

**Phenology, growth and physiological responses to photoperiod and elevated [CO₂] of
boreal white birch (*Betula papyrifera* Marsh): implications to climate change-induced
northward migration**

By

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Abstract

With the increase in average global temperature due to rising atmospheric carbon dioxide concentration ($[\text{CO}_2]$), rapid shifts of climate envelopes are expected to lead to northward migration for boreal tree species. As a result, trees will be exposed to new photoperiod regimes and higher $[\text{CO}_2]$. These factors will likely have interactive effects on the physiology, phenology, survival, and growth of tree species. The successful northward migration of trees will likely depend on their biological capacity (phenotypic plasticity) to cope with novel conditions. To better understand how boreal tree species will respond to changes in photoperiod and $[\text{CO}_2]$, it is critical to experimentally investigate the impact of potential changes on the physiology, phenology, and growth of individual species.

The main objective of this thesis was to examine how the phenology, growth, and physiology of white birch (*Betula papyrifera* Marsh) would respond to photoperiod and elevated $[\text{CO}_2]$ in the context of climate change-induced tree migration. To achieve this target, seedlings were exposed to ambient ($400 \mu\text{mol mol}^{-1}$) or elevated ($1000 \mu\text{mol mol}^{-1}$) $[\text{CO}_2]$, and four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58° N for two growing seasons. The results showed that a moderate increase in photoperiod increased growth and biomass, CO_2 elevation stimulated photosynthetic carbon uptake (P_n). However, white birch did not benefit further from even longer photoperiods. The lack of P_n stimulation by CO_2 elevation was associated with the downregulation of photosynthetic capacity, the maximum Rubisco activity (V_{cmax}) and maximum electron transport rate (J_{max}). The seedlings under longer photoperiod advanced budburst under elevated CO_2 , whereas photoperiod had opposite effects on budburst under ambient CO_2 . Further, CO_2 elevation lengthened the duration of the leaf senescence process due to an earlier

onset and later completion of leaf senescence. Interestingly, longer photoperiods at the two high latitudes induced an earlier onset of leaf senescence and achieved a higher level of cold hardiness than at the two lower latitudes under elevated [CO₂]. Further, the photoperiod at the latitude 10° N north of seed origin enhanced the maximum stem-specific hydraulic conductivity. The interactive effects of photoperiod and [CO₂] on the phenology, growth, and physiology of white birch seedlings indicate that novel conditions at higher latitudes may play a critical role in the success of climate change-induced migration.

Keywords: Climate change, White birch, Photoperiod, Elevated CO₂, Migration, Leaf senescence, Cold hardiness, Budburst, Photosynthesis, Growth.

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Abbreviations

A[CO₂] – Ambient CO₂ concentration

ANOVA – Analysis of variance

C_i/C_a – Intercellular to ambient CO₂ concentration

[C:N] – Carbon to Nitrogen ratio

DF – Degree of freedom

DOY – Day of year

E[CO₂] – Elevated CO₂ concentration

FT – Freeze-thaw

FV/FM – Potential maximum quantum efficiency of PSII

g_s – Stomatal conductance

H – Seedling height

IWUE – Instantaneous photosynthetic water use efficiency

J_{max} – Maximum rate of photosynthetic electron transport

J_{max}/*V_{cmax}* – Maximum rate of carboxylation to maximum rate of photosynthetic electron transport

LAR – Leaf area ratio

LMR – Leaf mass ratio

LT₅₀ – Lethal temperature for causing 50% electrolyte leakage

N – North

N – Sample number

[N] – Nitrogen concentration

PAR – Photosynthetically active radiation

PLC – Percent loss of conductivity

P_n – Net photosynthetic rate

RCD – Seedling root collar diameter

REL – Relative electrolyte leakage

RMR – Root mass ratio

RS – Root to shoot ratio

SLA – Specific leaf area

SMR – Stem mass ratio

TPU – Triose phosphate utilization rate

V_{cmax} – Maximum rate of carboxylation

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1 Chapter 1: General introduction

The continuous increase in average global temperature due to anthropogenic CO₂ emissions are causing a mismatch between trees and the environment to which they are adapted (Parmesan 2006). These changes have led to shifts in climate envelopes poleward (Parmesan 2006, Pachauri et al. 2014); as a result, several tree species in North American are expected to move northward (Walther et al. 2002, McKenney et al. 2007). Most mid to high latitude tree species show adaptive responses to the local environments (such as local photoperiods and temperatures) to synchronize their growth with the environment (Aitken et al. 2008). These adaptive strategies will likely affect growth and survival if trees migrate northwards (Way and Montgomery 2015). For example, the adaptive response of southern trees to shorter photoperiod may allow them to exploit the longer growing season of the north (Thomas and Vince-Prue 1997), however, it may influence phenological events that are important for the proper development of dormancy and mobilization of resources from senescing leaves to other storage tissues (Way and Montgomery 2015). In addition to photoperiod, traits that are critical for tree fitness, such as phenology, growth, physiology and cold tolerance, appear to be responsive to other climate variables, including [CO₂] elevations (Jach et al. 2001). Hence, the successful migration of trees northward will likely depend on the biological capacities essential in a species' ability to cope with novel conditions via physiological or phenological plasticity. While genetic adaptation to local conditions is often perceived as a distinct process from the alteration of phenological and physiological processes to match developmental processes with new site conditions, the former may constrain the range of plastic responses or responses thresholds.

Phenology, the study of recurring biological events (Lieth 1974), has long been a focus of plant ecology research, and recent scientific interest has been driven by climate change (Chmielewski and Rötzer 2001, Badeck et al. 2004, Richardson et al. 2013). Trees in the temperate and boreal regions evolved to have a precise timing of annual growth cycles (Thomas and Vince-Prue 1997). In autumn, carbon fixing tissues such as leaves of deciduous trees avoid adverse climatic conditions in winter by senescence (Ingvarsson et al. 2006). The process of leaf senescence, which involve leaf coloration due to the degradation of chlorophylls followed by leaf fall is a critical factor in controlling carbon uptake, and nutrient resorption in trees (Näsholm et al. 1998, Andersson et al. 2004, Keskitalo et al. 2005). In preparation for winter, tissues such as shoot meristems enter dormancy, under which growth is visibly cease. During dormancy, buds go through three main phases. From summer to early autumn (paradormancy), bud growth is suppressed by hormones (Horvath et al. 2003) while early autumn to mid-winter (endodormancy), bud development is inhibited by endogenous factors within the meristem (Cooke et al. 2012). During mid-winter to mid-spring (ecodormancy), at this stage bud development any longer suppressed by endogenous factors, however bud cell growth is inhibited by external environmental factors (Caffarra et al. 2011).

In the temperate and boreal regions, photoperiod controls various physiological and phenological processes/traits and serves as an environmental cue for the synchronization of plants' phenological and physiological processes with the seasonal climate conditions (Salisbury 1981, Thomas and Vince-Prue 1997, Lambers et al. 2008, Jackson 2009). In many tree species, growth cessation, the termination of apical elongation, is triggered during late summer and early autumn with the decrease in photoperiod (Heide 1974, Junttila 1982, Li et al. 2003). The initiation and development of resting buds in response to a certain photoperiod have been reported in many

deciduous trees, including *Populus* (Böhlenius et al. 2006), and *Betula* (Habjorg 1972, Downs and Bevington 1981, Li et al. 2003, Viherä-Aarnio et al. 2005), as well as in other species (Heide, 1974; Howe et al., 1995; Junttila, 1982). On the other hand, the influence of photoperiod on the resumption of bud growth during ecodormancy varies between species (Way and Montgomery 2015). However, both the chilling requirement and photoperiod regulation are viewed to safeguard temperature sensitive tissues from late frost damage (Way and Montgomery 2015). Tree species with a wide-range distribution often show ecotypic differences in the photoperiod required for the induction of autumnal phenological changes (Howe et al. 1995, Keller et al. 2012). These adaptive responses to the local environmental conditions may affect the response of trees to climate change-induced migration. For example, the northward migration or transfer of a species can delay autumnal phenology, such as budset and dormancy development as the threshold photoperiod will occur later, which can lead to damages by early frosts, less cold hardiness and reduced nutrient reabsorption during leaf senescence (Velling 1979, Junttila and Kaurin 1990, Viherä-Aarnio et al. 2005, Way and Montgomery 2015).

Photoperiod can also influence the physiology, growth, and productivity of plants via its effect on the timing of photoperiod-controlled phenological events such as bud burst in the spring and bud formation in autumn (Velling 1979, Junttila and Kaurin 1990, Basler and Körner 2012). Longer photoperiod can also stimulate growth (Oleksyn et al. 1992, Johnsen and Seiler 1996) and the rate of biomass accumulation (Stinziano and Way 2017), increases in leaf area and leaf mass ratios and specific leaf area (Hay 1990, Adams and Langton 2005). Photoperiod can also affect the biomass allocation between aboveground and belowground organs in trees (Li et al. 2015). However, results on the photoperiod influence over the biomass allocation in trees vary

with species and other factors (Heide 1974, Heide et al. 1985, Burdett and Yamamoto 1986, Hay 1990, Bigras and Daoust 1993, Stinziano and Way 2017).

In addition to photoperiod, several studies have demonstrated that elevated CO₂ influences the phenology of trees by altering the timing of growth cessation and bud burst (Murray et al. 1994, Ceulemans et al. 1995). Elevated CO₂ can affect bud phenology (Murray et al. 1994, Ceulemans et al. 1995), cold hardiness (Tinus et al. 1995, Jach et al. 2001), and leaf senescence (Taylor et al. 2008). The response in the timing of spring bud break to CO₂ elevations varies with species, ranging from advancing (Repo et al. 1996, Karnosky 2003), to delaying (Murray et al. 1994), to no response (Sigurdsson 2001, Karnosky 2003, Norby et al. 2003). CO₂ elevations can advance the timing of bud set, increase autumn cold hardiness (Murray et al. 1994) or decrease cold hardiness (Margolis and Vézina 1990). Growing under elevated [CO₂] can also lead to earlier leaf senescence in some plant species (Körner et al., 2005; Sigurdsson, 2001) but delay leaf senescence in other species (Karnosky, 2003; Körner et al., 2005; Li et al., 2000) or have no effect in still other species (Herrick and Thomas 2003).

Photosynthesis, a process that involves the conversion of light energy into chemical energy that can be incorporated into organic molecules (Lambers et al. 2008). This process can be broadly divided into light-harvesting and carbon fixation. In the light reactions of photosynthesis, which take place in photosystems II and I, light is absorbed by pigments in protein structures (photosystems) (Grossman et al. 1995). In the light harvesting complex, electrons flow from photosystem II to photosystem I through a series of electron donors and acceptors, which then reduces nicotinamide adenine dinucleotide phosphate (NADP⁺) to NADPH (Zanetti and Curti 1981). NADPH produced from light reactions is used in the Calvin cycle for CO₂ fixation

(Buchanan 1991). The light reactions also produce a proton motive force across the thylakoid membrane, and this proton gradient is used to synthesize adenosine triphosphate (ATP) which is then used for CO₂ fixation. ATP and NADPH produced during the light reactions provide the energy to drive carbon fixation in the Calvin cycle, which produce triose phosphates using carboxylation enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Lambers et al. 2008).

Photosynthetic carbon uptake responds to several environmental and resource conditions including nutrient, water, light, temperature, CO₂ (Lambers et al. 2008). Elevated [CO₂], generally, increase photosynthesis of C₃ plants, particularly when resources and other environmental conditions are favorable (Eamus and Jarvis 1989, Ainsworth and Long 2004, Leakey et al. 2009). The [CO₂] elevation stimulation of photosynthesis and its influence on stomatal conductance can reduce transpiration rate leading to enhanced water use efficiency (WUE) (Ainsworth and Rogers 2007). However, the carbon to nitrogen ratio (C/N) in the leaf often increases under elevated [CO₂] (Ellsworth et al. 2004, Ainsworth and Rogers 2007). An interesting question is how the longer period of photosynthesis associated with the longer photoperiod regime at higher latitudes will affect plant response to [CO₂] elevations. Further, [CO₂] elevation generally enhances the growth and influences biomass allocations (Ceulemans and Mousseau 1994, Rogers et al. 1994, Curtis and Wang 1998). Under elevated [CO₂], plants often tend to increase dry matter allocation to roots, particularly when water or nutrient resources are limited (Stulen and Den Hertog 1993, McGuire et al. 1995, Rogers et al. 1995, Curtis and Wang 1998, Saxe et al. 1998). While the influence of [CO₂] elevations and photoperiod on biomass allocation are relatively studied (Heide 1974, Burdett and Yamamoto 1986, Bigras and Daoust 1993), studies on the interaction effects area are generally scarce.

In addition to [CO₂], photoperiod also impacts the photosynthetic process. The response in photosynthetic rates to longer photoperiod varies with the magnitude of change in the photoperiod and species: ranging from enhancements (Kuppers et al. 1988, Souther et al. 2014, Benomar et al. 2016) to reductions in photosynthetic capacity, leading to feedback inhibition to photosynthetic rates and photosynthetic downregulation (Osborne and Beerling 2003, Royer et al. 2005, Equiza et al. 2006). However, the period of photosynthesis associated with the particular latitude is likely to affect plant response to [CO₂] elevations (Beerling and Osborne 2002).

As I have discussed above, both photoperiod and [CO₂] elevations alter various plant processes; however, the open question remains as to how the photoperiod regime of higher latitudes will affect plant response to [CO₂] elevations and vice versa. The interactive effects of photoperiod and [CO₂] will have implications for climate change-induced tree migration. Beerling and Osborne (2002) found that the CO₂ stimulation of photosynthesis in polar forest species is constrained by extended photoperiods at high-latitudes during the summer solstice while the level of stimulation increases later in the summer when the photoperiods get shorter; suggesting a photoperiodic effect on the seasonal variations of CO₂ stimulation of photosynthesis. An interesting question is can trees stimulate photosynthesis by [CO₂] elevations if they migrate to higher latitudes. In addition to how photoperiod and [CO₂] elevations may interactively affect plant phenological responses, it is equally unknown how much of the stimulation of carbon allocation to roots by [CO₂] elevations (McGuire et al. 1995) can be offset by the opposite effect of longer photoperiods associated with a northward migration (Johnsen and Seiler 1996, Gestel et al. 2005, Hamilton et al. 2016).

The overall purpose of this study is to understand the physiological, phenological, and growth and biomass responses of white birch to the interactive effects of photoperiod regimes and higher atmospheric carbon dioxide concentration ($[CO_2]$) under the scenario of climate change-induced northward migration. Specifically, this dissertation was compiled to investigate the following (1) how CO_2 elevation and photoperiod regimes interactively influence the timing of bud development, leaf senescence, cold hardiness, and bud break in white birch (*Betula papyrifera* Marsh.); (2) to investigate the photosynthetic response to changes in photoperiod regime and $[CO_2]$; (3) how growth, biomass, and biomass allocations respond to the interactive effects of photoperiod regimes and $[CO_2]$; and (4) how elevated $[CO_2]$ and photoperiod affect freeze-thaw induced xylem embolism and spring leaf-out. All experiments were done on plant material that was grown in the greenhouse and was exposed to the experimental treatments for two growing seasons except for the fourth chapter, which was based on one-year-old seedlings.

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2 Chapter 2: Photoperiod regimes at higher latitudes and CO₂ elevation change autumn and spring phenology as well as cold hardiness in boreal white birch

2.1 Abstract

Plants will be exposed to new photoperiod regimes and higher atmospheric carbon dioxide concentrations ([CO₂]) under the scenario of climate change-induced northward migration. These factors will likely have interactive effects on the physiology, phenology, survival, and growth of plants, particularly in the boreal region where climate change will be the most prominent. This study investigated how CO₂ elevation and photoperiod regimes interactively influence the timing of bud development, leaf senescence, cold hardiness, and bud break in white birch (*Betula papyrifera* Marsh.). Seedlings were exposed to ambient (AC= 400 μmol mol⁻¹) or elevated (EC= 1000 μmol mol⁻¹) [CO₂], and four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58° N for two growing seasons. I found that EC lengthened the process of leaf senescence in white birch by 30 days due to both an earlier start (23 days) and a later completion. Furthermore, leaf senescence started earlier under the photoperiod regimes of the high latitudes (55 and 58° N) than the lower latitudes under EC, but no such differences occurred under AC. Additionally, the temperature causing 50% electrolyte leakage was lower at higher latitudes (-46 °C at 55°, -60 °C at 58° N) under EC than other conditions (>-40 °C). [CO₂] and photoperiod also interactively influenced the timing of spring budburst. Budburst occurred earlier at 55 and 58° N than at 48 and 52° N under EC, but the trend was the opposite under AC. EC delayed spring budburst at 48 and 52° N, but advanced it at 55° N and had no significant impact at 58° N. The results suggest that interactions between photoperiod and [CO₂] can alter autumnal and spring phenology and thus should be considered in assessing species suitability for assisted migration or in predicting the future distribution and productivity of boreal trees.

2.2 Introduction

The climate change-driven northward migration of tree species will expose trees to different photoperiod regimes than what they are adapted to (Way and Montgomery 2015). Generally, when a tree species or genotype is moved to a higher latitude from their current location, they will be exposed to longer photoperiods in the growing season and faster rates of change in photoperiod during the transition between growing season and non-growing seasons (Thomas and Vince-Prue 1997). These changes can alter the timing, rate, and duration of phenological events that are critical for growth and survival (Junttila & Kaurin, 1990; Li, Junttila, Ernstsén, Heino, & Palva, 2003). For instance, the northward migration or transfer of a species can delay autumnal phenology, such as budset and dormancy development as the threshold photoperiod will occur later, which can lead to damages by early frosts, a low degree of cold hardiness and reduced nutrient reabsorption during leaf senescence (Velling 1979, Junttila and Kaurin 1990, Viherä-Aarnio et al. 2005, Way and Montgomery 2015).

Bud dormancy is a required growth transition consisting of a sequence of events such as the termination of apical elongation, the development of resting buds, an increase in frost tolerance, leaf senescence, and dormancy release which allows growth to resume under favorable conditions (Thomas and Vince-Prue 1997, Jackson 2009). Tree species with a wide range distribution often show ecotypic differences in the photoperiod required for the induction of autumnal phenological changes (Howe et al. 1995, Keller et al. 2012). The longer photoperiod requirement of genotype from high latitudes to prevent cessation of growth compared to genotypes from low latitude have been reported for *Betula* species including *B. papyrifera* (Downs and Bevington 1981), *B. pubescens* (Habjorg 1972), *B. pendula* (Li et al., 2003; Viherä-Aarnio et al., 2005) and other species (Heide, 1974; Howe et al., 1995; Junttila, 1982). In

addition to within-species variations in the timing to enter dormancy, studies have shown a close relation in the rate of dormancy development and cold acclimation with the progression of the local photoperiod (Howe et al. 2003, Savolainen et al. 2007, Chen et al. 2012, Alberto et al. 2013). While the adaptive response of southern trees to shorter photoperiod allows them to exploit the longer growing season of the north (Thomas and Vince-Prue 1997), it may also influence phenological events that are important for the proper development of dormancy and mobilization of resources from senescing leaves to other storage tissues (Way and Montgomery 2015). For example, southern *B. pedula* grown in a nursery in the north ceases height growth later in the fall, but fails to resorb nutrients from leaves and achieves lower cold hardiness, resulting in lower survival rates when planted in the field (Raulo 1976, Velling 1979, Viherä-Aarnio et al. 2005). Similarly, Junttila and Kaurin (1990) have reported that southern *Salix pentandra* grown in a nursery displayed late growth cessation and a slower rate of cold acclimation, and these trees were subsequently damaged by frosts when planted in the field. Despite providing valuable information on the effect of photoperiod on tree physiology and phenology, there are only a few studies examining how multiple changing environmental factors may interactively affect plant phenological responses in the context of climate change-induced migration. Newaz et al., (2016) have reported a significant advance in spring budburst of jack pine seedlings under the combination of elevated [CO₂] and photoperiod regime 10 degrees north of 48° N. Li et al., (2015) have found that nutrient deficiency negatively impacts the autumnal cold hardiness in black spruce and advance budburst in the spring.

CO₂ elevations can also affect bud phenology (Murray et al. 1994, Ceulemans et al. 1995), cold hardiness (Tinus et al. 1995, Jach et al. 2001), and leaf senescence (Taylor et al. 2008). The response in the timing of spring bud break to CO₂ elevations varies with species, ranging from

advancing (Repo et al. 1996, Karnosky 2003), to delaying (Murray et al. 1994), to no response (Sigurdsson 2001, Karnosky 2003, Norby et al. 2003). CO₂ elevations can advance the timing of bud set, increase autumn cold hardiness (Murray et al. 1994) or decrease cold hardiness (Margolis and Vézina 1990). Growing under elevated [CO₂] can also lead to earlier leaf senescence in some plant species (Körner et al., 2005; Sigurdsson, 2001) but delay leaf senescence in other species (Karnosky, 2003; Körner et al., 2005; Li et al., 2000) or have no effect in still other species (Herrick and Thomas 2003). The wide variations in response to elevated [CO₂] among tree species further complicate the prediction of interactive effects of elevated [CO₂] and photoperiod regimes on trees. A good understanding of such interactive effects on the phenology and physiology of trees is critically important in the context of northward migration under a changing climate.

