

INTERACTIVE EFFECTS OF ELEVATED CO₂ AND TEMPERATURE, NUTRITION, AND
ECTOMYCORRHIZAS ON MORPHOLOGICAL TRAITS AND SUMMER SHOOT
FORMATION IN JACK PINE

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A Master's Thesis Submitted in Partial Fulfillment of the
Requirements for the Master of Science in Forestry Degree

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ABSTRACT

Understanding the responses of trees, ectomycorrhizal fungi (ECM) and their association to climate change can be challenging due to their interactions with multiple environmental factors. The combined impacts of elevated CO₂ and elevated temperature on tree growth are mostly positive, but the impacts to ECM are less well understood. The objective of this study was to investigate how the elevations of CO₂ and temperature and soil nutrient supply interactively affect the formation and growth-enhancing efficiency of ECM, as well as the formation of summer shoots and morphology of jack pine seedlings. One-year old jack pine seedlings were exposed to ambient CO₂ and temperature treatment (ACT: 410 μmol/mol CO₂ and current average temperature) and elevated CO₂ and temperature (ECT: 750 μmol/mol CO₂ and current +6°C temperature) as a single treatment, two nutrient levels (low nutrient LN vs. very low nutrient VLN) and with or without *Hebeloma longicaudum* (Pers.) P. Kumm (yes ECM or no ECM) inoculation. The ECT treatment increased total seedling biomass, root biomass and root collar diameter (RCD), and most summer shoot formation and growth attributes. The VLN treatment reduced all growth attributes except for height increment, and height increment + lammas shoot length. An interactive effect of the CT and N treatment was observed for the RCD, suggesting that lower nutrient sites may limit the positive affects of elevated CO₂ and temperature on tree growth, and the interactive effect on specific leaf area (SLA) suggests that the lower nutrients limited the needles' ability to accumulate carbon at ECT. Another interactive effect observed on SLA suggests that ECM inoculation allowed for better nutrient uptake and caused seedlings to demonstrate a higher SLA at VLN under ECT. Both ECT and LN increased the probability of summer shoot formation. The results suggest that jack pine is less likely to produce summer shoots on nutrient poorer sites, and in current CO₂ and temperature growing conditions. Furthermore, we observed significant two-way and three-way interactions that suggest the ECT treatment increased the effectiveness of ECM inoculation in forming mycorrhizae clusters, and reduced the degree of nutrient suppression to mycorrhizal root cluster formation. The results of this study highlight the importance of incorporating multiple factors when evaluating tree responses to climate change, particularly in controlled-environment studies.

Keywords: biomass allocation, CO₂, ectomycorrhizal fungi, jack pine, morphology, soil nutrition, summer shoots, temperature.

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ACKNOWLEDGEMENTS

I would like to thank Dr Qing-Lai Dang for being my advisor and sharing his expertise on this topic. I would also like to thank him for supplying me with materials and financial support to complete the project.

I would also like to thank my committee member Dr. Leonard Hutchison for use of the pathology lab and associated material, and suggestions during project design, and valuable input when reviewing the thesis. Also thank you to my other committee member Lense Meyer for reviewing my thesis and providing feedback. Also thank you to my external reviewer Jian Wang for his comments.

I would also like to thank Keri Pigeon and Ryan Wilkie for their assistance in setup and maintenance of greenhouse conditions. Additionally thank you to Eric Searle for his aid in data analysis. Thank you to Eva Scollie for administrative support. A special thank you to friends and family for the encouragement while completing my experiment, data analysis and writing. Financial support for this thesis was provided through Mitacs Research Training Award, Dr Qing-Lai Dang, and various awards from Lakehead University.

ABREVIATIONS

ACT – Ambient CO₂ and temperature treatment

CT – CO₂ and temperature treatment

ECM – Ectomycorrhizal fungi

ECT – Elevated CO₂ and temperature treatment

LN – Low nutrient treatment

N – Nutrient level

No ECM – Seedlings not inoculated with ectomycorrhizal fungi

RCD – Root collar diameter

SLA – Specific leaf area (cm²/g)

VLN – Very Low Nutrient

Yes ECM – Seedlings inoculated with ectomycorrhizal fungi

INTRODUCTION

The association between ectomycorrhizal fungi and tree roots is one of the most important symbiotic relationships occurring in the forest. It is therefore important to understand how global climate change will affect this relationship (Pickles et al. 2012). The main environmental changes observed globally today include increases in average temperature, and atmospheric carbon dioxide (CO₂) concentration, which can have significant effects on the growth of ectomycorrhizal fungi and trees (Pendall et al. 2004). Carbon dioxide elevations generally increase photosynthesis, which in turn increases tree growth (Hyvonen et al. 2007). Warming has also been shown to promote plant growth, but the response is altered by other environmental factors (Way and Oren 2010). Tree seedling response to combined increases in CO₂ concentrations and temperature have been observed in many studies, and the response to the interactive effect is usually greater tree growth, but the response has varied with tree species (Cantin et al. 1997, King et al. 1997, Olszyk et al. 1998, Tjoelker et al. 1998, Hortal et al. 2016, Kurepin et al. 2018 and Dusenge et al. 2020). The relationship between elevated CO₂ and temperature, and ectomycorrhizal fungi are not yet well understood (Saxe et al. 1998, Drigo et al. 2008, Pickles et al. 2012, Bennett and Classen 2020). In greenhouse experiments, elevated CO₂ has shown to increase ectomycorrhizal colonization (O'Neill et al. 1986, Ineichen et al. 1995, Runion et al. 1997, Shier and McQuattie 1998, Treseder et al. 2004., Alberton et al. 2005 and Choi et al. 2005), as has the combination of elevated CO₂ and elevated temperature (Hortal et al. 2016). At the ecosystem-level, the role of mycorrhizas alleviating nutrient limitation that constrains positive plant growth response to elevated CO₂ has recently received attention (Terrer et al. 2016, Terrer et al. 2019 and Jiang et al. 2020) but requires more research.

Understanding the responses of trees, ectomycorrhizal fungi and their association to climate change can be challenging due to interactions with other environmental factors (Vincent and Declerck 2021). It has been predicted that nutrient availability may constrain the positive responses of trees to warmer temperatures and higher CO₂ levels (Oren et al. 2001, Finzi et al, 2002, Norby et al. 2010, Ellsworth et al. 2017 and Stinziano and Way 2014), so it is important to explore the response of trees at different nutrient levels. Root biomass and mycorrhizas are major plant components that are utilized to minimize environmental limitations (Lambers et al. 2008), and it is valuable to study their role. Jack pine (*Pinus banksiana* Lamb.) is a tree species that relies on ectomycorrhizal fungi for nutrient absorption (Danielson et al. 1994). The species is very important to the composition and ecological functions of boreal forest ecosystems and the forest industry of Canada. Jack pine has been known to form summer shoots, an undesirable occurrence that can negatively affect tree form and susceptibility to frost injury (Rudolph 1964, Rudolph and Laidly 1990). Warming (Kushida et al. 1999, Lee et al. 2007, Landis 2012, Kushida 2015, Chang et al. 2018 and Jung et al. 2020), and elevated CO₂ on a smaller scale (Alden 1970) can affect the occurrence of summer shoots in other species.

The boreal forest climate is changing at an alarming rate, but the development of appropriate adaptive policies and management strategies to ensure successful forest regeneration and growth have been lagging. The interactive effects of CO₂, temperature, nutrients, and ectomycorrhizal fungi may be critical to the better understanding of how the boreal forest will respond to a changing climate. The response of trees could include an increased frequency of summer shoot formation (Kushida et al. 1999, Lee et al. 2007, Landis 2012, Katrevis et al. 2018, Kushida 2015, Jung et al. 2020, Chang et al. 2020). Summer shoots have been studied in

the past, but neither the occurrence in boreal tree species in Canada, nor how climate change and its interaction with other environmental factors may affect the formation are well understood.

The objective of this study is to investigate how the elevations of CO₂ and temperature interact with soil nutrient supply in affecting the formation and growth-enhancing efficiency of ectomycorrhizae, as well as the formation of summer shoots and morphology of jack pine seedlings. In this study, I exposed jack pine seedlings to different combinations of CO₂, temperature, nutrient supply and mycorrhizal fungi, to investigate the interactive effects on biomass allocation, morphology, summer shoot formation, and the level of ectomycorrhizal colonization in jack pine seedlings. Since the combined impacts of elevated CO₂ and temperature are mostly positive (Cantin et al. 1995, King et al. 1997, Peltola et al. 2002, Hortel et al. 2016, Kurepin et al. 2018, Duan et al. 2018), and the presence of ectomycorrhizal fungi is known to increase tree growth, we hypothesize that the elevation of CO₂ and temperature, and ectomycorrhizal fungi inoculation would increase tree growth, but that tree growth would be reduced at the very low nutrient level. Because low soil fertility tends to increase mycorrhizal colonization (Gagnon et al. 1987, Saxe et al. 1997, Shier and McQuattie 1998), and the elevation of CO₂ and temperature can increase carbohydrate production and availability for mycorrhizae fungi (Shier and McQuattie 1998, Alberton 2007), we also hypothesize that the elevation of both CO₂ and temperature, and very low nutrient supply would increase the ectomycorrhizal colonization in jack pine seedling roots. In addition, since increased summer shoot formation has been observed in other tree species with elevated CO₂ and nutrients (Alden 1971), and higher temperatures (Landis 2012, Kushida et al. 1999, Lee et al. 2007, Kushida 2015 and Jung et al. 2020) and higher nutrients (Søgaard et al. 2011, Rikala and Huurinainen 1990), we hypothesized

that elevated CO₂ and temperature, and the low nutrient supply (containing more nutrients) would increase the probability of summer shoots forming on the jack pine seedlings

LITERATURE REVIEW

Climate Change

In September of 2019, global atmospheric CO₂ concentration reached 412 μmol/mol⁻¹, while the concentration was around 280 μmol/mol⁻¹ before the industrial revolution (Stein 2018). Since 2000, atmospheric CO₂ concentration has risen approximately 20 μmol/mol⁻¹ per decade. This is a sharp rate of increase, higher than any rate of atmospheric CO₂ increase in the last 800 000 years (Lüthi et al. 2008, Bereiter et al. 2015). If CO₂ emissions continue to increase at this rate, CO₂ is projected to reach between 750 μmol/mol⁻¹ to 1300 μmol/mol⁻¹ by 2100 (IPCC 2013). As CO₂ levels rise, more radiation is trapped, and global surface temperatures rise as a result.

As of 2017, human induced global warming caused by rising CO₂ concentrations had increased global temperatures by 1°C since the start of the industrial revolution (IPCC 2018). If the current rate of warming continues, global average surface temperatures could increase by 1.5°C by 2040, and by between 3°C and 5°C by 2100 (IPCC 2013), with the boreal forest and other higher latitude areas experiencing even higher temperature changes (Curry et al. 1995).

Boreal Forest and Industry

The boreal forest is both economically and ecologically important to Canada, contributing \$19.8 billion to the gross domestic product (NRC 2020). Being mainly comprised of conifer species, and a few deciduous species (Brandt et al. 2013), the boreal forest covers 270 million hectares within Canada, making it ecologically valuable to the Country's landscape (NRC 2021a). Approximately 50% of the forest that is clear cut in the boreal forest industry is artificially regenerated (NRC 2021b). The process of artificially regenerating includes harvesting

seeds from a specific seed zone and storing the seeds in a seed bank until needed, at which point they are grown in a greenhouse and finally planted in the clear cut blocks. Because climate change is occurring so rapidly, there is not enough time for the seed trees to adapt, since genetic changes take many generations (Lambers et al. 2008). As a result, seed trees can only acclimate, and make adjustments to compensate for the changes in their environment, which can enable them to maintain or even increase performance within the lifetime of the individual tree (Lambers et al. 2008). Since seeds used in the forest industry are grown from acclimated trees rather than from trees adapted to the changing climate, the trees used for forest renewal in the forest industry are not adapted to these changes in the climate. As the atmospheric CO₂ concentration increases, and the climate in northern latitudes warms at a higher rate than the rest of the world, it is important to understand how the projected changes will affect the regeneration of trees as we continue to harvest and replant them.

Silvics of Jack Pine

Jack pine is the most widely distributed pine of the Northern forests of Canada, and to a lesser extent the United States (Rudolph and Laidly 1990). It is a pioneer species and establishes in areas where mineral soil has been exposed by disturbances such as fire. Jack pine usually grows in even-aged, pure, or mixed stands and is often planted on less fertile and drier soil than conditions required by other native boreal species. It is usually found on sandy soils, but can also grow in very dry sandy or gravelly soils, or in clay soils, but does best in well drained, loamy sands (Rudolph and Laidly 1990).

