Tree Physiology 40, 917–927
doi:10.1093/treephys/tpaa044

Research paper

Photoperiod and CO_2 elevation influence morphological and physiological responses to drought in trembling aspen: implications for climate change-induced migration

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Received August 3, 2019; accepted April 3, 2020; handling Editor Roberto Tognetti

Past research suggests climate change will cause the climate envelopes of various tree species to shift to higher latitudes and can lead to a northward migration of trees. However, the success and scope of the migration are likely affected by factors that are not contained in the climate envelope, such as photoperiod and interactive effects of multiple environmental factors, and these effects are currently not well understood. In this study, we investigated the interactive effects of CO_2 concentrations ([CO_2]), photoperiod and soil moisture on the morphological and physiological traits of *Populus tremuloides* Michx. We grew seedlings under two levels of [CO_2] (ambient [CO_2] (AC) 400 vs elevated [CO_2] (EC) 1000 μmol mol^{-1}), four photoperiod regimes (growing season photoperiods at 48 (seed origin), 52, 55 and 58°N latitude) and two soil moisture regimes (high soil moisture (HSM) vs low soil moisture (LSM), −2 MPa) for two growing seasons in greenhouses. Both morphological and physiological responses were observed. Low soil moisture reduced leaf size, total leaf area and height growth by 33, 46 and 12%, respectively, and increased root/shoot ratio by 20%. The smaller leaf area and increased root/shoot ratio allowed the seedlings in LSM to maintain higher the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport for RuBP regeneration (J_{max}) than control seedlings (55 and 83% higher in July, 52 and 70% in August, respectively). Photoperiod and [CO_2] modified responses to LSM and LSM altered responses to photoperiod and [CO_2], e.g., the August photosynthetic rate was 44% higher in LSM than in HSM under EC but no such a difference existed under AC. The increase in V_{cmax} and J_{max} in response to LSM varied with photoperiod (V_{cmax}: 36% at 52°N, 22% at 55°N, 47% at 58°N; J_{max}: 29% at 52°N, 21% at 55°N, 45% at 58°N). Stomatal conductance and its reduction in response to LSM declined with increasing photoperiod, which can have significant implications for soil moisture effect on northward migration. This study highlights the need to consider the complex interactions of [CO_2], photoperiod and soil moisture when planning assisted migration or predicting the natural migration of boreal forests in the future.

Keywords: [CO_2], gas exchange, morphology, *Populus tremuloides* Michx., soil moisture, tree migration.

Introduction

The increase in atmospheric CO_2 concentration ([CO_2]) and change in water availability associated with increasing temperature and changes in precipitations are expected to substantially alter the performance of trees by affecting their physiology, phenology and growth (Keeling et al. 1996, Myneni et al. 1997, Huang et al. 2007, Delbart et al. 2008). These changes are expected to lead to the northward shift of the climate envelopes for boreal trees (McKenney et al. 2007, 2011). However, northward migration will expose trees to substantial changes in factors that are not associated with climate change such as photoperiod, as well as those that are associated with climate change (Pachauri et al. 2014, Wang et al. 2014). The new combinations of environmental factors can hinder or promote the survival and growth of trees and thus influence their geographic distribution (Thomas and Vince-Prue 1996, Jach et al. 2001). However, the physiological and growth responses of boreal tree species to these potential changes are not well understood.

If migrating to a higher latitude, the trees may need to acclimate to the environmental conditions physiologically and/or morphologically (Lafleur et al. 2010). For instance, the predicted more frequent and longer duration water stress in many regions of North America (Pachauri et al. 2014) may hamper the establishment of tree seedlings during the migration if the trees are unable to acclimate. Higher [CO_2], on the other hand, may lower the impact of water stress via increasing water-use efficiency and reducing the impact of drought on photosynthesis (Robredo et al. 2007). In addition, plants exposed to elevated [CO_2] tend to allocate a greater proportion of biomass to roots and hence have better access to soil moisture (Stulen and Den Hertog 1993, Saxe et al. 1998). Since the photoperiod is longer in the growing season at higher latitudes, the impact of low soil moisture (LSM) may be even greater because of the extended period of transpirational water loss. However, it is unknown whether the negative impact of longer photoperiod under drought stress can be compensated by the positive effect of [CO_2] elevation.

Photoperiod can regulate growth and phenology in photoperiod-sensitive plant species (Nelson et al. 2010). Long photoperiods can stimulate plant growth (Oleksyn et al. 1992, Johnsen and Seiler 1996) and increase resource allocation to aboveground organs (Thomas and Vince-Prue 1996). It has also been reported that longer photoperiods alter the rate of photosynthesis (Comstock and Ehleringer 1986). Photoperiod is determined by latitude and does not change with climate change, while moisture availability and other environmental factors are affected by climate change (Lafleur et al. 2010, Pachauri et al. 2014). However, photoperiod can modify plant responses to other environmental factors. Stomatal regulations can be used in response to short-term fluctuations in moisture (Passioura 1996); leaves developed under drought conditions may have modified structure/anatomy, resource allocation and photosynthetic capacity (Panković et al. 1999, Kitao et al. 2003, Aranda et al. 2005, Kitaoka and Koike 2005). Plants exposed to moisture stress generally allocate more biomass to roots to better access soil water (Poorter et al. 2012, Hertel et al. 2013). However, longer photoperiods can have opposite effects, i.e., reducing biomass allocation to roots (Thomas and Vince-Prue 1996). Since biomass allocation represents a trade-off between above- and belowground organs (Poorter and Nagel 2000, Craine 2009), an interesting question is how photoperiod and moisture stress will interactively affect biomass allocation, particularly under the higher [CO_2] in the future. Further, since higher [CO_2] generally increases photosynthetic carbon fixation in C_3 plants (Jach et al. 2001), elevated [CO_2] is expected to further increase biomass allocation to roots under water stress and could influence the interactive effect of photoperiod and moisture stress. A good understanding of the responses in the physiology, growth, biomass and biomass allocation of tree seedlings to simultaneous changes in photoperiod, water and CO_2 elevation is particularly important for understanding the potential performance of trees under the scenario of climate change-induced northward migration or seed transfer in the projected future conditions.