White birch (*Betula papyrifera* Marsh.) is one of the most widely distributed birch species in North America. Generally, *Betula* spp. have developed ecotypes with different responses to photoperiod in growth cessation, dormancy induction, and cold acclimation (Habjorg, 1972; Li et al., 2003). This study investigates how changes in photoperiod regime associated with climate changed-induced northward migration influence the autumnal and spring phenology responses to elevated [CO₂] in white birch. I hypothesize that the faster rate of change in photoperiod during the seasonal transition between growth and dormancy at higher-latitudes will negatively impact the development of cold hardiness in the fall because of the delayed arrival of the critical photoperiod for the initiation of cold hardening process, but CO₂ elevations will modify the degree of the response.

2.3 Materials and methods

2.3.1 Plant material

Mature catkins of white birch (*Betula papyrifera* Marsh) were collected from 12 natural trees in Thunder Bay (48.4215° N, 89.2619° W) in September 2016. Seeds were extracted manually, air dried and stored in plastic bags at room temperature. The experiment was conducted at the Lakehead University Forest Ecology Complex in Thunder Bay. Seeds were sown in germination trays (50cm × 25cm) filled with a 1:1 (v:v) peat moss and vermiculite mixture. The day/night temperatures and photoperiod during germination were set to 22/16 °C and 16 hours, respectively. Seedlings of relatively uniform size (2 cm average height) were transplanted into pots of 12 cm deep and 12/9.5 cm top/bottom diameter for the experiment. The growing medium was a mixture of vermiculite and peat moss (1:3, v:v).

2.3.2 Experimental design

The experiment was a split-plot design. The treatments were comprised of two levels of carbon dioxide concentration [CO₂] (AC 400 and EC 1000 μmol mol⁻¹) as the whole plot and four photoperiod regimes corresponding to the latitudes of 48 (seed origin), 52, 55, and 58° N as split-plots within each whole plot. There were fifteen seedlings in each treatment combination. The CO₂ elevation was achieved using natural gas CO₂ generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA). Each [CO₂] level was replicated twice using independent environment-controlled greenhouses. The photoperiod regime of each greenhouse was set to emulate the weekly average regime of the growing season for the longest photoperiod of the four treatments, and that photoperiod was shortened by manually applying black-out shade to emulate the photoperiod regime for each of the other three treatments. The photoperiods were adjusted weekly to emulate the weekly averages for the corresponding

latitudes. To facilitate the shading and to be consistent across treatments, a wooden frame was established around each split-plot. High-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada) were used to extend the natural photoperiod when the natural day-length in the greenhouse was shorter than required. The day and night temperatures were derived from the 10-year weekly averages of Environment Canada records for the seed origin location (Environment Canada, 2016). The daily temperature in each greenhouse was ramped at four set points at 4:00, 10:00, 16:00, and 22:00 hours. The set points were calculated from the Environment Canada data as described previously. The experiment was carried out for two growing cycles. The first cycle emulated the environmental conditions of June 7 to November 15 and the second cycle emulated the conditions of April 26 to November 12. The dates mentioned in the rest of the paper refer to the dates that were emulated.

The [CO₂], light, temperature, and humidity were all controlled and monitored using an Argus Titan Environment-control system (Argus Control Systems Ltd, Vancouver, BC, Canada). The volumetric water content of the growing medium was maintained around 50-60 % of the field capacity as determined using an HH2 Moisture Meter and ML2X Theta Probe (DELTA-T DEVICES, Cambridge, UK). The seedlings were fertilized twice a week with 50/81/30.3 mg L⁻¹ of N/P/K from April 26 to May 25; 150/65.2/125 mg L⁻¹ N/P/K at the rapid growth phase (May 26 to August 30); and finisher with 50/54.3/156.3 mg L⁻¹ N/P/K (September 1 to 25) (Plant Products Co Ltd, Brampton, Ont., Canada). The fertilization was stopped on September 25. At the end of the first growing season, the seedlings were stored in a cold store (-4 °C) before the initiation of the second growing season. The average seedling height and root collar diameter were 76 and 0.9 cm, respectively at the end of the first growing season, and 178 and 1.6 cm, respectively at the end of the second growing season. The seedlings were transferred into bigger

pots (18 cm deep, 16/14 cm top/bottom diameter) in the second growing season but the treatments were otherwise identical in the two growing seasons.

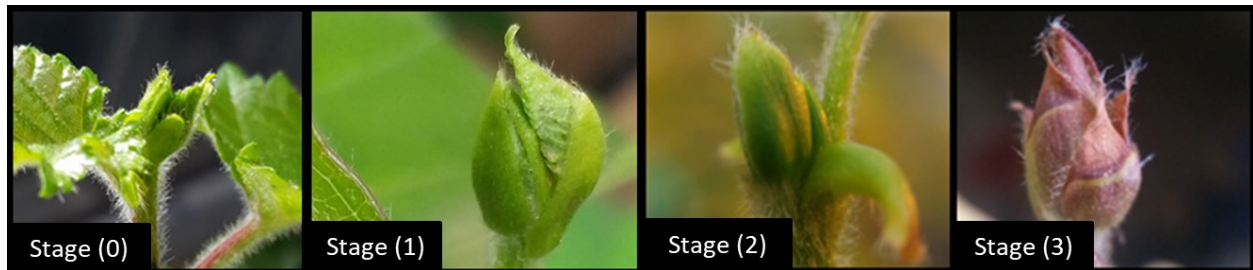


Figure 2.1. Stages of bud set as observed in white birch. Stage 0: no terminal bud is visible; Stage 1: bud visible for the first time; Stage 2: bud scales are closed but are still green in colour; Stage 3: bud-scales turn red-brown.

Observation of bud set and leaf senescence

A random sample of four seedlings per treatment combination (per replication) was used to monitor the timing of bud set and leaf senescence. Bud set was assessed in August and September (emulated dates). It was monitored using four stages of the terminal bud development by visual assessment (Figure 2.1). Bud set was considered complete when the terminal bud reached stage 2. The progress of leaf senescence was monitored by counting the number of green leaves at five-day intervals from August to November. The onset and end of leaf senescence for each seedling was defined as the dates when 10% and 90 % of the leaves fell or changed colour, respectively.

Cold hardiness test

In both growing seasons, cold hardiness was assessed at the end of leaf senescence by determining the level of injury caused by freezing temperatures using the ion-leakage technique (Sukumaran and Weiser 1972). Shoot tips of 10 cm without the terminal bud were taken from

four seedlings per treatment combination and cut into 2-cm sections. Each section from different seedlings was kept in a separate 50 mL Falcon Centrifuge Tubes with 30 mL of deionized distilled water overnight. All the sections were removed from the solution and frozen progressively at four temperatures; -5 , -15 , -30 and -45 °C using a programmable freezer (Model 45-6.8; ScienTemp Corp., Adrian, MI). The freezer temperature started from $+5$ °C and gradually lowered at a rate of 5° C per hour and held for one hour after each 5° C change. One hour after the testing temperature was reached, the samples were removed from the freezer and thawed at room temperature overnight in 30 mL of deionized distilled water. At this stage, the initial electrical conductivity of the solution was measured using an Accumet AR 20 electrical conductivity meter (Fisher Scientific, Ottawa, Canada). The samples subsequently were placed in an oven at 80° C for two hours, and then the final conductivity was measured. The relative electrolyte leakage (REL) due to freezing was calculated as a ratio of electrical conductivity of the solution after freezing and that after the sections were heat killed. I used a linear interpolation of the REL and the test temperature to estimate the lethal temperature for causing 50% electrolyte leakage (LT_{50}).

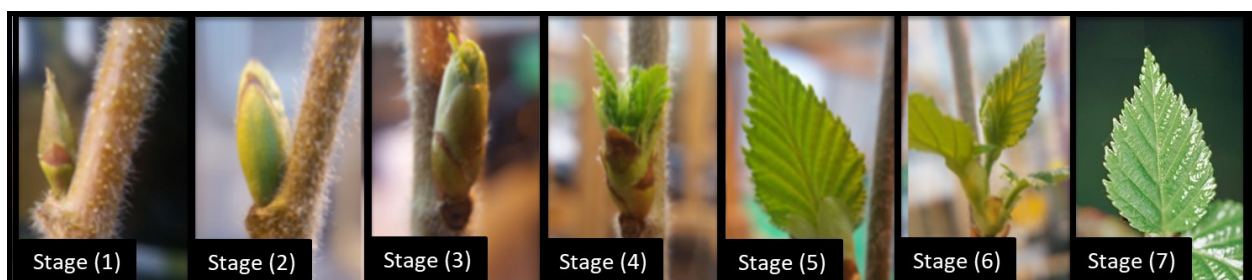


Figure 2.2. Stages of bud burst as observed in white birch. 1= the buds are completely closed; 2= the start of bud swelling, 3= the bud scales split open; 4= emergence of the first leaf from the bud; 5= the emergence of the base and petiole of the first leaf from the bud; 6= all leaves fully out of the bud; 7= completion of leaf expansion.

Timing of bud break

The spring bud break was monitored on the same seedlings that were used for the observation of bud set and leaf senescence. Since the seedlings were germinated from seeds in the first growing season, this observation was only conducted in the second growing season. For each seedling, I monitored five lateral buds immediately below the terminal bud. The observations were made visually every two days, from May to June. The bud phenology was divided into the following 7 phases (Figure 2.2) according to Linkosalo and Lechowicz (2006). For each seedling, the bud break was considered complete when at least three buds reached phase 5.

2.3.3 Statistical analysis

Two-way analyses of variance (ANOVA) were used to test treatment effects and their interactions. An effect was considered significant at $P \leq 0.05$ and marginally significant at $P \leq 0.1$. Fisher's LSD was used for post hoc pair-wise comparison when the interaction was significant. All data analyses were done using the R program (v. 3.5.0, R Core Team 2018). The data were transformed before analysis using the power transformation to meet the assumption of normality and homogeneity.

2.4 Results

Bud set and leaf senescence

Bud formation was not significantly affected by any of the treatments in either of the growing seasons (Table 2.1). Significant treatment effects on leaf senescence occurred only in the second growing season (Table 2.1). The CO₂ elevation advanced the onset of leaf senescence by an average of 23 days, but delayed the completion of senescence by 7 days, resulting in a much longer duration of leaf senescence than the ambient [CO₂] (Figure 2.1B). While leaf senescence

generally tended to start earlier at higher latitudes (55 and 58° N) than at lower latitudes (48 and 52 ° N) under the elevated [CO₂], no such a difference occurred under the ambient [CO₂] (Table 2.1, Figure 1A and C). There was no significant interactive effect or significant photoperiod effect on the duration or the end date of leaf senescence (Table 2.1).

Table 2.1. Summary of ANOVA (*P*-value and Degree of freedom (DF)) for the effects of [CO₂], photoperiod regime, and their interactions on the timing of bud formation, the onset, duration and end of leaf senescence, cold hardiness, and bud break. Seedlings were subjected to two [CO₂] (400 and 1000 μmol mol⁻¹) and four photoperiod regimes (corresponding to 48, 52, 55, and 58° N latitude) for two growing seasons in environment-controlled greenhouses. Significant (*P* ≤ 0.05) and marginal significant (*P* ≤ 0.1) *P*-values are bolded).

Parameters	Growing season	[CO ₂]	Photoperiod	[CO ₂] × Photoperiod
Bud formation	2016/17	0.47	0.461	0.782
	2017/18	0.902	0.248	0.564
Onset of senescence	2016/17	0.636	0.406	0.774
	2017/18	0.083	0.041	0.025
End of senescence	2016/17	0.693	0.859	0.795
	2017/18	0.040	0.74	0.378
Duration of senescence	2016/17	0.799	0.329	0.326
	2017/18	0.038	0.076	0.113
Cold hardiness	2016/17	0.163	0.195	0.635
	2017/18	0.693	0.009	0.038
Bud break	2017/18	0.193	0.852	0.003
DF		(DF=1)	(DF=3)	(DF=3)

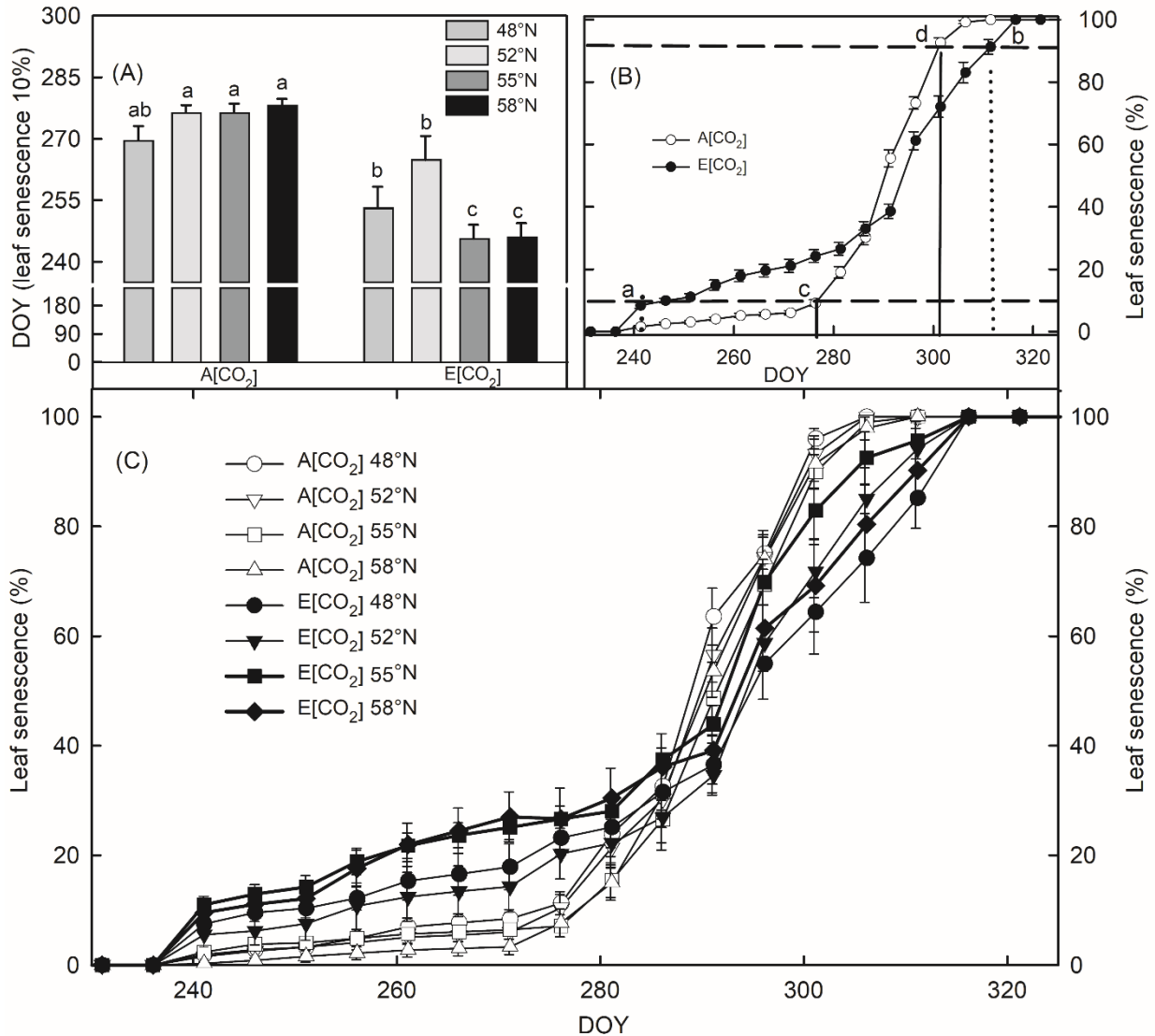


Figure 2.3. Effects of [CO₂] and photoperiod regime on the progression of leaf senescence in white birch seedlings in the second growing season. (A) day of year for 10% leaf senescence. (B) and (C) photoperiod treatment pooled, and photoperiod × CO₂, respectively. Seedlings were grown under photoperiod regimes corresponding to 48° (seed origin), 52°, 55°, and 58° N latitude and under 400 (ambient) and 1000 μmol mol⁻¹ (elevated) for two growing seasons. The vertical line a (dotted) and c (solid) in Figure B denotes the date of the onset of senescence, under the elevated and ambient [CO₂], respectively, while the vertical line b (dotted) and d (solid) represent the end of senescence at the elevated and ambient [CO₂], respectively. The horizontal broken lines in Figure B indicate 10 and 90% leaf senescence. (DOY) day of year. The value of each point represents the mean and standard error of 8 (A and C) and 32 (B) seedlings.

Cold hardiness

The treatments did not significantly affect the LT_{50} in the first growing season, but this parameter was significantly affected by the interaction between $[CO_2]$ and photoperiod regime in the second growing season (Table 2.1; Figure 2.2). The CO_2 elevation substantially increased the cold hardiness at the two higher latitudes (55 and 58° N): the average LT_{50} was -60 °C at 58° N and -46 °C at 55° N while the average LT_{50} was less than -40 in all other treatment combinations (Figure 2.2). In other words, under the elevated CO_2 , the seedlings endured much colder freezing temperatures under the photoperiod regimes corresponding to the two higher than those corresponding to the two lower latitudes. There was no significant photoperiod effect on LT_{50} under the ambient $[CO_2]$ or significant CO_2 effect at the photoperiod regimes corresponding to the two lower latitudes (Figure 2.2).

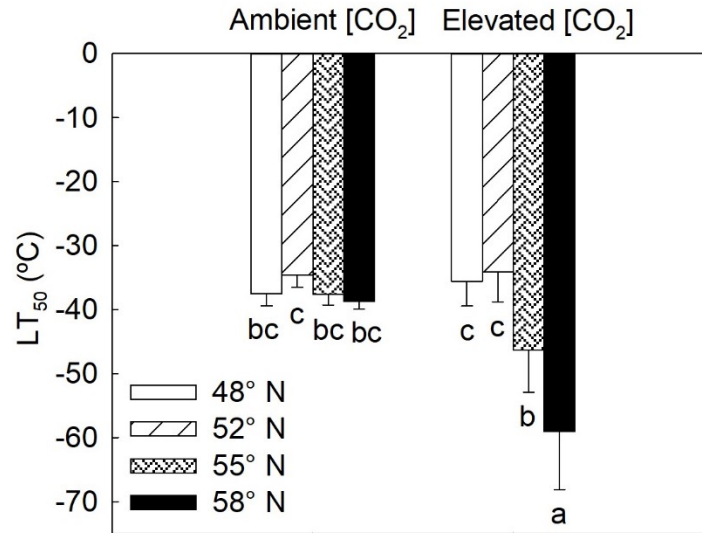


Figure 2.4. Freezing tolerance (LT_{50} = the lethal temperature for 50% tissue injury) of stem segments of white birch seedlings as affected by photoperiod regime and $[CO_2]$. Error bars represent 1 Standard error of the mean ($n = 8$). Means with different letters indicate significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Figure 2.1 for more explanations.

Bud break phenology

The interaction between $[CO_2]$ and photoperiod regime significantly affected the date of spring budburst (Table 2.1). Photoperiod regimes corresponding to 55 and 58° N significantly accelerated bud break under elevated $[CO_2]$, whereas they significantly delayed bud break under ambient $[CO_2]$ (Figure 2.3A-C). However, no significant differences were found between the photoperiod regime of the seed origin and that at 52° N or between 55 and 58° N (Figure 3A-C). Furthermore, the CO_2 elevation significantly delayed spring budburst at the photoperiod regimes corresponding to the two lower latitudes (48 and 52° N), significantly advanced at 55° N, but no significant effect at 58° N (Figure 2.3A-C).