Combined CO₂ and Temperature Impacts to Tree Growth

The effects of higher temperatures and elevations in CO₂ on trees have been extensively studied both separately, and on a smaller scale together. Even though CO₂ concentration and temperature increases occur concurrently, knowledge of the combined effect of elevated CO₂ and temperature on boreal trees is limited (Kurepin et al. 2018). Of the limited number of studies that have been done, most indicated variable responses of different growth attributes between different tree species (Cantin et al. 1995, Olszyk et al. 1998, Tjoelker and Reich 1998, Peltola et al. 2002, Sallas et al. 2003, Lewis et al. 2015, Hortal et al. 2016, Kurepin et al. 2018, Duan et al. 2018 and Dusenge et al. 2020). Studies that look at soil temperature instead of air temperature, coupled with CO₂ elevation (Zhang and Dang 2007) also found that different species have varying growth responses. Therefore, it is important to test all important boreal tree species. Cantin et al. (1995), Hortal et al. (2016) and Duan et al. (2018) found that total seedling biomass increased under a combined elevated CO₂ and elevated temperature treatment, while Dusenge (2020) and Olszyk et al. (1998) saw a decrease in seedling biomass. Kurepin et al. (2018) observed that only shoot biomass and height were increased, and Peltola et al. (2002) found that RCD increased in 10-year old *Pinus sylvestris* L. King et al. (1997) found root systems of *Pinus taeda* L. and *Pinus ponderosa* Dougl. ex P. & C. Laws increased in size with elevated CO₂ and temperature. Lewis et al. (2015) found that growth may increase with rising CO₂, but increasing temperature may offset the increased growth in *Wollemia nobilis* W.G.Jones, K.D.Hill, & J.M.Allen. trees. At higher CO₂ levels, trees can increase carbon assimilation rates since the rate it is not saturated at current atmospheric CO₂ levels (Lambers et al. 2008). Therefore, trees can demonstrate a higher rate of photosynthesis, which usually translates into increase tree growth (Saxe et al. 1998, Ceulmans et al. 1999, Eamus and Ceulemans 2001). Photosynthetic down-

regulation, where carbon assimilation rate decreases after exposure to elevated CO₂ can occur when seedlings are exposed to elevated CO₂ (Lambers et al. 2008), but this usually only occurs if the root volume is limited (Curtis & Wang 1998 and Eamus and Ceulemans 2001) and/or when nutrients are limiting (Ceulemans et al. 1999). Additionally, a warmer temperature is known to alleviate photosynthetic down-regulation when it occurs at elevated CO₂ (Li et al. 2014).

Increase in air temperature can result in greater tree growth by stimulating the CO₂ assimilation rate, while acclimating respiration rates to the higher temperature, which reduces carbon loss through respiring (, Way and Oren 2010, Way and Yamori 2014).

Nutrient Levels

Understanding how variable environmental conditions affect tree growth responses to a higher temperature and CO₂ concentration is crucial to address how boreal forests will respond to a changing climate. Nutrient availability in forests will be impacted by changing CO₂ and temperature, due to CO₂ and temperature's impact on nutrient cycling (Hyvönen et al. 2006 and Lukac et al. 2010). Sigurdsson et al. (2013) concluded that low nutrient availability in the boreal forest is likely to limit the forest's ability to respond to an increase in temperature and CO₂ concentrations, and multiple studies have found that low nutrient soils limit various tree species productivity at elevated CO₂ conditions (Norby et al. 2010 and Ellsworth et al. 2017). On the other hand, rising temperature may enhance organic matter decomposition rates and mineralization and mineral weathering rates (Lukac et al. 2010), as well as cause higher nitrogen deposition (Hyvönen et al. 2006) which could alleviate nutrient limitation in some areas (Lukac et al. 2010). Lukac et al. (2010), Stinziano and Way (2014), Way et al. (2015) and suggest multifactor experiments that manipulate CO₂ and temperature, along with water and nutrient supply are necessary to fully address this issue. Testing a low nutrient level versus a high

nutrient level along with climate change variables is one way to observe how trees respond to climate change when soil nutrient conditions vary (Dang et al. 2020), which is the method this experiment uses. Low nutrient supply has been found to reduce the positive effects of elevated CO₂ (Oren et al. 2001, Norby et al. 2010, Lukac et al. 2010), and a meta-analysis by Curtis & Wang (1998) found that the increase in seedling biomass commonly found with elevated CO₂ is reduced at low nutrient availabilities. As well, elevated CO₂ and temperature have been found to increase a tree's demand for nutrients (Oren et al. 2001, Lukac et al. 2010, Li et al. 2013 and Li et al. 2014). Additionally, a higher quantity of nutrients may be necessary to sustain the positive effects on growth of elevated CO₂ (Lukac et al. 2010, Li et al. 2013, Danyagri and Dang 2014). In addition biomass allocation to roots has been known to increase as a result of CO₂ elevation alone (Eamus and Ceulemans 2001, Alberton et al. 2007 and Newaz et al. 2016), low nutrient on its own (Li et al. 2013 and Dang et al. 2020), low nutrient and elevated CO₂ (Curtis et al. 1994, Shier and McQuattie 1998, Saxe et al. 1998, Sigurdsson et al. 2013), the combination of CO₂ and temperature elevation (Cantin et al. 1995, King et al. 1997 and Hortal et al. 2016). Increasing biomass allocation to roots improves a tree's ability to explore more regions of the soil, and allows more surface area to increase transport and storage of nutrients (Eamus and Ceulemans 2001, Lambers et al. 2008). As well, increased growth at ECT when nutrients are not limiting could improve carbon sequestration at the forest stand level (Oren et al. 2001, Hyvönen et al. 2006).

Ectomycorrhizal Fungi

Another important factor that experiments do not usually include when testing the effects of various climate factors on tree growth is the role of ectomycorrhizal fungi (ECM) (Stinziano and Way 2014). Saxe et al. (1998) and Eamus and Ceulemans (2001) conclude that the

inoculation or control of mycorrhizas must be included in studies of elevated CO₂ and forest trees. Ectomycorrhizal fungi are extremely important to ecosystem processes across the globe (Courty et al. 2010). When growing in the natural environment of the boreal forest, ectomycorrhizal fungi supply nutrients and water to trees that their own roots would otherwise not be able to access. Ectomycorrhizal fungi are able to supply these nutrients (such as nitrogen, phosphorus and potassium) due to their ability to mobilize nutrients from soil organic matter, and weather minerals present in the soil that the ECM can transfer to the tree to utilize (Courty et al. 2010). In exchange, ECM retrieve carbohydrates that are essential for the development of the fungi (Allen et al. 2003). The responses of ECM to elevated temperatures and CO₂ levels are not fully clear (Saxe et al. 1998, Drigo et al. 2008, Pickles et al. 2012, Bennet and Classen 2020 and Vincent and Declerck 2021). Experiments related to ectomycorrhizal fungi and global climate change have looked mainly at CO₂ individually (O'Neill 1986, Ineichen et al. 1995, Shier and McQuattie 1998, Runion et al. 1997, Treseder 2004 and Choi et al. 2005) and only a limited number have investigated CO₂ and temperature together (Hortal et al. 2016). Hortal et al. (2016) found that CO₂ on its own negatively impacted ectomycorrhizal development, but higher temperatures positively affected it, and combined, the impact was positive.

Choi et al. (2005) found that ECM inoculation causes significant increase in biomass and root collar diameter of seedlings grown at both ambient and elevated CO₂, and at the elevated CO₂, the rate of mycorrhizal inoculation was higher. At elevated CO₂ conditions, O'Neill (1986), Runion et al. (1995) and Shier and McQuattie (1998) found percentage mycorrhizal short roots were higher, and Ineichen et al. (1995) found that the number of mycorrhizae clusters per tree was significantly higher. Treseder (2004) concluded that quantity of ECM generally increases under elevated CO₂ concentrations, and the quantity declines in response to increased nitrogen

and phosphorus availability. Alberton (2005) concludes that as CO₂ concentration increases, the plant's need for N and P will increase, which fungi may be able to provide, and the fungi will receive more carbon for its development from increased rates of photosynthesis. Some studies investigating climate change effects on tree species do include mycorrhizal fungi, but none incorporate CO₂, temperature, different nutrient levels, and the effect inoculation of an ECM has on the trees growing in those conditions. Mohan et al. (2014) noted that most experiments investigating mycorrhizae's role on ecosystem responses to climate change factors only address one environmental factor, and that the few studies that do address more than one factor find that the response of the ecosystem is different when another factor is included in the study, highlighting the importance of multifactor experiments. Since the presence of ectomycorrhizal fungi can alter the morphology of jack pine seedlings (Browning and Whitney 1990), it's important to incorporate their presence when studying jack pine growth response. In addition, mycorrhizal associations and benefits depend on available nutrients (Gagnon et al. 1987, Landis et al. 1990, Saxe et al. 1998) therefore multi-factor experiments that look at different nutrient levels are also valuable. The fungus *Hebeloma longicaudum* (Pers.)P.Kumm. is known to associate with boreal conifer trees, and is an early stage fungus. To the best of our knowledge, no study to date has examined the effects of mycorrhizal fungi inoculation on jack pine growth response under conditions of different nutrient levels, in combination with elevated CO₂ and temperature conditions.

Summer Shoots

Some tree species, including jack pine, are able to produce a summer shoot, a burst of growth late in the growing season from recently formed buds which were not expected to open until the following year (Kramer and Kozlowski 1979 and Rudolph and Laidly 1990). These

shoots can either be lammas shoots, growing from the terminal bud, or proleptic shoots that grow from the lateral buds at the base of the terminal bud, which would have become branches the following year (Kramer and Kozlowski 1979).

The occurrence of summer shoots in seedlings is not ideal for the forest industry (Søgaard et al. 2011). Proper tree form, or apical dominance is required to produce logs. Both types of summer shoots are often injured in the winter because the shoots may not harden off adequately for the cold months, which can lead to poor tree form (Kramer and Kozlowski 1979). In addition, when both lammas and proleptic shoots are formed, or when only proleptic shoots are formed, competition for apical dominance will occur, which can lead to stem forking. When a fork is developed in the stem, the tree is not suitable to become a log (Kramer and Kozlowski 1979). In a study by Søgaard et al. (2011), the occurrence of lammas shoots increased the probability of Norway spruce (*Picea abies* (L.) Karst.) trees developing fork in their stems the following year. The presence of summer shoots can also alter the root:shoot biomass ratio of the of seedlings by significantly increasing the shoot growth relative to the root growth (McKown et al. 2016).

Factors Affecting the Formation of Summer Shoots

The formation of either type of summer shoot can be stimulated by various environmental conditions, and is also influenced by the genetics of the tree. Genetic factors are found to be associated with the occurrence of lammas growth (Laidly and Rudolph 1990, Steffenrem et al. 2008, Skrøppa and Steffenrem 2019). Summer shoot growth is often observed when there are warmer growing conditions and adequate or higher soil moisture (Kushida et al. 1999 and Landis 2012). Lee et al. (2007), Kushida (2015) and Jung et al. (2020) found that higher temperatures increased both the occurrence and length of lammas shoots. Chang et al. (2018) found that warming led to more developed summer shoots (length and amount of needle

growth on the shoot) but did not affect the occurrence rate of the shoots. High summer temperatures affect the occurrence of summer shoots by increasing the number of leaf primordia that lead to their formation (Kushida 2015). There may be a temperature threshold for summer shoot occurrence, as a higher temperature treatment in an experiment with Chang et al. (2020) showed when it limited the production of summer shoots on *Pinus koraiensis*, Siebold & Zucc. whereas a smaller increase in temperature increases the occurrence rate of summer shoots. Olszyk et al. (1998) found that an increase of 4°C inhibited the occurrence of summer shoots of *Pseudotsuga menziesii* (Mirb.) Franco seedlings. Greater nutrient availability also increases the occurrence of summer shoots (Rikala and Huurinainen 1990, Søggaard et al. 2011 and Landis 2012). Aldan (1970) demonstrated that favourable environmental conditions (CO₂, water and nutrients) during the first year of growth resulted in a low number of seedlings with summer shoots in their second year of growth. Conversely, a high number of seedlings with summer shoots occur when poor environmental conditions are applied during the first year of growth. Alden's work (1970) suggests that CO₂, water and nutrient levels can contribute to lammas growth. Other factors also affect summer shoot formation. For example forest stands with no competition produce a higher proportion of trees with lammas shoots (Katrevis et al. 2018), and lammas growth has been found to occur in planted sites more often than in naturally regenerated sites (Granus et al. 2019).

