   standard blackout techniques used in tree nurseries. All the seedlings were fertilized biweekly

356   with N:P:K fertilizer (7.14, 0.56 and 2.12 mmol per liter of water, respectively, during the rapid

357   growth phase and 1.78, 2.20 and 4.64 mmol per liter of water, respectively during the hardening

358   phase) (Scarratt, 1986). The moisture level was maintained at 60–70% of the field water

359   capacity. The moisture content of the growing medium was monitored daily using a Delta-T

360   ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). In all the greenhouses

361   the relative humidity was maintained at 55–60% during the rapid growth phase (initial 130 days)

362   and reduced to 45–50% during the hardening phase (later 60 days). The day and night air

363   temperatures were maintained mimicking the mean biweekly day and night air temperatures at

364   the seed origin. Relative humidity and air temperature were controlled automatically using the

365   Argus Environment Control System.

366   **2.2.4 Measurements**

367   Height and root collar diameter (RCD) of each individual seedling were measured at the

368   beginning of the experiment. Ten seedlings from each treatment combination were randomly

369   chosen to investigate bud break at the beginning of the experiment and bud setting during the

370   cold hardening phase. Bud break was determined by visual observation of the terminal meristem

371   of seedlings. Bud break was considered completed when bud scales were mostly fallen, with tips

372   of needles protruding about 2 mm (Bigras & Bertrand, 2006). Days to bud break was counted

373   from the beginning of the treatment (November 20, 2013). Bud set was determined by visual

374   observation and considered accomplished when bud scales were completely closed (Bigras &

375   Bertrand, 2006). Bud setting time was counted from April 16, 2014, when the day length and day

376   and night air temperature were reduced to 12 hours and 14º C and 8°C, respectively.

377   Heights and RCDs of three randomly chosen seedlings from each treatment combination

378   were measured on May 28, 2014. The seedlings were then separated into foliage, stem (including

379   branches) and roots. The needles were scanned with WinSeedle system (Regent Instruments Inc,

380   Quebec, Canada) to determine projected leaf area. The roots were scanned to analyze root traits

381   using a WinRhizo system (Regent Instruments Inc, Quebec, Canada). The foliage, stem and

382   roots were subsequently oven-dried at 70º C for 48 hours and weighed on an analytical balance

383   (0.001g precision) to determine dry mass. Stem mass ratio (ratio of stem mass to total biomass,

384   SMR), root mass ratio (ratio of root mass to total biomass, RMR), leaf mass ratio (ratio of leaf

385   mass to total biomass, LMR), shoot to root mass ratio (ratio of above ground to belowground

386   mass, SRR) were used as indices of biomass allocation. Specific leaf area (SLA) was determined

387   from projected leaf area and leaf dry mass. Specific root length (SRL) and specific root surface

388   area (SRA) were determined as root length per unit root dry mass and root surface area per unit

389   root dry mass, respectively. Root length to leaf area ratio (RLA) was calculated to estimate the

390   water supply/demand index. The stem volume (V) was calculated from height (H) and RCD (D)

391   using the equation (van den Driessche, 1992): $V = (\pi D^2/4) H/3$. The relative growth rate of

392   height (HT), root-collar diameter (RCD) and volume (Vol) were determined by dividing the

393   increments by the corresponding initial values.

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397 **2.2.5 Statistical analysis**

398   All data were analyzed using Analysis of Variance (ANOVA). Before the ANOVA test,

399   Shapiro-Wilk and Bartlett tests were conducted to test the normality of distribution and

400   homogeneity of variance, respectively. Whenever necessary, suitable transformation was done to

401   normalize the data. Tukey's HSD Post-hoc comparisons were carried out when ANOVA showed

402   a significant interaction ($P < 0.05$). In the analyses, $[CO_2]$, soil temperature and photoperiod were

403   treated as fixed factors. All analyses were done using R 3.1.3 (R Core Team 2015).

404 **2.3 Results**

405   On the average, budburst advanced for 5 days by elevated $[CO_2]$ (C_e) and 2 days by

406   warmer soil temperature (T_e) compared to those in current $[CO_2]$ (C_a) and soil temperature (T_s)

407   (Figures 2.1A and 2.1B). The effect of soil temperature was mostly in C_e (significant CxT

408   interaction ($P<0.05$) see Table 2.1 and Figure 2.1D). The total day to budburst was significantly

409   less with the photoperiod regimes associated with northward migration: 34.63 ± 0.55, 32.63 ± 0.55

410   and 29.69 ± 0.49 days for P_s, P_{nm1} and P_{nm2}, respectively (Figure 2.1C). The examination of the 3-

411   way interaction ($P<0.05$) indicated the longest days to budburst for C_a x T_s x P_s treatment

412   combination (38.7 ± 0.874) and the shortest for C_e x T_e x P_{nm2} combination (25.7 ± 0.7) (Figure

413   2.1E). The total day to bud set increased with photoperiod from P_s (32.16 ± 0.2 days) to P_{nm2}

414   (34.58 ± 0.157 days) ($p<0.001$, Figure 2.1F) and the variation among C_a x T_e within P_s was

415   marginally significant effect ($p=0.087$).

416

Table 2.2: ANOVA P-values for the effects of $[CO_2]$ (C), soil temperature (T), photoperiod (P) and their interactions on total days to budburst and bud set, relative growth rate of height, root collar diameter and volume (RGR_H, RGR_D and RGR_V, respectively), total biomass (M), total leaf area (projected) per seedling (LA), stem mass ratio (SMR), root mass ratio (RMR), leaf mass ratio (LMR), shoot to root mass ratio (SRR), specific leaf area (SLA), specific root length (SRL), specific root surface area (SRA) and root length to leaf area ratio (RLA). Seedlings were grown under two levels of $[CO_2]$, two levels of soil temperature and three photoperiod regimes.

Response variables	Treatment effects						
	C	T	P	C*T	C*P	T*P	C*T*P
Budburst	**0.028**	**0.013**	**0.044**	**0.020**	0.999	0.633	**0.045**
Budset	0.152	0.134	**<0.001**	0.895	0.107	0.325	0.087
RGR_H	0.927	0.726	**0.019**	0.359	0.250	0.769	0.240
RGR_D	**0.040**	0.128	0.395	**0.010**	0.567	0.275	0.423
RGR_V	**0.013**	0.394	0.709	0.083	0.494	0.711	0.511
M	**0.005**	0.062	0.315	0.145	0.081	0.981	0.709
SLA	**0.020**	0.071	0.120	0.633	0.497	0.588	0.553
SMR	0.613	0.071	0.205	0.167	0.399	0.802	0.552
RMR	0.055	0.147	0.136	0.831	0.851	0.608	0.943
LMR	**0.013**	0.565	0.236	0.155	0.714	0.376	0.300
SRR	**0.042**	0.117	0.084	0.713	0.730	0.609	0.963
SLA	0.950	0.624	0.322	0.714	0.807	0.790	0.914
SRL	**0.033**	0.168	0.774	0.168	0.130	0.104	0.277
SRA	**0.012**	0.093	0.650	0.078	0.256	0.639	0.534
RLA	0.346	**0.034**	0.052	0.090	0.762	0.921	0.436

Note: Significant effects ($p \leq 0.05$) are shown in bold.
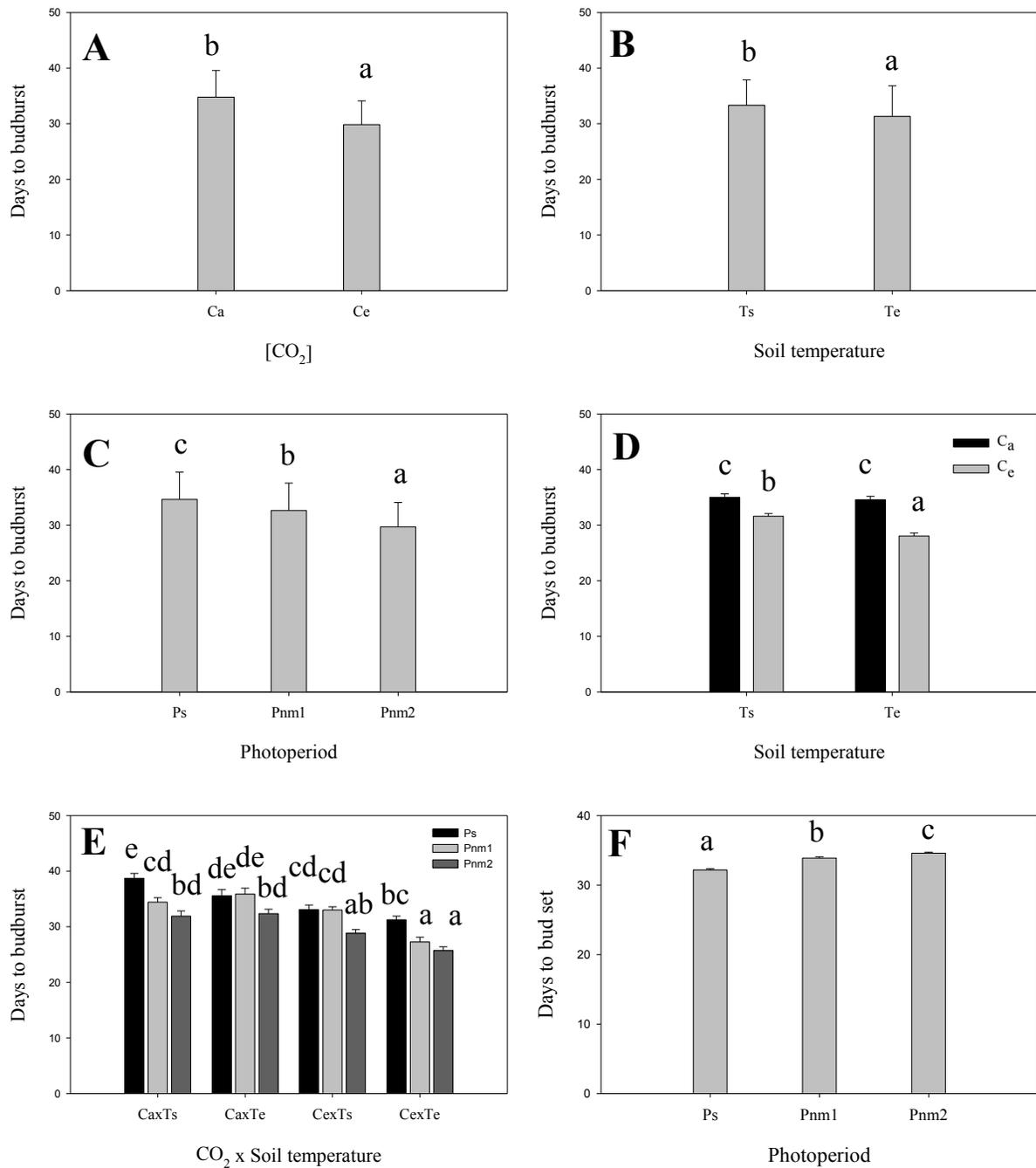
Figure 2.1: Least square means (+SE) of total days to budburst and bud set in jack pine seedlings grown under two levels of $[CO_2]$ (C_a = 400 & C_e = 950 µmol mol^{-1}), two soil temperature (T_s, seed origin and T_e, 5° warmer than T_s) and three photoperiods (P_s, seed origin and P_{nm1} & P_{nm2}, values for 5° & 10° northward migration, respectively). Different lowercase letters indicate statistically significant differences at p≤0.05.
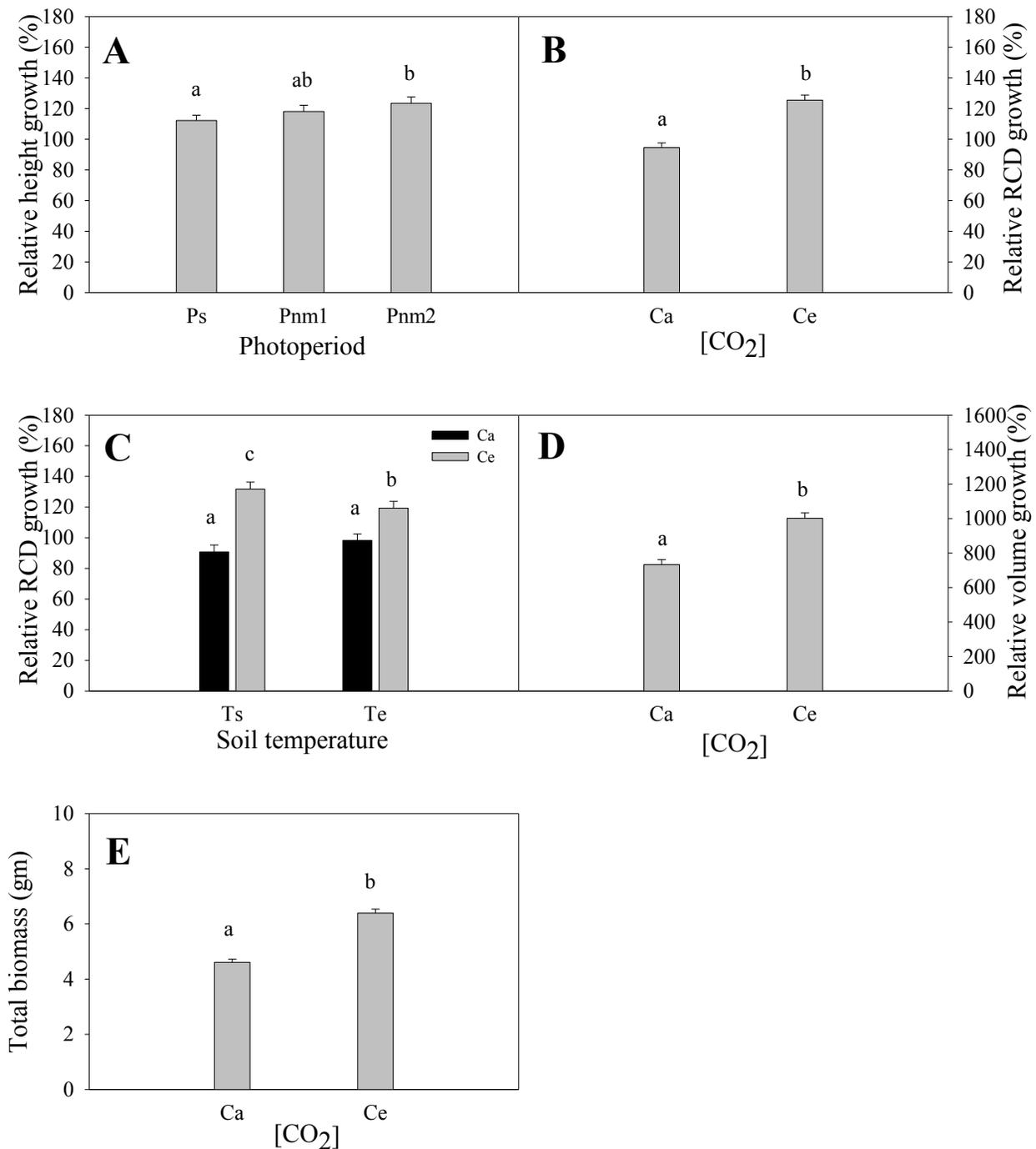
Figure 2.2: Least square means (+SE) of relative growth for height, root collar diameter (RCD) and stem volume, and total seedling biomass in jack pine seedlings grown under two levels of $[CO_2]$ (C_a = 400 & C_e = 950 µmol mol^{-1}), two soil temperature (T_s, seed origin and T_e, 5° warmer than T_s) and three photoperiods (P_s, seed origin and P_{nm1} & P_{nm2}, values for 5° & 10° northward migration, respectively). Different lowercase letters indicate statistically significant differences at p≤0.05.
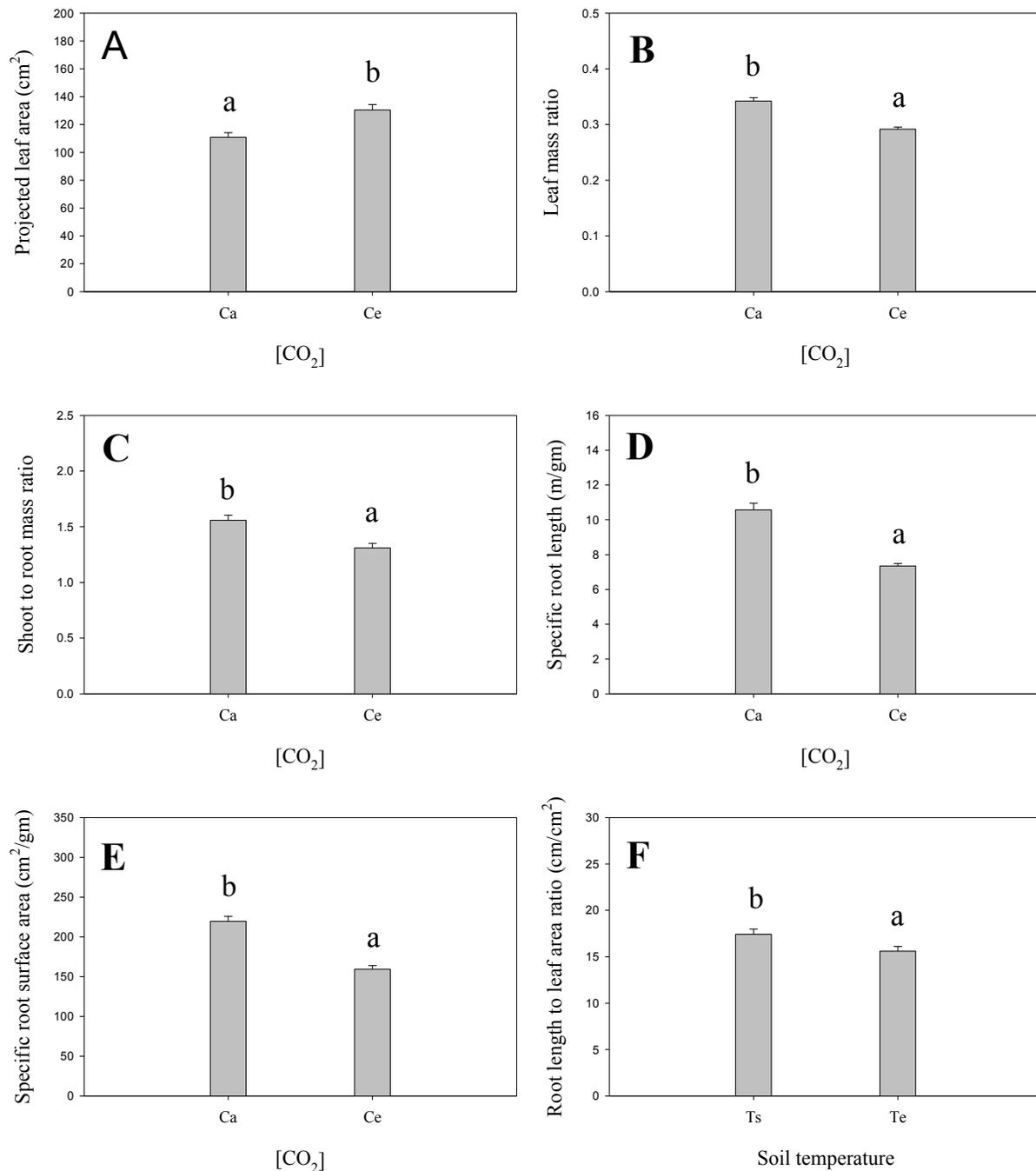
Figure 2.3: Least square means (+SE) of total leaf area, leaf mass ratio, shoot to root mass ratio, specific root length, specific root surface area and root length to leaf area ratio in jack pine seedlings grown under two levels of $[CO_2]$ (C_a = 400 & C_e = 950 µmol mol^{-1}), two soil temperature (T_s, seed origin and T_e, 5° warmer than T_s) and three photoperiods (P_s, seed origin and P_{nm1} & P_{nm2}, values for 5° & 10° northward migration, respectively). Different lowercase letters indicate statistically significant differences at p≤0.05.