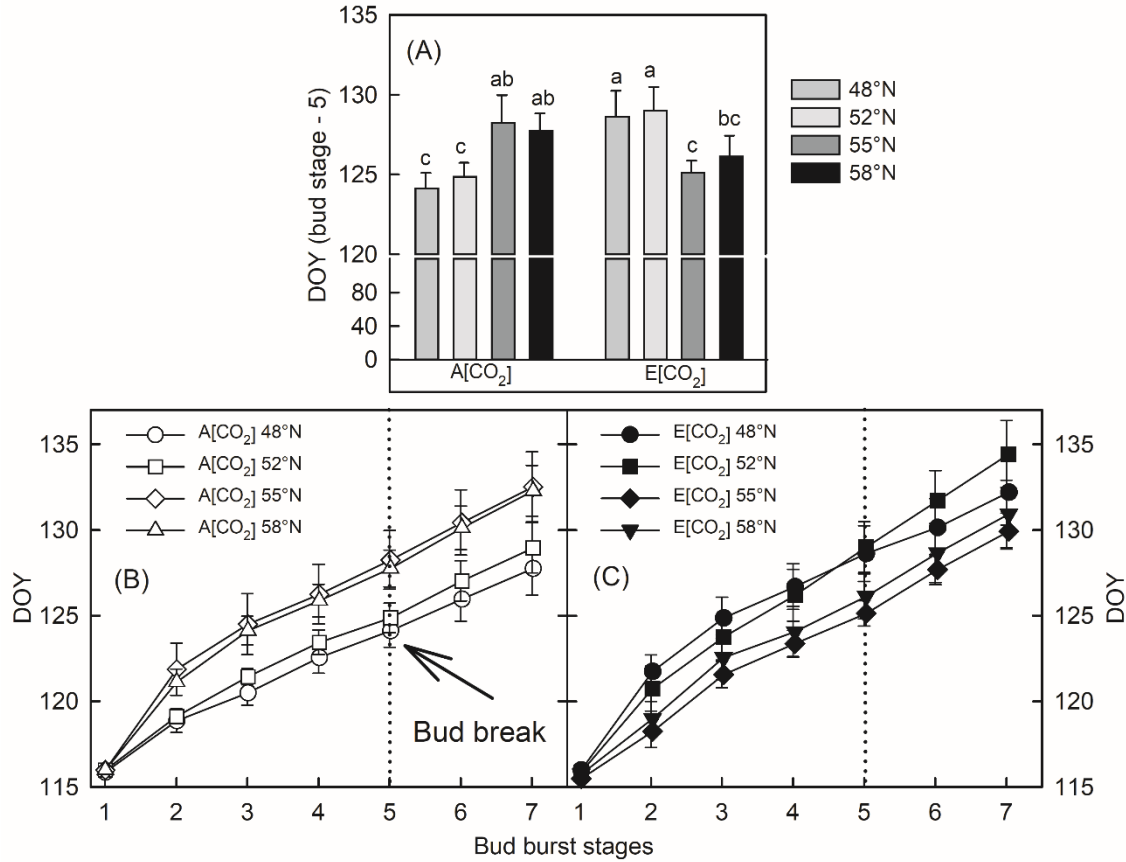


Figure 2.5. The progression of spring budburst in white birch seedlings subjected to 4 photoperiod regimes and two [CO₂] for two growing seasons. (A) day of year for budburst (Stage-5). (B, ambient and C, elevated CO₂) photoperiod × CO₂ interaction. The value of each point represents the mean and standard error of 8 seedlings. See Figure 2.1 for more explanations.

2.5 Discussion

The most interesting finding of this study is that the CO₂ elevation lengthened the duration of leaf senescence in white birch by as much as 30 days. This lengthening is due to both the earlier start and later completion of the senescence process, with the earlier start date as the primary contributor. The process of leaf senescence under elevated [CO₂] started 23 days earlier than that under ambient [CO₂]. Although there are variations in the method of assessing leaf senescence, empirical observations on deciduous trees have shown an advanced onset of senescence by [CO₂] elevations (Mousseau et al. 1995, Sigurdsson 2001, Körner et al. 2005, Warren et al.

2011). However, there are also opposite results in the literature. In the study of Godbold et al. (2014), CO₂ elevation delayed the onset of leaf senescence in *B. pendula*, and prolonged the completion of leaf senescence by ca. 12 days. Elevated CO₂-induced early onset of senescence has been explained by its indirect effect on the biochemistry and physiology of leaves (Jach et al. 2001). The increases in starch and sugar content and decreases in leaf nitrogen in plants grown under elevated [CO₂] (Ainsworth and Long 2004) is believed to trigger an earlier onset of leaf senescence (Wingler et al. 2006). Such a response can enhance nitrogen availability to young expanding leaves by freeing nitrogen invested in the Rubisco of old leaves (Wingler et al. 2006). While the later completion of leaf senescence process under elevated [CO₂] can prolong the duration of carbon fixation, it is unknown how much the delayed ending of leaf senescence contributes to the overall productivity in birch species (Goulden et al. 1996) or how much of the positive effect is offset by decreases in photosynthesis associated with the earlier onset of leaf senescence. Any increase in carbon fixation after growth cessation can have a positive effect on bud development, winter storage, and spring leaf-out (Pollard 1970, Horwath et al. 1994). However, delayed completion of leaf senescence may lead to the risk of premature leaf-fall from a sudden temperature decline in the fall which may affect autumnal freezing tolerance, nutrient resorption, and growth in the following growing season (Way and Montgomery 2015).

Another interesting finding is that under elevated [CO₂] the process of leaf senescence in white birch started earlier under the photoperiod regimes of the two high latitudes (55 and 58° N) than those at the two low latitudes (48 and 52° N) whereas no such differences occurred under ambient [CO₂]. Barr et al. (2004) and Fracheboud et al. (2009) have presented evidence that photoperiod controls the onset of leaf senescence in the fall and found that the leaf senescence in *Populus tremuloides* starts on the same date each year. Such a strong association of leaf

senescence with photoperiod has been manifested in both greenhouse and field experiments for *B. pendula* (Velling 1979). However, the results of this study suggest that this response can be modified by CO₂ elevations. While photoperiod regime did not affect the timing of leaf senescence in ambient [CO₂] (11:46 hours photoperiod), the process of leaf senescence under the elevated [CO₂] started at longer photoperiods at the two high latitudes (average 13:46 hours at 55 and 58° N) than at the two low latitudes (average 12:35 hours at 48 and 52° N). While the mechanism of the effect is unknown, the result may have significant implications on the prediction of forest productivity in the context of climate change induced northward migration. For example, the growing season may not be as long as we think it would be when trees migrate northward under elevated [CO₂]. Further research efforts are warranted in this area.

My results indicate that under elevated [CO₂], white birch would be able to tolerate much colder winter temperatures at higher latitudes. Under elevated [CO₂], the temperature that caused 50% freezing damage as indicated by electrolyte leakage was -46 and -60 °C, respectively for the photoperiod regimes corresponding to 55 and 58° N, while it was above -40 °C for the two lower latitudes and there were no such differences under the ambient [CO₂]. Wayne et al. (1998) have also reported a significant enhancement of freezing tolerance in *B. allaghanensis* seedlings grown under elevated [CO₂]. Although the physiological mechanism of CO₂ stimulation of freezing tolerance remains to be clarified, the enhancement of freezing tolerance by CO₂ elevations has been linked with enhanced carbohydrate production during cold acclimation and the production and roles of cryoprotective sugars in determining freezing tolerance (Castonguay et al. 1995, Poorter et al. 1997). The combination of the longer photoperiods at the two high latitudes (55 and 58° N) and elevated CO₂ may have led to a higher production of photosynthetic assimilates (Kuppers et al. 1988, Körner and Miglietta 1994, Gandin et al. 2011) and hence

greater cold hardiness (Repo et al. 1996). Furthermore, the longer process of leaf senescence under the photoperiod regimes of the two high latitudes and elevated [CO₂] might have played a role in improving freezing tolerance in this study. Bigras & Bertrand (2006) have found that the earlier start of fall phenological events in *Picea mariana* seedlings exposed to elevated [CO₂] is associated with improved freezing tolerance.

Photoperiod regimes had opposite effects on the timing of spring budburst in white birch under the two different [CO₂], and the effects of [CO₂] also varied with photoperiod regimes. Spring budburst in seedlings grown in the photoperiod regimes corresponding to the high latitudes (55 and 58° N) occurred earlier than those grown under the photoperiod regimes of the two lower latitudes (48 and 52° N) under elevated [CO₂], but the trend was the opposite under ambient [CO₂]. Further, elevated CO₂ delayed spring budburst at the two lower latitudes while it either advanced it or had no significant impact under the photoperiod regimes at the higher latitudes. Although temperature is believed to be the primary factor controlling spring budburst (Barr et al. 2004, Laube et al. 2014), exposure to longer photoperiods can reduce the accumulated temperature sum required for bud burst (Basler & Körner, 2014; Heide, 1993a, 1993b; Laube et al., 2014). In the present study, accelerated bud break at the longer photoperiods was apparent only under elevated [CO₂], but no such role was observed under the ambient [CO₂]. Increases in the availability of soluble sugars can play a crucial role in supplying the energy need at the time of budburst (Oren et al. 1988). Given the roles of CO₂ elevation and longer photoperiod in improving the production and storage of soluble sugars (Kuppers et al. 1988, Körner and Miglietta 1994, Gandin et al. 2011) and consequently freezing tolerance (Repo et al. 1996), it is possible that the combination of these two treatments was better able to satisfy the high demand of carbon for budburst in the spring. While it is believed that these responses could increase the

risk of late frost damage if trees are not synchronized with the season transitions at the new location (Aitken and Hannerz 2001), the simultaneous increase in cold hardiness in the treatment combinations suggest potentially higher rates of growth at higher latitudes under future elevated [CO₂] (Jach et al. 2001).

In summary, the individual and interactive effect of photoperiod and [CO₂] on white birch seedling reflect their critical role on the process of leaf senescence, freezing tolerance and spring bud break, which will, in turn, affect the duration of leaf presence, timing of growth onset, and tree survival under the scenario of climate change-induced northward migration. The results suggest that interactions between photoperiod and [CO₂] can alter autumnal and spring phenology and should be considered in assessing species suitability for assisted migration or in predicting the future distribution and productivity of boreal trees.

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3 Chapter 3: Photosynthesis of white birch (*Betula papyrifera* Marsh) will respond negatively to northward migration or seed transfer under the scenario of climate change

3.1 Abstract

Trees migrating to higher latitudes in response to climate change will encounter novel conditions at new locations, including changes in photoperiod regime and higher atmospheric carbon dioxide concentration ($[\text{CO}_2]$). These factors can interactively affect photosynthetic physiology and carbon uptake. In this study, I used simulated future environmental conditions to investigate the photosynthetic response to changes in photoperiod regime (seed origin (48°N), 52, 55, and 58°N) and $[\text{CO}_2]$ (ambient, 400 and elevated, 1000 $\mu\text{mol mol}^{-1}$) in white birch (*Betula papyrifera* Marsh) seedlings. My results show that $[\text{CO}_2]$ elevation did not affect the net photosynthetic rate (P_n) in seedlings grown under photoperiod regimes of much higher latitudes (55 and 58° N), but stimulated P_n under the photoperiod regimes of the seed origin or at a latitude 4° north of the seed origin. The CO_2 elevation resulted in the downregulation of maximum Rubisco activity (V_{cmax}) under the photoperiod regimes 55 and 58° N latitudes, maximum electron transport rate (J_{max}) and triose phosphate utilization (TPU) under the photoperiod regime at 58° N while it enhanced J_{max} and TPU under the photoperiod regimes of the lower latitudes. The higher instantaneous water-use efficiency (IWUE) under the photoperiod regimes of lower latitudes was primarily attributable to the stimulation of P_n by the elevated $[\text{CO}_2]$, while the higher IWUE under the photoperiod regimes of 55 and 58° N latitudes was achieved mainly by g_s control of water loss. My results show that the effects of photoperiod regimes depended on $[\text{CO}_2]$ level: the photoperiod regimes of 55 and 58° N generally suppressed P_n (relative to the seed origin) under elevated $[\text{CO}_2]$ but stimulated P_n under ambient $[\text{CO}_2]$. My study suggests that the

photosynthesis of white birch will likely respond negatively to northward migration or seed transfer in response to climate change.

3.2 Introduction

With the continuous increase in average global temperature (Pachauri et al. 2014), the rapid shift of climate envelopes are expected to move species geographic ranges poleward (McKenney et al. 2007). While trees that are able to migrate to locations with climates similar to their current environment which may reduce the effects of the stresses associated with climate change, plants will encounter new environmental conditions that they have not been adapted to (Griffith and Watson 2006), e.g., new photoperiod regimes (Way and Montgomery 2015) and higher atmospheric carbon dioxide concentration ($[CO_2]$). Both of these factors play a significant role in regulating plant phenological and physiological processes (Thomas and Vince-Prue 1997, Jach et al. 2001, Lambers et al. 2008). For instance, a change in photoperiod regime can influence photosynthesis and nitrogen content in the leaf (Comstock and Ehleringer 1986) and elevated $[CO_2]$ generally stimulates photosynthesis and carbohydrate production (Jach et al. 2001). Recently, there have been increased interests in understanding the effects of changes in photoperiod regime on plant phenology and growth in the context of climate change-induced tree migration (Li et al. 2015, Way and Montgomery 2015, Newaz et al. 2016). However, the interactive effects of photoperiod regime and $[CO_2]$ on photosynthetic physiology are still not well understood.

Small to moderate movement to a higher latitude may enhance the growth of plants even under the current climate conditions. For instance, moving a genotype of *Picea glauca* from 46 to 48° N enhances the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of

electron transport for RuBP regeneration (J_{\max}), leading to an increase in the rate of photosynthesis (P_n) (Benomar et al. 2016). Moving a genotype of *Eriophorum vaginatum* L from 65 to 68° N increases the light-saturated photosynthetic rate (A_{\max}) (Souther et al. 2014). The distance trees must move in order to stay within their climate envelope, however, will possibly be greater than 1100 kilometers (as much as 10 degrees) (McKenney et al. 2007, Loarie et al. 2009). Therefore, a dramatic change in the photoperiod regime is expected (Way and Montgomery 2015). Such a change may influence physiological processes, such as those involved in photosynthetic carbon assimilation. Exposure to extended photoperiod in the summer at higher latitudes can lead to reductions in V_{\max} , J_{\max} , and the efficiency of photosystem II when the use of carbohydrates cannot keep pace with the increase in production, leading to feed-back inhibition to P_n and photosynthetic downregulation (Osborne and Beerling 2003, Royer et al. 2005, Equiza et al. 2006). Photosynthetic downregulation or acclimation under longer photoperiods has been reported for various species (Dorais et al. 1996, Stutte et al. 1996, Llorens et al. 2009, Gandin et al. 2011).

It is well established that tree physiology is strongly influenced by [CO_2] elevation (Curtis and Wang 1998, Norby et al. 1999). While the effect of [CO_2] elevations on the photosynthesis of C_3 plants varies with species and other environmental conditions, elevated [CO_2] generally increase P_n , particularly when resources and other environmental conditions are favorable (Eamus and Jarvis 1989, Ainsworth and Long 2004, Leakey et al. 2009). Increased P_n and lower stomatal conductance (g_s) and transpiration rate (E) in response to elevated [CO_2] lead to enhanced water use efficiency (WUE) (Ainsworth and Rogers 2007). However, the carbon to nitrogen ratio (C/N) in the leaf often increases under elevated [CO_2] (Ellsworth et al. 2004, Ainsworth and Rogers 2007). An interesting question is how the longer period of photosynthesis associated with

the longer photoperiod regime at higher latitudes will affect plant responses to [CO₂] elevations. Theoretically, increases in the duration of photosynthesis without a corresponding increase in demand for carbohydrates will lead to photosynthetic downregulation (Long et al. 2004). Such a response may negate any stimulation to photosynthesis by elevated [CO₂]. Beerling and Osborne (2002) found that the CO₂ stimulation of P_n in polar forest species is constrained by extended photoperiods at high latitudes during the summer solstice while the level of the stimulation increases later in the summer when the photoperiods get shorter. The seasonal variation in the degree of CO₂ stimulation to photosynthesis is likely attributable to the seasonal change in photoperiod. The interactive effects of photoperiod regimes and [CO₂] may have implications to trees that migrate northward in response to climate change.

White birch (*Betula papyrifera* Marsh.) is one of the most widely distributed pioneer tree species in North America (Burns et al. 1990, Farrar 1995). It requires high water and nutrient availability and grows rapidly at the juvenile stage (Simard and Vyse 1992). In this study, I examined the photosynthetic responses of white birch to CO₂ elevation and photoperiod regimes. I hypothesized that the CO₂ stimulation of photosynthesis would decline with increases in photoperiod associated with higher latitudes.

3.3 Materials and methods

3.3.1 Plant material

I collected mature catkins of white birch from 12 natural trees in autumn of 2016 from Thunder Bay (48.4215° N, 89.2619° W). The seeds were then extracted manually from the catkins, air-dried and stored in a plastic bag until sowing time. The experiment was set up at the Lakehead University Forest Ecology Complex in Thunder Bay. Seeds were sown in germination trays

(50cm × 25cm) filled with a 1:1 (v:v) peat moss and vermiculite mixture. The day/night temperatures and photoperiod during germination were set to 22/16 °C and 16 hours, respectively. The seedlings of relatively uniform size (2 cm average height) were transplanted into pots of 12 cm deep and 12/9.5 cm top/bottom diameter for the experiment. The growing medium was a mixture of vermiculite and peat moss 1:3 (v:v).

3.3.2 Experimental design

The experiment was arranged in a split-plot design with four greenhouses (whole plots) accommodating two levels of [CO₂] treatment (ambient 400 and elevated 1000 μmol mol⁻¹) and four photoperiod regimes corresponding to 48°, 52°, 55°, and 58° N latitudes as the split-plot. There were ten seedlings in each treatment combination. The desired [CO₂] level was achieved in the greenhouses using natural gas CO₂ generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA). The four photoperiod regimes were set to simulate the natural weekly average regimes of the growing season for the four latitudes. The photoperiod regime for each greenhouse was set for the longest photoperiod of the four treatments and the shorter photoperiods for the other three treatments were achieved by manually applying black-out shades. The photoperiods were reset each week to the weekly average photoperiods for the four latitudes. To facilitate the shading operation, wooden frames were installed around the seedlings of each photoperiod treatment. High-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada) were used to extend the natural photoperiod when the natural day-length in the greenhouse was shorter than required. The day/night temperatures were derived from the 10-year weekly averages of Environment Canada records for the seed origin location (Environment Canada, 2016). The daily temperature in the greenhouses was ramped at four set points at 4:00, 10:00, 16:00, and 22:00 hours. The set points were calculated from the Environment Canada data

as described previously. The experiment was carried out for two growing cycles. The first cycle emulated the environmental conditions of June 7 to November 15 and the second cycle emulated the conditions of April 26 to November 12. The dates mentioned in the rest of the paper refer to the dates that were emulated.

The environmental conditions in the greenhouses ($[\text{CO}_2]$, temperature, and humidity) were monitored and controlled with an Argus control system (Argus Control Systems Ltd, Vancouver, BC, Canada). The volumetric water content of the growing medium was maintained around 50-60 % of the field capacity as determined using an HH2 Moisture Meter and ML2X Theta Probe (DELTA-T DEVICES, Cambridge, UK). The seedlings were fertilized twice a week with 50/81/30.3 mg L⁻¹ N/P/K from April 26 to May 25, 150/65.2/125 mg L⁻¹ N/P/K from May 26 to August 30, and 50/54.3/156.3 mg L⁻¹ N/P/K afterward (September 1 to 25). The seedlings were not fertilized after September 25. At the end of the first growing season, the seedlings were stored in a cold store (-4 °C) for six months before the initiation of the second growing season. The average seedling height and root collar diameter were 76 and 0.9 cm, respectively at the end of the first growing season, and 178 and 1.6 cm, respectively at the end of the second growing season. The seedlings were transplanted into bigger pots (18 cm deep, 16/14 cm top/bottom diameter) in the second growing season but the treatments were otherwise identical in the two growing seasons.

Chlorophyll fluorescence and A/Ci curve measurements

The measurements were taken in June, July, and August of the second growing season. Three seedlings, randomly each time from each treatment combination were selected, and a sun-leaf (5th to 7th from the top) of each tree was used for both chlorophyll fluorescence and gas

exchange measurements. Chlorophyll fluorescence was measured using a Hansatech FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd. Norfolk, UK). The minimum (F_o) and maximum (F_m) fluorescence yields were measured after the leaves were dark-adapted for at least 40 minutes. The potential maximum quantum efficiency of photosystem II (F_v/F_m) was determined as $(F_m - F_o)/F_m$.

The gas exchange was measured using a PP-Systems CIRAS-3 open gas exchange system (Amesbury, MA, USA). The response of net CO_2 assimilation rate to intercellular CO_2 concentrations (C_i) (A/C_i) were measured at 22 °C air temperature, 50% RH, under saturating light intensity $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ PAR and the following CO_2 concentrations: 400, 300, 250, 200, 150, 100, 50, 400, 450, 550, 650, 750, 850, 1000, 1200, 1500, and 1800 $\mu\text{mol mol}^{-1}$. Tests showed that gas exchange measurements were stable between 9:30 AM and 2:30 PM, and thus, all the measurements were made during this period. The net photosynthetic rate at treatment $[\text{CO}_2]$ (P_n), and ambient $[\text{CO}_2]$ (P_{n400}) were derived from the A/C_i curve of each seedling. Stomatal conductance (g_s), the rate of transpiration (E), and instantaneous water use efficiency (IWUE, determined by dividing P_n by transpiration rate E) were computed at their respective treatment $[\text{CO}_2]$. The ratio of intercellular to ambient $[\text{CO}_2]$ was computed for each seedling at their respective growth $[\text{CO}_2]$ conditions.

A/C_i curve fitting

The biochemical model of photosynthesis (Farquhar et al. 1980) was used to derive the following photosynthetic parameters from the A/C_i data for each seedling: the maximum rate of Rubisco carboxylation (V_{cmax} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), the maximum rate of electron transport for RuBP regeneration under saturating light (J_{max} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the rate of triose phosphate

utilization (TPU; $\mu\text{mol m}^{-2} \text{s}^{-1}$). The analysis was done using the “fitaci” function of the “plantecophys” package (Duursma, 2015) and R 3.5.0 (R Development Core Team, 2018). The “default” fitting method was used to estimate V_{cmax} , and J_{max} , whereas the “bilinear” fitting method was used to estimate TPU (Duursma, 2015).

