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CO$_2$ stimulation and response mechanisms vary with light supply in boreal conifers

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Abstract

Aims Black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) are congeneric species. Both are moderately shade tolerant and widely distributed across North American boreal forests.

Methods To understand light effects on their ecophysiological responses to elevated CO$_2$, 1-year-old seedlings were exposed to 360 μmol mol^{-1} and 720 μmol mol^{-1} CO$_2$ at three light conditions (100%, 50% and 30% of full light in the greenhouse). Foliar gas exchanges were measured in the mid- and late-growing season.

Important Findings Elevated CO$_2$ increased net photosynthesis (P_n) and photosynthetic water use efficiency, but it reduced stomatal conductance and transpiration. The stimulation of photosynthesis by elevated CO$_2$ was greatest at 50% light and smallest at 100%. Photosynthesis, maximum carboxylation rate (V_{cmax}) and light-saturated rate of electron transport (J_{max}) all decreased with decreasing light. Elevated CO$_2$ significantly reduced V_{cmax} across all light treatments and both species in mid-growing season. However, the effect of elevated CO$_2$ became insignificant at 30% light later in the growing season, with the response being greater in black spruce than in white spruce. Elevated CO$_2$ also reduced J_{max} in white spruce in both measurements while the effect became insignificant at 30% light later in the growing season. However, the effect on black spruce varied with time. Elevated CO$_2$ reduced J_{max} in black spruce in mid-growing season in all light treatments and the effect became insignificant at 30% light later in the growing season, while it increased J_{max} later in the season at 100% and 50% light. These results suggest that both species benefited from elevated CO$_2$, and that the responses varied with light supply, such that the response was primarily physiological at 100% and 50% light, while it was primarily morphological at 30% light.

Keywords: black and white spruce, boreal forest, CO$_2$–light–species interaction, ecophysiological response, photosynthesis, respiration, water use efficiency

北方针叶树CO$_2$的刺激和响应机制随光强而变化

摘要：黑云杉（*Picea mariana* [Mill.] B.S.P.）和白云杉（*Picea glauca* [Moench] Voss.）是同属物种，两者都是适度耐阴，并且在北美北方针叶林中广泛分布。为了了解光照对CO$_2$ 浓度升高的生理生态反应的影响，在三种光照条件下（温室中光照设置为100%、50%和30%）将一年生的两种幼苗暴露在360和720 μmol mol^{-1}浓度的CO$_2$环境中，测定了其中后期叶面气体交换量。研究结果表明，CO$_2$的浓度升高提高了净光合速率

(P_n)和光合水分利用效率，但降低了气孔导度和蒸腾作用。CO_2对光合作用的刺激在50%光照下最大，在100%光照下最小。光合作用、最大羧化速率(V_{cmax})和光饱和电子传递速率(J_{max})均随光照强度的降低而降低。升高的CO_2在所有光照处理中显著降低了V_{cmax}，在生长季节中期，两种云杉的V_{cmax}均显著降低，但在生长季节后期，当光照达到30%时，这一影响变得不明显，而且黑云杉的响应大于白云杉。CO_2浓度升高也降低了白云杉的J_{max}，但在生长季后期30%光照时，这种影响变得不显著。但CO_2浓度升高对黑云杉的影响随时间而变化。在所有光照处理中，CO_2浓度升高降低了黑云杉生长中期的J_{max}，且在生长后期30%光照时影响不显著，但在100%和50%光照时，J_{max}升高。这些研究结果表明，两个树种植物都受益于CO_2浓度的升高，但它们的响应机制随着光照的增加而变化：即在100%和50%光照下，它们的响应主要是生理上的，而在30%光照下，它们的响应主要是形态上的。

关键词：黑云杉，白云杉，北方针叶林，CO_2 -光-物种相互作用，生理生态响应，光合作用，呼吸作用，水利用效率

INTRODUCTION

The continuous increase of atmospheric carbon dioxide concentration (CO_2) from the pre-industrial revolution (IPCC 2019) is expected to have profound impacts on the physiology, growth and geographic distribution of plants (Curtis and Wang 1998; Ward and Strain 1999). Elevated CO_2 concentration enhances photosynthesis and biomass production at least in the short term (Ainsworth and Long 2005; Bigras and Bertrand 2006; Huang *et al.* 2007; Zhang and Dang 2005, 2006, 2007; Zhang *et al.* 2006), due to the stimulation to Rubisco carboxylation and suppression of photorespiration (Bigras and Bertrand 2006; Drake *et al.* 1997; Lambers and Oliveira 2019; Osborne *et al.* 1997). Elevated CO_2 is also known to lower stomatal conductance and transpiration and therefore improve water and light use efficiencies (Drake *et al.* 1997; Körner 2000), along with possible reduction in dark respiration during photosynthesis and increase in the quantum yield of photosynthesis (Hymus *et al.* 2001; Saxe *et al.* 1998).

