



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]



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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 refilling of embolized xylem conduits early in the spring, resulting in an earlier bud break. In this study, we 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. We 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 the 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.

1. 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 (Cochard et al., 2001; Sperry and Sullivan, 1992; Walker, 1990; Ward and Stephens, 1997) and freezing injuries (Charrier et al., 2013; Sakai and Larcher, 2012). 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 Sullivan, 1992; Sperry and Tyree, 1988). The size of air bubbles formed during freeze-thaw events depends primarily on the diameter of xylem conduits (Hacke and Sauter, 1996; Sperry and Sullivan, 1992). 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

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freeze-thaw-induced embolism (Davis et al., 1999; Pittermann and Sperry, 2003; Sperry and Sullivan, 1992). 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 (Pockman and Sperry, 1996; Sperry et al., 1994; Wang et al., 1992; 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 (Hacke and Sauter, 1996; Sperry et al., 1994). 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 (Hunter and Lechowicz, 1992; Lechowicz, 1984).

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; Kostianinen 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; 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, 1964, 1962). The combination of longer photoperiods and elevated [CO₂] can synergistically increase the production of photosynthetic assimilates (Gandin et al., 2011; Körner and Miglietta, 1994; Kupperts et al., 1988), 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, we examine the interactive effects of elevated [CO₂] and photoperiod regime on freeze-thaw induced xylem embolism and spring budburst in white birch. We 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 and has wide distributions in the boreal forest (Burns et al., 1990; Farrar, 1995; Sperry et al., 1994).

2. Materials and methods

2.1. 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 (50 cm × 25 cm) 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 h, 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).

2.2. 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. The split-plot design was organized as a completely randomized design (CRD) where the main plot treatment was assigned to the 4 greenhouses (whole plots: 2 greenhouses (replicates) for each CO₂). 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 h. The set points were calculated from the Environment Canada data as described previously. The experiment was carried out for one growing cycle. 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 1–25) (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.

2.3. Freeze-thaw experiment

We 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.

2.4. 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 we did not measure the vessel lengths, the literature has shown that a 15 cm 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 (20 cm 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 20 mM/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 twice (initial conductivity and maximum conductivity measured after gas trapped within the conduits has been flushed under high pressure) as the mass flow rate (kg s^{-1}) per unit of functional xylem area (the cross-sectional area of the xylem (K_s : xylem specific conductivity)). 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 0.5 m above the specimen and the measurement of 10 kPa pressure-induced flow rate measured (5 min) after the water tank was pressurized 10 kPa. 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.

2.5. 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 the cold storage and thawed while overnight at room temperature. The following day one of the groups was exposed to freeze-thaw cycle treatment as described previously while the control group was maintained at +4 °C. After the freeze-thaw treatment, both groups were moved to the greenhouses where spring conditions were simulated.

2.6. Phenological observation

The spring phenology of budburst was monitored on the seedlings of both groups. For each seedling, we 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, the bud break was considered complete when at least three buds

reached phase 5.

2.7. Statistical analysis

We used 3-way analysis of variance (ANOVA) for split-split plot design with two levels of CO₂ at the whole plot level, four levels of photoperiod at the sub-plot level and two levels of freeze-thaw treatment at the sub-sub plot level for PLC measured after three months in cold storage (FT-CS) and the date of spring budburst (DOY). In our study, budburst date was defined as the date when the base and petiole of the first leaf emerged from the bud (stage -5) and was used as a dependent variable. 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.

3. 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 1 Panel A). However, PLC measured after 3 months of cold storage was 11 % greater in seedlings subjected to the freeze-thaw treatment than control seedlings (Table 1 Panel A; Fig. 1B). Furthermore, the PLC value was substantially greater after than before the cold storage (95 % vs. 10 %, Table 1 Panel A).