Leaf nitrogen assay

After the completion of all the gas exchange measurements, leaves were harvested and subsequently over-dried at 80 °C for 48 hours and weighed. Mass-based leaf nitrogen [N] and carbon concentration [C] were determined by the dry combustion method (Horneck and Miller 1997) with a CNS-2000 (LECO Corp., St. Joseph, MI, USA). Mass-based leaf N concentration was converted to area-based leaf [N] by multiplying by the leaf area:mass ratio.

3.3.3 Statistical analysis

I used 3-way split-split-plot analysis of variance (ANOVA) to analyze all the data, with an independent month of the measurement considered as a time factor. However, two-way ANOVA was used for [N], [C], and C/N ratio. All the analyses were carried out with the R program (v. 3.5.0, R Core Team 2018). Power transformation was applied to meet the assumptions of normality on J_{max} , TPU, C_i/C_a , E , IWUE, [N], and F_v/F_m . The Fisher’s LSD post hoc pair-wise comparison was conducted when the effect of time or their photoperiod or interaction was significant ($P \leq 0.05$) or marginally significant ($P \leq 0.10$).

3.4 Results

Chlorophyll fluorescence and gas exchange

There was no significant treatment effect on the quantum efficiency of photosystem II (Fv/Fm) (Table 3.1). However, the effect of photoperiod \times [CO₂] was significant on P_n , and the effect was marginally affected by the time of the measurement (Table 3.1). The photoperiod regimes associated with the higher latitudes (55 and 58° N) significantly suppressed P_n under elevated [CO₂] (as compared with the seed origin), except at the July measurement where P_n under the photoperiod regime at 55° N was significantly lower than that at 52° N (Figure 3.1A).

In contrast, P_n was significantly higher at the two higher latitudes than the lower latitudes (48 and 52° N) in all the measurement in the ambient [CO₂] (Figure 3.1A). Moreover, the [CO₂] elevation did not stimulate P_n at the higher latitudes, but it did at the lower latitudes and the effect was similar in all three measurements (Figure 3.1A). Additionally, both g_s and E significantly declined over the measurement times, and g_s was marginally affected by [CO₂] \times photoperiod interaction (Table 3.1; Figure 3.2). The elevation of [CO₂] reduced g_s at the higher latitudes, but not at the lower latitudes (Figure 3.2).

Table 3.1. Summary of ANOVA (P-value and Degree of freedom (DF)) for the effects of [CO₂], photoperiod regime, time, and their interactions on the maximum quantum efficiency of PSII (Fv/Fm), net photosynthetic rate at treatment [CO₂] (P_n), and ambient [CO₂] (P_{n400}), stomatal conductance (gs), transpiration rate (E), instantaneous water-use efficiency (IWUE), internal to ambient CO₂ concentration ratio (Ci/Ca), maximum carboxylation rate (V_{cmax}), light-saturated electron transport rate (J_{max}), triose phosphate utilization (TPU), and the ratio of J_{max} to V_{cmax} (J_{max}/V_{cmax}). The effects of [CO₂], photoperiod regime, and their interactions on nitrogen [N] and carbon to nitrogen [C:N]. Seedlings were subjected to two [CO₂] (400 and 1000 μmol mol⁻¹), four photoperiod regimes (corresponding to 48, 52, 55, and 58° N latitude) and three measurement times. Significant (P ≤ 0.05) and marginal significant (P ≤ 0.1) P-values are bolded.

Variable		[CO ₂]	Photoperiod	[CO ₂] × Photoperiod	Time	[CO ₂] × Time	Photoperiod × Time	[CO ₂] × Photoperiod × Time
Fv/Fm	F	2.06	1.86	0.39	2.56	2.33	0.86	0.25
	P	0.29	0.24	0.77	0.11	0.13	0.55	0.96
P _n	F	23.25	5.15	35.69	41.91	17.02	1.19	2.24
	P	0.04	0.04	<0.01	<0.01	<0.01	0.36	0.09
gs	F	1.77	0.16	3.73	24.56	0.14	1.24	0.81
	P	0.32	0.92	0.07	<0.01	0.87	0.34	0.58
E	F	1.72	0.19	2.50	19.06	1.08	1.20	0.86
	P	0.32	0.90	0.16	<0.01	0.36	0.36	0.54
IWUE	F	24.87	12.13	12.47	1.88	5.97	3.78	4.89
	P	0.04	<0.01	<0.01	0.19	0.01	0.02	<0.01
Ci/Ca	F	27.78	31.19	13.45	21.76	6.59	2.95	3.66
	P	0.03	<0.01	<0.01	<0.01	<0.01	0.04	0.02
V _{cmax}	F	10.55	12.89	35.14	123.91	4.35	0.36	2.48
	P	0.08	<0.01	<0.01	<0.01	0.03	0.90	0.07
J _{max}	F	0.03	4.50	17.10	66.43	2.20	0.37	0.43
	P	0.89	0.05	<0.01	<0.01	0.14	0.89	0.85
TPU	F	0.29	4.08	15.98	70.54	2.86	0.37	0.41
	P	0.65	0.07	<0.01	<0.01	0.09	0.89	0.86
P _{n400}	F	10.30	7.35	33.43	41.05	4.61	1.93	2.00
	P	0.08	0.02	<0.01	<0.01	0.03	0.14	0.13
J _{max} /V _{cmax}	F	60.86	0.62	3.89	82.29	14.53	1.55	0.77
	P	0.02	0.63	0.07	<0.01	<0.01	0.23	0.60
DF		1	3	3	2	2	6	6
N	F	5.30	0.48	1.06				
	P	0.15	0.71	0.44				
C:N	F	12.50	0.83	1.29				
	P	0.08	0.53	0.36				
DF		1	3	3				

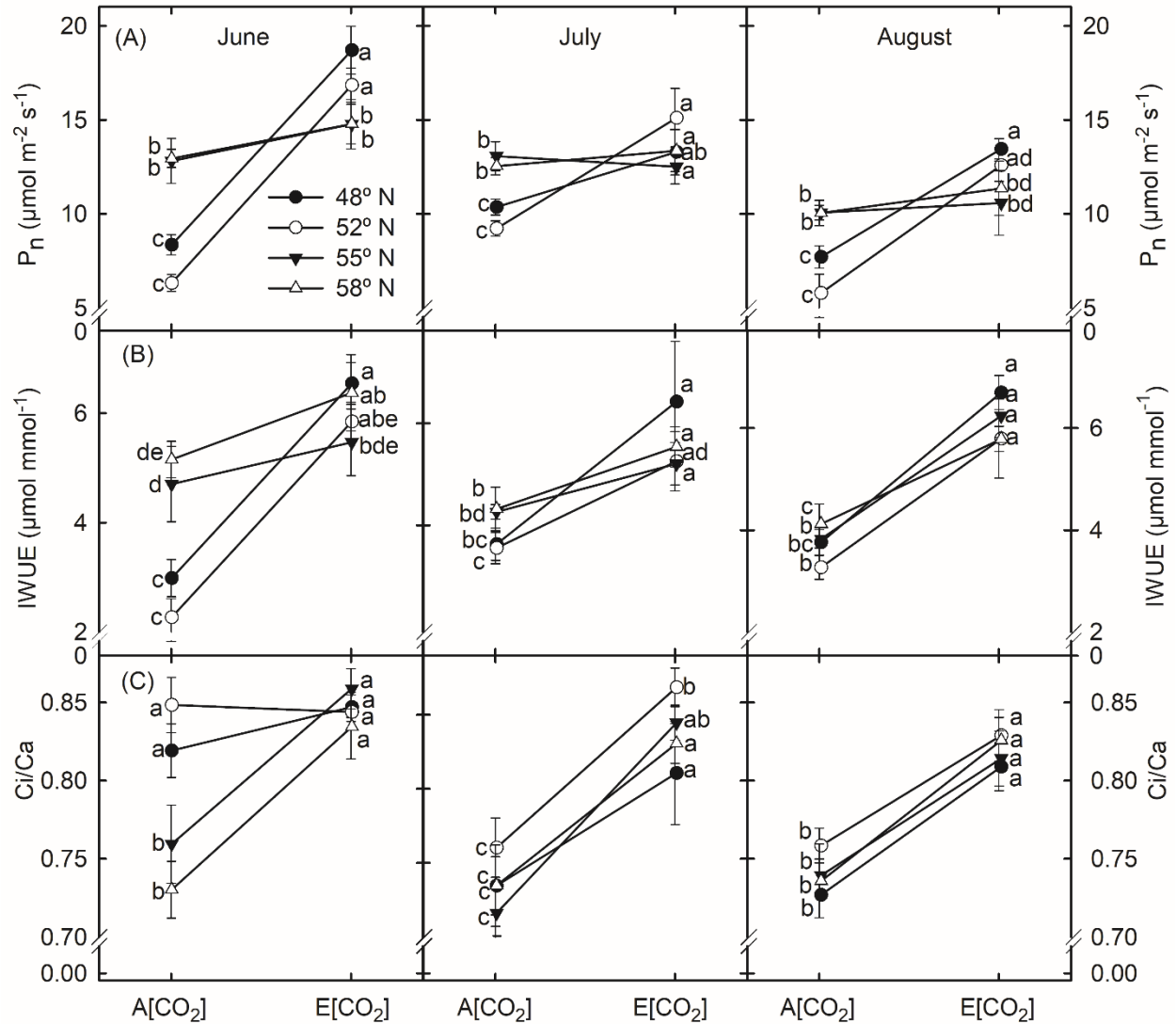


Figure 3.1. Effects of $[\text{CO}_2]$, photoperiod regime and measurement times and their interactions on the net photosynthetic rate at treatment $[\text{CO}_2]$ (P_n), instantaneous water use efficiency (IWUE), and internal to ambient $[\text{CO}_2]$ ratio (Ci/Ca) in white birch seedlings in the second growing season. Seedlings were grown under photoperiod regimes corresponding to 48° (seed origin), 52°, 55°, and 58° N latitude and under 400 (ambient, $\text{A}[\text{CO}_2]$) and 1000 $\mu\text{mol mol}^{-1}$ (elevated, $\text{E}[\text{CO}_2]$) for two growing seasons. The measurements were taken in June, July, and August. Data are presented as Mean \pm SE ($N = 6$: 3 seedlings per treatment combination \times 2 replications per combination). Means with different letters for each measurement time are significantly different from each other ($P \leq 0.1$, Fisher's LSD).

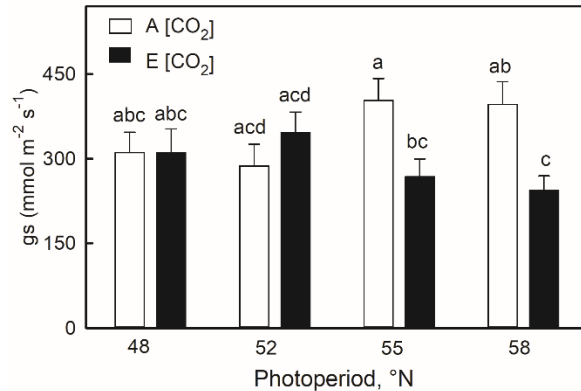


Figure 3.2. Stomatal conductance (g_s) of white birch seedlings as affected by photoperiod regime and $[CO_2]$. The data are presented as Mean + SE ($N= 18$: 3 seedlings per treatment combination and 2 replications per combination; the data were pooled for the 3 measurement times because the 3-way interaction was not statistically significant, $3 \times 2 \times 3 = 18$). Means with different letters indicate significant difference from each other ($P \leq 0.1$, Fisher's LSD). See Figure 1 for more explanations.

The interaction between $[CO_2]$ and photoperiod significantly affected both IWUE and C_i/C_a , and the effects varied over the measurement times (Table 3.1). The IWUE was lower at $55^\circ N$ than at $48^\circ N$ in the elevated $[CO_2]$ in the June measurement, but no photoperiod effect was found in subsequent measurements (Figure 3.1B). In contrast, while the photoperiod regimes of the higher latitudes enhanced IWUE compared with the lower latitudes in ambient $[CO_2]$ in the June and July measurement, no significant difference was found between 48 and $58^\circ N$ in the July measurement (Figure 3.1B). In the subsequent time, the differences were restricted to only between 58 and $52^\circ N$ in ambient $[CO_2]$. The effect of photoperiod was largely insignificant on C_i/C_a in elevated $[CO_2]$, apart from an increased at $52^\circ N$ compared with the $48^\circ N$ and $58^\circ N$ at the second measurement (Figure 3.1C). C_i/C_a was lower at higher latitudes under the ambient $[CO_2]$ in the June measurement, but there was no longer a significant photoperiod effect in the July and August measurement (Figure 3.1C). Both IWUE and C_i/C_a generally increased in response to $[CO_2]$ elevation under all the photoperiod regimes, but no significant $[CO_2]$ effect

was detected on IWUE at 55° N or on Ci/Ca at the lower latitudes in the June measurement (Figure 3.1B and C).

Photosynthetic capacity

There was a significant effect of photoperiod \times [CO₂] on V_{cmax} , and the effects were marginally affected by the time of measurement (Table 3.1). The photoperiod regime at 58° N reduced V_{cmax} in elevated [CO₂] only in the June measurement, but no significant photoperiod effect was detected under elevated [CO₂] in subsequent measurements (Figure 3.3). In contrast, the photoperiod regimes at the higher latitudes generally increased V_{cmax} in the ambient [CO₂], but the difference between the 48° N and 58° N at the July measurement was not significant (Figure 3.3).

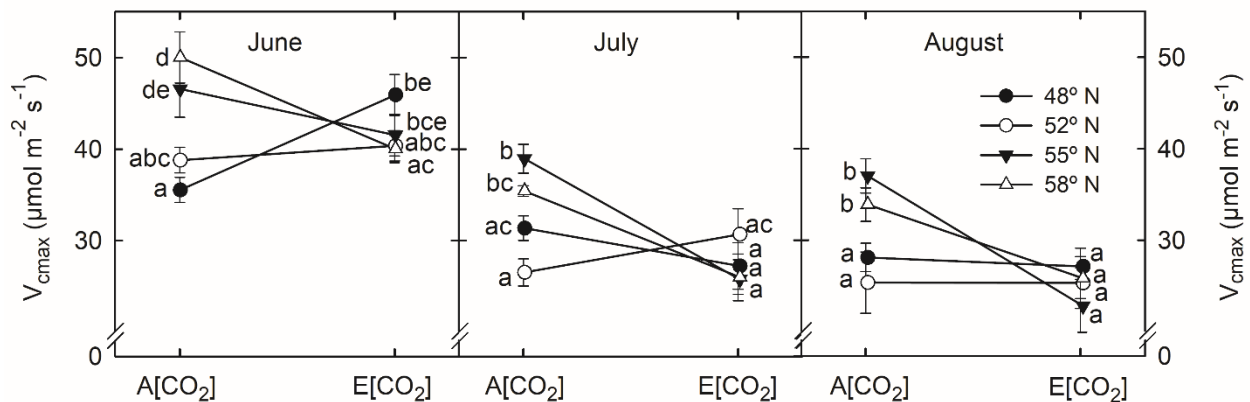


Figure 3.3. Effects of [CO₂], photoperiod regime, and measurement time on the maximum carboxylation rate (V_{cmax}). The data are presented as Mean \pm SE (N= 6). Means with different letters indicate significant difference from each other ($P \leq 0.1$, Fisher's LSD). See Figure 1 for more explanations.

Moreover, the [CO₂] elevation reduced V_{cmax} at the higher latitudes in all three measurements, but the decline at 55° N was not significant for the June measurement (Figure 3.3). With the exception of the June measurement where [CO₂] elevation enhanced V_{cmax} at 48° N, the [CO₂] elevation had no significant impact on V_{cmax} at the two lower latitudes (Figure 3.3).

There was a significant interactive effect of photoperiod \times $[\text{CO}_2]$ on J_{max} and TPU (Table 3.1).

The photoperiod regime at 58° N decreased J_{max} and TPU compared with 48° N in elevated $[\text{CO}_2]$ while both parameters increased by the higher latitudes in ambient $[\text{CO}_2]$ (Figure 3.4A and B).

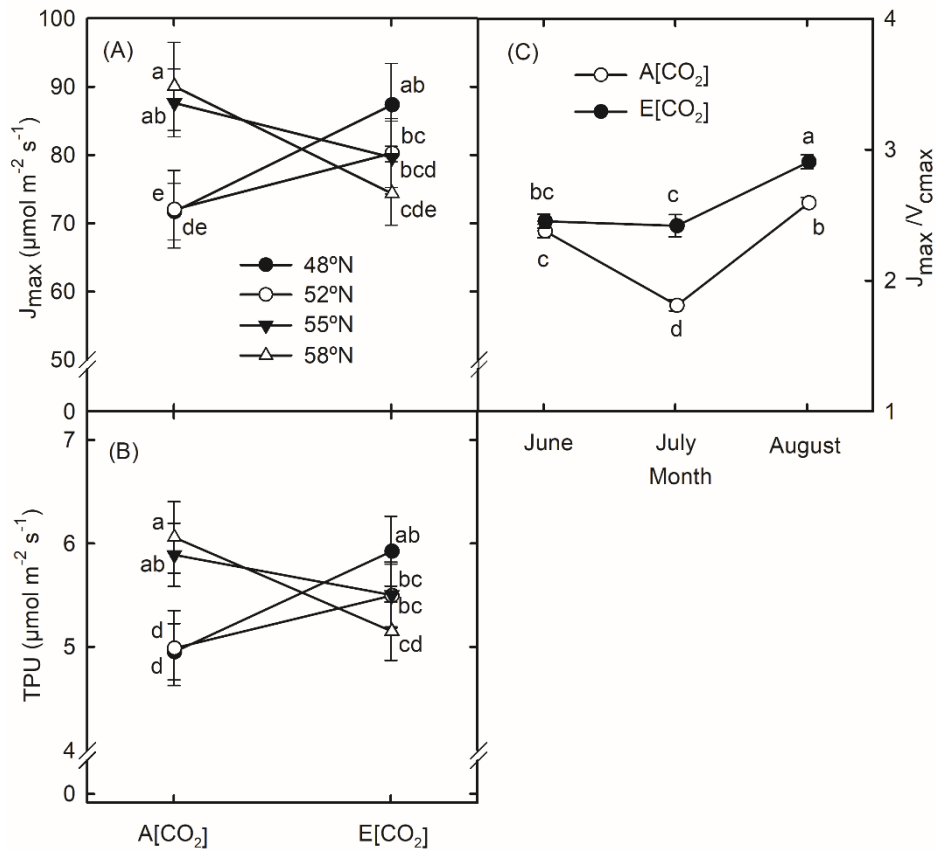


Figure 3.4. Effects of $[\text{CO}_2]$, and photoperiod regime on (A) the light-saturated electron transport rate (J_{max}), (B) triose phosphate utilization (TPU), and effects of $[\text{CO}_2]$ and measurement time on (C) the ratio of J_{max} to V_{cmax} . Data are presented as Mean \pm SE (N= 18 for (A) and (B) as explained in Figure 2; N= 24 for (C): data were pooled for the 4 photoperiods because the 3-way interaction was not statistically significant, $3 \times 2 \times 4 = 24$). Means with different letters indicate significant difference from each other ($P \leq 0.05$, Fisher's LSD). See Figure 1 for more explanations.

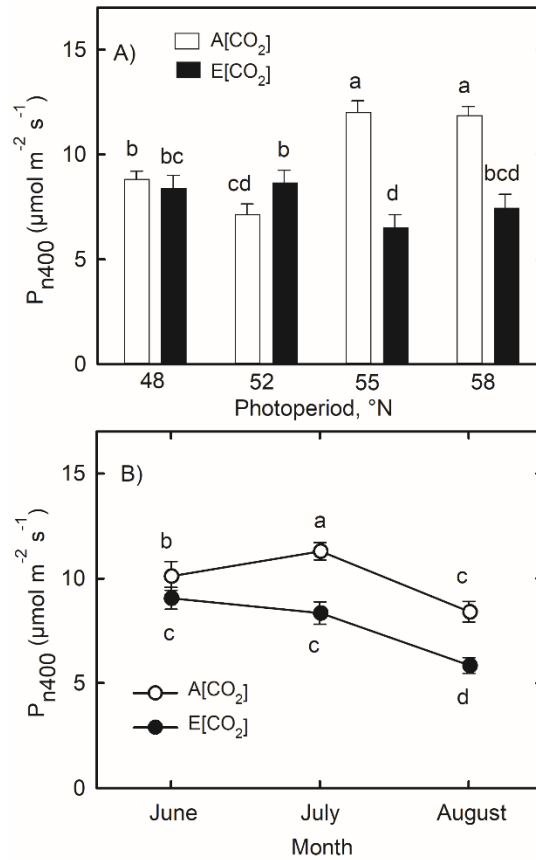


Figure 3.5. Effects of [CO₂] and photoperiod regime (A), and [CO₂] and measurement time (B) on the net photosynthetic rate at ambient [CO₂] (P_{n400}). Data are presented as Mean \pm SE (N= 18 for A); N= 24 for B); see Figure 4 for explanations). Means with different letters indicate significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Figure 1 for more explanations.

