Nordic Journal of Botany 35: 633–643, 2017
doi: 10.1111/njb.01325, ISSN 1756-1051

Subject Editor: Jens-Christian Svenning. Editor-in-Chief: Torbjörn Tyler. Accepted 7 July 2017

Eco-physiological potential of jack pine (*Pinus banksiana*) for assisted northward migration: interactions among photoperiod, [CO_2] and moisture stress

Shah Newaz, Qing-Lai Dang and Rongzhou Man

S. Newaz and Q.-L. Dang (http://orcid.org/0000-0002-5930-248X) (qdang@lakeheadu.ca), Faculty of Natural Resources Management, Lakehead Univ., Thunder Bay, Canada. – R. Man,Ontario Ministry of Natural Resources, Ontario Forest Research Inst., Sault Ste. Marie, Canada.

Climate change will cause northward shifts of climate envelopes for boreal plants, however, the different photoperiod and soil moisture regimes at higher latitudes will likely influence the success of species migrations (natural and assisted). The objective of this study was to assess the effects of photoperiod regime and its interactions with soil moisture and carbon dioxide concentration ([CO_2]) on the morpho-physiological processes in jack pine *Pinus banksiana*. One-year old seedlings were exposed to two [CO_2] (400 and 950 µmol mol^{-1}), two soil moistures (60–70% and 30–40% of field water capacity) and three photoperiod regimes (photoperiods at seed origin, 5° and 10° north of the seed origin) in environment controlled greenhouses. The impacts of photoperiod, soil moisture and elevated [CO_2] on growth and physiological processes in the seedlings were examined. The results suggest that the response of jack pine to climate change will be complex under the interactive effects of northward migration associated longer photoperiod, soil moisture stress and elevated [CO_2]. The longer photoperiod associated with higher latitudes under elevated [CO_2] significantly advanced the budburst at both high and low soil moisture regimes, which may likely increase the risk of late spring frosts damage prior to and during budburst. The interactive effects of longer photoperiod and low soil moisture significantly increased the water use efficiency under elevated [CO_2]. However, the significant 2- and 3-way interactions suggest that drought and longer photoperiods with northward migration will limit the positive effects of elevated [CO_2] on growth and physiological processes in the species. These results might have important implications in assisted migration/seed transfer of the species following climate change.

Forests have migrated across landscapes in response to past climate changes (Thomas 2000, Bunnell and Kremsater 2012). The continued increases in global atmospheric CO_2 will cause continued increases in temperature (Cao and Caldeira 2010, IPCC 2014) and alter the amount, intensity, frequency and type of precipitation. Such changes can aggravate soil water shortage in some regions (Allison and Treseder 2008, Johnston et al. 2009). The climate change will affect plant morpho-physiological processes, growth and survival (Volder et al. 2007, Domec et al. 2009) and the impacts will likely be greater in the boreal region (Serreze et al. 2000, Barnett et al. 2005, IPCC 2007, Pachauri and Reisinger 2007) because the magnitude of climate change will be greater in the boreal region than the global average (Christensen et al. 2007, Eskelin et al. 2011, Montzka et al. 2011). In response to the continued climate change, the climate envelopes of 130 North American tree species have been predicted to shift 10 degrees (approximately 1000 km) northward between 2071 and 2100 (McKenney et al. 2007, 2011). While the predicted shift in climate envelopes offers an opportunity to expand the species range northward, the migrations (human assisted or natural) will expose the plants to new environmental conditions where factors other than temperature and precipitation may be quite different from the conditions that the species have adapted to. Therefore the spatial extent and success of such migration will depend on the capability of the migrating species to acclimate to the physical environment of their new habitats (Thomas and Vince-Prue 1997). For example, they will face a different photoperiod regime. The photoperiodism for a population has evolved through genetic modifications over hundreds or even thousands of years (Thomas and Vince-Prue 1997). The length of photoperiod affects plant phenological and physiological events (Thomas and Vince-Prue 1997, Lambers et al. 2008, Soolanayakanahally et al. 2013) and net assimilation rate by changing the percentage of total nitrogen in dry matter (Lambers et al. 2008). It regulates the phenotypic processes in boreal plants through synchronization with the natural seasonal changes in environmental conditions (Lambers et al. 2008). Changes in photoperiod regimes associated with migration may affect the timing of photoperiod-controlled growth events and lead to phenological responses such as earlier bud burst in the spring or delayed bud formation and reduced cold hardiness in the fall, all of which may reduce plant resistance to adverse environmental conditions and affect the survival and growth (Man et al. 2009). Since the photoperiod at higher latitudes is longer in the summer, shorter in the winter and thus a faster rate of photoperiod change during seasonal transitions, the ability of a species to acclimate to the new photoperiod regime may be a key determinant for the success of northward tree migration and/or seed transfer. However, the effect of changes in photoperiod regime on ecophysiological traits in trees has not been well studied.

Elevated [CO_2] has diverse and complex effects on physiological and growth performance of plants (Lawlor and Mitchell 1991, Bowes 1993). It usually stimulates photosynthesis and promotes growth and yield (Ainsworth and Long 2005, Marfo and Dang 2009) and alters the structure and physiology of plants through changes in carbon allocation (Koch et al. 1986, Janssens et al. 1998, Tingey et al. 2000). Improved water–use efficiency under elevated [CO_2] (Long et al. 2004, Keenan et al. 2013) resulting from the reduced stomatal conductance and increased photosynthesis due to the depression of photorespiration by increased internal [CO_2] (Ainsworth and Rogers 2007) can indirectly reduce the impact of drought on photosynthesis and biomass production (Hungate et al. 2002). However, the effects are variable and subject to environmental feedback (Gunderson et al. 2002, Leakey et al. 2006). Elevated [CO_2] can also accelerate the bud formation and cold hardening of trees in the fall (Bigras and Bertrand 2006).

Soil moisture conditions can affect the physiological and morphological characteristics of plants that influence the response to CO_2 and environmental conditions associated with climate change. For example, the elevated [CO_2] induced decline in stomatal conductance is smaller under soil moisture stress (Mishra et al. 1999, Ambebe and Dang 2009, de Miguel et al. 2012). Soil moisture significantly modifies the effects of [CO_2] on net photosynthesis (Mishra et al. 1999, Robredo et al. 2007). Low soil moisture minimizes the enhancing effects of elevated soil temperature on net photosynthetic rate and stomatal conductance (Ambebe and Dang 2009). However, photosynthetic biochemistry and photochemistry are not affected by moisture stress unless the stress is extreme (Richardson et al. 2004, Ennahli and Earl 2005). Drought conditions inhibit water uptake and reduce transpiration (Brodribb et al. 2005). Plants acclimate to drought by modifying the sizes and shapes of leaves and increasing the root/shoot ratio (Marron et al. 2002, Warren et al. 2005). By reducing the specific leaf area, plants can adjust the balance between the light capturing capacity and the capacity of CO_2 assimilation (Sefton et al. 2002).

