

Longer photoperiods negate the CO₂ stimulation of photosynthesis in *Betula papyrifera* Marsh: Implications to climate change-induced migration

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Funding information

Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: 203198-2013-RGPIN; Lakehead University; NSERC

Edited by: I. Ensminger

Abstract

In response to global warming, trees are expected to shift their distribution ranges to higher latitudes. The range shift will expose them to novel environmental conditions, such as new photoperiod regimes. These factors can interact with rising atmospheric CO₂ ([CO₂]) to affect trees' physiology and growth. This study simulated future environmental conditions to investigate photosynthetic responses to changes in photoperiod regimes (seed origin [48°N], 52, 55, and 58°N) and [CO₂] (ambient 400 vs. elevated 1000 μmol mol⁻¹) in white birch (*Betula papyrifera* Marsh.) seedlings. Our results show that elevated [CO₂] stimulated leaf photosynthesis (P_n) at the two lower latitudes (48 and 52°N). However, this stimulation by elevated [CO₂] was lost in the two higher latitudes (55 and 58°N). Elevated [CO₂] led to the downregulation of maximum Rubisco activity (V_{cmax}) for the two higher latitudes, and maximum electron transport rate (J_{max}) and triose phosphate utilization (TPU) at 58°N, while it enhanced J_{max} and TPU for the two lower latitudes. Increased instantaneous water-use efficiency (IWUE) for the two lower latitudes was primarily attributed to the CO₂ stimulation of P_n while the higher IWUE under the photoperiod regimes of 55 and 58°N latitudes was explained by reduced water loss. Photoperiod effects varied with [CO₂]: P_n increased at the photoperiod regimes of 55 and 58°N in ambient [CO₂] while it tended to decline under these photoperiods in elevated [CO₂]. Our study suggests that the photosynthesis of white birch will likely respond negatively to northward migration or seed transfer in response to climate change.

1 | INTRODUCTION

As the climate gets warmer, the geographical positions of tree bioclimate envelopes are expected to shift rapidly, potentially moving trees to higher latitudes or altitudes (McKenney et al., 2007; Pachauri et al., 2014). While migrating according to the movement of their pre-

adapted climatic conditions (i.e., temperature and water regime) may reduce the potential impacts of climate change, plants will encounter new environmental conditions that they have not experienced in their recent history (Griffith & Watson, 2006), e.g., new photoperiod regimes (Way & Montgomery, 2015) and higher atmospheric carbon dioxide concentrations ([CO₂]). Both of these factors play a significant role in regulating plant phenological and physiological processes (Jach et al., 2001; Lambers et al., 2008; Thomas & Vince-Prue, 1996). For instance, a change in photoperiod regime can influence the photosynthesis and nitrogen content of the leaf (Comstock & Ehleringer, 1986) and elevated [CO₂] generally stimulates photosynthesis and carbohydrate production (Jach et al., 2001). In recent years, there has been

Abbreviations: [N], foliar nitrogen concentration; AC, ambient CO₂ concentration; C:N, carbon to nitrogen ratio; Ci/Ca, intercellular to ambient CO₂ concentration; EC, elevated CO₂ concentration; Fv/Fm, potential maximum quantum efficiency of PSII; g_s , stomatal conductance; IWUE, instantaneous photosynthetic water-use efficiency; J_{max} , maximum rate of photosynthetic electron transport; PAR, photosynthetically active radiation flux density; P_n , net photosynthetic rate; TPU, rate of triose phosphate utilization; V_{cmax} , maximum rate of Rubisco carboxylation.

increasing interest in understanding the effects of changes in the photoperiod regime on plant phenology and growth in the context of climate change-induced tree migration (Inoue et al., 2019, 2020; Li et al., 2015; Newaz et al., 2016; Tedla et al., 2019, 2020a, 2020b; Way & Montgomery, 2015). However, the interactive effects of photoperiod regime and $[\text{CO}_2]$ on photosynthetic physiology are still not well understood.

Small to moderate-scale migration to higher latitudes 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 (Benomar et al., 2016). Similar results have been observed in *Eriophorum vaginatum* L, where light-saturated photosynthetic rate (A_{max}) increased when genotypes were moved from 65 to 68°N (Souther et al., 2014). However, the distance that trees must move in order to stay within their climate envelope will possibly be greater (Loarie et al., 2009). As a result, a dramatic change in the photoperiod regime is expected (Way & Montgomery, 2015). Such a change will likely influence physiological processes, such as those involved in photosynthetic carbon assimilation. At higher latitudes, exposure to an extended photoperiod in the summer can lead to reductions in V_{cmax} , J_{max} , and the efficiency of photosystem II when carbohydrate utilization cannot keep pace with the increase in carbohydrate production. The imbalance between carbon acquisition and use may lead to a feedback inhibition of P_n and photosynthetic downregulation (Equiza et al., 2006; Osborne & Beerling, 2003; Royer et al., 2005). The downregulation of photosynthetic capacity under longer photoperiods has been reported for various species in controlled experiments (Dorais et al., 1996; Gandin et al., 2011; Llorens et al., 2009; Stutte et al., 1996).

It is well established that tree physiology is strongly influenced by elevated $[\text{CO}_2]$ (Curtis & Wang, 1998; Norby et al., 1999). While the P_n response of C3 plants to elevated $[\text{CO}_2]$ varies with species and other environmental conditions, the response is generally positive, particularly when other resources and environmental conditions are favorable (Ainsworth & Long, 2004; Eamus & Jarvis, 1989; Leakey et al., 2009). Increased P_n and reduced stomatal conductance (g_s) and transpiration rate (E) in response to elevated $[\text{CO}_2]$ lead to enhanced water-use efficiency (Ainsworth & Rogers, 2007). Photosynthetic response to elevated $[\text{CO}_2]$ in trees can also vary across the growing season due to seasonal variations in growth and resources, such as temperature, light, water, and nutrients (Gunderson & Wullschleger, 1994; Medlyn et al., 1999). The down-regulation of photosynthesis in plants grown under elevated $[\text{CO}_2]$ is often attributed to insufficient sink strength to utilize the additional carbohydrate produced as a result of the increased carbon fixation (Ainsworth & Long, 2004; Leakey et al., 2009). An interesting question is how the longer period of photosynthesis associated with a longer photoperiod at a higher latitude will affect the P_n of plants exposed to elevated $[\text{CO}_2]$. Several studies have demonstrated that increases in the duration of photosynthesis without a corresponding increase in demand for carbohydrates will lead to photosynthetic downregulation

(Ainsworth & Rogers, 2007; Long et al., 2004). Such a response may negate the stimulation of photosynthesis by elevated $[\text{CO}_2]$. Beerling and Osborne (2002) have found that the CO_2 stimulation of P_n in polar species is limited by extended photoperiods at high latitudes during the summer solstice while the level of the stimulation increases later in the summer when photoperiods become shorter. While other environmental factors that change concurrently with photoperiod, such as temperature, may contribute to the seasonal variation in photosynthesis, photoperiod itself can be a critical driver. The interactive effects of photoperiod regime and $[\text{CO}_2]$ may have implications for trees that migrate northward in response to climate change.

