INTERACTIVE EFFECTS OF PHOTOPERIOD, SOIL MOISTURE AND CARBON DIOXIDE ON ECOPHYSIOLOGICAL TRAITS OF YELLOW BIRCH (*BETULA ALLEGHANIENSIS*)

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Abstract

Climate change is expected to drive tree migration, a phenomenon of significant ecological importance. The projected northward migration of boreal and temperate trees by 10°N in the next century, due to increasing atmospheric carbon dioxide [(CO_2)] concentration, will likely expose them to new set of environmental conditions. These conditions, such as photoperiod regime and soil moisture availability, will likely influence eco-physiological traits in trees. The ability of these migrating trees to acclimate to these new conditions will be essential in determining the limit and success of their migration. In this study, I investigated the interactive effects of (1) elevated carbon dioxide and photoperiod and (2) elevated carbon dioxide and soil moisture regime on the eco-physiological response of yellow birch (*Betula alleghaniensis*) seedlings, research that holds significant implications for our understanding of tree species migration and climate change.

Seedlings were exposed to factorial combination of two levels of [CO_2] (400 vs. 1000 μmol mol^{-1}) and two soil moisture regimes (well-watered (WW) vs. drought stress (DS)), and factorial combinations of two levels of CO_2 and three photoperiods (45° (seed origin) 50° and 55°N latitudes)). In the first set of experiments, we observed that elevated carbon dioxide and longer photoperiod significantly decreased electron transport rate (J_{max}) and ratio of electron transport rate to carboxylation rate (J_{max}/Vc_{max}). Total leaf area (TLA), specific leaf area (SLA), leaf dry mass (LEAFDM) and total seedling dry mass (TSDM) were significantly increased under elevated carbon dioxide and longer photoperiod. Furthermore, the phenological study of yellow birch revealed that the timing of bud set and leaf senescence was delayed at (P_{50}) and advanced at the longest photoperiod of 10°N (P_{55}). Yellow birch might not tolerate freezing temperatures when exposed to (-45°C). This could reduce the cold hardiness performance of yellow birch in response to a changing climate. These findings open new avenues for future research in understanding the complex interplay between climate change, tree physiology, and species migration.

In the second experiment, a significant interaction effect was observed between soil moisture and carbon dioxide on height growth and specific leaf area (SLA). Drought stress significantly decreased tree height and *SLA* under elevated carbon dioxide. Also, drought stress and elevated carbon dioxide significantly increased root dry mass (ROOTDM), root mass ratio (RMR), maximum electron transport rate (J_{max}) and ratio of electron transport rate to carboxylation rate (J_{max}/Vc_{max}). These findings underscore the potential implications of climate change on tree species survival, advocating immediate action to mitigate its effects.

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Chapter 1: General Introduction

Since the 18th century, industrialization has significantly increased greenhouse gas concentrations in the atmosphere (IPCC, 2014). Climate change, primarily caused by rising greenhouse gas emissions, significantly impacts the environment. It leads to altered precipitation patterns, increasing average temperatures, and more frequent extreme weather events, such as drought (Masson-Delmotte et al., 2021; Steffen et al., 2015). As a result, atmospheric carbon dioxide concentrations have increased significantly compared to levels before the industrial era (Friedlingstein et al., 2022; Masson-Delmotte et al., 2021). By the end of the 21st century, concentrations of atmospheric carbon dioxide are expected to reach approximately 1000 μmol mol^{-1} under the Representative Concentration Pathway (RCP) 8.5 scenario (Allan et al., 2023; Masson-Delmotte et al., 2021; Meinshausen et al., 2011; Riahi et al., 2017; Van Vuuren et al., 2011). In response to climate change, many tree species have developed ways to acclimate to the local environment to facilitate their growth (Adger et al., 2005; Kremer et al., 2012; Saxe et al., 2001). However, in North America, trees are expected to show increased sensitivity to global climate change and migrate northward (Gray & Hamann, 2013; Iverson & McKenzie, 2013; Lafleur et al., 2010; McKenney et al., 2007). As trees migrate, they may encounter various environmental constraints, including shifts in photoperiod regime and soil moisture availability, which can influence their morphological, physiological, and phenological traits (Chuine, 2010; Kramer et al., 2000; Vitasse et al., 2021; Way & Montgomery, 2015). Understanding how trees respond to environmental factors in their new environment is crucial for predicting their future distribution, especially under increasing atmospheric carbon dioxide concentration.

Significant research has focused on the physiological and morphological responses of trees to elevated carbon dioxide concentrations (EC) (Cao et al., 2008; Haworth et al., 2013; Leakey et al., 2009; Norby et al., 2003; Pritchard et al., 1999). One of the most consistent effects of elevated carbon dioxide (EC) on trees is an increase in the rate of photosynthesis, provided there are no other limiting environmental factors (Ainsworth & Long, 2005; Leakey et al., 2009; Norby et al., 2003; Way & Montgomery, 2015). Furthermore, carbon dioxide concentrations play a crucial role in controlling the opening of stomatal pores (Harrison et al., 2020; Lawson, 2009; Mansfield & Freer-Smith, 1984; Medlyn et al., 2001), which facilitates gas exchange between trees and their environment (Haworth et al., 2011; Jarvis et al., 1999; Wang et al., 2015). Therefore, trees regulate the degree of stomatal opening to optimize photosynthesis while minimizing water loss (Ainsworth & Rogers, 2007; Harrison et al., 2020; Jarvis et al., 1999). In C_3 plants, EC increases growth rate (Danyagri & Dang, 2014; Wang et al., 2015), reduce stomatal conductance (Ainsworth & Long, 2021; Warren et al., 2011a), improve water use efficiency (Ainsworth & Long, 2005, 2021; Bunce, 2004; Flexas et al., 2013), and reduce transpiration rate (Allen Jr, 2000; Jarvis et al., 1999; Reddy et al., 1995). EC increased total leaf area (Ainsworth & Long, 2005, 2021; Rogers et al., 2017) and leaf weight (Ainsworth & Long, 2005; Poorter et al., 2009; Rogers et al., 2017). Root biomass, root length and root branching increased in response to elevated carbon dioxide (Beidler et al., 2015; Mackay et al., 2020; Norby et al., 2024; Rogers et al., 2017). While numerous studies have explored how elevated carbon dioxide enhances growth and physiological traits in trees, their responses to EC are complex and influenced by various environmental factors (Ainsworth & Long, 2021; Norby et al., 1999), such as photoperiod (Newaz et al., 2017; Tedla et al., 2019) and water availability (Berg & Sheffield, 2018; Inoue et al., 2020b, 2020a).

Photoperiod is an environmental signal that regulates growth and physiological responses, in trees (Chuine, 2010; Way & Montgomery, 2015). The ability to respond to photoperiod allow trees to anticipate environmental changes that typically occur at the same time each year and at specific latitudes, regardless of climate variations (Chuine, 2010; Franks et al., 2007; Thomas & Vince-Prue, 1997; Way & Montgomery, 2015). As climate change is expected to shift the climate envelopes of trees in boreal and temperate forests northward (Iverson & McKenzie, 2013; Pearson & Dawson, 2003), the growing season at higher latitudes may lengthen, potentially allowing trees to remain photosynthetically active for a longer period of time (Gunderson et al., 2012; Vitasse et al., 2009). For example, previous studies have shown that longer photoperiods enhance photosynthesis in trees (Adams & Langton, 2005; Bauerle et al., 2012; Wang et al., 1997) and increase the overall transpiration rate when water availability and nutrients are not limiting (Wang et al., 1997; Zhao et al., 2017). The combined effect of elevated carbon dioxide (EC) and longer photoperiods often increases photosynthetic capacity, leading to faster growth rates (Adams & Langton, 2005; Allen Jr, 1994; Inoue et al., 2020a; Tedla et al., 2021) increased leaf biomass, leaf size, and leaf area (Adams & Langton, 2005; Inoue et al., 2020a; Tedla et al., 2021; Zhao et al., 2017), as well as enhanced root growth (Inoue et al., 2020a; Tedla et al., 2019). In addition, this synergistic effect could increase tree growth (Newaz et al., 2016) by increasing water use efficiency (Zhao et al., 2017). However, photosynthetic downregulation under elevated carbon dioxide (EC) could occur due to decreased Rubisco carboxylation (Vc_{max}) and insufficient nitrogen supply (Jach & Ceulemans, 2000; Medlyn et al., 1999). When tree species move across different photoperiod regimes, it is important to understand their capacity to respond to the prolonged process of carbon fixation (Chuine, 2010; Vitasse et al., 2009; Way & Montgomery, 2015). Phenological events in boreal and temperate forests, such as growth cessation, bud set, leaf senescence, and leaf shedding, are also controlled by photoperiod (Gunderson et al., 2012; Norby et al., 2003; Vitasse et al., 2009, 2021).

Phenology generally refers to the timing of seasonal events in trees, such as spring leaf out, bud set, and leaf senescence (Cleland et al., 2007; Gunderson et al., 2012; Menzel et al., 2006; Piao et al., 2019). For deciduous temperate tree species, the timing of phenological events affects annual growth and competitive abilities (Chuine, 2010; Kramer et al., 2000; Vitasse et al., 2009). Consequently, phenology impacts tree fitness and distribution, especially in North America (Chen et al., 2011; Chuine, 2010; Parmesan, 2007). For over twenty years, there has been renewed interest in the overall effects of phenological shifts on forest ecosystems due to climate change, which led to new insights in interpreting environmental factors driving phenology (Norby et al., 2003; Piao et al., 2019; Way & Montgomery, 2015). The combined effects of elevated carbon dioxide and longer photoperiods on spring and autumn phenology have been less explored than their individual impacts (Vitasse et al., 2010, 2011; Way & Montgomery. Existing research indicates that these factors can interact in complex ways, depending on species, environmental conditions, and physiological and morphological traits (Newaz et al., 2016; Tedla et al., 2020; Way & Montgomery, 2015). Elevated carbon dioxide and longer photoperiods could significantly influence tree phenology by altering the timing and duration of various growth stages, such as bud burst, growth cessation, and leaf senescence (Basler & Körner, 2012, 2014; Tedla et al., 2020). Photoperiod regulates spring phenology in many temperate and boreal trees by signalling the end of winter dormancy (Hamilton et al., 2016; Maurya & Bhalerao, 2017). Experimental studies from Partanen et al. (1998), Heide 1993a, 1993b, Myking and Heide (1995) indicate that the timing of budburst in temperate and boreal trees is governed by a complex interplay among winter chilling, spring forcing temperatures, and photoperiod. Winter chilling is required to release buds from endodormancy and establish full growth competence (Hänninen et al., 1990a; Hänninen, 1995), after which thermal forcing initiates the ontogenetic processes leading to budburst. Under conditions where chilling is sufficient, photoperiod primarily serves to modulate the seasonal progression, helping to synchronize bud development with favourable environmental conditions. Conversely, other studies on *Fagus sylvatica* (Heide, 1993a, 1993b), *Pinus sylvestris* L. (Jensen & Gatherum, 1965), Betula *Pendula* Roth and *Betula pubescens* have demonstrated that, under suboptimal chilling, longer daylengths can partially compensate for chilling deficits by advancing the timing of budburst (Heide, 1993, 1993b, Myking and Heide, 1995).

Although elevated carbon dioxide has a limited direct effect on budburst, it may influence carbohydrate reserves and water use efficiency, potentially indirectly altering responses to photoperiod (Jach et al., 2001; Nord & Lynch, 2009; Tedla et al., 2020). Trees that strongly rely on photoperiod cues may be less sensitive to elevated carbon dioxide regarding budburst timing (Hänninen, 1995, 2016; Montgomery et al., 2020). However, tree species with weaker photoperiod sensitivity could experience minor phenological shifts due to CO_2-driven physiological changes (Heide, 2008). In contrast, a decreasing photoperiod is the primary signal for leaf senescence and abscission, surpassing the effects of temperature and EC (Estiarte & Peñuelas, 2015; Lang et al., 2019). Research suggests that the senescence-delaying effects of elevated carbon dioxide are limited by photoperiod, as trees prioritize photoperiodic cues for seasonal transitions (Way & Montgomery, 2015). For species with flexible photoperiodic responses, elevated CO_2 could lengthen the growing season by slightly delaying senescence, particularly under extended photoperiods (Li et al., 2000; Taylor et al., 2008; Warren et al., 2011b). However, the interactive effect of EC and longer photoperiod on tree phenology in the context of tree migration is unknown. Considering the impact of photoperiod on a tree's physiological processes, the duration of the photoperiod serves as a crucial signal for acclimation to low temperatures associated with seasonal changes. Developing a high degree of cold hardiness is critical for trees to survive severe winter conditions (Kalberer et al., 2006; Lorenzetti et al., 1971; Tedla et al., 2020; Vitasse et al., 2009; Wisniewski et al., 2014). A shorter photoperiod during winter and a reduced dormancy period may influence the development of cold hardiness and affect tree performance in the following growing season (Vitasse et al., 2009; Wu et al., 2013). Understanding how photoperiod interacts with EC to influence phenological events is essential for predicting future patterns of tree migration and growth under climate change. Empirical studies quantifying hardwood species are limited, although they are consistent in predicting the northward expansion of these species under expected climate change (Boisvert-Marsh, 2021; Gray & Hamann, 2013; Lafleur et al., 2010; McKenney et al., 2007). Going by the predicted changes in precipitation patterns, global climate change will undoubtedly alter soil moisture, thereby impacting the growth of temperate and boreal trees (Berg & Sheffield, 2018; Price et al., 2013; Saxe et al., 2001).

As the climate changes, water availability in trees of many Canada's forest regions is expected to decrease (Bonan, 2008; Gauthier et al., 2015; Price et al., 2013), as shown by a climatic moisture index that considers mean annual precipitation and potential evapotranspiration (Hogg, 1997; Zhang et al., 2000). Reduced water availability (drought) is recognized as the most significant abiotic factor limiting tree growth and development (Ahluwalia et al., 2021; Allen et al., 2010; Berg & Sheffield, 2018; Buras et al., 2020). Under drought stress, reduced water availability is the primary driver of physiological changes that lead to stomatal closure (Chaves, 1991; Chaves et al., 2003; Flexas et al., 2013; Martin-StPaul et al., 2017), which reduces stomatal conductance and limits CO_2 uptake, thereby affecting photosynthesis (Brodribb & Holbrook, 2003; Chaves et al., 2003; Flexas et al., 2006). Drought reduces leaf growth and leaf area (Anyia & Herzog, 2004; Liu & Stützel, 2004; Meier & Leuschner, 2008), limiting the photosynthetic capacity in trees (Chaves et al., 2003; Cornic & Massacci, 1996; Flexas et al., 2006; Siddique et al., 2016). An important adaptive response of trees to drought is increased biomass allocation to roots (Brunner et al., 2015; Mackay et al., 2020; Mohammadi Alagoz et al., 2023; Walters et al., 2023), which enhances water absorption (Nejad, 2011; Poorter et al., 2012). Drought also limit trees' response to other factors, such as carbon dioxide (Ainsworth & Long, 2005, 2021; Leakey et al., 2009), with elevated carbon dioxide (EC) known to significantly increase photosynthetic rates (Ainsworth & Long, 2021; Avila et al., 2020; Leakey et al., 2009). As increased water use efficiency (WUE) under elevated CO_2 enhances tree drought tolerance (Ainsworth & Long, 2021; Flexas et al., 2013; Keenan et al., 2013; Zhou et al., 2014), frequent drought events could still have severe impact on forest productivity (Adams et al., 2017; Allen et al., 2010; Choat et al., 2018; Hartmann et al., 2018; McDowell et al., 2022). The impact of drought on photosynthetic activity and tree growth under increasing carbon dioxide remains uncertain.

Yellow birch (*Betula alleghaniensis*) is one of Canada's valuable commercial tree species (Drobyshev et al., 2014; Erdmann, 1990; Gillis et al., 2005). It is an essential component of several major temperate forest types (Drobyshev et al., 2014; Gasser et al., 2010). In addition to its high-quality wood, yellow birch produces bioactive compounds with applications in various industries, including food, pharmaceuticals, and cosmetics (Clausen, 1973; Erdmann, 1990; Raymond & Dumais, 2023). Few studies have been conducted on yellow birch, particularly regarding height and growth cessation (Cheng, 2007; Gasser et al., 2010) and leaf morphology (Maloney et al., 2024; Rasheed & Delagrange, 2016). Yellow birch, a shade-intolerant species, may exhibit different physiological (Cheng, 2007; Erdmann, 1990), morphological (Gaucher et al., 2005; Raymond & Dumais, 2023), and phenological responses to environmental factors (Gasser et al., 2010; Morin et al., 2007), particularly when migrating northward (Drobyshev et al., 2014). However, it is important to understand the combined effects of elevated carbon dioxide and longer photoperiod on yellow birch in the context of northward migration. As trees encounter new environmental stressors, understanding their plastic responses is essential for predicting and managing native species like yellow birch in the face of climate change. Phenotypic plasticity is recognized as a key mechanism enabling trees to adjust to environmental variability. The extent to which plasticity supports survival in changing conditions remains largely uncertain. For example, yellow birch can adjust its photosynthetic machinery during seasonal transitions to enhance its efficiency in response to varying light and temperature conditions (Way et al., 2015). Sugar maple has developed traits like frost hardiness and shade tolerance, enabling it to thrive in northern temperate forests (Goldman et al., 1990). On the contrary, bur oak has adapted to drier environments by growing deep taproots and thick leaves (Abrams, 1990). Additionally, white oaks respond to drought stress by quickly closing their stomata to minimize water loss through transpiration (McDowell et al., 2008). The interplay of these mechanisms could determine how North American deciduous trees, such as yellow birch, cope with the challenges posed by climate change (Way & Montgomery, 2015; Richard et al., 2010).

This research aims to examine yellow birch's physiological, phenological, and morphological responses to the interactions between carbon dioxide concentration (CO_2), photoperiod regime, and soil moisture in the context of climate change-induced northward migration. There are three specific objectives:

- Objective 1: The interactive effects of elevated carbon dioxide concentration and photoperiod on yellow birch's physiological and morphological traits (Chapter 2).
- Objective 2: The interactive effects of elevated carbon dioxide concentration and photoperiod regime on the phenological response of yellow birch to autumn phenology. (Chapter 3).

- Objective 3: Examined yellow birch's physiological and morphological responses to elevated carbon dioxide concentration and drought (Chapter 4).

The following hypotheses were tested:

- Under elevated carbon dioxide concentration, photoperiod regime at higher latitude, will increase growth rate and biomass production and allocation to above-ground organs.
- Elevated carbon dioxide concentration and photoperiod regime at higher latitudes will increase the net photosynthetic rate, and this proportional increase might lead to photosynthetic downregulation.
- Elevated carbon dioxide and photoperiod regime at higher latitude will delay bud set and leaf senescence phenology because of the prolonged growing season. Since shorter days are the primary cue for trees to prepare for winter, elevated carbon dioxide and higher photoperiod regime will synergistically delay dormancy induction in yellow birch, which could reduce cold hardiness.
- Drought stress will limit the stimulation of photosynthetic activity, reduce tree growth and total biomass, while elevated carbon dioxide will ameliorate the effect of drought stress
- Elevated carbon dioxide and drought stress will induce more biomass allocation to the root, increase the root-to-shoot ratio.

1.1 References

Adams, S., & Langton, F. (2005). Photoperiod and plant growth: A review. *The Journal of Horticultural Science and Biotechnology*, *80*(1), 2–10.

Adger, W. N., Arnell, N. W., & Tompkins, E. L. (2005). Successful adaptation to climate change across scales. *Global Environmental Change*, *15*(2), 77–86.

Ahluwalia, O., Singh, P. C., & Bhatia, R. (2021). A review on drought stress in plants: Implications, mitigation and the role of plant growth-promoting rhizobacteria. *Resources, Environment and Sustainability*, *5*, 100032. Ainsworth, E. A., & Long, S. P. (2021). 30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation? *Global Change Biology*, *27*(1), 27–49.

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist*, *165*(2), 351–372.

Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. *Plant, Cell & Environment*, *30*(3), 258–270.

Allan, R. P., Arias, P. A., Berger, S., Canadell, J. G., Cassou, C., Chen, D., Cherchi, A., Connors, S. L., Coppola, E., & Cruz, F. A. (2023). Intergovernmental panel on climate change (IPCC). Summary for policymakers. In *Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change* (pp. 3–32). Cambridge University Press.

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D. D., & Hogg, E. T. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, *259*(4), 660–684.

Allen Jr, L. (2000). Evapotranspiration responses of plants and crops to carbon dioxide and temperature. *Journal of Crop Production*, *2*(2), 37–70.

Allen Jr, L. H. (1994). Carbon dioxide increase: Direct impacts on crops and indirect effects mediated through anticipated climatic changes. *Physiology and Determination of Crop Yield*, 425–459.

Anyia, A., & Herzog, H. (2004). Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *European Journal of Agronomy*, *20*(4), 327–339.

Avila, R. T., de Almeida, W. L., Costa, L. C., Machado, K. L., Barbosa, M. L., de Souza, R. P., Martino, P. B., Juárez, M. A., Marçal, D. M., & Martins, S. C. (2020). Elevated air [CO_2] improves photosynthetic performance and alters biomass accumulation and partitioning in drought-stressed coffee plants. *Environmental and Experimental Botany*, *177*, 104137.

Basler, D., & Körner, C. (2014). Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology*, *34*(4), 377–388.

Basler, D., & Körner, C. (2012). Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology*, *165*, 73–81.

Bauerle, W. L., Oren, R., Way, D. A., Qian, S. S., Stoy, P. C., Thornton, P. E., Bowden, J. D., Hoffman, F. M., & Reynolds, R. F. (2012). Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. *Proceedings of the National Academy of Sciences*, *109*(22), 8612–8617.

Beidler, K. V., Taylor, B. N., Strand, A. E., Cooper, E. R., Schönholz, M., & Pritchard, S. G. (2015). Changes in root architecture under elevated concentrations of CO_2 and nitrogen reflect alternate soil exploration strategies. *New Phytologist*, *205*(3), 1153–1163.

Berg, A., & Sheffield, J. (2018). Climate change and drought: The soil moisture perspective. *Current Climate Change Reports*, *4*, 180–191.

Boisvert-Marsh, L. (2021). *Patterns and processes affecting northward migration of tree species in a changing climate*.

Bonan, G. B. (2008). Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. *Science*, *320*(5882), 1444–1449.

Brodribb, T. J., & Holbrook, N. M. (2003). Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology*, *132*(4), 2166–2173.

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in Plant Science*, *6*, 547.

Bunce, J. A. (2004). Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. *Oecologia*, *140*, 1–10.

Buras, A., Rammig, A., & Zang, C. S. (2020). Quantifying impacts of the 2018 drought on European ecosystems in comparison to 2003. *Biogeosciences*, *17*(6), 1655–1672.

Cao, B., Dang, Q.-L., Yü, X., & Zhang, S. (2008). Effects of [CO_2] and nitrogen on morphological and biomass traits of white birch (Betula papyrifera) seedlings. *Forest Ecology and Management*, *254*(2), 217–224.

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought—From genes to the whole plant. *Functional Plant Biology*, *30*(3), 239–264.

Chaves, M. (1991). Effects of water deficits on carbon assimilation. *Journal of Experimental Botany*, *42*(1), 1–16.

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026.

Cheng, S. (2007). Elevated CO_2 changes the moderate shade tolerance of yellow birch seedlings. *Journal of Environmental Sciences*, *19*(4), 502–507.

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, *558*(7711), 531–539.

Chuine, I. (2010). Why does phenology drive species distribution? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3149–3160.

Clausen, K. E. (1973). *Genetics of yellow birch* (Vol. 18). US Department of Agriculture.

Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A., & Schwartz, M. D. (2007). Shifting plant phenology in response to global change. *Trends in Ecology & Evolution*, *22*(7), 357–365.

Cornic, G., & Massacci, A. (1996). Leaf photosynthesis under drought stress. In *Photosynthesis and the Environment* (pp. 347–366). Springer.

Danyagri, G., & Dang, Q.-L. (2014). Effects of elevated carbon dioxide concentration and soil temperature on the growth and biomass responses of mountain maple (Acer spicatum) seedlings to light availability. *Journal of Plant Ecology*, *7*(6), 535–543.

Drobyshev, I., Guitard, M.-A., Asselin, H., Genries, A., & Bergeron, Y. (2014). Environmental controls of the northern distribution limit of yellow birch in eastern Canada. *Canadian Journal of Forest Research*, *44*(7), 720–731.

Erdmann, G. (1990). Betula alleghaniensis Britton. *Silvics of North America*, *2*, 133–147.

Estiarte, M., & Peñuelas, J. (2015). Alteration of the phenology of leaf senescence and fall in winter deciduous species by climate change: Effects on nutrient proficiency. *Global Change Biology*, *21*(3), 1005–1017.

Flexas, J., Bota, J., Galmés, J., Medrano, H., & Ribas-Carbó, M. (2006). Keeping a positive carbon balance under adverse conditions: Responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, *127*(3), 343–352.

Flexas, J., Niinemets, Ü., Gallé, A., Barbour, M. M., Centritto, M., Diaz-Espejo, A., Douthe, C., Galmés, J., Ribas-Carbo, M., & Rodriguez, P. L. (2013). Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynthesis Research*, *117*, 45–59.

Franks, S. J., Sim, S., & Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, *104*(4), 1278–1282.

Friedlingstein, P., O'sullivan, M., Jones, M. W., Andrew, R. M., Gregor, L., Hauck, J., Le Quéré, C., Luijkx, I. T., Olsen, A., & Peters, G. P. (2022). Global carbon budget 2022. *Earth System Science Data*, *14*(11), 4811–4900.

Gasser, D., Messier, C., Beaudet, M., & Lechowicz, M. J. (2010). Sugar maple and yellow birch regeneration in response to canopy opening, liming and vegetation control in a temperate deciduous forest of Quebec. *Forest Ecology and Management*, *259*(10), 2006–2014.

Gaucher, C., Gougeon, S., Mauffette, Y., & Messier, C. (2005). Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. *Tree Physiology*, *25*(1), 93–100. https://doi.org/10.1093/treephys/25.1.93

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A. Z., & Schepaschenko, D. G. (2015). Boreal forest health and global change. *Science*, *349*(6250), 819–822.

Gillis, M. D., Omule, A., & Brierley, T. (2005). Monitoring Canada’s forests: The national forest inventory. *The Forestry Chronicle*, *81*(2), 214–221.

Gratani, L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in botany*, *2014*(1), 208747.

Gray, L. K., & Hamann, A. (2013). Tracking suitable habitat for tree populations under climate change in western North America. *Climatic Change*, *117*(1), 289–303.

Gunderson, C. A., Edwards, N. T., Walker, A. V., O’Hara, K. H., Campion, C. M., & Hanson, P. J. (2012). Forest phenology and a warmer climate–growing season extension in relation to climatic provenance. *Global Change Biology*, *18*(6), 2008–2025.

Hamilton, J. A., El Kayal, W., Hart, A. T., Runcie, D. E., Arango-Velez, A., & Cooke, J. E. (2016). The joint influence of photoperiod and temperature during growth cessation and development of dormancy in white spruce (Picea glauca). *Tree Physiology*, *36*(11), 1432–1448.

Hänninen, H. (2016). Boreal and temperate trees in a changing climate. *Biometeorology. Springer, Dordrecht, Netherlands*.

Hänninen, H. (1995). Effects of climatic change on trees from cool and temperate regions: An ecophysiological approach to modelling of bud burst phenology. *Canadian Journal of Botany*, *73*(2), 183–199.

Hänninen, H., Häkkinen, R., Hari, P., & Koski, V. (1990). Timing of growth cessation in relation to climatic adaptation of northern woody plants. *Tree Physiology*, *6*(1), 29-39.

Harrison, E. L., Arce Cubas, L., Gray, J. E., & Hepworth, C. (2020). The influence of stomatal morphology and distribution on photosynthetic gas exchange. *The Plant Journal*, *101*(4), 768–779.

Hartmann, H., Moura, C. F., Anderegg, W. R., Ruehr, N. K., Salmon, Y., Allen, C. D., Arndt, S. K., Breshears, D. D., Davi, H., & Galbraith, D. (2018). Research frontiers for improving our understanding of drought-induced tree and forest mortality. *New Phytologist*, *218*(1), 15–28.

Haworth, M., Elliott-Kingston, C., & McElwain, J. C. (2011). Stomatal control as a driver of plant evolution. *Journal of Experimental Botany*, *62*(8), 2419–2423.

Haworth, M., Elliott-Kingston, C., & McElwain, J. C. (2013). Co-ordination of physiological and morphological responses of stomata to elevated [CO_2] in vascular plants. *Oecologia*, *171*, 71–82.

Heide, O. M. (1993a). Dormancy release in beech buds (Fagus sylvatica) requires both chilling and long days. *Physiologia Plantarum*, *89*(1), 187-191.

Heide, O. M. (1993b). Daylength and thermal time responses of budburst during dormancy release in some northern deciduous trees. *Physiologia plantarum*, *88*(4), 531-540.

Hogg, E. H. (1997). Temporal scaling of moisture and the forest-grassland boundary in western Canada. *Agricultural and Forest Meteorology*, *84*(1–2), 115–122.

IPCC. (2014). Climate change 2014 synthesis report. *IPCC: Geneva, Szwitzerland*, 1059–1072.

Inoue, S., Dang, Q. L., Man, R., & Tedla, B. (2020a). Photoperiod and CO2 elevation influence morphological and physiological responses to drought in trembling aspen: implications for climate change-induced migration. *Tree Physiology*, *40*(7), 917-927.