The effects of climate change on trees reflect the interactive response of multiple factors (Johnston et al. 2009). The combined effects of interacting factors can be quite different from the summation of their individual effects. For example, the effects of elevated [CO_2] vary considerably with changes in other environmental conditions (Mishra et al. 1999, Zhang and Dang 2006, 2007, Robredo et al. 2007, Marfo and Dang 2009). Moisture stress reduces the positive effects of elevated [CO_2] on leaf area, leaf dry weight and stem dry weight (Mishra et al. 1999). However, the interactive effects of multiple factors on plant responses to climate change are not well understood.

Jack pine *Pinus banksiana* is distributed over a wide range of environmental conditions (Rudolph and Laidly 1990). This species has a superior tolerance to drought, a plant trait that is increasingly important with increasing climatic drought under rising temperature and changes in the distribution of precipitation. The main objective of this study was to investigate the interactive effects of photoperiod, soil moisture and [CO_2] on the physiological and morphological traits of jack pine that can influence the potential migration and/or seed transfer of the species. We hypothesized that elevated [CO_2] and northward migration associated changes in photoperiod will accelerate the growth and physiological processes in jack pine but their interactions with soil moisture stress will complicate and limit those responses.

Material and methods

Plant materials

The experiment was conducted using one-year old jack pine *Pinus banksiana* seedlings raised from seeds collected in Kakabeka, Ontario (48°57′N, 90°44′W). A total of 448 seedlings of relatively uniform sizes (average height/root collar diameter = 13.05 cm/ 0.21 cm) were transplanted in plastic pots (15 cm in height and 13 cm in diameter) filled with a mixture of premium grade vermiculite and peat moss (1:1, v/v).

Experimental design

The experiment was carried out in four greenhouses at Lakehead Univiversity Thunder Bay campus. The treatments consisted of two levels of [CO_2] (400 and 950 μmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field capacity) and three photoperiod regimes (P_s, P_{nm1} and P_{nm2}, representing the photoperiod at the seed origin, 5° and 10° north of the seed origin, respectively). The experiment was a split plot design, with [CO_2] as the whole plot, soil moisture as the sub-plot and photoperiod as the sub-sub plot. Two levels of [CO_2] were assigned to four independent greenhouses randomly with two replicates of each. Two levels of soil moisture were applied within each greenhouse and three photoperiod regimes were incorporated within each soil moisture treatment.

Growing conditions

The experiment was conducted between 20 Nov 2013 and 31 May 2014, but the environmental conditions in the greenhouse were controlled to mimic the actual day lengths and temperatures of the seed origin for the period of 16 Apr to 31 Oct. Desired levels of CO_2 were maintained in the greenhouses using electronic ignition natural gas CO_2 generators (model GEN-2E, Custom Automated Products Inc., Riverside, CA). Circulation fans were used to ensure even distribution of CO_2 throughout each of the greenhouses. The [CO_2] in each greenhouse was monitored and controlled automatically with an Argus CO_2 Control System (Argus, Vancouver, BC, Canada). The moisture content of the growing medium was measured daily with a HH2 moisture meter and a ML2x Theta probe (Delta-T Devices, Cambridge, UK) and the desired moisture levels were maintained by adding water when required. The photoperiod for each treatment was adjusted biweekly to mimic the actual biweekly average photoperiods (16 Apr to 31 Oct) at the corresponding locations. The desired photoperiod lengths were achieved by extending the natural day lengths using high-pressure sodium lamps or shortening the natural day lengths through manual shading of the seedlings. To avoid the possible influence of light from longer photoperiod treatments, trees in shorter photoperiods were shaded all around using blackout tarp (silver color on one side and black on the other side) during dark periods. All the seedlings were fertilized biweekly with N:P:K fertilizer (7.14, 0.56 and 2.12 mmol per liter of water, respectively during the rapid growth phase and 1.78, 2.20 and 4.64 mmol per liter of water, respectively during the hardening phase) (Scarratt 1986). In all the greenhouses relative humidity was maintained at 55–60% during the rapid growth phase and reduced to 45–50% during the hardening phase. The day and night air temperatures were set biweekly to mimic the mean biweekly day and night air temperatures at the seed origin and the regime was the same in all the treatments. Relative humidity and air temperature were controlled automatically using an Argus control system.

Growth measurements and observation of bud break and bud formation

At the time of transplanting, the height and root collar diameter (RCD) of each seedling were measured. Ten seedlings were selected randomly from each treatment combination to investigate the bud break and bud formation at the beginning of the experiment and during the cold hardening phase, respectively. The terminal meristems of the seedlings were regularly checked visually for bud break. Bud break was considered completed when most of the bud scales fell, with tips of needles protruding about 2 mm (Bigras and Bertrand 2006). Days to bud break were counted from the beginning of the treatments (20 Nov 2013). Bud set was also determined by visual observation and was considered accomplished when bud scales were completely closed (Bigras and Bertrand 2006). Bud formation was completed in all treatments by 16 Apr 2014 (corresponding to the autumn equinox when the day length was reduced to 12 h). The bud set time was the number of days from the day when bud formation observation started (3 Mar).

Gas exchange measurement

Foliar gas exchange was measured on the current year foliage of three randomly chosen seedlings from each treatment combination using a PP-Systems CIRAS-3 open gas exchange system (PP System Inc., Amesbury, MA, USA). All measurements were conducted between 09:30 and 14:30 h during the active growing phase (102 to 120 d of treatment). The photosynthetic responses to CO_2 concentration (A/C_i curves) were measured at eight [CO_2]: 50, 150, 250, 400, 550, 730, 900 and 1200 μmol mol^{-1} under 800 μmol m^{-2}s^{-1} photosynthetically active radiation (measured to be above the light saturation point), 25°C air temperature and 50% RH. The rate of CO_2 assimilation (A_n), stomatal conductance (g_s), transpiration rate (E), and the instantaneous water use efficiency (iWUE) were calculated according to Farquhar et al. (1980). The A/C_i response curves were analyzed to estimate V_{cmax} (maximum rate of carboxylation), J_{max} (maximum rate of electron transport) and TPU (triose phosphate utilization) according to Sharkey et al. (2007).

Measurements of biomass and leaf and root traits

At the end of the experiment (28 May 2014) the height and RCD on three randomly chosen seedlings from each treatment combination were measured again and the relative growth rates of height and RCD were determined. The stem volume was calculated according to van den Driessche (1992) ($V = (\pi D^2/4)\ H/3$).The seedlings were harvested when all the measurements were completed and separated into foliage, stem (including branches) and roots. The projected leaf area (PLA) was determined by scanning all live needles with WinSeedle (Regent Instruments Inc, Quebec, Canada) for determining the specific leaf area. The roots were scanned to analyze root traits using WinRhizo (Regent Instruments Inc, Quebec, Canada). The foliage, stem and roots were subsequently oven-dried at 70°C for 48 h and weighed on an analytical balance (0.001 g precision) to determine dry mass. Stem mass ratio (ratio of stem mass to total biomass, SMR), root mass ratio (ratio of root mass to total biomass, RMR), leaf mass ratio (ratio of leaf mass to total biomass, LMR), and shoot to root mass ratio (ratio of above ground to belowground mass, SRR) were used as indices of biomass allocation. Specific leaf area (SLA) was determined from the projected leaf area and leaf dry mass. Specific root length (SRL) and specific root surface area (SRA) were determined as root length per unit root dry mass and root surface area per unit root dry mass, respectively.