Trembling aspen (*Populus tremuloides* Michx.) is the most abundant deciduous tree species in Canada's boreal forest (Perala 1990, Hogg et al. 2002) and a commercially important source of wood. The species also has great cultural values for indigenous people and is an important species for wildlife and biodiversity. The decline and dieback of trembling aspen have been observed across large areas of west-central Canada due to recent severe drought spell coupled with other stresses such as insect outbreaks and pathogens (Hogg et al. 2008, Marchetti et al. 2011, Worrall et al. 2013). The fact that trembling aspen is very sensitive to water availability and the boreal region is predicted to be drier in the future (Seager et al. 2014, Sherwood and Fu 2014, Wang et al. 2014) suggests that managing trembling aspen will be more challenging in the future. In this study, we investigated whether longer photoperiods would exacerbate the negative effect of moisture stress on the physiology and growth of trembling aspen and whether [CO_2] elevation could ameliorate the negative effect of moisture stress and/or moderate the effect of longer photoperiod on biomass partitioning. We also attempted to determine what would be the primary mechanism or mechanisms responsible for the response or acclimation of the species to the simultaneous changes in these three factors.

Materials and methods

Plant materials

We collected seed catkins from 10 free-pollinated trembling aspen (*P. tremuloides* Michx.) in the Thunder Bay region on 9 June 2016 (48.38°N, 89.25°W). The catkins were air-dried at room temperature for 5 days, and seeds were extracted using the method of Moench (1999). All the seeds were mixed and stored in a sealed glass bottle at −4 °C.

The seeds were sown in horticultural trays filled with a 1:1 (v/v) mixture of peat moss and vermiculite in the greenhouse at the Thunder Bay campus of Lakehead University. The environmental conditions in the greenhouse were 24/14 (±2) °C (day/night) temperature and 16-h photoperiod. The natural photoperiod was extended to 16 h by using high-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada). All trays were well-watered. Eighteen days following the completion of germination, the seedlings were individually transplanted to circular pots (12 cm tall and 12 cm top and 9.5 cm bottom diameter) filled with peat moss and vermiculite (7:3 v/v). The seedlings were transplanted into larger-sized pots (18 cm tall and 16 cm top and 14 cm bottom diameter) in the second growing season to accommodate the increased need of the larger trees (Poorter et al. 2012, Kawaletz et al. 2014) and to minimize pot limitation.

Experiment design

The experiment was conducted in four greenhouses. The treatments comprised two levels of [CO_2] (ambient [CO2] (AC) 400 vs elevated [CO2] (EC) 1000 μmol mol^{-1}); four photoperiod regimes corresponding to 48°N (seed origin), 52, 55 and 58°N latitude and two soil moisture regimes (high vs low). The experiment design was a split-split plot design with CO_2 as the main plot, photoperiod as sub-plot and soil moisture as sub-sub-plot. Each CO_2 treatment was randomly assigned to two of the four greenhouses (i.e., two replications). The photoperiod treatment was nested within CO_2 treatment and soil moisture nested within the photoperiod. There were three seedlings per treatment combination (total: 3 × 2 (moisture) × 4 (photoperiod) × 2 (CO_2) × 2 (CO_2 replicates) = 96).

Treatments and the greenhouse environment controls

The environmental condition of each greenhouse was individually controlled using the Argus Titan Environment Control System (Argus Controls Systems Ltd, Surrey, BC, Canada). The CO_2 elevation was achieved with the model GEN2E gas CO_2 generators (Custom Automated Products Inc., Riverside, CA, USA). The photoperiod of each greenhouse was set to the longest photoperiod of the four treatments and shorter photoperiods were attained by shading with neutral density shading cloth. Wooden frames were installed to facilitate the complete shading of treatment so that the seedlings within the treatment were not affected by external light. High-pressure sodium lamps (Model LR48877, P.L. Systems) were used to extend the natural photoperiod and to supplement the sunlight on cloudy days. The photoperiod setting was adjusted weekly based on the calculated photoperiod for each of the four latitudes. The volumetric water content (VWC) of the growing medium was maintained between 46 and 58% (field capacity = 77%) for the high soil moisture (HSM) treatment and between 10 and 15% for the LSM treatment. Before starting the experiment, we conducted a preliminary study to determine the appropriate soil moisture content for the treatments, in which the growing medium was let dry to a range of VWC and the midday water potential of seedlings was measured. The average midday water potential corresponding to the VWC of 10–15% (i.e., LSM) was −2.0 MP, which is considered as moderate drought for trembling. Cai and Tyree (2010) have reported that a water potential of −2.25 MPa will lead to a 50% loss of hydraulic conductivity in aspen seedlings. We also determined that adding 100 ml water to the container would bring the VWC from 46 to 58% and adding 40 ml water would bring the water content from 10 to 15%. During that climate the experiment, we monitored the VWC daily using a Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). When the measurement reached around 46% in the HSM treatment, 100 ml water was added to each container. When the measurement in the LSM treatment was around 10%, 40 ml water was added.

The experiment was run for two growing seasons. The environmental conditions in the greenhouses were set to emulate the average weekly natural conditions of June 7 to November 20 in the first growing season and those of April 26 to November 7 in the second growing season. The reason for not starting and ending the two growing seasons on the same emulated dates was that the first growing season was started with new germinants but the second season was started with dormant seedlings. The dates referred to hereafter are the emulated natural dates. Temperature adjustments were synchronized with photoperiod based on the weekly averages of hourly temperatures in the past 10 years at each latitude (Environment Canada records for Thunder Bay, ON). The daily temperature in each greenhouse was ramped at 6-h set points. The seedlings were fertilized with a fertilizer solution of 33 mg N l^{-1}, 73 mg P l^{-1} and 55 mg K l^{-1} during the establishment phase (June 7–21 in the first growing season and April 26–May 25 in the second growing season); 100 mg N l^{-1}, 60 mg P l^{-1}, 150 mg K l^{-1}, 80 mg Ca l^{-1}, 40 mg Mg l^{-1} and 60 mg S l^{-1} during the fast-growing phase (June 22–August 31 in and May 26–August 31 for the growing seasons); and of 33 mg N l^{-1}, 44 mg P l^{-1} and 83 mg K l^{-1} during the hardening phase (September 1–25) Landis (1989).

At the end of the first growing season, the seedlings (in pots) were stored in walk-in cold storage at −4 °C in the dark. The seedlings were covered with plastic sheets to avoid desiccation during the storage. To start the second growing season, the seedlings were transplanted into larger pots (18 cm in height and 16 cm top and 14 cm bottom diameter) and thawed gradually under the emulated spring conditions in the greenhouses as described previously.