444   The relative growth rate of height (RGR_H) increased (P<0.05) with the length of

445   photoperiod (123.51±4.14 for P_{nm2} vs. 112.20±3.40 for P_s, Figure 2.2A). Similarly, the relative

446   growth rate of RCD (RGR_D), relative growth rate of stem volume growth (RGR_V), and seedling

447   total biomass (M) became greater by elevated [CO_2] (Figures 2.2B, 2.2D, 2.2E). The significant

448   CxT interaction (P<0.05) on RGR_D indicated greater [CO_2] effect under current soil temperature

449   (Table 2.2, Figure 2.2C).

450   The biomass allocation of jack pine seedlings was significantly affected by atmospheric

451   [CO_2] and soil temperature (P<0.05, Table 2.2). The total projected leaf area was significantly

452   greater (Figure 2.3A), and leaf mass ratio (0.34±0.00 vs. and 0.29±0.00), shoot to root mass ratio

453   (1.56±0.05 vs. 1.31±0.04) (Figures 2.3B and 2.3C), specific root length (10.57±0.38 vs.

454   7.35±0.15 m/gm, and specific root area (219.63±6.02 vs 159.29±4.52 cm^2/gm) (Figures 2.3D &

455   2.3E) were smaller under elevated [CO_2]. The root length to leaf area ratio reduced with the

456   increase of soil temperature (15.59±0.52 vs. 17.41±0.57 cm/cm^2, Table 2.2, Figure 2.3F).

2.4 Discussion

457

458   The advance of budburst in jack pine seedlings with [CO_2] elevation, soil warming and

459   photoperiod increase is consistent with the results of many predictions (Basler & Körner, 2012;

460   Heide, 1993a; Heide, 1993b; Körner & Basler, 2010; Laube et al., 2014; Myking & Heide, 1995;

461   Partanen et al., 1998; Zohner & Renner, 2014; Zohner & Renner, 2015), but different from

462   others including delayed or no responses to elevated [CO_2] ((Jach et al., 2001; Slaney et al.,

463   2007; Apple et al., 1998; Olszyk et al., 1998; Bigras & Bertrand, 2006; Roberntz, 1999; Bergh &

464   Linder, 1999; Domisch et al., 2001; Vapaavuori et al., 1992; Lyr & Garbe, 1995). As budburst is

465   primarily controlled by temperature and longer photoperiod promotes dormancy release and

466   budburst only in some species (Laube et al., 2014; Zohner & Renner, 2014; Basler & Körner,

24

467   2014; Hänninen, 1990; Partanen *et al.*, 1998; Zohner & Renner, 2015), the advance of budburst

468   by P_{nm2} may be more due to the greater thermal accumulation under longer daytime hours in

469   spring time. The individual effects of elevated $[CO_2]$, soil temperature and longer growing

470   season photoperiods appeared to be additive; the budburst in the C_e x T_e x P_{nm2} combination was

471   13 days earlier than that in the C_a x T_s x P_s treatment combination.

472   The lack of treatment effect by $[CO_2]$ and soil temperature levels on bud set timing is

473   supported by the general believe that bud set is induced largely by shortening photoperiod

474   (Dalen, 1998, Centritto *et al.*, 1999), although a positive effect of elevated $[CO_2]$ has been

475   reported on some tree species including black spruce (Bigras & Bertrand, 2006) and Sitka spruce

476   (Murray *et al.*, 1994). However, the delayed bud set in jack pine seedlings by the photoperiod

477   regimes under northward migration is probably associated with the concentrations of endogenous

478   abscisic acid (ABA) and indole-3-acetic acid (IAA), which play a role in the photoperiodic

479   control of bud dormancy (Li *et al.*, 2003).

480   As expected, the elevated $[CO_2]$ increased the relative growth rates of root collar diameter

481   (RGR_D) and stem volume (RGR_V) as well as total seedling biomass (TB). In C_3 plants the rate of

482   net CO_2 assimilation is not CO_2-saturated at the current level of atmospheric $[CO_2]$ (Lambers *et*

483   *al.*, 2008) and higher $[CO_2]$ would positively affect growth if nutrients are not limiting (as would

484   be in this study). The enhanced growth by elevated $[CO_2]$ may also be related to the reductions in

485   dark respiration and earlier budburst and therefore longer growing season (Surano *et al.*, 1986;

486   Guehl *et al.*, 1994; Ceulemans *et al.*, 1995; Johnsen & Seiler, 1996; Curtis & Wang, 1998;

487   DeLucia, 2000; Bigras & Bertrand, 2006; Zhang & Dang, 2006 and Marfo & Dang, 2009).

488   Similarly, RGR_H was greater under photoperiod regimes associated with higher latitudes in jack

489   pine seedlings, possibly due to longer hours of photosynthesis. There was no significant

490   increases of RGR_H with elevated $[CO_2]$ and seedling growth with warmer soils, as has been

491   reported by others (Ceulemans *et al.*, 1995; Pushnik *et al.*, 1995; Jach & Ceulemans, 1999;

492   Ambebe *et al.*, 2013; Dawes *et al.*, 2011; Peng & Dang, 2003; Walker *et al.*, 2006). I speculate

493   that the soil temperature at seed origin is not too low to affect the physiological activities and

494   growth of jack pine seedlings, as indicated by others that soil temperature effect is more species-

495   specific (Butler *et al.*, 2012; Danyagri & Dang, 2013; Dawes *et al.*, 2011; Melillo *et al.*, 2011).

496   As found by others (Huang *et al.*, 2007; Lukac *et al.*, 2010; Curtis & Wang, 1998; Rogers

497   *et al.*, 1994; Zhang *et al.*, 2006; Huang *et al.*, 2007 and Cao *et al.*, 2008), elevated $[CO_2]$ affected

498   biomass allocation and functional traits of leaf and root in jack pine seedlings such as lower leaf

499   mass ratio (LMR), shoot to root ratio (SRR), specific root length (SRL) and specific root surface

500   area (SRA), even though not all of the treatment effects (e.g., specific leaf area (SLA)) reached

501   the level of significance.

502   Though the main effects on phenological traits, growth and biomass allocation were mostly

503   significant, interactive effects were generally statistically insignificant with the exception of

504   budburst and RCD. The lack of interaction effects suggest that soil temperature and northward

505   migration associated changes in photoperiod will limit the enhancing effects of elevated $[CO_2]$

506   on the growth and phenotypic traits in jack pine. However, advanced budburst at photoperiod

507   regime of 10º north of the seed origin under elevated $[CO_2]$ and soil temperature will likely

508   increase the risk of spring frost damage, which may be an obstacle to the northward migration of

509   the species.

510

511

Chapter 3: Both CO$_2$ elevation and soil warming reduce the cold hardiness of jack pine seedlings

3.1 Introduction

Physiological traits often determine the ability of a species to establish and grow under the physical environment of a particular site (Lambers *et al.*, 2008). Climate changes may modify the environmental conditions and the availability of resources that are crucial to plant performance. Plants respond to these changes by making phenotypic adjustments in short and medium term or by migrating to a new location having similar environmental conditions that they are adapted to, or by combinations of the two (Aitken *et al.*, 2008). Since it is almost impossible to get exactly the same environment to migrate to, the most common response of plants to climate change might be the combination of migration and acclimation, which depends on the magnitude of climate change and the plasticity of the species. Therefore, the physiological plasticity of plants will be critical in their response to climate change with or without migration.

In response to climate changes forests have moved across landscapes (Bunnell & Kremsater, 2012). The climate envelopes of 130 North American tree species have been predicted to shift 10 degrees (approximately 1000 kilometers) northward between 2071 and 2100 in response to the climate change (McKenney *et al.*, 2007; McKenney *et al.*, 2011). If a species migrates naturally or with human assistance according to the predicted shift in their climate envelope, they will face a new environment with many factors that are very different from the conditions as their current habitat, for example, photoperiod and soil temperature. Changes in photoperiod associated with climate change induced northward migration will likely affect the

534 phenotypic and physiological processes in plants (Pothier & Prevost, 2002) by regulating the

535 chemical composition and the ratio of active and inactive forms of phytochrome. Since

536 photoperiods at higher latitudes are longer in the summer and shorter in the winter, the ability of

537 trees to acclimate to the new photoperiod regime may be a key determinant for the success of

538 tree migration or seed transfer northward. A 10° northward migration or seed transfer under

539 doubled atmospheric [CO_2] may increase growth, but the cold hardiness and the timing of bud

540 flush may not be properly synchronized with the change of the season, exposing trees to frost

541 damage (Li *et al.*, 2015). A 10° northward migration may be too much a leap for trees to

542 acclimate to. There might be a threshold photoperiod regime beyond which the tree will not have

543 the capacity to acclimate.

544   It is predicted that the average global air temperature will increase 1.8 - 4.0° C by the end

545 of this century (IPCC, 2007). Regions at high latitudes are predicted to warm up substantially

546 faster and to a greater extent (IPCC, 2007; Pachauri & Reisinger, 2007). For example, the mean

547 annual temperature increase in the boreal region is likely to be as high as $5–7^{\circ}$ C (Christensen *et*

548 *al.*, 2007; Eskelin *et al.*, 2011; Heimann & Reichstein, 2008; Montzka *et al.*, 2011). Such

549 increases in temperatures will likely bring major changes in ecosystem structure and functions as

550 well as shifts in species' geographical ranges (IPCC, 2007). Since the extent of climate change is

551 likely to be greater in the boreal region, the impacts on boreal plants may also be greater (Barnett

552 *et al.*, 2005; Juday *et al.*, 2005; Serreze *et al.*, 2000).

553   The global atmospheric CO_2 is predicted to increase to as high as 950 μmol mol^{-1} by the

554 end of the 21st century (Cao & Caldeira, 2010; IPCC, 2001; Luthi *et al.*, 2008). Elevated

555 atmospheric [CO_2] affects the growth and physiological performance of plants by its influence

556 on plant physiology and phenology (Long *et al.*, 2004). Elevated atmospheric [CO_2] generally

557   stimulates photosynthesis in C_3 plants with a concomitant increase in biomass (Eamus &

558   Ceulemans, 2001) but long term exposure to high atmospheric [CO_2] can reduce the extent of the

559   stimulation due to the down regulation of photosynthetic capacity (Cui & Nobel, 1994). Stomatal

560   conductance under elevated [CO_2] typically reduces (Bunce, 2004; Gunderson *et al.*, 2002;

561   Leakey *et al.*, 2006; Marchi *et al.*, 2004; Medlyn *et al.*, 2001; Morgan *et al.*, 2004). Increased

562   atmospheric [CO_2] may affect bud phenology through changes in tree biochemistry and

563   physiology. Changes in starch or hormonal concentrations due to increased atmospheric [CO_2]

564   may alter dormancy status and growth patterns by shifting the timing and duration of the

565   vegetative growth (Cannell, 1990).

566   Soil temperature is likely to increase with the increase in air temperature under the

567   predicted changes of climate in the boreal forests where net photosynthesis and tree growth are

568   generally restricted by low soil temperatures (Aphalo *et al.*, 2006; Ensminger *et al.*, 2008). As a

569   result, the increased soil temperature should improve the establishment and early growth of

570   conifer seedlings (Smith, 1985) and reduce stresses on newly planted seedlings by enhancing

571   water relations, gas exchange and root growth (Grossnickle, 2000). Soil temperature can

572   effectively modify the morphological and physiological traits (Cai & Dang, 2002; Dang &

573   Cheng, 2004) and influence the stomatal conductance and water uptake in plants (Alvarez-Uria

574   & Körner, 2007; Ambebe *et al.*, 2010; Frechette *et al.*, 2011; Zhang & Dang, 2007). However, a

575   high soil temperature reduces photosynthetic rates and growth (Ambebe *et al.*, 2010; Way &

576   Sage, 2008b; Way & Sage, 2008a) and a low soil temperature can inhibit nutrient and water

577   uptake and influence the physiological processes in plants (Grossnickle, 2000; Peng & Dang,

578   2003; Stathers & Spittlehouse, 1990).