Statistical analysis

All data analyses were carried out using the R programming environment 3.1.3 (R Core Team). Before conducting the ANOVA, normality of distribution and homogeneity of variance were assessed using the Shapiro-Wilk and Bartlett tests, respectively. The total seedling biomass (TB), SRL, root to leaf area ratio (RLA), relative growth rate of stem volume (RGR_V), photosynthetic rate measured at growth [CO_2] ($A_{n\text{-}growth}$)and g_s data were transformed using natural logarithm function and iWUE data using the reciprocal of its square root to meet the ANOVA assumptions. When ANOVA showed a significant interaction or photoperiod effect, Tukey's HSD post-hoc comparisons were carried out. The threshold probability was 0.95 for all the analyses. [CO_2], soil temperature and photoperiod were treated as fixed effects.

Table 1. P-values for the effects of CO_2 concentration (C), soil moisture (M), photoperiod (P) and their interactions on the phenological and growth characteristics of jack pine seedlings. Seedlings were grown under two levels of [CO_2] (400 and 950 µmol mol^{-1}), two levels of soil moisture (60–70% and 30–40% of field water capacity) and three levels of photoperiod (seed origin, 5° and 10° north of seed origin).

	Treatment effects						
Response variables	C	M	P	C × M	C × P	M × P	C × M × P
Budburst	0.062	0.816	**0.037**	0.973	0.984	0.159	**0.025**
Bud set	0.089	0.440	0.189	0.702	0.487	**<0.001**	0.088
PLA	0.125	0.532	0.126	0.195	0.499	0.722	0.613
SLA	0.648	0.107	0.069	0.349	0.078	0.934	0.756
SMR	0.159	0.840	0.270	0.594	0.462	0.111	0.276
SRL	**0.010**	**0.029**	**0.003**	0.180	0.066	0.167	0.567
SRA	**0.004**	0.055	**0.002**	0.415	**0.011**	0.334	0.753
LMR	**0.023**	**0.025**	0.726	0.845	0.749	0.792	0.944
RLA	0.485	0.137	0.082	0.237	0.606	0.470	0.987
SRR	**0.018**	0.078	0.371	0.283	0.896	0.923	0.851
RMR	**0.013**	0.113	0.227	0.509	0.795	0.861	0.901
RGR_H	0.252	0.501	0.115	0.718	0.052	0.637	0.545
RGR_D	**0.024**	0.097	0.686	0.118	0.410	0.832	0.791
RGR_V	**0.013**	0.204	0.949	0.345	0.245	0.636	0.456
TB	**0.013**	0.802	0.575	0.189	0.614	0.729	0.936

Abbreviations: PLA (projected leaf area), SLA (specific leaf area), SMR (stem mass ratio), SRL (specific root length), SRA (specific root surface area), LMR (leaf mass ratio), RLA (root length to leaf area ratio), SRR (shoot to root ratio), RMR (root mass ratio), RGR_H, RGR_D and RGR_V (relative height, root collar diameter and stem volume growth rate, respectively), TB (total biomass).

Results

Morphological and biomass characteristics

The impacts of photoperiod on budburst and bud set depended on soil moisture and/or [CO_2] (significant 3- or 2-way interactions in Table 1). Budburst generally advanced with the increase in photoperiod toward north. The effect was more obvious under the elevated [CO_2] (Fig. 1A). Budburst was generally earlier in elevated [CO_2], particularly at the high soil moisture and P_{nm2} photoperiod (Fig. 1A). Bud set was the earliest at P_{nm2} under low soil moisture but

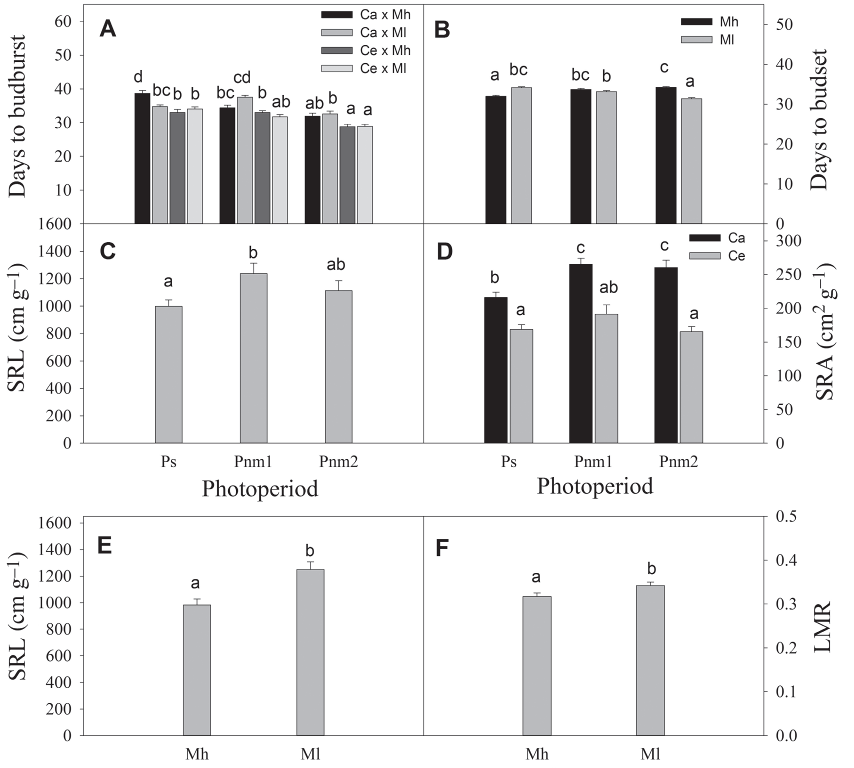

Figure 1. Mean (± SE) days to budburst and bud formation, specific root length (SRL), specific root surface area (SRA), leaf mass ratio (LMR), root mass ratio (RMR), shoot to root ratio (SRR), relative growth rate of root collar diameter (RGR_D) and volume (RGR_V) and total biomass production in jack pine seedlings grown under two levels of [CO_2] (Ca = ambient, Ce = elevated), two levels of soil moisture (Mh = high, Ml = low) and three levels of photoperiod (Ps = at seed origin, Pnm1 = 5° north and Pnm2 = 10° north of seed origin). Means with different letters were significantly different from each other (p = 0.05).