Inoue, S., Dang, Q.-L., Man, R., & Tedla, B. (2020b). Photoperiod, [CO_2] and soil moisture interactively affect phenology in trembling aspen: Implications to climate change-induced migration. *Environmental and Experimental Botany*, *180*, 104269.

Iverson, L. R., & McKenzie, D. (2013). Tree-species range shifts in a changing climate: Detecting, modeling, assisting. *Landscape Ecology*, *28*, 879–889.

Jach, M., Ceulemans, R., & Murray, M. (2001). Impacts of greenhouse gases on the phenology of forest trees. *The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems. CAB International, Wallingford*, 193–235.

Jach, M., & Ceulemans, R. (2000). Effects of season, needle age and elevated atmospheric CO_2 on photosynthesis in Scots pine (Pinus sylvestris). *Tree Physiology*, *20*(3), 145–157.

Jarvis, A., Mansfield, T., & Davies, W. J. (1999). Stomatal behaviour, photosynthesis and transpiration under rising CO_2. *Plant, Cell & Environment*, *22*(6), 639–648.

Jensen, K. F., & Gatherum, G. E. (1965). Effects of temperature, photoperiod, and provenance on growth and development of Scotch pine seedlings. *Forest Science*, *11*(2), 189-199.

Kalberer, S. R., Wisniewski, M., & Arora, R. (2006). Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts. *Plant Science*, *171*(1), 3–16.

Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., & Richardson, A. D. (2013). Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*, *499*(7458), 324–327.

Kramer, K., Leinonen, I., & Loustau, D. (2000). The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: An overview. *International Journal of Biometeorology*, *44*, 67–75.

Kremer, A., Ronce, O., Robledo-Arnuncio, J. J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J. R., Gomulkiewicz, R., Klein, E. K., & Ritland, K. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*, *15*(4), 378–392.

Lafleur, B., Pare, D., Munson, A. D., & Bergeron, Y. (2010). Response of northeastern North American forests to climate change: Will soil conditions constrain tree species migration? *Environmental Reviews*, *18*(NA), 279–289.

Lang, W., Chen, X., Qian, S., Liu, G., & Piao, S. (2019). A new process-based model for predicting autumn phenology: How is leaf senescence controlled by photoperiod and temperature coupling? *Agricultural and Forest Meteorology*, *268*, 124–135.

Lawson, T. (2009). Guard cell photosynthesis and stomatal function. *New Phytologist*, *181*(1).

Leakey, A. D., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., & Ort, D. R. (2009). Elevated CO_2 effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. *Journal of Experimental Botany*, *60*(10), 2859–2876.

Li, J., Dijkstra, P., Hymus, G., Wheeler, R., Piastuch, W., Hinkle, C., & Drake, B. (2000). Leaf senescence of Quercus myrtifolia as affected by long-term CO_2 enrichment in its native environment. *Global Change Biology*, *6*(7), 727–733.

Liu, F., & Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (Amaranthus spp.) in response to drought stress. *Scientia Horticulturae*, *102*(1), 15–27.

Lorenzetti, F., Tyler, B., Cooper, J., & Breese, E. (1971). Cold tolerance and winter hardiness in Lolium perenne: I. Development of screening techniques for cold tolerance and survey of geographical variation. *The Journal of Agricultural Science*, *76*(2), 199–209.

Mackay, D. S., Savoy, P. R., Grossiord, C., Tai, X., Pleban, J. R., Wang, D. R., McDowell, N. G., Adams, H. D., & Sperry, J. S. (2020). Conifers depend on established roots during drought: Results from a coupled model of carbon allocation and hydraulics. *New Phytologist*, *225*(2), 679–692.

Maloney, A., Dang, Q., Godakanda, P., & Thomson, A. (2024). Genetic variation in growth and leaf traits associated with local adaptation to climate in yellow birch (Betula alleghaniensis Britton). *Botany*, *102*(4), 198–210.

Mansfield, T., & Freer-Smith, P. (1984). The role of stomata in resistance mechanisms. *Gaseous Air Pollutants and Plant Metabolism*, 131–146.

Martin-StPaul, N., Delzon, S., & Cochard, H. (2017). Plant resistance to drought depends on timely stomatal closure. *Ecology Letters*, *20*(11), 1437–1447.

Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., & Gomis, M. (2021). Climate change 2021: The physical science basis. *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, *2*(1), 2391.

Maurya, J. P., & Bhalerao, R. P. (2017). Photoperiod-and temperature-mediated control of growth cessation and dormancy in trees: A molecular perspective. *Annals of Botany*, *120*(3), 351–360.

McKenney, D. W., Pedlar, J. H., Lawrence, K., Campbell, K., & Hutchinson, M. F. (2007). Potential impacts of climate change on the distribution of North American trees. *BioScience*, *57*(11), 939–948.

Medlyn, B. E., Badeck, F., De Pury, D., Barton, C., Broadmeadow, M., Ceulemans, R., De Angelis, P., Forstreuter, M., Jach, M., & Kellomäki, S. (1999). Effects of elevated [CO_2] on photosynthesis in European forest species: A meta-analysis of model parameters. *Plant, Cell & Environment*, *22*(12), 1475–1495.

Medlyn, B. E., Barton, C., Broadmeadow, M., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S., Kellomäki, S., & Laitat, E. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: A synthesis. *New Phytologist*, *149*(2), 247–264.

Meier, I. C., & Leuschner, C. (2008). Leaf size and leaf area index in Fagus sylvatica forests: Competing effects of precipitation, temperature, and nitrogen availability. *Ecosystems*, *11*, 655–669.

Meinshausen, M., Smith, S. J., Calvin, K., Daniel, J. S., Kainuma, M. L., Lamarque, J.-F., Matsumoto, K., Montzka, S. A., Raper, S. C., & Riahi, K. (2011). The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Climatic Change*, *109*, 213–241.

Menzel, A., Sparks, T. H., Estrella, N., & Roy, D. (2006). Altered geographic and temporal variability in phenology in response to climate change. *Global Ecology and Biogeography*, *15*(5), 498–504.

Mohammadi Alagoz, S., Zahra, N., Hajiaghaei Kamrani, M., Asgari Lajayer, B., Nobaharan, K., Astatkie, T., Siddique, K. H., & Farooq, M. (2023). Role of root hydraulics in plant drought tolerance. *Journal of Plant Growth Regulation*, *42*(10), 6228–6243.

Montgomery, R. A., Rice, K. E., Stefanski, A., Rich, R. L., & Reich, P. B. (2020). Phenological responses of temperate and boreal trees to warming depend on ambient spring temperatures, leaf habit, and geographic range. *Proceedings of the National Academy of Sciences*, *117*(19), 10397–10405.

Morin, X., Augspurger, C., & Chuine, I. (2007). Process-based modeling of species'distributions: what limits temperate tree species'range boundaries? *Ecology*, *88*(9), 2280–2291.

Mura, C., Charrier, G., Buttò, V., Delagrange, S., Surget-Groba, Y., Raymond, P., ... & Deslauriers, A. (2025). Local conditions have greater influence than provenance on sugar maple (Acer saccharum Marsh.) frost hardiness at its northern range limit. *Tree Physiology*, *45*(1), tpae167.

Myking, T., & Heide, O. M. (1995). Dormancy release and chilling requirement of buds of latitudinal ecotypes of Betula pendula and B. pubescens. *Tree physiology*, *15*(11), 697-704.

Newaz, S., Dang, Q., & Man, R. (2017). Eco-physiological potential of jack pine (Pinus banksiana) for assisted northward migration: Interactions among photoperiod, [CO_2] and moisture stress. *Nordic Journal of Botany*, *35*(6), 633–643.

Newaz, S., Dang, Q.-L., & Man, R. (2016). Morphological response of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod. *American Journal of Plant Sciences*, *7*(6), 879–893.

Norby, R. J., Loader, N. J., Mayoral, C., Ullah, S., Curioni, G., Smith, A. R., ... & MacKenzie, A. R. (2024). Enhanced woody biomass production in a mature temperate forest under elevated CO2. *Nature Climate Change*, *14*(9), 983-988.

Norby, R. J., Hartz-Rubin, J. S., & Verbrugge, M. J. (2003). Phenological responses in maple to experimental atmospheric warming and CO_2 enrichment. *Global Change Biology*, *9*(12), 1792–1801.

Norby, R. J., Wullschleger, S. D., Gunderson, C. A., Johnson, D. W., & Ceulemans, R. (1999). Tree responses to rising CO_2 in field experiments: Implications for the future forest. *Plant, Cell & Environment*, *22*(6), 683–714.

Nord, E. A., & Lynch, J. P. (2009). Plant phenology: A critical controller of soil resource acquisition. *Journal of Experimental Botany*, *60*(7), 1927–1937.

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, *13*(9), 1860–1872.

Partanen, J., Koski, V., & Hänninen, H. (1998). Effects of photoperiod and temperature on the timing of bud burst in Norway spruce (Picea abies). *Tree physiology*, *18*(12), 811-816.

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography*, *12*(5), 361–371.

Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., & Zhu, X. (2019). Plant phenology and global climate change: Current progresses and challenges. *Global Change Biology*, *25*(6), 1922–1940.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, *182*(3), 565–588.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30–50.

Price, D. T., Alfaro, R. I., Brown, K., Flannigan, M. D., Fleming, R. A., Hogg, E. H., Girardin, M. P., Lakusta, T., Johnston, M., & McKenney, D. W. (2013). Anticipating the consequences of climate change for Canada's boreal forest ecosystems. *Environmental Reviews*, *21*(4), 322–365.

Pritchard, S. G., Rogers, H. H., Prior, S. A., & Peterson, C. M. (1999). Elevated CO_2 and plant structure: A review. *Global Change Biology*, *5*(7), 807–837.

Rasheed, F., & Delagrange, S. (2016). Acclimation of Betula alleghaniensis Britton to moderate soil water deficit: Small morphological changes make for important consequences in crown display. *Tree Physiology*, *36*(11), 1320-1329.

Raymond, P., & Dumais, D. (2023). Ecological Silviculture for Yellow Birch–Conifer Mixedwoods in Eastern Canada. *Ecological Silvicultural Systems: Exemplary Models for Sustainable Forest Management*, 112.

Reddy, V., Reddy, K., & Hodges, H. (1995). Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpiration, and water-use efficiency. *Field Crops Research*, *41*(1), 13–23.

Riahi, K., Van Vuuren, D. P., Kriegler, E., Edmonds, J., O'neill, B. C., Fujimori, S., Bauer, N., Calvin, K., Dellink, R., & Fricko, O. (2017). The Shared Socioeconomic Pathways and their energy, land use, and greenhouse gas emissions implications: An overview. *Global Environmental Change*, *42*, 153–168.

Rogers, A., Medlyn, B. E., Dukes, J. S., Bonan, G., Von Caemmerer, S., Dietze, M. C., Kattge, J., Leakey, A. D., Mercado, L. M., & Niinemets, Ü. (2017). A roadmap for improving the representation of photosynthesis in Earth system models. *New Phytologist*, *213*(1), 22–42.

Saxe, H., Cannell, M. G., Johnsen, Ø., Ryan, M. G., & Vourlitis, G. (2001). Tree and forest functioning in response to global warming. *New Phytologist*, *149*(3), 369–399.

Siddique, Z., Jan, S., Imadi, S. R., Gul, A., & Ahmad, P. (2016). Drought stress and photosynthesis in plants. *Water Stress and Crop Plants: A Sustainable Approach*, *1*, 1–11.

Steffen, W., Richardson, K., Rockström, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., De Vries, W., & De Wit, C. A. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science*, *347*(6223), 1259855.

Taylor, G., Tallis, M. J., Giardina, C. P., Percy, K. E., Miglietta, F., Gupta, P. S., Gioli, B., Calfapietra, C., Gielen, B., & Kubiske, M. E. (2008). Future atmospheric CO_2 leads to delayed autumnal senescence. *Global Change Biology*, *14*(2), 264–275.

Tedla, B., Dang, Q. L., & Inoue, S. (2021). Longer photoperiods negate the CO_2 stimulation of photosynthesis in Betula papyrifera Marsh: Implications to climate change-induced migration. *Physiologia Plantarum*, *172*(1), 106-115.

Tedla, B., Dang, Q.-L., & Inoue, S. (2020). CO_2 elevation and photoperiods north of seed origin change autumn and spring phenology as well as cold hardiness in boreal white birch. *Frontiers in Plant Science*, *11*, 506.

Thomas, B., & Vince-Prue, D. (1997a). Daylength perception in short-day plants. *Photoperiodism in Plants*, 85–117.

Van Vuuren, D. P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G. C., Kram, T., Krey, V., & Lamarque, J.-F. (2011). The representative concentration pathways: An overview. *Climatic Change*, *109*, 5–31.

Vitasse, Y., Baumgarten, F., Zohner, C. M., Kaewthongrach, R., Fu, Y. H., Walde, M. G., & Moser, B. (2021). Impact of microclimatic conditions and resource availability on spring and autumn phenology of temperate tree seedlings. *New Phytologist*, *232*(2), 537–550.

Vitasse, Y., Bresson, C. C., Kremer, A., Michalet, R., & Delzon, S. (2010). Quantifying phenological plasticity to temperature in two temperate tree species. *Functional Ecology*, *24*(6), 1211–1218.

Vitasse, Y., Delzon, S., Dufrêne, E., Pontailler, J.-Y., Louvet, J.-M., Kremer, A., & Michalet, R. (2009). Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? *Agricultural and Forest Meteorology*, *149*(5), 735–744.

Vitasse, Y., Francois, C., Delpierre, N., Dufrêne, E., Kremer, A., Chuine, I., & Delzon, S. (2011). Assessing the effects of climate change on the phenology of European temperate trees. *Agricultural and Forest Meteorology*, *151*(7), 969–980.

Walters, M. B., Kunkle, J. M., Kobe, R. K., & Farinosi, E. J. (2023). Seedling drought responses governed by root traits, site-soil moisture regimes and overstory competition-facilitation. *Forest Ecology and Management*, *544*, 121159.

Wang, H., Xiao, W., Niu, Y., Chai, R., Jin, C., & Zhang, Y. (2015). Elevated carbon dioxide induces stomatal closure of Arabidopsis thaliana (L.) Heynh. Through an increased production of nitric oxide. *Journal of Plant Growth Regulation*, *34*, 372–380.

Wang, Z., Yuan, Z., & Quebedeaux, B. (1997). Photoperiod alters diurnal carbon partitioning into sorbitol and other carbohydrates in apple. *Functional Plant Biology*, *24*(5), 587–597.

Warren, J. M., Norby, R. J., & Wullschleger, S. D. (2011a). Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology*, *31*(2), 117–130.

Warren, J. M., Pötzelsberger, E., Wullschleger, S. D., Thornton, P. E., Hasenauer, H., & Norby, R. J. (2011b). Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO_2. *Ecohydrology*, *4*(2), 196–210.

Way, D. A., & Montgomery, R. A. (2015). Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment*, *38*(9), 1725–1736.

Way, D. A., & Oren, R. A. M. (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.

Wisniewski, M., Nassuth, A., Teulieres, C., Marque, C., Rowland, J., Cao, P. B., & Brown, A. (2014). Genomics of cold hardiness in woody plants. *Critical Reviews in Plant Sciences*, *33*(2–3), 92–124.

Wu, C., Gough, C. M., Chen, J. M., & Gonsamo, A. (2013). Evidence of autumn phenology control on annual net ecosystem productivity in two temperate deciduous forests. *Ecological Engineering*, *60*, 88–95.

Zhang, X., Vincent, L. A., Hogg, W., & Niitsoo, A. (2000). Temperature and precipitation trends in Canada during the 20th century. *Atmosphere-Ocean*, *38*(3), 395–429.

Zhao, Y., Wang, Z., Wei, H., Bao, Y., & Gu, P. (2017). Effect of prolonged photoperiod on morphology, biomass accumulation and nutrient utilization in post-transplant Taxus cuspidata seedlings. *Pakistan Journal of Botany*, *49*(4), 1285–1290.

Chapter 2: Interactive Effects of Elevated Carbon Dioxide and Photoperiod Regime at Higher Latitudes on the Physiology and Morphology of Yellow Birch

2.1 Abstract

Trees are expected to expand their range toward higher latitudes in response to climate change. But the success of this migration will depend on their ability to adapt to the new environmental conditions of their new locations. Understanding eco-physiological acclimation to varying photoperiod regimes under elevated carbon dioxide ([CO_2]) levels is crucial for predicting tree migration driven by climate change.

This study conducted a greenhouse experiment to evaluate the morphological and physiological responses of yellow birch (*Betula alleghaniensis*) seedlings to photoperiod regimes associated with three latitudes (45°N (seed origin), 50°N and 55°N) under both ambient and elevated [CO_2] (AC = 400; EC= 1000 mol mol^{-1}) for one growing season.

The findings revealed that the interaction of elevated carbon dioxide and longer photoperiod associated with 10°N of seed origin significantly decreased the electron transport rate (J_{max}) and the ratio of electron transport rate to rubisco carboxylation rate (J_{max}/Vc_{max}). Total leaf area (TLA), specific leaf area (SLA), leaf dry mass (LEAFDM) and total seedling dry mass (TSDM) were significantly increased under elevated carbon dioxide and photoperiod regime at higher latitudes.

In summary, this result showed that the growth and biomass of yellow birch seedlings will likely benefit from the longer photoperiod regime associated with migration. The result also suggests that climate change associated with an increase in atmospheric carbon dioxide and a longer photoperiod at higher latitude will likely lead to photosynthetic down regulation of yellow birch.

2.2 Introduction

In recent years, the impact of climate change on northern forests has become increasingly significant (IPCC, 2014; Masson-Delmotte et al., 2021). Human activities, including fuel combustion and land use changes, are raising atmospheric carbon dioxide levels ([CO_2]) and other greenhouse gases (Canadell et al., 2023; Friedlingstein et al., 2022; IPCC, 2014). This increase in greenhouse gas emissions impacts the earth's climate, and future projections indicate that global emissions may exceed the high greenhouse gas emission scenario (RCP) 8.5 (Friedlingstein et al., 2022; IPCC, 2014; Meinshausen et al., 2011). In response to climate change, global temperature is projected to increase by 1.5°C by the year 2100 based on RCP pathway projections (Masson-Delmotte et al., 2021), with changes in precipitation patterns. Natural or human-assisted migration may become an inevitable response for trees to adapt to climate change (Davis & Shaw, 2001; Parmesan, 2007), and fluctuation in precipitation patterns. Based on climate envelope predictions across North America, a northward shift of approximately 10°N latitude (about 1,100 km) is expected to occur before the year 2100 (Iverson & McKenzie, 2013; McKenney et al., 2007). Consequently, trees are expected to migrate to higher latitudes and face new environmental conditions, such as photoperiod regimes (Aitken et al., 2008). These environmental changes may significantly influence tree physiology and morphology (Canadell et al., 1999; Chaudhry & Sidhu, 2022; Gray & Brady, 2016). Therefore, we need to understand if these new environmental factors will influence the success of tree performance.

Rapid environmental changes driven by climate change could create conditions that outpace the natural migration abilities of tree species (Aitken et al., 2008; Davis & Shaw, 2001; Pearson & Dawson, 2003). As a result, trees may struggle to shift their ranges quickly enough to adjust to these changing conditions (Chen et al., 2011; Gray & Hamann, 2013; McKenney et al., 2007). This imbalance between the rapid pace of environmental change and the slower rate of tree migration is expected to constrain tree populations in tracking shifting climatic conditions, resulting in delayed or incomplete northward range shifts (Boisvert-Marsh, 2021; Kremer et al., 2012; Lafleur et al., 2010; Wang et al., 2019; Way & Montgomery, 2015b). Photoperiod is a consistent and reliable cue for annual phenological events, as it remains unchanged yearly (Basler & Körner, 2012; Way & Montgomery, 2015). Photoperiod is longer in summer and shorter in winter (Jackson, 2009). As climate change drives shifts in seasonal patterns, this can result in an extended growing season (Basler & Körner, 2014). Photoperiod regimes can influence tree growth and performance, particularly photoperiod-sensitive ones (Basler & Körner, 2014; Way & Montgomery, 2015a). However, climate envelope models do not consider photoperiod (Anderson et al., 2013). As trees migrate naturally or with human assistance, they may be exposed to substantial changes in factors associated with latitudes, such as photoperiod (Morin et al., 2007; Way & Montgomery, . Trees may likely be unable to acclimate to the photoperiod regimes at their new location.

Atmospheric carbon dioxide concentration is a key factor influencing tree's physiological processes (Ainsworth & Long, 2005, 2021; Norby et al., 2005). The increase in atmospheric (CO_2) promotes net photosynthesis (A_n) (Ainsworth & Long, 2021; Bowes, 1996; Reddy et al., 1995), changes in carbon allocation (Ainsworth & Long, 2021; Reddy et al., 2010; Saxe et al., 1998), and growth (Alonso et al., 2009). However, due to physiological and morphological modifications, trees may experience growth decline (Poorter et al., 2012; Vitasse et al., 2009). For example, photosynthetic downregulation could occur when species are exposed to elevated carbon dioxide concentration (EC) over a long period as trees acclimate to the new environment (Lambers et al., 2008, . The process of photosynthetic downregulation mostly occur to prevent accumulation of excess carbon in the leaves when the demand for carbon does not match with the increase in supply (Ainsworth & Long, 2005; Bauerle et al., 2012; Equiza et al., 2006; Keenan et al., 2023; Saxe et al., 1998). It has been suggested that an accumulation of excess carbon in the leaves may lead to a reduction in the levels of Rubisco (Krapp et al., 1993) and other photosynthetic enzymes (Matt et al., 2002; Stitt et al., 2010), reflecting an imbalance between photosynthate formation and utilization at EC (Rogers et al., 2017). EC reduces stomatal conductance in trees (Ainsworth & Rogers, 2007; Field et al., 1995; Haworth et al., 2011; Jarvis et al., 1999), and this reduction may restrict transpiration rate per unit leaf area (Jarvis et al., 1999; Reddy et al., 1995). Furthermore, the reduction in stomatal aperture in response to EC led to a trade-off between water conservation and photosynthetic carbon assimilation (Ainsworth & Rogers, 2007; Crawley, 2009; Pastore et al., 2019; Saban et al., 2019; Sperry et al., 2017). A positive effect of EC on water-use efficiency (WUE) has been widely acknowledged (Ainsworth & Long, 2021; Bunce, 2004; Keenan et al., 2013; Reddy et al., 1995). *WUE* refers to the ratio of carbon gained through photosynthesis to the water lost through transpiration. Since the growing season may be extended at higher latitudes, which would result in more time available for accumulation of non-structural carbohydrates for growth the following season (Gunderson et al., 2012; Parmesan, 2007; Way & Montgomery, 2015a), this could improve the tree fitness and survival (Ainsworth & Long, 2021; Long et al., 2006). Therefore, gaining a deeper understanding of the synergistic effect of photoperiod regime at higher latitude and elevated carbon dioxide on the physiology of deciduous trees is crucial in addressing tree response to climate change.

With the predicted shift in climate envelopes for North American species in response to climate change (Iverson & McKenzie, 2013; McKenney et al., 2007, 2011), morphological response may differ in trees (Aubin et al., 2016; Morin et al., 2007; Rasheed & Delagrange, 2016;

Zhu et al., 2021). EC concentrations could stimulate carbon fixation (Leakey et al., 2009; Norby et al., 2005) and increase tree growth (Bader et al., 2010; Jach & Ceulemans, 1999; Poorter et al., 2012; Rais et al., 2014). EC increases leaf area (Zhang et al., 2020b; Zhu et al., 2021), leaf size in deciduous trees (DeLucia & Thomas, 2000; Norby et al., 2005), and thicker leaves (de Ávila Silva et al., 2021; Maloney et al., 2024; Poorter et al., 2009), which supports the expansion of tree crowns. Trees may also display increased root biomass and improved root-to-shoot ratio under EC (Drake et al., 1997; Norby et al., 2005; Pritchard et al., 1999; Wang et al., 2020). Photoperiod, on the other hand, promotes shoot growth (Zhang et al., 2020b), enlarges tree leaves (Aubin et al., 2016; Zhu et al., 2021), and could indirectly influence root growth (Poorter et al., 2012; Wang et al., 2020). When there is a shift in the climate envelope, we need to know how the morphological traits of deciduous trees will respond to elevated carbon dioxide and longer photoperiods at higher latitudes.

Yellow Birch (*B. alleghaniensis* Britt.) is an ecologically and economically important deciduous tree species in the north temperate zone and mixed-forest regions (Delagrange et al., 2004, 2006). It adapts to various sites, primarily moist soils (Burns & Honkala, 1990; Clausen, 1973; Drobyshev et al., 2014). It is used for flooring, high-quality furniture, woodwork, and railway ties (Burns & Honkala, 1990; Raymond & Dumais, 2023). In recent years, there is increased interest in understanding how photoperiod affects yellow birch photosynthesis and growth as trees migrate northward due to climate change (Inoue et al., 2020a; Newaz et al., 2017; Tedla et al., 2019, 2021). However, it is still unclear how carbon dioxide and photoperiod regime at higher latitude may interact to affect photosynthesis, stomatal conductance, biomass production and allocation in many tree species. For example, Tedla et al. (2019) discovered that white birch growth, biomass and A_n were stimulated by two photoperiod regimes (52°N & 55°N) north of the seed origin, while the farthest photoperiod regime (58°N) did not stimulate growth or A_n. On the contrary, Inoue et al. (2020) reported increased A_n and growth in trembling aspen seedlings at the farthest latitude (58°N). Therefore, it is important to understand how yellow birch will respond to increased carbon dioxide with a longer photoperiod at higher latitudes. This study investigated the interactive effect of carbon dioxide and photoperiod on the physiological and morphological traits of yellow birch seedlings. Under three photoperiod regimes, I explored how elevated carbon dioxide could modify the growth and physiology of yellow birch in response to climate change. Given EC enhances photosynthetic rates, increases tree growth and biomass production, and that longer photoperiod may further stimulate the process of photosynthesis and tree growth, I tested the following hypothesis:

(i) Elevated carbon dioxide will enhance the photosynthetic rate with an increase in

Photoperiod regime. However, longer periods of photosynthetic activity could lead to downregulation at the farthest latitude.

(ii) Elevated carbon dioxide and a longer photoperiod at higher latitude will increase growth rate and enhance biomass production.

(iii) Elevated carbon dioxide and a longer photoperiod at higher latitude will allocate more biomass above ground since there are no limitations in other environmental factors.

2.3 Materials and Methods

2.3.1 Plant Materials

Seeds of yellow birch (*Betula alleghaniensis*) were sourced from the National Tree Seed Centre in Fredericton. The seeds were collected from ten individual trees in a forest situated at Prosser Brook, New Brunswick, Canada (45.80°N, 64.92°W). The current climate conditions of this site include an annual mean temperature of 5.6°C, a January mean temperature of -9.4°C, a July mean temperature of 19.3°C, an annual mean precipitation of 1077mm, and an average growing season length of 130 days (Environment Canada, 2023). Seeds were stored in a sealed glass jar at -4°C, followed by stratification for three weeks according to standard procedures (Bonner & Karrfalt, 2008) at Lakehead University's Ecophysiology laboratory. Seeds were sown (at the greenhouse, building, Lakehead University) in germination trays (50 cm x 25 cm x 5 cm) containing a 1:1 (v/v) mix of peat moss and vermiculite as the growing medium. Germination occurred under temperature conditions of 25/16°C (day/night) with a 16-hour photoperiod. High-pressure sodium lamps were used to supplement natural light when the ambient photosynthetically active radiation (PAR) fell below 500 μmol m^{-2} s^{-1}. Seedlings reaching an average height of 2 cm were transplanted into pots (15 cm high and 13 cm in diameter), which hold a 2:1 (v/v) combination of peat moss and vermiculite (ten days after germination was completed).

2.3.2 Experimental Design

The experiment was conducted in four greenhouses at Lakehead University, Thunder Bay, Ontario, Canada. A split-plot design was employed, with the atmospheric carbon dioxide (CO_2) treatment (AC: 400 μmol mol^{-1} vs EC: 1000 μmol mol^{-1}) as the main plot and three photoperiod regimes, corresponding to 45°N, 50°N, and 55°N latitudes as the sub-plot. The photoperiod treatments stand for the photoperiod at the seed origin (P_{45}), at 5° (P_{50}) and 10° (P_{55}) north of seed origin, respectively. The 10°N of seed origin simulates the predicted future northward shift in climate envelopes for temperate and boreal tree species (McKenney et al., 2007, 2011), and the 5°N represents the midpoint between the species' current and predicted future distribution. Each (CO_2) treatment had two independent replicates, randomly assigned to separate greenhouses. For each treatment-replicate combination, 12 seedlings were used, resulting in a total of 144 seedlings (12 seedlings × 2 CO_2 replicates × 2 CO_2 levels × 3 photoperiods = 144 seedlings).