Foliar gas exchange measurements and chlorophyll fluorescence

Foliar gas exchange was measured using a PP-Systems CIRAS-3 open gas exchange system with PLC3 Universal Leaf Cuvette (PP Systems, Amesbury, MA, USA) in July (12–22) and again in August (9–19) of the second growing season. A healthy and fully expanded leaf (8th or 9th from the top) was measured for three randomly selected seedlings from each treatment-replicate combination under the following conditions: 22 °C air temperature, 50% relative humidity, 800 μmol m^{-2} s^{-1} photosynthetically active radiation and sequentially 400, 300, 250, 200, 150, 100, 50, 400, 450, 550, 650, 750, 850, 1000, 1200, 1500 and 1800 μmol mol^{-1} CO_2. The measurements were carried between 8:00 a.m. and 3:00 p.m. when our tests showed relatively stable gas exchange measurements. The net photosynthetic rate at growth [CO_2] (P_n) and P_n at the ambient [CO_2] (P_{n400}) were extracted the A/C_i data for each sample tree. Stomatal conductance (g_s), transpiration rate (E), instantaneous water-use efficiency (iWUE) and intercellular/ambient [CO_2] ratio at the corresponding growth [CO_2] were also extracted. Seedlings used for gas exchange measurements were picked randomly at a given time to distribute the effect of measurement timing across samples.

Chlorophyll fluorescence was measured in August of the second growing season using a Hansatech FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd, Norfolk, UK) on the same leaf of which the gas exchange was measured. The leaves were dark-adapted for at least 40 min for the measurement. The potential maximum quantum efficiency of photosystem II (PSII) was determined as $(F_m - F_o)/F_m$.

Growth and biomass allocation

The height and root collar diameter (RCD) of the seedlings for the physiological measurements were measured at the end of the second growing season. The sample trees were then harvested and separated into leaves, stem and roots. The total leaf area and the number of leaves per tree were determined using the Regent Instruments WinFolia system (Quebec, Canada). Leaf, stem and root dry masses were determined after being oven-dried at 80 °C for 48 h. The following variables were determined: specific leaf area (SLA = total leaf area/total leaf dry mass), leaf area ratio (LAR = total leaf area/total seedling dry mass), leaf mass ratio (LMR = leaf mass/seedling dry mass), stem mass ratio (SMR = stem dry mass/total seedling dry mass), root mass ratio (RMR = root dry mass/total seedling dry mass) and root-to-shoot ratio (RSR = root dry mass/total leaf and stem dry mass).

A/C_i curve fitting

The A/C_i data were analyzed using the 'fitaci' function of the 'plantecophys' package (Duursma 2015) on the R software package 3.5.0 (R Development Core Team 2018). The 'default' fitting method was used to estimate the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport for RuBP regeneration under saturating light (J_{max}) for each seedling.

Data analysis

The data were examined graphically for the normality of distribution (probability plots of residuals) and homogeneity of variance (scatter plots) using the R software (Version 3.5.0, R Development Core Team 2018) before being subjected to the analysis of variance (ANOVA). The effects of [CO_2], photoperiod and soil moisture were considered as 'fixed effect' in the ANOVA. An effect was considered significant if $P \leq 0.05$ or marginally significant if $P \leq 0.1$. When ANOVA showed a significant ($P \leq 0.05$) photoperiod effect or a significant interaction, the Fisher's least significant difference post hoc test was used to compare individual means. All the analyses were performed using the R 3.5.0 (R Development Core Team 2018).

Results

Growth and biomass allocation

Seedling height generally increased with increases in photoperiod although the difference was only statistically significant between the shortest (seed origin) and the longest photoperiod (10°N of the seed origin) (Table 1 and Figure 1a). The LSM reduced the height growth, RCD, leaf size and total leaf area by 32.5, 32.5, 49.6 and 86.3%, respectively (Figure 1a–d). Low soil moisture reduced the leaf, stem, root and total biomass by 95, 101.3, 63.6 and 87.8%, respectively (Table 1 and Figure 2a). However, the reduction of root biomass by LSM was smaller under EC than AC (39 vs 96.1%, Figure 2a). In relative terms, LSM reduced biomass allocation to stem (SMR) but increased the allocation to roots (RMR), leading to a higher root–shoot ratio (RSR) (Table 2 and Figure 2b).

Foliar gas exchange and chlorophyll fluorescence

The photoperiod treatment did not significantly affect the net photosynthetic rate (P_n) measured at treatment [CO_2] in either measurement, whereas the EC increased the August measurement of P_n only in the LSM treatment. Interestingly, the LSM treatment significantly increased P_n in both [CO_2] treatments in July but only in EC in August (Table 2 and Figure 3a). However, there were no significant interactive effects of soil moisture and photoperiod or of [CO_2], photoperiod and soil moisture on any of the gas exchange parameters (Table 2). The LSM significantly reduced stomatal conductance (g_s) and transpiration rate (E) in all photoperiod and [CO_2] treatments in July but the magnitude of the effect on g_s declined with increasing photoperiod in August (Table 2, Figure 3b and c). It is interesting to note that g_s showed a general decreasing trend with increasing growing season photoperiod or latitude (from 48 to 58°N) in seedlings grown under the HSM, but such a trend did not exist in seedlings grown under the LSM (Figure 3b). The LSM increased iWUE in both [CO_2] treatments, but the effect was greater under EC (Figure 3d). The EC increased iWUE in both soil moisture treatments in July, but the effect was not significant in the HSM in August (Table 2 and Figure 3d). The LSM reduced the ratio of internal to ambient [CO_2] (C_i/C_a) in all photoperiod and [CO_2] treatments (Table 2 and Figure 3e).

Table 1. ANOVA results (*P* values) for the effects of [CO$_2$] (C), photoperiod (P), soil moisture (SM) and their interactions on height, RCD, leaf size, total leaf area (total LA), SLA, leaf biomass (leaf DW), stem biomass (stem DW), root biomass (root DW), LMR, shoot mass ratio (SMR), RMR and shoot root ratio (SRR). The seedlings were exposed to two levels of [CO$_2$] (400 vs 1000 μmol mol^{-1}), four photoperiod regimes (at 48 (seed origin), 52, 55 and 58°N) and two soil moisture regimes (high vs low). The numbers in bold are significant at <0.05.