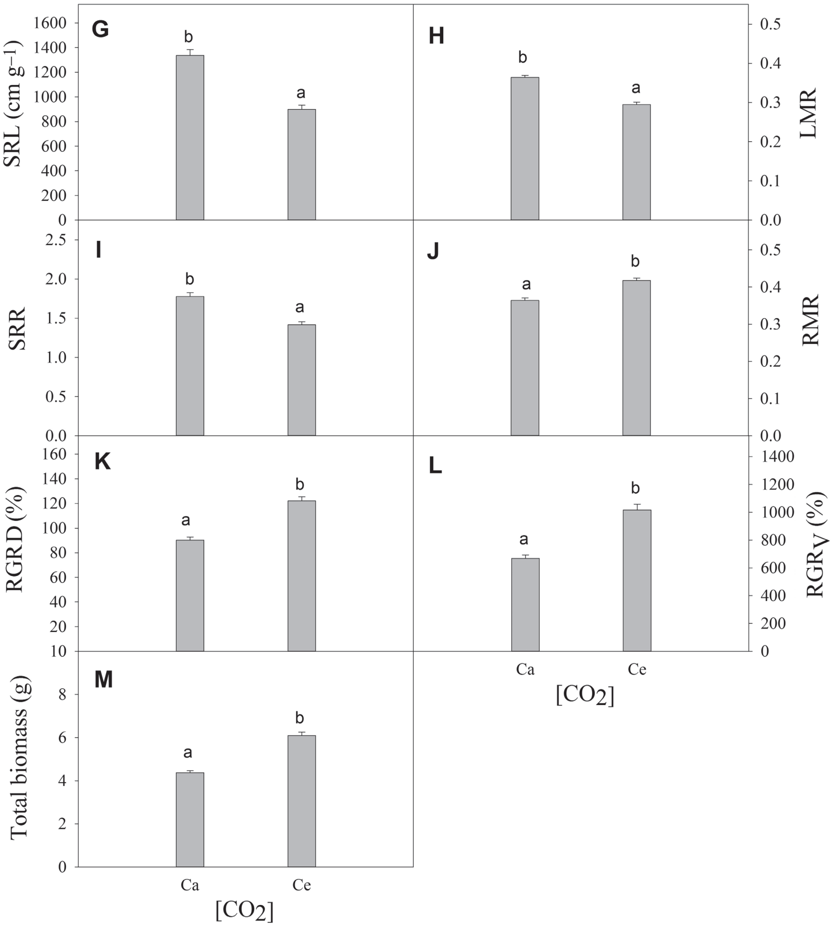

Figure 1. Continued.

the trend was reversed at the high soil moisture (Fig. 1B). The low moisture treatment delayed bud formation under the photoperiod regime of the seed origin but expedited it under the photoperiod regime 10° north of the seed origin (Fig. 1B).

The specific root length (SRL) was generally lower in the photoperiod regime of the seed origin (P_s) but the difference was only statistically significant between P_s and P_{nm1} (Fig. 1C). Photoperiod also had significant effect on the specific root surface area (SRA), but the effect was dependent on [CO_2] (Table 1). The SRA at the P_s was significantly smaller than the other two photoperiod regimes under the ambient [CO_2] but the effect was not significant under the elevated [CO_2] (Fig. 1D).

Soil moisture had significant effects on SRL and leaf mass ratio (LMR) (Table 1). Low soil moisture regime increased SRL by 27% and LMR by 8% (Fig. 1E, F). Elevated [CO_2] significantly decreased the SRL (Fig. 1G), LMR (Fig. 1H) and the shoot to root ratio (SRR) (Fig. 1I), but significantly increased the root mass ratio (RMR) (Fig. 1J). Relative diameter growth rate (RGR_D) and relative volume growth rate (RGR_V) were significantly higher under elevated [CO_2] (Fig. 1K, L), which resulted in significantly higher total biomass (TB) production under elevated [CO_2] (Fig. 1M).

Ecophysiological traits

The net CO_2 assimilation rates measured at the ambient [CO_2] ($A_{n\text{-}400}$) were not significantly affected by any of the treatments (Table 2). However, the photosynthesis at growth [CO_2] ($A_{n\text{-}growth}$) was significantly higher in the elevated [CO_2] treatment compared to the ambient [CO_2] and the effect was greater in the high moisture treatment (Fig. 2A). The high moisture treatment significantly increased $A_{n\text{-}growth}$ only under elevated [CO_2] (Fig. 2A). Photoperiod regime had no significant effect on any of the physiological processes measured (Table 2). However, it had significant interactive effects with soil moisture and [CO_2] on the photosynthetic water use efficiency (iWUE) (a 3-way interaction). In the P_{nm1} and P_{nm2}, iWUE increased with elevated [CO_2] and decrease in soil moisture. In the P_s, however, soil moisture did not significantly affect iWUE but it did affect its response to [CO_2], i.e., the CO_2 effect was significantly greater in the high than in the low soil moisture treatment (Fig. 2B). In all combinations of soil moisture and photoperiod regime, iWUE was higher at elevated [CO_2]. The lower soil moisture treatment significantly reduced g_s and E only at the ambient [CO_2] (Fig. 2C, D) and the elevated [CO_2] significantly reduced g_s and E only in the high moisture treatment (Fig. 2C, D).

Table 2. P-values for the effects of CO_2 concentration (C), soil moisture (M), photoperiod (P) and their interactions on net photosynthetic rate (A_n), instantaneous water-use efficiency (iWUE), stomatal conductance (g_s), transpiration rate (E), maximum rate of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}), triose phosphate utilization (TPU) and internal to ambient [CO_2] ratio (Ci/Ca). A_n was analyzed for a common [CO_2] of 400 µmol mol⁻¹ CO_2 ($A_{n\text{-}400}$) as well as growth CO_2 ($A_{n\text{-growth}}$). All other analyses were done for growth CO_2. Seedlings were grown under two levels of [CO_2] (400 and 950 µmol mol⁻¹), two levels of soil moisture (60–70% and 30–40% of field water capacity) and three levels of photoperiod (seed origin, 5° and 10° north of seed origin).

	Treatment effects						
Response variables	C	M	P	C × M	C × P	M × P	C × M × P
$A_{n\text{-}400}$	0.073	0.135	0.800	0.103	0.925	0.279	0.853
$A_{n\text{-growth}}$	**0.005**	0.231	0.523	**0.047**	0.608	0.424	0.723
iWUE	**0.003**	**0.028**	0.300	0.784	0.400	0.069	**0.027**
g_s	0.055	**0.006**	0.568	**0.011**	0.198	0.210	0.121
E	**0.010**	**0.008**	0.535	**0.004**	0.728	0.364	0.921
V_{cmax}	**0.034**	0.818	0.324	0.127	0.153	0.588	0.481
J_{max}	**0.019**	0.428	0.752	**0.024**	0.396	0.939	0.894
TPU	**0.004**	0.402	0.893	**0.041**	0.357	0.721	0.962
Ci/Ca	**0.054**	0.086	0.453	0.703	0.636	0.953	0.266