579   While environmental variables, e.g. [CO_2], soil temperature and photoperiod, have their

580   individual impacts on the growth and biomass allocations in trees (Huang *et al.*, 2007; Lukac *et*

581   *al.*, 2010), interactive effects among them could be quite different (Curtis *et al.*, 2000; Nowak *et*

582   *al.*, 2004). For example, responses of plants to elevated [CO_2] are influenced by air temperature,

583   soil temperature and light regime (Allen *et al.*, 1990; Marfo & Dang, 2009; Mishra *et al.*, 1999;

584   Robredo *et al.*, 2007; Zebian & Reekie, 1998). Although the effects of individual environmental

585   factors or bi-factor effects on boreal tree growth are well documented (DeLucia & Smith, 1987;

586   Fraser, 1962; Li *et al.*, 2015; Peng & Dang, 2003; Soolanayakanahally *et al.*, 2013; Way & Sage,

587   2008b; Way & Sage, 2008a), our understanding on the interactive effects of multiple

588   environmental variables, e.g., [CO_2], soil temperature and photoperiods, is still limited. The

589   objective of this study was to assess the interactive effects between photoperiod and elevated soil

590   temperature on the physiological responses of jack pine to CO_2 elevation. Since jack pine grows

591   in a wide range of climatic conditions with large fluctuations in most climatic variables (Rudolf,

592   1965), I hypothesize that warmer soil temperature and photoperiod regimes at higher latitudes

593   than seed origin under elevated [CO_2] would accelerate the physiological processes in jack pine

594   seedlings but reduce cold hardiness and make seedlings prone to frost damage.

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3.2 MATERIALS AND METHODS

3.2.1 Plant materials

One-year old jack pine (*Pinus banksiana* Lamb.) seedlings were obtained from the Boreal Tree Seedling Nursery near Thunder Bay. The seedlings were raised from seeds collected from Kakabeka region ($48^{\circ}57^{'}$ N & $90^{\circ}44^{'}$ W). A total of 608 seedlings, relatively uniform in height and diameter, were planted in pots of 15 cm in height and 13 cm in diameter filled up with a mixture of premium grade vermiculite and peat moss in a ratio of 1:1 (v/v).

3.2.2 Experimental design

The experiment was conducted in four green houses at Lakehead University's Thunder Bay campus following a split plot design, with [CO_2] as the whole plot, soil temperature as the sub-plot and photoperiod as the sub-sub plot. The treatments were consisted of two levels of [CO_2] (400 and 950 µmol mol^{-1}), two soil temperatures (soil temperature at seed origin, and 5ºC warmer) and three photoperiod regimes (photoperiod at seed origin, 5º and 10º north of the seed origin). Two levels of [CO_2] were randomly assigned to four independent greenhouses with two replicates of each. The two levels of soil temperature were set up within each level of [CO_2] and three levels of photoperiod were assigned within each soil temperature.

3.2.3 Environmental controls

The [CO_2] in the greenhouses was elevated using electronic ignition natural gas CO_2 generators (model GEN-2E, Custom Automated Products Inc, Riverside, CA). Circulation fans were used to distribute CO_2 evenly throughout each of the greenhouses. The [CO_2] in each

621 greenhouse was monitored and controlled automatically with an Argus CO_2 Control System

622 (Argus, Vancouver, BC, Canada). The soil temperatures were derived from the biweekly mean

623 soil temperatures of 2012 and 2013 at Kakabeka region (seed origin). Average temperatures of

624 depths 5 cm and 20 cm were used. Soil temperatures were adjusted biweekly based on the

625 biweekly average, using the soil temperature control system designed by Cheng *et al.* (2000).

626 The lengths of the photoperiod for active growing and cold hardening phases for each of the

627 three latitudes were adjusted biweekly based on the biweekly averages at corresponding

628 locations. The photoperiods were extended using high-pressure sodium lamps when the natural

629 day length was shorter than the set values or shortened through manual shading when the natural

630 day length were longer than the set values. Seedlings were fertilized biweekly at 7.14N, 0.56P

631 and 2.12 mmol K per litre of water during the rapid growth phase and 1.78 mmol N, 2.20 mmol

632 P and 4.64 mmol K per liter of water during the hardening phase (Scarratt, 1986). The soil

633 moisture level was maintained at 60–70% of the field water capacity, monitored daily using a

634 Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). In all the

635 greenhouses the relative humidity was maintained at 55–60% during the rapid growth phase and

636 45–50% during the hardening phase. The day and night air temperatures were controlled to

637 mimic the mean biweekly day and night air temperatures at the seed origin. Relative humidity

638 and air temperature were controlled automatically using the Argus Control System referred to

639 previously.

640 **3.2.4 Gas exchange measurements**

641   Foliar gas exchange measurements started after 102 days of the treatments on three

642 seedlings randomly selected from each treatment combination. All measurements were made

643 between 0930 and 1430 h. The gas exchange was measured on current year foliage using a PP-

644   Systems CIRAS-3 open gas exchange system (PP System Inc., Amesbury, MA, USA).

645   Photosynthetic responses to CO_2 concentration (A/C_i curves) were measured at 50, 150, 250,

646   400, 550, 730, 900 and 1200 µmol mol^{-1} CO_2 at photosynthetically active radiation of 800 µmol

647   m^{-2}s^{-1}, 25º C air temperature and 50% relative humidity. The net rate of carbon dioxide

648   assimilation (A_n), stomatal conductance (g_s) and transpiration rate (E) were calculated according

649   to Farquhar *et al.* (1980). The A/C_i response curves were analyzed to estimate V_{cmax} (maximum

650   rate of carboxylation), J_{max} (maximum rate of electron transport) and *TPU* (triose phosphate

651   utilization) according to Sharkey *et al.* (2007). The light saturated A_n at the growth [CO_2] (400

652   and 950 µmol mol^{-1}) and at the ambient [CO_2] for both CO_2 treatments (A_{n-400}) were derived

653   from the *A/Ci* curves. g_s, E and *WUE* measured at the corresponding growth [CO_2] were used in

654   data analyses.

655   **3.2.5 Electrical conductivity and index of injury**

656   The cold hardiness of shoot tips was assessed at the end of the experiment (May 2014)

657   following the method by Colombo *et al.* (1989). Terminal shoot tips of 3 cm in length were

658   collected from nine seedlings randomly selected from each treatment combination and divided

659   among three testing temperatures (-5, -15 and -30 ºC). Each shoot tip was rinsed with distilled

660   water and placed in a test tube containing 15 ml distilled water and allowed to incubate at room

661   temperature (20–25 ºC) for 24 hours. After shaking the test tubes vigorously, the control

662   electrical conductivity (ECC) of the bathing solution was determined using a Fisher Accumet AR

663   29 electrical conductivity meter (Fisher Scientific, Ottawa, Canada). The bathing solution was

664   then separated and the wet shoot tips in stoppered test tubes were cooled down to each testing

665   temperature at a rate of 5 ºC per hour using a programmable freezer (Foster Refrigeration Ltd.,

666   King's Lynn Norfolk, U.K.). After 70 minutes at the testing temperature, the temperature was

667 gradually raised (5 ℃ per hour) to room temperature and the samples were transferred to an

668 insulated box at 5 ℃ and left overnight. The previously separated bathing solution was returned

669 to the corresponding test tubes and the samples were allowed to incubate at room temperature for

670 24 hours. At this stage, electrical conductivity of the bathing solutions was measured again as the

671 freezing electrical conductivity (ECF). The samples were then placed in a drying oven at 80 ℃

672 for two hours to kill the tissues and left in room temperature for another 24 hours. The electrical

673 conductivity of the bathing solution was measured again as the killed electrical conductivity

674 (ECK).

675 The index of injury (I_i), which is inversely related to cold hardiness (Flint *et al.*, 1967), was

676 calculated as follows (Colombo *et al.*, 1989):

677 $$I_i = \frac{\frac{ECF}{ECK} - \frac{ECC}{ECK}}{1 - \frac{ECC}{ECK}} X100$$

678 Where, I_i = index of injury (%)

679 ECC = control electrical conductivity

680 ECF = electrical conductivity of the bathing solution measured following freezing of the

681 shoot tips, and

682 ECK = electrical conductivity of the bathing solution measured after killing the shoot tips.

683

684 **3.2.6 Statistical analysis**

685 All data were subjected to Analysis of Variance (ANOVA). Before the ANOVA test,

686 Shapiro-Wilk and Bartlett tests were conducted to test the normality of distribution and

687 homogeneity of variance, respectively. The J_{max}, *TPU* and R_d data were transformed using

688   natural logarithm function, g_s using square root and *WUE* using reciprocal of square root to meet

689   the ANOVA assumptions. Tukey's HSD Post-hoc comparisons were carried out when ANOVA

690   tests showed a significant interaction or photoperiod effect ($P \leq 0.05$). In the analyses, $[CO_2]$,

691   soil temperature and photoperiod were treated as fixed variables. All analyses were done using R

692   3.1.3 (R Core Team, 2015).

693

694   **3.3 Results**

695   While the net CO_2 assimilation rate at ambient $[CO_2]$ (A_{n-400}) was not affected by any of

696   the treatments (Table 3.1), elevated $[CO_2]$ significantly increased the net CO_2 assimilation rate at

697   growth $[CO_2]$ ($A_{n-growth}$) (Figure 3.1A) and photosynthetic water-use efficiency (*WUE*) (Figure

698   3.1B). The maximum rate of carboxylation (V_{cmax}) was significantly higher with elevated $[CO_2]$,

699   but had different patterns of variation with photoperiod regimes under different $[CO_2]$ levels

700   (significant CO_2, photoperiod, and CO_2 by photoperiod interaction (Figure 3.1C and Table 3.1).

701   Although the triose phosphate utilization (*TPU*) was generally greater with elevated $[CO_2]$, but

702   the differences varied with soil temperature and photoperiod regime (significant 3-way

703   interaction (Figure 3.1D and Table 3.1). Elevated $[CO_2]$ marginally increased the light saturated

704   rate of electron transport (J_{max}) ($P = 0.079$, Table 3.1).

705   The index of injury (I_i) was significantly higher with elevated $[CO_2]$ at all three testing

706   temperatures (Figures 3.2A, 3.2B and 3.2C) and with warmer soil at -15 and -30 ºC (Figures

707   3.2D and 3.2E). The index of injury was not affected by photoperiod or any interactions (Table

708   3.1).

709

710 **Table 3.1:** ANOVA P-values for the effects of carbon dioxide concentration (C), soil
711 temperature (T), photoperiod (PP) and their interactions on net photosynthetic rates measured at
712 a common $[CO_2]$ (A_{n-400}) and corresponding growth $[CO_2]$ ($A_{n-growth}$), stomatal conductance (g_s),
713 transpiration rate (E), photosynthetic water-use efficiency (WUE), maximum rate of
714 carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}), triose phosphate utilization
715 (TPU), day time dark respiration (R_d) and index of injury (I_i) at test temperatures of -5, -15 and -
716 30 ºC in jack pine seedlings. Seedlings were grown under two levels of $[CO_2]$, two levels of soil
717 temperature and three photoperiod regimes.

Response variables	Treatment effects						
	C	T	PP	C*T	C*PP	T*PP	C*T*PP
An-400	0.300	0.113	0.896	0.937	0.825	0.908	0.844
An-growth	**0.013**	0.938	0.865	0.342	0.853	0.465	0.424
g_s	0.234	0.691	0.603	0.905	0.366	0.453	0.862
E	0.131	0.599	0.709	0.722	0.699	0.530	0.838
WUE	**0.010**	0.416	0.916	0.756	0.366	0.887	0.905
V_{cmax}	**0.003**	0.067	**0.004**	0.764	**0.006**	0.649	0.343
J_{max}	0.079	0.948	0.335	0.433	0.616	0.727	0.087
TPU	**0.038**	0.706	0.552	0.457	0.697	0.379	**0.031**
R_d	0.391	0.365	0.518	0.818	0.746	0.883	0.078
I_i at -5 degree	**0.025**	0.201	0.471	0.273	0.466	0.775	0.167
I_i at -15 degree	**0.028**	**0.020**	0.727	0.235	0.403	0.840	0.650
I_i at -30 degree	**0.010**	**0.047**	0.513	0.710	0.477	0.718	0.756

718
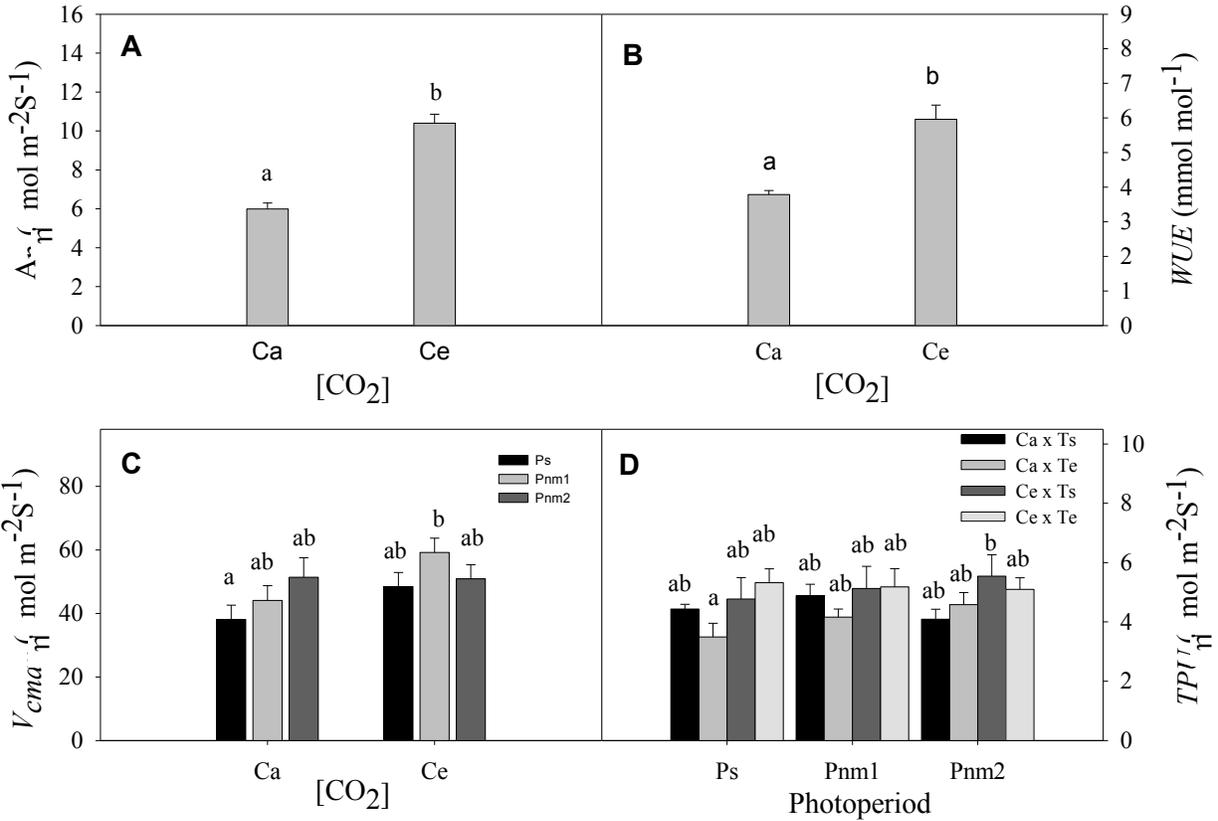
Figure 3.1: Mean (± SE) net photosynthetic rate at growth CO_2 ($A_{n\text{-}growth}$), photosynthetic water-use efficiency (*WUE*), maximum rate of carboxylation (V_{cmax}) and triose phosphate utilization (*TPU*) in jack pine seedlings grown under two levels of [CO_2], two levels of soil temperature and three levels of photoperiod. Means with different letters were significantly different from each other (p≤0.05). C_a & C_e = ambient & elevated [CO_2], respectively, T_s & T_e = high & low soil temperature, respectively, P_s, P_{nm1} & P_{nm2} = photoperiods at seed origin, 5° & 10° north of seed origin, respectively.
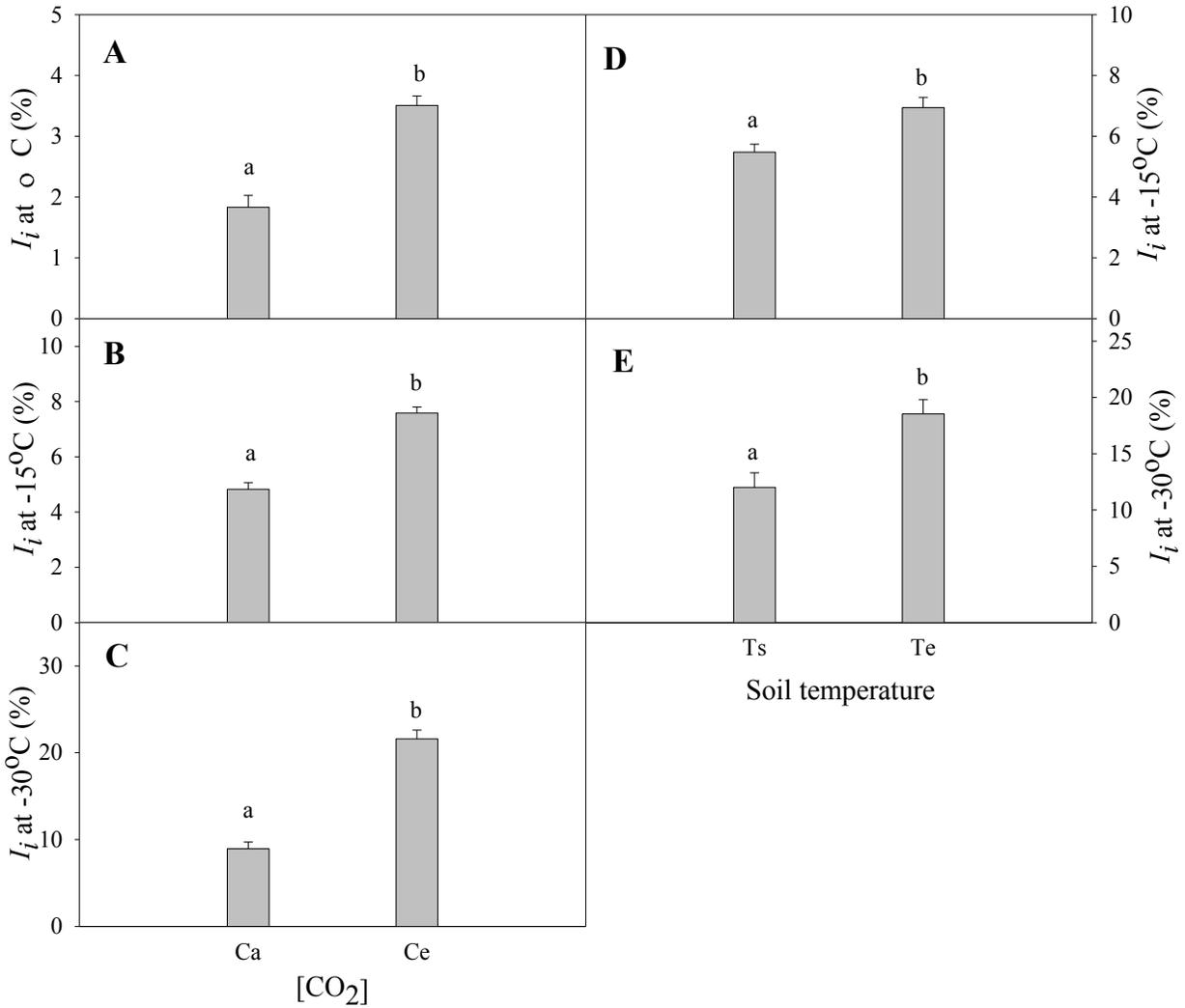
Figure 3.2: Index of injury (mean ± SE) at -5, -15 and -30° C in jack pine seedlings. Seedlings were grown under two levels of [CO_2], two levels of soil temperature and three levels of photoperiod. Means with different letters were significantly different from each other (p≤0.05).