Our previous study investigated the influence of photoperiod regime on the growth, biomass, and biomass allocation of white birch (*Betula papyrifera* Marsh.) under different CO_2 concentrations (Tedla et al., 2019). White birch is a widely distributed pioneer tree species in North America (Burns et al., 1990; Farrar, 1995). Our results have shown that growth, biomass production, and biomass allocations are influenced by photoperiod regime but not by $[\text{CO}_2]$. While the photoperiod 4°N of the seed origin stimulated growth and biomass, further increases in photoperiod did not lead to additional increases in growth and total biomass. Leaf and stem biomass, however, increased with further increases in photoperiod (Tedla et al., 2019). In contrast, biomass allocation to roots declined with increases in photoperiod. In the present study, we examine possible physiological adjustments associated with the morphological responses. We hypothesize that elevated $[\text{CO}_2]$ would stimulate photosynthesis and the degree of stimulation would decline with increases in photoperiod.

2 | MATERIALS AND METHODS

2.1 | Plant material

We collected mature catkins of white birch from 12 natural trees in autumn 2016 from Thunder Bay (48.4215°N, 89.2619°W). Seeds were extracted manually, air-dried, and stored in a plastic bag before the start of the experiment. The experiment was set up at the Lakehead University Forest Ecology 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). The seedlings were transplanted into bigger pots (18 cm deep, 16/14 cm top/bottom diameter) in the second growing season.

2.2 | Experimental design

The experiment was set up as a split-plot design consisting of two levels of $[\text{CO}_2]$ treatment (ambient 400 and elevated

1000 $\mu\text{mol mol}^{-1}$) as main plots and four photoperiod regimes corresponding to 48°, 52°, 55°, and 58°N latitudes nested within each CO_2 treatment as split plots. There were two independent replications (greenhouses) for each CO_2 treatment and 10 seedlings within each treatment-replicate combination. A total of 160 seedlings (2 $\text{CO}_2 \times 4$ photoperiods \times 2 replicates \times 10 seedlings in each combination) were used for the study. The desired $[\text{CO}_2]$ level was achieved in the greenhouses using natural gas CO_2 generators (model GEN-2E; Custom Automated Products Inc.). The four photoperiod regimes were set to simulate the natural weekly average regimes of the growing season for the four latitudes. The overall photoperiod regime in 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 shade. 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) were used to extend the natural photoperiod when the natural day-length in the greenhouse was shorter than required. The lamps provided about 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR at the canopy level, with less blue light than sunlight but a similar R/FR ratio. 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 h. The set points were calculated from the Environment Canada weather records of the past 10 years. 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). The volumetric water content of the growing medium was maintained at around 50–60% of the field capacity as determined using an HH2 Moisture Meter and ML2X Theta Probe (DELTA-T DEVICES). The seedlings were fertilized twice a week with 50/81/30.3 mg l^{-1} N/P/K at the establishment stage from April 26 to May 25; 150/65.2/125 mg l^{-1} N/P/K at the rapid growth phase from May 26 to August 30; and with 50/54.3/156.3 mg l^{-1} N/P/K at the hardening phase from September 1 to September 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 with identical treatments as the first one.

2.3 | Chlorophyll fluorescence and A/Ci curve measurements

Leaf chlorophyll fluorescence and gas exchange were measured in June, July, and August of the second growing season. These

measurements were made on the youngest fully expanded leaf (fifth to seventh from the top) of three randomly selected seedlings per treatment combination. Chlorophyll fluorescence was measured using a Hansatech FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd. Norfolk) after the leaves were dark-adapted for at least 40 min. The potential maximum quantum efficiency of PSII was determined as $(F_m - F_o)/F_m$.

Immediately after chlorophyll fluorescence measurements, foliar gas exchange was measured on the same leaves using a PP-Systems CIRAS-3 open gas exchange system (Amesbury). A/Ci curves were measured at 22°C air temperature, 50% RH, 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 $\mu\text{mol mol}^{-1}$. It generally took 3–5 min for the readings to stabilize at each step and steady-state readings were recorded. 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 time period. Net photosynthetic rate at treatment $[\text{CO}_2]$ (P_n) and at ambient $[\text{CO}_2]$ (P_{n400}) was derived from the A/Ci curve of each seedling. Stomatal conductance (g_s), the rate of transpiration (E), Ci/Ca ratio, and instantaneous water-use efficiency (IWUE) were computed at their respective treatment $[\text{CO}_2]$.

2.4 | A/Ci curve fitting

The biochemical model of photosynthesis (Farquhar et al., 1980) was used to derive the following photosynthetic parameters from the A/Ci 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) under R Version 3.5.0. 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).

2.5 | Leaf nitrogen assay

After the completion of all the gas exchange measurements, leaves were harvested and subsequently oven-dried at 80°C for 48 h and weighted. Mass-based leaf nitrogen [N] and carbon concentration [C] were determined by the dry combustion method (Horneck & Miller, 1997) with a CNS-2000 (LECO Corp.). Mass-based leaf N concentration was converted to area-based leaf [N] by multiplying by the specific leaf area.

2.6 | Statistical analysis

[N], [C] and C/N ratio were subjected to two-way analysis of variance (factors: CO_2 and photoperiod) and all other variables were

analyzed using three-way ANOVA (factors: CO₂, photoperiod and measurement time). The analyzes were done using the “AOV for split plot design” function of the open-source statistical package R Version 3.5.0. To address the issue that experiments with small sample sizes generally have a greater probability to fail to detect real treatment effects (type II error), we used the Protected Fisher’s LSD Test to compare means when ANOVA showed a significant effect, as recommended by Klockars and Sax (1986). The assumptions of normality and homogeneity were assessed with the Shapiro tests and residual plots, respectively. Power transformation was applied to J_{\max} , TPU, Ci/Ca, E, IWUE, [N], and Fv/Fm in order to meet the ANOVA assumptions.