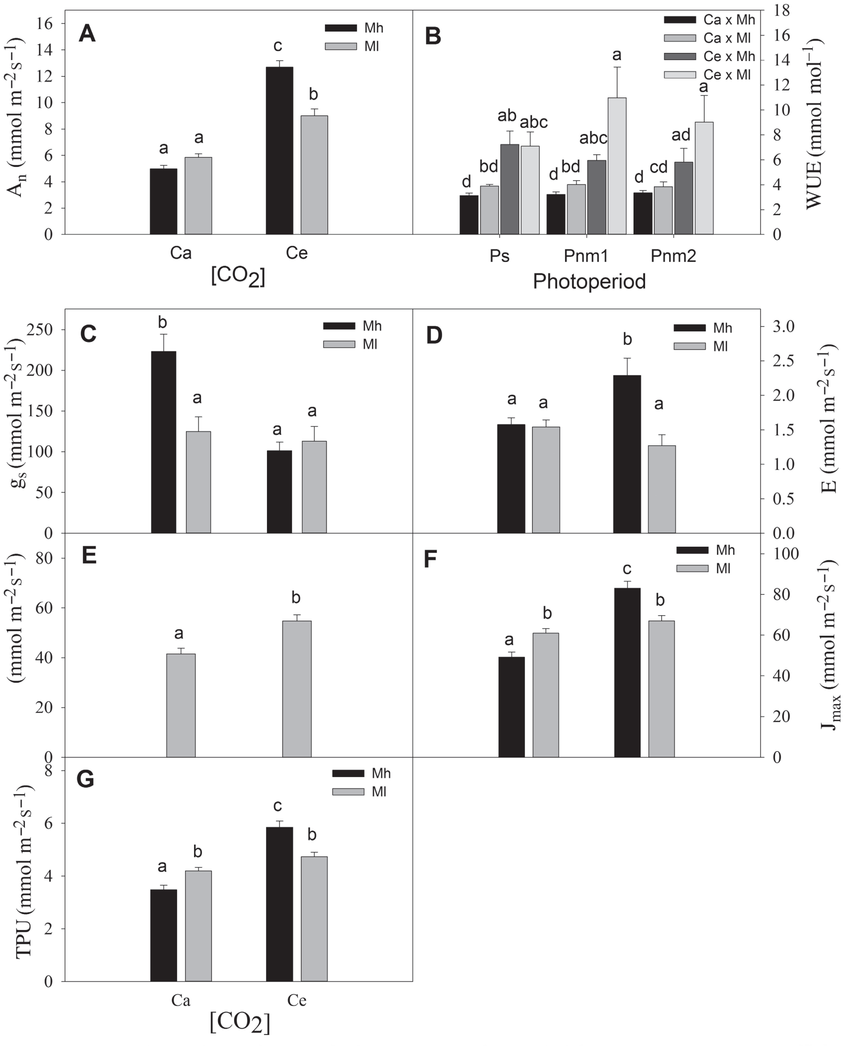

Figure 2. Mean (+ SE) net photosynthetic rate at growth CO_2 (A_n),instantaneous water-use efficiency (WUE), stomatal conductance (g_s),transpiration rate (E), maximum rate of carboxylation (V_{cmax}), light saturated rate of electron transport (J_{max}),and triose phosphate utilization (TPU) in jack pine seedlings grown under two levels of [CO_2] (Ca = ambient, Ce = elevated), two levels of soil moisture (Mh = high, Ml = low) and three photoperiod regimes (Ps = at seed origin, Pnm1 = 5° north and Pnm2 = 10° north of seed origin). A_n was analyzed for a common [CO_2] of 400 µmol mol⁻¹ CO_2 ($A_{n\text{-}400}$) as well as growth CO_2 ($A_{n\text{-growth}}$). All other analyses were done for growth CO_2. Means with different letters were significantly different from each other ($p \leq 0.05$).

Elevated [CO_2] significantly increased V_{cmax} in both soil moisture treatments (Fig. 2E), but it increased J_{max} and TPU significantly only in the high moisture treatment (Table 2, Fig. 2F, G). The soil moisture treatments had opposite effect on J_{max} and TPU under different [CO_2]: the low soil moisture treatment significantly increased J_{max} and TPU under the ambient [CO_2] but decreased both under the elevated [CO_2] (Fig. 2F, G). The internal to ambient [CO_2] ratio (Ci/Ca) was not affected by any of the treatments or their interactions (Table 2, Fig. 3A–G).

Discussion

Both photoperiod and soil moisture regimes influenced the ecophysiological responses of jack pine to elevated [CO_2]. For example, elevated [CO_2] advanced budburst under high soil moisture in the photoperiod regime of the seed origin, but in the photoperiod regimes of 5° and 10° north of the seed origin the elevated [CO_2] advanced budburst only under the low soil moisture treatment. Further, the elevated [CO_2] stimulated J_{max} and TPU but reduced g_s and E only in the high moisture treatment. These results suggest that soil moisture can promote or delay budburst and modify the physiological processes in plants, depending on the photoperiod and [CO_2] levels that influence seedling physiological activities and phenological development. Advanced budburst in jack pine under the longer photoperiod might suggest that the longer photoperiods may have reduced the accumulated temperature sum required for bud burst (Heide 1993a, b, Caffarra and Donnelly 2011, Basler and Körner 2014, Laube et al. 2014, Zohner and Renner, 2015). Basler and Körner (2012) stated that shorter photoperiods delay budburst in late successional species and do not have much impact in early successional species while Caffarra and Donnelly (2011) found significant photoperiod effects on budburst in