3.4 Discussion

The greater net photosynthetic rate ($A_{n-growth}$) with elevated atmospheric [CO_2] ('CO_2 fertilization effect') is consistent with the findings of previous studies on various tree species (Danyagri & Dang, 2014; DeLucia & Thomas, 2000; Tjoelker $et\ al.$, 1998; Zhang & Dang, 2005). The increase is probably due to the increased availability of substrate (CO_2) and enhanced photosynthetic enzyme activity (Zhang & Dang, 2006), as the CO_2 assimilation is not CO_2-saturated in C_3 plants at current CO_2 level (Lambers $et\ al.$, 2008) and nutrient limitations should not occur under well-fertilized conditions. Photosynthetic water-use efficiency (WUE) was also greater with elevated [CO_2], as found by others (Keenan $et\ al.$, 2013; Long $et\ al.$, 2004; Zhang & Dang, 2005). The increased WUE under elevated [CO_2] might be associated with decreased leaf area to root mass ratio as stated by Norby & O'Neill (1991) and Poorter (1999).

In general, warmer soil temperature increases $A_{n-growth}$ by reducing root resistance to water uptake and thus increasing water absorption and leaf water potential (Dang & Cheng, 2004; Day $et\ al.$, 1991) at certain threshold levels (Dang & Cheng 2004). High soil temperatures could impair root growth and activity and limit water supply to shoots causing stomatal closure and decline in A_n (Xu & Huang, 2000), as reported by Ishida $et\ al.$ (1999), Pons & Welschen (2003), Haldimann & Feller (2004), Souza $et\ al.$ (2005) and Ambebe & Dang (2009).

No photosynthetic down-regulation was observed in this study, which are consistent with the observations by Osborne $et\ al.$ (1997), Liang $et\ al.$ (2001), Zhang & Dang (2006), Darbah $et\ al.$ (2010), Danyagri & Dang (2013), but different from the observations by Ellsworth $et\ al.$ (2004), Lewis $et\ al.$ (2004), Nowak $et\ al.$ (2004), Tissue & Lewis (2010) and Watanabe $et\ al.$ (2011). Photosynthetic down-regulations are usually associated with nutrient supply, particularly

764 nitrogen, sink strength and the leaf area index (Jach & Ceulemans, 1999). Growth at elevated

765 [CO_2] with low N supply results in decreased carboxylation capacity and reduced amount of

766 Rubisco protein (Lambers *et al.*, 2008). In this study, jack pine seedlings were well fertilized and

767 leaf [N] limitation on photosynthesis was unlikely, despite of greater photosynthesis rate, growth

768 and demand for nitrogen under elevated [CO_2]. Moreover, the seedlings were actively growing

769 and leaf area was increasing at the time of the measurement, indicating a strong sink. The

770 stimulated photosynthesis at elevated [CO_2], however, may not be maintained for long due to

771 feedback within plants and ecosystem (Luo & Reynolds, 1999), as well as the change of sink

772 activity with developmental stage and leaf age in plants (Long *et al.*, 2004). The reported

773 changes in V_{cmax} by elevated [CO_2] are generally due to the changes of leaf [N] (Lewis *et al.*,

774 2004), as would be the impacts of fertilization. Enhanced carboxylase activity at elevated [CO_2]

775 may be associated with needles of the young seedlings examined as young leaves enhance the

776 response of RuBP carboxylase to elevated [CO_2] (Hicklenton & Jolliffe, 1980; Koch *et al.*, 1986;

777 Peet *et al.*, 1986; Porter & Grodzinski, 1984).

778   The results of the study indicate that both elevated [CO_2] and soil temperature significantly

779 reduced the cold hardiness of jack pine, which are in line with the findings of Repo *et al.* (1996),

780 Guak *et al.* (1998), Lutze *et al.* (1998) and Barker *et al.* (2005). Ice nucleation takes place at a

781 higher temperature in plants grown under elevated [CO_2] (Lutze *et al.*, 1998), which might be a

782 potential reason of reduced cold hardiness in seedlings grown under elevated [CO_2]. High soil

783 temperature would influence plant temperature and therefore the level of cold hardiness, as

784 winter hardening, induced by shortening photoperiod, develops with the decrease of

785 temperatures (Dalen & Johnsen, 2004). High temperatures affects development of cold hardiness

786 by changing membrane fluidity, reducing calcium influx to the cytosol and thus disrupting

787   calcium signalling (Monroy & Dhindsa, 1995). Elevated $[CO_2]$ and soil temperature could also

788   affect xylem sap pH and therefore abscisic acid concentration, which acts as a signal for the

789   perception of cold temperatures (Hwei-Hwang *et al.*, 1983). However, the lack of the

790   photoperiod and interaction effects on cold hardiness in jack pine might be associated with rapid

791   shortening of the day length toward north. To initiate the cold acclimation process, some species

792   respond to the combination of minimum temperature and shortening of day length rather than

793   relying on low temperature alone (Dalen & Johnsen, 2004).

794   The findings of the experiment suggest that predicted elevated atmospheric $[CO_2]$ will

795   accelerate the physiological processes in jack pine, but both elevated $[CO_2]$ and soil temperature

796   reduces the cold hardiness of the species. The lack of treatment effects by photoperiod suggests

797   that the enhanced physiological processes by elevated $[CO_2]$ and reduced cold hardiness by

798   elevated $[CO_2]$ and soil temperature in jack pine seedlings will not change with northward

799   migration in response to climate change. However, since the study was conducted on the current

800   foliage after a short period of growth in environment controlled greenhouses, the results may not

801   accurately reflect the response of plants after long exposure to field conditions.

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Chapter 4: Eco-physiological potential of jack pine for northward migration: interactions among photoperiod, [CO_2] and moisture stress

4.1 Introduction

Forests have migrated across landscapes in response to past climate changes (Bunnell & Kremsater, 2012; Thomas, 2000). The continued increase in global atmospheric CO_2 will cause continued increase in temperature (Cao & Caldeira, 2010; IPCC, 2001; Luthi *et al.*, 2008) and alter the amount, intensity, frequency and type of precipitation. Such changes can aggravate soil water shortage in some regions (Allison & Treseder, 2008; Johnston *et al.*, 2009). The climate change will affect plant morpho-physiological processes, growth, and survival (Domec *et al.*, 2009; Meinzer, 2002; Volder *et al.*, 2007) and the impacts will likely be greater in the boreal region (Barnett *et al.*, 2005; IPCC, 2007; Juday *et al.*, 2005; Pachauri & Reisinger, 2007; Serreze *et al.*, 2000) because the magnitude of climate change will be greater in the boreal region than the global average (Christensen *et al.*, 2007; Eskelin *et al.*, 2011; Heimann & Reichstein, 2008; Montzka *et al.*, 2011). In response to the continued climate change, the climate envelopes of 130 North American tree species have been predicted to shift 10 degrees (approximately 1000 kilometers) northward between 2071 and 2100 (McKenney *et al.*, 2007; McKenney *et al.*, 2011). The predicted northward migration will expose the migrating plants to environmental conditions that may be quite different from the conditions they have adapted to (photoperiod regime, for example). Therefore the spatial extent and success of such migration will depend on the capability of the migrating species to acclimate to the physical environment of their new habitats (Thomas & Vince-Prue, 1997).

The photoperiodism of a species develops through genetic modifications over hundreds or even thousands of years (Thomas & Vince-Prue, 1997). The length of photoperiod affects plant phenological and physiological events (Soolanayakanahally *et al.*, 2013; Thomas & Vince-Prue, 1997) and net assimilation rate by changing the percentage of total nitrogen in dry matter (Lambers *et al.*, 2008). It regulates the phenotypic processes in boreal plants through synchronization with the natural seasonal changes in environmental conditions (Lambers *et al.*, 2008). Changes in photoperiod regimes associated with northward migration may affect the timing of photoperiodically controlled growth events and lead to untimely phenological responses like earlier bud burst in the spring or delayed bud setting and reduced cold hardiness in the fall, all of which may expose plants to adverse environmental conditions and affect their survival and growth (Man *et al.*, 2009). Since the photoperiod at higher latitudes is longer in the summer, shorter in the winter and thus a faster rate of photoperiod change during seasonal transitions, the ability of a species to acclimate to the new photoperiod regime may be a key determinant for the success of tree migration and/or seed transfer northward. However, the effect of changes in photoperiod regime has not been well understood.

Elevated [CO_2] has diverse and complex effects on physiological and growth performance of plants (Bowes, 1993; Lawlor & Mitchell, 1991). It usually stimulates photosynthesis and promotes growth and yield (Ainsworth & Long, 2005; Marfo & Dang, 2009; Norby *et al.*, 1999) and alters the structure and physiology of plants through changes in carbon allocation (Janssens *et al.*, 1998; Koch *et al.*, 1986; Rogers *et al.*, 1994; Tingey *et al.*, 2000). The improved water–use efficiency under elevated [CO_2] (Field *et al.*, 1995; Keenan *et al.*, 2013; Long *et al.*, 2004) resulting from the reduced stomatal conductance (Ainsworth & Rogers, 2007) indirectly stimulates photosynthesis in dry environments by reducing the impact of drought on

photosynthesis (Hungate *et al.*, 2002). However, the effects are variable and subject to environmental feedback (Gunderson *et al.*, 2002; Leakey *et al.*, 2006; Morgan *et al.*, 2004). Elevated [CO_2] also affects the growth rhythm of trees by altering the timing of bud burst and growth cessation (Bigras & Bertrand, 2006; Ceulemans *et al.*, 1995).

The soil moisture conditions can affect the physiological and morphological characteristics of plants and influence their response to the changes of CO_2 and environmental conditions associated with climate change. For example, the CO_2 elevation induced decline in stomatal conductance is smaller under soil moisture stress (Ambebe & Dang, 2009; de Miguel *et al.*, 2012; Mishra *et al.*, 1999); low soil moisture minimizes the enhancing effects of elevated soil temperature on net photosynthetic rate and stomatal conductance (Ambebe & Dang, 2009). However, photosynthetic biochemistry and photochemistry are not affected by moisture stress unless the stress is extreme (Richardson *et al.*, 2004; Ennahli & Earl, 2005). Drought conditions inhibit water uptake and reduce transpiration (Brodribb *et al.*, 2005; Wang *et al.*, 2003). Plants acclimate to drought by modifying the sizes and shapes of leaves and increasing the root/shoot ratio (Ibrahim *et al.*, 1997; Marron *et al.*, 2002; Pallardy, 2008; Warren *et al.*, 2005), in order to achieve balances between the capture of light and CO_2 and the limitations imposed by water loss (Sefton *et al.*, 2002).

The effects of climate change on trees reflect the interactive response of multiple factors (Johnston *et al.*, 2009). The combined effects of interacting factors can be quite different from the summation of their individual effects (Curtis *et al.*, 2000; Nowak *et al.*, 2004). For example, the effects of CO_2 elevation vary considerably with changes in other environmental conditions (Allen *et al.*, 1990; Cao *et al.*, 2007; Gunderson & Wullschleger, 1994; Marfo & Dang, 2009; Mishra *et al.*, 1999; Poorter, 1993; Robredo *et al.*, 2007; Zhang & Dang, 2007; Zebian & Reekie,

1998; Zhang & Dang, 2006). Moisture stress reduces the positive effects of CO_2 elevation on leaf area, leaf dry weight and stem dry weight (Mishra *et al.*, 1999). However, the interactive effects on plant responses to climate change are not well understood.

Jack pine is distributed over a wide range of environmental conditions (Rudolph & Laidly, 1990). This species has a strong tolerance to drought, a plant trait that is increasingly important with increasing climatic drought under rising temperature and changes in the distribution of precipitation. The main objective of this study was to investigate the interactive effects of photoperiod, soil moisture and $[CO_2]$ on the physiological and morphological traits of jack pine and examine the influences of these factors on the potential migration and/or seed transfer of the species. I hypothesized that elevated $[CO_2]$ and northward migration associated longer photoperiod during summer will accelerate the growth and physiological processes in jack pine but their interactions with soil moisture stress will complicate and limit those responses.

4.2 Materials and methods

4.2.1 Plant materials

The experiment was conducted using one-year old jack pine (*Pinus banksiana* Lamb.) seedlings raised from seeds collected in Kakabeka region (48º57′ N & 90º44′ W). A total of 448 seedlings of relatively uniform sizes (height and root collar diameter) were transplanted in plastic pots (15cm in height and 13cm in diameter) filled up with a mixture of premium grade vermiculite and peat moss (1:1, v/v).

4.2.2 Experimental design

The experiment was carried out in four greenhouses at Lakehead University's Thunder Bay campus. The treatments consisted of two levels of $[CO_2]$ (400 and 950 µmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field capacity) and three photoperiod regimes (P_s, P_{nm1} and P_{nm2}, representing the photoperiod at the seed origin, 5° and 10° north of the seed origin, respectively). The experiment was a split plot design, with $[CO_2]$ as the whole plot, soil moisture as the sub-plot and photoperiod as the sub-sub plot. Two levels of $[CO_2]$ were assigned to four independent greenhouses randomly with two replicates of each. Two levels of soil moisture were applied within each greenhouse and three photoperiod regimes were incorporated within each soil moisture treatment.