2.3.3 Environmental conditions

Environmental conditions in each greenhouse were monitored and controlled independently using an Argus Titan System (Argus Controls Systems Ltd., Surrey, BC, Canada). The CO_2 concentration in the EC treatment was increased to 1000 µmol mol^{-1} using GEN-2E CO_2 generators (Custom Automated Products Inc., Riverside, California, USA). The photoperiod in all the greenhouses was set to the longest of the three regimes P_{55} (17 hours day length), with high-pressure sodium lamps used to extend the natural day length when necessary. The shorter photoperiods at P_{45} (15 hours) and P_{55} (16 hours) were achieved by manually applying neutral-density shading cloth, which effectively blocked light, as confirmed by a 0 µmol m^{-2} s^{-1} reading on a Li-Cor LI-250 light meter and LI-190 quantum sensor (LI-COR Inc., USA). Photoperiod adjustment was synchronized with the temperature setting and was weekly adjusted to the calculated photoperiod for each of the three latitudes. Temperature settings were adjusted weekly according to the previous seven years' averages (2016 – 2022, Environment Canada (2023) to emulate seasonal variation from June 11 to November 4. The temperature was adjusted at 6-hour steps (12 am, 6 am, 12 pm, 6 pm) in each greenhouse to emulate the diurnal variation. The experiment was conducted for one growing season. Seedlings were watered every two days to the drip point. Fertilizer (Plant Products Co. Ltd., Brampton, Ontario, Canada) was applied weekly, with 50 mg/l N // 81.3 mg/l P // 29 mg/l K during the establishment stage (the first 21 days of the experiment), followed by 100mg/l N // 43.63 mg/l P // 99.6 mg/l K during the rapid growing stage with micronutrients (55 days after establishment) (Landis & Nisley, 1990). Fertilization was stopped on September 16 (98 days after treatments began) after growth measurements had been taken.

2.3.4 Gas Exchange measurements and parameter determination

Preliminary diurnal variation measurement showed that photosynthetic activity was most stable between 8:00 am and 3:30 pm. Therefore, all gas exchange measurements were taken during this period. Foliar gas exchange was measured on three randomly selected seedlings from each treatment combination during the rapid growth stage (77th–82nd day of treatment). These measurements were taken on the first fully expanded leaf from the top of each seedling using a LI-6800 portable photosynthesis system (LICOR, Lincoln, NE, USA) Li-Cor 6800 (Li-cor, Inc.). The photosynthetic response curve to internal CO_2 concentration (A/C_i curves) was measured at 23°C air temperature, 50% relative humidity, photosynthetically active radiation (PAR) of 650μmolm^{-2}s^{-1}, and the following CO_2 concentrations (CO_2):400, 40, 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 700, 900, 1000, 1,300 and 1,500 μmol mol^{-1}. The fitaci function from the Plantecophys package (Duursma, 2015) in R programming software (2024, version 4.4.2) was used to determine the maximum carboxylation rate of ribulose-1,5-bisphosphate (Vc_{max}, μmol m^{-2}s^{-1}) and the maximum electron transport rate of photosynthesis (J_{max}, μmol m^{-2} s^{-1})

The net photosynthetic rate (A_n), stomatal conductance (g_s) and transpiration (E) under growth conditions (ambient and elevated CO_2: 400 and 1000 μmol mol^{-1}) were extracted from the A/C_i data for each seedling. Photosynthetic water use efficiency (WUE) was estimated as the ratio of net photosynthesis (A_n) to transpiration (E) for each seedling.

2.3.5 Seedling Growth and Biomass Measurement

The sample seedlings' overall height and root collar diameter were measured following the gas exchange measurement. These seedlings were then harvested and separated into leaves, stems and roots. Total leaf area (*TLA*) was measured using the Regent Win Folia system (Regent Inc, Quebec, Canada). The dry mass of stem, leaf, and root was determined using an electronic balance with 0.00g precision, after being oven-dried at 80°C for 48 hours. Biomass allocation was assessed by calculating the following variables: root-to-shoot ratio (RSR= root dry mass/ total leaf and stem dry mass), stem mass ratio (SMR= stem dry mass/ total seedling dry mass), root mass ratio (RMR= root dry mass/total seedling dry mass), leaf mass ratio (LMR= leaf dry mass/seedling dry mass) and specific leaf area (SLA = total leaf area of the seedling/total leaf dry mass).

2.3.6 Statistical analysis

The data were assessed for normality of distribution with the Shapiro-Wilk test and for variance homogeneity using the Bartlett test. Two-way analysis of variance (ANOVA) was used to assess the effect of CO_2, photoperiod and their interactions. Tukey's LSD post hoc was applied to compare individual means when significant main effect or interactions were found ($P \leq 0.05$). Root collar diameter and transpiration rate data were transformed using square root transformation prior to analysis to ensure normality and homogeneity assumptions were met. All statistical analyses were done using the R programming software (2024, version 4.4.2).

2.4 Results

2.4.1 Gas exchange

The interaction between (CO_2) and photoperiod (PP) showed a significant effect on the maximum rate of electron transport (J_{max}) and the ratio of the maximum electron transport rate to the maximum rate of Rubisco carboxylation (J_{max}/Vc_{max}) ratio (Table 2.1). J_{max} consistently decreased compared to EC than AC across all photoperiod regimes. This decrease was more noticeable at P_{45} and P_{50} (i.e 24% decrease) indicating a possible constraint on electron transport efficiency, but smallest at P_{55} (i.e 14% decrease) suggesting a possible acclimation or other compensatory mechanism (Fig. 1A). J_{max}/Vc_{max} ratio decreased by 38.1% at P_{45}, 20.0% at P_{50} and 35.3% at P_{55} under EC compared with AC (Fig. 1B). EC significantly increased net photosynthetic (A_n) and photosynthetic water-use efficiency (WUE). This increased by 21% and 22% for A_n and *WUE,* respectively (Table 2.1, Fig. 1C and 1D) shows a strong positive effect of carbon dioxide enrichment.

Table 2.1: Summary of ANOVA results (F value, P-value, and degree of freedom (DF)) for the effects of carbon dioxide concentration ([CO_2]), photoperiod (PP), and their interactions on the maximum rate of electron transport (J_{max}), maximum rate of Rubisco carboxylation (Vc_{max}), ratio of maximum rate of electron transport to maximum rate of carboxylation (J_{max}/Vc_{max}), net photosynthetic rate (A_n), water-use efficiency (WUE), stomatal conductance (g_s), and transpiration rate (E) of yellow birch seedlings grown under two levels of (CO_2) (AC = 400 Vs. EC = 1000 µmol mol^{-1}) and three photoperiod regimes corresponding to 45°N (Seed origin), 50°N and 55°N latitude. Significant p-values (≤ 0.05) are boldfaced.

RESPONSE VARIABLE	CO2 (DF = 1)	PP (DF = 2)	CO2*PP (DF = 2)
J_{max}	0.04^{ns}	39.83**	30.15**
Vc_{max}	3.29^{ns}	0.20^{ns}	0.02^{ns}
J_{max}/Vc_{max}	0.2^{ns}	1.71**	0.66**
A_n	36.45**	4.15^{ns}	0.99^{ns}
WUE	9.25**	1.01^{ns}	0.8^{ns}
g_s	4.03^{ns}	0.91^{ns}	0.82^{ns}
E	1.22^{ns}	0.05^{ns}	1.59^{ns}

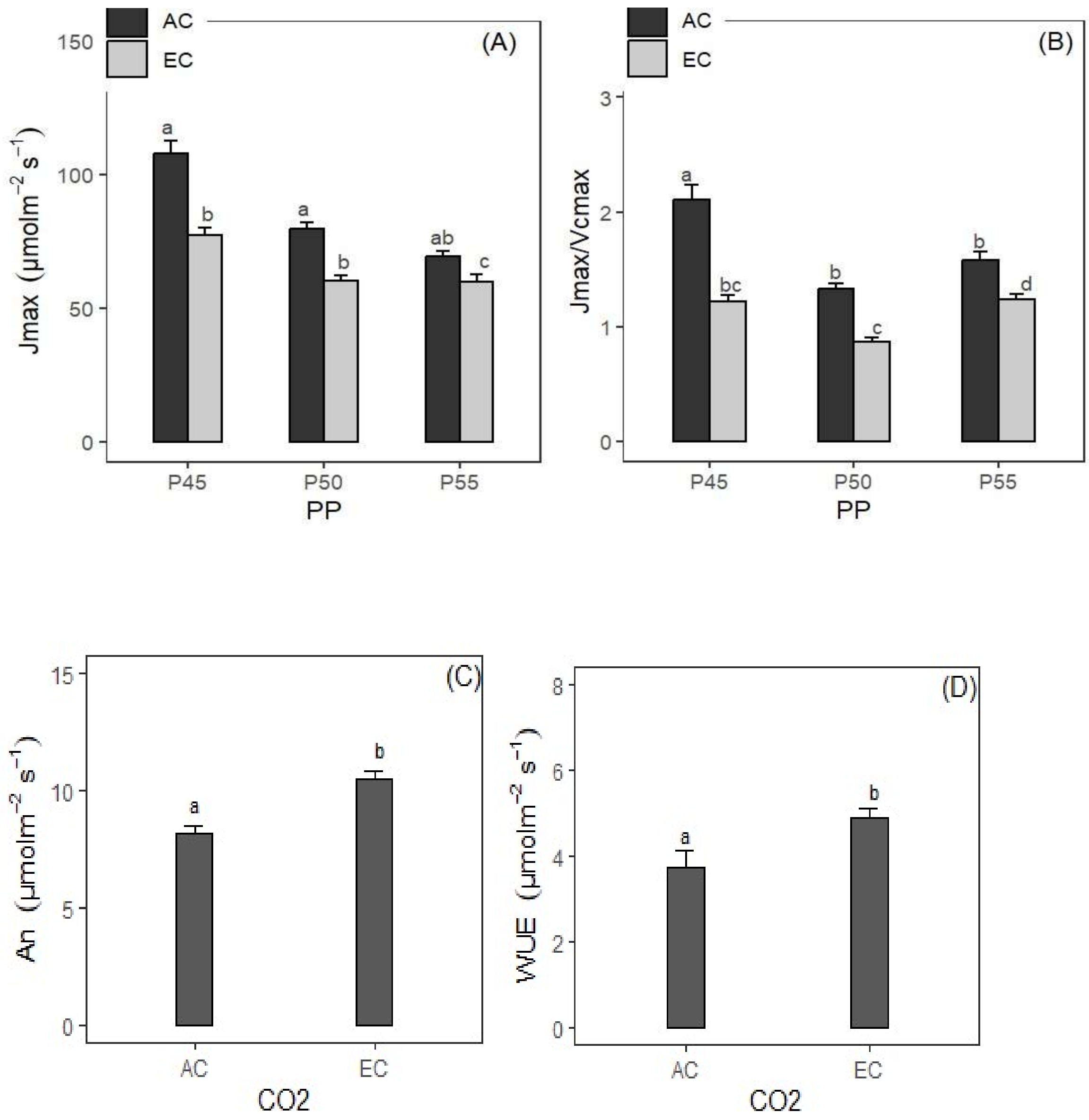


Figure 1: Mean (+SE) values for (A) the maximum rate of electron transport (J_{max}), (B) the ratio of maximum rate of electron transport to maximum rate of Rubisco carboxylation (J_{max}/Vc_{max}), (C) net photosynthetic rate (A_n) and (D) water use efficiency (WUE) of yellow birch seedlings grown at two levels of CO_2 (ambient (AC) and elevated (EC)) and three photoperiod regimes (P_{45}, P_{50} & P_{55}). Data were pooled across (CO_2) and photoperiod (PP) for (A) and (B) (n = 36, three seedlings per treatment replicate, 3 × $2CO_2$ × 3PP × 2 replicate) and (CO_2) for (C) and (D) (n = 12, three seedlings per treatment replicate, 3 × $2CO_2$ × 2 replicate). Different lowercase letters indicate statistically significant differences at $P < 0.05$.

2.4.2 Growth

Both (CO_2) and PP had a significant interactive effect on seedling height, total leaf area (TLA) and specific leaf area (SLA) (Table 2.2). EC increased tree height by 20% at P_{45}, while a slight decrease of 3.8% was observed at P_{50} (Fig. 2A). This suggests that the effect of EC on growth diminished at P50. EC generally increased *TLA* across all photoperiod regimes, with the increase becoming more pronounced as the photoperiod regime lengthened (6% at P_{45}, 10% at P_{50}, and 31.8% at P_{55}). However, the effect was not statistically significant at P_{45} (Table 2.2, Fig. 2C). *SLA* was influenced by the interaction between (CO_2) and PP (Table 2.2). EC reduced *SLA* by 37% at P_{45} and 27% at P_{50}, while at P_{55}, EC significantly increased *SLA* by 26% (Fig. 2D). The significant shift in *SLA* at P_{55} suggests a possible shift in leaf development under CO_2-enriched conditions.

Table 2.2: Summary of ANOVA results (F value, P-value and degree of freedom (DF)) for the effects of carbon dioxide concentration [CO_2], photoperiod (PP) and their interactions on height, root collar diameter (RCD), specific leaf area (SLA) and total leaf area (TLA) of yellow birch seedlings grown at under two levels of [CO_2] (AC = 400 Vs. EC= 1000 µmol mol^{-1}) and three photoperiod regimes corresponding to 45°N (photoperiod at seed origin), 50°N and, 55N latitude. Significant values ($P \leq 0.05$) are boldfaced.

RESPONSE VARIABLE	CO2 (DF = 1)	PP (DF = 2)	CO2*PP (DF = 2)
HEIGHT	1.36^{ns}	18.33**	3.15*
RCD	1.43^{ns}	1.4^{ns}	1.25^{ns}
TLA	37.36**	30.13**	12.98**
SLA	39.34**	30.3**	47.35**

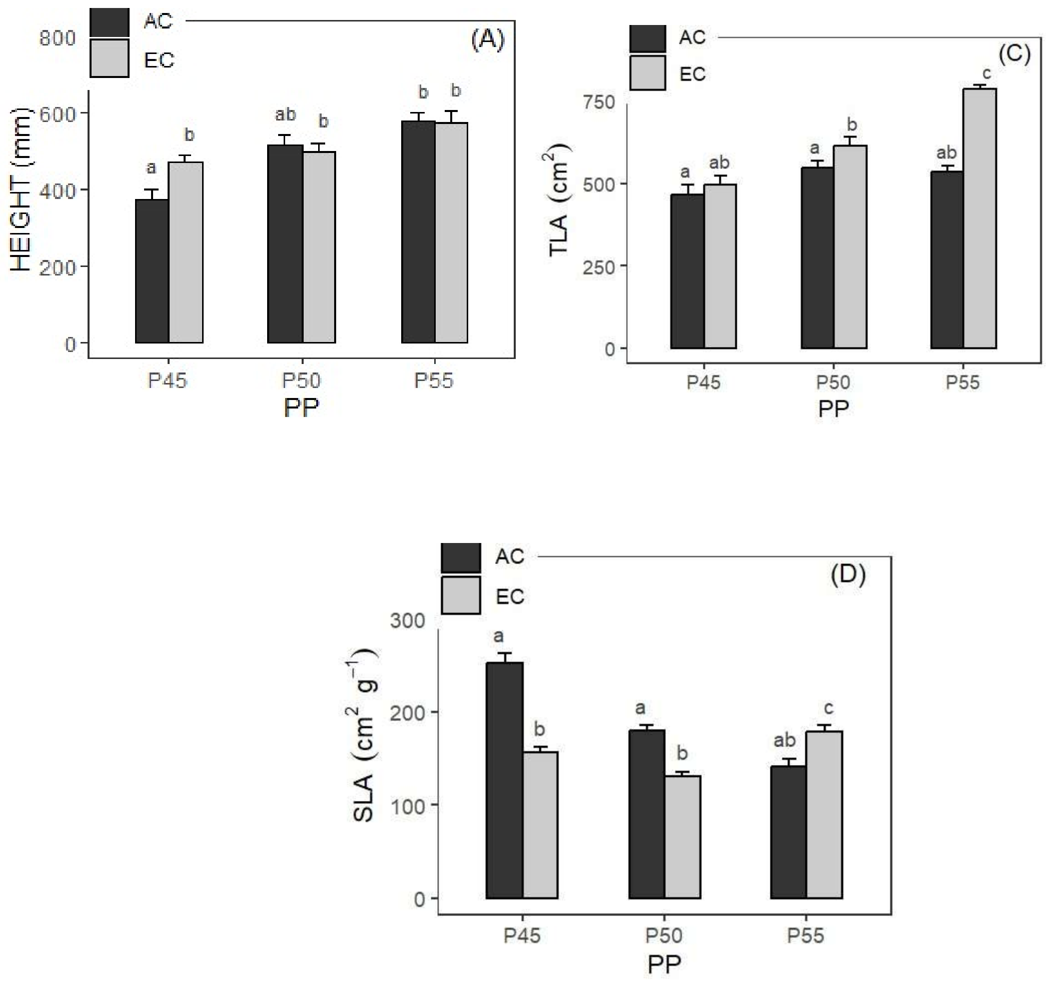


Figure 2: Mean (+SE) values for (A) height, (B) total leaf area (TLA), and (C) specific leaf area (SLA) of yellow birch seedlings grown at two levels of (CO$_2$) (ambient (AC) and elevated (EC)) and three photoperiod regimes (P$_{45}$, P$_{50}$ & P$_{55}$). The total number of seedlings was n=36, three seedlings per treatment replicate, 3 × 2CO$_2$ × 3PP × 2 replicates). Different lowercase letters show statistically significant differences at ($P < 0.05$).

2.4.3 Biomass and Biomass Allocation

The interaction between (CO_2) and PP had a significant effect on leaf dry mass (LEAFDM) and total seedling dry mass (TSDM) (Table 2.3). EC significantly increased *LEAFDM* by 41% at P_{45}, 34% at P_{50} and 12% at P_{55} (Fig. 3A). This suggests that trees under EC treatment grew more leaves than those under AC. However, the growth was more effective at P_{50}. Furthermore, *TSDM* increased under EC by 40% at P_{45}, 29% at P_{50} and 14% at P_{55} (Fig. 3F). The most rapid growth occurred at P_{50}, suggesting a critical period for biomass accumulation.

EC consistently increased both stem dry mass (STEMDM) and root dry mass (ROOTDM) by 27% and 24% respectively (Fig. 3B and 3D). Furthermore, PP significantly stimulated *STEMDM*, with an increase of 29% at P_{50} and 47% at P_{55}, as well as *ROOTDM*, with an increase of 38% at P_{50} and 42% at P_{55} compared to seed origin (Fig. 3C and 3E).

Table 2.3: Summary of ANOVA results (F value, P-value, and degree of freedom (DF)) for the effects of carbon dioxide concentration (CO_2), Photoperiod (PP), and their interactions on root dry mass (ROOTDM), leaf dry mass (LEAFDM), stem dry mass (STEMDM), total seedling dry mass (TSDM), root mass ratio (RMR), leaf mass ratio (LMR), stem mass ratio (SMR) and root to shoot ratio (RSR) of yellow birch seedlings grown at two (CO_2) levels (AC = 400 Vs. EC= 1000 μmol mol^{-1}) and three photoperiod regimes corresponding to 45°N (photoperiod at seed origin), 50°N and 55°N. Significant values ($P \leq 0.05$) are boldfaced.

RESPONSE VARIABLES	CO2 (DF = 1)	PP (DF = 2)	CO2*PP (DF = 2)
LEAFDM	46.62***	33.731***	3.49**
STEMDM	33.32***	44.09***	0.46^{ns}
ROOTDM	35.28***	46.24***	1.31^{ns}
TSDM	119.90***	128.93***	4.47**
LMR	0.18^{ns}	0.76^{ns}	0.27^{ns}
SMR	0.25^{ns}	3.89^{ns}	0.02^{ns}
RMR	0.40^{ns}	1.52^{ns}	0.163^{ns}
RSR	0.53^{ns}	1.29^{ns}	0.15^{ns}

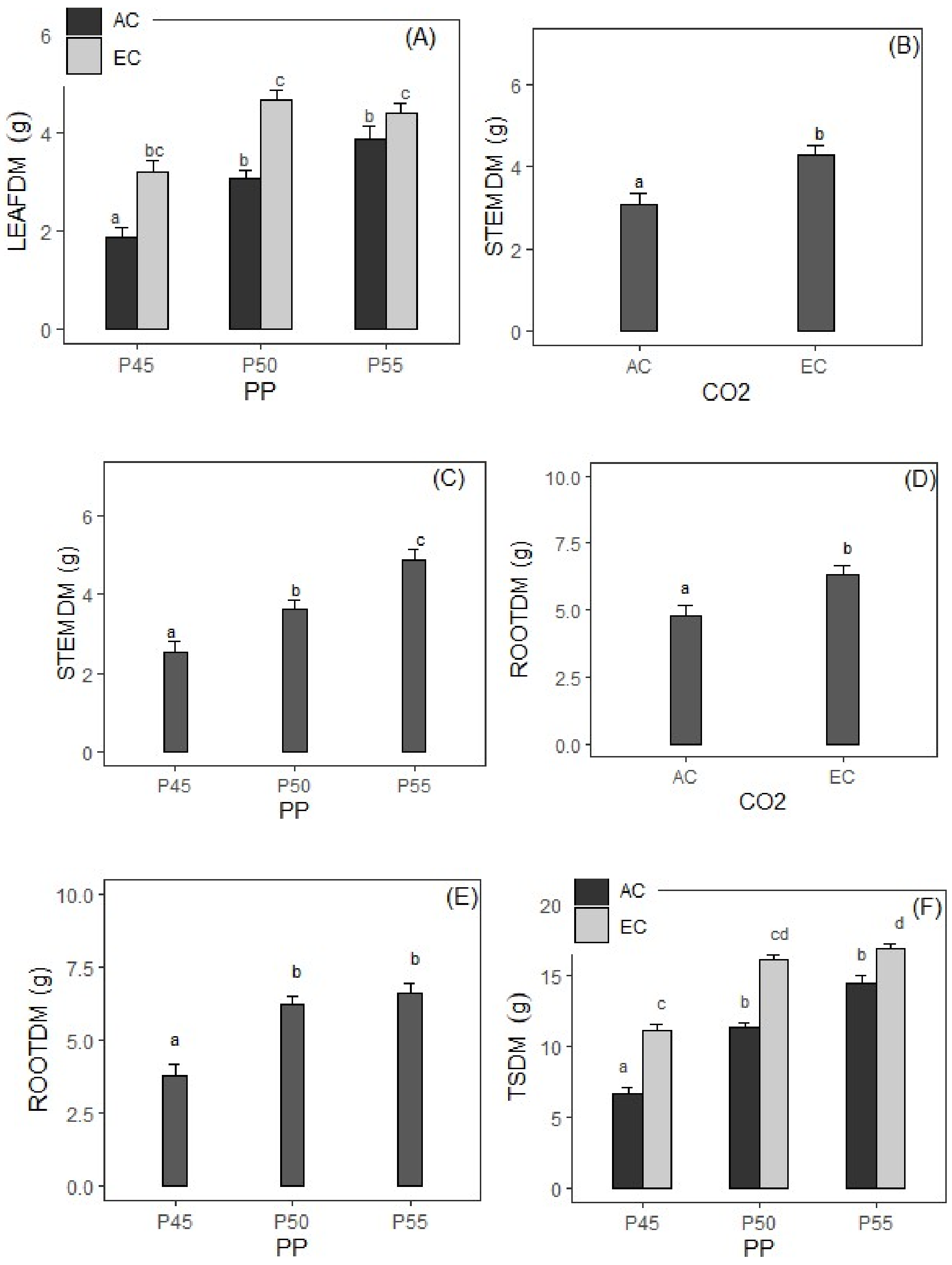


Figure 3: Mean (+SE) values for (A) leaf dry mass (*LEAFDM*), (B, C) stem dry mass (*STEMDM*), (D, E) root dry mass (*ROOTDM*) and (F) total seedling dry mass (*TSDM*) of yellow birch seedlings grown at two (CO_2) levels (ambient (AC) and elevated (EC)) and three photoperiod regimes (P_{45}, P_{50} & P_{55}). Data were pooled across (CO_2) and photoperiod replicate,(A) and (F) (n=36, three seedlings per treatment replicate, 3 × $2CO_2$ × 3PP × 2 replicate), (CO_2) for (B) and (D) (n= 12, three seedlings per treatment replicate, 3 × $2CO_2$ × 2 replicate) and (PP) for (C) and (E) (n= 18 three seedlings per treatment replicate, 3 × 3PP × 2 replicate). Different lowercase letters indicate statistically significant differences at P-value ($<$ 0.05).

2.5 Discussion

Effect of elevated carbon dioxide on net photosynthetic rate.

This result does not support the hypothesis that elevated carbon dioxide (EC) and longer photoperiod (PP) would synergistically enhance the net photosynthetic rate (A_n). EC and PP had no detectable interactive effect on the net photosynthetic rate. This could be that the trees were limited in their capacity to use or store extra carbohydrates (sink limitation), resulting in feedback inhibition of photosynthesis. However, the net photosynthetic rate A_n increased in response to EC. The findings reported by Long et al. (2004), Ainsworth and Rogers (2007), and Yang et al. (2016) support the idea that various tree species experience increased photosynthesis under elevated conditions. The increase is likely due to an enhanced availability of (CO_2) as a substrate, as well as enhanced carboxylation capacity (Zhang & Dang, 2006).

Interactive effect of elevated carbon dioxide and longer photoperiod at higher latitudes on J_{max} and the ratio of J_{max}/Vc_{max}

In this study, EC significantly reduced both J_{max} and the J_{max}/Vc_{max} ratio, with a decrease of approximately 24% observed at photoperiods P_{45} and P_{50} and a smaller reduction of 14% at P_{55}. This suggests that under EC, the capacity for electron transport is downregulated, particularly under shorter photoperiods. The J_{max}/Vc_{max} ratio is often used as an indicator of the balance between RuBP regeneration (driven by electron transport) and carboxylation capacity (associated with rubisco activity) (Smith & Keenan, 2020; Dusenge et al., 2021). A lower ratio under EC implies that electron transport becomes a more limiting factor relative to rubisco capacity, potentially reflecting a shift in photosynthetic control or resource allocation (Smith & Keenan, 2020; Dusenge et al., 2021). This finding is consistent with reports that EC can lead to downregulation of photosynthetic machinery, particularly when sink strength is insufficient to accommodate the increased carbohydrate supply (Ainsworth & Rogers, 2007; Long et al., 2004). The higher reductions at shorter photoperiods may reflect less time for carbon assimilation and reduced demand for higher J_{max}/Vc_{max} activity, compounding the effect of EC-induced acclimation. The smaller reduction at P_{55} may suggest that longer photoperiods provide more time for carbohydrate use or export, particularly mitigating EC-induced downregulation of $J_{max.}$

Acclimation is often associated with the gradual development of carbon (C) sink limitations. When non-carbon resources do not restrict growth, the demand for photosynthetically produced carbon compounds decreases relative to their supply, resulting in C sink limitation. In this context, the accumulation of photosynthates could have reduced the levels of Rubisco, the nitrogen-rich photosynthetic enzyme (Long & Bernacchi, 2003).

Interactive effect of elevated carbon dioxide and longer photoperiod at higher latitudes growth, biomass production and allocation

Elevated carbon dioxide increased height growth, total leaf area (TLA), leaf biomass, and overall seedling biomass, especially under longer photoperiod (Zhang et al., 2021). This result is consistent with the findings of Tedla et al. (2019). An increase in [CO_2] enhances tree growth and biomass accumulation through its direct positive effect on photosynthesis under adequate nutrients and water conditions (Bazzzaz & Miao, 1993; Curtis & Wang, 1998; Drake et al., 1997). The increased photosynthesis under EC at higher latitudes (P_{55} in this study) may have boosted carbohydrate production that translated into seedling growth (Liu et al., 2021; Poorter et al., 2022; Zhang et al., 2022). Most studies report that a longer photoperiod leads to greater carbon uptake

(Keenan et al., 2023; Richardson et al., 2013). However, it remains unclear whether longer photoperiods might impose constraints on the physiology of fully developed leaves, particularly when other factors are limiting. The need to consider the photoperiodic influence on tree carbon fluxes is not just about having a longer season for leaf retention but must also account for other physiological and morphological processes (Way & Montgomery, 2015).

Specific leaf area (SLA), has a significant influence on tree growth rate (Fellner et al., 2016; Wilson et al., 1999). Species with higher *SLA* often exhibit increased photosynthetic capacity per unit leaf mass, leading to accelerated growth (Poorter & Bongers, 2006; Reich et al., 1997). Higher *SLA* indicates thinner leaves, which are associated with faster growth rates (Poorter et al., 2009). However, species with lower *SLA* values tend to have thicker leaves, which are commonly associated with resource conservation strategies and slower growth rates (Niinemets, 2001). EC concentrations can influence specific leaf area *SLA*, but the effect may be species-specific. For instance, studies have shown that EC decreased the leaf area in certain species, such as *Quercus myrtifolia.* However, growth rates were positively correlated with *SLA* (Avalos, 2023; Hilty et al., 2021; Liu et al., 2021; Xi et al., 2021; Zhang et al., 2021b). Consequently, EC treatment significantly increased *SLA* at the highest latitude in this study (i.e., 10°N of seed origin P_{55}) but decreased it at P_{50}. The *SLA* reduction at P_{50} could result from the accumulation of carbohydrate in the leaf at that location (Zhang et al., 2006), this could be that thicker leaves associated with low *SLA* have more mesophyll cells per unit area (de Ávila Silva et al., 2021; Wang et al., 2020; Zhang et al., 2020b). The observed increase at P_{55}, could indicate a shift in leaf morphology under extended photoperiod exposure and that yellow birch exhibited faster growth, leading to thinner leaves due to enhanced carbohydrate production under projected (CO_2) levels and at higher latitudes with longer photoperiods (Tenkanen, 2023). Both EC and longer photoperiods independently enhanced root dry mass (ROOTDM) and stem dry mass (STEMDM). This further aligns with the observation that EC promotes biomass and growth, which in turn enhances the strength of carbon sinks and raises the demand for photosynthetic products (Xu et al., 2025).