While both J_{max} and TPU declined in response to the CO₂ elevation at 58° N, they increased in response to the CO₂ elevation at 48 and 52 ° N (Figure 3.4A and B). There was a linear relationship between J_{max} and V_{cmax} across all treatments with a slope of 1.987 ($P < 0.001$; $R^2 = 0.70$, data not shown). The $J_{\text{max}}/V_{\text{cmax}}$ ratio was significantly higher in August than in June and July under the elevated [CO₂] and [CO₂] elevation significantly increased $J_{\text{max}}/V_{\text{cmax}}$ in July and August, but not in June (Table 3.1, Figure 3.4C). The [CO₂] elevation significantly reduced P_{n400} (photosynthesis measured at a common, ambient [CO₂]) at 55 and 58° N in all three measurements but the effect was greater in July and August than June (Figure 3.5A and B).

While the $[\text{CO}_2]$ elevation did not have a significant effect on P_{n400} at 48°N , it increased P_{n400} at 52°N (Figure 3.5A). Also, the elevated $[\text{CO}_2]$ marginally increased C:N ratio (Table 3.1).

3.5 Discussion

My results generally support the hypothesis that the stimulation of photosynthesis by CO_2 elevations would diminish with increases in photoperiod associated with northward migration. In fact, the photosynthesis of birch seedlings under the photoperiod regimes associated with the two highest latitudes (55 and 58°N) was not stimulated at all by the $[\text{CO}_2]$ elevation and the rates of photosynthesis under those two photoperiod regimes and elevated $[\text{CO}_2]$ were significantly lower than the comparable rates at the two lower latitudes. Photosynthesis under the photoperiod regimes of the two lower latitudes (48 and 52°N) was stimulated by the CO_2 elevation by an average of 91%. Furthermore, the lack of P_n stimulation at the two higher latitudes persisted in all three measurement periods from June to August, which is in contrast to the seasonal variations of CO_2 stimulation to photosynthesis reported by other researchers, e.g., Beerling and Osborne (2002). It has been suggested that the greater P_n stimulation in the mid-summer is due to the higher utilization of photosynthetic products when plants are growing more vigorously (Long 1991, Gunderson and Wullschlegel 1994, Lewis et al. 1996, Tissue et al. 1997, Quentin et al. 2015). However, the seasonal pattern of the response may vary with latitude. At a very high latitude of 69°N , P_n in trees is only stimulated by elevated $[\text{CO}_2]$ in early spring and late summer (Beerling and Osborne 2002, Osborne and Beerling 2003).

My results suggest that the lack of P_n stimulation by the elevated $[\text{CO}_2]$ at the two higher latitudes resulted from the downregulation of photosynthetic capacity as indicated by the reductions in V_{cmax} and J_{max} . In contrast, no such downregulations occurred under the

photoperiod regimes associated with the two lower latitudes. CO₂ elevation-induced decreases in V_{cmax} and J_{max} at mid and high latitudes are often attributed to reductions in foliar nitrogen content and sink strength (Gunderson and Wullschleger 1994, Medlyn et al. 1999) and associated reduction in the amount of Rubisco (Tissue et al. 1999, Griffin et al. 2000, Rogers and Ellsworth 2002). However, the down-regulation of V_{cmax} and J_{max} in this study was not coupled with a decrease in leaf nitrogen content. Under the photoperiod regimes at the two lower latitudes, in contrast, there were photosynthetic upregulations in response to the CO₂ elevation, particularly in J_{max} , which should have contributed to the stimulation of photosynthesis by the elevated CO₂.

The results of this study suggest that the photosynthetic downregulation at the higher latitudes in response to the CO₂ elevation was related to a possible feedback inhibition to photosynthesis. A feedback inhibition occurs when the rate of photosynthetic carbohydrate production exceeds the rate of utilization by growth and respiration and the subsequent accumulation of triose phosphate in the cytosol will lead to a short supply of inorganic phosphate for the photosynthetic Calvin cycle, triggering the downregulation of photosynthetic machinery (Lambers et al. 2008). Indeed, the rate of triose phosphate utilization (TPU) in seedlings grown under the photoperiod regime of 58° N latitude declined significantly in response to the CO₂ elevation. Sage (1994) has reported that declines in starch and sucrose synthesis could reduce the rate of RuBP regeneration because the metabolization of triose phosphate cannot keep up with its production, leading to sink limitation (Foyer and Spencer 1986).

Another interesting finding of this study is that photoperiod regimes had opposite effects on P_n in white birch under the two different [CO₂]. The rate of photosynthesis in seedlings grown in the photoperiod regimes corresponding to the high latitudes (55 and 58° N) was higher than those

grown under the photoperiod regimes of the two lower latitudes (48 and 52° N) under the ambient [CO₂], but the trend was the opposite under the elevated [CO₂]. Further, the response patterns of J_{\max} and TPU were similar to those of P_n . A longer duration of photosynthetic activity under a longer photoperiod will increase the daily photosynthetic production of carbohydrates if the photosynthetic rate remains the same (Kuppers et al. 1988). However, such an increase in photosynthesis can lead to an accumulation of assimilates in the leaves if there is not a synchronized increase of demand for assimilates, leading to feedback inhibition of photosynthesis (Stutte et al. 1996, Gestel et al. 2005). CO₂ elevations are known to increase the rate of photosynthesis (Ainsworth and Long 2004, Leakey et al. 2009) and thus can further aggravate the feedback inhibition, which may provide a plausible explanation for the interactive effects of [CO₂] and photoperiod regimes in this study (Kuppers et al. 1988, Körner and Miglietta 1994, Gandin et al. 2011).

The CO₂ elevation significantly increased the photosynthetic water use efficiency of white birch in all the photoperiod treatments, but the physiological mechanisms of the IWUE varied with photoperiod regime. For seedlings grown under the photoperiod regimes of 48 and 52° N latitudes, the CO₂ elevation induced enhancement in IWUE was primarily attributed to the CO₂ stimulation to photosynthesis rather than a reduction in transpiration, which is in contrast to the common conclusion in the literature (Ainsworth and Long 2004, Ainsworth and Rogers 2007). Under the photoperiod regimes of higher latitudes of 55 and 58° N, however, the improvement in IWUE was primarily a result of the reduction in transpiration that was triggered by the CO₂ elevation. In conifers, empirical observations appear to indicate a differential mechanism in maintaining the expected stimulation of WUE from CO₂ elevation under extended photoperiod. Llorens et al. (2009) reported that acclimation in photosynthetic capacity negates any stimulation

to photosynthesis by elevated [CO₂] in deciduous conifer, swamp cypress (*Taxodium distichum*) when grown at the photoperiod regime of 69° N latitude. However, the positive effect of [CO₂] elevation on IWUE was maintained throughout the growing season due to a decline in stomatal conductance, which reduced transpiration. In contrast, stimulation of IWUE in coastal redwood (*Sequoia sempervirens*) and dawn redwood (*Metasequoia glyptostroboides*) was limited to early in the spring and later in the summer when the photoperiods get shorter and level of the CO₂ stimulation of P_n increases (Llorens et al. 2009).

In summary, the results of this study suggest that photoperiod regimes can substantially affect the photosynthetic responses of boreal trees to CO₂ elevations and such effects are highly relevant to the northward migration or seed transfers of boreal tree species under the scenario of climate change. Much more research efforts are warranted in this research topic. Furthermore, the effects of photoperiod regimes should be considered in future efforts to evaluate carbon sequestration under climate change-induced northward migration.

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4 Chapter 4: White birch has limited phenotypic plasticity to take advantage of increased photoperiods at higher latitudes north of the seed origin

4.1 Abstract

Global warming induced northward migration will expose trees to longer photoperiod regimes during the growing season. The phenotypic ability of trees to take advantage of the longer photoperiods and elevated atmospheric CO₂ concentration ([CO₂]) will likely be a critical factor for determining their success and performance at the new locations. I investigated how growth, biomass, and biomass allocations respond to the interactive effects of photoperiod regimes and [CO₂] in white birch (*Betula papyrifera* Marsh.). Seedlings were exposed to ambient (400 μmol mol⁻¹) or elevated concentration (1000 μmol mol⁻¹) [CO₂], and four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58° N latitude for two growing seasons. My results show that growth, biomass, and biomass allocation were affected by photoperiod regime but not by [CO₂]. Seedling growth and biomass were stimulated by the three photoperiod regimes north of the seed origin. Plants under the photoperiod regime of 52° N were 15% higher in growth and 18% higher in biomass than under the photoperiod regime of the seed origin (48° N). However, increases in photoperiod regimes beyond that of 4° north of the seed origin did not lead to an additional increase in growth and biomass. The differences in biomass components among the three longer photoperiods were insignificant, but the leaf biomass and stem biomass were higher under the longer photoperiods relative to the seed origin. While the differences between two adjacent photoperiods were not always statistically significant during the two growing seasons, biomass allocated to roots showed a general decreasing trend with increases in photoperiod from the seed origin to 58° N latitude. My results suggest that despite the limited plasticity in growth and biomass displayed in much higher latitudes, white birch will likely benefit from the longer

photoperiod regimes during the growing season associated with migration or seed transfer to higher latitudes.

4.2 Introduction

The predicted shift in tree species distribution in response to the ongoing climate change requires the successful establishment of seedlings in the new location which depends on, among other factors, the physiological and/or morphological plasticity of the species or population to acclimate to the climatic and edaphic conditions of the new location which may have positive or negative effects on survival and growth (Pitelka and Group 1997, Chen et al. 2011). For example, a northward range shift of tree species in quest of suitable habitat will expose them to new photoperiod regimes. Photoperiod regimes can have a significant influence on the survival, growth, and productivity of trees and influence the distribution of tree species (Thomas and Vince-Prue 1997). Therefore, a good understanding of how changes in photoperiod regimes may interact with the future, elevated atmospheric CO₂ concentration in affecting the growth, biomass and biomass allocations of trees, particularly in the context of climate-change-induced migration, will likely be critical for understanding and predicting the distribution, composition and productivity of future forests (Saikkonen et al. 2012).

The CO₂ concentration in the atmosphere has increased dramatically in the last century and will continue to increase for the rest of this century (Pachauri et al. 2014). The increase in atmospheric [CO₂] generally enhances the photosynthetic activities and growth of C₃ plants and influences biomass allocations (Ceulemans and Mousseau 1994, Rogers et al. 1994, Curtis and Wang 1998). Under elevated [CO₂], plants often tend to increase dry matter allocation to roots, particularly when water or nutrient resources are limited (Stulen and Den Hertog 1993, McGuire

et al. 1995, Rogers et al. 1995, Curtis and Wang 1998, Saxe et al. 1998). While the influence of [CO₂] elevations on biomass allocation can be influenced by other environmental factors, such as photoperiod regimes, research in this area is generally scarce, particularly in the context of climate change induced northward migration of boreal trees.

In the temperate and boreal zones, photoperiod controls various physiological and phenological processes/traits and serves as an environmental cue for the synchronization of plants' phenological and physiological processes with the seasonal climate conditions (Salisbury 1981, Thomas and Vince-Prue 1997, Lambers et al. 2008, Jackson 2009). A change in photoperiod regime can affect the growth and productivity of tree species in different ways. Photoperiod can influence growth and productivity via its effect on the timing of photoperiod-controlled phenological events such as bud burst in the spring and bud formation in autumn (Velling 1979, Junttila and Kaurin 1990, Basler and Körner 2012). Photoperiod can also alter plant growth rate and biomass production (Hay 1990, Thomas and Vince-Prue 1997). Longer photoperiods have been reported to stimulate growth (Oleksyn et al. 1992, Johnsen and Seiler 1996) and the rate of biomass accumulation (Stinziano and Way 2017). Increases in leaf area and leaf mass ratios and specific leaf area have also been reported for plants grown under extended photoperiods (Hay 1990, Adams and Langton 2005). Photoperiod can also affect the biomass allocation between aboveground and belowground organs in trees (Li et al. 2015). However, the results on the photoperiod influence over the biomass allocation in trees vary with species and other factors, and past studies are primarily focused on coniferous trees, grass and herbaceous species (Heide 1974, Heide et al. 1985, Burdett and Yamamoto 1986, Hay 1990, Bigras and Daoust 1993, Stinziano and Way 2017). Furthermore, the combined effect of two or more interacting factors is generally different from the summation of their individual effects. For example, it is unknown

how much of the stimulation of carbon allocation to roots by [CO₂] elevations (McGuire et al. 1995) can be offset by the opposite effect of longer photoperiods associated with a northward migration (Johnsen and Seiler 1996, Gestel et al. 2005, Hamilton et al. 2016). Therefore, a good understanding of such potential interactive effects may play an important role in predicting the success of northward migration of tree species under a changing climate.

White birch (*Betula papyrifera* Marsh.) is a widely distributed pioneer tree species in North America (Burns et al. 1990, Farrar 1995). It requires high water and nutrient supplies and grows rapidly in the juvenile stage (Simard and Vyse 1992). This study investigated the interactive effects of photoperiod regime and CO₂ elevation on the growth and biomass allocation of white birch in the context of climate change induced migration. I exposed white birch seedlings to the photoperiod regimes at 48 (seed origin), 52, 55, and 58° N latitudes and two CO₂ concentrations (400 μmol mol⁻¹ and 1000 μmol mol⁻¹). I have tested the following hypotheses: (1) CO₂ elevation will increase the overall seedling growth and biomass allocation to roots; (2) A longer photoperiod will stimulate aboveground growth and increase biomass allocation to the shoot; and (3) CO₂ elevation and longer photoperiod will cancel each other's effect on biomass allocation between shoot and roots because their effects are opposite of each other.

4.3 Materials and methods

4.3.1 Plant materials

Catkins of white birch (*Betula papyrifera* Marsh.) were collected from 12 natural trees in Thunder Bay (48.4215° N, 89.2619° W). The seed trees were at least 115 m apart from each other and from the same population. Seeds were extracted manually, air dried and stored in plastic bags at room temperature before sowing. The experiment was conducted at the Lakehead

University Forest Ecology Complex in Thunder Bay. Seeds were germinated in a tray (50cm × 25cm) filled with peat moss and vermiculite (1:1, v:v). During the germination, the temperature and photoperiod were set to 22/16 °C day/night and 16 hours day-length, respectively. Seedlings of relatively uniform size (2 cm average height) were transplanted into pots of 12 cm deep and 12/9.5 cm top/bottom diameter filled with a mixture of vermiculite and peat moss (1:3, v:v).

4.3.2 Experimental design

The treatments consisted of two [CO₂] (ambient 400 and elevated 1000 μmol mol⁻¹) and four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58° N latitude in a split-plot design with [CO₂] as the whole plot and photoperiod regime as the split-plot. There were 20 seedlings per treatment combination. The CO₂ elevation was achieved using natural gas CO₂ generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA). Each [CO₂] was replicated twice using independently environment-controlled greenhouses. The photoperiod in each greenhouse was adjusted weekly to emulate the weekly average regime of the growing season for the longest photoperiod of the four treatments, and that photoperiod regime was shortened by manually applying black-out shade to emulate the photoperiod regime for each of the other three treatments. A wooden frame was established around each split-plot to facilitate the shading and to maintain consistency across treatments. High-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada) was used to extend the natural photoperiod when the natural day-length in the greenhouse was shorter than required. The air temperatures were derived from the 10-year weekly averages of Environment Canada records for the seed origin location (Environment Canada, 2016). The temperature in each greenhouse was ramped at four set points at 4:00, 10:00, 16:00, and 22:00 hours according to the 10-year average temperatures for the corresponding hour. The experiment was carried out for two growth cycles. The first

cycle emulated the environmental conditions of June 7 to November 15 and the second cycle emulated the conditions of April 26 to November 12. The dates mentioned in the rest of the paper refer to the dates that were emulated.

The [CO₂], temperature, and humidity were controlled using an Argus Titan Environment-control system (Argus Control Systems Ltd, Vancouver, BC, Canada). During the growing period, the water content of the growing medium was maintained around 50-60 % of the field capacity as determined using an HH2 Moisture Meter and ML2X Theta Probe (DELTA-T DEVICES, Cambridge, UK). All the seedling were fertilized twice a week with a fertilizer solution containing 50/81/30.3 mg L⁻¹ of N/P/K from April 26 to May 25; 150/65.2/125 mg L⁻¹ N/P/K at the rapid growth phase (May 26 to August 30); and 50/54.3/156.3 mg L⁻¹ N/P/K during the hardening phase (September 1 to 25) (Plant Products Co Ltd, Brampton, Ont., Canada). The fertilizer application was discontinued on September 25. The seedlings were all cold hardened and set buds at the end of the growing season when the environment conditions in the greenhouses were emulating the average natural environmental conditions of mid-November for the region as described previously. The dormant seedlings were moved to a dark cold storage room (-4 °C) where they were kept for 5 months and 11 days before the initiation of the second growing season when the average natural environmental conditions for the region were emulated starting from those of April 26. The seedlings were transferred into bigger pots (18 cm deep, 16/14 cm top/bottom diameter) in the second growing season but the treatments were otherwise identical in the two growing seasons.

Growth and biomass measurement

I took measurements of seedling height and root collar diameter on five randomly chosen seedlings per treatment combination per replication at the end of each growth cycle when the

buds were fully formed, 267 Julian days (around the autumnal equinox, no significant difference among different photoperiod treatments). Following growth measurement, the seedlings were harvested and separated into leaves, stems, and roots. The samples were then oven dried at 70°C for 48 h and weighed on an analytical balance (0.001 g precision) to determine biomass for leaf, stem and root. The total leaf area (LA) per seedling was determined using WinFolia (Regent Instrument Inc., Quebec, Canada). I then calculated the specific leaf area (SLA= leaf area/dry leaf mass), leaf area ratio (LAR= total leaf area/seedling dry mass), root to shoot ratio (R:S= root mass/shoot mass), leaf mass ratio (LMR= leaf mass/seedling dry mass), stem mass ratio (SMR= stem mass/seedling mass), and root mass ratio (RMR= root mass/seedling mass).

4.3.3 Statistical analysis

Statistical analyses were performed using the R program (v. 3.5.0, R Core Team 2018). Photoperiod, [CO₂] and their interactive effects were tested by two-way analysis of variance (ANOVA). The two levels of CO₂ (n=2) were randomly assigned to the whole plots (four greenhouses) as a completely randomized design (CRD) and the sub-plots (n=4) were treated as nested within the whole plot in ANOVA. ANOVA was run separately for the two growth cycles. The total number of individuals (N) was 80: 5 seedlings per treatment combination, 2 levels of CO₂ with 2 replicates each, and 4 photoperiods nested within CO₂. Mean differences were considered significant at $P \leq 0.05$ and marginally significant at $P \leq 0.1$. Significant differences among photoperiod regimes and significant interactions were further evaluated using the Fisher's LSD test. The data were transformed before analysis using the power transformation to meet the assumption of normality and homogeneity.

4.4 Results

Growth and biomass production

Growth and biomass were not affected by [CO₂] (Table 4.1). The height of seedlings grown under the three photoperiod regimes north of the seed origin was generally significantly greater than that under the photoperiod regime of the seed origin (Table 4.1, Figure 4.1A and B). The response pattern of seedling diameter was similar to that of height in the first growing season (Figure 4.1C), but the differences became statistically insignificant in the second growing season (Table 4.1).

Leaf biomass was significantly affected by photoperiod regime and the response patterns were different between the two growing seasons: it generally increased with increasing latitude from the seed origin in the first growing season (Table 4.1, Figure 4.2A), but the response to photoperiod regime was significantly affected by [CO₂] in the second growing season (Table 4.1). Under the elevated [CO₂], leaf biomass was smaller at 52° N than other photoperiods; Under the ambient [CO₂], leaf biomass was greater at 52 and 58° N than at the other photoperiod regimes (Figure 4.3). Further, the [CO₂] elevation generally enhanced leaf biomass at all photoperiod except at 52° N (Figure 4.3).

Table 4.1. Summary of ANOVA (*P*-value and Degree of freedom (DF)) for the effects of [CO₂], photoperiod regime, and their interactions on height (H), root-collar diameter (RCD), biomass components, total plant biomass, biomass ratio, and leaf area ratio (LAR) and specific leaf area (SLA). Seedlings were subjected to two [CO₂] (400 and 1000 μmol mol⁻¹) and four photoperiod regimes (corresponding to 48, 52, 55, and 58° N latitude) for two growing seasons in environment-controlled greenhouses. Significant (*P* ≤ 0.05) and marginal significant (*P* ≤ 0.1) *P*-values are bolded.