4.2.3 Growing conditions

The experiment was conducted between November 20, 2013 and May 31, 2014 mimicking the actual day lengths and temperatures of the seed origin for the period of April 16 to October 31. Desired levels of CO_2 were maintained in the greenhouses using electronic ignition natural gas CO_2 generators (model GEN-2E, Custom Automated Products Inc, Riverside, CA). Circulation fans were used to ensure even distribution of CO_2 throughout each of the greenhouses. The $[CO_2]$ in each greenhouse was monitored and controlled automatically with Argus CO_2 Control System (Argus, Vancouver, BC, Canada). The moisture content of the growing medium was measured daily with a HH2 moisture meter and a ML2x Theta probe (Delta-T Devices, Cambridge, UK) and the desired moisture levels were maintained by adding water when required. The photoperiod for each treatment was adjusted biweekly to mimic the actual biweekly average photoperiods (April 16 to October 31) at the corresponding locations.

The desired photoperiod lengths were achieved by extending the natural day lengths using high-pressure sodium lamps or shortening the natural day lengths through manual shading of the seedlings. All the seedlings were fertilized biweekly with N:P:K fertilizer (7.14, 0.56 and 2.12 mmol per litre of water, respectively during the rapid growth phase and 1.78, 2.20 and 4.64 mmol per liter of water, respectively during the hardening phase) (Scarratt, 1986). In all the greenhouses relative humidity was maintained at 55–60% during the rapid growth phase and reduced to 45–50% during the hardening phase. The day and night air temperatures were set biweekly to mimic the mean biweekly day and night air temperatures at the seed origin. Relative humidity and air temperature were controlled automatically using an Argus Control System.

4.2.4 Growth measurements and observation of bud break and bud setting

At the time of transplanting, the height and root collar diameter (RCD) of each seedling were measured. Ten seedlings were selected randomly from each treatment combination to investigate the bud break and bud setting at the beginning of the experiment and during the cold hardening phase, respectively. The terminal meristems of the seedlings were checked visually every day for bud break. Bud break was considered completed when most of the bud scales fell, with tips of needles protruding about 2 mm (Bigras & Bertrand, 2006). Days to bud break were counted from the beginning of the treatments (November 20, 2013). Bud set was also determined by visual observation and was considered accomplished when bud scales were completely closed (Bigras & Bertrand, 2006). Bud setting time was counted from April 16, 2014, when the day length and day/night air temperature were reduced to 12 hours and 14/8 ºC, respectively.

4.2.5 Gas exchange measurement

Foliar gas exchange was measured on the current year foliage of three randomly chosen seedlings from each treatment combination using a PP-Systems CIRAS-3 open gas exchange system (PP System Inc., Amesbury, MA, USA). All measurements were conducted between 0930 and 1430 h during the active growing phase (102 to 120th days of treatment). The photosynthetic responses to CO_2 concentration (A/C_i curves) were measured at eight [CO_2]: 50, 150, 250, 400, 550, 730, 900 and 1200 $\mu mol\ mol^{-1}$ under 800 $\mu mol\ m^{-2}s^{-1}$ photosynthetically active radiation, 25 ºC air temperature and 50% RH. Readings were taken at each increment allowing a 5-6 minutes acclimation period. The rate of CO_2 assimilation (A_n), stomatal conductance (g_s), transpiration rate (E), water use efficiency (*WUE*) were calculated according to Farquhar *et al.* (1980). The A/C_i response curves were analyzed to estimate V_{cmax} (maximum rate of carboxylation), J_{max} (maximum rate of electron transport) and *TPU* (triose phosphate utilization) according to Sharkey *et al.* (2007).

4.2.6 Measurements of biomass and leaf and root traits

At the end of the experiment (May 28, 2014) the height and RCD on three randomly chosen seedlings from each treatment combination were measured again and the relative growth rates of height and RCD were determined. The stem volume was calculated according to van den Driessche (1992) ($V = (\pi D^2/4)\ H/3$).The seedlings were harvested when all the measurements were completed and separated into foliage, stem (including branches) and roots. The projected leaf area (PLA) was determined by scanning all live needles with WinSeedle (Regent Instruments Inc, Quebec, Canada) for determining the specific leaf area. The roots were scanned to analyze root traits using WinRhizo (Regent Instruments Inc, Quebec, Canada). The foliage,

stem and roots were subsequently oven-dried at 70 ºC for 48 hours and weighed on an analytical

balance (0.001g precision) to determine dry mass. Stem mass ratio (ratio of stem mass to total

biomass, SMR), root mass ratio (ratio of root mass to total biomass, RMR), leaf mass ratio (ratio

of leaf mass to total biomass, LMR), shoot to root mass ratio (ratio of above ground to

belowground mass, SRR) were used as indices of biomass allocation. Specific leaf area (SLA)

was determined from the projected leaf area and leaf dry mass. Specific root length (SRL) and

specific root surface area (SRA) were determined as root length per unit root dry mass and root

surface area per unit root dry mass, respectively.

4.2.7 Statistical analysis

All data analyses were carried out using the R programming environment 3.1.3 (R Core

Team, 2015). Before conducting the ANOVA, normality of distribution and homogeneity of

variance were assessed using the Shapiro-Wilk and Bartlett tests, respectively. The TB, SRL,

RLA, RGR_V, $A_{n\text{-}growth}$, R_d and g_s data were transformed using natural logarithm function and

WUE data using the reciprocal of its square root to meet the ANOVA assumptions. When

ANOVA showed a significant interaction or photoperiod effect, Tukey's HSD Post-hoc

comparisons were carried out. The threshold probability was 0.95 for all the analyses. $[CO_2]$, soil

temperature and photoperiod were treated as fixed effects.

4.3 Results

4.3.1 Morphological and biomass characteristics

The impacts of photoperiod on budburst and bud set depended on soil moisture and/or

$[CO_2]$ (significant 3- or 2-way interactions in Table 4.1). Budburst generally advanced with the

increase in photoperiod toward north, particularly under the elevated $[CO_2]$ (Figure 4.1A). The

effect of soil moisture on budburst was limited to the ambient $[CO_2]$, either delayed or

accelerated by high soil moisture at Ps and P_{nm2} (Figure 4.1A). Bud set was the earliest at P_{nm2}

under low soil moisture but the trend was reversed at the high soil moisture (Figure 4.1B).

Between the two soil moisture levels the low moisture treatment delayed bud setting under the

photoperiod regime of the seed origin but expedited it under the photoperiod regime 10° north of

the seed origin (Figure 4.1B).

Table 4.1: P values for the effects of CO_2 concentration (C), soil moisture (M), photoperiod (P) and their interactions on the phenological and growth characteristics of jack pine seedlings. Seedlings were grown under two levels of $[CO_2]$ (400 and 950 µmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field water capacity) and three levels of photoperiod (seed origin, 5° and 10° north of seed origin).

Response variables	Treatment effects						
	C	M	P	C*M	C*P	M*P	C*M*P
Budburst	0.062	0.816	**0.037**	0.973	0.984	0.159	**0.025**
Bud set	0.089	0.440	0.189	0.702	0.487	**<0.001**	0.088
PLA	0.125	0.532	0.126	0.195	0.499	0.722	0.613
SLA	0.648	0.107	0.069	0.349	0.078	0.934	0.756
SMR	0.159	0.840	0.270	0.594	0.462	0.111	0.276
SRL	**0.010**	**0.029**	**0.003**	0.180	0.066	0.167	0.567
SRA	**0.004**	0.055	**0.002**	0.415	**0.011**	0.334	0.753
LMR	**0.023**	**0.025**	0.726	0.845	0.749	0.792	0.944
RLA	0.485	0.137	0.082	0.237	0.606	0.470	0.987
SRR	**0.018**	0.078	0.371	0.283	0.896	0.923	0.851
RMR	**0.013**	0.113	0.227	0.509	0.795	0.861	0.901
RGR$_H$	0.252	0.501	0.115	0.718	0.052	0.637	0.545
RGR$_D$	**0.024**	0.097	0.686	0.118	0.410	0.832	0.791
RGR$_V$	**0.013**	0.204	0.949	0.345	0.245	0.636	0.456
M	**0.013**	0.802	0.575	0.189	0.614	0.729	0.936

Abbreviations: PLA (projected leaf area), SLA (specific leaf area), SMR (stem mass ratio), SRL (specific root length), SRA (specific root surface area), LMR (leaf mass ratio), RLA (root length to leaf area ratio), SRR (shoot to root ratio), RMR (root mass ratio), RGR$_H$, RGR$_D$ and RGR$_V$ (relative height, root collar diameter and volume growth rate, respectively), M (total biomass).
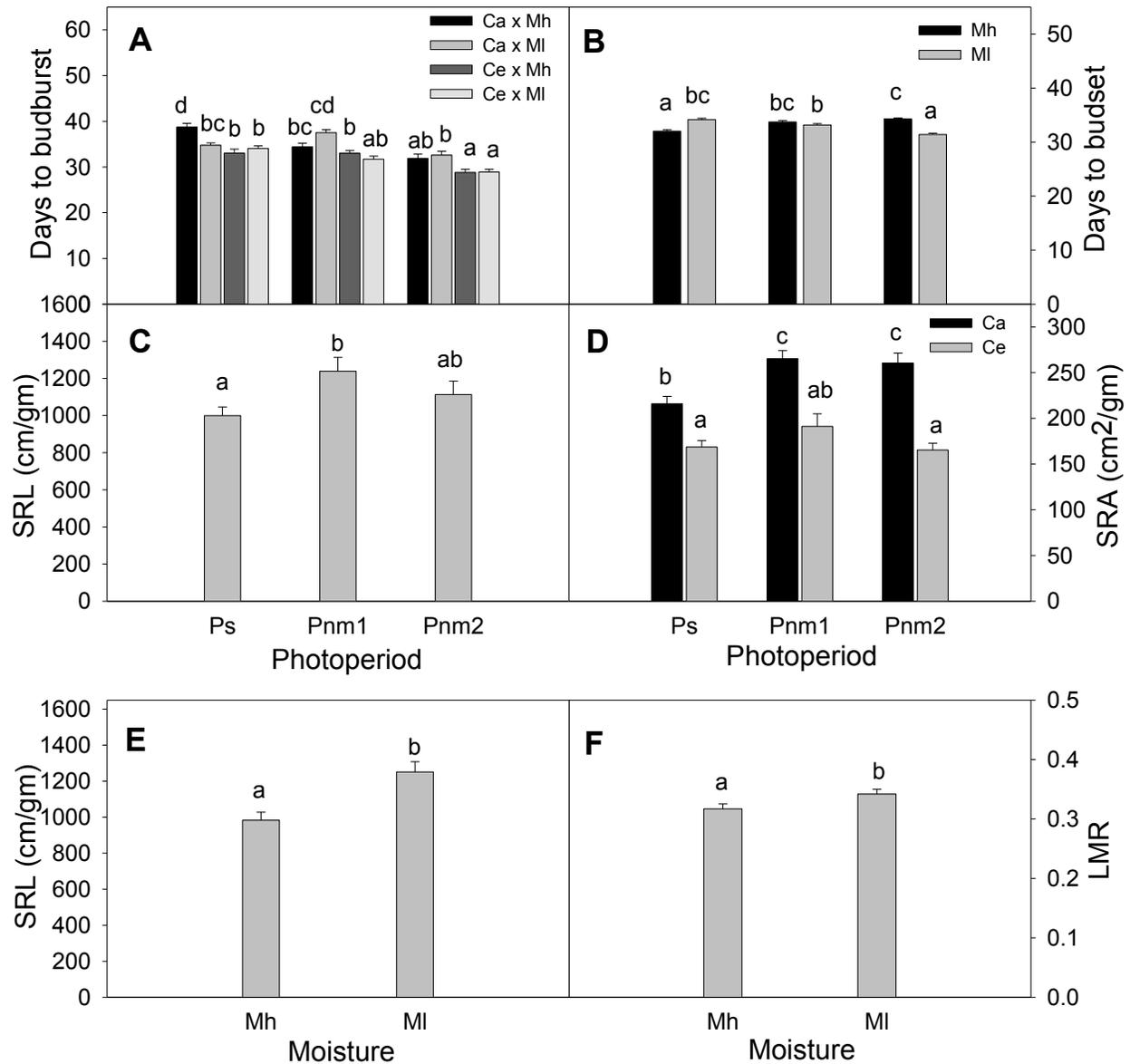
Figure 4.1: Mean (+SEM) days to budburst and bud set, specific root length (SRL), specific root surface area (SRA), leaf mass ratio (LMR), root mass ratio (RMR), shoot to root ratio (SRR), relative growth rate of root collar diameter (RGR_D) and volume (RGR_V) and total biomass production in jack pine seedlings grown under two levels of [CO_2], two levels of soil moisture and three levels of photoperiod. Means with different letters were significantly different from each other (P≤0.05). C_a & C_e = ambient & elevated [CO_2], respectively, M_h & M_l = high & low soil moisture, respectively, P_s, P_{nm1} & P_{nm2} = photoperiods at seed origin, 5° & 10° north of seed origin, respectively.
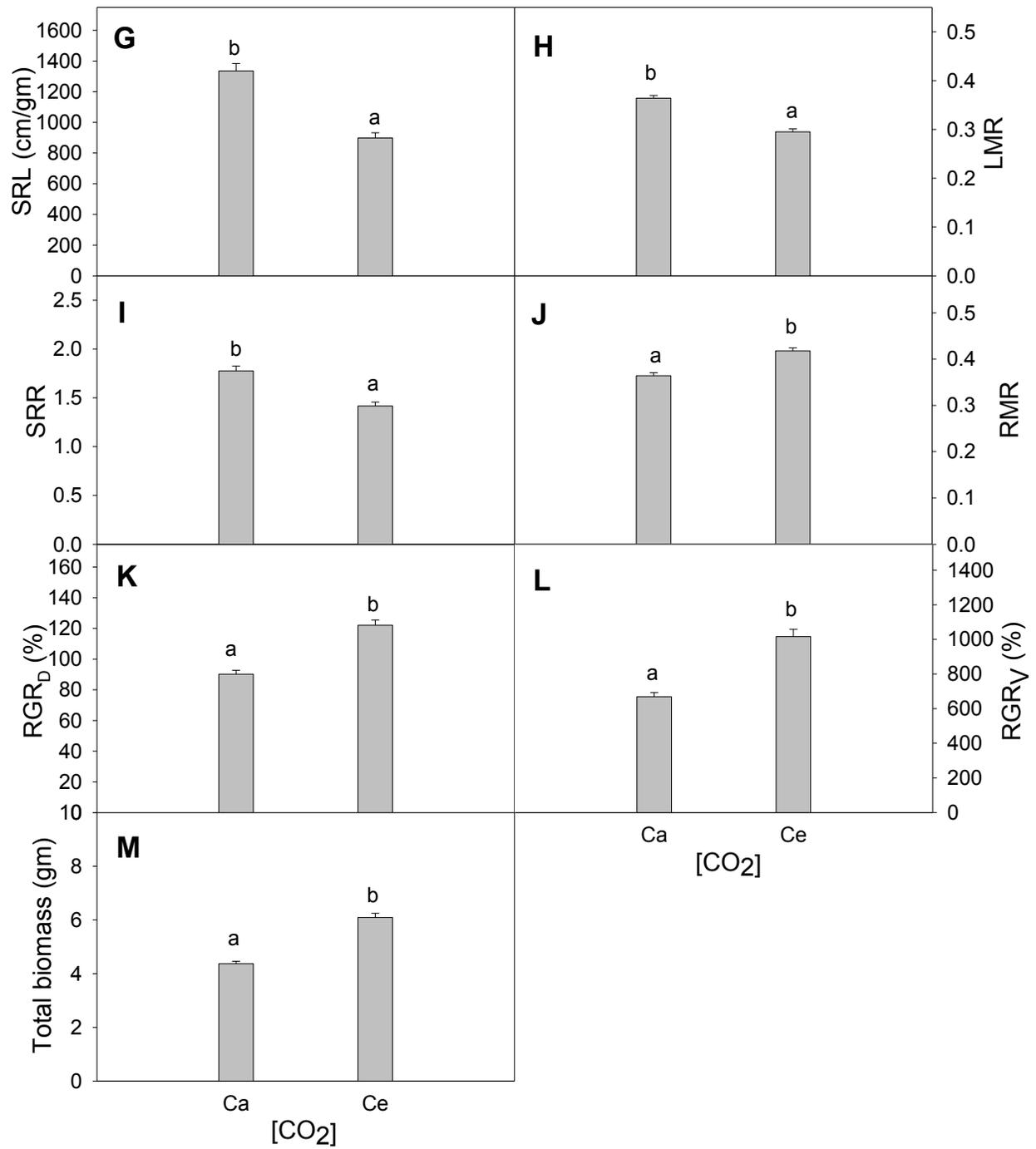
Figure 4.1 (Contd.):

The specific root length (SRL) was generally lower in the photoperiod regime of the seed origin (P_s) but the difference was significant only between P_s and P_{nm1} (Table 4.1, Figure 4.1C). The specific root surface area (SRA) was also affected by photoperiod, but the effect was [CO_2] dependent (Table 4.1). The SRA at P_s was significantly smaller than other two photoperiod regimes under ambient [CO_2] but the effect was not significant under elevated [CO_2] (Figure 4.1D).