The results of this study suggest that under future climate scenarios with EC and changing photoperiods, yellow birch may experience physiological down-regulation, particularly in electron transport capacity J_{max}, at photoperiod regimes at higher latitudes. Successful regeneration may depend on targeting areas with longer growing seasons or managing stand conditions to optimize light availability. Assisted migration towards northern altitudes could help align species needs with future climate and photoperiod trends (Pedlar et al., 2012). Furthermore, integrating photoperiod sensitivity into management planning will improve the resilience of hardwood forests facing climate change.

2.6 References

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist*, *165*(2), 351–372.

Ainsworth, E. A., & Long, S. P. (2021). 30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation? *Global Change Biology*, *27*(1), 27–49.

Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. *Plant, Cell & Environment*, *30*(3), 258–270.

Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., & Curtis-McLane, S. (2008). Adaptation, migration or extirpation: Climate change outcomes for tree populations. *Evolutionary Applications*, *1*(1), 95–111.

Alonso, A., Pérez, P., & Martínez-Carrasco, R. (2009). Growth in elevated CO_2 enhances temperature response of photosynthesis in wheat. *Physiologia Plantarum*, *135*(2), 109–120.

Anderson, J. J., Gurarie, E., Bracis, C., Burke, B. J., & Laidre, K. L. (2013). Modeling climate change impacts on phenology and population dynamics of migratory marine species. *Ecological Modelling*, *264*, 83-97.

Aubin, I., Munson, A., Cardou, F., Burton, P., Isabel, N., Pedlar, J., Paquette, A., Taylor, A., Delagrange, S., & Kebli, H. (2016). Traits to stay, traits to move: A review of functional traits to assess sensitivity and adaptive capacity of temperate and boreal trees to climate change. *Environmental Reviews*, *24*(2), 164–186.

Avalos, G. (2023). Specific leaf area (SLA) serves as a proxy to predict total carbon content in understory individuals of the neotropical canopy palm Socratea exorrhiza. *Trees*, *37*(6), 1831–1840.

Bader, M. K.-F., Siegwolf, R., & Körner, C. (2010). Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. *Planta*, *232*, 1115–1125.

Basler, D., & Körner, C. (2014). Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology*, *34*(4), 377–388.

Bauerle, W. L., Oren, R., Way, D. A., Qian, S. S., Stoy, P. C., Thornton, P. E., Bowden, J. D., Hoffman, F. M., & Reynolds, R. F. (2012). Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. *Proceedings of the National Academy of Sciences*, *109*(22), 8612–8617.

Bazzaz, F. A., & Miao, S. L. (1993). Successional status, seed size, and responses of tree seedlings to CO_2, light, and nutrients. *Ecology*, *74*(1), 104-112.

Boisvert-Marsh, L. (2021). *Patterns and processes affecting northward migration of tree species in a changing climate*.

Bonner, F. T., & Karrfalt, R. P. (2008). *The woody plant seed manual* (Issue 727). Forest Service.

Bowes, G. (1996). Photosynthetic responses to changing atmospheric carbon dioxide concentration. *Photosynthesis and the Environment*, 387–407.

Bunce, J. A. (2004). Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. *Oecologia*, *140*, 1–10.

Burns, R. M., Honkala, B. H., & Coordinators, T. (1990). Silvics of North America: Volume 2. Hardwoods. United States Department of Agriculture (USDA), Forest Service. *Agriculture Handbook*, *654*, 1990.

Canadell, J. G., Monteiro, P. M., Costa, M. H., Cotrim da Cunha, L., Cox, P. M., Eliseev, A. V., Henson, S., Ishii, M., Jaccard, S., & Koven, C. (2023). Intergovernmental Panel on Climate Change (IPCC). Global carbon and other biogeochemical cycles and feedbacks. In *Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change* (pp. 673–816). Cambridge University Press.

Canadell, H. M., Chapin III, F., Ehleringer, J., Korner, C., McMurtrie, R., Parton, W., Pitelka, L., & Schulze, E. (1999). Ecosystem physiology responses to global change. *The Terrestrial Biosphere and Global Change: Implications for Natural and Managed Ecosystems*, *4*, 141.

Chaudhry, S., & Sidhu, G. P. S. (2022). Climate change-regulated abiotic stress mechanisms in plants: A comprehensive review. *Plant Cell Reports*, *41*(1), 1–31.

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026.

Clausen, K. E. (1973). *Genetics of yellow birch* (Vol. 18). US Department of Agriculture.

Crawley, M. J. (2009). *Plant ecology*. John Wiley & Sons.

Curtis, P. S., & Wang, X. (1998). A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia*, *113*, 299-313.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, *292*(5517), 673-679.

de Ávila Silva, L., Omena-Garcia, R. P., Condori-Apfata, J. A., Costa, P. M. de A., Silva, N. M., DaMatta, F. M., Zsögön, A., Araújo, W. L., de Toledo Picoli, E. A., & Sulpice, R. (2021). Specific leaf area is modulated by nitrogen via changes in primary metabolism and parenchymal thickness in pepper. *Planta*, *253*, 1–13.

Delagrange, S., Messier, C., Lechowicz, M. J., & Dizengremel, P. (2004). Physiological, morphological and allocational plasticity in understory deciduous trees: Importance of plant size and light availability. *Tree Physiology*, *24*(7), 775–784.

Delagrange, S., Montpied, P., Dreyer, E., Messier, C., & Sinoquet, H. (2006). Does shade improve light interception efficiency? A comparison among seedlings from shade-tolerant and-intolerant temperate deciduous tree species. *New Phytologist*, *172*(2), 293–304.

DeLucia, E. H., & Thomas, R. B. (2000). Photosynthetic responses to CO_2 enrichment of four hardwood species in a forest understory. *Oecologia*, *122*, 11–19.

Drake, B. G., Gonzàlez-Meler, M. A., & Long, S. P. (1997). More efficient plants: A consequence of rising atmospheric CO_2? *Annual Review of Plant Biology*, *48*(1), 609–639.

Drobyshev, I., Guitard, M.-A., Asselin, H., Genries, A., & Bergeron, Y. (2014). Environmental controls of the northern distribution limit of yellow birch in eastern Canada. *Canadian Journal of Forest Research*, *44*(7), 720–731.

Dusenge, M. E., Ward, E. J., Warren, J. M., Stinziano, J. R., Wullschleger, S. D., Hanson, P. J., & Way, D. A. (2021). Warming induces divergent stomatal dynamics in co-occurring boreal trees. *Global Change Biology*, *27*(13), 3079–3094.

Duursma, R. A. (2015). Plantecophys-an R package for analysing and modelling leaf gas exchange data. *PloS one*, *10*(11), e0143346.

Equiza, M. A., Day, M. E., Jagels, R., & Li, X. (2006). Photosynthetic downregulation in the conifer Metasequoia glyptostroboides growing under continuous light: The significance of carbohydrate sinks and paleoecophysiological implications. *Botany*, *84*(9), 1453–1461.

Fellner, H., Dirnberger, G. F., & Sterba, H. (2016). Specific leaf area of European Larch (Larix decidua M ill.). *Trees*, *30*, 1237-1244.

Field, C. B., Jackson, R. B., & Mooney, H. A. (1995). Stomatal responses to increased CO_2: Implications from the plant to the global scale. *Plant, Cell & Environment*, *18*(10), 1214–1225.

Friedlingstein, P., O'sullivan, M., Jones, M. W., Andrew, R. M., Gregor, L., Hauck, J., Le Quéré, C., Luijkx, I. T., Olsen, A., & Peters, G. P. (2022). Global carbon budget 2022. *Earth System Science Data*, *14*(11), 4811–4900.

Gray, S. B., & Brady, S. M. (2016). Plant developmental responses to climate change. *Developmental Biology*, *419*(1), 64–77.

Gray, L. K., & Hamann, A. (2013). Tracking suitable habitat for tree populations under climate change in western North America. *Climatic Change*, *117*(1), 289–303.

Gunderson, C. A., Edwards, N. T., Walker, A. V., O'Hara, K. H., Campion, C. M., & Hanson, P. J. (2012). Forest phenology and a warmer climate–growing season extension in relation to climatic provenance. *Global Change Biology*, *18*(6), 2008–2025.

Haworth, M., Elliott-Kingston, C., & McElwain, J. C. (2011). Stomatal control as a driver of plant evolution. *Journal of Experimental Botany*, *62*(8), 2419–2423.

Hilty, J., Muller, B., Pantin, F., & Leuzinger, S. (2021). Plant growth: The what, the how, and the why. *New Phytologist*, *232*(1), 25–41.

Inoue, S., Dang, Q.-L., Man, R., & Tedla, B. (2020a). Photoperiod and CO_2 elevation influence morphological and physiological responses to drought in trembling aspen: Implications for climate change-induced migration. *Tree Physiology*, *40*(7), 917–927.

IPCC. (2014). Climate change 2014 synthesis report. *IPCC: Geneva, Szwitzerland*, 1059–1072.

Iverson, L. R., & McKenzie, D. (2013). Tree-species range shifts in a changing climate: Detecting, modeling, assisting. *Landscape Ecology*, *28*, 879–889.

Jach, M. E., & Ceulemans, R. (1999). Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (Pinus sylvestris) seedlings after two years of exposure in the field. *Tree Physiology*, *19*(4–5), 289–300.

Jackson, S. D. (2009). Plant responses to photoperiod. *New Phytologist*, *181*(3), 517–531.

Jarvis, A., Mansfield, T., & Davies, W. J. (1999). Stomatal behaviour, photosynthesis and transpiration under rising CO_2. *Plant, Cell & Environment*, *22*(6), 639–648.

Keenan, T. F., Luo, X., Stocker, B. D., De Kauwe, M. G., Medlyn, B. E., Prentice, I. C., Smith, N., Terrer, C., Wang, H., & Zhang, Y. (2023). A constraint on historic growth in global photosynthesis due to rising CO_2. *Nature Climate Change*, *13*(12), 1376–1381.

Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., & Richardson, A. D. (2013). Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*, *499*(7458), 324–327.

Krapp, A., Hofmann, B., Schäfer, C., & Stitt, M. (1993). Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation'of photosynthesis?. *The plant journal*, *3*(6), 817-828.

Kremer, A., Ronce, O., Robledo-Arnuncio, J. J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J. R., Gomulkiewicz, R., Klein, E. K., & Ritland, K. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*, *15*(4), 378–392.

Lafleur, B., Pare, D., Munson, A. D., & Bergeron, Y. (2010). Response of northeastern North American forests to climate change: Will soil conditions constrain tree species migration? *Environmental Reviews*, *18*(NA), 279–289.

Lambers, H., Chapin III, F. S., & Pons, T. L. (2008). *Plant physiological ecology*. Springer Science & Business Media.

Lambers, H., Oliveira, R. S., Lambers, H., & Oliveira, R. S. (2019a). Growth and allocation. *Plant Physiological Ecology*, 385–449.

Leakey, A. D., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., & Ort, D. R. (2009). Elevated CO_2 effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. *Journal of Experimental Botany*, *60*(10), 2859–2876.

Liu, F., Wang, C., & Wang, X. (2021). Sampling protocols of specific leaf area for improving accuracy of the estimation of forest leaf area index. *Agricultural and Forest Meteorology*, *298*, 108286.

Long, S., Ainsworth, E., Bernacchi, C., Davey, P., Morgan, P., Hymus, G., Leakey, A., & Osborne, C. (2006). Long-Term Responses of Photosynthesis and Stomata to Elevated [CO_2] in Managed Systems. *Managed Ecosystems and CO_2: Case Studies, Processes, and Perspectives*, 253–270.

Long, S. P., Ainsworth, E. A., Rogers, A., & Ort, D. R. (2004). Rising atmospheric carbon dioxide: Plants FACE the future. *Annu. Rev. Plant Biol.*, *55*(1), 591–628.

Long, S. P., & Bernacchi, C. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany*, *54*(392), 2393–2401.

Maloney, A., Dang, Q., Godakanda, P., & Thomson, A. (2024). Genetic variation in growth and leaf traits associated with local adaptation to climate in yellow birch (Betula alleghaniensis Britton). *Botany*, *102*(4), 198–210.

Masson-Delmotte, V., Zhai, P., Pirani, S., Connors, C., Péan, S., Berger, N., Caud, Y., Chen, L., Goldfarb, M., & Scheel Monteiro, P. M. (2021). *Ipcc, (2021). Summary for policymakers. In: Climate change 2021: The physical science basis. Contribution of working group i to the sixth assessment report of the intergovernmental panel on climate change*.

Matt, P., Krapp, A., Haake, V., Mock, H., & Stitt, M. (2002). Decreased Rubisco activity leads to dramatic changes of nitrate metabolism, amino acid metabolism and the levels of phenylpropanoids and nicotine in tobacco antisense RBCS transformants. *The Plant Journal*, *30*(6), 663–677.

McKenney, D. W., Pedlar, J. H., Lawrence, K., Campbell, K., & Hutchinson, M. F. (2007). Potential impacts of climate change on the distribution of North American trees. *BioScience*, *57*(11), 939–948.

McKenney, D. W., Pedlar, J. H., Rood, R. B., & Price, D. (2011). Revisiting projected shifts in the climate envelopes of North American trees using updated general circulation models. *Global Change Biology*, *17*(8), 2720–2730.

Meinshausen, M., Smith, S. J., Calvin, K., Daniel, J. S., Kainuma, M. L., Lamarque, J.-F., Matsumoto, K., Montzka, S. A., Raper, S. C., & Riahi, K. (2011). The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Climatic Change*, *109*, 213–241

Morin, X., Augspurger, C., & Chuine, I. (2007). Process-based modeling of species'distributions: what limits temperate tree species'range boundaries? *Ecology*, *88*(9), 2280–2291.

Newaz, S., Dang, Q., & Man, R. (2017). Eco-physiological potential of jack pine (Pinus banksiana) for assisted northward migration: Interactions among photoperiod,[CO_2] and moisture stress. *Nordic Journal of Botany*, *35*(6), 633–643.

Niinemets, Ü. (2001). Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, *82*(2), 453-469.

Norby, R. J., DeLucia, E. H., Gielen, B., Calfapietra, C., Giardina, C. P., King, J. S., Ledford, J., McCarthy, H. R., Moore, D. J., & Ceulemans, R. (2005). Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*, *102*(50), 18052–18056.

Pastore, M. A., Lee, T. D., Hobbie, S. E., & Reich, P. B. (2019). Strong photosynthetic acclimation and enhanced water-use efficiency in grassland functional groups persist over 21 years of CO_2 enrichment, independent of nitrogen supply. *Global Change Biology*, *25*(9), 3031–3044.

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, *13*(9), 1860–1872.

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography*, *12*(5), 361–371.

Pedlar, J. H., McKenney, D. W., Aubin, I., Beardmore, T., Beaulieu, J., Iverson, L., ... & Ste-Marie, C. (2012). Placing forestry in the assisted migration debate. *BioScience*, *62*(9), 835-842.

Poorter, L., & Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, *87*(7), 1733-1743.

Poorter, H., Knopf, O., Wright, I. J., Temme, A. A., Hogewoning, S. W., Graf, A., Cernusak, L. A., & Pons, T. L. (2022). A meta-analysis of responses of C3 plants to atmospheric CO_2: Dose–response curves for 85 traits ranging from the molecular to the whole-plant level. *New Phytologist*, *233*(4), 1560–1596.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30–50.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, *182*(3), 565–588.

Pritchard, S. G., Rogers, H. H., Prior, S. A., & Peterson, C. M. (1999). Elevated CO_2 and plant structure: A review. *Global Change Biology*, *5*(7), 807–837.

Rais, A., van de Kuilen, J.-W. G., & Pretzsch, H. (2014). Growth reaction patterns of tree height, diameter, and volume of Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) under acute drought stress in Southern Germany. *European Journal of Forest Research*, *133*, 1043–1056.

Rasheed, F., & Delagrange, S. (2016). Acclimation of Betula alleghaniensis Britton to moderate soil water deficit: Small morphological changes make for important consequences in crown display. *Tree Physiology*, *36*(11), 1320–1329.

Raymond, P., & Dumais, D. (2023). Ecological Silviculture for Yellow Birch–Conifer Mixedwoods in Eastern Canada. *Ecological Silvicultural Systems: Exemplary Models for Sustainable Forest Management*, 112.

Reddy, A. R., Rasineni, G. K., & Raghavendra, A. S. (2010). The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. *Current Science*, 46–57.

Reddy, V., Reddy, K., & Hodges, H. (1995). Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpiration, and water-use efficiency. *Field Crops Research*, *41*(1), 13–23.

Reich, P. B., Ellsworth, D. S., Walters, M. B., Vose, J. M., Gresham, C., Volin, J. C., & Bowman, W. D. (1999). Generality of leaf trait relationships: a test across six biomes. *Ecology*, *80*(6), 1955-1969.

Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology*, *169*, 156–173.

Rogers, A., Medlyn, B. E., Dukes, J. S., Bonan, G., Von Caemmerer, S., Dietze, M. C., Kattge, J., Leakey, A. D., Mercado, L. M., & Niinemets, Ü. (2017). A roadmap for improving the representation of photosynthesis in Earth system models. *New Phytologist*, *213*(1), 22–42.

Saban, J. M., Chapman, M. A., & Taylor, G. (2019). FACE facts hold for multiple generations, Evidence from natural CO_2 springs. *Global Change Biology*, *25*(1), 1–11.

Saxe, H., Ellsworth, D. S., & Heath, J. (1998). Tree and forest functioning in an enriched CO_2 atmosphere. *The New Phytologist*, *139*(3), 395–436.

Smith, N. G., & Keenan, T. F. (2020). Mechanisms underlying leaf photosynthetic acclimation to warming and elevated CO_2 as inferred from least-cost optimality theory. *Global Change Biology*, *26*(9), 5202–5216.

Sperry, J. S., Venturas, M. D., Anderegg, W. R., Mencuccini, M., Mackay, D. S., Wang, Y., & Love, D. M. (2017). Predicting stomatal responses to the environment from the optimization of photosynthetic gain and hydraulic cost. *Plant, Cell & Environment*, *40*(6), 816–830.

Stitt, M., Sulpice, R., & Keurentjes, J. (2010). Metabolic networks: How to identify key components in the regulation of metabolism and growth. *Plant Physiology*, *152*(2), 428–444.

Tedla, B., Dang, Q., & Inoue, S. (2021). Longer photoperiods negate the CO2 stimulation of photosynthesis in Betula papyrifera Marsh: Implications to climate change-induced migration. *Physiologia Plantarum*, *172*(1), 106–115.

Tedla, B., Dang, Q.-L., & Inoue, S. (2019). White birch has limited phenotypic plasticity to take advantage of increased photoperiods at higher latitudes north of the seed origin. *Forest Ecology and Management*, *451*, 117565.

Tenkanen, A. (2023). *Latitude-related variation in silver birch provenances in a changing climate: Growth, biomass allocation and photosynthesis-associated leaf-traits in the field and in growth chamber trials* (Doctoral dissertation, Itä-Suomen yliopisto).

Vitasse, Y., Delzon, S., Bresson, C. C., Michalet, R., & Kremer, A. (2009). Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research*, *39*(7), 1259–1269.

Wang, N., Gao, G., Wang, Y., Wang, D., Wang, Z., & Gu, J. (2020). Coordinated responses of leaf and absorptive root traits under elevated CO_2 concentration in temperate woody and herbaceous species. *Environmental and Experimental Botany*, *179*, 104199.

Wang, W. J., Thompson III, F. R., He, H. S., Fraser, J. S., Dijak, W. D., & Jones-Farrand, T. (2019). Climate change and tree harvest interact to affect future tree species distribution changes. *Journal of Ecology*, *107*(4), 1901–1917.

Way, D. A., & Montgomery, R. A. (2015a). Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment*, *38*(9), 1725–1736.

Xi, N., Adler, P. B., Chen, D., Wu, H., Catford, J. A., van Bodegom, P. M., Bahn, M., Crawford, K. M., & Chu, C. (2021). Relationships between plant–soil feedbacks and functional traits. *Journal of Ecology*, *109*(9), 3411–3423.

Xu, K., Zou, G., & Hu, H. (2025). Forest Carbon Sequestration Functions and Mitigation Strategies for Global Climate Change.

Yang, N., Zavišić, A., Pena, R., & Polle, A. (2016). Phenology, photosynthesis, and phosphorus in European beech (Fagus sylvatica L.) in two forest soils with contrasting P contents. *Journal of Plant Nutrition and Soil Science*, *179*(2), 151–158.

Zhang, H., Yuan, F., Wu, J., Jin, C., Pivovaroff, A. L., Tian, J., ... & McDowell, N. G. (2021). Responses of functional traits to seven-year nitrogen addition in two tree species: coordination of hydraulics, gas exchange and carbon reserves. *Tree physiology*, *41*(2), 190–205.

Zhang, L., Shipley, B., & Zhou, S. (2020a). Exploring the relationship between functional structure and ecosystem multifunctionality requires intraspecific trait variability. *bioRxiv*, 2020–11.

Zhang, L., Yang, L., & Shen, W. (2020b). Dramatic altitudinal variations in leaf mass per area of two plant growth forms at extreme heights. *Ecological Indicators*, *110*, 105890.

Zhang, S., & Dang, Q.-L. (2006). Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. *Tree Physiology*, *26*(11), 1457–1467.

Zhu, J., Cao, Y., He, W., Xu, Q., Xu, C., & Zhang, X. (2021). Leaf functional traits differentiation in relation to covering materials of urban tree pits. *BMC Plant Biology*, *21*, 1–11.

Chapter 3: Phenological Response of Yellow Birch to the Interactive Effect of Photoperiod and Elevated Carbon Dioxide at Higher Latitude.

3.1 Abstract

Climate change is gradually altering the timing of plant phenological events, potentially affecting their geographic distribution and ecosystem function. Previous research has focused on the impact of climate change on spring phenology, and to date, the impact on autumn phenology remains poorly understood, especially in the context of climate change-induced northward migration. This study investigated the phenological response of yellow birch to the effect of photoperiods and elevated carbon dioxide in the context of climate change-induced northward migration. We conducted a greenhouse experiment to assess the interactive effects of carbon dioxide levels (AC = 400; EC= 1000 mol mol^{-1}), and photoperiod regimes associated with three latitudes (45°N (seed origin), 50°N and 55° N) on growth cessation, bud formation, leaf senescence, and cold hardiness in yellow Birch (*Betula alleghaniensis*) seedlings. The combination of elevated carbon dioxide and longer photoperiod associated with 10°N of seed origin was found to significantly advance bud set phenology. Furthermore, longer growing season photoperiods north of seed origin made the seedlings more prone to frost injury, as indicated by a higher frost injury index (*Ii* -45°C). The combined influence of these environmental signals on bud phenology highlights the need to incorporate photoperiods into predictive models assessing the impact of climate change on northern forests.

3.2 Introduction

Climate change is modifying the phenology of temperate trees in North America (Piao et al., 2019). For two decades, there has been interest in the overall impact of phenological shifts in forest ecosystems due to climate change (Cleland et al., 2007; Hänninen, 1995). Climate change has altered precipitation, temperature patterns, and climate seasonality (IPCC, 2014; Masson-Delmotte et al., 2021). The effects of climate change on tree phenology have inspired discussions on whether trees can cope with the predicted rate of climate change (Aitken et al., 2008; Cleland et al., 2007; Saxe et al., 2001). Phenological synchronization with changing seasons is critical for the survival and fitness of temperate and boreal trees (Cleland et al., 2007; Jach et al., 2001; Kramer et al., 2000). These species have evolved to use environmental cues such as photoperiod to regulate their phenological (Flynn & Wolkovich, 2018; Jackson, 2009; Way & Montgomery, 2015). Because phenology is crucial to tree distribution and conservation (Chuine, 2010; Piao et al., 2019), understanding the phenological responses to climate change is essential for projecting the future distribution of trees and forests (Parmesan et al., 2013). Research has shown that the climate envelopes for most North American tree species will shift northward by as much as 10°N (Aitken et al., 2008; McKenney et al., 2007, 2011). Thus, environmental factors such as photoperiod could influence a shift or migration of tree species (Ettinger et al., 2021; Saikkonen et al., 2012; Zohner et al., 2016). For instance, a change in the photoperiod regime can modify the timing of phenological events (Adams & Langton, 2005; Ettinger et al., 2021; Flynn & Wolkovich, 2018). While the photoperiodic signal is stable for any day of the year (Way & Montgomery, 2015), temperate forest zones are characterized by a seasonal variation of photoperiod that increases with latitude (Ettinger et al., 2021; Parmesan, 2007). Temperate forest trees have mostly adapted to the photoperiod of their local habitat (Dreiss & Volin, 2020; Hänninen, 2016; Jackson, 2009), and this controls their phenological events and physiological processes (Nord & Lynch, 2009; Way & Montgomery, 2015). Therefore, when trees migrate to higher latitudes, it is unclear how photoperiodic changes will affect the phenological response in a changing climate.

Photoperiod is one of the critical factors controlling the annual growth cycles in temperate and boreal tree species (Adams & Langton, 2005; Flynn & Wolkovich, 2018; Jackson, 2009). For example, the photoperiod at higher latitudes is longer in the summer but shorter during winter (Vitasse et al., 2009; Wielgolaski &, 2003). Thus, trees may delay or advance their growth processes in response to photoperiod after migrating up north (Jackson, 2009; Way & Montgomery, 2015). Progressively, longer photoperiod may delay growth cessation, bud set, leaf senescence and development of dormancy in the autumn (Hamilton et al., 2016; Heide, 2008; Maurya & Bhalerao, 2017). Delayed dormancy may expose them to freeze damages in winter (Charrier et al., 2015; Vitasse et al., 2014; Wisniewski et al., 2014). Several studies have revealed that warmer temperatures, rather than photoperiod, control the timing of leaf out (bud break) (Basler & Körner, 2014; Flynn & Wolkovich, 2018; Menzel et al., 2006). However, others have found that bud break in spring is controlled by winter chilling and photoperiod (Partanen et al., 1998; Pletsers et al., 2015). The development of sufficient cold hardiness is essential for plant survival in regions with harsh winter (Kalberer et al., 2006; Wisniewski et al., 2014). A shorter winter season and a reduced dormancy period could impact tree growth for the following growing season (Hamilton et al., 2016; Harrington et al., 2010, 2010; Rohde & Bhalerao, 2007). The effect of photoperiod and elevated carbon dioxide on spring phenology has been extensively studied (Huber et al., 2021; Inoue et al., 2020b; Newaz et al., 2017; Reekie et al., 1997).

Elevated carbon dioxide (EC) is known to have altered the growth rhythm of forest trees by modifying the timing of growth cessation, autumnal leaf senescence and bud formation (Jach et al., 2001; Richardson et al., 2013; Tedla et al., 2020). From recent research into the autumnal phenophase of trees in response to carbon dioxide, it was observed that EC could delay growth cessation (Hamilton et al., 2016), promote earlier leaf senescence (Estiarte & Peñuelas, 2015; Li et al., 2000), initiate earlier bud formation, and increase cold hardiness (Kalberer et al., 2006; Tedla et al., 2020). When trees migrate to a new climate (Aitken et al., 2008; Boisvert-Marsh, 2021), these species may likely be unable to acclimate to the photoperiod regimes (Ettinger et al., 2021). In addition, faster changes in photoperiod during summer-autumn transitions at higher latitudes may affect bud formation and cold hardening (Inoue et al., 2020b; Rapacz et al., 2014). A rapid response to these environmental cues helps synchronize phenological life-history transitions (Cleland et al., 2007; Gunderson et al., 2012; Richardson et al., 2013; Way, 2011). This could play a crucial role in the health and survival of tree species in north-temperate and boreal regions (Aubin et al., 2016; Dreiss & Volin, 2020; Morin et al., 2007). Therefore, further research on how photoperiod affects the timing of phenological events under EC could improve our understanding and predictions of tree response to climate change.

Phenological sensitivity reflects the capability of trees to track climate change and indicate if trees will persist or decline in response to climate change (Cleland *et al.*, 2012). Changes in autumn phenology could alter the productivity of trees and forest ecosystems (Gillis et al., 2005; Richardson et al., 2013; Wu et al., 2013). Autumn phenology has received less attention in climate change research on temperate and boreal trees (Amanda et al., 2015). Furthermore, the timing of autumn phenological events is essential for the survival and distribution of temperate and boreal species (Gill et al., 2015; Kramer et al., 2000; Lang et al., 2019). Few studies have compared the cold hardiness of yellow birch to other temperate hardwood species (such as sugar maple, American beech, or northern). For instance, Calme et al. (1994) subjected yellow birch, northern red oak and sugar maple seedlings to outdoor freezing trials. They observed that yellow birch was the most frost-tolerant species, with a 100% seedlings survival at the end of the trial. Yellow birch roots remained alive at -33°C. Although the interactive effects of environmental factors on the phenology of temperate deciduous trees have been studied (Inoue et al., 2020b; Man et al., 2014; Tedla et al., 2020), our understanding of these environmental cues on the autumnal phenology of yellow birch is limited. Yellow birch grows larger than other eastern birches and is one of the principal hardwoods used in the distillation of wood alcohol, acetate of lime, charcoal, tar and soils. It is a characteristic tree species of the northern Appalachians and the hemlock hardwood forests of Great Lakes region. Yellow birch occurs on moist, well-drained soils of various types of uplands and mountain ravines. Yellow birch is intermediate in shade tolerance. The objective of this study was to assess the interactive effect of elevated carbon dioxide and a longer photoperiod on the phenological response of yellow birch. Many temperate trees like yellow birch rely partly on shorter day length to trigger leaf senescence. Since EC can delay budset, and a longer photoperiod can delay the process of photosynthesis could delay leaf senescence in autumn.