Variable	2017			2018			
	[CO ₂]	Photoperiod	[CO ₂] × Photoperiod	[CO ₂]	Photoperiod	[CO ₂] × Photoperiod	
H	<i>F</i>	7.866	13.439	0.201	1.74	5.096	0.063
	<i>P</i>	0.107	0.005	0.892	0.318	0.044	0.978
RCD	<i>F</i>	0.201	3.151	8.372	1.128	2.892	0.226
	<i>P</i>	0.218	0.015	0.213	0.399	0.124	0.875
Leaf	<i>F</i>	1.335	11.385	0.669	1.272	8.498	5.833
	<i>P</i>	0.367	0.007	0.601	0.377	0.014	0.033
Root	<i>F</i>	0.175	1.119	0.248	1.708	2.308	0.014
	<i>P</i>	0.716	0.413	0.86	0.321	0.176	0.998
Stem	<i>F</i>	2.765	8.246	0.395	2.537	6.374	0.322
	<i>P</i>	0.238	0.015	0.761	0.252	0.027	0.810
Total biomass	<i>F</i>	1.026	5.343	0.460	1.977	4.65	0.549
	<i>P</i>	0.418	0.039	0.720	0.295	0.052	0.667
Shoot	<i>F</i>	2.063	9.472	0.471	1.908	6.406	1.175
	<i>P</i>	0.287	0.011	0.713	0.301	0.027	0.395
LMR	<i>F</i>	0.120	4.944	0.051	0.654	10.794	7.181
	<i>P</i>	0.762	0.046	0.983	0.504	0.008	0.021
SMR	<i>F</i>	0.206	11.969	0.058	0.714	5.964	1.725
	<i>P</i>	0.694	0.006	0.980	0.487	0.031	0.261
RMR	<i>F</i>	0.226	12.217	0.054	1.068	5.525	1.283
	<i>P</i>	0.682	0.006	0.982	0.41	0.037	0.362
RS	<i>F</i>	0.232	12.268	0.056	0.895	6.127	1.378
	<i>P</i>	0.678	0.006	0.981	0.444	0.029	0.337
LAR	<i>F</i>	0.211	2.748	0.184	1.553	0.62	0.849
	<i>P</i>	0.691	0.135	0.904	0.339	0.627	0.516
SLA	<i>F</i>	0.038	0.151	0.575	0.919	1.463	0.544
	<i>P</i>	0.864	0.925	0.652	0.439	0.316	0.670
DF	1	3	3	1	3	3	

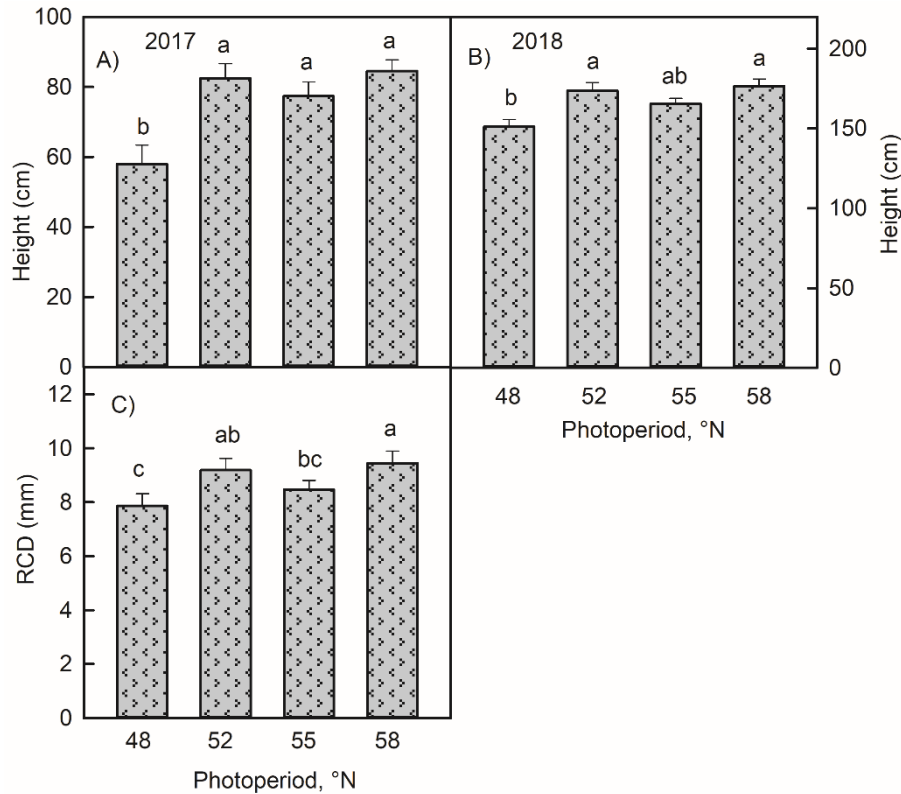


Figure 4.1. Total height and root-collar diameter (RCD) in white birch seedlings in 2017 (left panel) and in 2018 (right panel) growing seasons. Seedlings were grown under the photoperiod regimes corresponding to 48° (seed origin), 52°, 55°, and 58° N latitude and under 400 (ambient) and 1000 $\mu\text{mol mol}^{-1}$ CO₂ (elevated) for two growing seasons. Data are presented as Mean \pm SE ($N = 20$: 5 seedlings per treatment combination \times 2 replications per combination; the data were pooled for the [CO₂] treatment because the interaction was not significant, $5 \times 2 \times 2 = 20$). Means with different letters for each measurement time are significantly different from each other ($P \leq 0.1$, Fisher's LSD).

Root mass was not significantly affected by any of the treatments in either of the growing seasons (Table 4.1). The stem biomass and total seedling biomass at the seed origin were generally smaller than those at the photoperiod regimes at higher latitudes with the exception of 55° N at which the stem and total seedling biomass were not significantly different from those at the seed origin (Figure 4.2E-H). However, no significant differences were detected among the photoperiod regimes at 52, 55, and 58° N latitude on stem or total biomass in either growing season (Figure 4.2E-H).

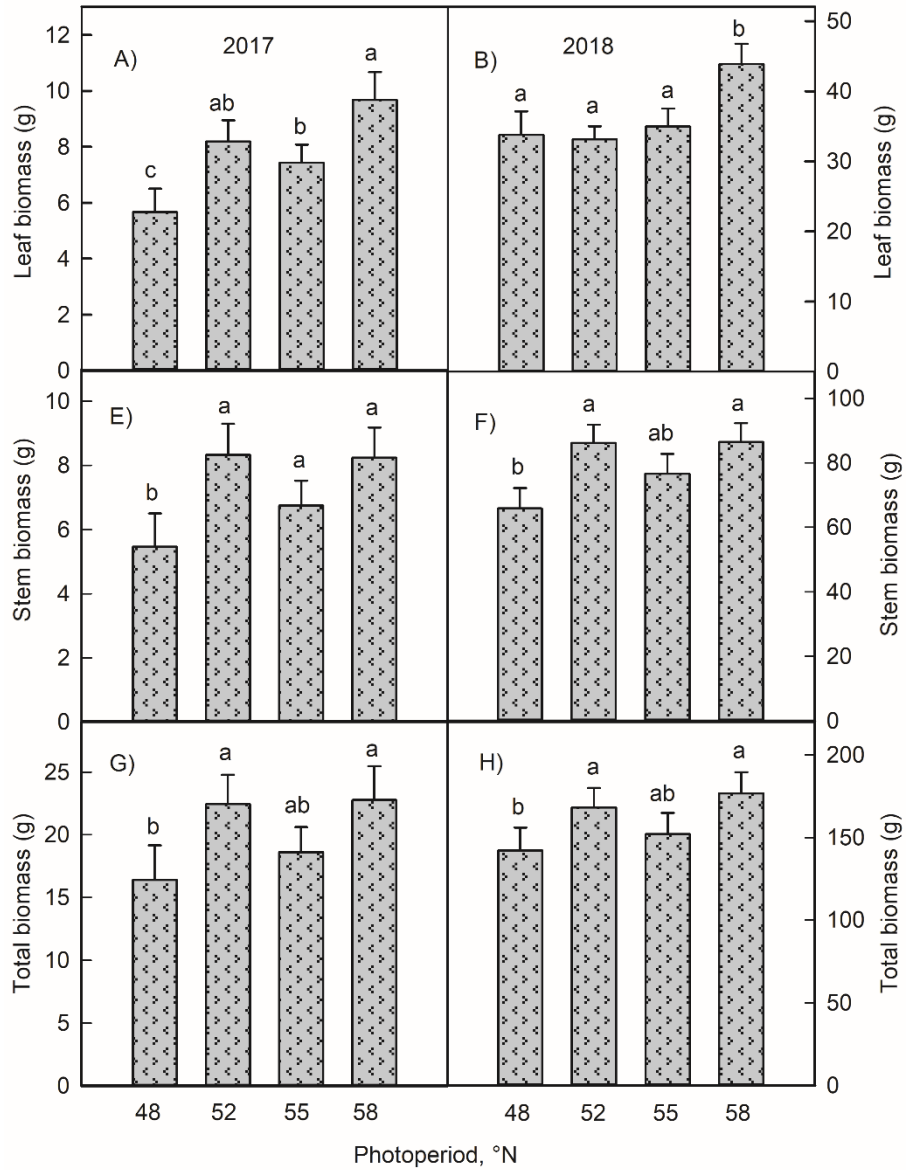


Figure 4.2. Leaf biomass, stem biomass and total plant biomass of white birch seedlings as affected by photoperiod regime in 2017 (left panel) and 2018 (right panel) growing seasons. Data are presented as Mean \pm SE ($N=20$ as explained in Figure 1). Means with different letters indicate significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Figure 4.1 for more explanations.

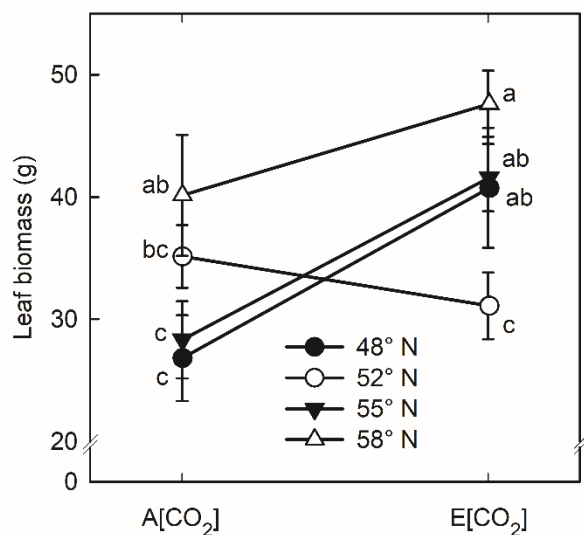


Figure 4.3. Leaf biomass of white birch seedlings as affected by photoperiod regime and [CO₂] (ambient, A[CO₂]; elevated, E[CO₂]) in 2018 growing season. Data are presented as Mean ± SE ($N = 10$: 5 seedlings per treatment combination x 2 replications per combination. Means with different letters indicate significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Figure 4.1 for more explanations.

Biomass allocation

Photoperiod regime significantly affected biomass allocation to leaf, stem, and roots (Table 4.1).

In the first growing season, the leaf mass ratio (LMR) under the photoperiod regime of 58° N was significantly greater than under the photoperiod of the seed origin and 52° N primarily at the expense of reduced root mass ratio (RMR, Figure 4.4A). The stem mass ratio (SMR) under the photoperiod regime of the seed origin was significantly lower than those under other photoperiod regimes (Figure 4.4A). The response patterns, however, changed in the second growing season (Figure 4.4B): LMR was significantly smaller under the photoperiod of 52° N than under the other three photoperiod regimes, and the SMR at 52 and 55° N was significantly greater than the seed origin. Also, the biomass allocated to leaf was lowered by the photoperiod of 52° N under elevated [CO₂], and the [CO₂] elevation increased this parameter at 48° N but decreased it at 52° N (data not shown). The biomass allocation to roots and root/shoot ratio decreased with increases

in latitude, but the difference between 52 and 55° N, or between 55 and 58° N were insignificant (Figure 4.4A-D).

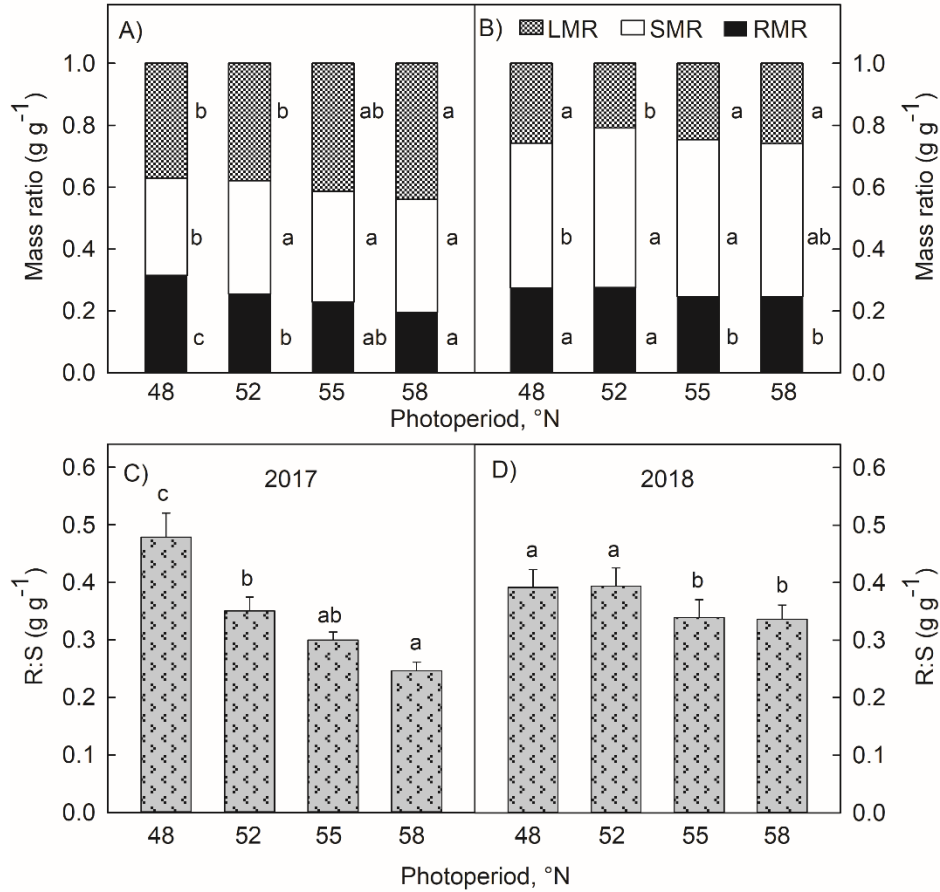


Figure 4.4. Leaf, stem and root mass ratios (A and B) and root to shoot ratio (C and D) of white birch seedlings as affected by photoperiod regime. Left panel and right panels indicate 2017 and 2018 growing seasons, respectively. Data are presented as Mean ± SE ($N=20$ as explained in Figure 1). Means with different letters indicate significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Figure 4.1 for more explanations.

4.5 Discussion

My results supported the hypothesis that longer photoperiods would stimulate growth and increase biomass allocation to above-ground parts but did not support the hypothesis that [CO₂] elevation would offset the effect of longer photoperiods on biomass allocation. The results suggest that the photoperiod regime of 4° north of the seed origin (52 vs. 48° N) increased the growth and biomass of white birch. However, further increases in photoperiod regimes associated with even higher latitudes did not lead to much further increase in growth, suggesting that white birch may not be able to take advantage of longer photoperiods in the growing season at much higher latitudes. The seedlings grown under the photoperiod regime 4° north of the seed origin latitude were 15% higher in growth and 18% higher in biomass than the seedlings grown under the photoperiod regime of the seed origin. However, there was no significant difference either in seedling growth or biomass between 52° N and the two higher latitudes. Although, the contribution of leaf biomass weakened at the final harvest for plants grown under 52° N, increased total seedling biomass at longer photoperiods was primarily attributed to increases in stem biomass and leaf biomass. At the end of the experiment period, I observed a net increase in shoot biomass (leaf + stem) of 20% at 52° N, 5% at 55° N and 9% at 58° N latitude, as compared to the seed origin. The corresponding increases in total light period during the growing season at the three latitudes were 65, 107 and 116 hours, respectively. Apparently, more hours of light did not lead to much further increase in growth. While the positive responses in growth and biomass to longer photoperiods found in this study are in general agreement with the results on other tree species in the literature (Bigras and Daoust 1993, Johnsen and Seiler 1996, Abeli et al. 2015), this is the only study that has identified the magnitude of increase in photoperiod at which the maximum phenotypic plasticity is reached. Oleksyn et al. (1992) have reported significant

increases in height growth and total biomass when scots pine (*P. sylvestris* L.) is grown under a photoperiod regime corresponding to a 10° increase in latitude from the seed origin. Similar results are found in a field experiment by Schreiber et al. (2013) where trembling aspen (*Populus tremuloides* Michx.) is moved 7° north from the latitude of the seed origin (54 vs. 47° N).

The most likely reason for the increased growth of white birch grown under longer photoperiods is the lengthened periods of photosynthesis and subsequent increases in carbohydrate production (Stinziano and Way 2017). There are several mechanisms for growth increases under longer photoperiods. Longer photoperiods can expedite budburst and leaf expansion in the spring (Thomas and Vince-Prue 1997, Basler and Körner 2012) and delay leaf senescence in the fall (Raulo 1976, Velling 1979, Viherä-Aarnio et al. 2005), leading to a longer growing season. In this study, the longer photoperiods did not affect the timing of leaf senescence in the fall (Tedla et al., unpublished). However, the spring budburst did occur earlier at 55 and 58° N than at 48 and 52° N under the elevated [CO₂], but the trend was opposite under the ambient [CO₂] (Tedla et al., unpublished). Longer photoperiods can also increase biomass and growth by increasing leaf area ratio and specific leaf area (Hay 1990, Adams and Langton 2005), but photoperiod did not significantly affect either of them in this study. Longer photoperiods can affect the decline of photosynthetic capacity during summer to autumn transition as the photoperiod become shorter and temperatures lower (Bauerle et al. 2012). The physiological measurements of this study show that the photoperiod regimes of 55 and 58° N generally did not stimulate photosynthetic rate (relative to the seed origin) under the elevated [CO₂] but substantially increased photosynthetic rate under the ambient [CO₂] (Tedla et al., unpublished). Stinziano and Way (2017) also suggest that both the long hours of photosynthesis and increased efficiency of light energy conversion into biomass are major contributors to the increased growth of white spruce

(*Picea glauca* (Moench) Voss) under a longer photoperiod regime. However, the current and other controlled-environment studies cannot give any indication on the effects of the declined solar elevation and increased lateral shading within and between trees at higher latitudes on the physiology, phenology and growth of trees in the field.

Growing under longer photoperiod regimes reduced the biomass allocation to roots in white birch. Although the differences between two adjacent treatment levels were not always statistically significant, there is a general trend that the proportion of biomass allocated to roots declined with increases in photoperiods associated with increases in latitude from 48 (seed origin) to 58° N. This trend seems counter-intuitive. Since longer photoperiods increase carbohydrate production as stated previously, growth should become more limited by water and nutrients from the soil, and logically, the biomass allocation to roots should increase rather than decrease. However, all the seedlings were well watered and supplied with ample of nutrients in this study. Presumably, the trees did not experience drought or nutrient stress in any of the treatments. It is, therefore, reasonable to conclude that the decrease in root mass ratio with increasing photoperiods reflected the stimulation of shoot growth by the increased carbohydrate production in trees growing under longer photoperiods (Keyes and Grier 1981). However, because of the relatively small sample size and short term nature of this study, this study may not have been able to detect all the changes in biomass allocation to aboveground parts.

Nevertheless, the leaf mass ratio and stem mass ratio were significantly higher under the photoperiod regime at 58° N than the seed origin (48° N). Similar trends are reported for other tree species, e.g., *P. glauca* (Bigras and Daoust 1993), lodgepole pine (*Pinus contorta* Dougl.) (Burdett and Yamamoto 1986), and Norway Spruce (*Picea abies*) (Heide 1974). However, there

are also results in the literature that photoperiods have no effects on biomass partitioning (Burdett and Yamamoto 1986, Stinziano and Way 2017).

White birch has an indeterminate growth pattern and is highly plastic in morphology and physiology in response to environmental conditions (Li et al. 1996, Ashton et al. 1998, Wang et al. 1998). Although the main effect of photoperiod on growth and biomass allocation were mostly significant, the interactive effect of photoperiod and [CO₂] was generally statistically insignificant in this study. Our results suggest that white birch may benefit from the longer photoperiods associated with a northward migration or seed transfer of up to 4° north of the seed origin. However, the current study is a short-term greenhouse experiment on a single population of the species. There may be variations in the response between different populations. Therefore, larger scale studies are warranted to investigate possible genetic variations in the response.

4.6 Reference

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5 Chapter 5: Freeze-thaw events delay spring budburst and leaf expansion while longer photoperiods have opposite effect under different [CO₂] in white birch: advance it under elevated but delay it under ambient [CO₂]

5.1 Abstract

Past studies indicate that narrower conduits such as those in diffuse-porous species are less vulnerable to freeze-thaw (FT) induced embolism and also facilitate the re-filling of embolized xylem conduits early in the spring, resulting in an earlier bud break. In this study, I investigated if a novel environmental condition associated with climate change-induced northward migration will affect the vulnerability to FT-induced embolism and spring phenology in white birch. Seedlings were grown under ambient (400 $\mu\text{mol mol}^{-1}$) or elevated CO₂ concentration (1000 $\mu\text{mol mol}^{-1}$), and four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58° N latitude. I found that the longest photoperiod (corresponding to 58° N latitude) significantly increased the maximum specific hydraulic conductivity of the stem. CO₂ concentration ([CO₂]) and photoperiod had no significant impact on the vulnerability to FT-induced embolism. The treatment of 5 freeze-thaw cycles (+5 to -20 °C) led to an 11% loss of hydraulic conductivity in dormant seedlings that had been stored at -4 °C for 3 months while the effect of such a treatment in the fall was much smaller. This result suggests that freeze-thaw events in late winter or spring can impair the hydraulic conductivity of the xylem which in turn may negatively affect the physiology of the trees. Indeed, the FT treatment in this study delayed budburst and leaf expansion in the spring. It is interesting to note that photoperiods had opposite effect on budburst under different [CO₂]: longer photoperiods led to earlier budburst in the spring under elevated [CO₂], but delayed budburst under ambient [CO₂]. The synergistic effect of longer photoperiods and CO₂ elevation suggests that the growing season for white birch may be

longer than what we predict from either factor alone at a migration site in the future when [CO₂] will be much higher.