Table 1

Panel A: two-way ANOVA on the effects of CO₂ and photoperiod on the percent loss of hydraulic conductivity (PLC) measured in the fall (FT-Fall) and three-way ANOVA on the effects of CO₂, photoperiod and freeze-thaw treatment on PLC measured after 3-month cold storage (FT-CS). Panel B: three-way ANOVA in the effects of CO₂, photoperiod and time of measurement (FT-Fall vs. FT-CS) on PLC. Seedlings were subjected to two [CO₂] (400 and 1000 $\mu\text{mol mol}^{-1}$) and four photoperiod regimes (corresponding to 48, 52, 55, and 58 °N latitude) for one growing season in environment-controlled greenhouses. Significant ($P \leq 0.05$) *P*-values are bolded. Abbreviations: df = degrees of freedom; F = F value; P = probability.

Panel A: Before (FT-Fall) and after 3 months of cold storage (FT-CS)							
Measurement time	FT-Fall				FT- CS		
factor	df	F	P	Parameter	df	F	P
CO ₂	1	1.13	0.4	CO ₂	1	0.43	0.58
P	3	1.19	0.39	P	3	2.49	0.16
CO ₂ × P	3	0.8	0.53	CO ₂ × P	3	2.67	0.14
				FT	1	23.38	< 0.01
				CO ₂ × FT	1	2.07	0.19
				P × FT	3	2.01	0.19
				CO ₂ × P × FT	3	0.72	0.57
Panel B: Comparison between FT-Fall and FT-CS							
factor	df	F	P				
CO ₂	1	2.19	0.28				
P	3	1.15	0.4				
CO ₂ × P	3	0.37	0.78				
Time	1	1364.6	< 0.01				
CO ₂ × Time	1	0.7	0.43				
P × Time	3	0.15	0.93				
CO ₂ × P × Time	3	1.33	0.33				

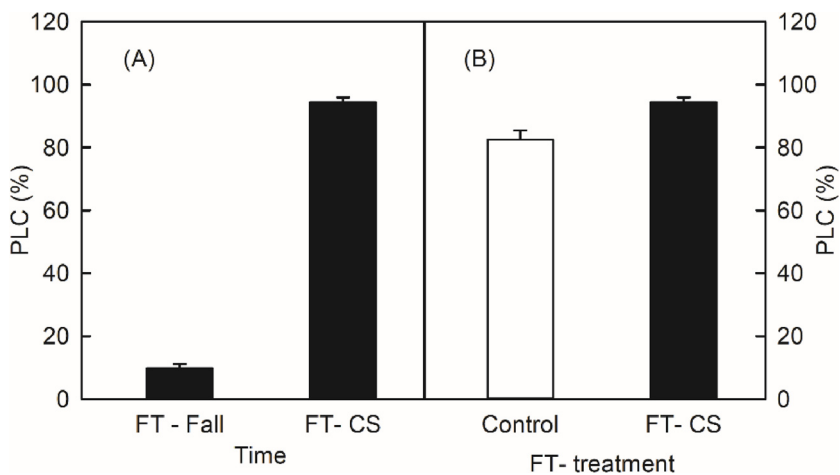


Fig. 1. Percent loss of hydraulic conductivity (PLC) as affected by season (A) and freeze-thaw treatment after 3-months of cold storage (B). The measurements were taken in the fall (FT-Fall) and after 3-months of cold storage (FT-CS). Measurement was made on 15 cm stem segments of white birch seedlings. 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 $[\text{CO}_2]$ because the interaction was not statistically significant, $3 \times 2 \times 4 \times 2 = 48$).