METHODS

Plant Materials

One-year old jack pine seedlings were obtained from Hill's Greenhouse Ltd. in Kakabeka Falls, Ontario and stored at -3°C. The seedlings were grown from seeds from Stanley Jack Pine Orchard (seed zone 13, Stanley, Ontario). The seedlings were relatively uniform in size at the start of the experiment (average height = 15.58cm and average diameter = 3.02mm). The seedlings were planted in circular plastic pots (20cm diameter by 25cm height) with 4 drainage holes in the bottom. The growing medium was a mixture of peat moss and vermiculite (3:1 v/v).

Fungal Material

The ectomycorrhizal fungus *Hebeloma longicaudum* (Pers.)P.Kumm., an early-stage fungal colonizer was used in this experiment as it is known to associate with boreal conifer trees. The culture of *Hebeloma longicaudum* (isolate 723) was obtained from the Lakehead University Mycological Herbarium Fungal Culture Collection. A preliminary experiment was conducted in July 2019 to test an inoculation methodology. All trees inoculated with mycelial plugs of *Hebeloma longicaudum* in the preliminary experiment were harvested in October 2019 and found to have successfully formed mycorrhizae. On October 26th, 2019, fresh cultures were transferred into 8 plastic Petri dishes (90mm diameter) containing modified Melin-Norkrans Agar. On December 4th, four mycelial plugs, (7mm diameter) were aseptically added to 80-250mL-flasks containing 125mL of vermiculite, and 70mL of modified Melin-Norkrans solution (See Appendix 2.1) with 2g/L glucose, which had previously been sterilized in an autoclave. The vermiculite-fungus mixture was left to grow in the flask in the dark at 20°C for 2 months before being used in the experiment. Seedlings subject to the ectomycorrhizal treatment were inoculated

at the time of planting by placing the vermiculite-fungus mixture from the flask onto the roots. One-hundred and twenty-five mL of vermiculite with no fungal inoculum was added to the roots of each control seedling at the time of planting, so that mycorrhizal and non-mycorrhizal seedlings were grown in otherwise comparable growing medium.

Experimental Design

The experiment was executed in the four experimental greenhouses in the Forest Ecology Complex at Lakehead University's Thunder Bay campus. The treatments consisted of two combined CO₂-temperature levels (ACT: ambient CO₂ and temperature: 410 µmol/mol⁻¹ and current temperature versus ECT: elevated CO₂ and temperature: 750 µmol/mol⁻¹ and current +6°C temperature), two nutrient supply levels (low nutrient: LN versus very low nutrient: VLN), and ectomycorrhizal fungus *Hebeloma longicaudum* (ECM) inoculation (Yes ECM: inoculated versus No ECM: not inoculated). The experiment was a split plot design. The two CO₂ and temperature combined treatment was the main plot assigned at the greenhouse level, with two replicates each. The factorial combinations of nutrient level and ectomycorrhizal fungi inoculation were the sub-plots, nested within the CO₂ and temperature treatment (see Appendix 2.2 for experiment layout). Each treatment combination had 10 seedlings (2 levels of CO₂ and temperature, 2 nutrient levels, 2 ectomycorrhizal treatments) and 2 replications per treatment, $10 \times 2 \times 2 \times 2 \times 2 = 160$).

Greenhouse environment controls

The environmental conditions in the greenhouse (temperature, CO₂, light and humidity) were controlled by an Argus Titan system (Argus Control Systems Ltd. Surrey, British Columbia). Carbon dioxide concentration was maintained at either 410 µmol/mol⁻¹ for the ACT

treatment or $750 \mu\text{mol}/\text{mol}^{-1}$ for the ECT treatment by using the model GEN2E gas CO_2 generators (Custom Automated Products Inc., Riverside, California, USA). The photoperiod and temperature settings were updated weekly. The temperature for the ACT was determined by the 10-year average hourly temperatures at the seed source (Environment Canada Weather Records) while the temperature for the ECT treatment was always 6°C warmer than that in the ACT treatment. The diurnal temperature was ramped at 3-hour intervals to emulate actual diurnal changes in temperature at the seed source.

The nutrient treatments were formulated based on three considerations: firstly, the Container Tree Nursery Manual (Landis et al. 1989) recommends $100 \mu\text{mol}/\text{mol}^{-1}$ of nitrogen with 20-7-19 (N-P-K) nutrient ratio for growing conifer seedlings in containers during rapid growth phase; secondly, jack pine generally grows on sites with lower fertility (Rudolph and Laidly 1990); and thirdly higher nutrient levels in the growing medium suppress ectomycorrhiza formation (Gagnon et al. 1987, Landis et al. 1989 and Smith and Reid 1997). Therefore, the nutrient treatment should be lower than $100 \mu\text{mol}/\text{mol}^{-1}$. Since some mycorrhizae species respond differently to soil fertility (Landis et al. 1989), we conducted a preliminary experiment in which 1 year-old jack pine seedlings were inoculated with several ectomycorrhizal fungi and grown at $50 \mu\text{mol}/\text{mol}^{-1}$ N with the 20-7-19 nutrient ratio. We found that *Hebeloma longicaudum* formed the most mycorrhizae per seedling among all the fungus species that we tested and was chosen for the study. We chose $50 \mu\text{mol}/\text{mol}^{-1}$ N (20-7-19 N-P-K ratios) as the LN treatment and 1/10 of the strength as the very low nutrient treatment (VLN). The composition and concentration of the fertilizer for the LN treatment were $35 \mu\text{mol}/\text{mol}^{-1}$ NH_4 , $58 \mu\text{mol}/\text{mol}^{-1}$ NO_3 , $7 \mu\text{mol}/\text{mol}^{-1}$ NH_2 (which comprises the $50 \mu\text{mol}/\text{mol}^{-1}$ Nitrogen) $35 \mu\text{mol}/\text{mol}^{-1}$ P_2O_5 , $95 \mu\text{mol}/\text{mol}^{-1}$ K_2O , and micronutrients comprised of $0.15 \mu\text{mol}/\text{mol}^{-1}$ B, $0.3 \mu\text{mol}/\text{mol}^{-1}$ Cu, 2

$\mu\text{mol}/\text{mol}^{-1}$ Fe, $0.3 \mu\text{mol}/\text{mol}^{-1}$ Mn, $0.025 \mu\text{mol}/\text{mol}^{-1}$ Mo, $0.3 \mu\text{mol}/\text{mol}^{-1}$ Zn, $4 \mu\text{mol}/\text{mol}^{-1}$ MgO. See Appendix 2.3 for detailed fertilizer composition and concentrations. The seedlings were fertilized weekly.

The light level in the greenhouses was maintained above $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at the seedling canopy level and high-pressure sodium lamps (Model LR48877, P.L. Systems, Grimsby, ON, Canada) were used when the natural light level was too low and/or the natural day length was too short for the season that was being emulated. For the last 3 weeks of the experiment, the natural day length was longer than the growing season being emulated, so the photoperiod was shortened by draping opaque plastic sheets around the seedlings. The seedlings were watered when the volumetric water content of the growing medium dropped to approximately 30%, as determined using a Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). The trees were grown for four months, with photoperiod and temperatures emulating the conditions of June 1st to September 14th. To minimize potential effects of any ununiform conditions within the greenhouses, the seedlings were rotated within the greenhouse three times per week.

Growth Measurements

The morphological traits of all seedlings were measured at the end of the experiment. Height and root collar diameter were measured the day the seedlings were planted, and again the day when the seedlings were harvested to determine net growth. In addition, the length of all summer shoots were recorded (lammas and/or proleptic on apical stem, and/or branches). Once harvested, the root systems were gently washed to remove peat and vermiculite, and to prevent damage to fine short roots and mycorrhizae, and then scanned with WinRHIZO Pro (Regent Instruments Inc., Quebec City, Quebec, Canada). A visual inspection of the roots

was completed for the formation of ectomycorrhizae, which could be easily identified by the fungal sheath that envelope feeder roots (Landis et al. 1989). A randomly selected portion of roots were analyzed with WinRHIZO Pro to determine the proportion of short roots that were mycorrhizal by tallying regular short roots and bifurcate short roots using the short root analysis tool. The total and average (AA) projected areas were determined using WinSEEDLE (Regent Instruments Inc., Quebec City, Quebec, Canada) separately for current year needles (needles that grew on the main stem or branches during the experiment) and lammas and proleptic needles (needles that grew on summer shoots that formed during the experiment).

Biomass Measurements

The seedlings were separated into root, stem and needles (current year, lammas, and proleptic separately) and oven-dried at 70°C for 48 hours. Once oven-dried, the dry mass of root, stem and needles was determined. Biomass allocation, specific leaf area (SLA= total leaf area / total leaf dry mass), and root:shoot ratio (RSR = root dry mass/shoot dry mass) were determined subsequently.

Statistical Analysis

ANOVA for split-plot design for all measured and calculated variables was conducted using the following linear model:

$$Y_{ijkl} = \mu + CT_i + W_{(i)j} + \delta_{(ij)} + N_k + F_l + NF_{kl} + CTN_{(ik)} + CTF_{(il)} + CTNF_{ikl} + N_{m(i)jk} + F_{m(i)jl} + NF_{m(i)jkl} + \varepsilon_{(ijklm)}$$

Where CT(i) = 2 levels of CO₂ and temperature, j = error of whole plot factor (greenhouse error), δ = restriction due to randomization, N(k) = 2 nutrient levels, F(l) = ectomycorrhizal fungus inoculation and m = error of the split plot factors.

All analyses were conducted using ‘R’ software (‘R’, Geneva, Switzerland, Version 3.5.0, R Development Core Team 2020). The normality and homogeneity of data was ensured by reviewing diagnostic plots (scatter plot, residual plot, Q-Q plot, and density plot) for all dependent variable, and completing a Shapiro-Wilks, and Levene’s test for most dependent variables. When necessary, suitable transformation was done to normalize the data. CT, nutrient level and ectomycorrhizal fungus inoculation were treated as fixed effect factors in the ANOVA. When ANOVA showed a significant interaction ($p \leq 0.05$), an emmeans table was produced in R to examine the means, and a post-hoc analysis was completed using Tukey’s adjustment to compare differences between individual means. Several of the response variables (summer shoot biomass, and proleptic needle biomass, SLA and AA) were subjected to a model that did not include the mycorrhizal treatment as a factor due to the lack of sufficient summer shoots each treatment combination. Since even less lammas shoots occurred, the lammas needle biomass, SLA and AA were pooled for both nutrient and inoculation treatments.

RESULTS

Growth

The height increment, and the height increment plus apical lammas shoot was greater at ECT, regardless of the nutrient and ECM treatment, (Figures 1a & 1c), but the differences were not statistically significant (Table 1). The total shoot length (height increment of apical stem, plus all apical summer shoots (lammas and/or proleptic) was significantly greater in the LN than VLN (10% increase). Root collar diameter (RCD) was significantly affected by CT, N and their interactions (Table 1). The LN treatment significantly increased RCD under both ACT and ECT conditions, but the increase was greater in the ECT treatment than ACT (30% versus 19%). ECT significantly increased RCD under LN, but had no significant under VLN (Figure 1d).

Table 1. ANOVA results (p values) for the effects of CO₂-Temperature combination (CT), nutrient level (N), ectomycorrhizal fungi inoculation (ECM) and their interactions on height increment (Height), height increment plus apical lammas shoot (Height + Lammas), the height increment plus all apical summer shoots (Total Shoot Length), root collar diameter (RCD), current year needle biomass (Current Yr. Needle Bio.), current year needle specific leaf area (Current Yr. Needle SLA), current year needle average area (Current Yr. Needle AA), total biomass of all needles (Total Leaf Bio), total biomass of shoot (Total Shoot Bio), total biomass of root (Root Bio), total biomass of seedling (Total Bio), Root:Shoot Ratio, percentage of short roots mycorrhizal (% short root ECM) and number of mycorrhizal clusters (# of ECM clusters). The values in bold text are significant at <0.05.