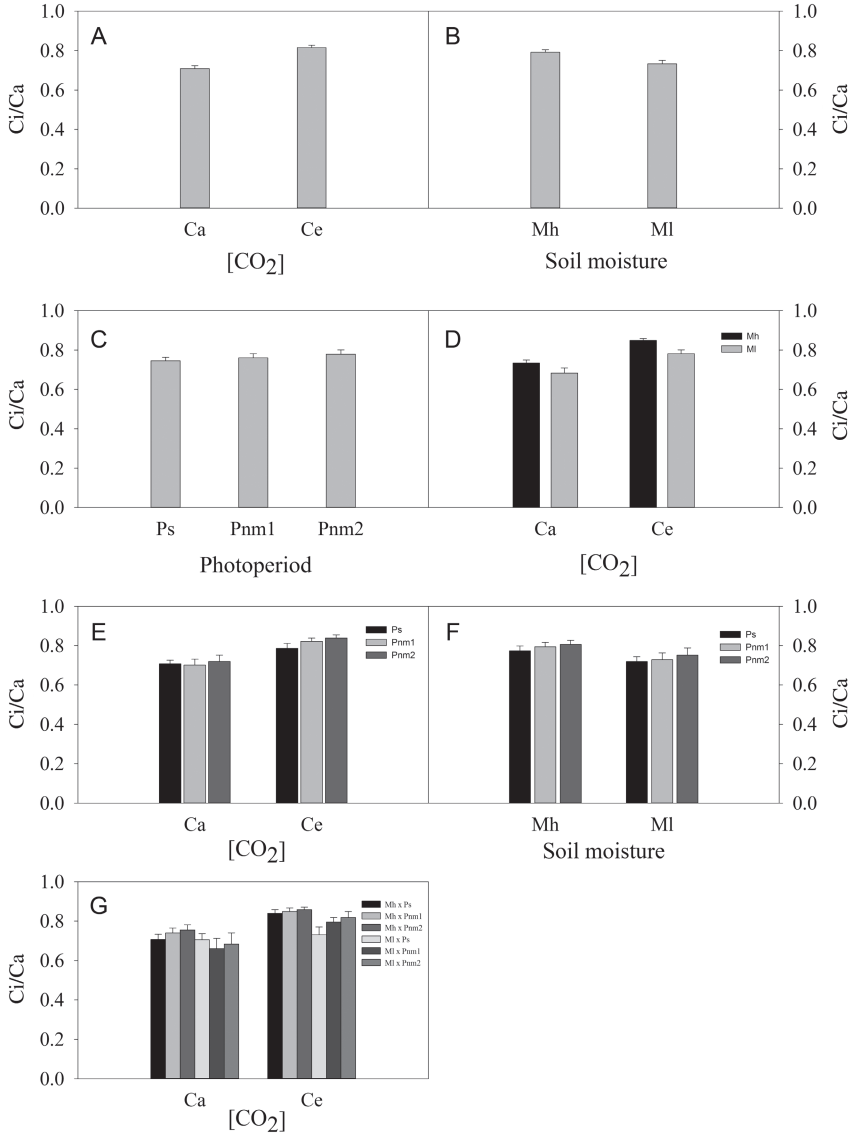

Figure 3. Mean (± SE) internal to ambient CO_2 concentrations ratio (Ci/Ca) in jack pine seedlings at different levels of [CO_2] (Ca = ambient, Ce = elevated), soil moisture (Mh = high, Ml = low), photoperiod regimes (Ps = at seed origin, Pnm1 = 5° north and Pnm2 = 10° north of seed origin) and their interactions.

both late-successional and early successional species, which is consistent with our findings. The effect of soil moisture E and g_s were lessened, but the effects on $A_{n\text{-growth}}$, J_{max} and TPU became greater under the elevated [CO_2], further demonstrating the interactive effects of elevated [CO_2] and soil moisture on physiological traits, as suggested by Duan et al. (2013). These results suggest that the acclimation or response to one stress may limit or magnify the ability of the plant to respond to another stress or stresses. Such an effect may even change the mechanisms of response. For example, the photosynthesis was co-limited by CO_2 (as indicated by V_{cmax}) and RuBP regeneration (as indicated by J_{max}) under the ambient [CO_2] when the jack pine seedlings were well watered, but limited only by V_{cmax} under the drought condition in this study.

Photoperiod regimes and elevated [CO_2] affected the ecophysiological responses of jack pine to soil moisture. For example, the low soil moisture treatment delayed the bud formation in the fall in the photoperiod regime of the seed origin but expedited it under the photoperiod regime 10° north of the seed origin. Under the ambient [CO_2], the low soil moisture treatment reduced g_s and E while it reduced $A_{n\text{-growth}}$, J_{max} and TPU only under the elevated [CO_2]. Bud formation in the fall advanced with increasing latitude when the soil moisture was low, but the trend was the opposite when the growing medium was well supplied with water. The significant soil moisture and photoperiod interaction on bud set suggests that the role of soil moisture in bud formation can be totally different, depending on the timing of threshold photoperiod. The results of this study suggest that the influence of soil moisture on the physiological responses to photoperiod regime should be taken into consideration in predicting tree migrations and the environmental conditions of the specific site should be considered in seed transfer programs or studies. This study further suggests that there are complicated interacting effects of different environmental factors on bud formation that are currently not understood and thus further research is warranted.

The effect of soil moisture on net assimilation ($A_{n\text{-growth}}$) was dependent on the CO_2 level. Low soil moisture reduced $A_{n\text{-growth}}$ only under the elevated [CO_2]. However, at both levels of soil moisture, elevated [CO_2] increased $A_{n\text{-growth}}$ significantly. The increased $A_{n\text{-growth}}$ under elevated [CO_2] might be associated with increased availability of substrate (CO_2) (Zhang and Dang 2006) and efficiency of photosystem II (Tjoelker et al. 1998, McGrath et al. 2010) and reduced photorespiration. We did not observe significant photosynthetic down-regulation in jack pine seedlings based on the increased V_{cmax} and J_{max} under elevated [CO_2], as has been often observed in response to elevated [CO_2] (Gunderson and Wullschleger 1994, Medlyn et al. 1999). This might be attributed to high nitrogen fertilization during the active growth phase of the seedlings, as shown by the increases of J_{max} and TPU with increasing N (Maier et al. 2008) and P (Wykoff et al. 1998, Watanabe et al. 2011) fertilization. Inorganic phosphate (P_i) concentration in cytosol largely determines the exportation of triose phosphate to cytosol or storage in chloroplasts (Lambers et al. 2008) and thus affects both J_{max} and TPU. The up-regulations of V_{cmax}, J_{max} and TPU observed in this study are consistent with the findings of Long et al. (2004), Darbah et al. (2010) and Danyagri and Dang (2014a) but are in contrast to the down-regulations that are commonly reported in the literature, e.g. Tissue and Lewis (2010) and Watanabe et al. (2011). Zhang and Dang (2005) report that the responses vary with seedling age and soil temperature. The increased $A_{n\text{-growth}}$ under elevated [CO_2] are consistent with the findings of other researchers (Zhang and Dang 2005, Cao et al. 2007, Danyagri and Dang 2014b).

The effect of elevated [CO_2] on the instantaneous water use efficiency (iWUE) in jack pine was influenced by both photoperiod and soil moisture regimes. Under the photoperiod of seed origin, elevated [CO_2] enhanced the iWUE only at high soil moisture treatment. However, under the photoperiod of 5° north of the seed origin it increased the iWUE at both high and low soil moisture treatments and under 10° north the effect was significant only at low soil moisture treatment. The increased iWUE at elevated [CO_2] was a result of decreased stomatal conductance (marginally significant) which tends to have greater effect on transpiration than on photosynthesis, as found by others (Medlyn et al. 2001, Long et al. 2004, Zhang and Dang 2005, Keenan et al. 2013), and an increase in A_n as a result of increased Ci under a nearly constant ratio of internal to ambient [CO_2] (Ci/Ca) and elevated [CO_2]. Our results also confirmed that low soil moisture reduces the transpiration (E) and stomatal conductance (g_s) and increases the iWUE as observed by others (Medlyn et al. 2001, Ambebe and Dang 2009, de Miguel et al. 2012, Keenan et al. 2013). Drought reduces water uptake for transpiration use (Brodribb et al. 2005) and therefore enhances iWUE by conservative use of available water through stomatal closure (Marron et al. 2002, Irvine et al. 2005). The effects of elevated [CO_2] and low soil moisture on iWUE at longer photoperiod indicate a higher iWUE of jack pine with possible northward migration.

The effect of photoperiod regime on the morphological traits in jack pine was influenced by the CO_2 treatment. For example, the response of SRA to photoperiod regime was modified by the CO_2 treatment. The longer photoperiod of 5° north of the seed origin increased the SRA significantly only under the ambient [CO_2]. However, further increase of photoperiod did not produce additional increase in SRA, indicating a possible threshold photoperiod beyond which [CO_2] induced A_n enhancement may be restricted by water and nutrient limitations. The higher SRA at longer photoperiod are consistent with the findings of Mozafar et al. (1993).