I hypothesize that

(i) Elevated carbon dioxide and photoperiod regime at higher latitudes will delay bud set and leaf senescence as a result of a prolonged growing season.

(ii) Since shorter day lengths are the primary cue for trees to prepare for winter, elevated carbon dioxide and a higher photoperiod regime will synergistically delay dormancy induction in yellow birch, which could reduce cold hardiness.

3. 3 Materials and Methods

3.3.1 Plant Materials

The yellow birch (*Betula alleghaniensis*) seeds used for this were collected from ten individual trees in a forest situated at Prosser Brook, New Brunswick, Canada (45.80°N, 64.92°W). Seeds were stored in a sealed glass jar before being stratified at -4°C for three weeks (Bonner & Karrfalt, 2015) at Lakehead University's eco-physiology laboratory. The seeds were sown in germination trays (50 cm x 25 cm x 5 cm) filled with a 1:1 (v/v) mixture of vermiculite and peat moss as the growing medium. Germination occurred under temperature conditions of 25/16°C (day/night) with a 16-hour photoperiod. Natural light was enhanced with high-pressure sodium lamps when the photosynthetically active radiation (PAR) fell below 500 µmol m^{-2} s^{-1}. Seedlings reaching an average height of 2 cm were transplanted into larger pots (15 cm high and 13 cm in diameter) and a 2:1 (v/v) mixture of peat moss and vermiculite ten days after germination.

3.3.2 Experimental Design

The experiment was conducted in four greenhouses at Lakehead University, Thunder Bay, Ontario, Canada. A split-plot design was employed, with the atmospheric carbon dioxide [(CO_2)] treatment (AC: 400 µmol mol^{-1} vs EC: 1000 µmol mol^{-1}) as the main plot and photoperiod regimes corresponding to 45 °N, 50 °N, and 55 °N latitudes as the sub-plot. Each (CO_2) treatment had two independent replicates, randomly assigned to separate greenhouses. The photoperiod treatments represented the photoperiod at the seed's origin (P_{45}), at 5° north (P_{50}) and the 10° north of the seed origin (P_{55}). The 10° north simulates the predicted northward shift in climate envelopes for temperate and boreal tree species (McKenney et al., 2007, 2011), and 5° north represents the midpoint between the species' current range and predicted future distribution. For each treatment-replicate combination, 12 seedlings were used, resulting in a total of 144 seedlings (12 seedlings × 2 CO_2 replicates × 2 CO_2 levels × 3 photoperiods = 144 seedlings).

3.3.3 Environmental Controls

Environmental conditions in each greenhouse were independently monitored and regulated using an Argus Titan System (Argus Controls Systems Ltd, Surrey, BC, Canada). The photoperiod in all the greenhouses was set to the longest of the three regimes, with high-pressure sodium lamps used to extend the natural day length when necessary. The shorter photoperiods were achieved by manually applying neutral-density shading cloth, which effectively blocked light, as confirmed by a 0 $\mu mol\ m^{-2}\ s^{-1}$ reading on a Li-Cor LI-250 light meter and LI-190 quantum sensor (LI-COR Inc., USA). The (CO_2) concentration in the EC treatment was increased to 1000 $\mu mol\ mol^{-1}$ using GEN-2E CO_2 generators (Custom Automated Products Inc., Riverside, California, USA). Temperature settings were adjusted weekly to hourly temperature measurements at the seed origin (seven years (2016 – 2022) averages) (Environment Canada (2023). The temperature was set at 6-hour intervals (12 am, 6 am, 12 pm, 6 pm) in each greenhouse. The experiment was conducted for one growing season from November 13, 2023, to April 4, 2024, emulating actual day lengths and temperatures from June 11 to November 4. Fertilizer (Plant Products Co. Ltd., Brampton, Ontario, Canada) was applied weekly, with 50ppm N / 81.3 ppm P / 29 ppm K during the establishment stage (the first 21 days of the experiment), followed by 100ppm N / 43.63 ppm P /99.6 ppm K during the rapid growing stage with micronutrients (55 days after establishment) (Landis & Nisley, 1990). Fertilization was stopped on September 16 (98 days after treatments began), and growth measurements were taken. The relative humidity of the greenhouses was maintained at 50 – 53% during the active growth phase and 45 -50% during the cold hardening stage.

3.3.4 Measurement of Autumnal budset and Leaf Senescence

Three samples were randomly selected per treatment replicate combination which was used to observe the timing of budset and leaf senescence in August and September of emulated date. The terminal bud development was divided into four stages (Tedla et al., 2020). Stage 0- no bud visible, stage 1- bud vivisble, stage 2 – bud scale closed but still green, stage 3- bud scale turned brown. The process of leaf senscence was observed at the initial stage when 10% of the leaves (Marien et al., 2019 and Vitasse et al., 2009).

3.3.5 Cold hardening test

The electrolyte leakage method was carried out to determine the cold hardiness after the completion of leaf senescence. A 10 cm shoot segment from each sample seedling was cut into five 2 cm-long sections and washed with distilled water (DW). Each section was placed into a separate 50 ml falcon tube and stored at 4 °C for 24 hours (Hamilton et al., 2016; Tedla et al., 2020; Man et al., 2014). The stem sections were frozen to different temperatures, +5 (control- non-freezing), -10°C, -20°C, -30°C and -45°C (Man et al., 2014). While the non-freezing samples remained in the refrigerator at 5 °C for the duration of freezing test, the other samples were exposed to -10°C, -20°C, -30°C and -45°C in a programmable freezer (Model: 45-6.8, Scientemp Corporation, Adrian, MI, USA). The freezer's temperature was reduced to 0 °C at the first hour and maintained for 1 hour at equilibrium, then lowered at a constant rate of 5 °C h^{-1}. The samples were maintained at each target temperature for 1 hour between 0 °C and -45°C before they were removed from the freezer. Upon removal from the freezer, samples were thawed at room temperature (22°C) for 24 hrs in 20ml of distilled water. The samples were then shaken 1hr prior to the initial electrical conductivity of the solution, which was measured with an Accumet AR 20 electrical conductivity meter (Fisher Scientific, Ottawa, Canada) at room temperature. The falcon tubes containing the solution and samples were then heated in a dry oven for 1 hour at 95 °C, cooled to room temperature, and shaken at 5 °C for 24 hours. The second electrical conductivity measurement of the sample was measured at room temperature. Flint's index injury I_t (Flint et al., 1967; Hamilton et al., 2016) was calculated as a percentage of injury as follows:

$$I_t = 100\ (Rt - Ro) / (1 - Ro)$$

Where $R_t = L_t/L_k$ and $R_o = L_o/L_d$, estimated from a ratio of conductivity between frozen samples preceding (L_t) and following heat kill (L_k) and between unfrozen control samples preceding (L_o) and following heat kill (L_d). The analysis was done using variance analysis (ANOVA) to test for the interactions of carbon dioxide and photoperiod on the difference in cold injury.

3.3.6 Statistical Analysis

The data were assessed for normality using the Shapiro-Wilk test and homogeneity of variance using the Levene Test (car package). A two-way analysis of variance (ANOVA) was performed to evaluate the effect of CO_2, photoperiod and their interactions. Tukey's LSD post hoc was applied to compare means when significant treatment interactive effects were found ($P \leq 0.05$). All statistical analyses and graph constructions were done using the analytical software RStudio version 4.4.2 (R Development Core Team 2024).

3.4 Results

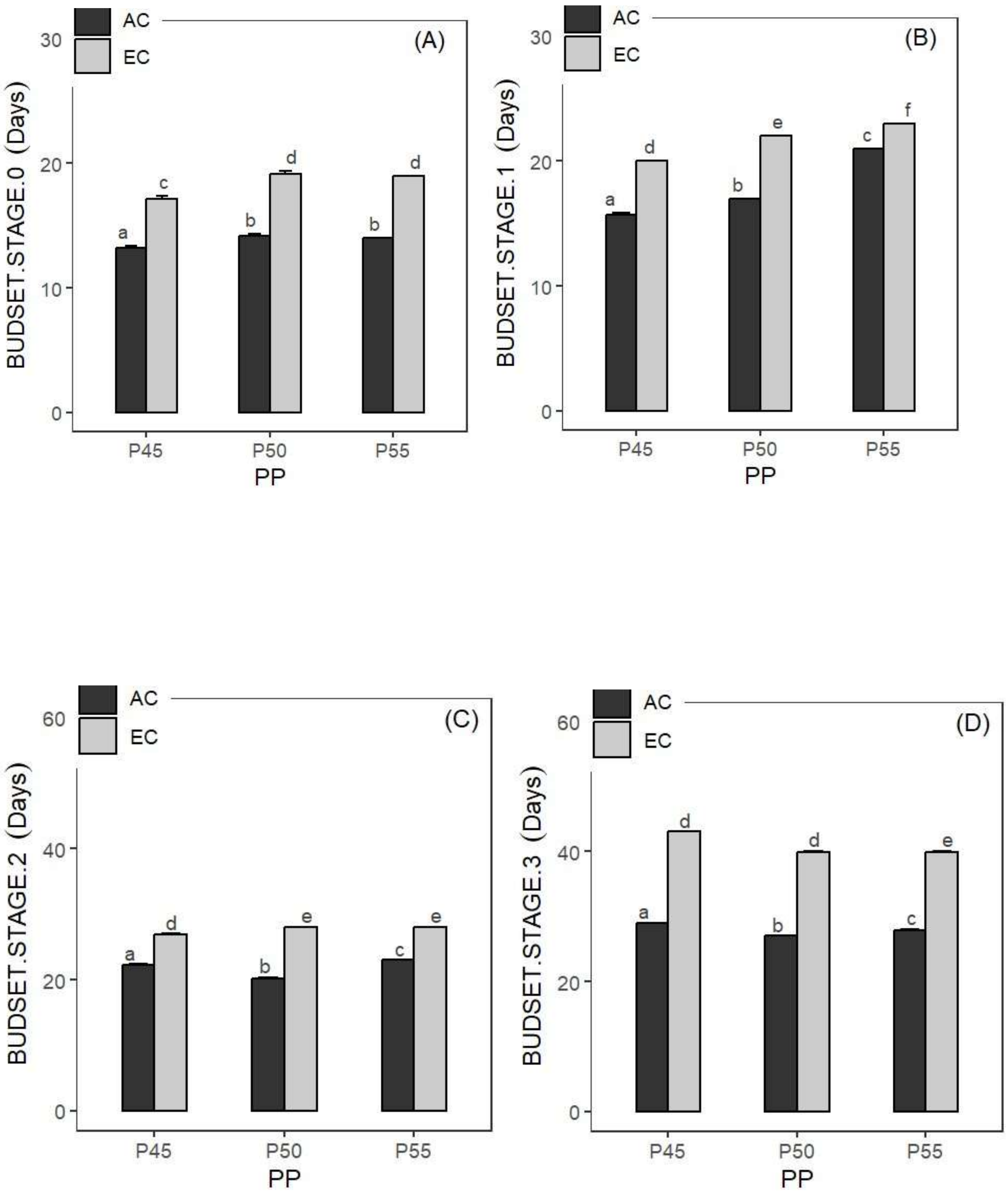
3.4.1 Bud Set Phenology and Cold Hardiness

CO_2 and photoperiod [PP] had a significant interactive effect on bud set phenology. EC generally delayed bud set at all the stages, but the number of days delayed varied with photoperiod (Table 3.1). EC delayed bud set stage 0 (i.e., 33% of leaf fall) by 4 days at P_{45}, 5 days at P_{50} and 1 day at P_{55} (Fig. 4A). At stage 1 (66% leaf fall), EC delayed bud set by 4 days at P_{45}, 5 days at P_{50} and 2 days at P_{55} (Fig 4B). Also, for stage 2 (100% leaf fall), EC delayed bud set by 9 days at P_{45}, 8 days at P_{50} and 5 days at P_{55} (Fig. 4C). For stage 3, EC was delayed by 14 days at P_{45}, 13 days at P_{50} and 12 days at P_{55} consecutively (Table 3.1, Fig 4D). The average duration of bud set phenology observed in this study is 30 days. EC generally delayed the initiation and completion of leaf senescence with an increase in photoperiod at higher latitudes (Table 4A).

The freezing injury index (*Ii*) at -45°C was significantly higher at the longest photoperiod [PP] than at the other two PP (Table 3.1, Fig 4G), while EC significantly reduced the injury index at freezing temperature -30°C (Table 3.1, Fig 4H). There was no significant interactive or individual treatment effect at freezing temperatures *Ii* (-20°C) and *Ii* (-10°C).

Table 3.1: Summary of ANOVA results (F value, P-value and degree of freedom (DF)) for the effects of carbon dioxide concentration [CO$_2$], photoperiod (PP) and their interactions on bud set stage 0, bud set stage 1, and bud set stage 2, bud set stage 2, 10% leaf senescence initiation, 90% leafs senescence initiation, injury index *I*i (-45°C), injury index *I*i (-30°C), injury index *I*i (-20°C), injury index *I*i (-10°C), of yellow birch seedlings grown at under two levels of [CO$_2$] (AC = 400 Vs. EC= 1000 μmol mol^{-1}) and three photoperiod regimes corresponding to 45°N (photoperiod at seed origin), 50°N and, 55°N latitude. Significant values (P ≤0.05) are boldfaced.

RESPONSE VARIABLES	**CO2 (DF = 1)**	**PP (DF = 2)**	**CO2*PP (DF = 2)**
BUD SET STAGE 0	446.3***	293.2***	70.9***
BUD SET STAGE 1	289***	118.77***	167.5***
BUD SET STAGE 2	128.7***	505.3***	345.7***
BUD SET STAGE 2	202.3***	279.3***	399.1***
10% INITIAL SENESCENCE	219***	126.77***	137.5***
90% COMPLETE SENESCENCE	207.3***	295.3***	364.1***
Ii (-45°C)	1.56^{ns}	5.68**	2.01^{ns}
Ii (-30°C)	21.59***	0.88ns	1.619^{ns}
Ii (-20°C)	3.234^{ns}	2.668^{ns}	2.202^{ns}
Ii (-10°C)	3.468^{ns}	0.062^{ns}	3.056^{ns}


(A)
AC
EC
BUDSET.STAGE.0 (Days)
0
10
20
30
a
c
b
d
b
d
P45
P50
P55
PP
(B)
AC
EC
BUDSET.STAGE.1 (Days)
0
10
20
30
a
d
b
e
c
f
P45
P50
P55
PP
(C)
AC
EC
BUDSET.STAGE.2 (Days)
0
20
40
60
a
d
b
e
c
e
P45
P50
P55
PP
(D)
AC
EC
BUDSET.STAGE.3 (Days)
0
20
40
60
a
d
b
d
c
e
P45
P50
P55
PP

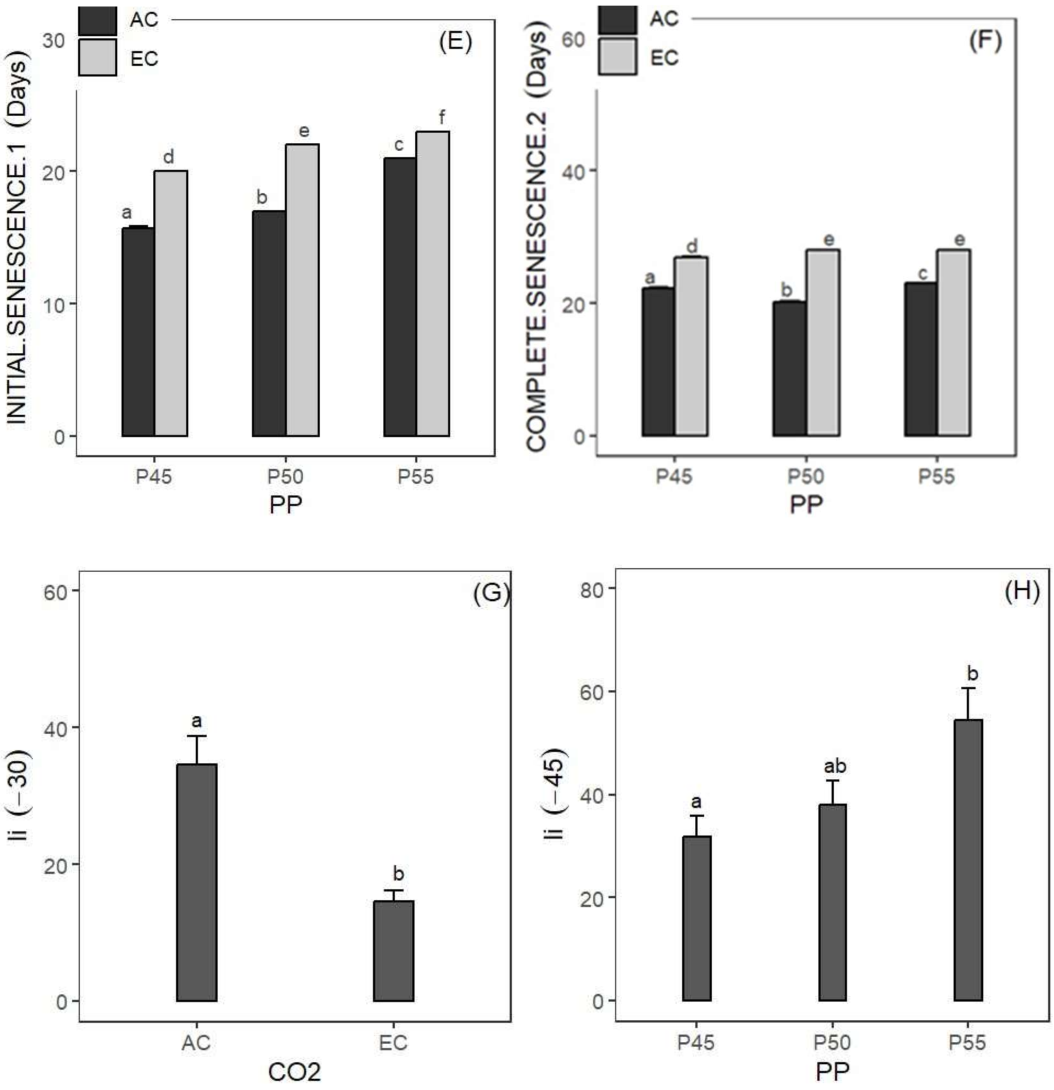


Figure 4: Mean (+SE) values for (A) bud set stage 1, (B) bud set stage 2, (C) bud set stage 3, (D) bud set stage 4, (E) 10% initial leaf senescence (F) 90% complete leaf senecence (G) injury index *I*i (-30°C), and (H) injury index *I*i (-45°C), of yellow birch seedlings grown at two levels of CO$_2$ (ambient (AC) and elevated (EC)) and three photoperiod regimes (P$_{45}$, P$_{50}$ & P$_{55}$). Data were pooled across [CO$_2$] and photoperiod [PP] for (A), (B), (C), (D) (E) (F) (n = 36, three seedlings per treatment replicate, 3 × 2CO$_2$ × 3PP × 2 replicate), [CO$_2$] for (G) (n = 12, three seedlings per treatment replicate, 3 × 2 CO$_2$ × 2 replicate), and [PP] for (H) (n = 18, three seedlings per treatment replicate, 3 × 3PP × 2 replicate). Different lowercase letters indicate statistically significant differences at $P < 0.05$.

3.5 Discussion

This experiment provided an opportunity to investigate the relationship between elevated carbon dioxide, photoperiod, and phenological shifts associated with tree growth in temperate regions. The first hypothesis and the result showed that the timing of bud set completion was delayed under elevated carbon dioxide across photoperiod regimes. However, this delay (number of days) was smaller at the longest photoperiod (P_{55}) than at the seed origin. In response to shorter photoperiod, leaves gradually reduce photosynthetic capacity after the summer solstice despite retaining their green colour (Bauerle et al., 2012). Declining radiation during autumn may further enhance the limitation on autumn photosynthesis (Zhang et al., 2020). The timing of bud set in autumn might influence the risk of frost damage late in the growing season, which could, in turn, affect nutrient resorption (Norby et al., 2000; Fracheboud et al., 2009). The prolonged process of bud set phenology under the combination of elevated CO_2 and longer photoperiod may indicate that deciduous seedlings had longer periods to develop cold hardiness (Tedla et al., 2020). On the contrary, it was discovered that EC led to an earlier start of autumn phenological events and better frost tolerance in *Picea mariana* seedlings (Bigras & Bertrand, 2006). Recent works on forest tree species showed that populations from northern latitudes exhibited greater photosynthetic efficiency than populations from lower latitudes (Elferjani et al., 2016; Oleksyn et al., 1998; Tenkanen et al., 2021).

Photoperiod sensitivity is important in regulating the transition between active growth and dormancy in hardwood trees (Garner & Allard, 1923; Kramer, 1936; Olsen, 2010; Petterle et al., 2013). Dormancy is an adaptive trait that enables perennial plants to survive seasonal environmental changes (Rohde & Bhalerao, 2007). The ability to grow while reducing the risk of winter injury due to delays in the development of dormancy and dormancy-related traits represents a critical trade-off essential for the health and survival of north-temperate and boreal tree species (Morgenstern, 1996; Howe et al., 2003; Tanino et al., 2010). However, some are of the opinion that shorter photoperiods in autumn typically mostly lead to the acquisition of cold hardiness and the formation of buds during autumn (Howe et al., 2003). The prediction that elevated carbon dioxide and longer interesting to note that yellow birch seedlings could not tolerate much colder freezing temperatures, as seen at (-45°C) with the general increase in freezing injury across the photoperiod regime. On the other hand, yellow birch significantly reduced the injury index under elevated carbon dioxide at freezing temperature (-30°C). This suggests that yellow birch can possess increased cold hardiness up to (-30°C), and a further decrease in temperature might lead to reduced cold hardiness. It has been shown that cold acclimation is mainly driven by temperature and photoperiod (Li et al., 2003). However, Wayne et al. (1998) discovered that elevated CO_2 enhanced freezing temperature of yellow birch seedlings. The enhancement of freezing tolerance by EC may be linked to an increase in the production of carbohydrates and sugars (Gandin et al., 2011). Many temperate tree species exhibit high resistance to cold, usually to -70°C during winter, and their distribution seems unrelated to temperature extremes. Species have developed strategies to adapt their level of hardiness to environmental conditions (Weiser, 1970).

In conclusion, a longer photoperiod regime will limit the scope of yellow birch migration. Yellow birch trees may not be able to survive very cold temperature such as -45°C if they migrate 10°N or more. Since there was no testing for migrations between -30 °C and -45 °C, it is possible that the scope of migration and the temperature they can withstand are somewhere in between those ranges. This study has identified a few of the primary environmental drivers of autumn phenology. However, there are other gaps in our knowledge of how climate change impacts autumn phenology. Another area of interest will be to compare the extent to which autumn responses to climate change are more genetic than plastic. Ecologists are encouraged to study the effects of climate change on autumn phenology, as very many studies have been conducted for spring phenology.

3.6 Reference

Basler, D., & Körner, C. (2012). Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology*, *165*, 73–81.

Basler, D., & Körner, C. (2014). Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology*, *34*(4), 377–388.

Beaudet, M., & Messier, C. (1998). Growth and morphological responses of yellow birch, sugar maple, and beech seedlings growing under a natural light gradient. *Canadian Journal of Forest Research*, *28*(7), 1007–1015.

Bradshaw, W. E., Zani, P. A., & Holzapfel, C. M. (2004). Adaptation to temperate climates. *Evolution*, *58*(8), 1748–1762.

Brunner, A. M., Varkonyi-Gasic, E., & Jones, R. C. (2017). Phase change and phenology in trees. *Comparative and Evolutionary Genomics of Angiosperm Trees*, 227–274.

Calmé, S., Bigras, F. J., Margolis, H. A., & Hébert, C. (1994). Frost tolerance and bud dormancy of container-grown yellow birch, red oak and sugar maple seedlings. *Tree Physiology*, *14*(12), 1313–1325.

Charrier, G., Ngao, J., Saudreau, M., & Améglio, T. (2015). Effects of environmental factors and management practices on microclimate, winter physiology, and frost resistance in trees. *Frontiers in Plant Science*, *6*, 259.

Delagrange, S. (2011). Light-and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. *Environmental and Experimental Botany*, *70*(1), 1–10.

Dreiss, L. M., & Volin, J. C. (2020). Forests: Temperate evergreen and deciduous. In *Terrestrial Ecosystems and Biodiversity* (pp. 213–226). CRC Press.

Elferjani, R., DesRochers, A., & Tremblay, F. (2016). Plasticity of bud phenology and photosynthetic capacity in hybrid poplar plantations along a latitudinal gradient in northeastern Canada. *Environmental and Experimental Botany*, *125*, 67–76.

Ettinger, A., Buonaiuto, D., Chamberlain, C., Morales-Castilla, I., & Wolkovich, E. (2021). Spatial and temporal shifts in photoperiod with climate change. *New Phytologist*, *230*(2), 462–474.

Flynn, D., & Wolkovich, E. (2018). Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist*, *219*(4), 1353–1362.

Gill, A. L., Gallinat, A. S., Sanders-DeMott, R., Rigden, A. J., Short Gianotti, D. J., Mantooth, J. A., & Templer, P. H. (2015). Changes in autumn senescence in northern hemisphere deciduous trees: A meta-analysis of autumn phenology studies. *Annals of Botany*, *116*(6), 875–888.

Gunderson, C. A., Edwards, N. T., Walker, A. V., O'Hara, K. H., Campion, C. M., & Hanson, P. J. (2012). Forest phenology and a warmer climate–growing season extension in relation to climatic provenance. *Global Change Biology*, *18*(6), 2008–2025.

Hamilton, J. A., El Kayal, W., Hart, A. T., Runcie, D. E., Arango-Velez, A., & Cooke, J. E. (2016). The joint influence of photoperiod and temperature during growth cessation and development of dormancy in white spruce (Picea glauca). *Tree Physiology*, *36*(11), 1432–1448.

Hänninen, H. (2016). Boreal and temperate trees in a changing climate. *Biometeorology. Springer, Dordrecht, Netherlands*.

Hänninen, H., & Hänninen, H. (2016). Climatic adaptation of boreal and temperate tree species. *Boreal and Temperate Trees in a Changing Climate: Modelling the Ecophysiology of Seasonality*, 1–13.

Harrington, C. A., Gould, P. J., & Clair, J. B. S. (2010). Modeling the effects of winter environment on dormancy release of Douglas-fir. *Forest Ecology and Management*, *259*(4), 798–808.

Heide, O. M. (1974). Growth and dormancy in Norway spruce ecotypes (Picea abies) I. Interaction of photoperiod and temperature. *Physiologia Plantarum*, *30*(1), 1–12.

Helm, B., Ben-Shlomo, R., Sheriff, M. J., Hut, R. A., Foster, R., Barnes, B. M., & Dominoni, D. (2013). Annual rhythms that underlie phenology: Biological time-keeping meets environmental change. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130016.

Huber, B. M., Louws, F. J., & Hernández, R. (2021). Impact of different daily light integrals and carbon dioxide concentrations on the growth, morphology, and production efficiency of tomato seedlings. *Frontiers in Plant Science*, *12*, 615853.

Inoue, S., Dang, Q.-L., Man, R., & Tedla, B. (2020). Photoperiod and CO2 elevation influence morphological and physiological responses to drought in trembling aspen: Implications for climate change-induced migration. *Tree Physiology*, *40*(7), 917–927.

Kalberer, S. R., Wisniewski, M., & Arora, R. (2006). Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts. *Plant Science*, *171*(1), 3–16.

Kramer, K., Leinonen, I., & Loustau, D. (2000). The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: An overview. *International Journal of Biometeorology*, *44*, 67–75.

Liu, Q., Piao, S., Campioli, M., Gao, M., Fu, Y. H., Wang, K., He, Y., Li, X., & Janssens, I. A. (2020). Modeling leaf senescence of deciduous tree species in Europe. *Global Change Biology*, *26*(7), 4104–4118.

Lorenzetti, F., Tyler, B., Cooper, J., & Breese, E. (1971). Cold tolerance and winter hardiness in Lolium perenne: I. Development of screening techniques for cold tolerance and survey of geographical variation. *The Journal of Agricultural Science*, *76*(2), 199–209.

Man, R., Colombo, S., Lu, P., Li, J., & Dang, Q.-L. (2014). Trembling aspen, balsam poplar, and white birch respond differently to experimental warming in winter months. *Canadian Journal of Forest Research*, *44*(12), 1469–1476.

McKENNEY, D. W., Pedlar, J. H., Rood, R. B., & Price, D. (2011). Revisiting projected shifts in the climate envelopes of North American trees using updated general circulation models. *Global Change Biology*, *17*(8), 2720–2730.

Montgomery, R. A., Rice, K. E., Stefanski, A., Rich, R. L., & Reich, P. B. (2020). Phenological responses of temperate and boreal trees to warming depend on ambient spring temperatures, leaf habit, and geographic range. *Proceedings of the National Academy of Sciences*, *117*(19), 10397–10405.

Moser, L., Fonti, P., Büntgen, U., Esper, J., Luterbacher, J., Franzen, J., & Frank, D. (2010). Timing and duration of European larch growing season along altitudinal gradients in the Swiss Alps. *Tree Physiology*, *30*(2), 225–233.