Response Variable	CT	N	ECM	CT*N	CT*ECM	N*ECM	CT*N*ECM
Height	0.418	0.180	0.405	0.275	0.694	0.791	0.861
Height + Lammas	0.379	0.287	0.441	0.253	0.709	0.651	0.849
Total Shoot Length	0.395	0.013	0.344	0.613	0.614	0.254	0.660
RCD	0.043	<0.001	0.827	0.006	0.323	0.698	0.650
Current Yr. Needle Bio.	0.248	<0.001	0.817	0.701	0.207	0.181	0.215
Current Yr. Needle SLA	0.201	0.007	0.087	0.001	0.593	0.042	0.358
Current Yr. Needle AA	0.085	<0.001	0.294	0.412	0.214	0.900	0.349
Total Leaf Bio	0.093	<0.001	0.514	0.189	0.853	0.323	0.066
Total Shoot Bio	0.083	<0.001	0.706	0.327	0.818	0.347	0.058
Root Bio	0.037	<0.001	0.660	0.506	0.872	0.452	0.347

Table 1 Continued:

Response Variable	CT	N	ECM	CT*N	CT*ECM	N*ECM	CT*N*ECM
Total Bio	0.044	<0.001	0.946	0.400	0.850	0.376	0.143
Root : Shoot Ratio	0.336	0.021	0.285	0.502	0.407	0.886	0.219
% short root ECM	0.061	<0.001	<0.001	0.364	0.297	0.078	0.053
# of ECM clusters	0.360	<0.001	<0.001	0.439	0.009	0.286	0.005

Table 2. ANOVA results (p values) for the effects of CO₂-Temperature combination (CT), nutrient level (N), and their interaction on the total needle biomass of all summer shoots (Summer Shoot Needle Bio), total needle biomass of all proleptic shoots (Proleptic Needle Bio), proleptic needle specific leaf area (Proleptic Needle SLA) and average area of proleptic needles (Proleptic Needle AA), lammas needle biomass (Lammas Needle Bio), lammas needle specific leaf area (Lammas Needle SLA), and lammas needle average area (Lammas Needle AA). The values in bold text are significant at <0.05. Please note: Lammas Needle Bio, Lammas Needle SLA and Lammas Needle AA data were pooled for nutrient due to insufficient observations (see Text for further explanations).

Response Variable	CO ₂ T	Nutrient	CO ₂ T*Nutrient
Summer Shoot Needle Bio	0.002	<0.001	0.144
Proleptic Needle Bio	<0.001	<0.001	0.175
Proleptic Needle SLA	0.246	0.666	0.693
Proleptic Needle AA	0.009	<0.001	0.111
Lammas Needle Bio	0.070	-	-
Lammas Needle SLA	0.168	-	-
Lammas Needle AA	0.039	-	-

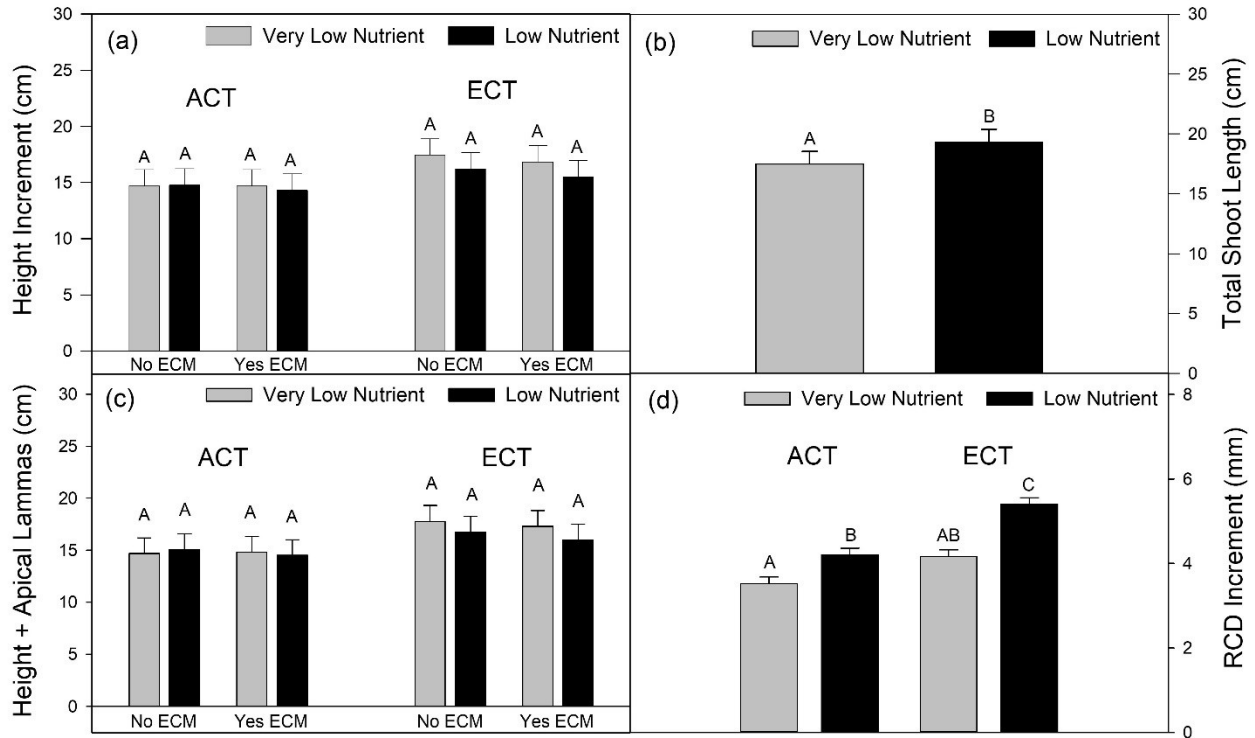


Figure 1. The effects of CO₂-Temperature combination (CT), nutrient level (N), ectomycorrhizal fungi inoculation (ECM) and their interactions on the mean (+SE) of height increment (a), total shoot length (b), height increment plus lammas shoot (c), root collar diameter increment (d) of jack pine seedlings. Data has been pooled across N for (b), and CT and N for (d), since those interactions were significant. Different letters indicate means that were statistically different from each other at $p \leq 0.05$.

The low nutrient treatment significantly increased total leaf biomass (by 28%) and shoot biomass (by 26%) (Figure 2b). The root biomass was also significantly increased by LN (17%), as well as ECT (20% increase) (Figure 2b). Similarly, total biomass was significantly increased by LN (19%), and ECT (18%) (Figure 2a). The root:shoot ratio decreased with LN (6%) (Figure 2d).

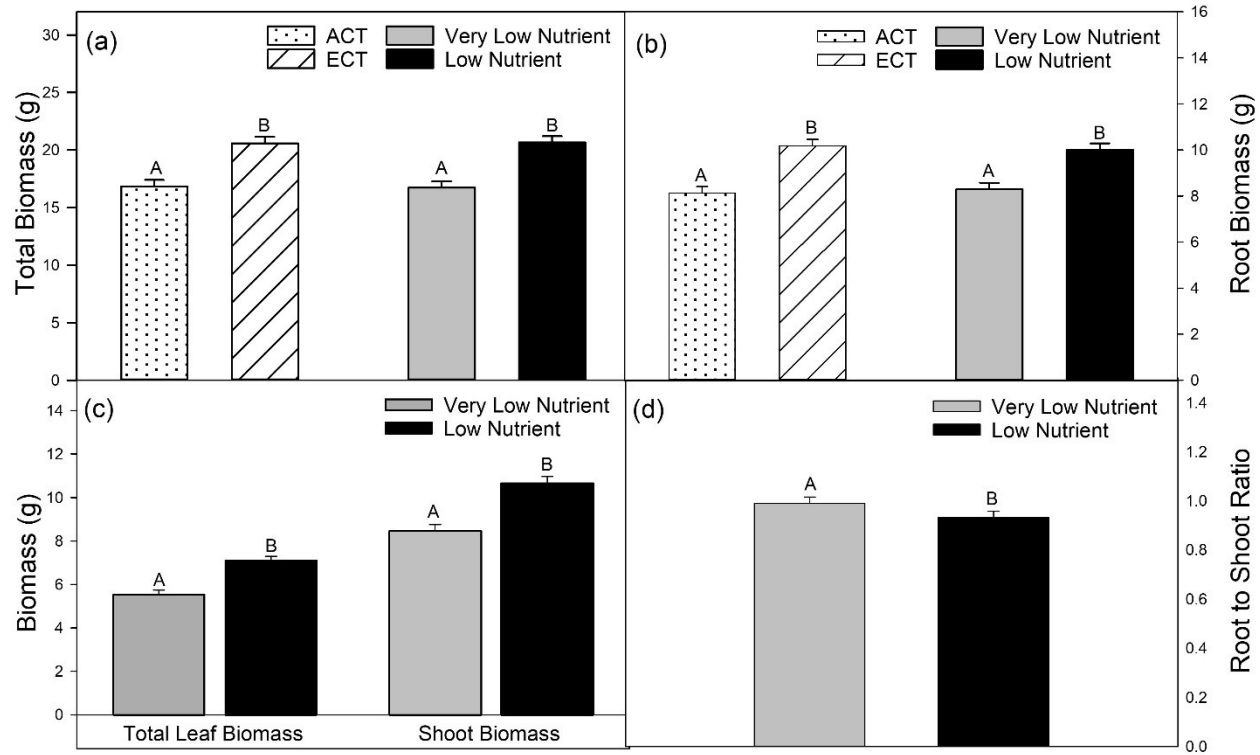


Figure 2. The effects of CO₂-Temperature combination (CT), nutrient level (N), ectomycorrhizal fungi inoculation (ECM) and their interactions on the mean (+SE) of total seedling biomass (a) root biomass (b), total leaf biomass and shoot biomass (c), and root:shoot ratio (d) of jack pine seedlings. Data has been pooled across N for (c) and (d), and N and CT separately for (a) and (b), since those interactions were significant. Different letters indicate means that were statistically different from each other at $p \leq 0.05$.

The biomass and average area of current year needles were significantly increased by LN (by 20% and 10%, respectively) (Figures 3a and 3b). Specific leaf area (SLA) of current year needles at ACT was not significantly affected by nutrient level, but at ECT LN significantly decreased the SLA by 8% (Figure 3c). The total biomass of needles on all summer shoots (lammas and/or proleptic on apical stem and/or lateral branch) was significantly increased by 88% by ECT and 102% by LN level (Figure 3d).

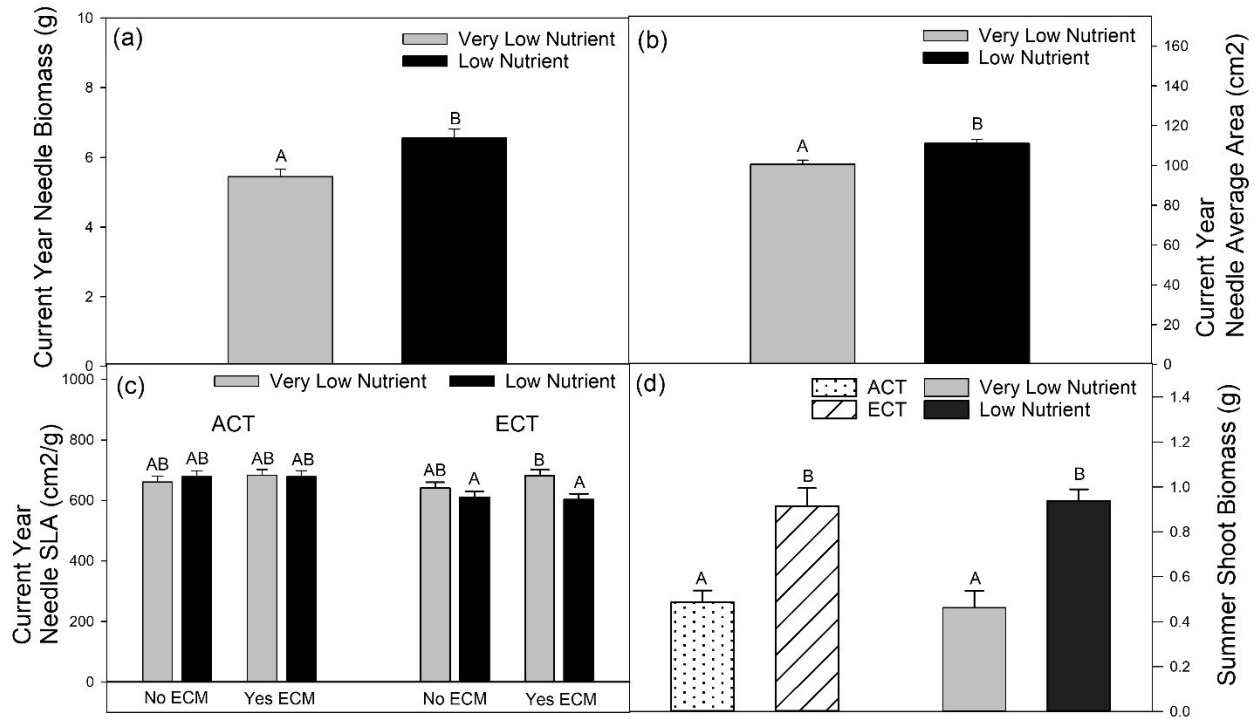


Figure 3. The effects of CO₂-Temperature combination (CT), nutrient level (N), ectomycorrhizal fungi inoculation (ECM) and their interactions on the mean (+SE) of total current year needle biomass (a), current year needle average area (b), current year needles specific leaf area (SLA) (c), and summer shoot needle biomass (d) of jack pine seedlings. Data has been pooled across N for (a) and (b), and CT and N separately for (d), since those interactions are significant. Different letters indicate means that are statistically different at $p \leq 0.05$.