Variable	C (DF = 1)	P (DF = 3)	SM (DF = 1)	C × P (DF = 3)	C × SM (DF = 1)	P × SM (DF = 3)	C × P × SM (DF = 3)
Height	0.680	**0.043**	<**0.001**	0.438	0.262	0.662	0.553
RCD	0.266	0.923	<**0.001**	0.959	0.631	0.940	0.545
Leaf size	0.711	0.191	<**0.001**	0.127	0.059	0.885	0.232
Total LA	0.463	0.159	<**0.001**	0.283	0.115	0.475	0.511
SLA	0.250	0.240	0.552	0.392	0.122	0.275	0.452
Leaf DW	0.295	0.186	<**0.001**	0.269	0.826	0.314	0.808
Stem DW	0.399	0.694	<**0.001**	0.408	0.239	0.793	0.102
Root DW	0.549	0.639	<**0.001**	0.173	**0.043**	0.999	0.204
Total DW	0.435	0.875	<**0.001**	0.275	0.189	0.805	0.168
LMR	0.888	0.184	0.368	0.352	0.073	0.078	0.062
SMR	0.847	0.149	**0.007**	0.892	0.751	0.493	0.180
RMR	0.998	0.118	**0.024**	0.096	0.376	0.773	0.815
RSR	0.966	0.150	**0.025**	0.107	0.391	0.776	0.827

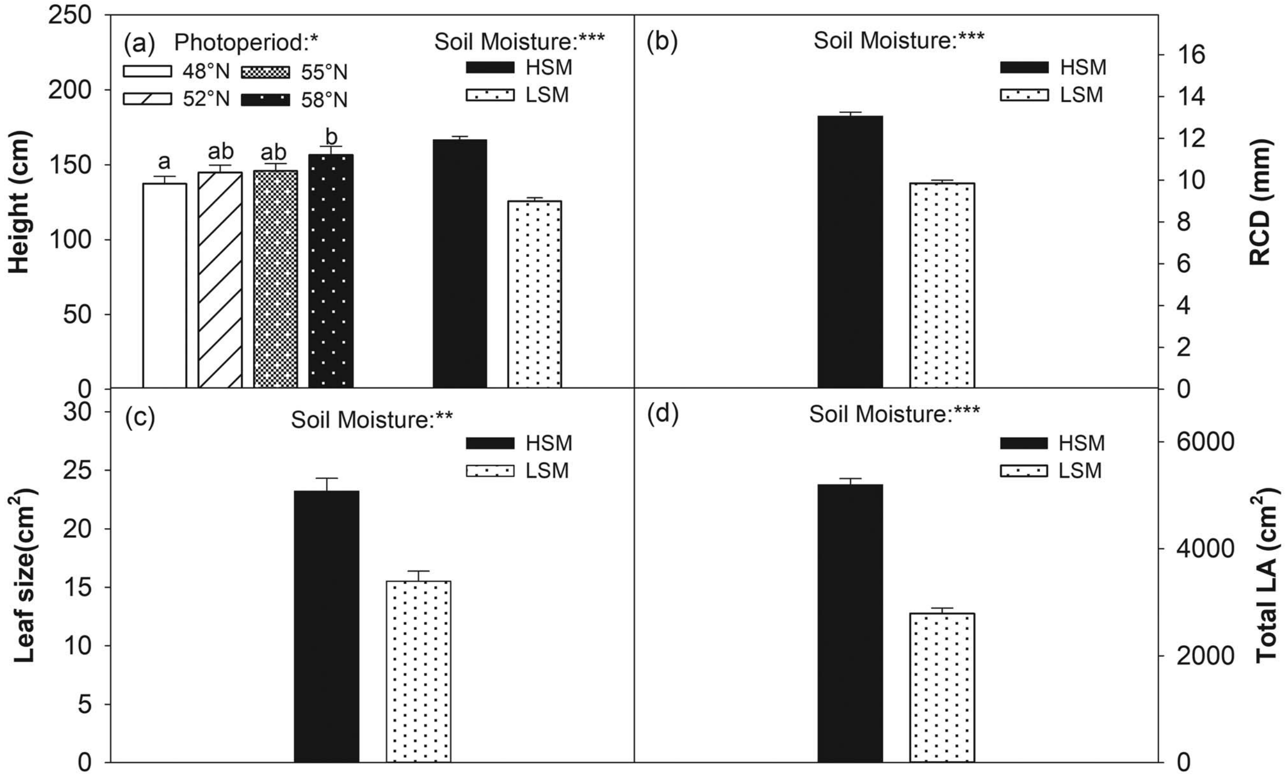

Figure 1. Mean (+SE) height growth (a), RCD (b), leaf size (c) and total LA (d) of trembling aspen seedlings grown under two levels of [CO$_2$] (ambient vs elevated), four photoperiod regimes (at 48 (seed origin), 52, 55 and 58°N) and two levels of soil moisture (high vs low) for two growing seasons. Data are pooled across [CO$_2$] and soil moisture for (a)-left ($N = 24$: three seedlings with two replications per treatment, two levels of [CO$_2$] and two levels of soil moisture, $3 \times 2 \times 2 \times 2 = 24$), and [CO$_2$] and photoperiod for (a)-right, (b), (c) and (d) ($N = 48$: three seedlings with two replications per treatment, two levels of [CO$_2$] and four levels of photoperiod, $3 \times 2 \times 2 \times 4 = 48$), because the three-way interaction was not statistically significant. Means with different letters in (a) are significantly different from each other based on Fisher's least significant difference post hoc test ($P < 0.05$). Significance levels for each effect: *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ are indicated on the top.

The LSM significantly increased the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport for RuBP regeneration (J_{max}) in both July and August, but the magnitude of increase varied with photoperiod or latitude in August (Table 2, Figure 4a and b). The August measurements of V_{cmax} and J_{max} showed a general increasing trend with increasing latitude or growing-season photoperiod in seedlings grown under the LSM, but no such a trend was revealed in