Newaz, M. S., Dang, Q.-L., & Man, R. (2016). Morphological response of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod. *American Journal of Plant Sciences*, *7*(6), 879–893.

Newaz, S. M., Dang, Q.-L., & Man, R. (2021). CO2 elevation and soil warming reduce cold hardiness of jack pine under photoperiods of seed origin and latitudes of potential migration. *New Forests*, *52*(5), 777–790.

Oleksyn, J., Modrzýnski, J., Tjoelker, M., Z· ytkowiak, R., Reich, P. B., & Karolewski, P. (1998). Growth and physiology of Picea abies populations from elevational transects: Common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology*, *12*(4), 573–590.

Parmesan, C., Burrows, M. T., Duarte, C. M., Poloczanska, E. S., Richardson, A. J., Schoeman, D. S., & Singer, M. C. (2013). Beyond climate change attribution in conservation and ecological research. *Ecology Letters*, *16*, 58–71.

Partanen, J., Koski, V., & Hänninen, H. (1998). Effects of photoperiod and temperature on the timing of bud burst in Norway spruce (Picea abies). *Tree Physiology*, *18*(12), 811–816.

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography*, *12*(5), 361–371.

Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., & Zhu, X. (2019). Plant phenology and global climate change: Current progresses and challenges. *Global Change Biology*, *25*(6), 1922–1940.

Pletsers, A., Caffarra, A., Kelleher, C. T., & Donnelly, A. (2015). Chilling temperature and photoperiod influence the timing of bud burst in juvenile Betula pubescens Ehrh. And Populus tremula L. trees. *Annals of Forest Science*, *72*, 941–953.

Prentice, I. C., Farquhar, G., Fasham, M., Goulden, M. L., Heimann, M., Jaramillo, V., Kheshgi, H., Le Quéré, C., Scholes, R. J., & Wallace, D. W. (2001). The carbon cycle and atmospheric carbon dioxide. *Climate Change 2001: The Scientific Basis, Intergovernmental Panel on Climate Change*.

Rapacz, M., Ergon, Å., Höglind, M., Jørgensen, M., Jurczyk, B., Østrem, L., Rognli, O. A., & Tronsmo, A. M. (2014). Overwintering of herbaceous plants in a changing climate. Still more questions than answers. *Plant Science*, *225*, 34–44.

Reekie, J., Hicklenton, P., & Reekie, E. (1997). The Interactive Effects of Carbon Dioxide Enrichment and Daylength on Growth and Development inPetunia hybrida. *Annals of Botany*, *80*(1), 57–64.

Richardson, A. D., Andy Black, T., Ciais, P., Delbart, N., Friedl, M. A., Gobron, N., Hollinger, D. Y., Kutsch, W. L., Longdoz, B., & Luyssaert, S. (2010). Influence of spring and autumn phenological transitions on forest ecosystem productivity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3227–3246.

Rohde, A., & Bhalerao, R. P. (2007). Plant dormancy in the perennial context. *Trends in Plant Science*, *12*(5), 217–223.

Saikkonen, K., Taulavuori, K., Hyvönen, T., Gundel, P. E., Hamilton, C. E., Vänninen, I., Nissinen, A., & Helander, M. (2012). Climate change-driven species' range shifts filtered by photoperiodism. *Nature Climate Change*, *2*(4), 239–242.

Singh, R. K., Bhalerao, R. P., & Eriksson, M. E. (2021). Growing in time: Exploring the molecular mechanisms of tree growth. *Tree Physiology*, *41*(4), 657–678.

Singh, R. K., Svystun, T., AlDahmash, B., Jönsson, A. M., & Bhalerao, R. P. (2017). Photoperiod-and temperature-mediated control of phenology in trees–a molecular perspective. *New Phytologist*, *213*(2), 511–524.

Tenkanen, A., Suprun, S., Oksanen, E., Keinänen, M., Keski-Saari, S., & Kontunen-Soppela, S. (2021). Strategy by latitude? Higher photosynthetic capacity and root mass fraction in northern than southern silver birch (Betula pendula Roth) in uniform growing conditions. *Tree Physiology*, *41*(6), 974–991.

Visser, M. E., Caro, S. P., Van Oers, K., Schaper, S. V., & Helm, B. (2010). Phenology, seasonal timing and circannual rhythms: Towards a unified framework. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3113–3127.

Vitasse, Y., Lenz, A., & Körner, C. (2014). The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in Plant Science*, *5*, 541.

Vitasse, Y., Porté, A. J., Kremer, A., Michalet, R., & Delzon, S. (2009). Responses of canopy duration to temperature changes in four temperate tree species: Relative contributions of spring and autumn leaf phenology. *Oecologia*, *161*, 187–198.

Wang, S., Wu, Z., Gong, Y., Wang, S., Zhang, W., Zhang, S., De Boeck, H. J., & Fu, Y. H. (2022). Climate warming shifts the time interval between flowering and leaf unfolding depending on the warming period. *Science China Life Sciences*, *65*(11), 2316–2324.

Way, D. A. (2011). Tree phenology responses to warming: Spring forward, fall back? *Tree Physiology*, *31*(5), 469–471.

Way, D. A., & Montgomery, R. A. (2015). Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment*, *38*(9), 1725–1736.

Wielgolaski, F. E., & Inouye, D. W. (2003). High latitude climates. *Phenology: An Integrative Environmental Science*, 175–194.

Wisniewski, M., Nassuth, A., Teulieres, C., Marque, C., Rowland, J., Cao, P. B., & Brown, A. (2014). Genomics of cold hardiness in woody plants. *Critical Reviews in Plant Sciences*, *33*(2–3), 92–124.

Wu, C., Gough, C. M., Chen, J. M., & Gonsamo, A. (2013). Evidence of autumn phenology control on annual net ecosystem productivity in two temperate deciduous forests. *Ecological Engineering*, *60*, 88–95.

Wuebbles, D. J., Fahey, D. W., Hibbard, K. A., Arnold, J. R., DeAngelo, B., Doherty, S., Easterling, D. R., Edmonds, J., Edmonds, T., & Hall, T. (2017). *Climate science special report: Fourth national climate assessment (NCA4), Volume I*.

Zohner, C. M., Benito, B. M., Svenning, J.-C., & Renner, S. S. (2016). Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. *Nature Climate Change*, *6*(12), 1120–1123.

Chapter 4: Interactive effect of drought and increasing carbon dioxide on the physiology and morphology of yellow birch

4.1 Abstract

In temperate and boreal regions, climate change is anticipated to increase the frequency of prolonged summer droughts. Increasing atmospheric carbon dioxide levels associated with climate change are known to influence how various forest tree species respond to drought conditions. In this study, physiological and morphological traits were assessed. We exposed yellow birch (*Betula alleghaniensis*) seedlings to the current and predicted future carbon dioxide levels (400 vs. 1000 μmol m^{-1}) in the greenhouse, and our results revealed that maximum photosynthetic electron transport rate (J_{max}) and the ratio of electron transport rate to carboxylation rate (J_{max}/Vc_{max}) were significantly increased under drought stress and elevated carbon dioxide. This contradicts the reduction trend in photosynthesis machinery. Drought stress significantly reduced specific leaf area (SLA) and height growth in response to elevated carbon dioxide. Biomass allocation was higher for the root system with increased root dry mass (ROOTDM) and root mass ratio (RMR) under drought stress and elevated carbon dioxide. Overall, these findings indicate that the interactive effect of drought and elevated carbon dioxide enhanced some aspects of photosynthesis machinery, while reducing growth and shifting biomass belowground. This suggests an adaptive strategy focused more on stress tolerance than productivity, which could influence its regenration survival performance under future climate change scenarios.

4.2 Introduction

Since the Industrial Revolution, atmospheric carbon dioxide and other greenhouse gases have increased substantially (IPCC, 2014; Masson-Delmotte et al., 2021). Increased carbon dioxide regulates global climate, influencing temperature and precipitation patterns (Berg & Sheffield, 2018; Wuebbles et al., 2017). Moreover, burning fossil fuels and land-use changes, driven by population growth have been identified as key contributors to climate change (Canadell et al., 2023). Rising atmospheric carbon dioxide, also known as elevated carbon dioxide (EC), is expected to contribute to climate warming, which may likely result in more frequent and intense drought events (Allen et al., 2010; Boisvenue & Running, 2006). Under these conditions, significant changes in plant physiology and morphology are expected, as reduced water availability may limit tree growth and survival (Adams et al., 2017; Berg & Sheffield, 2018; Rais et al., 2014; Siddique et al., 2016). The response to drought stress under elevated carbon dioxide is expected to vary depending on the tree species (Avila et al., 2020; McDowell et al., 2022; Walters et al., 2023; Warren et al., 2011)Understanding the trade-offs among tree species' functional traits is crucial for adapting to a changing climate and its associated stressors. Therefore, gaining deeper insight into the combined impacts of elevated carbon dioxide (EC) and drought on tree physiology and morphology is essential.

Drought affects various aspects of tree morphology (Allen et al., 2010; Bréda et al., 2006; Chaves et al., 2003; Choat et al., 2018), such as leaves. Leaf functional traits are important indicators of a tree's ability to acquire, utilize and conserve resources under environmental stress (Delagrange, 2011; Zhu et al., 2021). Under drought conditions, many trees reduce total leaf area (TLA) to minimize water loss (Dobbertin et al., 2010; Liu & Stützel, 2004; Rötzer et al., 2017). Drought can also increase biomass allocation to fine root growth (Brunner et al., 2015; Nejad, 2011; Rötzer et al., 2017; Walters et al., 2023). It is well established that drought affects several physiological processes, such as photosynthetic activity (Bréda et al., 2006; Cornic & Massacci, 1996; Siddique et al., 2016). Under drought conditions, reduced stomatal conductance (g_s) limits [CO_2] diffusion into the leaf, subsequently decreasing net photosynthesis (A_n) and potentially imposing carbon limitations on growth (Damour et al., 2010; Field et al., 1995; Sperry et al., 2017). With the projected increase of [CO_2] and drought conditions, we need to know how trees will respond to these environmental factors.

Elevated carbon dioxide (EC) can enhance biomass production through improved water use efficiency, reduced stomatal conductance and altered carbon allocation patterns (Bunce, 2004; Damour et al., 2010; Medlyn et al., 2001; Mitchell et al., 2014; Ruehr et al., 2019). It was reported that EC enhanced biomass allocation to below-ground tissues, leading to an increased root-to-shoot ratio (Thompson et al., 2017; Wang et al., 2020). Also, EC enhances photosynthesis in many tree species by increasing the substrate for the Calvin cycle (Bowes, 1996; Reddy et al., 2010). Though the degree of stimulation varies among tree species. This variation underscores the importance of understanding how specific species, such as yellow birch respond to drought under EC conditions. EC enhance water use efficiency (WUE) by inducing stomatal closure and reducing transpiration while stimulating photosynthesis (Long et al., 2004). In the temperate forests of North America, yellow birch (*Betula alleghaniensis* Britton) relies on sufficient water availability for successful regeneration (Gasser et al., 2010; Raymond & Dumais, 2023). This tree species thrives best in mesic forest environments with full sun exposure and well-drained soils (Cheng, 2007). However, the extent of its adaptation to water deficits after regeneration and the processes driving this adaptation remain uncertain. Trends of growth decline and mortality have been associated with severe drought stress (Adams et al., 2017; Allen et al., 2010; Hartmann et al., 2018). Trees have developed mechanisms to either prevent or withstand drought through different adaptive responses (Bréda et al., 2006; Chaves et al., 2003; Oguz et al., 2022; Walters et al., 2023; Yang et al., 2021). Studies have shown that EC increases leaf area under well watred soil moisture, but this increase remains the same under drought stress. Also, some believe that a change in leaf area under EC and drought stress may not be synchronous, which can affect water use efficiency. However, some studies suggest that EC could alleviate the adverse effects of drought on photosynthesis and biomass production (Wang et al., 2022). Therefore, we need to know how leaf gas exchange, water use efficiency and tree morphological structures respond to drought stress and elevated carbon dioxide concentration, especially for temperate deciduous trees like yellow birch.

This study evaluated yellow birch's physiological and morphological responses to drought stress under increasing [CO_2] elevation. Morphological response and gas exchange parameters, including net photosynthetic rate (A_n), transpiration rate E and stomatal conductance (g_s), were observed and analyzed.

My underlying hypotheses were that:

1) The combined effect of drought and elevated carbon dioxide will reduce tree growth and reduce biomass production.
2) Elevated carbon dioxide and drought stress will allocate more biomass to the root, increase the root-to-shoot ratio and reduce leaf area.
3) The combined effect of drought and elevated carbon dioxide will reduce photosynthetic activity.

4.3 Materials and Methods

4.3.1 Planting Material and Growth Treatment

Yellow birch seeds were sourced from the National Tree Seed Center in Fredericton and were stored at 4°C in the laboratory. The seeds were originally from Prosser Brook, New Brunswick, Canada (45.80°N, 64.92°W). The current mean annual temperature is 5.6 °C, and mean January and July temperatures are -9.4°C and 19.3°C, respectively. Mean annual precipitation is 10777.7mm, and the average growing season is 130 days (Environment Canada, 2023). According to standard procedure, yellow birch seeds were stratified for three weeks (Bonner & Karrfalt, 2015). Following stratification, seeds were sown in germination trays (50 cm × 25 cm × 5 cm) with a mixture of vermiculite and peat moss (1:1, v: v) at Lakehead University's growth chamber in Thunder Bay, Ontario. The temperature and photoperiod during germination were 25/16°C (day/night) and 16-hours photoperiod. Natural lights were enhanced with high-pressure sodium lamps when the flux density of photosynthetically active radiation (PAR) was below 500 µmol $m^{-2}s^{-1}$. The seedlings were transplanted into bigger pots filled with vermiculite and peat moss (1:2, v: v) after the first two leaves sprouted (10 days after germination). Potted seedlings were moved to the treatment room three days after transplanting.

4.3.2 Experimental Design

The experiment was conducted in four greenhouses at Lakehead University, Thunder Bay, Ontario, Canada. A split-plot design was employed, with the (CO_2) treatment (AC: 400 µmol mol^{-1} vs EC: 1000 µmol mol^{-1}) as the main plot and soil moisture regime (well-watered [WW] and drought stress [DS]) as the sub-plot. Each (CO_2) had two independent replicates randomly assigned to four separate greenhouses. Twelve seedlings of relatively uniform height were assigned to each of the two (CO_2) levels and two soil moisture regimes with treatment replicate combination (12 × 2 CO_2 × 2 soil moisture × 2 replicate = 96 seedlings).

4.3.3 Environment Controls

The environmental conditions in each greenhouse were independently monitored and regulated using the Argus Titan Environment Control System (Argus Controls Systems Ltd., Surrey, BC, Canada). The ambient (CO_2) treatment, which represented the current atmospheric CO_2 concentration, had no additional (CO_2) added. The elevated treatment held the (CO_2) concentration at 1000ppm, mimicking the projected (CO_2) concentration by the end of this century (IPCC, 2014). The carbon dioxide elevation was carried out using the model GEN2E gas generators made by Custom Automated Products Inc. (Riverside, California, USA). The photoperiod in each greenhouse was set according to the photoperiod of Proser Brooks, New Brunswick. High-pressure sodium lamps (Model LR48877, P.L. Systems, Grimsby, ON, Canada) were used to supplement ambient light during cloudy conditions early mornings and late evenings. Drought stress was initiated 30 days after the start of the experiment. The soil moisture content in the drought treatment was maintained at 55% - 60% of the field capacity as measured with a Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). A preliminary test showed that the permanent wilting point was at a volumetric water content of 5%. The well-watered and the drought treatments were allowed to dry down to 25% and 10% VWC, respectively. The treatments were then watered to 60% and 20%, respectively. The soil volumetric water content was measured throughout the experiment. The experiment was run from June 11 to September 20. The temperature and photoperiod in all the greenhouses emulated the seasonal and diurnal conditions of an average growing season at the seed origin. The temperature and photoperiod were adjusted biweekly based on the weekly averages of the past three years for seed origin (Environment Canada, 2023). The daily temperature was ramped at 6-hour intervals (12 am, 6 am, 12 pm, 6 pm) in each greenhouse. Fertilization was done weekly with 50 mg/L N // 81.3 mg/L P // 9 mg/L K at the establishment stage (for 21 days after the start of the experiment), and 100 mg/L N // 43.6 mg/L P // 99.6 mg/L K at the rapid growing stage (for 55 days after the establishment stage). Fertilization stopped 98 days from the start of treatment after growth measurements were made.

4.3.4 Growth Measurement and Biomass Partitioning

Three seedlings were randomly chosen from each treatment combination and height (mm) and root collar diameter (mm) recorded prior to the start of drought treatment and at the end of the experiment. The seedlings were harvested and sectioned into leaves, stems and roots. The total leaf area of each seedling was determined using a Regent winFolia system (Regent Instruments Inc., Quebec City, Canada). The specific leaf area was determined as the ratio of total leaf area per seedling to total leaf dry mass. All plant sections were oven-dried at 80°C for 48h to obtain dry mass of the leaf, stem, root and the biomass ratios among plant organs.

4.3.5 Gas Exchange Measurement

The photosynthetic response curve to internal [CO_2] concentration (A/C_i curves) was measured on three randomly selected seedlings from each treatment-replication combination. For selected seedlings, leaf gas exchange measurements were performed (47th – 52nd day after drought initiation) on fully expanded leaf (5^{th} or 6^{th} from the apex) using a Li-6800 gas exchange system (Li-Cor, Lincoln, NE, USA). Hourly measurements from each treatment combination showed that gas exchange parameters were relatively stable between 8:00 am and 3:30 pm. Therefore, all subsequent measurements were carried out during this period. The measurements were conducted at 23°C air temperature, 50% relative humidity, at 800 µmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR), and the following [CO_2]: 400*, 40, 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 700, 900, 1000*, 1,300 and 1,500 ppm µmol mol^{-1} [CO_2]. The *A/C_i curves* were analyzed using the fitaci function of the plantecophys package (Duursma, 2015) in R 4.4.2 (Programming software, 2024) to determine the maximum carboxylation rate of ribulose-1,5-bisphosphate (RuBP) (Vcmax, µmol $m^{-2}s^{-1}$), and the maximum electron transport rate photosynthesis (J_{max}, µmol m^{-2} s^{-1}) (Duursma, 2015). The light-saturated (PAR 650 µmol $m^{-2}s^{-1}$) net photosynthetic rate (A_n) measured at growth CO_2 (400 and 1000 µmol mol^{-1} was extracted from the A/C_i data for each tree. Other physiological parameter such as stomatal conductance (g_s), transpiration (E) and Water use efficiency (WUE*)* were measured at corresponding [CO_2] levels.

4.3.6 Statistical Analysis

The normality of distribution and homogeneity of variance of the data sets were tested with Shapiro-Wilk test and Bartlett test. Interactive effects of carbon dioxide and soil moisture were assessed using two-way Anova. The Analyses were conducted in R programming software (4.4.2) (2004).

4.4 Results

4.4.1 Growth Analysis

A significant interaction was observed between soil moisture and carbon dioxide effects on height growth and *SLA* (Table 4.1). Drought stress significantly decreased tree height under elevated carbon dioxide by 25%, but the reduction is less severe when compared to ambient carbon dioxide treatment (Fig. 5A). Furthermore, drought stress significantly reduced *SLA* by 24.2% under elevated carbon dioxide and by 51% under ambient carbon dioxide (Fig. 5B). This reduction may indicate the formation of thicker and denser leaves to conserve water. Drought stress significantly reduced total leaf area by 28% (Fig 5C) compared with the well-watered treatment. EC significantly increased *RCD* by 1.4% (Table 4.1, Fig. 5D).

Table 4.1: Summary of ANOVA results (F value, P-value and degree of freedom (DF) for the effects of carbon dioxide concentration [CO_2], photoperiod [PP] and their interactions on Height, specific leaf area (*SLA*), total leaf area (*TLA*) and root collar diameter (*RCD*) of yellow birch seedlings grown under two levels of CO_2 (AC = 400 Vs. EC = 1000 µmol mol^{-1}) and two soil moisture regimes (drought stress and well-watered) at seed origin. Significant values ($P \leq 0.05$) are boldfaced.

RESPONSE VARIABLE	CO2 (DF = 1)	SM (DF = 1)	CO2*SM (DF = 1)
HEIGHT	68.92***	55.65***	4.3**
SLA	33.99***	95.17***	28.14***
RCD	14.16***	0.13^{ns}	1.85^{ns}
TLA	1.72^{ns}	33.87***	0.54^{ns}

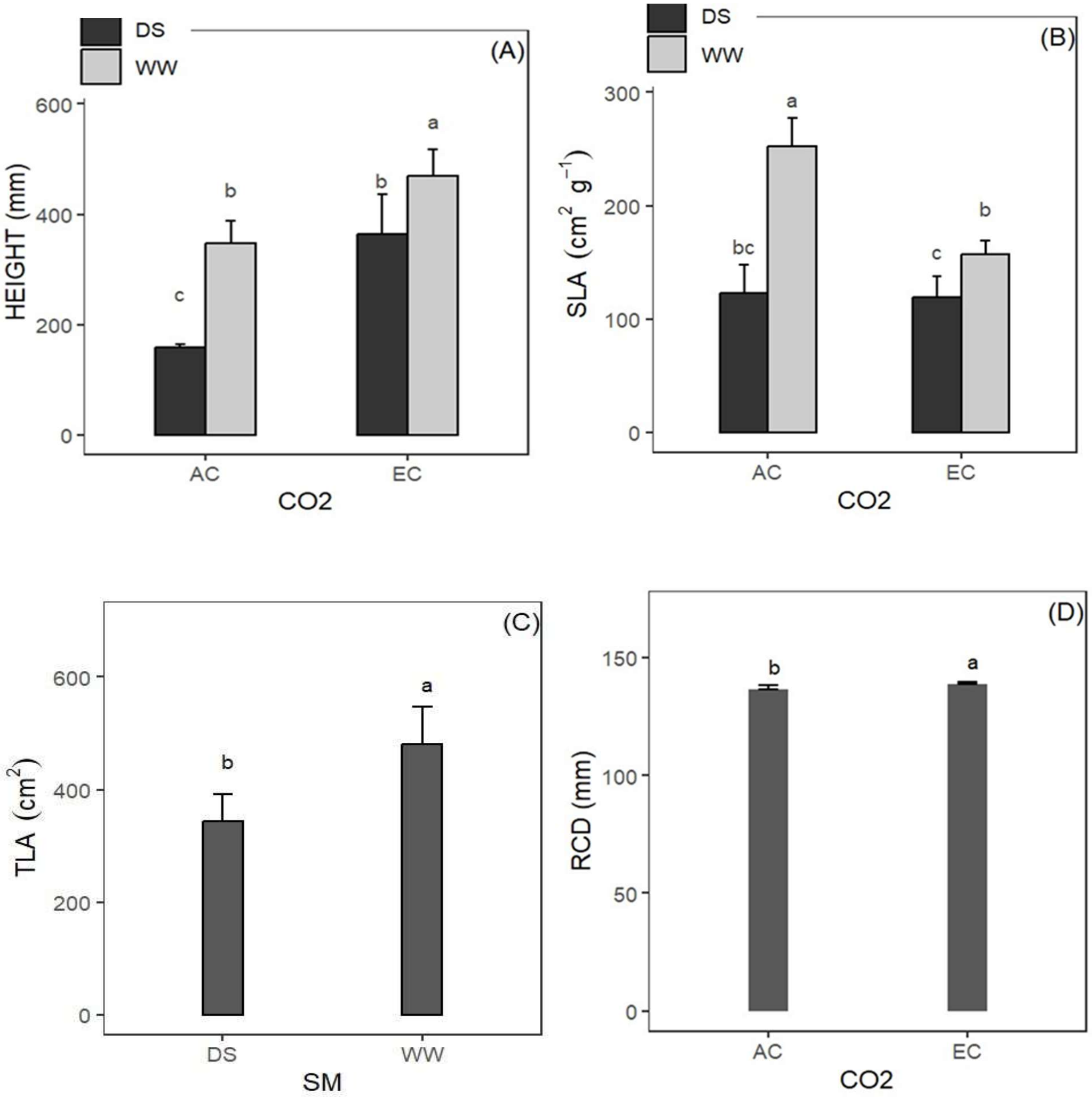


Figure 5: Mean (+SE) values for (A, B) Height, (C) specific leaf area (*SLA*), (D) total leaf area (*TLA*) and (E) root collar diameter (*RCD*) of yellow birch seedlings grown under two levels of [CO_2] (ambient (AC) and elevated (EC)) and two levels of soil moisture regime (DS and WW). Data were pooled across [CO_2] and soil moisture replicate, (C) (n = 24, three seedlings per treatment replicate, 3 × 2CO_2 × 2SM × 2 replicate), [CO_2] for (A) and (E) (n = 12, three seedlings per treatment replicate, 3 × 2CO_2 × 2 replicate) and SM for (B) and (D) (n = 12, three seedlings per treatment replicate, 3 × 2SM × 2 replicate). Different lowercase letters indicate statistically significant differences at (**$P < 0.05$).**

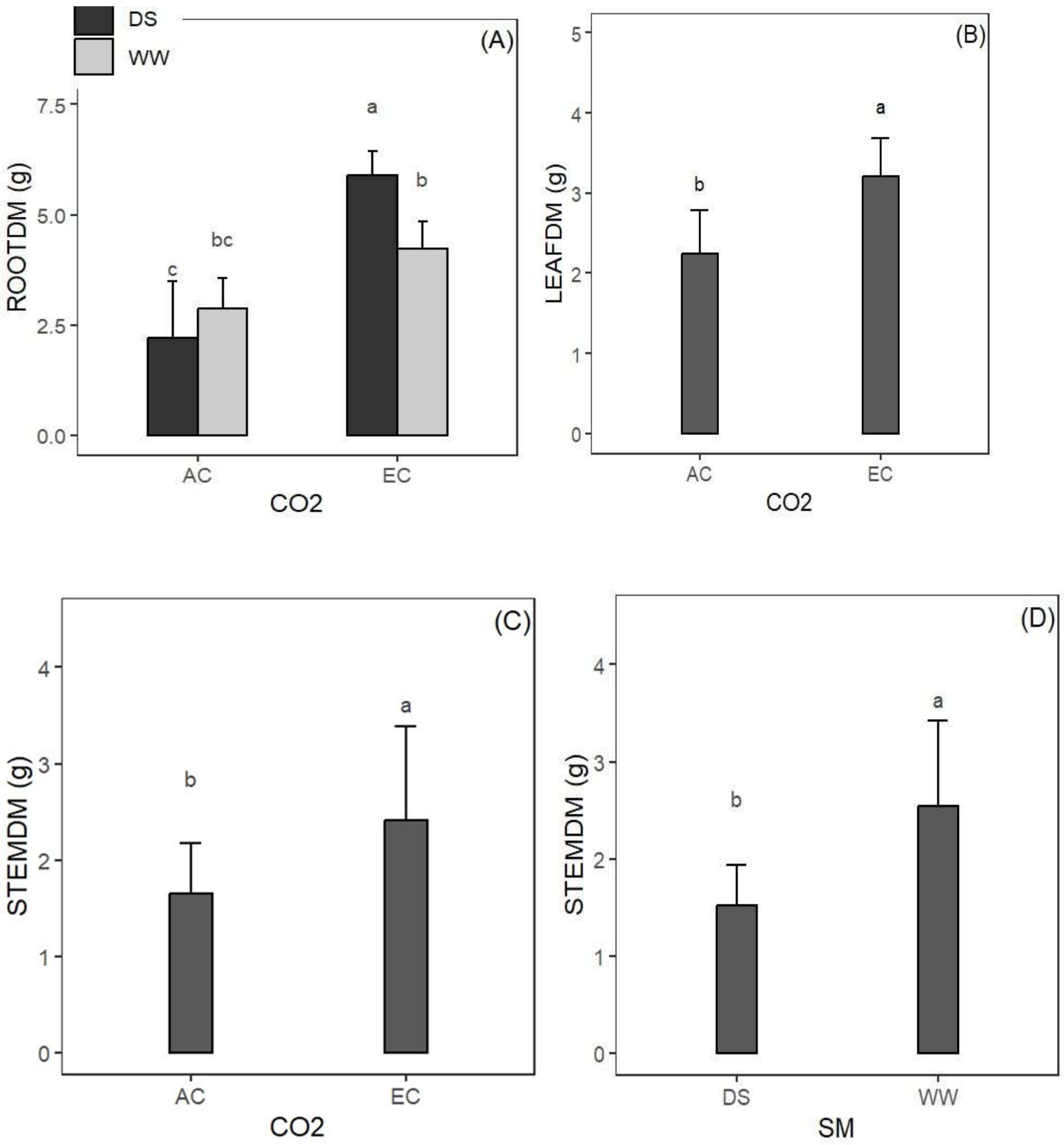
4.4.2 Biomass and Biomass Allocation

Soil moisture and carbon dioxide had a significant effect on root dry mass (ROOTDM) and root mass ratio (RMR) (Table 4.2). Drought stress significantly increased *ROOTDM* by 6.5% under elevated carbon dioxide (Fig. 6A), while *ROOTDM* was significantly reduced by 2.5% under ambient conditions. In addition, drought stress had a greater impact on *ROOTDM* under EC by (25%) than under the ambient CO_2 (Fig. 6F).