Table 2

Results of two-way ANOVA (performed on stem specific hydraulic conductivity) and three-way on the timing of budbreak (DOY) to test the effects of $[\text{CO}_2]$ and photoperiod (P) and freeze-thaw treatment (FT). Note: budbreak date (stage - 5) was defined as the stage in bud development when the base and petiole of the first leaf emerged from the bud. See Table 1 for more explanations.

factor	df	Stem specific hydraulic conductivity ($\text{Kg m}^{-1} \text{s}^{-1} \text{Mpa}^{-1}$)		Budbreak; stage - 5 (DOY)	
		F	P	F	P
CO_2	1	0.2	0.9	0.26	0.66
P	3	0.2	0.04	0.04	0.99
$\text{CO}_2 \times \text{P}$	3	4.98	0.18	26.33	< 0.01
FT	1			6.3	0.04
$\text{CO}_2 \times \text{FT}$	1			0.26	0.62
$\text{P} \times \text{FT}$	3			0.05	0.98
$\text{CO}_2 \times \text{P} \times \text{FT}$	3			1.4	0.31

Table 1 Panel B; Fig. 1A).

The seedlings differed substantially in their maximum potential hydraulic conductivity in response to the photoperiod regimes (Table 2; Fig. 2). 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

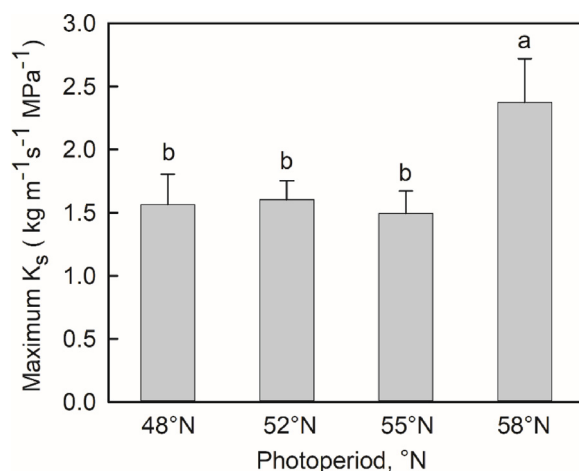


Fig. 2. Effects of photoperiod regime at different latitudes on maximum stem-specific hydraulic conductivity (mean $\pm$ SE, N = 12) of white birch seedlings. Means with different letters were significantly different from each other ($P \leq 0.05$, Fisher's LSD). See Fig. 1 for more explanations.

regimes (Table 2; Fig. 2). Seedlings under 58°N latitude increased hydraulic conductivity by an average of 52 % compared to the seed origin.

The timing of spring phenophases was affected by freeze-thaw treatment. The freeze-thaw treatment delayed the progress of budburst and leaf expansion in the spring (Table 2; Fig. 3). Moreover, the interaction between $[\text{CO}_2]$ and photoperiod significantly affect the progress and completion date of spring budburst (Table 2). Compared to other photoperiod regimes, the spring budburst progressed faster and completed sooner in the photoperiod regime of the seed origin under the ambient $[\text{CO}_2]$ (Fig. 4A and C), but the trend was the opposite under the elevated $[\text{CO}_2]$ (Fig. 4B and C). For example, the budburst of seedlings at the two longer photoperiod regimes (55 and 58°N) occurred on average 3.6 - 4 days earlier than at the seed origin in elevated $[\text{CO}_2]$, whereas, this variable was occurred 4 - 4.3 days later at the two shorter photoperiods (48 and 52°N) in ambient $[\text{CO}_2]$ (Fig. 4A-C). Furthermore, the increase in CO_2 supply significantly advanced spring budburst at the photoperiod regimes corresponding to the two higher latitudes, no significant effect at 52°N, but significantly delayed at the seed origin (Fig. 4C).