Photosynthetic enhancement by CO_2 elevation is particularly important to plants under low light conditions where maintaining a positive carbon balance is critical to survival and growth (Körner 2006; Messier *et al.* 1999; Saxe *et al.* 1998). Plants tend to lower the light compensation point of photosynthesis and increase light use efficiency in acclimation to a low light environment (Danyagri and Dang 2013; Herrick and Thomos 1999; Körner 2006; Long and Drake 1991; Man and Lieffers 1997; Ward and Strain 1999), particularly in shade tolerant species (Kubiske and Pregitzer 1996). Similar responses can also be triggered by CO_2 elevations (Kerstiens 1998; Osborne *et al.* 1997). However, the interactive effects of light and CO_2 on the photosynthesis of trees are largely unknown because past studies on CO_2 elevation are conducted on sun-acclimated trees/foliage, but a good understanding of such interactive effects are important for several reasons. Firstly, many tree species, particularly shade tolerant species, regenerate under the exiting canopy of large trees. The light level in the forest profile declines from the top of the canopy to the forest floor. In other words, the light level is the lowest for seedlings and increases as the trees grow taller. Therefore, the effects of lights on the physiological responses of seedlings to CO_2 elevation can be critically important for understanding forest regeneration in the future when the atmospheric CO_2 concentration will be much higher. Secondly, any differential effects of light on the responses of tree species with different shade tolerance to CO_2 elevation can differentially affect the regeneration potential of those species and affect the trajectory of forest succession and the species composition of the future forest. The literature indicates that CO_2 elevations can differentially affect plant species of different shade tolerance (Leakey *et al.* 2002) and the differential responses can lead to changes in the species composition and structure of plant communities (Bolker *et al.* 1995; Kerstiens 1998). However, there is no information in the literature on how different light levels affect the photosynthetic responses of tree species with different shade tolerance to CO_2 elevations. Thirdly, the knowledge of light effects on the responses of trees to CO_2 elevation can provide insights on how the response of trees to CO_2 elevation may change with developmental stages because light levels increase as trees grow taller and trees of different heights are exposed to different light conditions.

Black spruce and white spruce are economically important species in the boreal forest (Nienstaedt and Zasada 1990; Viereck and Johnston 1990). They can form pure stands or mix with each other or with other species (Viereck and Johnston 1990). Being congeneric, the two species have similar growth and morphological characteristics (Dudley 1996). While both species can tolerate certain levels of shade and regenerate under a forest canopy (Nienstaedt and Zasada 1990; Viereck and Johnston 1990), there are differences in low light thresholds at which they can realize their photosynthetic potentials. White spruce can reach maximum photosynthesis (Man and Lieffers 1997) and height growth (Lieffers and Stadt 1994) at 40%–60% of full sunlight, whereas black spruce can reach photosynthetic light saturation at 25%–50% full sunlight (Grossnickle 2000; Lamhamedi and Bernier 1994). Therefore, black spruce is more shade tolerant than white spruce. Given their differences in shade tolerance and the influence of shade tolerance on responses to CO_2, CO_2 elevations could differentially affect the relative performance of the two species. Our previous study shows that the relative enhancement of total biomass by CO_2 elevation increased with decreasing light level and was greater in black spruce than in white spruce (Marfo and Dang 2009). The greater biomass enhancement at lower light may be attributable to relatively greater enhancement to photosynthesis (Idso *et al.* 1993; Lambers and Oliveira 2019) and increased photosynthetic light use efficiency (Leakey *et al.* 2002; Saxe *et al.* 1998), and/or reduced respiration during photosynthesis (Lambers and Oliveira 2019; Teskey and Shrestha 1985). In this study, we examine the physiological responses of black spruce and white spruce to CO_2 elevation under different light conditions and test the hypothesis that CO_2 elevation will be more beneficial to trees growing under lower light conditions and to more shade tolerant species. The maximum Rubisco carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) are two important photosynthetic parameters that are used in process-based global vegetation models and ecosystem models to predict interactions between biosphere and atmosphere but information on the interactive effects of light, CO_2 and shade tolerance on those important parameters is lacking in the literature. The results on such interactive effects from this study will likely help to improve the precision of those models in projecting the potential interactions between biosphere and atmosphere under the predicted future climatic conditions.