5.2 Introduction

In temperate and boreal regions, freezing events are a critical determining factor for the survival, growth, and distribution of plants (Sakai and Larcher 2012). Freeze-thaw cycles can lead to increased winter damage to xylem conduits (Walker 1990, Sperry and Sullivan 1992, Ward and Stephens 1997, Cochard et al. 2001) and freezing injuries (Sakai and Larcher 2012, Charrier et al. 2013). With the predicted increases in temperature and temperature fluctuations due to climate change, freeze-thaw events are expected to increase both in duration and frequency (Solomon et al. 2007). In addition, because of the rapid shift of climate envelopes and a subsequent northward shift of species geographic ranges (McKenney et al. 2007), the plant hydraulic structure and functionality could be influenced by the novel environmental conditions at the new location such as a combination of higher [CO₂] and new photoperiod regime. However, the effects of such changes in the environmental conditions on hydraulic characteristics such as freeze-thaw induced xylem dysfunction remain poorly understood.

Water transport in plants can be disrupted when xylem conduits are embolized. Freeze-thaw events can result in the nucleation of air bubbles formed in the xylem conduits during the phase transition of water from liquid to ice and lead to xylem embolization (Sperry and Tyree 1988, Sperry and Sullivan 1992). The size of air bubbles formed during freeze-thaw events depends primarily on the diameter of xylem conduits (Sperry and Sullivan 1992, Hacke and Sauter 1996). While the relationship between vulnerability to winter embolism and conduit size is not always apparent (Mayr and Charra-Vaskou 2007), species that produce narrower conduits (e.g., diffuse-

porous species such as *Acer* spp, *Betula* spp, and *Poplar* spp) are generally more resistant to freeze-thaw-induced embolism (Sperry and Sullivan 1992, Davis et al. 1999, Pittermann and Sperry 2003). Xylem embolism and subsequent loss of hydraulic conductivity reduce plants' ability to take advantage of the favorable environmental conditions in the spring and can even lead to diebacks (Wang et al. 1992, Sperry et al. 1994, Pockman and Sperry 1996, Zhu et al. 2000). For most diffuse-porous trees including birch, winter xylem cavitation is reversed by positive root pressure in the spring which allows early transport of water before growth begins (Sperry et al. 1994, Hacke and Sauter 1996). However, the lack of efficient re-filling or a high degree of xylem cavitation may delay the timing of spring bud phenology and make plants unable to take advantage of the favorable growing condition in the spring (Lechowicz 1984, Hunter and Lechowicz 1992).

There is also evidence in the literature that changes in the growing conditions can alter xylem properties. For example, CO₂ elevation can prompt a plastic response of the xylem to allow the synchrony of water transport and hydraulic demand (Rico et al. 2013). Increases in hydraulic demand may induce the production of larger-diameter conduits and lead to greater xylem to stem cross-sectional area ratios (Lambers et al. 2008). Elevated [CO₂] can increase the rate of photosynthesis, radial growth, and the diameter and wall thickness of xylem vessels (Atkinson and Taylor 1996, Ainsworth and Rogers 2007, Kostianen et al. 2014). Elevated [CO₂] can also decrease the wall thickness to diameter ratio of the conduit (Medeiros and Ward 2013).

Hättenschwiler et al. (1996) have reported that CO₂ elevation can alter lignification, which plays a vital role in protecting cells from being damaged during ice formation. Increases in conduit diameter improve the efficiency of water transport as hydraulic conductance is proportional to

the 4th power of conduit radius (Lambers et al. 2008). However, conduits with a larger diameter become vulnerable to freeze-thaw induced cavitation.

Longer photoperiods generally increase growth and enhance leaf development (Hay 1990, Oleksyn et al. 1992, Johnsen and Seiler 1996, Adams and Langton 2005, Inoue et al. 2019, Tedla et al. 2019), which will require increases in hydraulic conductivity. Longer photoperiods have been found to increase the diameter of xylem tracheid in *Pinus resinosa* (Larson 1962, 1964). The combination of longer photoperiods and elevated [CO₂] can synergistically increase the production of photosynthetic assimilates (Kuppers et al. 1988, Körner and Miglietta 1994, Gandin et al. 2011), leading to expedited cell expansion and deposition of cell materials (Lambers et al. 2008), which in turn can improve hydraulic conductivity. However, such an increase in hydraulic conductivity may be accompanied by an increase in the probability of embolism during freeze-thaw events. In this study, I examine the interactive effects of elevated [CO₂] and photoperiod regime on freeze-thaw induced xylem embolism and spring budburst in white birch. I hypothesize that the positive impact of [CO₂] elevation and longer photoperiod on growth will enhance hydraulic conductivity and increase vulnerability to freeze-thaw induced embolism in white birch (*Betula papyrifera* Marsh.). White birch is a diffuse-porous deciduous tree species that has a wide distribution in the boreal forest (Burns et al. 1990, Sperry et al. 1994, Farrar 1995).

5.1. Materials and methods

5.1.1. Plant materials

White birch seeds (*Betula papyrifera* Marsh.) were collected from 12 natural trees in Thunder Bay region (48.4215° N, 89.2619° W). Mature catkins were collected in September 2016. Seeds

were extracted manually, air-dried, and stored in plastic bags at room temperature. The experiment was conducted at the Lakehead University greenhouse complex in Thunder Bay. Seeds were sown in germination trays (50cm × 25cm) filled with a 1:1 (v:v) peat moss and vermiculite mixture. The day/night temperatures and photoperiod during germination were set to 22/16 °C and 16 hours, respectively. Seedlings of relatively uniform size (2 cm average height) were transplanted into pots of 12 cm deep and 12/9.5 cm top/bottom diameter for the experiment. The growing medium was a mixture of vermiculite and peat moss (1:3, v:v).

5.1.2. Experimental design and treatments

The experiment was a split-plot design. The treatments were comprised of two levels of carbon dioxide concentration [CO₂] (ambient 400 and elevated 1000 μmol mol⁻¹) as the whole plot and four photoperiod regimes corresponding to the latitudes of 48 (seed origin), 52, 55, and 58° N as split-plots nested within each whole plot. There were 15 seedlings in each treatment combination. The CO₂ elevation was achieved using natural gas CO₂ generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA). Each [CO₂] level was replicated twice using independently environment-controlled greenhouses. The photoperiod regime of each greenhouse was set to emulate the weekly average regime of the growing season for the longest photoperiod of the four treatments, and that photoperiod was shortened by manually applying black-out shade to emulate the photoperiod regime for each of the other three treatments. The photoperiods were adjusted weekly to emulate the weekly averages for the corresponding latitudes. To facilitate the shading and to be consistent across treatments, a wooden frame was established around each split-plot. High-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada) was used to extend the natural photoperiod when the natural day-length in the greenhouse was shorter than required. The day and night temperatures were

derived from the 10-year weekly averages of Environment Canada records for the seed origin location (Environment Canada, 2016). The daily temperature in each greenhouse was ramped at four set points at 4:00, 10:00, 16:00, and 22:00 hours. The set points were calculated from the Environment Canada data as described previously. The experiment was carried out for one growing cycles. The growing cycle emulated the environmental conditions of June 7 to November 15. The dates mentioned in the rest of the paper refer to the dates that were emulated.

The [CO₂], light, temperature, and humidity were all controlled and monitored using an Argus Titan Environment-control system (Argus Control Systems Ltd, Vancouver, BC, Canada). The volumetric water content of the growing medium was maintained around 50-60 % of the field capacity as determined using an HH2 Moisture Meter and ML2X Theta Probe (DELTA-T DEVICES, Cambridge, UK). The seedlings were fertilized twice a week with 50/81/30.3 mg L⁻¹ of N/P/K from June 7 to June 21; 150/65.2/125 mg L⁻¹ N/P/K at the rapid growth phase (June 22 to August 31); and finisher with 50/54.3/156.3 mg L⁻¹ N/P/K (September 1 to 25) (Plant Products Co Ltd, Brampton, Ont., Canada). The fertilization was stopped on September 25. At the end of the first growing season, the seedlings were stored in a cold store (-4 °C) before the initiation of the second growing season. The average seedling height and root collar diameter were 76 and 0.9 cm, at the time of measurement.

Freeze-thaw experiment

I simulated freeze-thaw cycles in the fall (when the buds were fully formed (FT-Fall)) and again after the seedlings were cold-stored for three months (FT-CS). Whole trees in the pot were used in the freeze-thaw experiment. Three seedlings from each replication of each treatment-combination were taken from the greenhouses in the morning and wrapped up in a plastic bag to

minimize water loss during the treatment. A programmable freezer (Model 45-6.8; ScienTemp Corp., Adrian, MI) was used to simulate freeze-thaw events. In the first round of simulation (FT-Fall), the seedlings were exposed to five freeze-thaw cycles. For each freeze-thaw cycle, the freezer temperature started from +5 °C and was gradually lowered at a rate of 5 °C per hour and held for one hour after each 5 °C change until the temperature reached -20 °C after which the temperature was increased back to +5 °C in the same manner as it was decreased (Améglio et al. 2002). The second round of thaw treatment was made on seedlings that were in cold storage at -4 °C for three months (FT-CS). Six seedlings from each treatment-replication combination were taken and divided into two groups, i.e., freeze-thaw group and control group. The freeze-thaw group was subjected to five freeze-thaw cycles as described previously. The control seedlings were wrapped up in plastic bags and kept at +4 °C when the freeze-thaw treatment was in process.

Percent loss of hydraulic conductivity assessment

The percent loss of hydraulic conductivity caused by the freeze-thaw treatment was examined using 15-cm stem segments that were cut under water from 30-cm segments with a razor blade. While I did not measure the vessel lengths, the literature has shown that a 15cm segment covers the full length for 90% of the vessels in a related species *Betula* spp (Sperry and Sullivan 1992). Segments from the middle of the main stems (20cm above the base of the stem) were used to determine hydraulic conductivity. The bark at the end of the stem segment was removed with a razor blade and covered with parafilm before the stem segment was attached to a flexible tube connected to a solution tank containing 20mM/L Potassium Chloride in distilled water as the hydraulic solution. The hydraulic conductivity was measured according to Sperry et al. (1988).

The hydraulic conductivity was determined as the mass flow rate (kg s^{-1}) per unit of functional xylem area. The first hydraulic conductivity measurement was derived from the difference between the measurement of a gravity-induced flow rate measured (5 min) by using a water tank 0.5m above the specimen and the measurement of 10kPa pressure-induced flow rate measured (5 min) after the water tank was pressurized 10kPa. Then, the second or maximum hydraulic conductivity was measured the segment was flushed under high pressure of 175 kPa. The percent loss of hydraulic conductivity (PLC) was calculated from the difference between the first and second measurement. The maximum stem specific hydraulic conductivity determined in the FT-Fall was used to compare the maximum water transport capacity between the treatments.

Freeze-thaw and spring budburst experiment

This experiment was conducted to assess the effect of freeze-thaw events on the timing of spring budburst. At the beginning of the second growing season, two groups of seedlings from each treatment combination and replication (three per group) were taken from cold storage and thawed while overnight at room temperature. The following day one of the groups was exposed to a freeze-thaw cycle treatment as described previously while the control group was maintained at $+4^{\circ}\text{C}$. After the freeze-thaw treatment, both groups were moved to the greenhouses where spring conditions were simulated.

Phenological observation

The spring phenology of budburst was monitored on the seedlings of both groups. For each seedling, I monitored the phenology of the terminal bud as well as five lateral buds immediately below it. The observations were made every two days, from May to June. The bud phenology

was divided into the following 7 phases according to Linkosalo and Lechowicz (2006); 1= the buds are completely closed; 2= the start of bud swelling, 3= the bud scales split open; 4= emergence of the first leaf from the bud; 5= the emergence of the base and petiole of the first leaf from the bud; 6= all leaves fully out of the bud; 7= completion of leaf expansion (i.e., reach full-size). For each seedling, bud break was considered complete when at least three buds reached phase 5.

5.1.3. Statistical analysis

I used 3-way split-split-plot ANOVA with two levels of CO₂ (main-plot), four levels of photoperiod (sub-plot) and two levels of freeze-thaw treatment (sub-sub plot) for PLC measured after three months in cold storage (FT-CS) and budburst. Time was used as the sub-sub plot when testing the difference between the two seasons of freeze-thaw treatment when no control trees (seedlings not subjected to freeze-thaw treatment) were involved. However, two-way ANOVA was used to test the effects of CO₂ and photoperiod on the fall measurements of PLC and maximum stem-specific hydraulic conductivity. All data analyses were done using the R program (v. 3.5.0, R Core Team 2018). In order to meet the assumption of normality and homogeneity, proportional data and percent loss of hydraulic conductivity were subjected to arcsine transformation, while power transformation was used on hydraulic conductivity. When an interaction or photoperiod had a significant effect ($P \leq 0.05$), Fisher's LSD was used for post hoc pair-wise comparison.

5.2. Results

Photoperiod, [CO₂] and their interaction had no significant effect on the PLC that was measured following the freeze-thaw treatment either before or after the 3-month cold storage (Table 5.1).

However, the PLC value was substantially greater after than before the cold storage (95% vs. 10 %, Table 5.1, Figure 5.1A). Furthermore, PLC measured after 3 months of cold storage was 11% greater in seedlings subjected to the freeze-thaw treatment than control seedlings (Figure 5.1B).

Table 5.1. Summary of ANOVA (*P*- and *F*-value, and Degree of freedom (DF)) for the effects of [CO₂] (C), photoperiod regime (P), freeze-thaw treatment (FT) and their interactions on maximum stem-specific hydraulic conductivity (K_s) and percent loss of hydraulic conductivity (PLC) measured in the fall (FT-Fall), after 3-month cold storage (FT-CS) and at bud break. Time (T) was used as the third factor to compare FT-Fall and FT-CS. Seedlings were subjected to two [CO₂] (400 and 1000 μmol mol⁻¹) and four photoperiod regimes (corresponding to 48, 52, 55, and 58° N latitude) for one growing season in environment-controlled greenhouses. Significant (*P* ≤ 0.05) *P*-values are bolded.

DF	K _s		FT-Fall		FT-Fall and FT-CS			FT-CS			Bud break		
	Treat.	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	Treat.	<i>F</i>	<i>p</i>	Treat.	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
1	C	0.2	0.9	1.13	0.4	C	2.19	0.28	C	0.43	0.58	0.26	0.66
3	P	0.2	0.04	1.19	0.39	P	1.15	0.4	P	2.49	0.16	0.04	0.99
3	C×P	4.98	0.18	0.8	0.53	C×P	0.37	0.78	C×P	2.67	0.14	26.33	<0.01
1						T	1364.6	<0.01	FT	23.38	<0.01	6.3	0.04
1						C×T	0.7	0.43	C×FT	2.07	0.19	0.26	0.62
3						P×T	0.15	0.93	P×FT	2.01	0.19	0.05	0.98
3						C×P×T	1.33	0.33	C×P×FT	0.72	0.57	1.4	0.31

The maximum specific hydraulic conductivity of the stem was significantly greater in seedlings grown under the photoperiod regime of 58° N latitude than those grown under all other photoperiod regimes whereas there was no significant difference among other photoperiod regimes (Table 5.1, Figure 5.2).

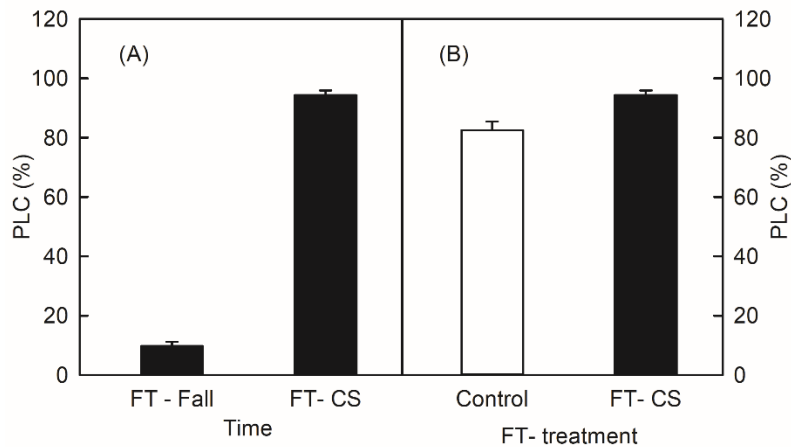


Figure 5.1. Percent loss of hydraulic conductivity (PLC) as affected by season (A) and freeze-thaw treatment after 3-months of cold storage (B). The measurements were taken in the fall (FT-Fall) and after 3-months of cold storage (FT-CS). Measurement was made on 15cm stem segments of white birch seedlings. Seedlings were grown under the photoperiod regimes corresponding to 48° (seed origin), 52°, 55°, and 58° N latitude and under 400 (ambient) and 1000 $\mu\text{mol mol}^{-1}$ CO_2 (elevated) for one growing season. Data are presented as Mean \pm SE (N= 48: data were pooled for the 4 photoperiods and 2 [CO_2] because the interaction was not statistically significant, $3 \times 2 \times 4 \times 2 = 48$).

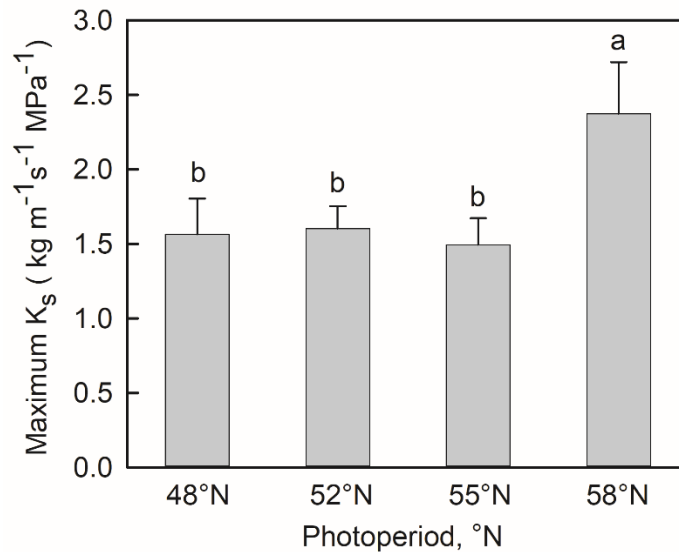


Figure 5.2. Effects of photoperiod regime at different latitudes on maximum stem-specific hydraulic conductivity (mean \pm SE, N= 12) of white birch seedlings. The data were pooled for [CO_2] because the interaction between the two was not significant. Means with different letters were significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Figure 1 for more explanations.

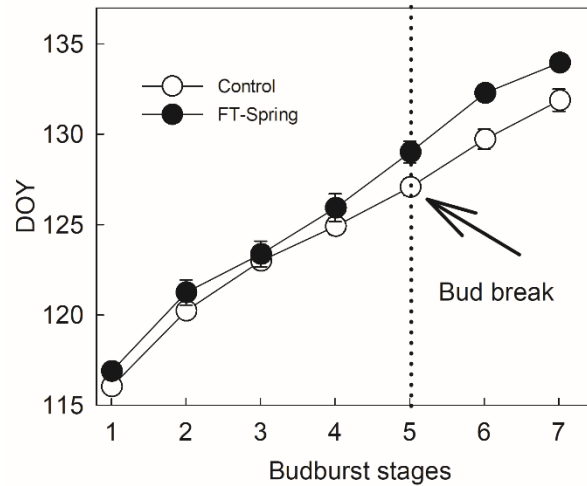


Figure 5.3. The progression of spring budburst in white birch seedlings that were subjected to five freeze-thaw cycles (FT-Spring) and control seedlings (Mean \pm SE, N= 48). (DOY) day of year. The data were pooled for photoperiod and CO₂ treatment because the interactions were not statistically significant (3 x 2 x 4 x 2= 48). See Figure 1 for more explanations.

The freeze-thaw treatment delayed the progress of budburst and leaf expansion in the spring (Table 5.1; Figure 5.3). The interaction between [CO₂] and photoperiod significantly affect the progress and completion date of spring budburst (Table 5.1). Compared to other photoperiod regimes, the spring budburst progressed faster and completed sooner in the photoperiod regime of the seed origin under the ambient [CO₂] (Figures 5.4A and C), but the trend was the opposite under the elevated [CO₂] (Figures 5.4B and C).