4. Discussion

Our 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

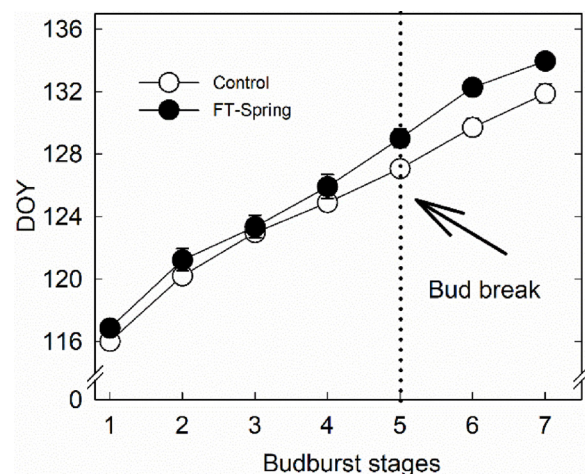


Fig. 3. The progression of spring budburst in white birch seedlings that were subjected to five freeze-thaw cycles (FT-Spring) and control seedlings (Mean $\pm$ SE, N = 48). (DOY) day of year. See Fig. 1 for more explanations.

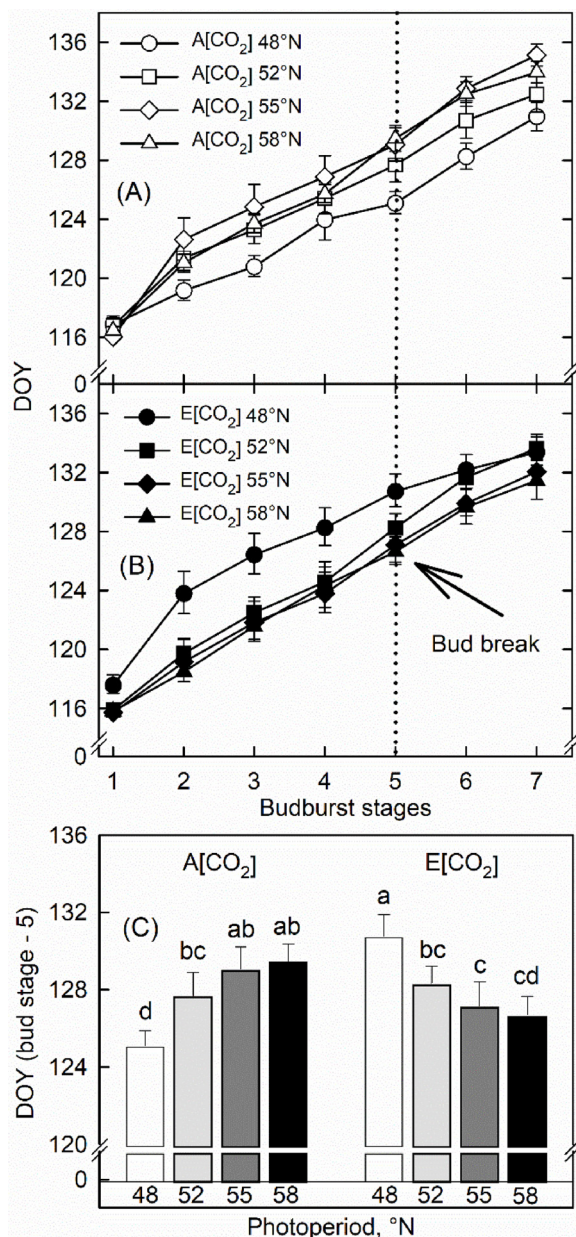


Fig. 4. The effect of photoperiod regime on the progression of spring budburst under ambient (A) and elevated [CO₂] (B). The interaction between photoperiod and CO₂ was significant ($P \leq 0.05$). The dotted vertical line in A and B indicate the completion of budburst (stage-5). Panel C, the interactive effect of CO₂ and photoperiod on the completion date (Day of the Year) of spring budburst with different letters within each CO₂ and between ambient and elevated CO₂ at a given photoperiod indicate significant difference from each other. The value of each point represents the average of 12 seedlings. See Fig. 1 for more explanations.