MATERIALS AND METHODS

Plant materials

One-year-old black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss.) were obtained from Boreal Tree Nursery (Thunder Bay, ON, Canada). The seedlings were selected such that they were relatively uniform in size (13 cm high) and form at the beginning of the experiment. The seedlings were planted in circular pots of 13.5 cm tall and 11 cm top diameter. The growing medium was a mixture (1/1, v/v) of premium grade peat moss and vermiculite.

Experimental design

The experiment was a split-plot design with the CO_2 concentration as the whole plot (360 $\mu mol\ mol^{-1}$ and 720 $\mu mol\ mol^{-1}$), light as the sub-plot (100%, 50% and 30% of the light in the greenhouse) and the two species was nested within the light–CO_2 combination. There were 8 seedlings per treatment combination. The study was conducted in four greenhouses with identical design at the Lakehead University Thunder Bay campus. CO_2 was elevated generally using GEN-2E CO_2 generators (Custom Automated Products Inc., Riverside, CA) but compressed CO_2 cylinders were used on hot days to eliminate the effect of CO_2 generation on temperature. Neutral density shade screens were used to provide 50% and 70% shade, or a 50% and 30% light. Other environmental conditions in the greenhouses were 16 h photoperiod, 55% ± 5% relative humidity (RH) and day/night temperature 25 ± 2 and 15 ± 2 °C, respectively. The natural light was supplemented on cloudy days, early mornings and late evenings using high-pressure sodium lamps. An Argus Classic Environment control system (Argus, Vancouver, BC, Canada) was used to monitor and control all the environmental conditions. The pots were watered when the volumetric water content dropped to 30% as determined using an HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, UK). All seedlings were fertilized weekly with a solution of 150 mg/L N, 60 mg/L P, 150 mg/L K, 40 mg/L Mg, 80 mg/L Ca and 60 mg/L S.

Gas exchange measurement

Foliar gas exchange was measured using a PP-Systems Ciras-1 open gas exchange system and a Parkinson leaf cuvette for conifers with an environment control system (PP-Systems, Amesbury, MA, USA). The environmental conditions in the cuvette were controlled at 25 °C temperature, 50% RH and 800 μmol m^{-2} s^{-1} PAR flux density (above the light saturation points for the two species). A–C_i curves (CO_2 assimilation rate A *vs.* sub-stomatal CO_2 concentration C_i) were measured at 50, 150, 250, 360, 450, 550, 700, 900 and 1100 μmol mol^{-1} CO_2. Test measurements showed that gas exchange measurements were relatively stable between 0900 h and 1500 h and therefore all measurements were taken during this time period to avoid the influence of diurnal variations. Three trees from each treatment combination were measured at their treatment locations. Measurements were taken on current year needles near the apex of the seedling after 2.5 and 4.5 months of treatments. Gas exchange parameters were expressed on a projected leaf area basis. Leaf area was measured using a Regent Winseedle System (Regent Instruments, Québec City, Canada). Net photosynthetic rate (P_n), transpiration rate (E) and stomatal conductance (g_s) at treatment CO_2 were estimated from the A–C_i curve. Instantaneous water use efficiency (IWUE) was calculated as the ratio of P_n/E. The maximum carboxylation rate of Rubisco (V_{cmax}) and light-saturated electron transport rate (J_{max}) were calculated using the Plantecophys package of R 4.0.2.

Statistical analysis

The data were analysed using the ANOVA function of R 4.0.2 for split-plot design. Multiple comparisons were conducted using the LSD function of R 4.0.2 when an interaction or light effect was significant ($P < 0.05$).

RESULTS

Net photosynthetic rate (P_n) increased with CO_2 concentration and light levels in both measurements and the responses were similar between the two species (Tables 1 and 2; Fig. 1a and b). The degree of the CO_2 enhancement varied with light treatment in the measurement after 2.5 months of treatments (Fig. 1a), but the interactive effect became non-significant after 4.5 months of treatment (Fig. 1b). The average CO_2 stimulation of photosynthesis was the highest under 50% light (81% for black spruce, 92% for white spruce) and lowest under full light (black spruce 29%, white spruce 53%) (Table 3).

The CO_2 elevation significantly decreased transpiration (E) but substantially increased the photosynthetic water use efficiency (IWUE) and the effect on IWUE was influenced by light level (Tables 1 and 2; Fig. 1c–f). The positive effect of CO_2 elevation on IWUE was largest at 50% light and smallest at 100% light, a similar trend as photosynthesis (Table 3). Both E and IWUE generally decreased with decreasing light (Fig. 1c–f).