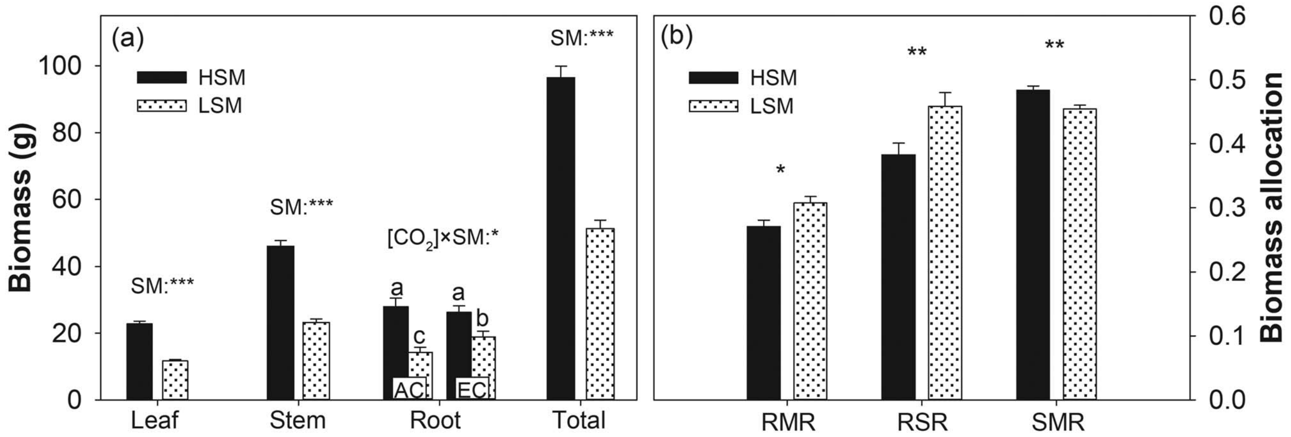

Figure 2. Mean (+SE) biomass (leaf, stem, root and total) (a) and biomass allocation (RMR, RSR and SMR) (b) of trembling aspen seedlings grown under two levels of $[CO_2]$ (ambient vs elevated) and two levels of soil moisture (high vs low) for two growing seasons. Data are pooled across $[CO_2]$ and photoperiod for (a)—except root biomass and (b) ($N = 48$ (see Figure 1 for explanations)), and data for root biomass in (a) are pooled across photoperiod ($N = 24$, three seedlings with two replications per treatment, four levels of photoperiod, $3 \times 2 \times 4 = 24$). The lower case letters indicate the interactive effect of $[CO_2]$ and soil moisture (SM). Means with different letters are significantly different from each other based on Fisher's least significant difference post hoc test ($P < 0.05$). Significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. ANOVA results (P values) for the effects of $[CO_2]$ (C), photoperiod (P), soil moisture (SM) and their interactions on net photosynthesis at growth condition (P_n) and ambient $[CO_2]$ (P_{n400}), stomatal conductance (g_s), transpiration rate (E), iWUE, maximal rate of Rubisco carboxylation (V_{cmax}), maximal RuBP regeneration rate (J_{max}), internal to ambient CO_2 concentration ratio (C_i/C_a) and the maximum quantum efficiency of PSII (F_v/F_m). The seedlings were exposed to two levels of $[CO_2]$ (400 and 1000 μmol mol^{-1}), four photoperiod regimes (at 48.38°N (seed origin), 51.68, 54.98 and 58.28°N) and two soil moisture regimes (HSM (75–45%v) and LSM (20%v–10%)). The numbers in bold are significant at <0.05.

Variable	Month	C (DF = 1)	P (DF = 3)	SM (DF = 1)	C × P (DF = 3)	C × SM (DF = 1)	P × SM (DF = 3)	C × P × SM (DF = 3)
P_n	July	0.171	0.817	**0.005**	0.458	0.305	0.404	0.991
	August	**0.014**	0.213	**0.002**	0.357	**0.001**	0.113	0.399
g_s	July	0.806	0.341	**0.001**	0.932	0.687	0.242	0.922
	August	0.495	0.343	<**0.001**	0.918	0.169	**0.046**	0.311
E	July	0.455	0.452	<**0.001**	0.821	0.496	0.357	0.976
	August	0.401	0.187	<**0.001**	0.311	0.880	0.271	0.687
iWUE	July	**0.002**	0.849	<**0.001**	0.438	**0.017**	0.063	0.664
	August	**0.075**	0.799	<**0.001**	0.604	**0.001**	0.677	0.991
V_{cmax}	July	0.792	0.368	<**0.001**	0.317	0.735	0.444	0.777
	August	0.092	**0.022**	<**0.001**	0.083	0.342	**0.004**	0.051
J_{max}	July	0.613	0.552	<**0.001**	0.448	0.835	0.304	0.608
	August	0.847	0.315	<**0.001**	0.302	0.577	**0.043**	0.289
J_{max}/V_{cmax}	July	0.201	0.410	<**0.001**	0.117	0.272	0.486	0.505
	August	0.137	0.186	**0.030**	0.328	**0.022**	0.761	0.551
P_{n400}	July	0.155	0.860	0.259	0.623	0.110	0.494	0.948
	August	0.060	**0.013**	0.265	0.176	0.120	**0.043**	0.213
C_i/C_a	July	0.138	0.799	<**0.001**	0.582	0.138	0.597	0.488
	August	0.114	0.513	<**0.001**	0.240	0.286	0.318	0.498
F_v/F_m	August	0.648	**0.015**	<**0.001**	**0.003**	0.168	0.258	0.988

seedlings grown under the HSM or in the July measurement of either moisture treatment (Figure 4a and b). However, the difference in V_{cmax} and J_{max} between two adjacent photoperiods was not always statistically significant (Figure 4). The LSM significantly increased J_{max}/V_{cmax} in July in both $[CO_2]$ treatments but only in the AC in the August measurement (Figure 4c). The LSM significantly decreased the net photosynthetic rate measured at the ambient $[CO_2]$ (P_{n400}) only in the photoperiod of seed origin in August, but no significant difference was found between soil moisture treatments in the photoperiods of higher latitudes (Figure 4d). Moreover, the August measurement of P_{n400} was marginally lower in the EC than AC, indicating a photosynthetic downregulation in response to the CO_2 elevation (Table 2). The LSM significantly increased the quantum efficiency of PSII (F_v/F_m) (Table 2 and Figure 5). The EC significantly reduced F_v/F_m in the photoperiod regimes