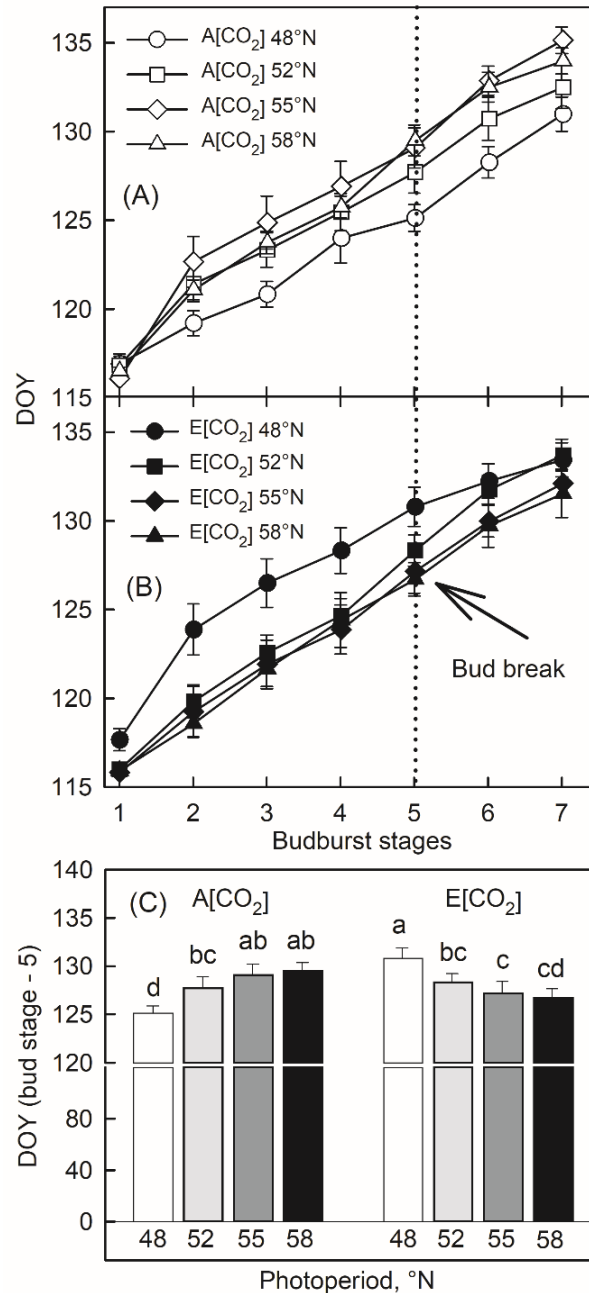


Figure 5.4. The effect of photoperiod regime on the progression of spring budburst under ambient (A) and elevated [CO₂] (B). The interaction between photoperiod and CO₂ was significant ($p < 0.05$). The dotted vertical line in A and B indicate the completion of budburst (stage-5). Figure C, the interactive effect of CO₂ and photoperiod on the completion date (Day of the Year) of spring budburst with different letters that indicate significant difference from each other. The value of each point represents the average of 8 seedlings. See Figure 1 for more explanations.

5.3. Discussion

My results partially supported the hypothesis that longer photoperiod would improve hydraulic conductivity, but did not support the hypothesis that the positive effect of photoperiod on hydraulic efficiency would make plants more vulnerable to freeze-thaw induced embolism. Our previous study (Tedla et al. 2019) find that longer photoperiods corresponding to latitudes north of the seed origin increase growth and biomass in white birch. Similar findings have been reported for other boreal tree species (Li et al. 2015, Newaz et al. 2016, Inoue et al. 2019). While the longest photoperiod in this study significantly increased maximum specific hydraulic conductivity of the stem, other photoperiod treatments had no significant impact on the hydraulic conductivity. However, none of the photoperiods had a significant effect on the vulnerability to freeze-thaw induced xylem cavitation in this study. I consider this result as having positive implications to the northward of white birch. Studies have indicated that the general warming trend of the global is accompanied by more temperature fluctuations and more freeze-thaw events (Man et al. 2015). My results suggest that at least the increased occurrence of freeze-thaw events would not add another stress to the hydraulic system of white birch. Newaz et al. (2017) report that increases in photoperiod associated with northward tree migration increase the vulnerability of black spruce to drought-induced xylem cavitation. The anatomic factors influencing the vulnerability to freeze-thaw induced xylem cavitation are different from those affecting resistance to drought-induced xylem cavitation: the vulnerability to freeze-thaw induced xylem cavitation is negatively related to the diameter of the xylem conduits whereas the vulnerability to drought-induced cavitation is affected by the properties of pit membrane (Lambers et al. 2008). Therefore, a good understanding of changes in the structure and anatomy

of xylem conduits may be key for understanding the influence of photoperiod and elevated CO₂ on the hydraulics of boreal trees in the context of climate changed induced northward migration.

My results imply a strong seasonal variation in xylem hydraulic conductivity and its vulnerability to freeze-thaw induced xylem cavitation. Although I did not measure the pre-freeze-thaw PLC in the fall, the combined total of freeze-thaw induced PLC and any PLC that existed before the freeze-thaw treatment was only a fraction of the value measured after 3-months of cold storage (corresponding to later winter or early spring) and less than the net value of freeze-thaw induced PLC (11%). Furthermore, the PLC measurement in the control seedlings was considerably greater than the PLC measured in the fall. These results suggest that xylem conduits had already lost a substantial amount of conductivity in the winter months and became much more vulnerable to freeze-thaw induced xylem cavitation later winter or early spring and that the PLC increased substantially over the winter even in the absence of any freeze-thaw stress. I wrapped the entire seedling in a polyethylene bag during the cold storage to minimize winter desiccation effect. Trees in the field experience much colder and drier conditions in the field than the seedlings in the cold storage. Therefore, the possibility that the spring measurements were artifacts is small. It is thus reasonable to conclude that trees may experience increased xylem cavitation throughout the winter season and that they may become more vulnerable to freeze-thaw induced xylem cavitation later in the dormant season. Several studies have reported observations of foliage, bud and even branch mortalities in boreal trees in the spring and have attributed the mortality to winter desiccation (Man et al. 2013, 2015). My results suggest that those mortalities may also be related to increases in vulnerability to freeze-thaw induced xylem cavitation. Freeze-thaw events are common in the spring (Man et al. 2015). A significant increase in PLC after storage has also been observed in yellow birch

(*Betula alleghaniensis* Britt.) (Zhu et al. 2001). Although I did not investigate other causes of xylem dysfunction such as infection, enzymatic degradation of vessel walls and debris have been reported to cause substantial cavitation due to direct vessel blockage (Venturas et al. 2017). The results of my study support the current understanding that freeze-thaw induced embolism can occur in diffuse-porous trees (Améglio et al. 2002, Sperry and Sullivan 1992).

My data suggest that xylem hydraulic conductivity is not always coupled with the growth rate. Seedlings grown under the photoperiod regime of 58° N latitude had the greatest maximum stem-specific hydraulic conductivity among all the photoperiod treatments. However, our previous study (Tedla et al. 2019) finds that the photoperiod at 58° N latitude does not stimulate the growth of white birch seedlings further than that at 52 or 55° N latitude does. Therefore, the large increase in hydraulic conductivity at 58° N observed in the current study was not associated with an increase in growth. Photoperiod may affect the hydraulic efficiency of plants in various ways. The relationship between the period of active photosynthesis and required water transport capacity may be involved. A longer duration of photosynthetic activity under a longer photoperiod regime likely demands a higher water transport capacity (Heide et al. 1985). Further, faster growth and higher carbon allocation to aboveground part of the plant under a longer photoperiod (Hay 1990, Oleksyn et al. 1992, Johnsen and Seiler 1996, Adams and Langton 2005) may require more efficient water transport to satisfy the increased demand. A high stem hydraulic conductivity allows faster water movement through the root-stem-leaves pathway to compensate for increases in transpiration water loss (Meinzer 2003). Such a response may reduce stomatal limitation to photosynthesis and enhance growth (Koch et al. 2004, Woodruff et al. 2004). These relationships, however, did not hold in the current study. However, there are also opposite results in the literature. Inverse relationships between vessel diameter and growth have

been observed in field experiments (Schreiber et al. 2011, 2013, 2015). It remains puzzling why the photoperiods at 52 and 55° N latitude did not but that at 58° N did increase the maximum hydraulic conductivity of white birch while they stimulated seedling growth similarly.

Interestingly, the freeze-thaw treatment delayed budburst and leaf expansion in the spring in white birch. Cavitated xylem vessels can be refilled by positive root pressure in the spring in diffuse-porous trees (Hacke and Sauter 1996, Cochard et al. 2001, Améglio et al. 2002, Mayr et al. 2007), which is often observed in *Betula* spp (Sperry et al. 1994). However, the result of our study suggests that the repair of the cavitated xylem in white birch did not occur fast enough or early enough in the spring to meet the demand of flushing buds and expanding leaves such that both budburst and leaf expansion were delayed. This effect essentially shortened the growing season. The potential effect of shortened growing season could be substantial if freeze-thaw events in the spring increase in the future as currently predicted (Solomon et al. 2007). However, this is a short term study conducted under controlled environmental conditions and only on one population of the species. More intensive and extensive studies are warranted.

It is also interesting to note that the spring budburst and leaf expansion of seedlings in the three longer photoperiods progressed faster and completed sooner than at the seed origin under elevated [CO₂], but the trend was the opposite under ambient [CO₂]. However, there was generally not much difference between the different photoperiods north of the seed origin under either [CO₂], which is consistent with the patterns of growth response that we have reported previously (Tedla et al. 2019). The acceleration of spring phenology at the longer photoperiods may be related to the faster rates of change in photoperiod during the season transition. It may also be a reflection of the faster temperature accumulation for budburst at longer light periods

(Basler & Körner, 2014; Heide, 1993a, 1993b; Laube et al., 2014). In either case, the lack of difference in the response between the three photoperiods north of seed origin indicates that white birch may have limited phenotypical plasticity in responding to longer photoperiod (Tedla et al. 2019). However, it is unclear why increase in longer photoperiods had opposite effects on spring budburst and leaf expansion under different [CO₂]. While the difference may be related to differences between different [CO₂] treatments in the accumulation of carbohydrates at the end of the previous season and subsequent availability for budburst and leaf expansion in the spring before the completion of development in leaf photosynthetic capacity, as suggested by Oren et al. (1988), the current study does not have data to permit a reasonable explanation on the mechanisms.

In conclusion, my study showed that longest photoperiod enhanced hydraulic efficiency in white birch while the interactive effect was generally statistically insignificant in the current study. The delayed progress of budburst and leaf expansion in the spring in response to freeze-thaw treatment imply that a delay in repair of the cavitated xylem can be a possible source of variation in spring phenology. Furthermore, the increase in hydraulic efficiency and an increase in growth (Tedla et al. 2019) appear to suggest that white birch may have favored productivity over safety under the scenario of northward migration, which makes sense as winter embolism may be less detrimental to a species capable of refilling embolized conduits (Sperry et al. 1994).

5.4. Reference

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6. General discussion

This dissertation aimed to understand the physiological, phenological, and growth responses of white birch to the interactive effects of photoperiod regimes and elevated [CO₂] under the scenario of climate change-induced northward migration. My research revealed the distinct response of *B. papyrifera* through simulation experiments which would not be possible in other experimental settings, such as a common garden. Common garden experiments that replicate across multiple environments can provide crucial information to understand how tree species will respond to environmental variabilities and for testing hypotheses related tree migration or seed transfer (Matyas 1996, Schreiber et al. 2013). In regard to climate change-induced migration, common garden experiments studies cannot capture the response of trees to novel conditions of the future, particularly for testing hypotheses on the interactive effects of [CO₂] and photoperiod due to logistic difficulties and considerable expenses in establishing common gardens.

The present data on the individual and interactive effect of photoperiod and [CO₂] in white birch seedlings reveal the critical roles that they may play in influencing the physiology, phenology, and growth, which will, in turn, affect tree survival and distribution. Below, I will summarize the key findings of my thesis. These findings should advance our understanding of how white birch, and deciduous boreal trees, in general, may respond to future climate conditions. I will also synthesize and discuss the major findings of this thesis and future research directions.

6.1. Key findings

The data in my second chapter demonstrate that CO₂ elevation lengthened the duration of the leaf senescence process in *B. papyrifera* primarily due to an earlier onset of leaf senescence as compared to later completion. Longer leaf retention can prolong the duration of carbon fixation

if leaves are physiologically active during the green leaf retention period. However, the earlier onset of senescence reduces the potential carbon uptake. Interestingly, longer photoperiods of the two high latitudes exhibited an earlier onset of leaf senescence than the two low latitude photoperiods under elevated [CO₂] conditions. These findings suggest under the scenario of climate change-induced migration, the photoperiod regime of higher latitudes could exert a certain control on autumnal senescence, likely preventing plants from benefiting from longer growing season. However, considerably more research is needed to elucidate the mechanism of changes in leaf senescence caused by the interactions of photoperiod and CO₂ concentration.

My data also show that autumnal cold hardiness and the timing of budburst in the spring were affected by the [CO₂]-photoperiod interaction. I found that the combination of the two longer photoperiods (55 and 58° N) and [CO₂] elevation enhanced cold hardiness in *B. papyrifera*. My findings suggest that climate change-induced migration to higher latitudes may enable sufficient development of freezing tolerance in *B. papyrifera* to protect the trees against cold winter temperatures. On the other hand, photoperiod had an opposing effect on the timing of spring budburst under different [CO₂]. While the two longer photoperiods advanced spring budburst under elevated [CO₂], they delayed budburst under the ambient [CO₂]. Since the differences in the budburst timing found were moderate, their impact on growth may be limited. However, such a response could increase the risk of late-spring frost damage that could injure or even kill new tissues and affect survival, if the response is to such an extent as to alter the proper synchronization of tree phenology with the season transition in environmental conditions at the new location. However, the simultaneous increase in cold hardiness may lessen the negative impact of a late frost.

In my third chapter, I provided evidence that the $[\text{CO}_2]$ elevation will not enhance P_n in seedlings grown under the photoperiod regimes of much higher latitudes, but stimulates P_n under the photoperiod regimes of the seed origin and at a latitude 4° north of the seed origin. I also found that the lack of CO_2 stimulation of P_n was due to the downregulation of V_{cmax} and J_{max} . In contrast, no such downregulations occurred under the photoperiod regimes associated with the two lower latitudes. The photosynthetic downregulation was related to a possible feedback inhibition to photosynthesis as indicated by the reductions in TPU at the higher latitude. This study has shown that photoperiod had contrasting effects on P_n in different $[\text{CO}_2]$: P_n increased in the higher photoperiod regimes in ambient $[\text{CO}_2]$, but the trend was the opposite in elevated $[\text{CO}_2]$. Taken together, these results suggest that the photosynthesis of white birch will possibly respond negatively to northward migration or seed transfer in response to climate change. My study also sheds light on the mechanisms of CO_2 elevation-induced increase in IWUE under different photoperiod regimes. The higher IWUE under the photoperiod regimes of lower latitudes was primarily attributed to the stimulated P_n by the elevated $[\text{CO}_2]$ while the higher IWUE under the photoperiod regimes of 55 and 58° N was achieved mainly by the g_s control of water loss.

My fourth chapter showed that growth and biomass of *B. papyrifera* responded positively to increases in the photoperiod of the growing season north of the seed origin. However, increases in photoperiod beyond an increase of more than of 4° in latitude north of the seed origin did not lead to an additional increase in growth and biomass. These results suggest that *B. papyrifera* may not be able to capitalize on the longer summer photoperiods if northward migration or seed transfer occurred at large scale. Further, the results also showed a correlation between biomass allocated to roots and photoperiod: a general decreasing trend with increases in photoperiod from

the seed origin to 58° N latitude. Overall, my experimental evidence suggests that despite the limited plasticity in growth and biomass displayed in response to much higher latitudes, white birch will likely benefit from the longer photoperiod regimes during the growing season associated with migration or seed transfer to higher latitudes.

The results in my last chapter showed that the longer day-length at 58° N latitude increased maximum stem-specific hydraulic conductivity. The data also showed that there was a substantial increase in PLC (up to 80%) following the three months of cold storage. The freeze-thaw cycle (FT) treatment resulted in an additional 11% increase in PLC. Another interesting result was that FT induced delay in the timing of spring budburst in my experiment.

Furthermore, budburst occurred earlier at longer photoperiods relative to the seed origin under elevated [CO₂], but the trend was the opposite under the ambient [CO₂]. The results suggest that freeze-thaw can be a possible cause for winter-embolism in deciduous trees and could affect the timing of spring phenology.

6.2. Thesis synthesis

As commonly observed in others deciduous trees (Eamus and Jarvis 1989, Ainsworth and Long 2004, Leakey et al. 2009), at current tree locations or with moderate change in photoperiod, CO₂ elevation stimulated P_n (Chapter 3) and increased growth and biomass (Chapter 4). For example, CO₂ elevation increased P_n at photoperiod regime 4° north of the seed origin. Similarly, the photoperiod regime at 4° north of the seed origin increased P_n relative to 10° N under elevated [CO₂]. White birch has an indeterminate growth pattern and is known to have high plasticity in morphology and physiology in response to environmental conditions (Li et al. 1996, Ashton et al. 1998, Wang et al. 1998). However, the lack of further increases in growth and biomass to use

the additional carbohydrates produced due to the longer period for photosynthesis at much higher latitude, means that the positive effect of CO₂ elevations diminishes (Chapter 3-4). This has led to a significant downregulation of photosynthetic capacity, as indicated by the reductions in V_{cmax} and J_{max} (Chapter 3). A feedback inhibition due to excess photosynthetic carbohydrate production exceeds increases in utilization by sinks, and the subsequent buildup of triose phosphate may have triggered the downregulation of photosynthetic machinery (Lambers et al. 2008). Indeed, the TPU of seedlings grown 10° north of the seed origin declined in response to the CO₂ elevation (Chapter 3). The decline in carbon allocation to roots with the increase in photoperiod (Chapter 4) and the lack of CO₂ stimulation of root biomass may have increased the source-sink imbalance to a greater extent, negating any CO₂ stimulation of P_n (Chapter 3).

The seasonal variation of photosynthetic response to photoperiods is not consistent with the findings of Beerling and Osborne (2002). They have found that longer photoperiods decrease the magnitude of P_n stimulation by CO₂ elevation in the major part of the growing season but increase the stimulation in early spring and fall. In my study, the lack of CO₂ P_n stimulation at the two higher latitudes persisted in all three measurement periods from June to August (Chapter 3). In other words, my results show that there will not be CO₂ stimulation of P_n if trees do migrate to higher latitude.

I found that seedlings under longer photoperiods advanced budburst under elevated CO₂, whereas the response was the opposite under the ambient CO₂ (Chapter 1). The longer photoperiod contribution in reducing the time required for budburst is entirely plausible (Basler & Körner, 2014; Heide, 1993a, 1993b; Laube et al., 2014). This may be related to the fact that the seedlings under longer photoperiod regimes were exposed to longer duration of day time

thermal accumulation despite the same temperatures (day/night) used in my study because light presumably increased the temperature of seedlings during the day. An earlier budburst in this study may not undoubtedly contribute to enhanced productivity. Seedlings that showed an earlier budburst was also accompanied by an earlier initiation of leaf senescence (23 days) (Chapter 1). In addition, the initiation of bud set by photoperiod has been described in *Betula* spp (Downs and Bevington 1981). Consistent with this, based on my data in Chapter 1 it appears that white birch bud set is triggered by a threshold photoperiod as shown by the consistent timing of bud set around the autumnal equinox in both growing seasons. However, the initiation of leaf senescence at the higher latitude photoperiod was advanced by an average of 20 days before growth was visibly ceased (Chapter 1). This result shows that leaf senescence is not controlled by photoperiod in white birch as seen in other broadleaf trees such as *Populus* spp (Barr et al. 2004, Fracheboud et al. 2009) and for *B. pendula* (Velling 1979). Furthermore, the earlier onset of leaf senescence before bud set may indicate that carbon uptake and use for growth was greatly reduced before growth ceases. Such responses of leaf senescence to longer photoperiod under [CO₂] elevation suggest that, if trees migrate to further north, the higher latitude photoperiods may impose limits on the physiology of photosynthetic leaves during the period with relatively favorable conditions. However, there is an alternative explanation for the earlier onset of senescence, i.e., C/N imbalance triggered the onset of leaf senescence to allow nitrogen translocated to young expanding leaves by freeing nitrogen invested in the Rubisco of old leaves (Wingler et al. 2006). This phenomenon may also explain the CO₂ enhanced cold hardiness at the two higher latitudes (Chapter 2 and 3). The increased carbohydrate production and roles of cryoprotective sugars in determining freezing tolerance have been reported in the past (Castonguay et al. 1995, Poorter et al. 1997). On the other hand, early budburst may increase the

probability of late frost damage. However, the simultaneous increase in cold hardiness in the treatment combinations suggest possible higher rates of growth at higher latitudes under future elevated [CO₂] (Chapter 1). While freeze-thaw induced dieback in young trees appears to be unlikely (Polgar and Primack 2011), the results of this study suggest that freeze-thaw events could be a source of variation in the timing of spring bud break in seedlings (Chapter 5). The increase in hydraulic efficiency appears to suggest that white birch would favor productivity over safety if migrating to higher latitudes because winter embolism may be less detrimental to a species capable of refilling embolized conduits whereas increased hydraulic efficiency can support growth, particularly in less water stressed conditions (Chapter 5).

6.3. Reference

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