3 | RESULTS

3.1 | Chlorophyll fluorescence and gas exchange

There were no significant treatment effects on the quantum efficiency of photosystem II (Fv/Fm) and the overall average was 0.8 (Table 1). Photosynthesis (P_n) was significantly higher at the elevated than ambient [CO₂] in the seedlings grown under the photoperiod regimes of the two lower latitudes (net increase was 72% at 48°N and 109% at 52°N). However, there was no significant CO₂ stimulation at the two higher latitudes (Figure 1A). P_n generally declined from June to August under the elevated CO₂ while it was the highest in July under the

TABLE 1 Summary of ANOVA (F -test; P = probability level, 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 (g_s), transpiration rate (E), instantaneous water-use efficiency (IWUE), internal to ambient CO₂ concentration ratio (Ci/Ca), maximum carboxylation rate (V_{\max}), light-saturated electron transport rate (J_{\max}), triose phosphate utilization (TPU), and the ratio of J_{\max} to V_{\max} (J_{\max}/V_{\max})

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
g_s	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_{\max}	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_{\max}	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				

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 -values ($P \leq 0.05$) are bolded.

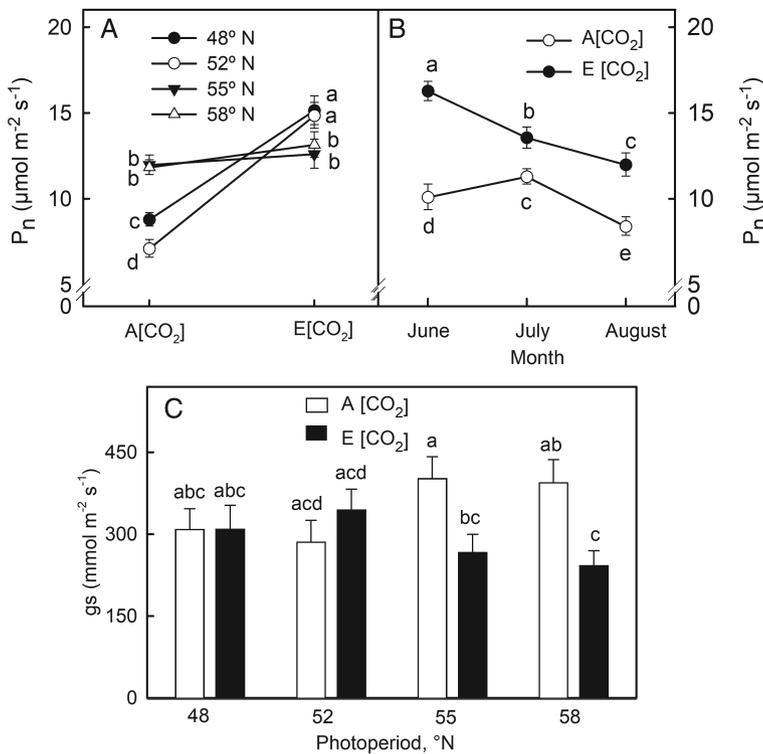


FIGURE 1 Effects of [CO₂] and photoperiod regime on (A) the net photosynthetic rate at treatment [CO₂] (P_n), and (C) stomatal conductance (g_s). Panel B, the effect of CO₂ and measurement times on P_n . Seedlings were grown under photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58°N latitude and under 400 (ambient, A[CO₂]) and 1000 $\mu\text{mol mol}^{-1}$ (elevated, E[CO₂]) for two growing seasons. The data are presented as mean + SE ($n = 18$ for panels A and C; $n = 24$ for panel B); the data were pooled for the three measurement times (A and C) and four photoperiod regimes (B) because the three-way interaction was not statistically significant. Different letters represent statistically significant difference between treatments

ambient [CO₂] (Figure 1B). The effect of photoperiod on P_n varied with CO₂ (Table 1). Under the elevated [CO₂], P_n was lower at the photoperiods of 55 and 58°N latitude than under that of the seed origin (by 17% and 13% respectively, Figure 1A). By contrast, P_n was ~35% higher under the photoperiods of 55 and 58°N latitudes compared to seed origin under the ambient [CO₂] (Figure 1A). g_s and E also generally declined from June to August (graph not shown) and with the increase in [CO₂] under the photoperiod regimes at the two higher latitudes, but not those at the lower latitudes (Table 1; Figure 1C).

The interaction between [CO₂] and photoperiod significantly affected both IWUE and Ci/Ca, and the effects varied over measurement time (Table 1). Across the season, the CO₂ elevation increased IWUE but the effect for the two higher latitudes was not statistically significant in June (Figure 2A). The effect of elevated [CO₂] on Ci/Ca was similar to that on IWUE but the effect was not significant in June for the two lower latitudes (Figure 2B). IWUE was significantly higher and Ci/Ca was significantly lower for the two higher latitudes compared to the two lower latitudes in June under the under ambient [CO₂] (Figure 2A,B).

3.2 | Photosynthetic capacity

The responses of V_{cmax} to [CO₂] varied with photoperiod treatment (Table 1; Figure 3A). The seasonal average V_{cmax} tended to be lower for the two higher latitudes when [CO₂] was elevated, e.g. the value under the 55°N photoperiod regime was ~10% lower than that under the photoperiod regime of seed origin but was similar to other

photoperiod treatments (Figure 3A). Under ambient [CO₂], in contrast, the longer photoperiod regimes at the two higher latitudes increased V_{cmax} (Figure 3A) and the average increase was 29% and 26% (compared to seed origin) for 55 and 58°N latitude, respectively, but there was no significant difference between the two lower latitudes or between the two higher latitudes (Figure 3A). Furthermore, the effect of elevated [CO₂] on V_{cmax} varied with photoperiod (Table 1): increased [CO₂] had no effects on V_{cmax} at the two lower latitudes, but reduced V_{cmax} by 26% and 23% at 55 and 58°N, respectively (Figure 3A). [CO₂] did not significantly affect V_{cmax} in June, but the elevated [CO₂] significantly reduced it in July and August (Figure 3B). V_{cmax} generally declined over time (data not shown).