ECT significantly increased the total needle biomass and average area on proleptic shoots, but not those of lammas needles (Figure 4a, b, e & f, Table 2). Low nutrient treatment significantly increased proleptic needle biomass and average area by 84% and 35%, respectively. Elevated CT decreased the SLA of both lammas and proleptic needles, but the effect was not statistically significant (Figures 4c, d, Table 2). The nutrient treatment did not significantly affect SLA of lammas or proleptic needles (Table 2).

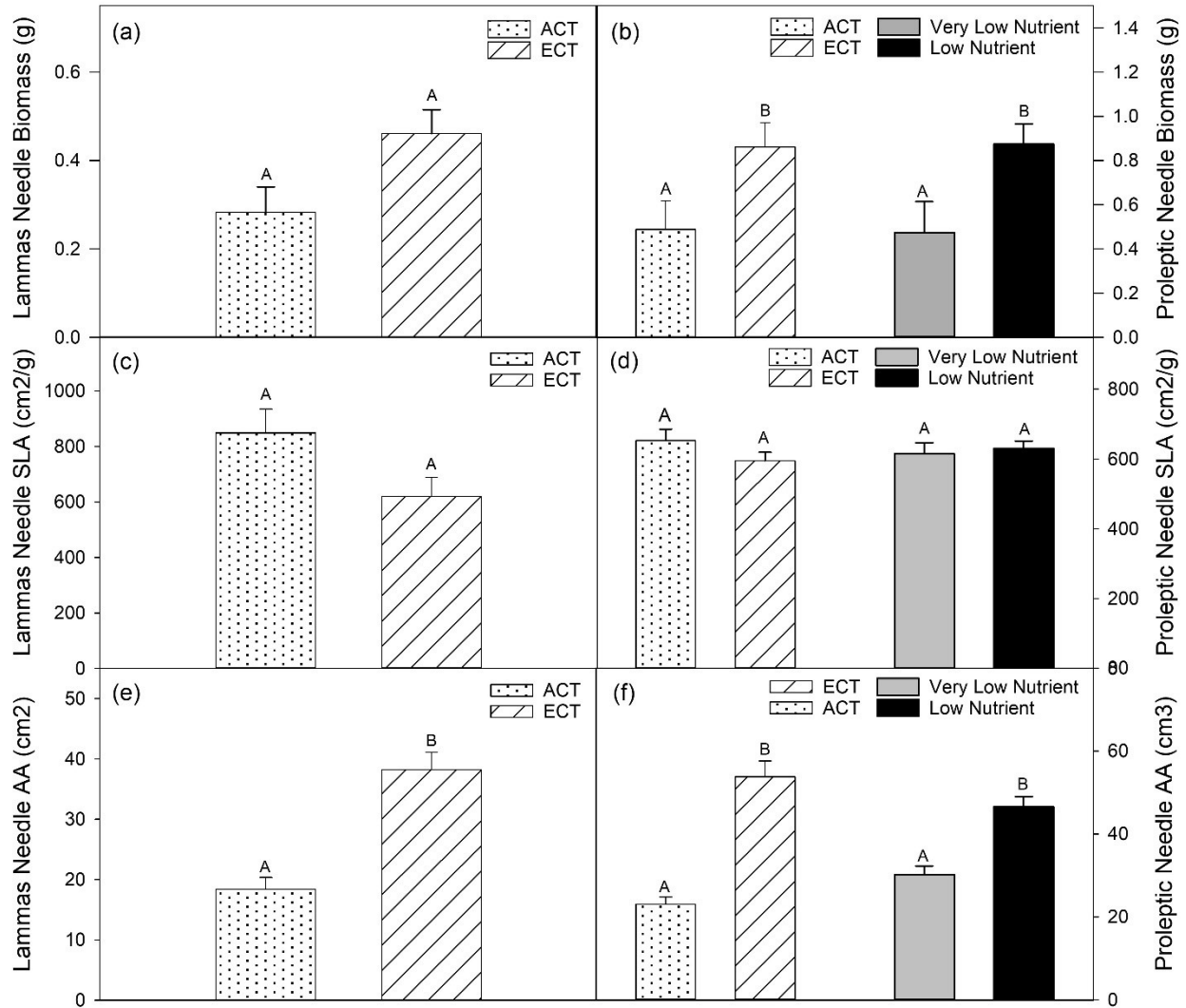


Figure 4. The effect of CO₂-Temperature combination (CT) on the mean (+SE) for lammas needle biomass (a), lammas needle specific leaf area (SLA) (b), lammas needle average area (c) of jack pine seedlings. Also, the effects of CT and N and their interactions on mean (+SE) for proleptic needle biomass (d), proleptic needle specific leaf area: SLA (e) and proleptic needle average area (f) of jack pine seedlings. Data has been pooled across N, and CT separately for (b) and (f) since those interactions are significant, and also (d) for easy interpretation of the figure. Different letters indicate means that are statistically different at $p \leq 0.05$.

Ectomycorrhizal fungus inoculation significantly increased short-root percentage in mycorrhizal roots (50%), and LN significantly decreased the percentage (37%) (Figure 5b). The inoculation also significantly increased the number of mycorrhizal clusters on roots at ECT, and LN reduced the clusters in both inoculation treatments (significant three-way interaction in Table

1, Figure 5a). ECM inoculation increased the count of mycorrhizal clusters by 47% at ACT, and 84% at ECT. Overall, VLN increased the count of ECM clusters by 638%.

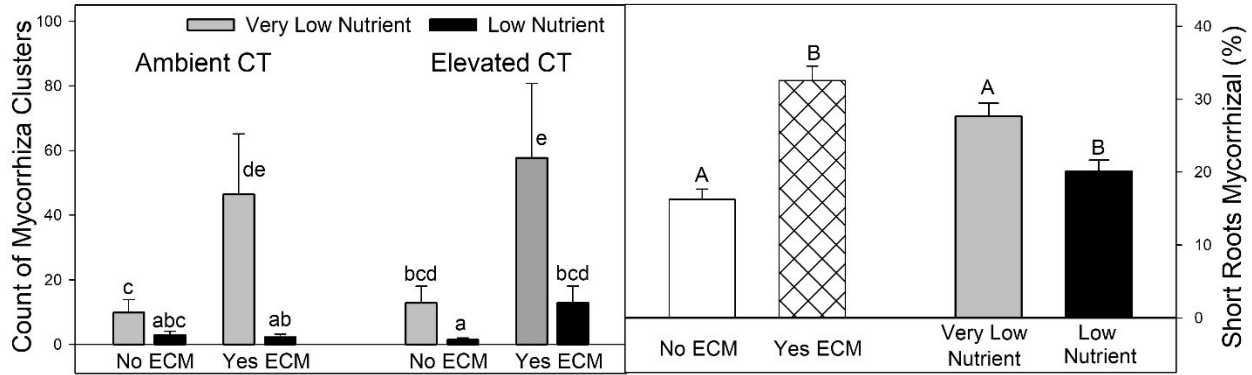


Figure 5. The effects of CO₂-Temperature combination (CT), nutrient level (N), ectomycorrhizal fungi inoculation (ECM) and their interactions on the mean (+SE) for the count of mycorrhizae clusters on the root system (a) and for percentage of short roots mycorrhizae (b) of jack pine seedlings grown. Data has been pooled across ECM and N treatment separately for (b) since those interactions were significant. Different letters indicate means that are statistically different from each other at $p \leq 0.05$.

Occurrence of Summer Shoots

Table 3. ANOVA results (p values) for the effects of the CO₂-Temperature combination (CT), nutrient level (N), and presence of ectomycorrhizal fungi (ECM) and their interaction on the probability of apical lammas shoots (A Lam Occurrence), apical proleptic shoots (A Pro Occurrence), any apical summer shoot (A SS Occurrence), and of any summer shoot (including apical stem and/or lateral branches)(All SS Probability). The values in bold are significant at <math><0.05</math>.

Response Variable	CT	N	ECM	CT*N	CT*ECM	N*ECM	CT*N*ECM
A Lam Occurrence	0.001	0.022	0.559	0.388	0.552	0.336	-
A Pro Occurrence	0.070	<0.001	0.862	0.709	0.816	0.399	0.601
A SS Occurrence	0.002	<0.001	0.240	0.427	0.374	0.148	0.654
All SS Probability	0.001	0.002	0.123	0.624	0.418	0.495	0.449

Both ECT and LN significantly increased the probability (out of 1) of lammas shoots formation on the apical stem, the probability of the formation of any type of summer shoot on the apical stem, and the probability of any summer shoot formation on both the apical stem and/or

lateral branches (Figures 6 a, c, d). The probability of proleptic shoot occurrence on apical stems was increased by ECT and LN, but only the effect of nutrient was significant (Figure 6 b).

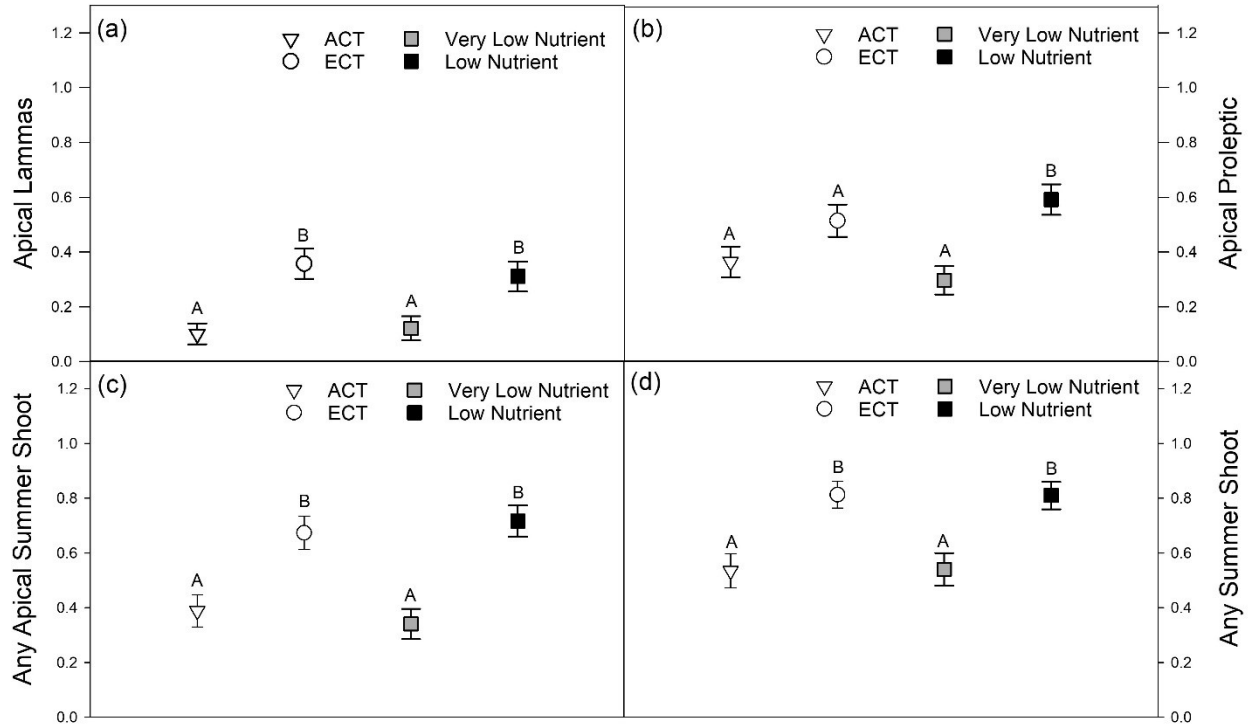


Figure 6. The effects of CO₂-Temperature combination (CT), nutrient level (N), ectomycorrhizal fungi inoculation (ECM) and their interactions on the probability value (out of 1) of apical lammas shoots (a), apical proleptic shoots (b), any apical summer shoot (c), or any summer shoot (d) occurring on jack pine seedlings grown. Data has been pooled across CT and N, separately for (a), (c) and (d) since those interactions are significant. Data was also pooled across CT and N for (b), to show the effects of both treatments, but only the N treatment was significant. Different letters indicate means that are statistically different at $p \leq 0.05$.