Elevated Carbon dioxide had a significant effect on leaf (LEAFDM), stem (STEMDM), and total seedling total (TSDM) dry mass (Table 4.2). EC significantly increased *LEAFDM* by 29.4% (Fig. 6B), *STEMDM* 40% and *TSDM by* 23% (Fig. 6C & 6E). Furthermore, soil moisture had significant effect on *STEMDM,* leaf mass ratio (LMR), and stem mass ratio (SMR). *STEMDM* and *SMR* significantly reduced under drought stress, showing a 33% and 45.5% decrease compared to well-watered conditions (Fig. 6D & 6H), respectively. However, *LMR* was significantly higher under drought stress, with a 28.6% increase when compared to well-watered conditions (Fig. 6G).

Table 4.2: Summary of ANOVA results (F value, P-value and Degree of freedom (DF) for the effects of carbon dioxide concentration [CO_2], photoperiod (PP) and their interactions on root dry mass (*ROOTDM*), leaf dry mass (*LEAFDM*), stem dry mass (*STEMDM*), total seedling dry mass (*TSDM*), root mass ratio *(RMR),* leaf mass ratio (*LMR*), shoot mass ratio (*SMR*) and root to shoot ratio (*RSR*) of yellow birch seedlings grown under two levels of CO_2 (AC = 400 Vs. EC = 1000 µmol mol^{-1}) and two soil moisture regimes (drought stress and well-watered) at seed origin. Significant values (P ≤0.05) are boldfaced.

RESPONSE VARIABLE	**CO2 (DF = 1)**	**SM (DF = 1)**	**CO2*SM (DF = 1)**
ROOTDM	53.90***	2.06^{ns}	11.58***
LEAFDM	21.29***	3.79^{ns}	3.52^{ns}
STEMDM	9.31**	36.93***	4.14^{ns}
TSDM	99.01***	0.14^{ns}	0.34^{ns}
RMR	6.50^{ns}	0.72^{ns}	12.21***
LMR	6.36^{ns}	1.38**	17.66^{ns}
SMR	4.92^{ns}	40.54***	0.23^{ns}
RSR	7.20^{ns}	2.95^{ns}	14.15^{ns}



DS
WW
(A)
ROOTDM (g)
7.5
5.0
2.5
0.0
a
b
c
bc
AC
EC
CO2
(B)
LEAFDM (g)
5
4
3
2
1
0
b
a
AC
EC
CO2
(C)
STEMDM (g)
4
3
2
1
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b
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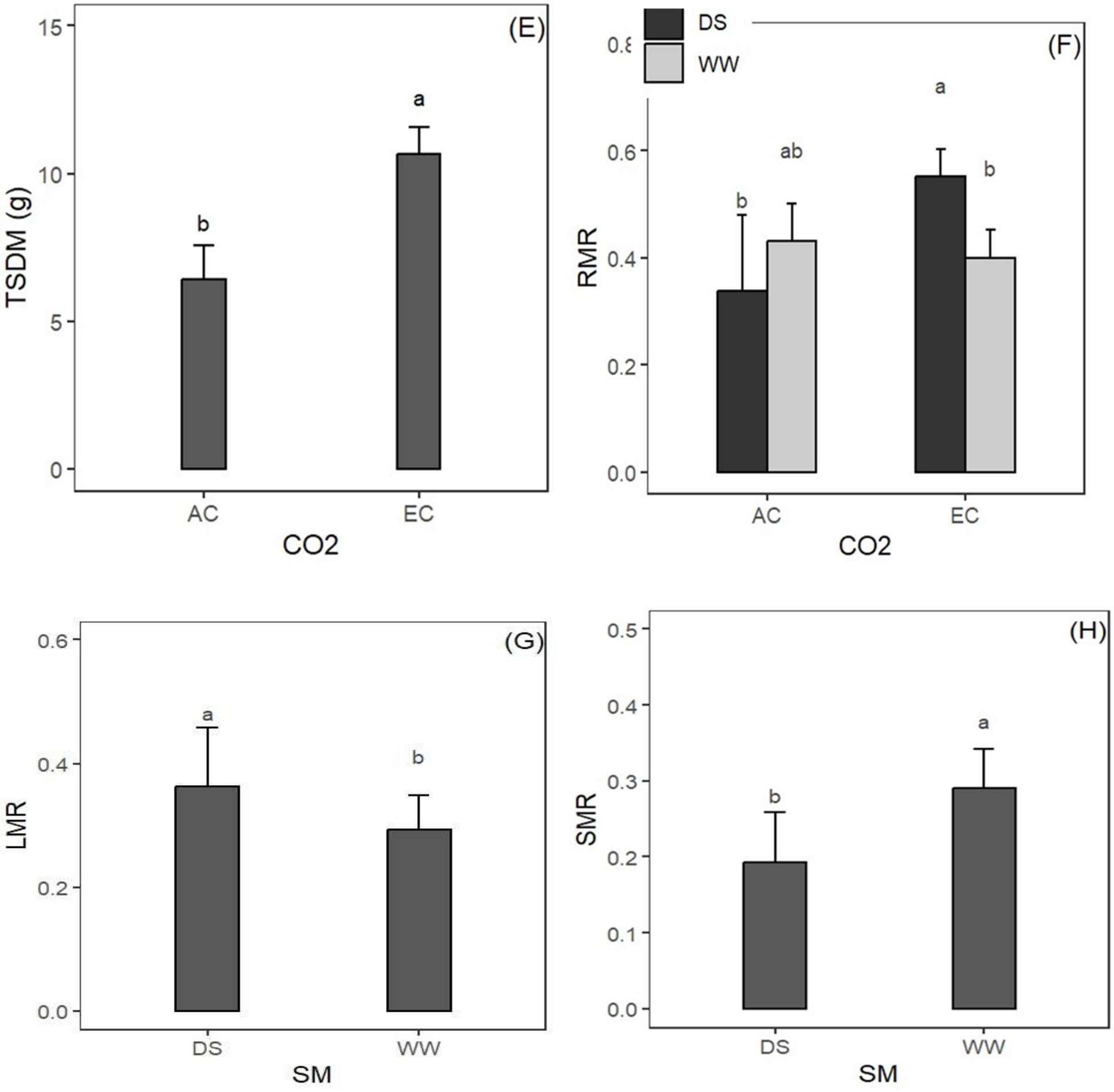


Figure 6: Mean (+SE) values for (A) root dry mass (*ROOT DM*), (B) leaf dry mass (*LEAF DM*), (C & D) stem dry mass (*STEMDM*), (E) total seedling dry mass (*TSDM),* (F) root mass ratio (*RMR*), (G) leaf mass ratio (*LMR*), and (H) stem mass ratio (*SMR*) of yellow birch seedlings grown under two levels of [CO_2] (ambient (AC) and elevated (EC)) and two levels of soil moisture regime (DS and WW). Data were pooled across [CO_2] and soil moisture replicate, (A, B, F) (n = 24, three seedlings per treatment replicate, 3 × 2CO_2 × 2SM × 2 replicate), [CO_2] for (C) and (E) (n = 12, three seedlings per treatment replicate, 3 × 2CO_2 × 2 replicate) and soil moisture for (D, G, H) (n= 12 three seedlings per treatment replicate, 3 × 2SM × 2 replicate). Different lowercase letters indicate statistically significant differences at ($P < 0.05$).

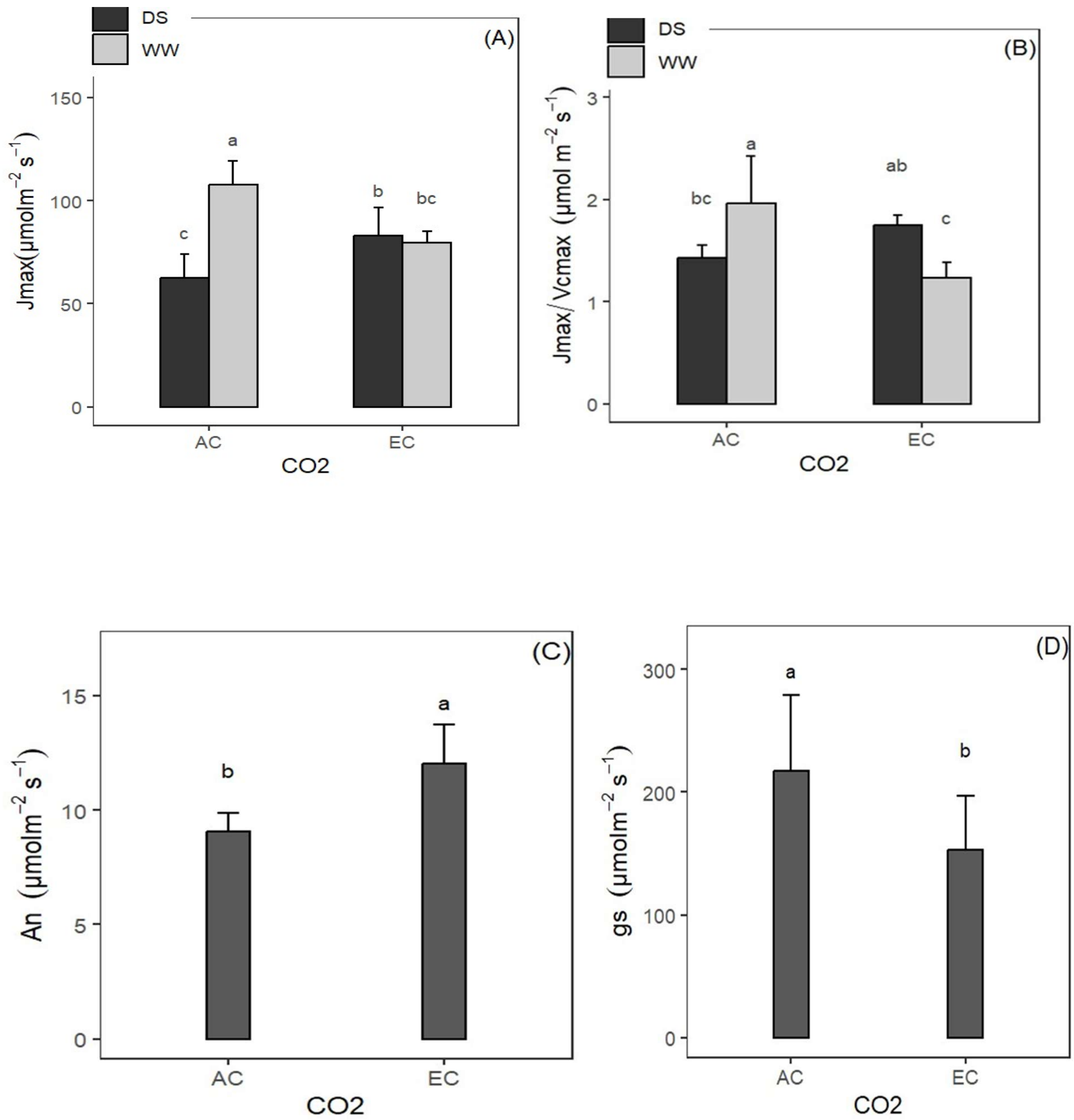
4.4.3 Foliar Gas Exchange

The interaction between carbon dioxide and soil moisture had a significant effect on the maximum electron transport rate (J_{max}) and ratio of electron transport rate to carboxylation rate (J_{max}/Vc_{max}) (Table 4.3). Drought stress significantly reduced J_{max} under ambient CO_2 by 31.7% but tended to increase it by 6.34% under EC (Fig. 7A). Drought stress significantly reduced the ratio of J_{max}/Vc_{max} under AC by 26.1% and increased it by 30.7 % under EC (Fig. 7B). EC significantly increased the net photosynthetic rate (A_n), water use efficiency (WUE) by 35% and 60.8% and reduced stomatal conductance (g_s) by 27.3% (Tables 4.3, Fig. 7C, 7G, 7D).

Under drought conditions, g_s and E significantly reduced, while *WUE* was significantly increased (Tables 4.3, Figs. 7E, 7F & 7H). Stomatal conductance was decreased by 33% and transpiration rate decreased by 28.6% under drought stress when compared to the well watered treatment. E under drought stress was reduced by 28.6% when compared to well-watered conditions. In conclusion, drought stress significantly enhanced *WUE* by 55%.

Table 4.3: Summary of ANOVA results (F value, P-value and Degree of freedom (DF) for the effects of carbon dioxide concentration [CO_2], photoperiod [PP] and their interactions on light-saturated rate of electron transport (J_{max}), maximum rate of rubisco carboxylation (V_{cmax}), ratio of electron transport rate to rate of rubisco carboxylation (J_{max}/Vc_{max}), the net rate of photosynthesis (A_n), stomatal conductance (g_S), transpiration rate (E), and photosynthetic water-use efficiency (WUE) of yellow birch seedlings grown under two levels of CO_2 (AC = 400 Vs. EC = 1000 µmol mol^{-1}) and two soil moisture regimes (drought stress and well-watered) at seed origin. Significant values (P ≤0.05) are boldfaced.

RESPONSE VARIABLE	CO2 (DF = 1)	SM (DF = 1)	CO2*SM (DF = 1)
J_{max}	0.72^{ns}	21.03***	29.04***
Vc_{max}	3.29^{ns}	0.19^{ns}	0.02^{ns}
J_{max}/Vc_{max}	3.90^{ns}	0.01^{ns}	25.34***
A_n	31.34***	2.74^{ns}	0.56^{ns}
g_s	11.75***	10.56***	0.03^{ns}
E	3.78^{ns}	7.60**	0.10^{ns}
WUE	22.65***	11.05***	1.45^{ns}


DS
WW
(A)
150
100
50
0
Jmax(µmolm^{-2}s^{-1})
a
c
b
bc
AC
EC
CO2
DS
WW
(B)
3
2
1
0
Jmax/Vcmax (µmol m^{-2}s^{-1})
a
bc
ab
c
AC
EC
CO2
(C)
15
10
5
0
An (µmolm^{-2}s^{-1})
b
a
AC
EC
CO2
(D)
300
200
100
0
gs (µmolm^{-2}s^{-1})
a
b
AC
EC
CO2

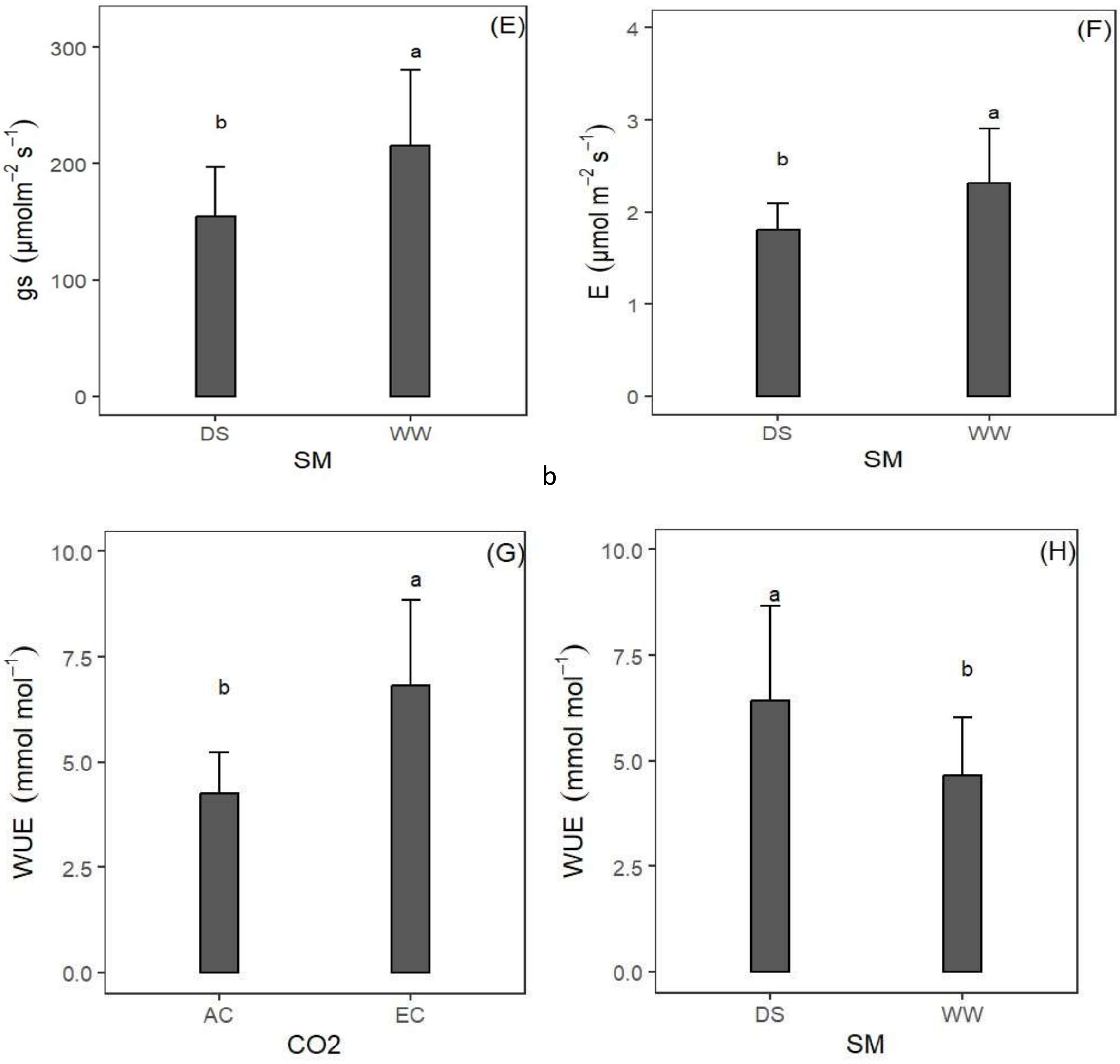


Figure 8: Mean (+SE) values for (A) for light-saturated rate of electron transport (J_{max}), (B) ratio of electron transport rate to the rate of rubisco carboxylation (J_{max}/Vc_{max}), (C) the net rate of photosynthesis (A_n), (D, E) stomatal conductance (g_s), (F) transpiration rate (*E*), and (G, H) photosynthetic water-use efficiency (WUE) of yellow birch seedlings grown under two levels of [CO_2] (ambient (AC) and elevated (EC)) and two levels of soil moisture regime (DS and WW). Data were pooled across [CO_2] and soil moisture replicate, (A, B) (n = 24, three seedlings per treatment replicate, 3 × 2CO_2 × 2SM × 2 replicate), [CO_2] for (C, D, G) (n = 12, three seedlings per treatment replicate, 3 2CO_2 ×2 replicate) and SM for (B, F, H) (n = 12, three seedlings per treatment replicate, 3 × 2SM × 2 replicate). Different lowercase letters indicate statistically significant differences at ($P < 0.05$).

4.5 Discussion

Interactive effect of drought and elevated carbon dioxide on growth, biomass and biomass allocation

The interaction of soil moisture and carbon dioxide concentration was a significant source of variation for height growth, specific leaf area *(*SLA), root dry mass (ROOTDM) and root mass ratio (RMR). Drought strongly limits tree growth, especially under ambient carbon dioxide. The negative effect of drought was alleviated under EC, in accordance with previous studies (Inoue et al., 2020; Newaz et al., 2017). This agrees with the first hypothesis of this study where EC mitigated the negative effects of drought stress on seedling height growth. The lower *SLA* under drought stress and EC reinforces the trend and formation of thicker leaves with reduced stomatal conductance to conserve water. Interestingly, drought did not interact with EC for total leaf area (TLA) and root collar diameter (RCD) observation. Drought stress significantly reduced *TLA*, which is likely due to reduced water availability affecting cell expansion. Trees under drought stress generally reduce leaf area to conserve water, and this might limit light interception and photosynthetic capacity (Beerling et al., 1996).

The interaction of drought and EC significantly enhanced *ROOTDM* and *RMR*. This also supports the second hypothesis of increased biomass production and more biomass allocation below ground. The higher increase observed in *ROOTDM* under drought conditions and EC may be due to root extension to access water or carbon allocation strategy, where trees exposed to EC may enhance resource allocation belowground under drought stress (Dickson & Tomlinson, 1996). It also indicates that under drought stress, sensitive tree species like yellow birch could express plasticity in biomass portioning to increase its drought tolerance.

No modification in root-to-shoot ratio (RSR) was observed in this study. An increase in *RSR* could limit stress when soil moisture is reduced and can be achieved by restricting shoot growth (Brunner et al., 2015). Notably, drought decreased stem dry mass and stem mass ratio by (33% and 45.5%) but increased leaf mass ratio by 28.6%. This indicates a strategic reallocation in response to drought, whereby the plant may allocate biomass to the leaves to maximize the photosynthesis process. Furthermore, lower biomass under drought stress is likely due to reductions in meristem activities such as slow cell elongation (Farooq et al., 2009). Drought generally reduced above-ground compartment in this study. In conclusion, during early developmental stages, total biomass production in yellow birch is susceptible to water deficit, especially leaf biomass production. This can be regarded as a stress-delaying strategy under water deficit to reduce total leaf area for transportation (Gaucher et al., 2005).

Interactive effect of drought and elevated carbon dioxide on photosynthetic capacity

EC generally have a positive effect on trees and ecosystem functions (Poorter et al., 2012). However, drought can inhibit the stimulating effect of EC (Jarvis et al., 1999; Long et al., 2004; Marchin et al., 2022). In this study, there was no significant interactive effect of drought and EC on the net photosynthesis of yellow birch seedlings. Under EC, drought stress slightly increased J_{max} compared to the well-water treatment in this study. The general reduction in J_{max} under EC when compared to Ambient conditions (AC) could indicate a downregulation of photosynthetic capacity in response to CO_2 or potentially due to stomatal regulation. Drought stress under EC maintains a relatively stable J_{max}/V_{cmax} which may indicate a different acclimation mechanism in drought-stressed trees. As observed in the experiment, trees under drought treatment had smaller leaves and reduced total leaf area than those under well-watered conditions. The low leaf area under drought possibly enabled the seedlings to maintain higher photosynthesis J_{max}/V_{cmax}, particularly under EC. The direct impact of drought on photosynthetic apparatus occurs by disrupting all major photosynthesis components, including the thylakoid electron transport, carbon reduction cycle and the stomatal conductance of the [CO_2] supply (Anjum et al., 2011). Although trees under drought and well-watered conditions were exposed to equal and adequate nutrient supply, no treatment effect was observed on carboxylation rate (V_{cmax}).

EC significantly increased net photosynthetic and this suggests that trees can fix more [CO_2], potentially leading to increased growth when other factors are not limited (Saxe et al., 1998). It has been established that the decrease in photosynthetic rate under water deficit is primarily due to reduced CO_2 assimilation and stomatal conductance (Cornic & Massacci, 1996). Blum (2011) reported the dependency of stomatal conductance, transpiration rate and CO_2 assimilation on water availability through guard cell turgor and abscisic acid changes. For individuals under water deficit, high stomatal sensitivity is associated with the ability to sustain growth during periods of low water availability (Chaves et al., 2003). Notwithstanding, further research is needed to understand the effects of rising atmospheric CO_2 concentrations on species with differing stomatal regulation strategies (Blum, 2011; Farooq et al., 2012; Fan et al., 2020).

In conclusion, as the climate progressively changes, the two-factorial experiments combining drought and elevated carbon dioxide still need to be studied. Our experiment shows that both drought and EC significantly modified the height growth, SLA, ROOTDM, RMR, J_{max}, and J_{max}/V_{cmax}. It is crucial to include such interactions in models predicting the growth and functioning of deciduous trees to predict future changes in tree growth and their capacity for carbon sequestration. Yellow birch is known to have a lateral deep root system when compared to other temperate deciduous tree species (Erdmann, 1990). As such, any improvement of the root system may enhance acclimation to soil water deficit. This study is limited to first-year seedlings' growth in response to [CO_2]. This experiment should be constantly repeated with 2-3-year-old seedlings to better understand the variation in physiological and morphological response displayed by the seedlings and to determine the effect of long-term exposure to drought.

4.6 References

Adams, H. D., Zeppel, M. J., Anderegg, W. R., Hartmann, H., Landhäusser, S. M., Tissue, D. T., Huxman, T. E., Hudson, P. J., Franz, T. E., & Allen, C. D. (2017). A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution*, *1*(9), 1285–1291.

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D. D., & Hogg, E. T. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, *259*(4), 660–684.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African journal of agricultural research*, *6*(9), 2026-2032.

Avila, R. T., de Almeida, W. L., Costa, L. C., Machado, K. L., Barbosa, M. L., de Souza, R. P., Martino, P. B., Juárez, M. A., Marçal, D. M., & Martins, S. C. (2020). Elevated air [CO2] improves photosynthetic performance and alters biomass accumulation and partitioning in drought-stressed coffee plants. *Environmental and Experimental Botany*, *177*, 104137.

Beerling, D. J., Heath, J., Woodward, F. I., & Mansfield, T. A. (1996). Drought—CO2 interactions in trees observations and mechanisms. *New Phytologist*, *134*(2), 235-242.

Berg, A., & Sheffield, J. (2018). Climate change and drought: The soil moisture perspective. *Current Climate Change Reports*, *4*, 180–191.

Blum, A. (2011). Drought resistance–is it really a complex trait?. *Functional Plant Biology*, *38*(10), 753-757.

Boisvenue, C., & Running, S. W. (2006). Impacts of climate change on natural forest productivity–evidence since the middle of the 20th century. *Global Change Biology*, *12*(5), 862–882.

Bowes, G. (1996). Photosynthetic responses to changing atmospheric carbon dioxide concentration. *Photosynthesis and the Environment*, 387–407.

Bréda, N., Huc, R., Granier, A., & Dreyer, E. (2006). Temperate forest trees and stands under severe drought: A review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, *63*(6), 625–644.

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in Plant Science*, *6*, 547.

Bunce, J. A. (2004). Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. *Oecologia*, *140*, 1–10.

Canadell, J. G., Monteiro, P. M., Costa, M. H., Cotrim da Cunha, L., Cox, P. M., Eliseev, A. V., Henson, S., Ishii, M., Jaccard, S., & Koven, C. (2023). Intergovernmental Panel on Climate Change (IPCC). Global carbon and other biogeochemical cycles and feedbacks. In *Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change* (pp. 673–816). Cambridge University Press.

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought—From genes to the whole plant. *Functional Plant Biology*, *30*(3), 239–264.

Cheng, S. (2007). Elevated CO2 changes the moderate shade tolerance of yellow birch seedlings. *Journal of Environmental Sciences*, *19*(4), 502–507.

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, *558*(7711), 531–539.

Cornic, G., & Massacci, A. (1996). Leaf photosynthesis under drought stress. In *Photosynthesis and the Environment* (pp. 347–366). Springer.

Damour, G., Simonneau, T., Cochard, H., & Urban, L. (2010). An overview of models of stomatal conductance at the leaf level. *Plant, Cell & Environment*, *33*(9), 1419–1438.

Delagrange, S. (2011). Light-and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. *Environmental and Experimental Botany*, *70*(1), 1–10.

Dickson, R. E., & Tomlinson, P. T. (1996). Oak growth, development and carbon metabolism in response to water stress. In *Annales des Sciences Forestieres* (Vol. 53, No. 2-3, pp. 181-196). EDP Sciences.

Dobbertin, M., Eilmann, B., Bleuler, P., Giuggiola, A., Graf Pannatier, E., Landolt, W., Schleppi, P., & Rigling, A. (2010). Effect of irrigation on needle morphology, shoot and stem growth in a drought-exposed Pinus sylvestris forest. *Tree Physiology*, *30*(3), 346–360.

Erdmann, G. G. (1990). Yellow birch. *Silvics of North America*, *2*, 133-147.

Fan, X., Cao, X., Zhou, H., Hao, L., Dong, W., He, C., Xu, M., Wu, H., Wang, L., & Chang, Z. (2020). Carbon dioxide fertilization effect on plant growth under soil water stress associates with changes in stomatal traits, leaf photosynthesis, and foliar nitrogen of bell pepper (Capsicum annuum L.). *Environmental and Experimental Botany*, *179*, 104203.

Farooq, M., Hussain, M., Wahid, A., & Siddique, K. (2012). Drought stress in plants: An overview. *Plant Responses to Drought Stress: From Morphological to Molecular Features*, 1–33.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. (2009). Plant drought stress: Effects, mechanisms and management. *Sustainable Agriculture*, 153–188.

Field, C. B., Jackson, R. B., & Mooney, H. A. (1995). Stomatal responses to increased CO2: Implications from the plant to the global scale. *Plant, Cell & Environment*, *18*(10), 1214–1225.

Gasser, D., Messier, C., Beaudet, M., & Lechowicz, M. J. (2010). Sugar maple and yellow birch regeneration in response to canopy opening, liming and vegetation control in a temperate deciduous forest of Quebec. *Forest Ecology and Management*, *259*(10), 2006–2014.

Gaucher, C., Gougeon, S., Mauffette, Y., & Messier, C. (2005). Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. *Tree Physiology*, *25*(1), 93-100.

Hartmann, H., Moura, C. F., Anderegg, W. R., Ruehr, N. K., Salmon, Y., Allen, C. D., Arndt, S. K., Breshears, D. D., Davi, H., & Galbraith, D. (2018). Research frontiers for improving our understanding of drought-induced tree and forest mortality. *New Phytologist*, *218*(1), 15–28.

IPCC. (2014). Climate change 2014 synthesis report. *IPCC: Geneva, Szwitzerland*, 1059–1072.

Inoue, S., Dang, Q.-L., Man, R., & Tedla, B. (2020). Photoperiod, [CO_2] and soil moisture interactively affect phenology in trembling aspen: Implications to climate change-induced migration. *Environmental and Experimental Botany*, *180*, 104269.