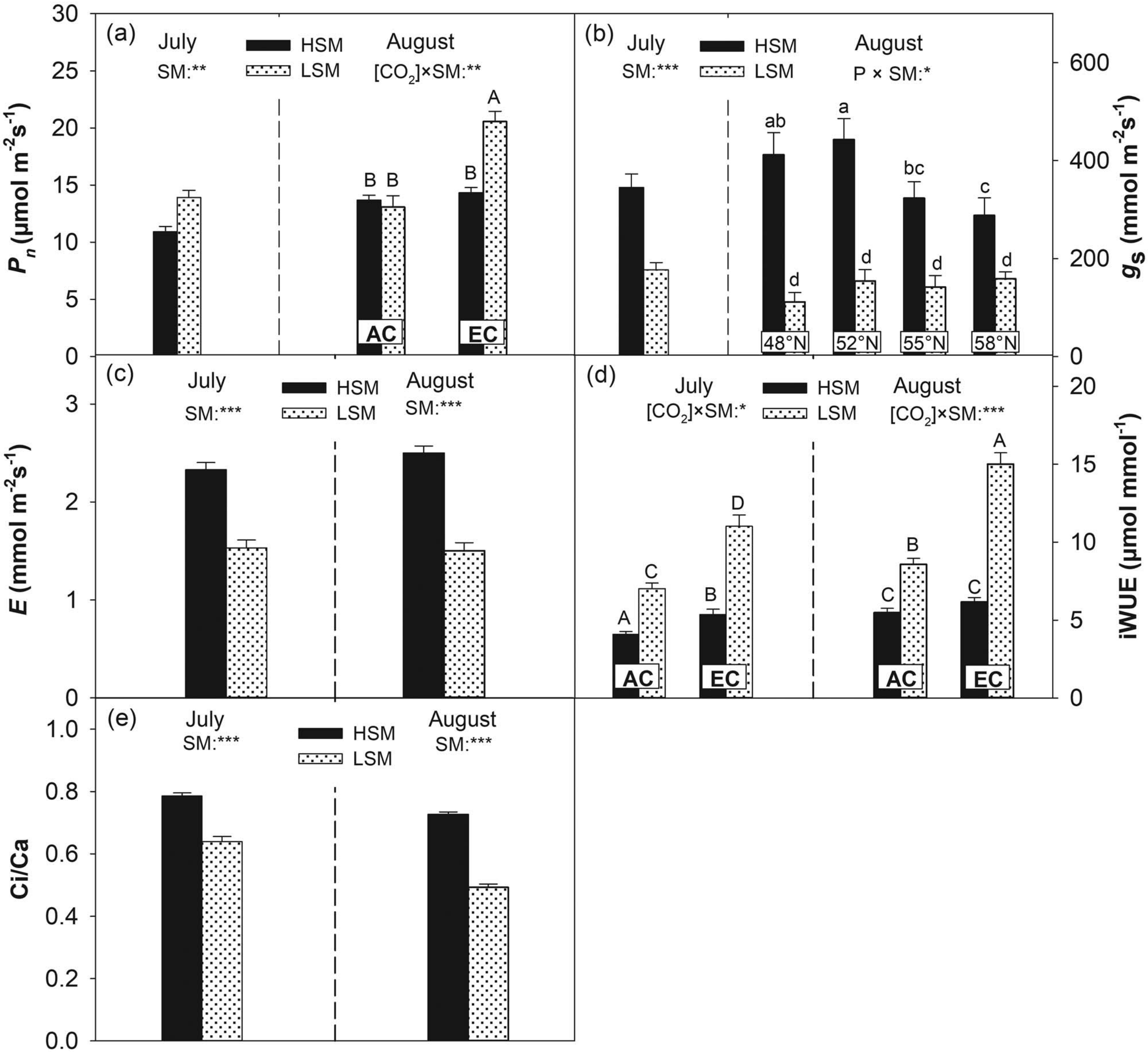

Figure 3. Mean (+SE) in situ rate of net photosynthesis (P_n) (a), stomatal conductance (g_s) (b), transpiration rate (E) (c), iWUE (d) and C_i/C_a of trembling aspen seedlings grown under two levels of [CO_2] (ambient vs elevated), four photoperiod regimes (at 48 (seed origin), 52, 55 and 58°N) and two levels of soil moisture (high vs low). Data are pooled across [CO_2] and photoperiod for (a)-left, (b)-left, (c), (e) ($N = 48$ (see Figure 1 for explanations)); photoperiod for (a)-right, (d) ($N = 24$ (see Figure 2 for explanations)) and [CO_2] for (b)-right ($N = 12$: three seedlings with two replications per treatment, and two levels of [CO_2], $3 \times 2 \times 2 = 12$). The measurements were carried out in July (left panel) and August (right panel). The lower case letters indicate the interactive effect of photoperiod (P) × soil moisture (SM) and the upper case letters indicate the interactive effect of [CO_2] × soil moisture (SM). Means with different letters are significantly different from each other based on Fisher's least significant difference post hoc test ($P < 0.05$). Significance levels: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

associated with the two lower latitudes but did not significantly affect it in the photoperiod regimes of the two higher latitudes (Figure 5). However, there were no clear trends in the response of F_v/F_m to the photoperiod regime (Figure 5).

Discussion

Photoperiod effect on growth

CO_2 concentration, photoperiod and their interactions were generally not a significant source of variation for most of the growth and biomass variables. The lack of treatment effect on those variables was similar to the results of some other studies where trees were exposed to elevated [CO_2] under different photoperiods (Johnsen and Seiler 1996, Newaz et al. 2016, 2017). However, the height growth of trembling aspen seedlings generally increased with increasing photoperiod. This result is in agreement with the findings of some other studies (Oleksyn et al. 1992, Johnsen and Seiler 1996). The higher height growth at longer photoperiod, particularly the longest photoperiod at 58°N latitude, was likely attributable to the