There were significant interactive effects of photoperiod \times [CO₂] on J_{max} and TPU (Table 1). The two higher latitudes had significantly higher J_{max} and TPU under ambient [CO₂] but not under the elevated [CO₂] and the elevated [CO₂] significantly reduced J_{max} and TPU for the two higher latitudes but the opposite occurred for the two lower latitudes (Figure 3C,E). This divergent reaction led to significantly higher J_{max} for 48°N compared to 58°N (Figure 3C,E).

The $J_{\text{max}}/V_{\text{cmax}}$ ratio was significantly higher in August than in June and July under the elevated [CO₂] and elevated [CO₂] significantly increased $J_{\text{max}}/V_{\text{cmax}}$ in July and August, but not in June (Table 1; Figure 3D). Elevated [CO₂] significantly reduced P_{n400} (photosynthesis measured at ambient [CO₂] in both CO₂ treatments) at 55 and 58°N (Figure 4A and B). While elevated [CO₂] did not have a significant effect on P_{n400} at 48°N, it increased P_{n400} at 52°N (Figure 4A). No significant effects of the experimental factors were observed on [N] content or C:N (Table 1).

FIGURE 2 Effects of [CO₂], photoperiod regime and measurement times on (A) the instantaneous water-use efficiency (IWUE), and (B) internal to ambient [CO₂] ratio (Ci/Ca) in white birch seedlings in the second growing season. The measurements were taken in June, July, and August. Data are presented as mean ± SE (n = 6: 3 seedlings per treatment combination × 2 replications per combination). Different letters indicate a two-way interaction ([CO₂] and photoperiod) for a given measurement time. See Figure 1 for more explanations

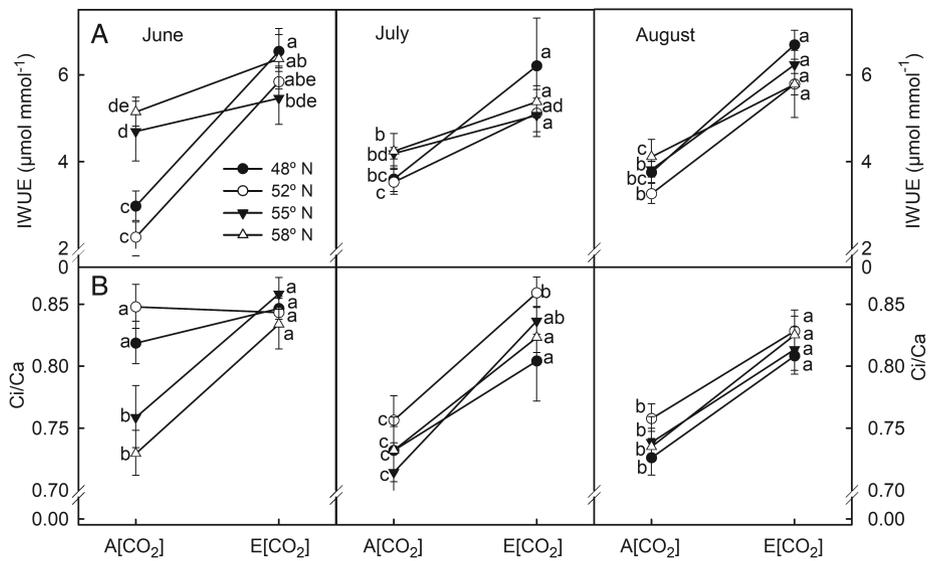
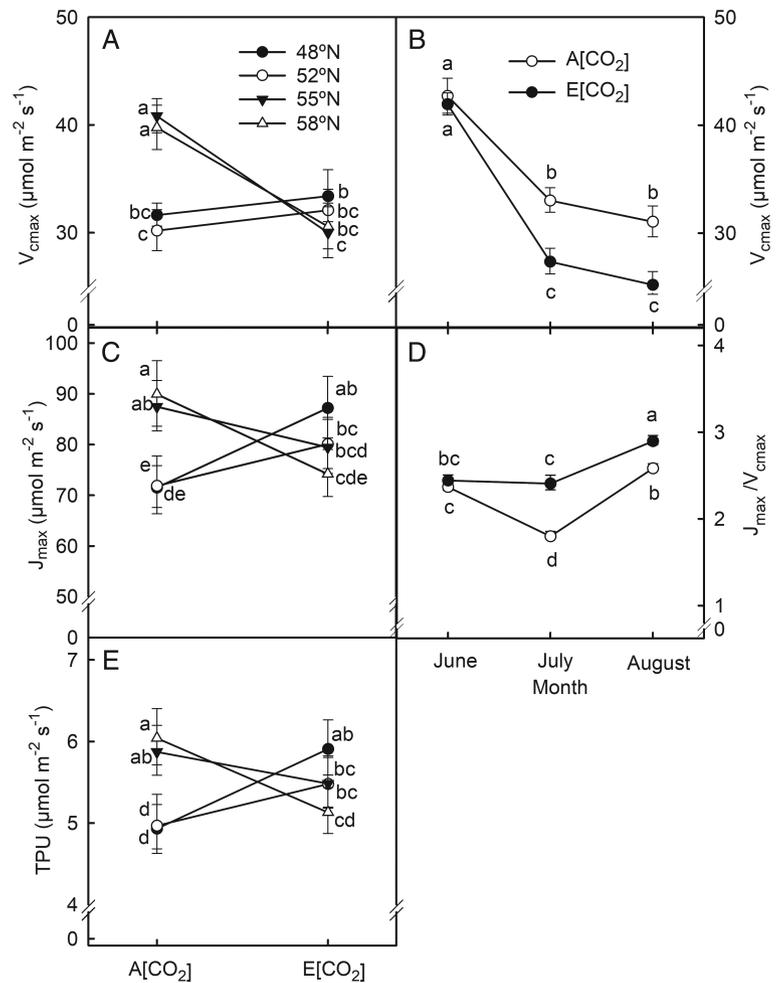