Soil moisture had significant effects on SRL and leaf mass ratio (LMR) (Table 4.1), with 27% increase in SRL and 8% increase in LMR at low soil moisture regime (Figures 4.1E and 4.1F). Elevated [CO_2] significantly decreased SRL (Figure 4.1G), LMR (Figure 4.1H) and shoot to root ratio (SRR) (Figure 4.1I) but increased root mass ratio (RMR) (Figure 4.1J). Relative diameter growth rate (RGR_D), relative volume growth rate (RGR_V), and total biomass (M) production were all significantly higher under elevated [CO_2] (Figures 4.1K, 4.1L and 4.1M).

4.3.2 Ecophysiological traits

The net CO_2 assimilation rates measured at the ambient [CO_2] (A_{n-400}) did not significantly differ with any of the treatments (Table 4.2). However, the photosynthesis at growth [CO_2] ($A_{n-growth}$) was significantly higher at the elevated [CO_2] and the effect was greater under the high moisture treatment (Figure 4.2A). Between the two soil moisture levels significant difference in $A_{n-growth}$ only occurred at the elevated [CO_2] (Figure 4.2A). The change in photoperiod from P_s to P_{nm1} and P_{nm2} did not affect any of the physiological variables measured (Table 4.2). However, it had significant interactive effects with soil moisture and [CO_2] on the photosynthetic water use efficiency (*WUE*) (significant 3-way interaction). In the P_{nm1} and P_{nm2}, *WUE* increased with CO_2 elevation and decrease in soil moisture. In the P_s, however, there was no significant difference

between the two soil moisture treatments although the CO_2 effect was greater in the high than in the low soil moisture treatment (Figure 4.2B). The significantly lower g_s and E by low soil moisture treatment only occurred at the ambient $[CO_2]$ and by elevated $[CO_2]$ only in the high moisture treatment (Figures 4.2C and 4.2D).

Table 4.2: P values for the effects of CO_2 concentration (C), soil moisture (M), photoperiod (P) and their interactions on net photosynthetic rate (A_n), water-use efficiency ($IWUE$), stomatal conductance (g_s), transpiration rate (E), maximum rate of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}), triose phosphate utilization (TPU) and day respiration (R_d). A_n was analyzed for a common $[CO_2]$ of 400 µmol mol^{-1} CO_2 (A_{n-400}) as well as growth CO_2 ($A_{n-growth}$). All other analyses were done for growth CO_2. Seedlings were grown under two levels of $[CO_2]$ (400 and 950 µmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field water capacity) and three levels of photoperiod (seed origin, 5º and 10º north of seed origin).

Response variables	Treatment effects						
	C	M	P	C*M	C*P	M*P	C*M*P
A_{n-400}	0.073	0.135	0.800	0.103	0.925	0.279	0.853
$A_{n-growth}$	**0.005**	0.231	0.523	**0.047**	0.608	0.424	0.723
WUE	**0.003**	**0.028**	0.300	0.784	0.400	0.069	**0.027**
g_s	0.055	**0.006**	0.568	**0.011**	0.198	0.210	0.121
E	**0.010**	**0.008**	0.535	**0.004**	0.728	0.364	0.921
V_{cmax}	**0.034**	0.818	0.324	0.127	0.153	0.588	0.481
J_{max}	**0.019**	0.428	0.752	**0.024**	0.396	0.939	0.894
TPU	**0.004**	0.402	0.893	**0.041**	0.357	0.721	0.962
R_d	**0.018**	0.309	0.820	0.544	0.174	0.967	0.835
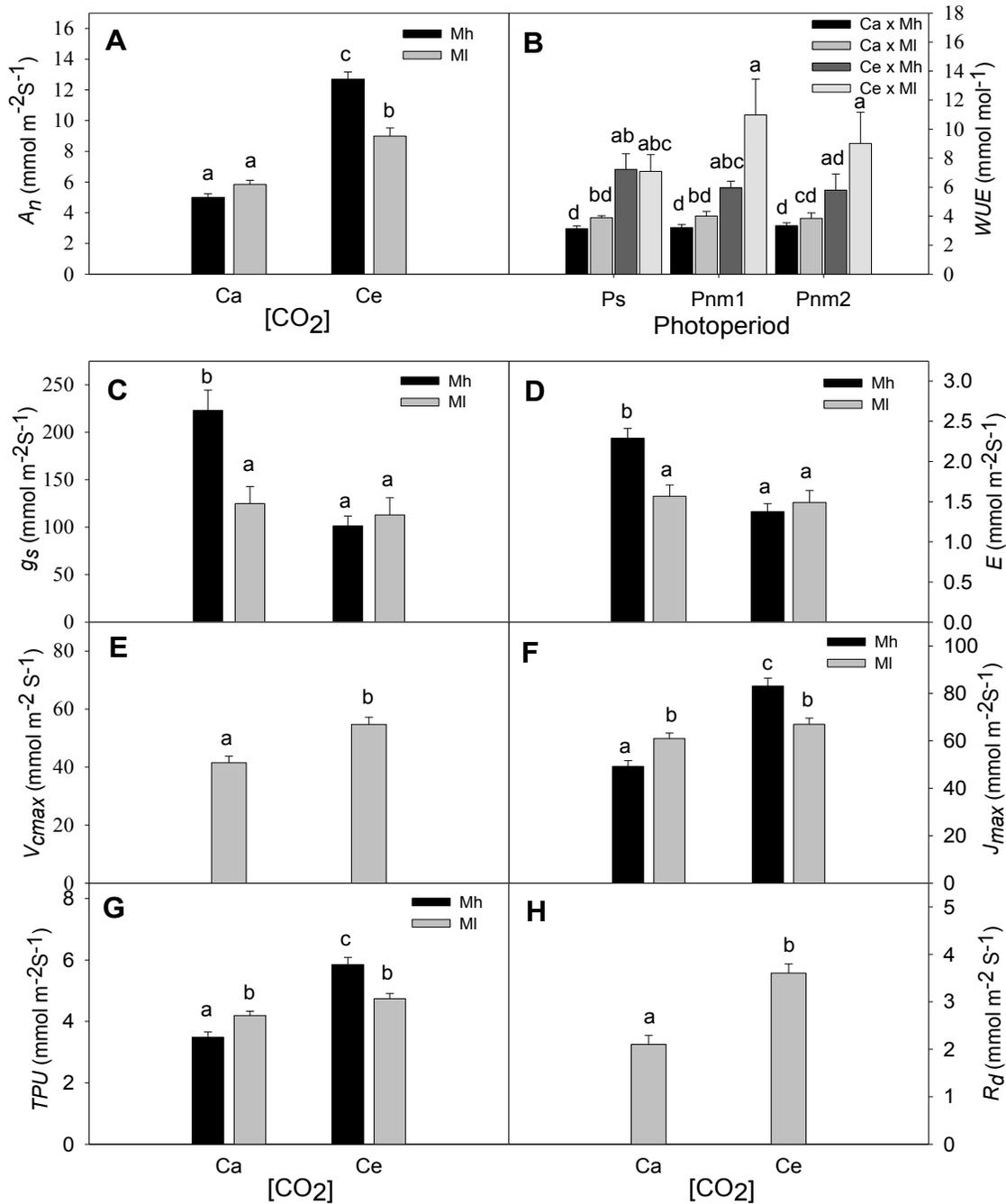
Figure 4.2: Mean (+SEM) net photosynthetic rate at growth CO_2 (A_n), water-use efficiency (*WUE*), stomatal conductance (g_s), transpiration rate (*E*), maximum rate of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}), triose phosphate utilization (*TPU*) and day respiration (R_d) in jack pine seedlings grown under two levels of [CO_2], two levels of soil moisture and three photoperiod regimes. A_n was analyzed for a common [CO_2] of 400 μmol mol^{-1} CO_2 (A_{n-400}) as well as growth CO_2 ($A_{n-growth}$). All other analyses were done for growth CO_2. Means with different letters were significantly different from each other (p≤0.05). C_a & C_e = ambient & elevated [CO_2], respectively, M_h & M_l = high & low soil moisture, respectively, P_s, P_{nm1} & P_{nm2} = photoperiods at seed origin, 5º & 10º north of seed origin, respectively.

Elevated $[CO_2]$ significantly increased V_{cmax}, R_d, J_{max} and TPU, but the impacts on J_{max} and TPU were restricted to the high moisture treatment (significant $[CO_2]$ by moisture interaction) (Figures 4.2E, 4.2H, 4.2F and 4.2G). Between the two soil moisture levels J_{max} and TPU were either greater with the low soil moisture treatment under the ambient $[CO_2]$ or with the high soil moisture treatment under the elevated $[CO_2]$ (Figures 4.2F and 4.2G).

4.4 Discussion

The results of this study suggest that soil moisture can promote or delay budburst and modify the physiological, morphological and phenological responses of plants to changes in photoperiod and $[CO_2]$ levels. Bud set is primarily determined by photoperiod (Chen *et al.*, 2012; Li *et al.*, 2003; Oleksyn *et al.*, 2001), while budburst by temperatures (Basler & Körner, 2014; Caffarra & Donnelly, 2011). The advanced budburst in jack pine under the longer photoperiod might be due to its influence on dormancy release during ecodormancy phase and a higher accumulated temperature sum needed to burst buds (Basler & Körner, 2014; Caffarra & Donnelly, 2011; Heide, 1993a; Heide, 1993b; Laube *et al.*, 2014; Way & Montgomery, 2015; Zohner & Renner, 2015). Though Basler & Körner (2012) stated that shorter photoperiod delays budburst in late successional species and does not have much impact in early successional species, Caffarra & Donnelly (2011) found significant photoperiod effects on budburst in both late-successional and early successional species, which is conducive to our findings. The effects of soil moisture were lessened in E and g_s, but intensified in $A_{n\text{-}growth}$, J_{max} and TPU under elevated $[CO_2]$, which suggests varying effects of $[CO_2]$ and soil moisture on different physiological parameters, as suggested by Duan *et al.* (2013). Such varying effects might be

associated with water stress that lowers leaf water potential (Dang & Cheng, 2004; Stinziano & Way, 2014) and the ability of leaves to cool down (Ainsworth & Long, 2005; Tozzi *et al.*, 2013).

Consistent with the findings of other researchers (DeLucia & Thomas, 2000; Zhang & Dang, 2005; Cao *et al.*, 2007; Danyagri & Dang, 2014), $A_{n\text{-growth}}$ increased with the increase of $[CO_2]$ level, likely due to increased availability of substrate (CO_2) (Zhang & Dang, 2006), leaf area index and efficiency of photosystem II (McGrath *et al.*, 2010; Tjoelker *et al.*, 1998). Again, soil moisture started to limit $A_{n\text{-growth}}$ under elevated $[CO_2]$. No photosynthetic down-regulation in jack pine seedlings was noticed based on the increased V_{cmax} and J_{max}, as has been often observed in response to elevated $[CO_2]$ (Gunderson & Wullschleger, 1994; Medlyn *et al.*, 1999). This might be attributed to high nitrogen fertilization during the active growth phase of the seedlings, as shown by the increases of J_{max} and *TPU* with increasing N (Maier *et al.*, 2008) and P (Watanabe *et al.*, 2011; Wykoff *et al.*, 1998) fertilization. Inorganic phosphate (P_i) concentration in cytosol largely determines the exportation of triose phosphate to cytosol or storage in chloroplasts (Lambers *et al.*, 2008) and thus affects both J_{max} and *TPU*. The findings in V_{cmax}, J_{max} and *TPU* are consistent with those by Long *et al.* (2004), Darbah *et al.* (2010) and Danyagri & Dang (2013) but opposite to those by Lewis *et al.* (2004), Tissue & Lewis (2010) and Watanabe *et al.* (2011). Zhang & Dang (2005) reported a mixed response depending on seedling age and soil temperature.

Similarly, my results showed that both photoperiod and soil moisture regimes influenced the responses of water use efficiency (*WUE*) to elevated $[CO_2]$ in jack pine. The increased *WUE* at elevated $[CO_2]$ might be associated with the decreased stomatal conductance (marginally significant), as found by others (Curtis & Wang, 1998; Keenan *et al.*, 2013; Long *et al.*, 2004; Medlyn *et al.*, 2001; Zhang & Dang, 2005), such that a near constant ratio of internal to ambient

[CO_2] (Ci/Ca) under elevated [CO_2] can be maintained for gas exchange regulation (Keenan *et al.*, 2013). The results also confirmed that low soil moisture reduces the transpiration (*E*) and stomatal conductance (g_s) and increases the *WUE* as observed by others (Ainsworth & Long, 2005; Ambebe & Dang, 2009; Curtis & Wang, 1998; de Miguel *et al.*, 2012; Keenan *et al.*, 2013; Long *et al.*, 2004; Medlyn *et al.*, 2001; Zhang & Dang, 2005). Drought reduces water uptake for transpiration use (Brodribb *et al.*, 2005; Wang *et al.*, 2003) and therefore enhances *WUE* by conservative use of available water through stomatal closure (Irvine *et al.*, 2005; Marron *et al.*, 2002). The effects of elevated [CO_2] and low soil moisture on *WUE* at longer photoperiod indicate a higher *WUE* of jack pine with possible northward migration.

Photoperiod regime also affected the morphological responses of jack pine CO_2 elevation. For example, the longer photoperiod of 5º north of the seed origin increased the SRA significantly only under the ambient [CO_2]. However, further increase of photoperiod did not produce additional increase in SRA, indicating a possible threshold photoperiod beyond which [CO_2] induced A_n enhancement may be restricted by water and nutrient limitations. The higher SRA at longer photoperiod are consistent with the findings of Mozafar *et al.* (1993) and Troughton (1961).

The observed higher leaf mass ratio (LMR) under low soil moisture, which is contrary to the findings of other researchers (Liu & Stützel, 2004; Zhao *et al.*, 2006; Ambebe & Dang, 2010), might be the result of physiological acclimation of jack pine seedlings to water stress. The higher LMR at low soil moisture might have resulted from the lower specific leaf area (SLA) and higher specific root length (SRL) observed under low soil moisture. The lower SLA at low soil moisture indicates thicker needles, a likely acclimation to reduce water loss under moisture stress condition (Warren *et al.*, 2005). On the other hand the increased SRL at low soil moisture

suggests that jack pine seedlings developed more extensive non-woody fine root systems to explore soil moisture and reduce moisture stress (Irvine *et al.*, 2005). As a result, there is a possibility of increasing the relative mass of leaf in comparison to the total mass of the seedlings.

There are morpho-physiological traits in jack pine seedlings that were not significantly affected by interactive effects of photoperiod, soil moisture, and $[CO_2]$ level. These include higher root mass ratio (RMR), relative growth of diameter (RGR_D) and volume (RGR_V), total biomass, maximum rate of carboxylation (V_{cmax}) and day respiration (R_d) and lower specific root length (SRL), leaf mass ratio (LMR) and shoot to root ratio (SRR) with elevated $[CO_2]$, and higher specific root length (SRL) at low soil moisture. The responses of these traits to elevated $[CO_2]$ and increasing drought will not depend on the changes of photoperiod with northward migration.

The findings of this study suggest that climate change-induced northward migration will be more complicated than what is predicted by the climate envelope models. The advanced budburst at longer photoperiod and elevated $[CO_2]$ may mean an increasing chance of damages from late spring frost for the northward migration of jack pine under predicted change of climate. Higher water use efficiency under longer photoperiod and low soil moisture regimes at elevated $[CO_2]$ indicates physiological adaptations of this species to increasing water stress under the warming climate. Finally, the significant interactive effects suggest that low soil moisture and northward migration associated changes in photoperiod will influence the enhancing effects of elevated $[CO_2]$ on growth and physiological processes in jack pine. These findings might be useful to assisted migration/seed transfer of jack pine towards north.