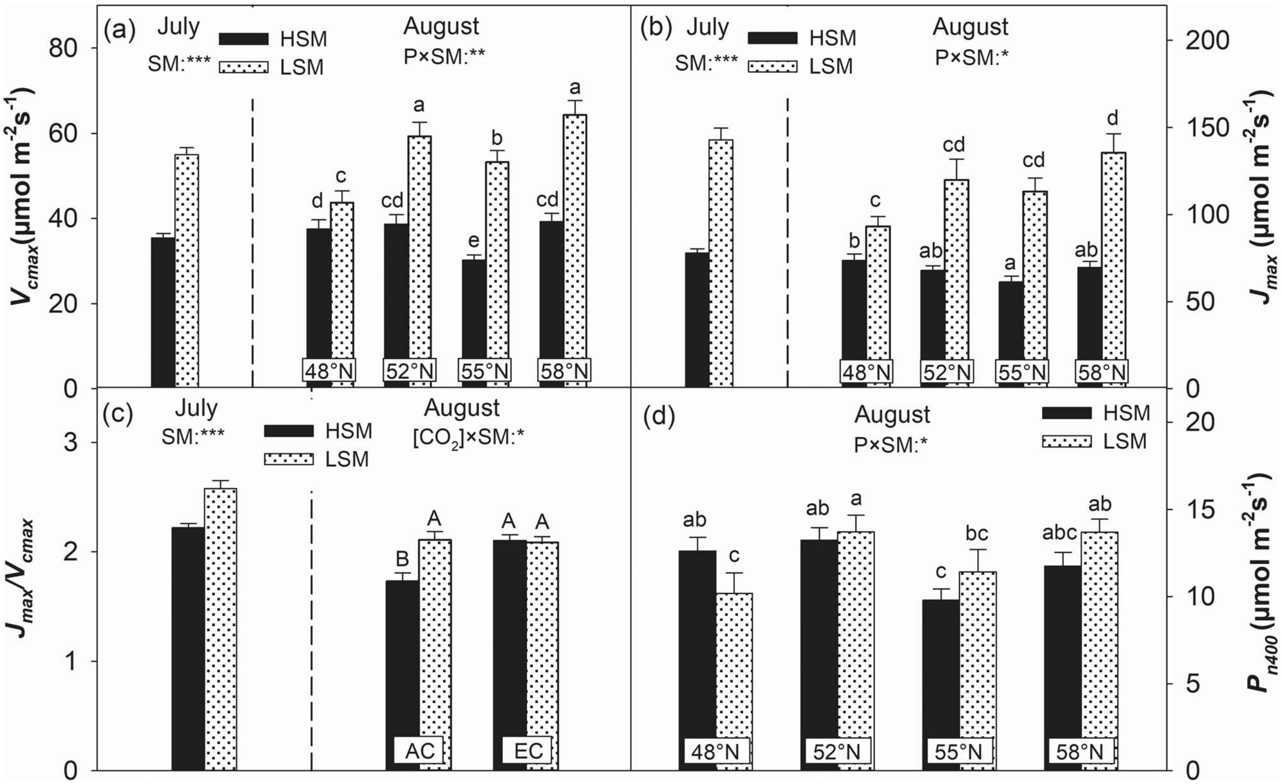

Figure 4. Mean (+SE) maximum rate of Rubisco carboxylation (V_{cmax}) (a), maximum rate of electron transport for RuBP regeneration (J_{max}) (b), $J_{\mathrm{max}}/V_{\mathrm{cmax}}$ ratio (c) and net photosynthetic rate at ambient [CO_2] (P_{n400}) (d) in trembling aspen seedlings grown under two levels of [CO_2] (ambient vs elevated), four photoperiod regimes (at 48 (seed origin), 52, 55 and 58°N) and two levels of soil moisture (high vs low), measured in July (left panel) and August (right panel). Data are pooled across [CO_2] and photoperiod for (a)-left, (b)-left, (c)-left ($N = 48$ (see Figure 1 for explanations)); [CO_2] for (a)-right (b)-right ($N = 12$ (see Figure 3 for explanations)); photoperiod for (c-right) ($N = 24$ (see Figure 1 for explanations)) and [CO_2] for (b)-right and (d)-right ($N = 12$ (see Figure 3 for explanations)). The lower case letters indicate the interactive effect of photoperiod × soil moisture (SM) and the upper case letters indicate the interactive effect of [CO_2] × of soil moisture. Means with different letters are significantly different from each other based on Fisher's least significant difference post hoc test ($P < 0.05$). Significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

longer periods of photosynthesis as well as longer growing season due to delays in growth cessation (Farmer 1993). The longer hours of photosynthesis and increased efficiency of light energy conversion into biomass under a longer photoperiod regime have been reported as major contributors to the increased growth of white spruce (*Picea glauca* (Moench) Voss) (Stinziano et al. 2017). Indeed, the cessation of height growth occurred later in the longer photoperiod regime of 58°N latitude than the shorter photoperiod regimes in this study (S. Inoue, Q-L. Dang, R. Man, B. Tedla, unpublished). The rate of height growth is an important determinant for the survival of pioneer species and frequently used as an indicator of fitness (Ying and Yanchuk 2006). A higher rate of height growth during the juvenile stage would give a species advantages in its competition for resources, particularly light (Ying et al. 1985, Simard 1992). Trembling aspen is a fast-growing species that is very intolerant of shade (Burns et al. 1990) and, having a faster rate of height growth than its competition species, may be critical for winning the competition and surviving at new locations if it migrates. However, the stimulation of height growth by longer photoperiods may diminish as the canopy of the young forest closes (Merritt and Kohl 1982, 1983, Burdett and Yamamoto 1986). But this should not reduce the significance of the effect on dominant trees because they are not shaded by neighboring trees, and they are the future of the forest stand. However, it is unknown whether the photoperiod effects on height growth will decline as trees get older or bigger. Koch and Fredeen (2005) have pointed out that increased height growth may increase trees' vulnerability to water stress because hydraulic resistance for water transport from roots to leaf increases with tree height.

Interactive effect of soil moisture, CO_2 and photoperiod on photosynthetic capacity

The seedlings in the LSM treatment had higher photosynthetic capacity than the well-watered seedlings, as indicated by the higher photosynthetic rate, greater V_{cmax} and greater J_{max}, and the increase was greater under elevated than ambient CO_2 and under the longer photoperiods of higher latitudes than at the seed origin. However, these results do not necessarily indicate an active photosynthetic upregulation. They may simply indicate

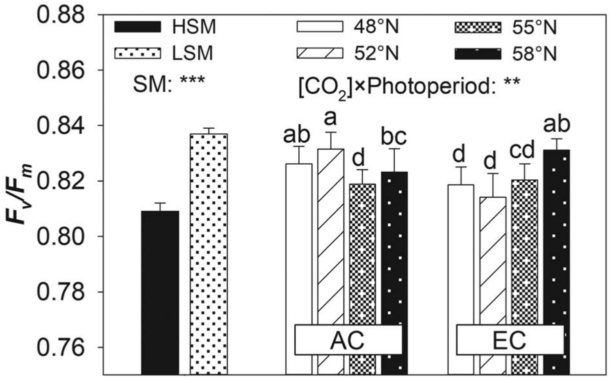

Figure 5. Quantum efficiency of photosystem II (F_v/F_m) (mean + SE) of trembling aspen seedlings grown under two levels of [CO$_2$] (ambient vs elevated), four photoperiod regimes (at 48 (seed origin), 52, 55 and 58°N) and two levels of soil moisture (high vs low) for two growing seasons. Data are pooled across [CO$_2$] and photoperiod for (left) ($N = 48$ (see Figure 1 for explanations)) and soil moisture for (right) ($N = 12$, three seedlings with two replications per treatment, and two levels of soil moisture, $3 \times 2 \times 2 = 12$). The lower case letters indicate the interactive effect of [CO$_2$] × photoperiod. Means with different letters are significantly different from each other based on Fisher's least significant difference post hoc test ($P < 0.05$). Significance levels: $^{**}P < 0.01$; $^{***}P < 0.001$.