DISCUSSION

The results of the study partially support our hypothesis that elevated CO₂, temperature, and the presence of ectomycorrhizal fungi would increase tree growth, but the tree growth would be reduced by low nutrients. The combination of elevated CO₂ and warmer temperature increased total seedling biomass, root biomass and root collar diameter, as well as most summer shoot growth attributes. The results of ECT treatment are consistent with the literature (Cantin et al. 1995, King et al. 1997, Peltola et al. 2002, Stinziano and Way 2014, Hortal et al. 2016 and Duan et al. 2018). Significant positive effects of the ECT were likely caused by a higher rate of photosynthesis at a higher CO₂ concentration; and the higher temperature positively affecting physiological processes. The rate of CO₂ assimilation is not saturated at the current level of CO₂ in the atmosphere, as reflected in the ACT treatment (Lambers et al. 2008). Therefore, trees under a ECT treatment containing 750 μmol/mol CO₂ should carry out a higher rate of photosynthesis, which usually translates into increased growth (Saxe et al. 1998, Ceulmans et al. 1999 and Eamus and Ceulemans 2001). An increase in air temperature can contribute to more tree growth by stimulating the CO₂ assimilation rate while the respiration rate acclimates to the higher temperature, and reduces carbon losses (Way and Yamori 2014, Way and Oren 2010). Kerepin et al. (2018) report that height growth, aboveground biomass, and biomass allocation to foliage in *Picea abies* all increase with elevated CO₂ concentrations and a 4°C increase in temperature, but the growth decreases when the temperature is increased by 8°C. Our results suggest that a 6°C warming from the current temperature can have positive effects on the growth and biomass of jack pine, since we did not observe a decrease to any variables at ECT compared to ACT. The lack of significant CO₂ and temperature effects on some aboveground variables in this experiment (height increment values, current year needle biomass and average area, total

leaf biomass, total shoot biomass, and root:shoot ratio) has been observed before in the response of other tree species to the combined effect of CO₂ and air temperature (Olszyk et al. 1998, Sallas et al. 2003, Lewis et al. 2015 and Dusenge et al. 2020). The fact that the root:shoot ratio was not significantly altered by ECT treatment in this study is supported by meta-analysis reviews that conclude CO₂ increases both aboveground and belowground biomass of seedlings (Curtis and Wang 1998, Tingey et al. 2000); and that other environmental factors such as water and nutrient supply alter the ratio (Saxe et al. 1998). Jack pine is a determinate species, meaning height growth is pre-determined in the previous growing season, so it is not surprising that the height increment seen in this experiment was not significantly different between treatments since we planted one-year old seedlings. This theory is supported by the fact that N level did not affect the height, or height plus apical lammas, but did affect every other variable, indicating that the determinate nature of jack pine limited any impact to the height.

Our results on the interactive effect of CT and N on RCD suggest that lower nutrient availability limited the positive effects of elevated CO₂ and temperature on seedling growth, which is consistent with the literature (Curtis and Wang 1998, Oren et al. 2001, Finzi et al. 2002, Lukac et al. 2010, Norby et al. 2010, Sigurdsson et al. 2013, Li et al. 2013, Stinziano and Way 2014 and Ellsworth et al. 2017). Li et al. (2013) saw a significant increase in RCD by elevated CO₂, and a lower nutrient supply treatment significantly reduced the amount of increase, which supports the findings in this study. The interactive effect between CT and N on other growth or biomass variables in our study was not statistically significant. The individual impact of ECT to the RCD suggests that at both low and very low nutrient levels jack pine are growing in, the RCD can be positively impacted by ECT. This is important to highlight, as a greater RCD could

lead to better carbon sequestration at the forest stand level, as investments to woody biomass will persist in the ecosystem for many years (Oren et al. 2001, Hyvönen et al. 2006).

While the VLN reduced needle and summer shoot needle characteristics, and ECT increased most summer shoot needle characteristics as expected, there was also significant interactive effect on specific leaf area between nutrient supply and CO₂ and temperature elevation, and between mycorrhizal fungus inoculation and nutrient supply. Lower nutrient availability generally decreases specific leaf area, resulting in a smaller leaf area for light interception and photosynthesis (Lambers et al. 2008). However, the results of this study indicated the opposite, i.e., the SLA was significantly greater in the lower nutrient treatment (VLN vs LN) under ECT. A possible reason for this result may be a greater level of carbohydrate accumulation in LN than in VLN at ECT conditions, which lead to a smaller SLA (thicker leaves) in the LN treatment. It is well documented that nutrient supply and elevated CO₂ concentration can interact and affect CO₂ assimilation into leaf (Ceuleman et al. 1999 and Li et al. 2013). Therefore, it is possible that the nutrient supply in the VLN treatment was so low that the photosynthesis was limited to a greater extent than in the LN treatment, resulting in less carbohydrate accumulation and a smaller leaf mass density (i.e., greater SLA) rather than a bigger leaf size in the SLA in the LN treatment. Furthermore, the VLN treatment significantly increased the SLA when subject to the Yes ECM treatment, but VLN did not alter the SLA for No ECM seedlings. This result was probably related to the fact that ECM-inoculated trees possessed a higher percentage of mycorrhizal short roots and clusters, which allowed for better nutrient uptake and faster growth, which, in turn led to a larger leaf size. Faster growing plants tend to have a higher SLA (Lambers et al. 2008, Ceulemans et al. 1999, Eamus and Ceulemans 2001). The SLA of needles growing on proleptic and lamma shoots were not affected by any

treatment, probably because the needles were relatively young; it was the second flush of the season, and there was not enough time for the treatments to significantly impact needle densities. Even though the current year needle biomass and average area were not significantly different between CT treatments, which disagrees with past experiments with jack pine needles (Cantin et al. 1995 and Newaz et al. 2016), our results hint that that ECT allowed more investment in needles, as seen in the increase in summer shoot needle biomass and average area, which provides more area for photosynthesis to take place. The summer shoot flushes may have started earlier with the higher temperatures (Chang et al. 2018), which allowed more time for the needles to grow, and elevated CO₂ conditions allowed more investment into their growth (Eamus and Ceulemans 2001 and Lambers et al. 2008).

The results of the VLN treatment support our hypothesis that lower nutrients would reduce the growth of the tree seedlings. The total biomass of the jack pine seedlings was significantly reduced by VLN, along with every other aboveground and belowground attribute measured in this study (except height and height+ apical lammas which was discussed earlier). While overall growth was reduced by VLN, biomass allocation was also altered, as observed in the increased root:shoot ratio at VLN. Our study suggests that low nutrient availability will result in greater investment belowground (root biomass, mycorrhizal clusters), and decreased investment aboveground (biomass of shoot, RCD, current year needles, summer shoot needles and higher probability of summer shoots forming). Other experiments have observed an increase in biomass allocation to roots with low nutrient treatment on its own (Tan and Hogan 1997, Li et al. 2013, Dang et al. 2020), and low nutrient and elevated CO₂ interactively (Curtis et al. 1994, Shier and McQuattie 1998, Saxe et al. 1998 and Stigurdson et al. 2013). The lower probability of summer shoot formation, and a decrease in the needle biomass and average area of the summer

shoots that did form at VLN contributed to less aboveground growth, and resulted in a higher root:shoot ratio). Higher nutrients are known to increase summer shoot formation (Søgaard et al. 2011, Rikala and Huurinainen 1990, Landis 2012). As well, the root:shoot ratio has been known to be reduced by the formation of summer shoots (McKowen 2016). Therefore, our study suggests that in sites of very poor nutrient quality, overall seedling growth of jack pine will be reduced, and the investment to belowground growth will be greater, while aboveground growth may decrease due to the reduced probability of summer shoots and associated leaf mass forming. Increasing biomass allocation to roots improves a tree's ability to explore the soil for nutrients and water (Lambers et al. 2008, Eamus & Cuelemans 2001), and would be helpful to improve a tree's ability to positively respond to elevated CO₂ and temperature (Sigurdsson et al. 2013).

The summer shoots observed in this study suggest that no matter the nutrient level, elevated CO₂ and temperature would increase the probability of summer shoots forming on the jack pine seedlings (though apical proleptic shoots were only increased marginally by ECT treatment (0.07)). The increasing probability of summer shoots with the ECT treatment fits with other studies, which indicate that warmer temperatures increase the occurrence of summer shoots (Kushida 2015, Lee et al. 2007, Jung et al. 2020), and that CO₂ alone can also play a role (Alden et al. 1970). However, there is a scarcity of studies observing the effect of CO₂ on summer shoot formation. It would have been beneficial to have separate elevated CO₂ and temperature treatments, to investigate the individual effect of CO₂ on summer shoot formation. The forest industry should monitor for seedlings producing summer shoots, especially since lammas growth has been found to occur in planted sites more often than in naturally regenerated sites (Granhus et al. 2019). Since poor tree form, stem forking and/or winter injury of the summer shoots can occur, forest managers should monitor for the presence of summer shoots and these negative

traits. Since studies have shown that too much warming can inhibit summer shoot occurrence (Chang et al. 2020 and Olszyk et al. 1998), and since we saw an increase in summer shoots at +6°C of warming, it can be assumed that +6°C does not meet the threshold for inhibiting summer shoot occurrence in jack pine. It would therefore be advantageous to investigate several levels of warming, to determine if jack pine negatively responds to more extreme levels of warming, i.e., higher than +6°C, and if less warming i.e., +4°C would show the same results as observed in this experiment.

The results partially support our hypothesis that elevated CO₂ and temperature and VLN would increase ectomycorrhizal colonization of jack pine seedling roots. CT treatment affected the responses in the number of clusters of ECM observed in the significant three-way interaction. While the ECM inoculation increased the percentage of mycorrhizal short roots and the number of clusters of mycorrhizae, consistent with the literature (Browning and Whitney 1990, Shier and McQuattie 1998), the increase was not as large as the results reported by them. The reason for the smaller increase in colonization and clusters in this study is that a large proportion of the uninoculated seedlings formed mycorrhizal short roots and clusters. While the source of ECM in the control treatment is uncertain, it is possible that the seedlings might have developed mycorrhizae during their first year of growth in the nursery or from spores present in the peat and vermiculite mixture. However, higher percentage of mycorrhizal short roots and a greater number of mycorrhizal clusters resulting from the VLN treatment is consistent with the idea that higher nutrient levels would suppress ectomycorrhizal formation (Landis et al. 1989, Schier and McQuattie 1998, Saxe et al. 1997). Situations of higher nutrient availability could occur with changes in climate due to increased nutrient cycling and mineralization caused by elevated CO₂ and temperature, but the likelihood of that occurring is unclear (Lukac et al. 2010).

The CT treatment did not affect the percentage of mycorrhizal short roots in this study. This contradicts the observations by other researchers that both elevated CO₂ alone (Choi et al. 2005, O'Neill 1986, Runion et al. 1995, Shier and McQuattie 1998) and the combination of elevated CO₂ and warmer temperature (Hortal et al. 2016) increase the percentage of mycorrhizal short roots. However, the observed two-way and three-way interaction suggests that the ECT treatment did increase the effectiveness of ECM inoculation in forming mycorrhizae clusters and reduced the degree of nutrient suppression to mycorrhizal root cluster formation. Such effect was likely due to the greater availability of carbohydrate to ECM fungi under ECT (Shier and McQuattie 1998, Alberton 2005).

The VLN treatment resulted in the greatest number of clusters, indicating the benefits to the tree of receiving more nutrients by associating with mycorrhiza was worth the carbon investment of allocating carbon to the fungi (Treseder 2004). The seedlings investment in a significantly higher number of clusters at LN, Yes ECM, ECT treatment combination suggests that even when nutrient availability is increased, the tree will still invest in mycorrhiza in warmer, higher CO₂ conditions; however, they will invest a lot less than when nutrients are very low. As stated earlier, the presence of No ECM seedlings possessing ectomycorrhizal roots likely diluted my results, but the higher number of clusters observed generally agree with the findings of Ineichin et al. (1995) and Schier and McQuattie (1998), where more clusters developed at an elevated CO₂ concentration. Further, in Schier and McQuattie's (1998) study, clusters formed at both high and low nutrient levels at elevated CO₂, when inoculated, which agrees with our observations.