FIGURE 3 Effects of [CO₂] and photoperiod regime on (A) maximum carboxylation rate (V_{cmax}), (C) the light-saturated electron transport rate (J_{max}), and (E) triose phosphate utilization (TPU). The effects of [CO₂] and measurement time on (C) V_{cmax} and (D) the ratio of J_{max} to V_{cmax}. Data are presented as mean ± SE (n = 18 for (A) and (B) as explained in Figure 1A; n = 24 for (C) as explained in Figure 1B). Different letters represent statistically significant difference between treatments. See Figure 1 for more explanations



4 | DISCUSSION

Our results generally support the hypothesis that the stimulation of photosynthesis by elevated [CO₂] would diminish with increases in photoperiod at higher latitudes. In fact, the photosynthesis of white

birch seedlings under the photoperiod regimes associated with the two highest latitudes (55 and 58°N) was not stimulated at all by elevated [CO₂], and the photosynthetic rates under these two photoperiod regimes were lower than those under the photoperiods of the two lower latitudes at elevated [CO₂] (Figure 1A). Photosynthesis

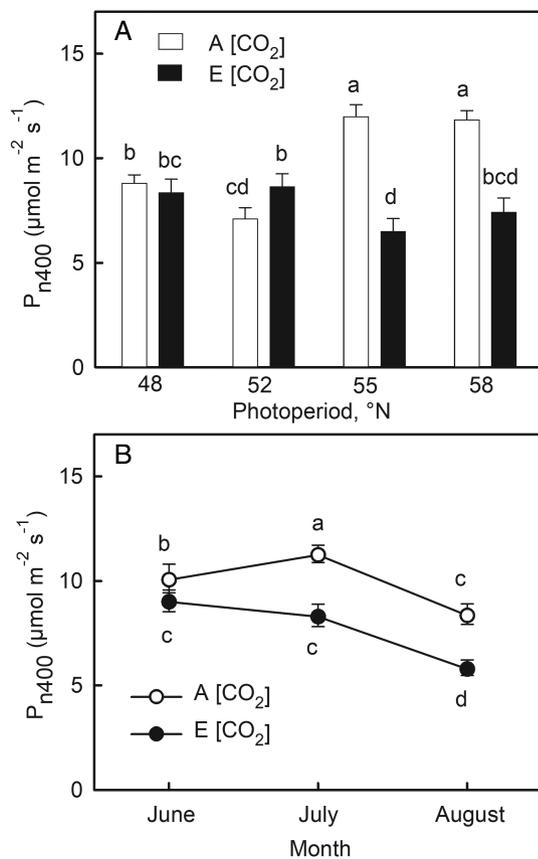


FIGURE 4 Effects of [CO₂] and photoperiod regime (A), and [CO₂] and measurement time (B) on the net photosynthetic rate at ambient [CO₂] (P_{n400}). Data are presented as mean \pm SE ($n = 18$ for A; $n = 24$ for B; see Figure 1 for explanations). Different letters represent statistically significant difference between treatments

under the photoperiod regimes for 48 and 52°N latitude was stimulated by elevated [CO₂] by an average of 91% (Figure 1A). No measurement period effect was observed on P_n response to photoperiod and [CO₂], which is in contrast to reports by 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 (Gunderson & Wullschleger, 1994; Lewis et al., 1996; Long, 1991; Quentin et al., 2015; Tissue et al., 1997). However, seasonal variations in the relative response of photosynthesis to elevated [CO₂] have also been shown to vary with latitude. At a high latitude of 69°N, trees grown in elevated [CO₂] exhibited greater P_n only in early spring and late summer (Beerling & Osborne, 2002; Osborne & Beerling, 2003).

Our results suggest that the lack of P_n stimulation by the elevated [CO₂] for the two higher latitudes resulted from the downregulation of photosynthetic capacity as indicated by the reductions in V_{cmax} and J_{max} (Figure 3A,C,E). In contrast, no such downregulation occurred under the photoperiod regimes associated with the two lower latitudes. Declines in V_{cmax} and J_{max} for trees grown in elevated [CO₂] at mid and high latitudes have been attributed to an insufficient capacity of sink organs and reduction in leaf nitrogen content (Gunderson &

Wullschleger, 1994; Medlyn et al., 1999), which can ultimately reduce the amount of Rubisco (Griffin et al., 2000; Rogers & Ellsworth, 2002; Tissue et al., 1999). However, the downregulation of V_{cmax} and J_{max} in this study was not coupled with a decrease in leaf nitrogen content because foliar [N] was not significantly affected by treatments in this study. Additionally, our results showed that the photochemical efficiency of Photosystem II was not affected by treatments in this study as indicated by the lack of treatment effects on F_v/F_m , suggesting that enhanced CO₂ concentrations and longer photoperiods may have greater impact on the biochemical reactions of photosynthesis. In contrast, there were photosynthetic upregulations in response to elevated [CO₂] under the photoperiod regimes for the two lower latitudes, particularly in J_{max} , which should have contributed to the stimulation of photosynthesis by elevated CO₂ (Figure 3A,C,E).

P_n responded little to high C_i in trees exposed to the elevated [CO₂], e.g. C_i values above 814 $\mu\text{mol mol}^{-1}$ in the longest photoperiod, which was below the operating C_i at the growth [CO₂] (Figure 5). When carbohydrate export from the Calvin–Benson–Bassham cycle cannot keep pace with production, TPU-utilization starts to limit photosynthesis and V_{cmax} and J_{max} are usually reduced to match the TPU capacity (Jensen et al., 1987). Thus, it is possible that photosynthesis at the highest latitude under the elevated [CO₂] was TPU-limited. However, it is difficult to discern the transition from RuBP regeneration limitation to TPU limitation from fitted A/C_i curves (Figure 5) as they usually co-exist (Bernacchi et al., 2013) and we do not have simultaneous PSII photochemical efficiency measurements to determine the transition. 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 leads to a deficient supply of inorganic phosphate for the Calvin–Benson–Bassham cycle, triggering the downregulation of the 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 elevated [CO₂] (Figure 3E). Sage (1994) has reported that declines in starch and sucrose synthesis could reduce the rate of RuBP regeneration because the metabolism of triose phosphate cannot keep up with its production, leading to sink limitation (Foyer & Spencer, 1986). Our previous finding that increases in photoperiod beyond 4° in latitude north of the seed origin do not lead to further increases in growth and biomass production (Tedla et al., 2019) indicates that there is a lack of increased carbohydrate demand for growth under the photoperiods associated with these high latitudes, providing indirect support for the conclusion that feedback inhibition induced downregulation in photosynthesis.