The observed higher leaf mass ratio (LMR) under low soil moisture, which is contrary to the findings of other researchers (Liu and Stützel 2004, Ambebe and Dang 2010), might be the result of physiological acclimation of jack pine seedlings to water stress. The higher LMR at low soil moisture might have some relationship with the lower specific leaf area (SLA) and higher specific root length (SRL) observed under low soil moisture. The lower SLA at low soil moisture indicates thicker needles and/or greater mass density, which might be an acclimation to reduce water loss under moisture stress condition (Warren et al. 2005). On the other hand the increased SRL at low soil moisture suggests that jack pine seedlings developed more extensive non-woody fine root systems to explore soil moisture and reduce moisture stress (Irvine et al. 2005).

The individual treatments had significant effects on some other morpho-physiological traits in jack pine seedlings. For example, elevated [CO_2] significantly increased the root mass ratio (RMR), relative growth of diameter (RGR_D) and volume (RGR_V), total biomass, maximum rate of carboxylation (V_{cmax}) and decreased the specific root length (SRL), leaf mass ratio (LMR) and shoot to root ratio (SRR). Both soil moisture stress and the photoperiod of 5° north of the seed origin increased the specific root length (SRL) significantly. However, the insignificant interactive effects indicate that northward migration will not affect those traits in jack pine.

The findings of this study suggest that climate change-induced northward migration will be more complicated than what is predicted by the climate envelope models. The advanced budburst at longer photoperiod and elevated [CO_2] may mean an increasing chance of damage from late spring frost for the northward migration of jack pine under the predicted climate change. Higher water use efficiency under longer photoperiod and low soil moisture regime at elevated [CO_2] indicates that the physiological processes of the species may be less limited by moisture stress at higher latitudes if northward migration does occur. Finally, the interaction effects suggest that soil moisture stress and changes in photoperiod regime associated with northward migration will likely limit the stimulating effects of elevated [CO_2] on growth and physiological processes in jack pine and increase the risk of damage by late spring frosts.

Acknowledgements – We are pleased to acknowledge Ms. Joan Lee, greenhouse manager and technologist, faculty of natural resources management, Lakehead Univ. for her logistic support and all out cooperation in the greenhouse operations. We have greatly appreciated the thorough review and constructive suggestions by two anonymous reviewers.

Funding – The study was supported by NSERC Discovery Grant to Q.-L. Dang (project no. 203198-2013-RGPIN).

References

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

Ainsworth, E. A. and Rogers, A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. – Plant Cell Environ. 30: 258–270.

Allison, S. D. and Treseder, K. K. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. – Global Change Biol. 14: 2898–2909.

Ambebe, T. F. and Dang, Q. L. 2009. Low moisture availability inhibits the enhancing effect of increased soil temperature on net photosynthesis of white birch (*Betula papyrifera*) seedlings grown under ambient and elevated carbon dioxide concentrations. – Tree Physiol. 29: 1341–1348.

Ambebe, T. F. and Dang, Q. L. 2010. Low moisture availability reduces the positive effect of increased soil temperature on biomass production of white birch (*Betula papyrifera*) seedlings in ambient and elevated carbon dioxide concentration. – Nord. J. Bot. 28: 104–111.

Barnett, T. P. et al. 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. – Nature 438: 303–309.

Basler, D. and Körner, C. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. – Agricult. Forest Meteorol. 165: 73–81.

Basler, D. and Körner, C. 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. – Tree Physiol. 34: 377–388.

Bigras, F. J. and Bertrand, A. 2006. Responses of *Picea mariana* to elevated CO_2 concentration during growth, cold hardening and dehardening: phenology, cold tolerance, photosynthesis and growth. – Tree Physiol. 26: 875–888.

Bowes, G. 1993. Facing the inevitable: plants and increasing atmospheric CO_2. – Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 309–332.

Brodribb, T. J. et al. 2005. Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. – New Phytol. 165: 839–846.

Bunnell, F. and Kremsater, L. L. 2012. Migrating like a herd of cats: climate change and emerging forests in British Columbia. – J. Ecosyst. Manage. 13: 1–24.

Caffarra, A. and Donnelly, A. 2011. The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. – Int. J. Biometeorol. 55: 711–721.

Cao, L. and Caldeira, K. 2010. Atmospheric carbon dioxide removal: long-term consequences and commitment. Environ. Res. Lett. 5: 024011.

Cao, B. et al. 2007. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO_2] in white birch seedlings. – Tree Physiol. 27: 891–899.

Christensen, J. H. H. B. et al. 2007. Regional Climate Projections. – In: Solomon, S. et al. (eds), The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge Univ. Press, pp. 1–94.

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

Danyagri, G. and Dang, Q. L. 2014b. Soil temperature and phosphorus supply interactively affect physiological responses of white birch to CO_2 elevation. – Am. J. Plant Sci. 5: 219–229.

Darbah, J. N. T. et al. 2010. Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. – Environ. Pollut. 158: 1008–1014.

de Miguel, M. et al. 2012. Functional and genetic characterization of gas exchange and intrinsic water use efficiency in a full-sib family of *Pinus pinaster* Ait. in response to drought. – Tree Physiol. 32: 94–103.

Domec, J. C. et al. 2009. Decoupling the influence of leaf and root hydraulic conductances on stomatal conductance and its sensitivity to vapour pressure deficit as soil dries in a drained loblolly pine plantation. – Plant Cell Environ. 32: 980–991.

Duan, H. et al. 2013. Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO_2] and elevated temperature. – Tree Physiol. 33: 779–792.

Ennahli, S. and Earl, H. J. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. – Crop Sci. 45: 2374.

Eskelin, N. et al. 2011. Assessing assisted migration as a climate change adaptation strategy for Ontario's forests: Project overview and bibliography. – Sault Ste Marie, ON, Ontario Forest Research Ins., OMNR. CCRR-19: 1–55.

Farquhar, G. D. et al. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. – Planta 149: 78–90.

Gunderson, C. A. and Wullschleger, S. D. 1994. Photosynthetic acclimation in trees to rising atmospheric CO_2: a broader perspective. – Photosynth. Res. 39: 369–388.

Gunderson, C. A. et al. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. – Plant Cell Environ. 25: 379–393.

Heide, O. M. 1993a. Daylength and thermal time responses of budburst during dormancy release in some northern deciduous trees. – Physiol. Plantarum 88: 531–540.

Heide, O. M. 1993b. Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. – Physiol. Plantarum 89: 187–191.

Hungate, B. A. et al. 2002. Evapotranspiration and soil water content in a scrub-oak woodland under carbon dioxide enrichment. – Global Change Biol. 8: 289–298.

IPCC 2007. Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. – Cambridge Univ. Press, pp 996.

IPCC 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. Core writing team, R. K. Pachauri and L. A. Meyer (eds). – IPCC, Geneva, Switzerland, pp. 151.

Irvine, J. et al. 2005. Coupling of canopy gas exchange with root and rhizosphere respiration in a semi-arid forest. – Biogeochemistry 73: 271–282.

Janssens, I. A. et al. 1998. Elevated atmospheric CO_2 increases fine root production, respiration, rhizosphere respiration and soil CO_2 efflux in Scots pine seedlings. – Global Change Biol. 4: 871–878.

Johnston, M. H. et al. 2009. Vulnerability of Canada's tree species to climate change and management options for adaptation: an overview for policy makers and practitioners. – Canadian Council of Forest Ministers, Ottawa, Ontario, pp 44.