The increased development of ectomycorrhizal fungi on tree roots has many benefits, and should be included in experiments regarding tree responses to climate change. A greater quantity of mycorrhizal clusters can result in the tree receiving more nutrients, which can positively

impact the growth of the tree and ensure its survival, especially in sites of very poor nutrient quality (Browning et al. 1990, Choi et al. 2005 and Drigo et al. 2008). The fact that we observed a significant interaction between Yes ECM and N in the current year needles SLA, indicates nutrients did transfer from fungi to the jack pine seedlings and altered tree growth, though that was the only impact to tree growth. This interaction supports the suggestion of Saxe et al. (1997) and Eamus and Ceulemans (2001) that experiments investigating tree responses to climate change should include ectomycorrhizal fungi as a treatment, as it can interact with other treatments and affect tree growth.

CONCLUSION

Jack pine seedlings growing in a climate with a higher CO₂ concentration and temperature level are likely to exhibit a favourable above and belowground response as demonstrated in the greater overall biomass, root biomass, RCD and summer shoot formation, and summer shoot needle biomass and average area. If jack pine seedlings grow on boreal forest sites with very low nutrients, our study suggests that overall biomass will be smaller and more biomass will be allocated to root systems, and the seedlings are less likely to produce summer shoots. The interactive effects observed in the RCD growth and SLA of current year needles suggest that the level of nutrients available to the tree can affect the tree's ability to increase growth with ECT. Further research should investigate this interactive concept with jack pine seedlings. While the increased probability of summer shoot formation at ECT and LN may contribute to a greater amount of shoot growth and needle biomass providing more area surface area for photosynthesis, there could be negative consequences that occur. The forest industry should monitor for seedlings producing summer shoots, and the associated negative tree form

that can occur as the tree matures, as this will affect the quality of lumber the industry can produce from the trees.

Even though the percentage of shoot roots colonized by ectomycorrhiza was not significantly increased, the development of ectomycorrhizal fungi into clusters was enhanced by elevated CO₂ and temperature, which even allowed a greater number of clusters when nutrients were higher. Since some seedlings we did not inoculate possessed ectomycorrhiza and clusters on short roots, this study may have underestimated the effect of ectomycorrhiza inoculation on tree growth. As a result, further research on jack pine, climate change and ectomycorrhizal fungi should be conducted to provide a clearer understanding the interactive relationship. Because there was one significant effect of ECM on growth of the seedlings that was interactive with N level, suggesting ECM can interact with other treatments and affect growth, climate change experiments should include ectomycorrhizal presence.

It would be beneficial to compare different degrees of warming with elevated CO₂, especially since seedling growth has been known to decline with too warm of temperatures, and the formation of summer shoots is affected. Since we designed the CT as a single treatment in our experiment, we cannot determine if this increase in temperature had any effect independently of the CO₂ concentration. Since this experiment was only a small fraction of a tree's life represented in a short-term experiment under a controlled environment, multiple growing seasons may be warranted to determine the long-term effect of ECT. Multiple growing seasons could also allow a more accurate observation of interactions among elevated CO₂ and temperature and ectomycorrhizal inoculation at the different nutrient levels, as the fungi would be more established on roots and may alter the relationship at that point. In addition, since height growth in jack pine is predetermined in the previous year, multiple growing seasons could allow

the examination of seedling height increment more accurately, as well as negative traits that may develop due to the formation of summer shoots. The results of this study highlight the importance of understanding tree responses to a changing global climate through observing the effects of CO₂, temperature, nutrient level and mycorrhizae on biomass allocation and both shoot and root morphology.

LITERATURE CITED

- Alberton, O., Kuyper, T.W. and Gorissen, A. 2005. Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO₂. *New Phytology*. 167:859–868.
- Alberton, O., Kuyper, T.W. and Gorissen, A. 2007. Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO₂. *Plant Soil*. 296:159-172.
- Aldén, T. 1971. Influence of CO₂, moisture and nutrients on the formation of Lammas growth and prolepsis in seedlings of *Pinus sylvestris* L. *Studia Forestalia Suecica* 93:1–21.
- Allen, M.F., Swenson, W., Querejeta, J.I., Egerton-Warburton, L.M., Treseder, K.K., 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology* 41: 271-303
- Archer, D. 2016. Near miss: the importance of the natural atmospheric CO₂ concentration to human historical evolution. *Climatic Change*. Vol 138:1-11.
- Bennett, A.E. and A.T. Classen. 2020. Climate change influences mycorrhizal fungal–plant interactions, but conclusions are limited by geographical study bias. *Ecology*. 101(4)1-11.
- Bereiter, B., Eggleston, S., Schmitt, J., Nehrbass-Ahles, C., Stocker, T.F., Fischer, H., Kipfstuhl, S., Chappelaz, J. 2015. Revision of the EPICA Dome C CO₂ record from 800 to 600-kyr before present. *Geophysical Research Letters*. 42(2):542-549.
- Brandt, J.P., Flannigan, M.D., Maynard, D.G., Thompson, I.D. and Volney, W.J.A. 2013. An introduction to Canada’s boreal zone: ecosystem processes, health, sustainability, and environmental issues. *Environmental Review*. 21: 207–226.
- Browning, M.H.R. and Whitney, R.D. 1990. Responses of jack pine and black spruce seedlings to inoculation with selected species of ectomycorrhizal fungi. *Canadian journal of forest research*. 21(5):701-706.
- Cantin, D., Tremblay, M.F. and Lechowicz, M.J. 1997. Effects of CO₂ enrichment, elevated temperature, and nitrogen availability on the growth and gas exchange of different families of jack pine seedlings. *Canadian Journal of Forest Research*. 27(4):510-520.
- Ceulemans, R., Janssens, I.A., and Jach, M.E. 1999. Effects of CO₂ enrichment on trees and forests: lessons to be learned in view of future ecosystem studies. *Annual Botany*. 84:577–590.
- Chang, H., Han, S.H., An, Kim, H., Kim, S. and Son, Y. 2020. Responses of Summer Shoots and Spring Phenology of *Pinus koraiensis* Seedlings to Increased Temperature and Decreased Precipitation. *Journal of Plant Biology*. 63:473–483.
- Chang, H., Han, S.H., An, J., Park, M.J. and Son, Y. 2018. Warming results in advanced spring phenology, delayed leaf fall, and developed abnormal shoots in *Pinus densiflora* seedlings. *Trees*, 32:1473-1479.

- Choi, D.S., Kayama, M., Chung, D.J., Jin, H.O., Quoreshi, A.M., Maruyama, Y. and Koike, T. 2005. Mycorrhizal activities in *Pinus densiflora*, *P. koraiensis* and *Larix kaempferi* native to Korea raised under high CO₂ concentrations and water use efficiency. *Phyton*. 45(4):139-144. *Soil Biology & Biochemistry*. 42:679-698.
- Courty, P.E., Buee, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S. and Garbaye, J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. *Soil Biology and Biochemistry*. 42(5): 679-698.
- Curry J.A., and Schramm J.L. and Ebert, E.E. 1995. Sea ice-albedo climate feedback mechanism. *Journal of Climate*. 8:240-247.
- Curtis, P.S. and Wang, X. 1998. A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia*. 13:299-313.
- Curtis P.S., Zak, D.R., Pregitzer, K.S. and Teeri, J.A. 1994. Above and belowground response of *Populus grandidentata* to elevated atmospheric CO₂ and soil N availability. *Plant Soil*. 165:45–51.
- Danielson, R.M. 1984. Ectomycorrhizal associations in jack pine stands in northeastern Alberta. *Canadian Journal of Botany*. 62:932-939.
- Dang, QL., Marfo, J., Du, F., Newaz, S. 2020. Nutrient supply and belowground interaction alter responses to CO₂ elevation in black spruce and white spruce. *Forest Ecology and Management*. 472:118271.
- Danyagri, G., Dang, QL., 2014. Soil temperature and phosphorus supply interactively affect physiological responses of white birch to CO₂ elevation. *American Journal of Plant Sciences*. 5:219–229.
- Drigo B., Kowalchuk, G.A. and van Veen JA. 2008. Climate change goes underground: effects of elevated atmospheric CO₂ on microbial community structure and activities in the rhizosphere. *Biology and Fertility of Soils*. 44:667–679.
- Duan, H., Chaszar, B., Lewis, J.D. and Smith, R.A. 2018. CO₂ and temperature effects on morphological and physiological traits affecting risk of drought-induced mortality. *Tree Physiology*. 38(8):1138-1151.
- Dusenge, M., Madhavji, S., and Way, D. 2020. Contrasting acclimation responses to elevated CO₂ and warming between an evergreen and a deciduous boreal conifer. *Global Change Biology*. 26:3639-3657.
- Eamus, D. and Ceulemans, R. 2001. Effects of greenhouse gases on the gas exchange of forest trees. pp17-20. In. *The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems: Report No. 3 of the IUFRO Task Force on Environmental Change*. Karnosky, D. CABI. 357pp.

- Ellsworth, D.S., Anderson, I., Crous, K.Y., Cooke, J., Drake, J.E., Gherlenda, A.N., Gimeno, T.E., Macdonald, C.A., Medlyn, B.E., Powell, J.R., Tjoelker, M.G. and Reich, P.B. 2017. Elevated CO₂ does not increase eucalypt forest productivity on a low-phosphorus soil. *Nature Climate Change*. 7:279–282.
- Finzi, A.C., DeLucia, E.H., Hamilton, J.D., Richter, D.D. and Schlesinger, W.H. 2002. The nitrogen budget of a pine forest under free air CO₂ enrichment. *Oecologia*. 132:567-578.
- Gagnon, J., Fortin, J.A. and Langlois, C.G. 1987. Growth of containerized jack pine seedlings inoculated with different mycorrhizal fungi under a controlled fertilization schedule. *Canadian Journal of Forestry Research*. 17:840-845.
- Granhus, A., Metslaid, M., Kvaalen, H. and Sjøgaard . 2019. Tree, stand and site characteristics affecting the occurrence of lammas growth and multiple tops in field-grown Norway spruce. *New Forests*. 50:291-305.
- Hortal, S., Powell, J.R., Plett, J.M., Simonin, A., Anderson, I.C. 2016. Intraspecific competition between ectomycorrhizal *Pisolithus microcarpus* isolates impacts plant and fungal performance under elevated CO₂ and temperature. *FEMS Microbiology Ecology*. 92(8):1-11.
- Hyvönen, R., Ågren, G.I., Linder, S., Persson, T., Cotrufo, M.F., Ekblad, A., Freeman, M., Grelle, A., Janssens, I.A., Jarvis, P.G., Kellomäki, S., Lindroth, A., Loustau, D., Lundmark, T., Norby, R.J., Oren, R., Pilegaard, K., Ryan, M.G., Sigurdsson, B.D., Strömberg, M., van Oijen, M. and Wallin, G. 2007. The likely impact of elevated CO₂, nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist*. 173:463-480.
- Ineichen, K., Wiemken, V. and Wiemken, A. 1995. Shoots, roots and ectomycorrhiza formation of pine seedlings at elevated atmospheric carbon dioxide. *Plant, Cell and Environment*. 18:703-707.
- IPCC. 2018. *Global Warming of 1.5°C. Chapter 1: Framing and context*. Cambridge, UK and New York, USA, Cambridge University Press.
- IPCC. 2013: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- Jiang, M., Caldararu, S., Zhang, H., Fleischer, K., Crous, K.Y., Yang, J., De Kauwe, M.G., Ellsworth, D.S., Reich, P.B., Tissue, D.T. and Zaehle, S. 2020. Low phosphorus supply constrains plant responses to elevated CO₂: A meta-analysis. *Global change Biology*. 26(10):5856-5873.