a light acclimation of the Rubisco carboxylation and maximum rate of electron transport for RuBP regeneration and/or the consequence of the stronger morphological acclimations. Although the stomata of trembling aspen are very sensitive to vapor pressure deficit in the air, the species is very tolerant of drought stress as that the wide variation of xylem water potential from −0.4 to −3.7 MPa has no significant effect on its photosynthetic rates (Dang et al. 1997). Furthermore, the trees in the low moisture treatment were smaller and had much smaller leaves, a smaller total leaf area and a relatively greater root system than those in the high moisture treatment, leading to a presumably more stable water balance, which in turn enabled the smaller trees to maintain higher photosynthesis, particularly under the elevated CO$_2$ concentration because higher CO$_2$ concentrations improve CO$_2$ diffusion into the leaf due to the greater concentration gradient (as indicated by the lower C_i/C_a) between inside and outside of the leaf (Saxe et al. 1998, Robredo et al. 2007). These in turn allowed the smaller trees with a lower leaf area (LSM) to maintain a higher V_{cmax} and J_{max} than larger trees (HSM) grown in the same-sized containers. Additionally, the trees in both low and high moisture treatments were exposed to the same nutrient supply, allowing the smaller trees in the low moisture treatment to maintain a higher concentration of nutrients in the leaf, particularly nitrogen, which is highly correlated to photosynthetic capacity (Evans 1989, Bungard et al. 1997, Walker et al. 2014).

The data show that the increase in photosynthetic capacity in the LSM treatment was greater under the longer photoperiods of higher latitudes than at the seed origin. Other studies have demonstrated that changes in photoperiod can alter leaf nitrogen concentration and photosynthesis and drive the seasonal variation in the photosynthetic capacity (Comstock and Ehleringer 1986, Bauerle et al. 2012). A genotype of *P. glauca* at 46°N latitude has increased its photosynthetic capacity when grown under the longer growing season photoperiod regime at 48°N latitude (Benomar et al. 2016). It is unknown what exactly was responsible for the above increased response in this study, but it may be a plausible to argue that the previously described benefits of smaller trees in the low moisture treatment lasted for more hours per day with increases in photoperiod and thus led to a greater accumulation.

Interactive effect of photoperiod and soil moisture on stomatal conductance

It is interesting to note that the magnitude of reduction in stomatal conductance by the LSM treatment declined with the increasing photoperiod. This response pattern was caused by the diverging trends of stomatal response to photoperiod under the two soil moisture conditions: under the HSM treatment, stomatal conductance generally declined with increasing photoperiod; in the LSM treatment, in contrast, stomatal conductance did not show a significant response to photoperiod, resulting in the difference in stomatal conductance between the two soil moisture treatments becoming smaller as photoperiod increased. Therefore, the understanding of the response pattern relies on the understanding of the two separate response trends in the two soil moisture treatments. There are three important factors that should be considered: vapor pressure deficit, internal water status and root-to-shoot signals. The stomata of trembling aspen tend to behave like those of isohydric species in that they are very responsive to changes in vapor pressure deficit (VPD) (Dang et al. 1997). While this behavior is sufficient to maintain tissue water potential relatively constant in isohydric species (Tardieu and Simonneau 1998, Tyree and Zimmermann 2002, Wilkinson and Davies 2002, Galvez et al. 2011), it is not the case in trembling aspen, the water potential of which fluctuates over a very wide range diurnally and seasonally (Dang et al. 1997). Furthermore, the VPD was similar in different photoperiod treatments in this study because photoperiods were nested in the greenhouse. Therefore, VPD could not be a contributing factor in this study. Soil moisture can influence stomatal conductance via root-to-shoot signals (hydraulic and/or chemical (abscisic acid)). The fact that stomatal conductance was relatively constant across the four photoperiods in the LSM treatment indicates that root signals probably played a vital role in controlling stomatal conductance to a minimal level under the LSM, making it unable to respond to photoperiod. However, internal water balance and water status were probably the primary factor for the declines in stomatal conductance with increasing photoperiod in the high moisture treatment. Since trees in different period treatments were exposed to similar soil moisture conditions (presumably similar root signals) and we have already ruled out vapor pressure deficit (VPD), the internal water condition was the only reasonable explanation left for the trends in the high moisture treatments. Since longer photoperiods keep the stomata open for more hours, it is plausible to infer that the trees under longer photoperiods lost more water and suffered deteriorated internal water conditions, leading to reduced stomatal conductance. Overall, the interaction between photoperiod and soil moisture condition suggests that the water relations of trembling aspen will be more prone to the influence of photoperiod on sites with better moisture conditions than dry sites if the species migrate to higher latitudes (naturally or by human intervention).

In conclusion, since more frequent and long-lasting water stresses in the future are predicted for many regions of North America, particularly the boreal region (Pachauri et al. 2014, Wang et al. 2014), trees will need effective strategies to cope with the increased level of stress, which may hinder their establishment and slow down their migration in response to climate change. Our results reveal that trembling aspen seedlings have the ability to acclimate morphologically to increased water stresses and to maintain photosynthetic capacity. Additionally, the response to LSM can be altered by CO_2 elevations and longer photoperiods, which will be associated with northward migration. This study highlights the need to contemplate the existence of complex interactive effects of $[CO_2]$, photoperiod and soil moisture when planning assisted migration or predicting shifts in the distribution of boreal forests in the future.

Acknowledgments

We acknowledge Ms Joan Lee, former Greenhouse Manager, and Ms Keri Pidgen, the current Greenhouse Manager of Lakehead University for their logistic support and other operational assistance during the experiments. We want to express our special thanks to three anonymous reviewers for their insightful and constructive comments and suggestions.

Conflict of interest

None declared.

Funding

The study was supported by the Natural Science and Engineering Research Council of Canada (NSERC). Discovery grant to Q.-L.D. (Project No. 203198-2013-RGPIN), an Ontario Trillium Scholarship to S.I., and Lakehead University Graduate Assistantships to S.I. and B.T.

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