Keenan, T. F. et al. 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. – Nature 499: 324–327.

Koch, K. E. et al. 1986. Growth, dry matter partitioning, and diurnal activities of RuBP carboxylase in citrus seedlings maintained at two levels of CO_2. – Physiol. Plantarum 67: 477–484.

Lambers, H. et al. 2008. Plant physiological ecology. – Springer.

Laube, J. et al. 2014. Chilling outweighs photoperiod in preventing precocious spring development. – Global Change Biol. 20: 170–182.

Lawlor, D. W. and Mitchell, R. A. C. 1991. The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. – Plant Cell Environ. 14: 807–818.

Leakey, A. D. B. et al. 2006. Long-term growth of soybean at elevated $[CO_2]$ does not cause acclimation of stomatal conductance under fully open-air conditions. – Plant Cell Environ. 29: 1794–1800.

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

Long, S. P. et al. 2004. Rising atmospheric carbon dioxide: plants FACE the future. – Annu. Rev. Plant Biol. 55: 591–628.

Maier, C. A. et al. 2008. Short-term effects of fertilization on photosynthesis and leaf morphology of field-grown loblolly pine following long-term exposure to elevated CO_2 concentration. – Tree Physiol. 28: 597–606.

Man, R. et al. 2009. A case of severe frost damage prior to budbreak in young conifers in northeastern Ontario: consequence of climate change? – For. Chron. 85: 453–462.

Marfo, J. and Dang, Q. L. 2009. Interactive effects of carbon dioxide concentration and light on the morphological and biomass characteristics of black spruce and white spruce seedlings. – Botany 87: 67–77.

Marron, N. et al. 2002. Physiological traits of two *Populus* x *euramericana* clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle. – Tree Physiol. 22: 849–858.

McGrath, J. M. et al. 2010. Spring leaf flush in aspen (*Populus tremuloides*) clones is altered by long-term growth at elevated carbon dioxide and elevated ozone concentration. – Environ. Pollut. 158: 1023–1028.

McKenney, D. W. et al. 2007. Potential impacts of climate change on the distribution of North American trees. – BioScience 57: 939–948.

McKenney, D. W. et al. 2011. Revisiting projected shifts in the climate envelopes of North American trees using updated general circulation models. – Global Change Biol. 17: 2720–2730.

Medlyn, B. E. et al. 1999. Effects of elevated $[CO_2]$ on photosynthesis in European forest species: a meta-analysis of model parameters. – Plant Cell Environ. 22: 1475–1495.

Medlyn, B. E. et al. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. – New Phytol. 149: 247–264.

Mishra, R. S. et al. 1999. Interactive effects of elevated CO_2 and moisture stress on the photosynthesis, water relation and growth of *Brassica* species. – J. Agron. Crop. Sci. 182: 223–230.

Montzka, S. A. et al. 2011. Non-CO_2 greenhouse gases and climate change. – Nature 476: 43–50.

Mozafar, A. et al. 1993. Photoperiod and root-zone temperature: interacting effects on growth and mineral nutrients of maize. – Plant Soil 153: 71–78.

Pachauri, R. K. and Reisinger, A. 2007. Climate change 2007: observed effects of climate change, synthesis report. – IPCC, Geneva, Switzerland, pp. 31–33.

Richardson, A. D. et al. 2004. Drought stress and paper birch (*Betula papyrifera*) seedlings: effects of an organic biostimulant on plant health and stress tolerance, and detection of stress effects with instrument-based, noninvasive methods. – J. Arboric. 30: 52–61.

Robredo, A. et al. 2007. Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. – Environ. Exp. Bot. 59: 252–263.

Rudolph, T. D. and Laidly, P. R. 1990. *Pinus banksiana* (Lamb.), Jack Pine. – In: Burns, R. M. and Honkala, B. H. (eds), Agriculture handbook 654. USDA Forest Service, Washington, DC, pp. 280–293.

Scarratt, J. B. 1986. An evaluation of some commercial soluble fertilizers for culture of jack pine container stock. – Canadian Forestry Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario. Information Report O-X-377, pp 21.

Sefton, C. A. et al. 2002. Anatomical variation in juvenile eucalypt leaves accounts for differences in specific leaf area and CO_2 assimilation rates. – Aust. J. Bot. 50: 301–310.

Serreze, M. C. et al. 2000. Observational evidence of recent change in the northern high-latitude environment. – Clim. Change 46: 159–207.

Sharkey, T. D. et al. 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. – Plant Cell Environ. 30: 1035–1040.

Soolanayakanahally, R. Y. et al. 2013. Timing of photoperiodic competency causes phenological mismatch in balsam poplar (*Populus balsamifera* L.). – Plant Cell Environ. 36: 116–127.

Thomas, B. and Vince-Prue, D. 1997. Photoperiodism in plants. – Academic Press.

Thomas, M. B. 2000. America's ancient forests: from the ice age to the age of discovery. – John Wiley and Sons.

Tingey, D. T. et al. 2000. Elevated CO_2 and conifer roots: effects on growth, life span and turnover. – New Phytol. 147: 87–103.

Tissue, D. T. and Lewis, J. D. 2010. Photosynthetic responses of cottonwood seedlings grown in glacial through future atmospheric [CO_2] vary with phosphorus supply. – Tree Physiol. 30: 1361–1372.

Tjoelker, M. G. et al. 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO_2 and temperature. – Tree Physiol. 18: 715–726.

van den Driessche, R. 1992. Absolute and relative growth of Douglas-fir seedlings of different sizes. – Tree Physiol. 10: 141–152.

Volder, A. et al. 2007. Effects of elevated atmospheric CO_2, cutting frequency, and differential day/night atmospheric warming on root growth and turnover of *Phalaris swards*. – Global Change Biol. 13: 1040–1052.

Warren, C. R. et al. 2005. Does rainfall explain variation in leaf morphology and physiology among populations of red ironbark (*Eucalyptus sideroxylon* subsp. *tricarpa*) grown in a common garden? – Tree Physiol. 25: 1369–1378.

Watanabe, M. et al. 2011. Growth and photosynthetic traits of hybrid larch F1 (*Larix gmelinii* var. japonica x *L. kaempferi*) under elevated CO_2 concentration with low nutrient availability. – Tree Physiol. 31: 965–975.

Wykoff, D. D. et al. 1998. The Regulation of photosynthetic electron transport during nutrient deprivation in *Chlamydomonas reinhardtii*. – Plant Physiol. 117: 129–139.

Zhang, S. and Dang, Q. L. 2005. Effects of soil temperature and elevated atmospheric CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. – Tree Physiol. 25: 523–531.

Zhang, S. and Dang, Q. L. 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. – Tree Physiol. 26: 1457–1467.

Zhang, S. and Dang, Q. L. 2007. Interactive effects of soil temperature and CO_2 on morphological and biomass traits in seedlings of four boreal tree species. – Forest Sci. 53: 453–460.

Zohner, C. M., Renner, S. S. 2015. Perception of photoperiod in individual buds of mature trees regulates leaf-out. – New Phytol. 208: 1023–1030.