Chapter 5: Interactive effects of photoperiod, soil moisture and [CO$_2$] on hydraulic conductivity and xylem vulnerability to embolism in jack pine seedlings

5.1 Introduction

Global warming will accelerate land surface drying and increase the frequency and severity of droughts by altering the amount, intensity, frequency and type of precipitation (Christensen *et al.*, 2007). Rain falls are likely to increase during autumn and spring over lands in middle and high latitudes of the Northern Hemisphere, diminishing water resources in summer (IPCC, 2007). Global warming might also be associated with changes in ecosystem water balance, leading to a higher vapor pressure deficit and increased evaporative demand and transpiration rate (Domec *et al.*, 2009; Meinzer, 2002; Way & Oren, 2010). Elevated soil temperature associated with global warming might further decrease soil water content (Allison & Treseder, 2008) and inevitably affect the movement of water through plants (Way & Oren, 2010). High tensions in the xylem under water stress might result in embolism if transpiration rates are not efficiently controlled (Lambers *et al.*, 2008). Embolism, a common event in nature resulting from water stress (Tyree & Sperry, 1989), reduces the ability to conduct water and limits plant growth when severe (Lambers *et al.*, 2008).

Physiological responses of trees to drought initially start with reductions in growth and photosynthesis and eventually hydraulic failure and xylem cavitation under severe droughts (McDowell *et al.*, 2011). As a short–term physiological control stomata indirectly respond to changes in vapor pressure deficit to regulate minimum leaf water potential to avoid excessive cavitation during drought periods (Baldocchi, 1997; Franks, 2004; Meinzer & Grantz, 1991).

When stomatal regulation capacity is exceeded, embolism occurs leading to shoot dieback (Tyree & Sperry, 1989). Again, if carbon uptake is severely suppressed by stomatal closure or the rate of xylem embolism exceeds the extent of repair and refilling, the risk of tree mortality increases (McDowell *et al.*, 2011). Species differ considerably in their vulnerability to embolism (Tyree & Sperry, 1989). Vulnerability of a species to embolism correlates with the xylem pressures (Tyree & Sperry, 1989). Generally plants growing under shade or in wetter sites are more vulnerable to embolism (Alder *et al.*, 1996; Cochard *et al.*, 1999). Species that tolerate extremely low temperatures are highly desiccation tolerant (Lambers *et al.*, 2008). The ability of a species to withstand embolism or to recover from it may significantly affect its ecological distribution (Tyree & Sperry, 1989).

Though water stress is the driving force in the process of embolism, atmospheric $[CO_2]$ and photoperiod also directly or indirectly influence the water potential in plants. Elevated atmospheric $[CO_2]$ directly influence the ecosystem processes and biosphere–atmosphere interactions in the boreal forests (Keenan *et al.*, 2013) and promotes plant growth and yield (Ainsworth & Long, 2005; Curtis & Wang, 1998; Norby *et al.*, 1999) by increasing photosynthesis (Long *et al.*, 2004) and improving the water–use efficiency (Field *et al.*, 1995). Under elevated $[CO_2]$ stomatal conductance typically reduces, though the effect is variable and subject to environmental feedback (Gunderson *et al.*, 2002; Leakey *et al.*, 2006; Long *et al.*, 2004; Marchi *et al.*, 2004). Elevated atmospheric $[CO_2]$ substantially increases the water-use efficiency in boreal forests through partial closure of stomata during gas exchange (Keenan *et al.*, 2013). The increased water-use efficiency results either in decreased transpiration or increased gross photosynthetic carbon uptake or both simultaneously and partially offset the effects of future droughts (Keenan *et al.*, 2013). The decrease in stomatal conductance indirectly

stimulates photosynthesis in dry environments by reducing the rate of soil drying and therefore the water limitation of photosynthesis (Hungate *et al.*, 2002). Elevated atmospheric $[CO_2]$ enhances root growth (Curtis & Wang, 1998; Rogers *et al.*, 1994) and thus enhances water availability to plants.

It has been predicted that by the end of the 21st century global atmospheric $[CO_2]$ will rise to 950 μmol mol^{-1} (Cao & Caldeira, 2010; IPCC, 2001; Luthi *et al.*, 2008) and average air temperature will increase by 1.8 - 4.0 °C (IPCC, 2001). The mean annual temperature increase in the boreal region is likely to be as high as 5–7 °C (Christensen *et al.*, 2007; Eskelin *et al.*, 2011; Heimann & Reichstein, 2008; Montzka *et al.*, 2011). Following the changes in atmospheric temperature, the pattern and intensity of precipitation will also be changed (IPCC, 2007). At the pace of such changes in the environmental parameters many of the boreal tree species might migrate further northward (McKenney *et al.*, 2007; McKenney *et al.*, 2011) and will be exposed to an altered photoperiod regime. Under altered environmental conditions, vulnerability of plants to embolism may also change (Lambers *et al.*, 2008) while elevated atmospheric $[CO_2]$ will partially offset it by reducing transpiration (Field *et al.*, 1995; Keenan *et al.*, 2013). But the interactive effects of altered photoperiod with water stress and elevated atmospheric $[CO_2]$ could be quite different from the total of their individual effects (Curtis *et al.*, 2000; Nowak *et al.*, 2004).

Jack pine (*Pinus banksiana* Lamb.) is one of the most dominant tree species in the boreal forests of Canada with great ecological and economic values (Chen & Popadiouk, 2002; Flannigan & Wotton, 1994; Forestry Canada, 1992; IPCC, 2001). The species grows well in diverse climatic conditions ranging from short warm to cool summers, very cold winters and low rainfall (Rudolf, 1965). Jack pine can withstand water stress conditions by shedding foliage to

reduce transpiration (Johnston *et al.*, 2009). It can withstand low soil moisture content for relatively long periods of times, but prolonged drought spell can lead to tree mortality (Johnston *et al.*, 2009). Though the species generally grows well in dry sites (Grossnickle & Blake, 1986), our knowledge on the vulnerability of the species to embolism under the interactive effects of predicted elevated atmospheric [CO_2], altered photoperiod and water stress is still limited.

In this study the vulnerability of jack pine seedling to embolism was investigated under the interactive influences of elevated atmospheric [CO_2], altered photoperiod and water conditions. The specific objectives were to investigate the influence of atmospheric [CO_2], photoperiod and soil moisture regime on the hydraulic conductivity and vulnerability to cavitation in jack pine seedling. Since hydraulic conductivity of trees is generally influenced by the internal and external moisture conditions and the diameter of xylem cells (Lambers et al. 2008) and accelerated growth rate increase the number of tracheids and the lumen area in jack pine (Krause et al. 2011), I hypothesize that northward migration associated longer photoperiod under elevated [CO_2] will enhance the hydraulic conductivity in the species and increase the vulnerability to cavitation as well.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials

One-year old jack pine (*Pinus banksiana* Lamb.) seedlings were raised by a commercial tree seedling nursery in Thunder Bay from seeds collected from matured jack pine stands in Kakabeka region ($48^{\circ}57'$ N & $90^{\circ}44'$ W). A total of 448 seedlings, relatively uniform in height and root collar diameter, were transplanted in plastic pots of 15 cm in height and 13 cm in

diameter filled up with a mixture of premium grade vermiculite and peat moss at a ratio of 1:1 (v/v).

5.2.2 Experimental design

The experiment was carried out in four green houses at Lakehead University's Thunder Bay campus within the frame of split-split plot design, with $[CO_2]$ as the whole plot, soil moisture as the sub-plot and photoperiod as the sub-sub plot. The treatments were consisted of two levels of $[CO_2]$ (400 and 950 µmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field water capacity) and three photoperiod regimes (photoperiods at seed origin, 5º north of the seed origin and 10° north of the seed origin). Two levels of $[CO_2]$ were randomly assigned to four independent greenhouses with two replicates of each. Two levels of soil moisture were nested within each level of $[CO_2]$ and three photoperiod regimes were nested within each level of soil moisture.

5.2.3 Growing conditions

Electronic ignition natural gas CO_2 generators (model GEN-2E, Custom Automated Products Inc, Riverside, CA) were used to generate CO_2 and the desired levels of $[CO_2]$ in the greenhouses were maintained using an Argus environment Control System (Argus, Vancouver, BC, Canada). Circulation fans were used to ensure even distribution of CO_2 within the greenhouse. The moisture content of the growing medium was measured daily with a HH2 moisture meter (Delta-T Devices, Cambridge, UK) and the desired levels were maintained by adding water when the water content fell below the set lower range. The photoperiods for each of the three locations were set periodically to synchronize with the actual biweekly averages at corresponding locations. The photoperiods were extended using high-pressure sodium lamps

when natural day lengths were shorter than the set values. Photoperiods shorter than the natural day lengths were achieved through manual shading. Seedlings were fertilized biweekly with N:P:K fertilizer (7.14, 0.56 and 2.12 mmol per litre of water, respectively during the rapid growth phase and 1.78, 2.20 and 4.64 mmol per liter of water, respectively during the hardening phase) (Scarratt, 1986). In all the greenhouses relative humidity was maintained at 55–60% during the rapid growth phase and reduced to 45–50% during the hardening phase. The day and night air temperatures were maintained at values mimicking the day and night air temperatures at the seed origin. Relative humidity and air temperature were controlled automatically using an Argus Control System.

5.2.4 Hydraulic conductivity measurement

Three branchless stem segments from each treatment combination were excised and placed in a water bath at 4 °C for 2 hours to reduce resin emission. Hydraulic conductivity (K_H) was measured following the methods of Sperry *et al.* (1988). Keeping submerged, both ends of the sample were cut using sharp clippers leaving 2 cm at each end of the pre-marked 15 cm segment. Rubber gaskets were used in both ends of the stem segment to ensure leak-proof fitting. Then both ends of the sample were trimmed with a fresh razor blade and rapidly attached to solution-filled tubing. At this stage perfusing solution was passed through the sample from supply tank under gravity, which was collected in a beaker sitting on an electronic balance (Traveler Scales, Ohaus Corporation, Pine Brook, NJ, USA) attached to a computer and the amount of fluid collected was recorded automatically. This flow rate was considered as the background flow rate. After that, the perfusing solution from the supply tank was passed through the sample under a hydrostatic pressure of 10 kPa and the pressurized flow rate was measured. Following the pressurized flow rate measurement, background flow rate measurement was repeated. The net

flow rate induced by the pressure was determined by subtracting the average background flow from the pressurized flow. The cross sectional area of the sample was determined and the specific hydraulic conductivity was determined by dividing the net flow rate by the pressure gradient along the stem.

5.2.5 Vulnerability to embolism

Bench-top dehydration is considered to be the standard method of generating vulnerability curves (Sperry et al. 2012; Hacke et al. 2015), but it is time consuming and requires multiple stems for the curve. Therefore, air injection technique was used to generate vulnerability curves, which is quick, requires a single stem for the curve and correlate well with the dehydration technique when sample segments are long enough (Cochard et al. 1992; Choat et al. 2010). Xylem cavitation was induced by successively increasing positive air pressures on stem segments inside a double-ended pressure chamber (1505D-EXP Pressure Chamber, PMS Instrument Company, Albany, OR, USA). Fifteen cm long stem segments were taken from three seedlings from each treatment combination. The segments were immersed in a water bath at 4 °C for 2 hours to reduce resin emission. At the beginning of each measurement the sample was flushed at a pressure of 175 kPa for half an hour to remove any existing emboli and the maximum hydraulic conductivity (K_{max}) was determined. After that, pressure in the pressure chamber was progressively increased to 0.3, 0.5, 0.8, 1.0, 1.3, 1.5, 1.8, 2.0, 2.3 and 2.5 MPa and hydraulic conductivity (K_h) was measured 10 minutes after the desired pressure value was reached at each pressure step. Percent loss in conductivity (PLC) following each pressurization was calculated as PLC = $100\{(K_{max} - K_h)/K_{max}\}$. Vulnerability curves for each treatment were constructed using Microsoft Excel (2010). Xylem pressures at 50% loss of conductivity (Ψ_{PLC50}) were calculated from the best-fit curves generated by using Weibull functions.

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5.2.6 Statistical analysis

Analysis of variance (ANOVA) was performed for the hydraulic conductivity and the xylem pressure at 50% loss of hydraulic conductivity data using R programming environment 3.1.3 (R Core Team, 2015). Before the ANOVA test, normality of distribution and homogeneity of variance were checked using Shapiro-Wilk and Bartlett tests, respectively. When ANOVA showed significant interactive effects or photoperiod effects, Tukey's HSD Post-hoc comparisons were carried out. In the analyses, $[CO_2]$, soil moisture and photoperiod were treated as fixed effects and the threshold probability was 0.05.

5.3 Results

5.3.1 Hydraulic conductivity

The hydraulic conductivity in jack pine seedlings differed by 34.48% between ambient and elevated $[CO_2]$ (Figure 5.1A) and by 14.47% between high and low soil moisture (Figure 5.1B), but was not significantly affected by photoperiod or interactions of the treatments (Table 5.1).

Table 5.1: P values for the effects of CO_2 concentration (C), soil moisture (M), photoperiod (P) and their interactions on the hydraulic conductivity (HC) and xylem pressure at 50% loss of conductivity (Ψ_{PLC50}) in jack pine seedlings. Seedlings were grown under two levels of $[CO_2]$ (400 and 950 μmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field water capacity) and three levels of photoperiod (seed origin, 5° and 10° north of seed origin).

Response variables	Treatment effects						
	C	M	P	C*M	C*P	M*P	C*M*P
HC	**0.043**	**0.041**	0.172	0.243	0.412	0.261	0.592
Ψ_{PLC50}	0.467	0.398	0.142	0.431	**0.037**	0.606	0.758

5.3.2 Vulnerability to embolism

The elevated $[CO_2]$ at photoperiod 10° north of the seed origin produced substantially smaller xylem pressure or less negative xylem water potential at which 50% of the hydraulic conductivity was lost (Ψ_{PLC50} = 0.8897 MPa), whereas other treatment combinations did not significantly differ from each other (Tables 5.1, Appendix 1; Figures 5.1C and 5.2 E). The percent loss of hydraulic conductivity with the increase of xylem pressures by different treatment combinations are shown in Figures 5.2A – 5.2G.
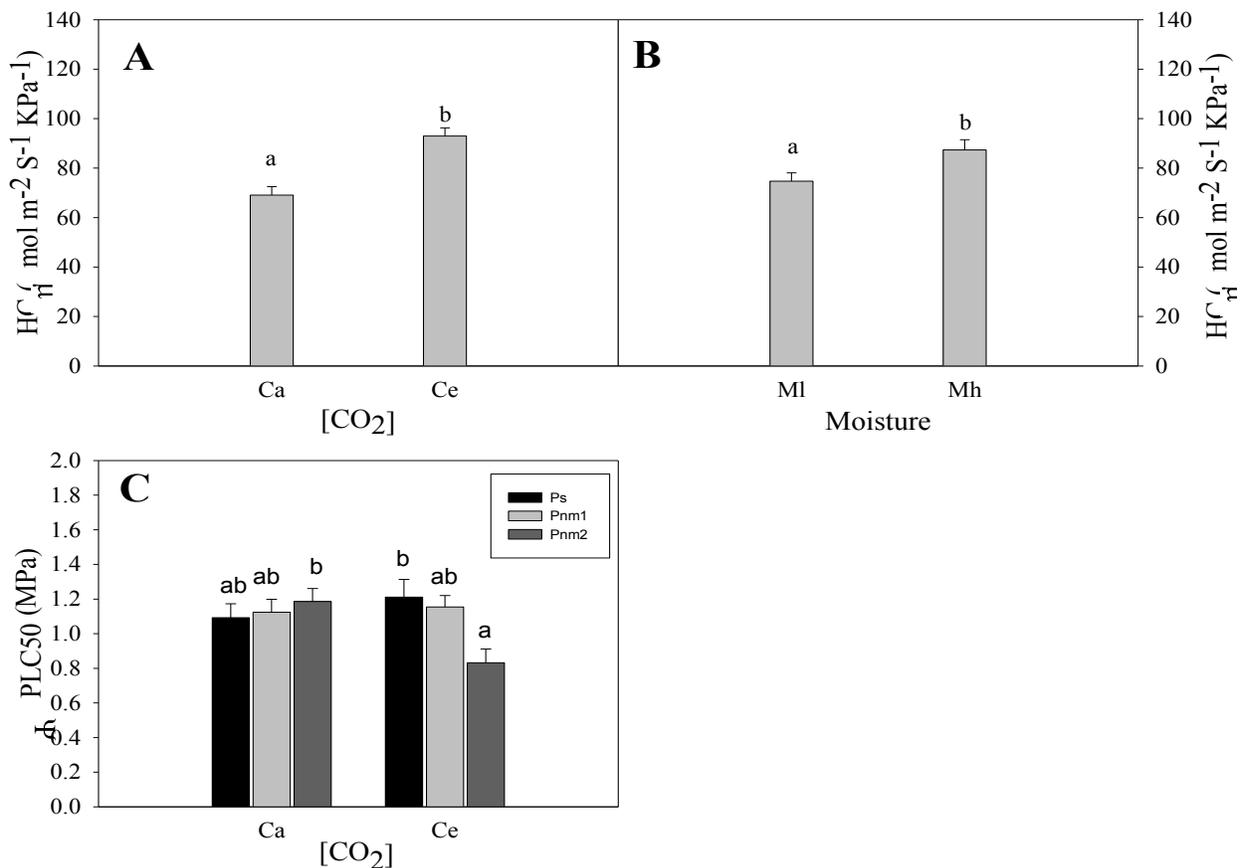
Figure 5.1: Specific hydraulic conductance of stem (HC) and xylem pressure at 50% loss of hydraulic conductivity (Ψ_{PLC50}) in jack pine seedlings grown under two levels of $[CO_2]$, two levels of soil moisture and three photoperiod regimes. P_s, P_{nm1} & P_{nm2} = photoperiods at seed origin, 5° & 10° north of seed origin, respectively.