Liu, F., & Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (Amaranthus spp.) in response to drought stress. *Scientia Horticulturae*, *102*(1), 15–27.

Long, S. P., Ainsworth, E. A., Rogers, A., & Ort, D. R. (2004). Rising atmospheric carbon dioxide: Plants FACE the future. *Annu. Rev. Plant Biol.*, *55*(1), 591–628.

Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., & Gomis, M. (2021). Climate change 2021: The physical science basis. *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, *2*(1), 2391.

McDowell, N. G., Sapes, G., Pivovaroff, A., Adams, H. D., Allen, C. D., Anderegg, W. R., Arend, M., Breshears, D. D., Brodribb, T., & Choat, B. (2022). Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. *Nature Reviews Earth & Environment*, *3*(5), 294–308.

Medlyn, B. E., Barton, C., Broadmeadow, Msj., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S., Kellomäki, S., & Laitat, E. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO2 concentration: A synthesis. *New Phytologist*, *149*(2), 247–264.

Mitchell, P. J., O'Grady, A. P., Tissue, D. T., Worledge, D., & Pinkard, E. A. (2014). Co-ordination of growth, gas exchange and hydraulics define the carbon safety margin in tree species with contrasting drought strategies. *Tree Physiology*, *34*(5), 443–458.

Nejad, T. S. (2011). Effect of drought stress on shoot/root ratio. *World Acad. Sci. Eng. Technol*, *81*, 598–600.

Newaz, S., Dang, Q., & Man, R. (2017). Eco-physiological potential of jack pine (Pinus banksiana) for assisted northward migration: Interactions among photoperiod,[CO2] and moisture stress. *Nordic Journal of Botany*, *35*(6), 633–643.

Oguz, M. C., Aycan, M., Oguz, E., Poyraz, I., & Yildiz, M. (2022). Drought stress tolerance in plants: Interplay of molecular, biochemical and physiological responses in important development stages. *Physiologia*, *2*(4), 180–197.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30–50.

Rais, A., van de Kuilen, J.-W. G., & Pretzsch, H. (2014). Growth reaction patterns of tree height, diameter, and volume of Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) under acute drought stress in Southern Germany. *European Journal of Forest Research*, *133*, 1043–1056.

Raymond, P., & Dumais, D. (2023). Ecological Silviculture for Yellow Birch–Conifer Mixedwoods in Eastern Canada. *Ecological Silvicultural Systems: Exemplary Models for Sustainable Forest Management*, 112.

Reddy, A. R., Rasineni, G. K., & Raghavendra, A. S. (2010). The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. *Current Science*, 46–57.

Rötzer, T., Biber, P., Moser, A., Schäfer, C., & Pretzsch, H. (2017). Stem and root diameter growth of European beech and Norway spruce under extreme drought. *Forest Ecology and Management*, *406*, 184–195.

Ruehr, N. K., Grote, R., Mayr, S., & Arneth, A. (2019). Beyond the extreme: Recovery of carbon and water relations in woody plants following heat and drought stress. *Tree Physiology*, *39*(8), 1285–1299.

Saxe, H., Ellsworth, D. S., & Heath, J. (1998). Tree and forest functioning in an enriched CO2 atmosphere. *The New Phytologist*, *139*(3), 395-436.

Siddique, Z., Jan, S., Imadi, S. R., Gul, A., & Ahmad, P. (2016). Drought stress and photosynthesis in plants. *Water Stress and Crop Plants: A Sustainable Approach*, *1*, 1–11.

Sperry, J. S., Venturas, M. D., Anderegg, W. R., Mencuccini, M., Mackay, D. S., Wang, Y., & Love, D. M. (2017). Predicting stomatal responses to the environment from the optimization of photosynthetic gain and hydraulic cost. *Plant, Cell & Environment*, *40*(6), 816–830.

Thompson, M., Gamage, D., Hirotsu, N., Martin, A., & Seneweera, S. (2017). Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: A perspective on root sugar sensing and hormonal crosstalk. *Frontiers in Physiology*, *8*, 578.

Walters, M. B., Kunkle, J. M., Kobe, R. K., & Farinosi, E. J. (2023). Seedling drought responses governed by root traits, site-soil moisture regimes and overstory competition-facilitation. *Forest Ecology and Management*, *544*, 121159.

Wang, Z., Wang, C., & Liu, S. (2022). Elevated CO2 alleviates adverse effects of drought on plant water relations and photosynthesis: a global meta-analysis. *Journal of Ecology*, *110*(12), 2836-2849.

Wang, B., Cai, W., Li, J., Wan, Y., Guo, C., Wilkes, A., You, S., Qin, X., Gao, Q., & Liu, K. (2020). Leaf photosynthesis and stomatal conductance acclimate to elevated [CO2] and temperature thus increasing dry matter productivity in a double rice cropping system. *Field Crops Research*, *248*, 107735.

Warren, J. M., Pötzelsberger, E., Wullschleger, S. D., Thornton, P. E., Hasenauer, H., & Norby, R. J. (2011). Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO2. *Ecohydrology*, *4*(2), 196–210.

Wuebbles, D. J., Fahey, D. W., Hibbard, K. A., Arnold, J. R., DeAngelo, B., Doherty, S., ... & Walsh, J. (2017). Climate science special report: Fourth national climate assessment (NCA4), Volume I.

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. (2021). Response mechanism of plants to drought stress. *Horticulturae*, *7*(3), 50.

Chapter 5: General Discussion and Conclusion

5.1 General Summary

Atmospheric Ocean General Circulation models predicted a 10°N shift (approximately 1000km) in the climate envelope of North American tree species. Consequently, temperate and boreal forest trees may move northward, exposing them to new environmental factors such as photoperiod (McKenney et al., 2011). The expected increase in climate variability will predictably lead to increased frequency, intensity and duration of extreme events (IPCC, 2014; Rummukainen, 2012; Seneviratne et al., 2012). Consequently, an increase in climate change events can profoundly affect perennial plants, especially trees, which are important for structuring many ecosystems, biodiversity, and carbon balance. Such changes in environmental conditions are likely to influence the eco-physiological response of migrating trees to EC.

Experimental manipulation of yellow birch seedlings' response to environmental factors such as CO_2 levels, photoperiod and drought during growing seasons helped in assessing overall tree productivity regarding photosynthetic capacity, growth and timing of seasonal changes. The experiment was conducted on young yellow birch seedlings. This study revealed that the interaction of elevated carbon dioxide and longer photoperiod associated with 10°N of seed origin significantly decreased J_{max}, J_{max}/Vc_{max}. *TLA, SLA, LEAFDM and TSDM* were significantly increased. The outcome of this study revealed that the growth and biomass of yellow birch seedlings may initially benefit from a longer photoperiod associated with northward migration but once [CO_2] is no longer limited, the treatment effect might not be beneficial.

The phenological study of trees is important in understanding the function of temperate deciduous forest ecosystems, specifically in the context of global change (Lui, 2016; Chen et al., 2018). Most phenological studies of deciduous forest ecosystems have investigated the onset of growing season, mainly by characterizing the timing of canopy development (Spring phenology). However, autumn phenology has been largely overlooked (Gallinat et al., 2015). Though the timing of autumn phenophase affects not only tree development (winter survival) and growth potential for the following year (Estiarte & Penuelas, 2015) but also fundamental ecosystem dynamics such as nutrient cycling, biomass production, carbon dioxide and water fluxes between forest and atmosphere (Richardson et al., 2010, 2013, Gallinat et al., 2015). Our study revealed that elevated carbon dioxide significantly delayed the timing of bud phenology and leaf senescence across all photoperiod regimes. However, advancement was observed at the longest photoperiod regime. To understand autumn phenology and its rational exploration in forestry, it is important to unravel the overlapping processes that co-occur in time and space. Trees must have completed a series of processes, including growth cessation, bud set and leaf senescence, before the first frost arrives or risk physical damage. Consequently, in many tree species, photoperiod is used as a signal to begin growth cessation, which is the first step toward reaching winter dormancy. The importance of photoperiodic cues in determining growth cessation varies between tree species (Howe et al., 1996); these signals have implications for forests in response to changing climate. While temperature may increase over time, photoperiod will remain unchanged, limiting the ability of photoperiod-controlled trees to extend their growing season.

Woody perennials in temperate climates develop cold hardiness in the fall. Adequately timed cold acclimation and de-acclimation are essential for trees to minimize freeze injury risks while also maximizing opportunities for growth and development. Dormancy is defined as the inability of a meristem to resume growth under favourable conditions (Nilson, 2022). The ability to grow competitively while reducing the risk of winter injury due to delays in the development of dormancy and dormancy-related traits represents a critical trade-off essential for the health and survival of north temperate deciduous tree species (Tanino et al., 2010). Photoperiod was discovered to be a major cue influencing the cold hardiness in woody species (Mahfoozi et al., 2000). Our result revealed that photoperiod regime will limit the scope of yellow birch migration. Trees will not be able to survive very cold temperature such as (-45°C) if they migrate 10°N northward. Since there were no tests for migration between 5°N and 10°N, the scope of migration and the temperature they can withstand may be somewhere in between. Across research domains, researchers require tools for evaluating cold hardiness that can be effectively repeated across diverse tree lineages while providing interpretable, mechanistic evaluations of cold hardiness traits.

Significant changes in forest productivity and composition are anticipated since water deficit is a critical factor affecting tree growth, productivity and survival (Allen et al., 2010). During water stress, trees may undergo critical physiological and morphological changes at the leaf level to whole trees (Chaves et al., 2009; Hamrick, 2004). However, the extent of their responses may differ largely among different tree species (Zhang et al., 2004), resulting in contrasting levels of acclimation to soil water deficit. Among the varieties of strategies, drought-adapted species generally increase the relative allocation to fine root growth and biomass (Chaves et al., 2003). Other acclimation strategies include increased stem reserves and decreased total leaf area *(TLA)* (Zhang et al., 2004). For some less drought-adapted species, other mechanisms have been reported, such as a reduction in the number of leaves and a decrease in leaf length and width (Breda, 2006). A major detrimental effect of drought stress is the reduction of photosynthesis caused by a decrease in leaf expansion, an impaired photosynthetic system and premature leaf senescence (Reddy, 2004). The direct impact of drought on photosynthetic apparatus occurs by disrupting all major photosynthesis components, including the thylakoid electron transport, carbon reduction cycle and the stomatal conductance of the CO_2 supply (Anjum et al., 2011). This study revealed that elevated carbon dioxide significantly increased J_{max} *and* J_{max}/Vc_{max} under drought conditions. This is contrary to the general trend of reduction. Also, elevated carbon dioxide increased *STEMDM, ROOTDM, RMR,* and *RSR* under drought stress. However, SLA, LMR and SMR reductions were observed in response to drought stress. These findings suggest that yellow birch seedlings can potentially increase tree growth and biomass, but severe drought might prove otherwise.

The observed responses might vary with the responses of trees grown under natural conditions, since the study was conducted in controlled environmental conditions, and some factors in the natural environment could vary. Moreover, the study used seedlings under manipulated environmental conditions that might differ from mature trees' responses. Various studies have reported significant variations of growth characteristics in yellow Birch, including biomass (Rasheed & Delagrange, 2016). A large sample size is generally required for ecophysiological response. Yellow birch potentially grows at mesic site conditions (Erdmann, 1990), causing the species to have a relatively narrow ecological niche. Lastly, a soil moisture deficit using more seeds would provide greater knowledge regarding yellow birch seedlings' responses to their environment. Including measurement of hydraulic failure and xylem conductivity would add significant value to a study involving water use efficiency and hydraulic conductivity. However, it is crucial for further understanding how yellow birch may respond to varying degrees of drought conditions.

5.2 Reference

Adopted, I. P. C. C. (2014). Climate change 2014 synthesis report. *IPCC: Geneva, Szwitzerland*, 1059-1072.

Allen, A. B., & Leary, M. R. (2010). Self-Compassion, stress, and coping. *Social and personality psychology compass*, *4*(2), 107-118.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African journal of agricultural research*, *6*(9), 2026-2032.

Bréda, N., Huc, R., Granier, A., & Dreyer, E. (2006). Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, *63*(6), 625-644.

Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of botany*, *103*(4), 551-560.

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought—from genes to the whole plant. *Functional plant biology*, *30*(3), 239-264.

Chen, Z., Badrinarayanan, V., Lee, C. Y., & Rabinovich, A. (2018, July). Gradnorm: Gradient normalization for adaptive loss balancing in deep multitask networks. In *International conference on machine learning* (pp. 794-803). PMLR.

Erdmann, G. G. (1990). Betula alleghaniensis Britton. *Silvics of North America*, *2*, 133-147.

Estiarte, M., & Peñuelas, J. (2015). Alteration of the phenology of leaf senescence and fall in winter deciduous species by climate change: effects on nutrient proficiency. *Global change biology*, *21*(3), 1005-1017.

Gallinat, A. S., Primack, R. B., & Wagner, D. L. (2015). Autumn, the neglected season in climate change research. *Trends in ecology & evolution*, *30*(3), 169-176.

Hamrick, J. L. (2004). Response of forest trees to global environmental changes. *Forest ecology and management*, *197*(1-3), 323-335.

Howe, G. T., Hackett, W. P., Furnier, G. R., & Klevorn, R. E. (1995). Photoperiodic responses of a northern and southern ecotype of black cottonwood. *Physiologia Plantarum*, *93*(4), 695-708.

Liu, Y., & Cao, X. (2016). Characteristics and significance of the pre-metastatic niche. *Cancer cell*, *30*(5), 668-681.

Mahfoozi, S., Limin, A. E., Hayes, P. M., Hucl, P., & Fowler, D. B. (2000). Influence of photoperiod response on the expression of cold hardiness in wheat and barley. *Canadian journal of plant science*, *80*(4), 721-724.

McKenney, D. W., Hutchinson, M. F., Papadopol, P., Lawrence, K., Pedlar, J., Campbell, K., ... & Owen, T. (2011). Customized spatial climate models for North America. *Bulletin of the American Meteorological Society*, *92*(12), 1611-1622.

Nilsson, O. (2022). Winter dormancy in trees. *Current Biology*, *32*(12), R630-R634.

Rasheed, F., & Delagrange, S. (2016). Acclimation of Betula alleghaniensis Britton to moderate soil water deficit: Small morphological changes make for important consequences in crown display. *Tree Physiology*, *36*(11), 1320-1329.

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of plant physiology*, *161*(11), 1189-1202.

Richardson, A. D., Andy Black, T., Ciais, P., Delbart, N., Friedl, M. A., Gobron, N., ... & Varlagin, A. (2010). Influence of spring and autumn phenological transitions on forest ecosystem productivity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3227-3246.

Rummukainen, M. (2012). Changes in climate and weather extremes in the 21st century. *Wiley Interdisciplinary Reviews: Climate Change*, *3*(2), 115-129.

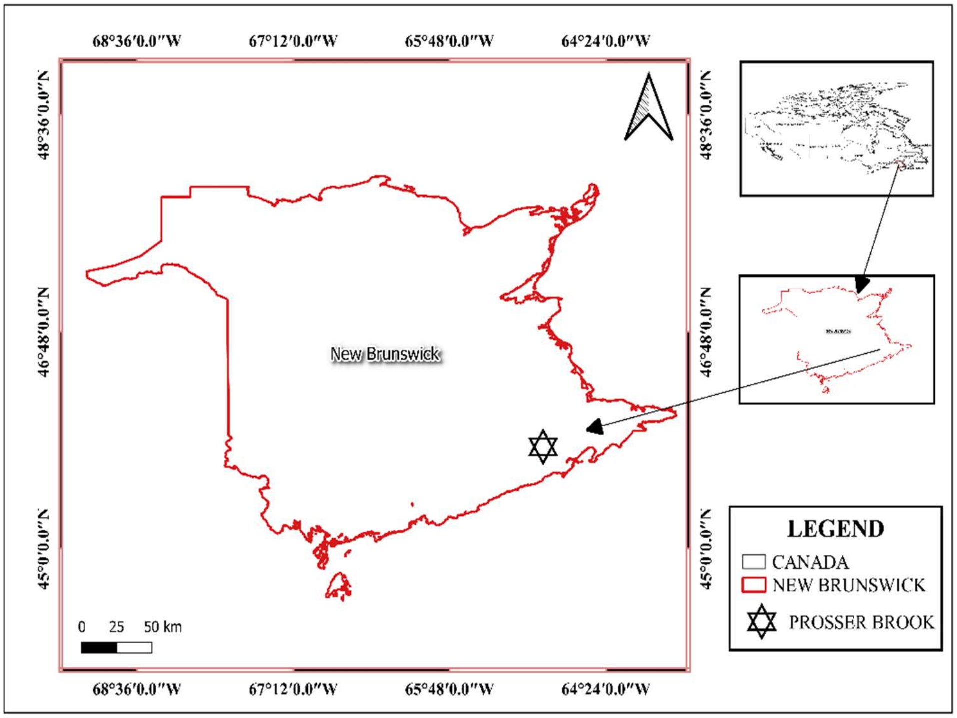
Seneviratne, S., Nicholls, N., Easterling, D., Goodess, C., Kanae, S., Kossin, J., ... & Zwiers, F. W. (2012). Changes in climate extremes and their impacts on the natural physical environment.

Tanino, K. K., Kalcsits, L., Silim, S., Kendall, E., & Gray, G. R. (2010). Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. *Plant molecular biology*, *73*, 49-65.

Zhang, M., He, J., Wang, B., Wang, S., Li, S., Liu, W., & Ma, X. (2013). Extreme drought changes in Southwest China from 1960 to 2009. *Journal of Geographical Sciences*, *23*, 3-16.

Appendices

A Geographical representation of seed location (Prosser Brook, New Brunswick)



B Schematic drawing of the Experiment Design (Chapter 2 &3).

AMBIENT CONDITIONS				ELEVATED CONDITIONS			
	P45	P45	P45	P45	P45	P45	
	P45	P45	P45	P45	P45	P45	
	P45	P45	P45	P45	P45	P45	
	P45	P45	P45	P45	P45	P45	
	P50	P50	P50	P50	P50	P50	
	P50	P50	P50	P50	P50	P50	
	P50	P50	P50	P50	P50	P50	
	P50	P50	P50	P50	P50	P50	
	P55	P55	P55	P55	P55	P55	
	P55	P55	P55	P55	P55	P55	
	P55	P55	P55	P55	P55	P55	
	P55	P55	P55	P55	P55	P55	
AMBIENT CONDITIONS (REPLICATE)				ELEVATED CONDITIONS (REPLICATE)			
	P45	P45	P45	P45	P45	P45	
	P45	P45	P45	P45	P45	P45	
	P45	P45	P45	P45	P45	P45	
	P45	P45	P45	P45	P45	P45	
	P50	P50	P50	P50	P50	P50	
	P50	P50	P50	P50	P50	P50	
	P50	P50	P50	P50	P50	P50	
	P50	P50	P50	P50	P50	P50	
	P55	P55	P55	P55	P55	P55	
	P55	P55	P55	P55	P55	P55	
	P55	P55	P55	P55	P55	P55	
	P55	P55	P55	P55	P55	P55	

C Schematic drawing of the Experiment Design (Chapters 4).

AC				EC			
WW	WW	WW	WW	WW	WW	WW	WW
WW	WW	WW	WW	WW	WW	WW	WW
WW	WW	WW	WW	WW	WW	WW	WW
DS	DS	DS	DS	DS	DS	DS	DS
DS	DS	DS	DS	DS	DS	DS	DS
DS	DS	DS	DS	DS	DS	DS	DS
AC REPLICATE				EC REPLICATE			
WW	WW	WW	WW	WW	WW	WW	WW
WW	WW	WW	WW	WW	WW	WW	WW
WW	WW	WW	WW	WW	WW	WW	WW
DS	DS	DS	DS	DS	DS	DS	DS
DS	DS	DS	DS	DS	DS	DS	DS
DS	DS	DS	DS	DS	DS	DS	DS

(AC) ambient carbon dioxide (EC) elevated carbon dioxide treatment, (WW) well-watered and (DS) drought stress treatment, with established replicates.

D Definition of Acronyms.

Acronyms	**Description**
A/C_i	Net photosynthesis rate vs intercellular CO_2 concentration-response curve
AC	Ambient Carbon dioxide concentration
A_n	Net photosynthetic rate
C_i	intercellular CO_2 compensation point
C_i/C_a	Intercellular to ambient CO_2 concentration
E	Transpiration rate
EC	Elevated Carbon dioxide concentration
g_s	Stomatal conductance
J_{max}	Maximum photosynthetic electron transport rate.
J_{max}/V_{cmax}	Maximum rate of carboxylation to maximum rate of photosynthetic Electron transport.
LEAFDM	Leaf Dry Mass
LMR	Leaf mass ratio
PAR	Photosynthetically active radiation
RCD	Root collar diameter
RMR	Root mass ratio
RSR	Root-to-shoot ratio
STEMDM	Stem Dry Mass
SMR	Stem Mass Ratio
ROOTDM	Root Dry Mass
TSDM	Total seedling Dry Mass
V_{cmax}	Maximum rate of RuBP carboxylation
WUE	Photosynthetic water use efficiency
TLA	Total leaf area
SLA	Specific leaf area

E Greenhouse Photoperiod settings

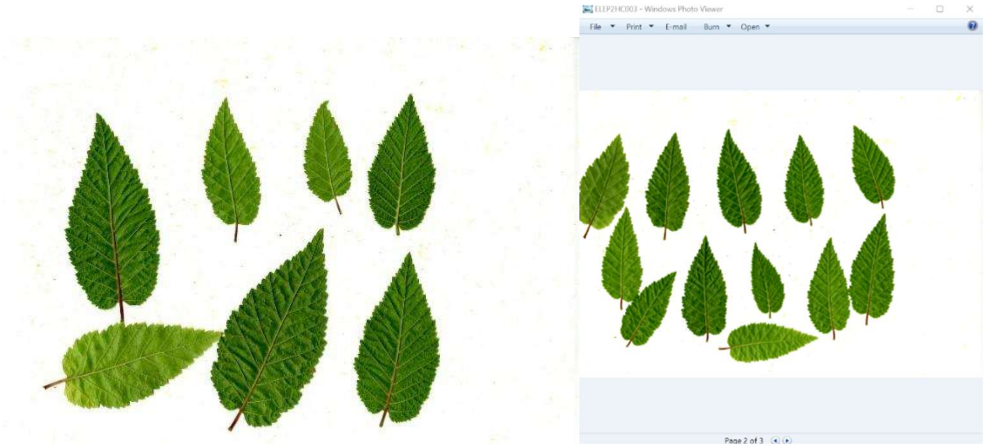
ACTUAL DATE	MIMICKED DATE	TEMP (°C) 12:00 AM	TEMP (°C) 6:00 AM	TEMP(°C) 12:00 PM	TEMP (°C) 6:00 PM	DAYLENGTH
JUNE 11-17	NOV 13-19	7.6	11.7	20.1	13.66	15 HRS
JUNE 18-24	NOV 20-26	9.5	12.6	25	14.1	15HRS
JUNE 25-JULY1	NOV 27- DEC3	11.3	14.36	26.6	16.4	16 HRS
JULY 2-JULY8	DEC4-10	12.7	15.4	26.4	17.26	16HRS
JULY9-15	DEC 11-17	12.2	13.04	26.4	16.92	16HRS
JULY 16-22	DEC18- 24	13.4	15.92	27.5	18.1	16HRS
JULY 23-29	DEC25- 31	14	19.5	27.4	21.9	16HRS
JULY 30 - AUG 5	JAN 1-7	13.2	18.36	28.7	22.01	15 HRS
AUG 6-12	JAN 8-14	13.2	18.2	28.7	24.32	15HRS
AUG 13-19	JAN 15-21	11.6	15.83	24.3	19.78	15HRS
AUG 20-26	JAN22-28	11.4	16.26	26	20.32	14 HRS
AUG 27- SEPT2	JAN 29-FEB 4	9	14.06	24.2	18.11	13 HRS
SEP 3-9	FEB 5-11	9.3	14.44	24.4	17.91	13HRS
SEP 10-16	FEB 12-18	9.1	14.3	24.7	12.67	13HRS
SEPT 17-23	FEB 19-25	7	11.03	19.1	13.78	13HRS
SEP 24-30	FEB 26-28	5.2	9.8	19	12.25	12 HRS
OCT 1-7	MAR 1-7	4.9	9.43	18.5	11.78	12HRS
OCT 8-14	MAR 8-14	0.5	7.8	16.16	11.71	12HRS
OCT 15- 21	MAR 15-21	5.76	9.66	17.46	12.88	12HRS
OCT 22 -28	MAR 22-28	5.8	10	13.3	9.13	12HRS
OCT 29 - NOV4	MAR 29-APR 04	0.5	5.86	11.92	7.81	10 HRS

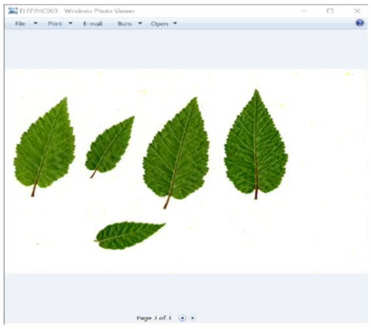
ACTUAL DATE	**MIMICKED DATE**	TEMP(°C) 12:00 AM	TEMP(°C) 6:00 AM	TEMP(°C) 12:00 PM	TEMP(°C) 6:00 PM	DAYLENGTH P1	DAYLENGTH P2	DAYLENGTH P3
JUNE 11-17	NOV 13-19	7.6	11.7	20.1	13.66	15 HRS	16 HRS	17 HRS
JUNE 18-24	NOV 20-26	9.5	12.6	25	14.1	15 HRS	16 HRS	17 HRS
JUNE 25-JULY1	NOV 27-DEC3	11.3	14.36	26.6	16.4	15 HRS	16 HRS	17 HRS
JULY 2-JULY8	DEC4-10	12.7	15.4	26.4	17.26	15 HRS	16 HRS	17 HRS
JULY9-15	DEC 11-17	12.2	13.04	26.4	16.92	15 HRS	16 HRS	17 HRS
JULY 16-22	DEC18- 24	13.4	15.92	27.5	18.1	15 HRS	16 HRS	17 HRS
JULY 23-29	DEC25- 31	14	19.5	27.4	21.9	15HRS	16 HRS	16 HRS
JULY 30 - AUG 5	JAN 1-7	13.2	18.36	28.7	22.01	15 HRS	15 HRS	16 HRS
AUG 6-12	JAN 8-14	13.2	18.2	28.7	24.32	15HRS	15 HRS	16 HRS
AUG 13-19	JAN 15-21	11.6	15.83	24.3	19.78	14HRS	15 HRS	15 HRS
AUG 20-26	JAN22-28	11.4	16.26	26	20.32	14 HRS	14 HRS	15 HRS
AUG 27-SEPT2	JAN 29-FEB 4	9	14.06	24.2	18.11	14 HRS	14 HRS	14 HRS
SEP 3-9	FEB 5-11	9.3	14.44	24.4	17.91	13HRS	13 HRS	14 HRS
SEP 10-16	FEB 12-18	9.1	14.3	24.7	12.67	13HRS	13 HRS	13 HRS
SEPT 17-23	FEB 19-25	7	11.03	19.1	13.78	13HRS	13 HRS	13 HRS
SEP 24-30	FEB 26-28	5.2	9.8	19	12.25	12 HRS	12 HRS	12 HRS
OCT 1-7	MAR 1-7	4.9	9.43	18.5	11.78	12HRS	12 HRS	12 HRS
OCT 8-14	MAR 8-14	0.5	7.8	16.16	11.71	11HRS	11 HRS	11 HRS
OCT 15- 21	MAR 15-21	5.76	9.66	17.46	12.88	11HRS	10 HRS	10 HRS
OCT 22 -28	MAR 22-28	5.8	10	13.3	9.13	10 HRS	10 HRS	10 HRS
OCT 29 - NOV4	MAR 29-APR 04	0.5	5.86	11.92	7.81	10 HRS	10 HRS	9 HRS

F Leaf Morphology (Win folia Scan)

Samples p_{45}

Samples P_{50}

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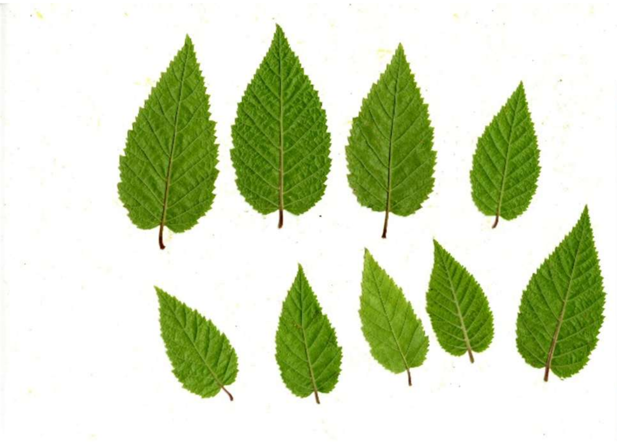
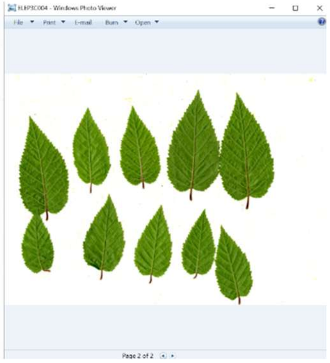
Samples P_{55}

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