Another interesting finding of this study is that photoperiod treatments had opposite effects on P_n under the two different [CO₂] levels. While P_n was significantly greater in the photoperiod regimes corresponding to the two higher latitudes (55 and 58°N) than those of the two lower latitudes under ambient [CO₂], the trend was the opposite under the elevated [CO₂], but these differences were smaller under the elevated than under the ambient [CO₂] (Figure 1A).

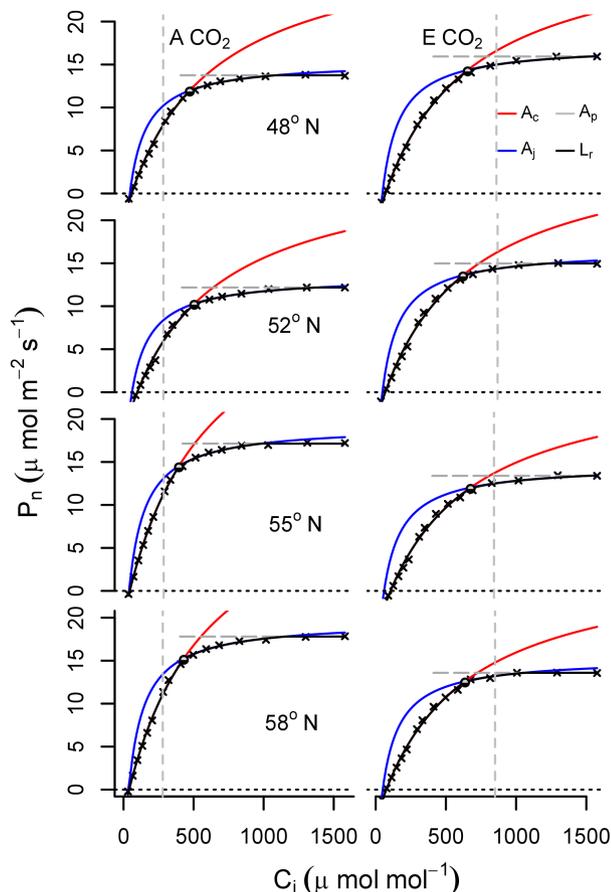


FIGURE 5 Mean A/Ci curves for different combinations of $[\text{CO}_2]$ and photoperiod (averaged over the growing season). Vertical dashed lines indicate the mean C_i value (280 and $850 \mu\text{mol mol}^{-1}$ for AC and EC, respectively) for the measurement of photosynthesis at treatment CO_2 (400 or $1000 \mu\text{mol mol}^{-1}$, respectively for AC and EC). Left panel: Ambient $[\text{CO}_2]$ (A CO_2), right panel: Elevated $[\text{CO}_2]$ (E CO_2). The data are presented as means ($n = 18$). $A_j = J_{\text{max}}$ -limited (blue), $A_c = V_{\text{cmax}}$ -limited (red), A_p = TPU-limited (gray), and L_r = limiting rate (black). The graphs were generated by the fitaci function of the R Plantecophys package (Duursma, 2015)

Furthermore, the response patterns of J_{max} and TPU were similar to those of P_n (Figure 3C,E). 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 a synchronized increase in demand for assimilates is not present, leading to a feedback inhibition of photosynthesis (Gestel et al., 2005; Stutte et al., 1996). Elevated $[\text{CO}_2]$ is known to increase the rate of photosynthesis (Ainsworth & Long, 2004; Leakey et al., 2009) and thus can further aggravate the feedback inhibition under the long photoperiods, which may provide a plausible explanation for the interactive effects of $[\text{CO}_2]$ and photoperiod regimes in this study (Gandin et al., 2011; Körner & Miglietta, 1994; Kuppers et al., 1988).

Elevated $[\text{CO}_2]$ significantly increased the photosynthetic water-use efficiency of white birch in all the photoperiod treatments

(Figure 2A), but the physiological mechanisms responsible for the increase varied with photoperiod. For seedlings grown under the photoperiod regimes of 48 and 52°N latitudes, the increase in IWUE can be attributed largely to the stimulation of photosynthesis by elevated $[\text{CO}_2]$ rather than reduced transpiration due to the reduction of stomatal conductance, which is in contrast to the common conclusion in the literature (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007). However, under the photoperiod regimes at the higher latitudes (55 and 58°N) the increase in IWUE may be primarily a result of the reduction in stomatal conductance, which causes greater reductions in transpiration than in photosynthesis (Lambers et al., 2008). The interactive effects of photoperiod and elevated $[\text{CO}_2]$ are also observed in other species. Llorens et al. (2009) have reported that reductions in transpiration due to reduced stomatal conductance are the primary contributing factor to elevated $[\text{CO}_2]$ induced increase in IWUE in swamp cypress (*Taxodium distichum*) growing under a photoperiod longer than that of the seed origin. In contrast, elevated $[\text{CO}_2]$ increased the IWUE of coastal redwood (*Sequoia sempervirens*) and dawn redwood (*Metasequoia glyptostroboides*) primarily via increasing P_n , which occurs in early spring and late summer when the photoperiod is shorter (Llorens et al., 2009).

In summary, the results of this study suggest that photoperiod regime can substantially affect the photosynthetic responses of boreal white birch to elevated $[\text{CO}_2]$ and such effects are highly relevant to the northward migration or seed transfers of boreal tree species under the scenario of climate change. A successful shift of distribution range for a tree species to higher latitudes in response to climate change will not only depend on their responses to elevated $[\text{CO}_2]$ and change in photoperiod regime but also depend on their ability to respond to changes in other biotic and abiotic factors that are not considered in our study. We used only the current temperatures of the seed origin in this study under the assumption that the migration of the species will be synchronized with the movement of its climate envelope and, therefore, the species will be growing under the same or very similar temperature and precipitation conditions as those in their current location. However, the reality can be very different because the shift of the climate envelope will essentially impose a range of temperature and precipitation onto a new geographic location with different edaphic and biotic conditions in addition to a new photoperiod regime, which can significantly impact the regeneration, survival, and growth of trees. Therefore, the most likely response of trees to the predicted shift of the climate envelope will be a combination of a slower migration than the shift of the climate envelope and acclimation to a warmer temperature and changed precipitations. This study provides insights on the potential effects of elevated $[\text{CO}_2]$ and changes in photoperiod regime on the potential of tree migration. The interactions of those factors with other biotic and abiotic conditions warrant further investigations in order to fully understand the ecophysiological responses and migration potential of trees. Furthermore, the results of this study suggest that photoperiod regimes should be considered in future efforts to evaluate carbon sequestration under climate change-induced northward migration.