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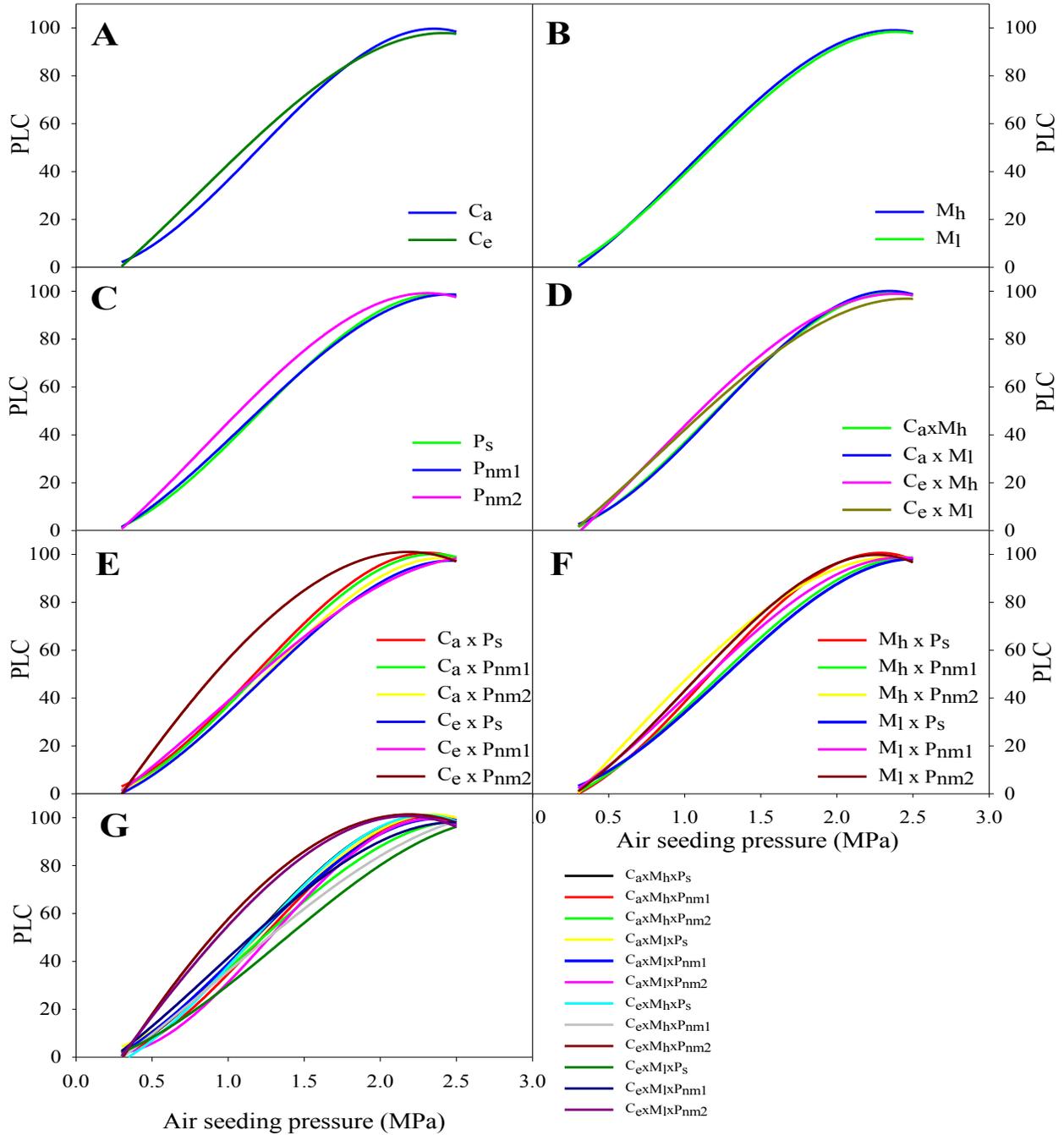
Figure 5.2: Vulnerability curves showing percentage loss of hydraulic conductivity (PLC) as a function of xylem pressure by different combinations of $[CO_2]$, soil moisture, photoperiod regime. C_a & C_e = ambient & elevated $[CO_2]$, respectively, M_h & M_l = high & low soil moisture, respectively, P_s, P_{nm1} & P_{nm2} = photoperiods at seed origin, 5º & 10º north of seed origin, respectively.

5.4 Discussion

The increased hydraulic conductivity at elevated CO_2 and decreased hydraulic conductivity at low soil moisture in jack pine seedlings are consistent with the changes of growth under these treatments (Chapter 4). The hydraulic conductivity of trees is influenced by both internal and external moisture conditions as well as by diameter of xylem cells which is in turn positively related to growth rates (Lambers et al. 2008). In jack pine accelerated growth rate has been found to increase the number of tracheids and the lumen area (Krause *et al.*, 2011), which in turn might increase the hydraulic conductivity of the species. Neufeld *et al.* (1992), Maherali & DeLucia (2000), Thomas *et al.* (2004), Cai & Tyree (2010) and Cai *et al.* (2014) indicate that larger tracheids/vessels are responsible for most of the water flow and increased tracheid/vessel diameter increases stem hydraulic conductivity.

There is a tradeoff between stem hydraulic conductivity and stem vulnerability to cavitation: stems with larger conduits are more conductive and more vulnerable to cavitation as well (Cai & Tyree, 2010). In this study a greater loss of hydraulic conductivity was observed in the seedlings grown under elevated [CO_2] with photoperiod 10º north of the seed origin, a treatment combination that produced more growth and therefore likely more tracheids and lumen area and greater vulnerability to cavitation. The pits on conduit walls and pit membranes have great influence on the vulnerability of xylem to cavitation (Wheeler *et al.*, 2005); higher mean lumen diameter or pit membrane surface area indicate greater total pit area and higher chance of embolism (Wheeler *et al.*, 2005). Tracheids with larger conduits usually cavitate more easily than those with smaller conduits (Hargrave *et al.*, 1994; LoGullo & Salleo, 1991; LoGullo *et al.*, 1995; Sperry & Tyree, 1990). However, Neufeld *et al.* (1992) did not observe any consistent relationship between the hydraulic conductivity or vessel diameter with xylem vulnerability.

Accelerated growth under elevated CO_2 and longer growing season photoperiod associated with northward migration might increase the risk of cavitation in jack pine and restrict the northward migration of the species at the pace of climate change.

Chapter 6: General Discussions

In response to climate change the climate envelopes of boreal trees are predicted to shift 10° (approximately 1000 kilometers) northward between 2071 and 2100 (McKenney *et al.*, 2007; McKenney *et al.*, 2011). If trees do migrate, they will face a new set of environmental conditions with many factors different from the conditions at their current habitats, for example, photoperiod, soil temperature and soil moisture. Such changes in environmental conditions will likely influence the ecophysiological responses of the migrating plants to elevated [CO_2].

The study revealed that the phenological responses of jack pine to CO_2 elevation were influenced by photoperiod regimes, soil temperature and soil moisture. For example, CO_2 elevation advanced budburst only in the photoperiod regime of the seed origin under the current soil temperature, while it advanced the budburst in all photoperiod regimes under elevated soil temperature. Again, at high soil moisture CO_2 elevation advanced budburst only in the photoperiod regime of the seed origin but at low soil moisture it advanced the budburst in the photoperiod regimes at both 5° and 10° north of the seed origin. Under elevated CO_2, photoperiod associated with 10° northward migration advanced the budburst at both levels of soil temperature and moisture. In both sets of experiment tri-factor interactions showed that budburst generally advanced with longer photoperiod towards north under elevated [CO_2]. In general budburst is primarily controlled by air temperature and/heat accumulation in the spring (Laube *et al.*, 2014; Zohner & Renner, 2014; Basler & Körner, 2014; Hänninen, 1990; Partanen *et al.*, 1998 and Zohner & Renner, 2015). Although the day and night temperature regimes were similar in different photoperiod treatments, the seedlings under longer photoperiod regimes were exposed to longer duration of day time temperatures, resulting in reaching the required total heat accumulation earlier. The magnitude of the advancement in budburst varied with interacting

treatment factors. For example, in the $[CO_2]$, soil temperature and photoperiod treatment combination earliest budburst occurred after 26 days of treatment (mean for C_e x T_e x P_{nm2}) while in the $[CO_2]$, soil moisture and photoperiod treatment combination earliest budburst occurred after 29 days (mean for C_e x M_h x P_{nm2}). However, the results suggest that irrespective of the changes in soil temperature or soil moisture, the photoperiod regime associated with 10° northward migration will significantly advance the budburst in jack pine and might expose the species to late spring frost injuries.

It is interesting to find that the effect of photoperiod regime on the timing of bud setting was opposite at dry and wet conditions in jack pine seedlings. The timing of bud setting was generally progressively delayed with changes in photoperiod regimes associated with increasing higher latitudes in the high moisture treatment, but the trend was the opposite in the low soil moisture treatment. Early bud setting towards higher latitudes in low soil moisture supports the general believe that bud set is induced largely by shortening photoperiod (Dalen, 1998, Centritto *et al.*, 1999). The reverse trend in high soil moisture might be associated with the concentrations of endogenous abscisic acid (ABA) and indole-3-acetic acid (IAA), which play a role in the photoperiodic control of bud dormancy (Li *et al*., 2003). Delayed bud set towards north under high moisture conditions may increase the risks of early fall frost damages to migrating jack pine.

Soil temperature, soil moisture and photoperiod also affected the responses of other morphological and physiological variables to elevated $[CO_2]$, including relative growth rates of root collar diameter (RGR_D) and specific root surface area (SRA), photosynthesis, and photosynthetic water use efficiency (*WUE*). Therefore, the interactions among photoperiod, soil

temperature and soil moisture need to be considered in predicting the responses of boreal trees to CO_2 elevations and future climate conditions.

My study indicated that jack pine will not have a vulnerability to cavitation under the predicted increase of $[CO_2]$, even with photoperiod 5° north of the seed origin. However, photoperiods associated with further northward migration will significantly increase the vulnerability of the species to cavitation, resulting primarily from substantial increase of growth. Seedlings grown under elevated $[CO_2]$ with the photoperiod regime 10° north of the seed origin had highest growth rate among all treatments and therefore could possibly have produced tracheids with larger conduits and pit membrane surface area, which might have increased the vulnerability to cavitation. Accelerated growth rates generally increase the number of tracheid and the lumen area in jack pine (Krause *et al.*, 2011) and tracheids with larger conduits or pit membrane surface area are more vulnerable to cavitation (Sperry & Tyree, 1990; Hargrave *et al.*, 1994; LoGullo *et al.*, 1995; Wheeler *et al.*, 2005). Therefore, there may be thresholds for northward migration beyond which increased vulnerability to cavitation and risks of spring frosts exceed the growth gain by elevated $[CO_2]$ and longer photoperiod in summer.

There was generally a lack of statistically significant 3-way interactions in this study. These results highlight the difficulties in detecting the interactive effects of multiple environmental variables and the generally small sample sizes in tree ecophysiological research rather than suggesting that there are no real interactions. As the number of treatment factors increase, the degree of freedom for the error term used to test interactions declines, causing a loss of the capability of the experiment to detect interactive effects. A remedy to the problem will be to increase the sample size. However, such a remedy often faces great logistical challenges. A

more practical approach for investigating the interactive effects of multiple factors is yet to be found.

The observed responses might vary with the responses of plants grown in natural conditions since the study was carried out in controlled environmental conditions and some factors in natural environment, e.g. N status, could be quite different. Moreover, in the study the response of one-year old seedlings to altered environmental conditions were investigated, which might be different from the responses of matured trees. For example, I observed 87% increase in net assimilation under elevated $[CO_2]$, while through a meta-analysis of the results of free-air CO_2 enrichment (FACE) experiments Ainsworth and Rogers (2007) reported an average increase of 31%. In the FACE experiments photosynthetic down regulations were observed, but I did not observe any photosynthetic down regulation as the seedlings were continuously fertilized. Despite the variations in responses, the findings of the experiment could be useful in assisted migration of jack pine. For example, photoperiod associated with 10˚ northward migration advanced budburst which might increase the risks of late spring frost damage. Such problem can be overcome to some extent by seed selection from the mid-range of the species as longer chilling hours required by the populations from mid-range might delay budburst. However, further research is required in this connection.

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Appendix 1: Xylem pressures at 50% loss of hydraulic conductivity (Ψ_{PLC50}) in jack pine seedlings at different levels of [CO_2], soil moisture and photoperiod and their interactions. The R^2-values are from the best-fit vulnerability curves constructed by using the Weibull functions.

Treatment	Ψ_{PLC50} (MPa)	R^2 value	Treatment	Ψ_{PLC50} (MPa)	R^2 value
C_a	1.2095	0.8872	M_h x P_s	1.1699	0.8767
C_e	1.1163	0.8257	M_h x P_{nm1}	1.2431	0.8919
M_h	1.1531	0.865	M_h x P_{nm2}	1.0442	0.8495
M_l	1.1774	0.844	M_l x P_s	1.2751	0.8287
P_s	1.2184	0.8481	M_l x P_{nm1}	1.1624	0.9048
P_{nm1}	1.2029	0.8962	M_l x P_{nm2}	1.1042	0.8245
P_{nm2}	1.0754	0.8354	C_a x M_h x P_s	1.1645	0.9035
C_a x M_h	1.2049	0.8925	C_a x M_h x P_{nm1}	1.2251	0.8982
C_a x M_l	1.2308	0.8821	C_a x M_h x P_{nm2}	1.2292	0.885
C_e x M_h	1.0989	0.8437	C_a x M_l x P_s	1.1866	0.8668
C_e x M_l	1.1350	0.809	C_a x M_l x P_{nm1}	1.1762	0.8981
C_a x P_s	1.1753	0.8844	C_a x M_l x P_{nm2}	1.2736	0.8908
C_a x P_{nm1}	1.2016	0.8972	C_e x M_h x P_s	1.1752	0.8538
C_a x P_{nm2}	1.2537	0.8853	C_e x M_h x P_{nm1}	1.2666	0.8969
C_e x P_s	1.2659	0.8209	C_e x M_h x P_{nm2}	0.8932	0.8866
C_e x P_{nm1}	1.2044	0.9005	C_e x M_l x P_s	1.3852	0.8207
C_e x P_{nm2}	0.8897	0.8519	C_e x M_l x P_{nm1}	1.1471	0.9143
			Ce x Ml x P_{nm2}	0.9299	0.8221

Note: C_a and C_e are ambient and elevated [CO_2], respectively; M_h and M_l are soil moistures at 60-70% and 30-40% of field water capacity, respectively; P_s, P_{nm1} and P_{nm2} are photoperiods at seed origin, 5º and 10º north of the seed origin, respectively.