efficiency would make plants more vulnerable to freeze-thaw induced embolism. In our previous study (Tedla et al., 2019), we found that longer photoperiods corresponding to latitudes north of the seed origin increased growth and biomass in white birch. Similar findings have been reported for other boreal tree species (Inoue et al., 2019; Li et al., 2015; Newaz et al., 2016). While the longest photoperiod in this study significantly increased the maximum specific hydraulic conductivity of the stem, other photoperiod treatments had no significant impact on the hydraulic conductivity (Fig. 2). However, none of the photoperiods had a significant effect on the vulnerability to freeze-thaw induced xylem cavitation in this study (Table 1). We consider this result as having

positive implications to the northward of white birch. Studies have indicated that the general global warming trend is accompanied by more temperature fluctuations and an increase in the frequency of freeze-thaw events (Man et al., 2015). Our 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) reported 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.

Our results imply a strong seasonal variation in xylem hydraulic conductivity and its vulnerability to freeze-thaw induced xylem cavitation. Although we 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 %) (Fig. 1A and B). Furthermore, the PLC measurement in the control seedlings was considerably greater than the PLC measured in the fall (Fig. 1A and B). 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. We wrapped the entire seedling in a polyethylene bag during the cold storage to minimize the 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., 2015, 2013). Our 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 we 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 our study support the current understanding that freeze-thaw induced embolism can occur in diffuse-porous trees (Améglio et al., 2002; Sperry and Sullivan, 1992).

Our 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 (Fig. 2). 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 (Fig. 2) 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; Johnsen and Seiler, 1996; Oleksyn et al., 1992) 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., 2015, 2013, 2011). 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 (Fig. 2) while they stimulated seedling growth similarly.

Interestingly, the freeze-thaw treatment delayed budburst and leaf expansion in the spring in white birch (Fig. 3). Cavitated xylem vessels can be refilled by positive root pressure in the spring in diffuse-porous trees (Améglio et al., 2002; Cochard et al., 2001; Hacke and Sauter, 1996; 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 (Fig. 3). Although the magnitude of the difference in days to budburst between the freeze-thaw treated and untreated seedlings is modest (Fig. 3), later and slower phenological events in the spring could reduce the risk of frost damage (Lenz et al., 2016; Vitasse et al., 2014a, 2014b). Alternatively, late budburst can also be a competitive disadvantage, especially for poleward migrating plants, because spring phenology at higher latitudes is strictly associated with favorable conditions for photosynthesis and growth where the resident individuals commonly need less heat accumulation for spring budbreak, allowing growth to be resumed at the earliest and thus maximize the growing period. Delayed spring phenology therefore may shorten the growing period and decrease productivity (Arora and Taulavuori, 2016). The potential effect of the 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 the elevated [CO₂], but the trend was the opposite under the ambient [CO₂] (Fig. 4). 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). Further, while the difference in spring phenology between the photoperiod treatments with each CO₂ appears to narrow at the completion of leaf expansion (Fig. 4), the early stages of leaf emergence are critical particularly in relation to the risk of frost damage. For migrating species in response to climate change, early spring phenology could maximize the competing ability, i.e., maximizing the use of favorable environmental conditions in early in the growing season. This advantage, however, may be accompanied by a greater risk of frost damage in late spring. The next result of this trade-off may vary from year to year, depending on the occurrence of frosts late in the spring. The acceleration of spring phenology at the longer photoperiods may be related to the faster rates of change in photoperiod during the season transition. Alternatively, it may be a reflection of the faster temperature accumulation for budburst at longer light

periods (Basler and 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 phenotypic plasticity in responding to longer photoperiod (Tedla et al., 2019). However, it is unclear why an increase in longer photoperiods had opposite effects on spring budburst and leaf expansion under different [CO₂] (Fig. 4). 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, our study showed that the 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 implies 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).

Author statement

All authors have made substantial contributions to all three of sections (1),(2) and (3) below:

- 1 the conception and design of the study, or acquisition of data, or analysis and interpretation of data
- 2 drafting the article or revising it critically for important intellectual content
- 3 final approval of the version to be submitted

Declaration of Competing Interest

The authors declare that the work submitted has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

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