- Jung, S.H., Kim, A.R., An, J.H., Lim, C.H., Lee, H. and Lee, C.S. 2020. Abnormal shoot growth in Korean red pine as a response to microclimate changes due to urbanization in Korea. *International journal of biometeorology*, 64(4):571-584.
- Katrevis, J., Neimane, U., Dzerina, B., Kitenberga, M., Jansons, J. and Jansons, A. (2018). Environmental factors affecting formation of lammas shoots in young stands of Norway spruce (*Picea abies* Karst.) in Latvia. *iForest*. 11:809-815.
- King, J.S., Thomas, R.B. and Strain, B.R. 1997. Morphology and tissue quality of seedling root systems of *Pinus taeda* and *Pinus ponderosa* as affected by varying CO₂, temperature, and nitrogen. *Plant and Soil*. 195: 107-119.
- Kramer, P.J. and Kozlowski, T.T. 1979. *Physiology of Woody Plants*. Academic Press. New York, NY. 926 pp.
- Kushida, T. 2015. Effect of High Summer Temperatures on Lammas Shoot Elongation and Flowering in Japanese Red Pine. *Phyton (Austria)*. 45(4):215-221.
- Kushida, T., Nakashima, A., Nagata, H. 1999. Lammas shoot induction in *Pinus densiflora* by heating in spring. *Journal of Japan Forestry Society*. 81(1):57–64.
- Kurepin, L.V., Stang, Z.R, Ivanov, A.G., Bui V., Mema, M., Hüner, N.P.A., Öquist, G., Way, D.A., Hurry, V. 2018. Contrasting acclimation abilities of two dominant boreal conifers to elevated CO₂ and temperature. *Plant Cell Environment*. 41:1331-1345.
- Lambers, H., Chapin III, F.S. and Pons, T.L. 2008. *Plant Physiological Ecology*. Second Edition. New York, NY.
- Landis, T.D., Tinus, R.W., McDonald, S.E., and Barnett, J.P. 1989. *The Container Tree Nursery Manual*. Volume 4. Seedling Nutrition and Irrigation. *Agricultural Handbook*. 674. Washington, DC. U.S. Department of Agriculture, Forest Service. 119pp.
- Landis, T.D. 2012. Lammas Shoots in Nurseries and Plantations. *Forest Nursery Notes*. 5-9.
- Lee, C.S., Song, H.G., Kim, H.S., Lee, B.N.R., Pi, J.H., Cho, Y.C., Seol, E.S., Oh, W.S., Park, S. and Lee, S.M. 2007. Which environmental factors caused lammas shoot growth of Korean red pine? *Journal of Ecology and Environment*, 30(1):101-105.
- Lewis, J.D., Phillips, N.G., Logan, B.A., Smith, R.A., Aranjuelo, I., Clarke, S., Offord, C.A., Frith, G.H., Barbour, M., Huxman, T. and Tissue D.T. 2015. Rising temperature may negate the stimulatory effect of rising CO₂ on growth and physiology of Wollemi pine (*Wollemia nobilis*). *Functional Plant Biology* 42: 836-850.
- Li, J., Dang, Q.L., Man, R. and Marfo, J. 2013. Elevated CO₂ alters N-growth relationship in spruce and causes unequal increases in N, P and K demands. *Forest Ecology and Management* 298:19–26.

- Li, L., Duan, B., Deng, D., Tu, W. and Zhang, Y. 2014. Soil salinity alters the sexual responses to elevated CO₂ and temperature in growth and leaf traits of a dioecious plant. *Canadian Journal of Forest Research*. 10:1292-1301.
- Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.M., Siegenthaler, U., Raynaud, D., Jouzel, J., Fischer, H., Kawamura, K., Stocker, T.F. 2008. High-resolution carbon dioxide concentration record 650,000-800,000 years before present. *Nature*. 453(7193):379-382.
- Lukac, M., Calfapietra, C., Lagomarsino, A. and Loreto, F. 2010. Global climate change and tree nutrition: effects of elevated CO₂ and temperature. *Tree Physiology* 30:1209
- McKown, A.D., Guy, R.D., Quamme, L.K. 2016. Impacts of bud set and lammas phenology on root:shoot biomass partitioning and carbon gain physiology in poplar. *Trees (Berlin, West)*. 30(6):2131-2141.
- Mohan, J.E., Cowden, C.C., Baas, P., Dawadi, A., Frankson, P.T., Helmick, K., Hughes, E., Khan, S., Lang, A., Machmuller, M., Taylor, M. and Witt, C.A. 2014. Mycorrhizal fungi mediation of terrestrial ecosystem responses to global change: mini-review. *Fungal Ecology*. 1-17
- Natural Resources Canada (NRC). 2020. Overview of Canada's forest industry. Government of Canada <https://www.nrcan.gc.ca/forests/industry/overview/13311>
- Natural Resources Canada (NRC). 2021a. Boreal Forest. Government of Canada <https://www.nrcan.gc.ca/our-natural-resources/forests/sustainable-forest-management/boreal-forest/13071>
- Natural Resources Canada (NRC). 2021b. Indicator: Forest Regeneration. Government of Canada. <https://www.nrcan.gc.ca/our-natural-resources/forests/state-canadas-forests-report/timber-being-harvested-sustainably/indicator-regeneration/16548>
- Newaz, M.S., Dang, Q.L., Man, R.Z., 2016. Morphological response of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod. *Am. J. Plant Sci*. 7:879–893.
- Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., and McMurtrie, R.E. 2010. CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America*. 107(45):19368–19373.
- Olszyk, D., Wise, C., VanEss, E., Apple, M. and Tingey, D. 1998. Phenology and growth of shoots, needles, and buds of Douglas-fir seedlings with elevated CO₂ and (or) temperature. *Canadian Journal for Forestry Research*. 76:1991-2001.
- O'Neill, E.G., Luxmoore, R.J., Norby, R.J. 1986. Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO₂ atmosphere. *Canadian Journal for Forestry Research*. 17:878-883.
- Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schäfer, K. V, McCarthy, H., Hendrey, G., McNulty, S.G., and Katul, G.G. 2001. Soil fertility limits

- carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature*. 411: 469–472.
- Peltola, H., Kilpelainen, A. and Kellomaki, S. 2002. Diameter growth of Scots pine (*Pinus sylvestris*) trees grown at elevated temperature and carbon dioxide concentration under boreal conditions. *Tree Physiology*. 22: 963-972.
- Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y., Megonigal, J.P., Olsrud, M. and Ryan, M.G. Wan, S. 2004. Below-ground process responses to elevated CO₂ and temperature: a discussion of observations, measurement methods, and models. *New Phytologist*. 162(2):311-322.
- Pickles, B.J., Egger, K.N. Massicotte, H.B. and Green, D.S. 2012. Ectomycorrhizas and climate change. *Fungal Ecology*. 5(1)73-84.
- Rikala, R. and Huurinainen, S. 1990. Effect of fertilization on the nursery growth and outplanting success of two-year old containerized Scots pine seedlings. *Folia Forestalia* 745(1-16)
- Rudolph, T. D. 1964. Lamm growth and prolepsis in jack pine in the Lake States. *Forest Science*, 10:1-70.
- Rudolph, T.D. and Laidly, P.R. 1990. *Pinus banksiana* (Lamb.), Jack Pine. In: Burns, R.M. and Honkala, B.H. (Eds.). *Agriculture Handbook 654*. USDA Forest Service. Washington, DC. pp. 280-293.
- Runion G.B., Mitchell R.J., Rogers H.H., Prior S.A., Counts T.K.. 1997. Effects of nitrogen and water limitation and elevated atmospheric CO₂ on ectomycorrhiza on longleaf pine. *New Phytologist* 137:681–689
- Sallas, L., Luomala, E-M., Utriainen, J., Kainulainen, P. and Holopainen, J.K. 2003. Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. *Tree Physiology*. 23:97-108.
- Saxe, H., Ellsworth, D.S., Heath, J. 1998. Tansley Review No. 98 Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytologist*. 139:395-436
- Shier, G.A. and McQuattie, C.J. 1998. Effects of carbon dioxide enrichment on response of mycorrhizal pitch pine (*Pinus rigida*) to aluminum: growth and mineral nutrition. *Trees*. 12(6):340-346.
- Sigurdsson, B.D., Medhurst, J.L., Wallin, G., Eggertsson, O., Linder, S. 2013. Growth of mature boreal Norway spruce was not affected by elevated [CO₂] and/or air temperature unless nutrient availability was improved. *Tree Physiology*. 33:1192-1205
- Smith, S. E. and Reid, D. 1997. *Mycorrhizal Symbiosis*, 2nd edition. Academic Press. London, England. 298-314.

- Søgaard, G., Fløistad, I.S., Granhus, A., Hanssen, K.H., Kvaalen, H., Skrøppa, T. & Steffenrem A. 2011. Lammas shoots in spruce – occurrence, genetics, and climate effects. In Granhus, A., Hanssen, K.H. & Søgaard, G.: Forest management and silviculture in the north – balancing future needs. Book of abstracts for the conference in Stjørdal, Norway, September 6-8, 2011. Rapport fra Skog og landskap 14/11. 57–58 pp.
- Skrøppa T. and Steffenrem A. 2019. Genetic variation in phenology and growth among and within Norway spruce populations from two altitudinal transects in Mid-Norway. *Silva Fennica*.53(1):19.
- Stein, T. 2018. Another Climate Milestone on Mauna Loa. The Office of Oceanic and Atmospheric Research (OAR). Silver Springs, MD. <https://research.noaa.gov/article/ArtMID/587/ArticleID/2362/Another-climate-milestone-falls-at-NOAA%E2%80%99s-Mauna-Loa-observatory>
- Steffenrem, A., Lindland, F., and Skrøppa, T. 2008. Genetic and environmental variation of internodal and whorl branch formation in a progeny trial of *Picea abies*. *Scandinavian Journal of Forest Research*. 23(4):290-298.
- Stinziano, J.R. and Way, D.A. 2014. Combined effects of rising CO₂ and temperature on boreal forests: growth, physiology and limitations. *Botany* 92: 425-436.
- Tan, W., Hogan, G.D. 1997. Physiological and morphological responses to nitrogen limitation in jack pine seedlings: potential implications for drought tolerance. *New Forests*. 14:19–31.
- Terrer, C., Vicca, S., Hungate, B. A., Phillips, R. P., & Prentice, I. C. 2016. Mycorrhizal association as a primary control of the CO₂ fertilization effect. *Science*. 353:72–74.
- Terrer, C., Jackson, R.B., Prentice, I.C., Keenan, T.F., Kaiser, C., Vicca, S., Fisher, J.B., Reich, P.B., Stocker, B.D., Hungate, B.A. and Peñuelas, J. 2019. Nitrogen and phosphorus constrain the CO₂ fertilization of global plant biomass. *Nature Climate Change*. 9(9):684-689.
- Tingey, D., Phillips, D., & Johnson, M. 2000. Elevated CO₂ and conifer roots: Effects on growth, life span and turnover. *New Phytologist*. 147(1):87-103.
- Tjoelker, M.G., Oleksyn, J., and Reich, P.B. 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO₂ and temperature. *Tree Physiology*. 18:715–726.
- Treseder, K.K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO₂ in field studies. *New Phytology*. 164:347e355.
- Vincent, B., Declerck, S. 2021. Ectomycorrhizal fungi and trees: brothers in arms in the face of anthropogenic activities and their consequences. *Symbiosis*. 84:337–351.
- Way, D.A., Oren, R and Kroner, Y. 2015. The space-time continuum: the effects of elevated CO₂ and temperature on trees and the importance of scaling. *Plant, Cell and Environment*. 38:991-1007

















- Way, D. A., & Oren, R. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree physiology*, 30(6):669-688.
- Way, D.A. and Yamori, W. 2014. Thermal acclimation of photosynthesis: on the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis research*. 119(1):89-100.
- Zhang, S. and Dang, QL. 2007. Interactive Effects of Soil Temperature and [CO₂] on Morphological and Biomass Traits in Seedlings of Four Boreal Tree Species. *Forest Science*. 53(3):453-460.

APPENDICES

Appendix Table 2.1. Recipe for Modified Melin-Norkran's Agar

Ingredient	Amount
Glucose	5g
Malt Extract	2g
Yeast Extract	1g
KH_2PO_4	0.5g
$(\text{NH}_4)_2\text{HPO}_4$	0.25g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.15g
CaCl_2	0.05g
NaCl	0.025g
FeCl_2	0.012g
Agar	15g
Distilled Water	1000mL

Appendix Table 2.2. Experimental Design

Treatment	Ambient CO2 and Temperature		Elevated CO2 and Temperature	
	No ECM	Yes ECM	No ECM	Yes ECM
Very Low Nutrient				
	Greenhouse 1		Greenhouse 3	
Low Nutrient				
Very Low Nutrient				
	Greenhouse 2		Greenhouse 4	
Low Nutrient				

Appendix Table 2.3. Fertilizer Chemical Formulation

Source of Nutrients	Low Nutrient Treatment (50 $\mu\text{mol/mol}$)	Very Low Nutrient Treatment (5 $\mu\text{mol/mol}$)
NH ₄	17.5	1.75
NO ₃	29	2.9
NH ₂	3.5	0.35
P ₂ O ₅	17.5	1.75
K ₂ O	47.5	4.75
B	0.075	0.0075
Cu	0.15	0.015
Fe	1	0.1
Mn	0.15	0.015
Mo	0.0125	0.00125
Zn	0.15	0.015
MgO	2	0.2
Amount of Fertilizer per Litre	0.25 g	0.025 g
Litres of solution added to pot	0.25 L	0.25 L