ACKNOWLEDGMENTS

The authors thank Ms. Joan Lee and Ms. Keri Pidgen (former and current greenhouse manager, respectively) for their technical support in setting up the greenhouse experiment. The study was supported by NSERC Discovery Grant to Q.-L. Dang (203198-2013-RGPIN) and Lakehead University Graduate Assistantship to B. Tedla. None of the authors have a conflict of interest in the publication of this manuscript.

AUTHOR CONTRIBUTIONS

Binyam Tedla performed the research and participated in all phases of the study. Qing-Lai Dang and Sahari Inoue contributed to experimental design, data interpretation, manuscript writing, and discussion of ideas. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated for this study may be provided upon request.

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REFERENCES

- Ainsworth, E.A. & Long, S.P. (2004) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂: Tansley review. *The New Phytologist*, 165, 351–372.
- Ainsworth, E.A. & Rogers, A. (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions: photosynthesis and stomatal conductance responses to rising [CO₂]. *Plant, Cell and Environment*, 30, 258–270.
- Beerling, D.J. & Osborne, C.P. (2002) Physiological ecology of Mesozoic Polar forests in a high CO₂ environment. *Annals of Botany*, 89, 329–339.
- Benomar, L., Lamhamedi, M.S., Rainville, A., Beaulieu, J., Bousquet, J. & Margolis, H.A. (2016) Genetic adaptation vs. ecophysiological plasticity of photosynthetic-related traits in young *Picea glauca* trees along a regional climatic gradient. *Frontiers in Plant Science*, 7, 48.
- Bernacchi, C.J., Bagley, J.E., Serbin, S.P., Ruiz-Vera, U.M., Rosenthal, D. M. & Vanloocke, A. (2013) Modelling C₃ photosynthesis from the chloroplast to the ecosystem. *Plant, Cell and Environment*, 36, 1641–1657.
- Burns, R.M., Honkala, B.H., Coordinators, T. (1990) Silvics of North America: volume 2. Hardwoods. U S Dep Agric USDA For Serv Agric Handb 654.
- Comstock, J. & Ehleringer, J.R. (1986) Photoperiod and photosynthetic capacity in *Lotus scoparius*. *Plant, Cell and Environment*, 9, 609–612.
- Curtis, P.S. & Wang, X. (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia*, 113, 299–313.
- Dorais, M., Yelle, S. & Gosselin, A. (1996) Influence of extended photoperiod on photosynthate partitioning and export in tomato and pepper plants. *New Zealand Journal of Crop and Horticultural Science*, 24, 29–37.
- Duursma, R. A. (2015) Plantecophys - an r package for analysing and modelling leaf gas exchange data. *PLOS ONE*, 10 (11), e0143346.
- Eamus, D. & Jarvis, P.G. (1989) The direct effects of increase in the global atmospheric CO₂ concentration on natural and commercial temperate trees and forests. In: Begon, M., Fitter, A.H., Ford, E.D. & MacFadyen, A. (Eds.) *Advances in ecological research*. Amsterdam, The Netherlands: Academic Press, pp. 1–55.
- Equiza, M.A., Day, M.E. & Jagels, R. (2006) Physiological responses of three deciduous conifers (*Metasequoia glyptostroboides*, *Taxodium distichum* and *Larix laricina*) to continuous light: adaptive implications for the early Tertiary polar summer. *Tree Physiology*, 26, 353–364.
- Farquhar, G.D., von Caemmerer, S. & Berry, J.A. (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149, 78–90.
- Farrar, J.L. (1995) *Trees in Canada*. Markham, Ontario, Canada: Canadian Forest Service and Fitzhenry and Whiteside Limited.
- Foyer, C. & Spencer, C. (1986) The relationship between phosphate status and photosynthesis in leaves. *Planta*, 167, 369–375.
- Gandin, A., Dizengremel, P. & Lapointe, L. (2011) Photoperiod has a stronger impact than irradiance on the source–sink relationships in the sink-limited species *Erythronium americanum*. *Botany*, 89, 763–770.
- Gestel, N.C.V., Nesbit, A.D., Gordon, E.P., Green, C., Paré, P.W., Thompson, L., et al. (2005) Continuous light may induce photosynthetic downregulation in onion – consequences for growth and biomass partitioning. *Physiologia Plantarum*, 125, 235–246.
- Griffin, K.L., Tissue, D.T., Turnbull, M.H. & Whitehead, D. (2000) The onset of photosynthetic acclimation to elevated CO₂ partial pressure in field-grown *Pinus radiata* D. Don. After 4 years. *Plant, Cell and Environment*, 23, 1089–1098.
- Griffith, T.M. & Watson, M.A. (2006) Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. *The American Naturalist*, 167, 153–164.
- Gunderson, C.A. & Wullschlegel, S.D. (1994) Photosynthetic acclimation in trees to rising atmospheric CO₂: a broader perspective. *Photosynthesis Research*, 39, 369–388.
- Horneck, D.A. & Miller, R.O. (1997) Determination of total nitrogen in plant tissue. In: *Handbook of reference methods for plant analysis*. New York, NY: CRC Press, pp. 85–93.
- Inoue, S., Dang, Q.-L., Man, R. & Tedla, B. (2019) Northward migration of trembling aspen will increase growth but reduce resistance to drought-induced xylem cavitation. *Botany*, 97, 627–638.
- Inoue, S., Dang, Q.-L., Man, R. & Tedla, B. (2020) Photoperiod and CO₂ elevation influence morphological and physiological responses to drought in trembling aspen: implications for climate change-induced migration. *Tree Physiology*, 40, 917–927.
- Jach, M.E., Ceulemans, R., Murray, M.B. (2001) Impacts of greenhouse gases on the phenology of forest trees. Impact Carbon Dioxide Greenh Gases For Ecosyst CAB Int Wallingford, 193–235.
- Jensen, R.G., Raynes, D.A. & Seftor, R.E.B. (1987) Regulation of CO₂ fixation by the ribulose 1, 5-Bisphosphate carboxylase in the chloroplast. In: *Progress in photosynthesis research*. The Netherlands: Springer, pp. 273–279.
- Klockars, A.J. & Sax, G. (1986) Multiple comparisons. In: *Quantitative applications in the social sciences*. Thousand Oaks, CA: SAGE Publications, Inc.
- Körner, C. & Miglietta, F. (1994) Long term effects of naturally elevated CO₂ on mediterranean grassland and forest trees. *Oecologia*, 99, 343–351.
- Kuppers, M., Koch, G. & Mooney, H. (1988) Compensating effects to growth of changes in dry matter allocation in response to variation in photosynthetic characteristics induced by photoperiod, light and nitrogen. *Australian Journal of Plant Physiology*, 15, 287.
- Lambers, H., Chapin, F.S. & Pons, T.L. (2008) *Plant physiological ecology*. New York, NY: Springer.
- Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. & Ort, D.R. (2009) Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany*, 60, 2859–2876.
- Lewis, J.D., Tissue, D.T. & Strain, B.R. (1996) Seasonal response of photosynthesis to elevated CO₂ in loblolly pine (*Pinus taeda* L.) over two growing seasons. *Global Change Biology*, 2, 103–114.

- Li, J., Dang, Q.-L. & Man, R. (2015) Photoperiod and nitrogen supply limit the scope of northward migration and seed transfer of black spruce in a future climate associated with doubled atmospheric CO₂ concentration. *American Journal of Plant Sciences*, 06, 189–200.
- Llorens, L., Osborne, C.P. & Beerling, D.J. (2009) Water-use responses of ‘living fossil’ conifers to CO₂ enrichment in a simulated Cretaceous polar environment. *Annals of Botany*, 104, 179–188.
- Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B. & Ackerly, D.D. (2009) The velocity of climate change. *Nature*, 462, 1052–1055.
- Long, S.P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: has its importance been underestimated? *Plant, Cell and Environment*, 14, 729–739.
- Long, S.P., Ainsworth, E.A., Rogers, A. & Ort, D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology*, 55, 591–628.
- McKenney, D.W., Pedlar, J.H., Lawrence, K., et al. (2007) Potential impacts of climate change on the distribution of North American trees. *Bioscience*, 57, 939–948.
- Medlyn, B.E., Badeck, F.-W., Pury, D.G.G.D., Barton, C.V.M., Broadmeadow, M., Ceulemans, R., et al. (1999) Effects of elevated [CO₂] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant, Cell and Environment*, 22, 1475–1495.
- Newaz, M.S., Dang, Q.-L. & Man, R. (2016) Morphological response of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod. *American Journal of Plant Sciences*, 7, 879.
- Norby, R.J., Wullschlegel, S.D., Gunderson, C.A., Johnson, D.W. & Ceulemans, R. (1999) Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant, Cell and Environment*, 22, 683–714.
- Osborne, C.P. & Beerling, D.J. (2003) The penalty of a long, hot summer. Photosynthetic acclimation to high CO₂ and continuous light in “living fossil” conifers. *Plant Physiology*, 133, 803–812.
- Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., et al. (2014) *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.
- Quentin, A.G., Crous, K.Y., Barton, C.V.M. & Ellsworth, D.S. (2015) Photosynthetic enhancement by elevated CO₂ depends on seasonal temperatures for warmed and non-warmed *Eucalyptus globulus* trees. *Tree Physiology*, 35, 1249–1263.
- Rogers, A. & Ellsworth, D.S. (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO₂ (FACE). *Plant, Cell and Environment*, 25, 851–858.
- Royer, D.L., Osborne, C.P. & Beerling, D.J. (2005) Contrasting seasonal patterns of carbon gain in evergreen and deciduous trees of ancient polar forests. *Paleobiology*, 31, 141–150.
- Sage, R.F. (1994) Photosynthetic response mechanisms to environmental change in C₃ plants. *Plant-Environment Interactions*. New York, NY: Marcel Dekker, 413–499.
- Souther, S., Fetcher, N., Fowler, Z., Shaver, G.R. & McGraw, J.B. (2014) Ecotypic differentiation in photosynthesis and growth of *Eriophorum vaginatum* along a latitudinal gradient in the Arctic tundra. *Botany*, 92, 551–561.
- Stutte, G.W., Yorio, N.C. & Wheeler, R.M. (1996) Interacting effects of photoperiod and photosynthetic photon flux on net carbon assimilation and starch accumulation in potato leaves. *Journal of the American Society for Horticultural Science*, 121, 264–268.
- Tedla, B., Dang, Q.-L. & Inoue, S. (2019) White birch has limited phenotypic plasticity to take advantage of increased photoperiods at higher latitudes north of the seed origin. *Forest Ecology and Management*, 451, 117565.
- Tedla, B., Dang, Q.-L. & Inoue, S. (2020a) 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]. *Environmental and Experimental Botany*, 173, 103982.
- Tedla, B., Dang, Q.-L. & Inoue, S. (2020b) CO₂ elevation and photoperiods north of seed origin change autumn and spring phenology as well as cold hardiness in boreal white birch. *Frontiers in Plant Science*, 11, 506.
- Thomas, B. & Vince-Prue, D. (1996) *Photoperiodism in plants*. Cambridge, MA: Academic Press.
- Tissue, D.T., Griffin, K.L. & Ball, J.T. (1999) Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO₂. *Tree Physiology*, 19, 221–228.
- Tissue, D.T., Thomas, R.B. & Strain, B.R. (1997) Atmospheric CO₂ enrichment increases growth and photosynthesis of *Pinus taeda*: a 4 year experiment in the field. *Plant, Cell and Environment*, 20, 1123–1134.
- Way, D.A. & Montgomery, R.A. (2015) Photoperiod constraints on tree phenology, performance and migration in a warming world: photoperiod limits on tree climate responses. *Plant, Cell and Environment*, 38, 1725–1736.

How to cite this article: Tedla B, Dang Q-L, Inoue S. Longer photoperiods negate the CO₂ stimulation of photosynthesis in *Betula papyrifera* Marsh: Implications to climate change-induced migration. *Physiologia Plantarum*. 2021;172:106–115. <https://doi.org/10.1111/ppl.13298>