There were significant interactive effects on g_s among CO_2, light and species after both 2.5 and 4.5 months of treatments (Tables 1 and 2). g_s was generally lower under elevated than ambient CO_2 and lower in white spruce than in black spruce (Fig. 1e and g). Light generally did not significantly affect g_s in white spruce but it did affect it in black spruce and the effect varied with CO_2 treatment:

Table 1: ANOVA P values for effects of CO_2, light, species (Sp) and interactions on P_n (net photosynthesis), E (transpiration rate), g_s (stomatal conductance), IWUE (instantaneous water use efficiency), V_{cmax} (maximum carboxylation rate of Rubisco) and J_{max} (light-saturated electron transport rate)

Parameter	CO_2	Light	CO_2 × light	Sp	CO_2 × Sp	Light × Sp	CO_2 × light × Sp
P_n	**<0.0001**	**<0.0001**	**0.0347**	0.1088	0.8521	0.2650	0.3176
E	**0.0036**	**0.0001**	0.5498	**0.0505**	0.7694	0.3683	0.8517
g_s	**0.0551**	0.6140	0.3160	**0.0035**	0.5023	0.7260	**0.0385**
IWUE	**0.0001**	**0.0008**	**0.0519**	**0.0321**	0.8370	0.0824	0.4422
V_{cmax}	**0.0044**	**0.0479**	0.3278	0.0633	**0.0067**	0.1814	0.6532
J_{max}	**0.0277**	**0.0279**	0.2216	**0.0021**	**0.0008**	**0.0029**	**0.0053**

One-year-old black spruce and white spruce seedlings were grown at two CO_2 concentrations (360 μmol mol^{-1} *vs.* 720 μmol mol^{-1}) and three light conditions (100%, 50% and 30% of the light in the greenhouse) for one growing season and the gas exchange was measured after 2.5 months of treatments. Significant effects are bold-faced.

Table 2: ANOVA P values for effects of CO_2, light, species (Sp) and interactions on P_n (net photosynthesis), E (transpiration rate), g_s (stomatal conductance), IWUE (instantaneous water use efficiency), V_{cmax} (maximum carboxylation rate of Rubisco) and J_{max} (light-saturated electron transport rate)

Parameter	CO_2	Light	CO_2 × light	Sp	CO_2 × Sp	Light × Sp	CO_2 × light × Sp
P_n	**0.0001**	**0.0001**	0.2107	**0.0541**	0.9101	0.8622	0.3484
E	**0.0094**	**0.0001**	0.1153	0.2988	0.1894	0.5794	0.8014
g_s	**0.0405**	0.6540	0.3530	**0.0064**	0.9443	0.2426	**0.0400**
IWUE	**0.0001**	**0.0007**	**0.0427**	0.1148	0.3807	0.4341	0.5140
V_{cmax}	0.7155	**0.0244**	0.5090	0.5541	**0.0085**	0.8055	**0.0438**
J_{max}	**0.0500**	**0.0139**	0.1749	0.9438	0.4434	0.9055	**0.0517**

One-year-old black spruce and white spruce seedlings were grown at two CO_2 concentrations (360 μmol mol^{-1} *vs*. 720 μmol mol^{-1}) and three light conditions (100%, 50% and 30% of the light in the greenhouse) for one growing season and the gas exchange was measured after 4.5 months of treatments. Significant effects are bold-faced.

g_s was highest under full light at ambient CO_2 but highest under 50% light elevated CO_2 (Fig. 1e and g).

The CO_2 elevation significantly reduced the maximum rate of Rubisco carboxylation (V_{cmax}) after 2.5 months of treatment (Table 1). The magnitude of the downregulation increased with decreasing light levels, for instance, the changes for black spruce were −37%, −41% and −67%, respectively, for the light treatments of 100%, 50% and 30% (Fig. 2a) and the downregulation was greater for black spruce than for white spruce (average of 3 light levels: −48% *vs*. −24%). V_{cmax} generally decreased with decreasing light (Fig. 2a). The CO_2 elevation also significantly reduced V_{cmax} after 4.5 months of treatment under 100% and 50% (Table 2; Fig. 2b) and the degree of downregulation decreased with decreasing light (black spruce: −22% and −8% at 100% and 50% light, respectively; white spruce: −36% and −12% at 100% and 50% light, respectively). Again V_{cmax} decreased with decreasing light (Fig. 2b). CO_2 concentration did not significantly affect V_{cmax} at 30% light in either species after 4.5 months of treatment (Fig. 2b).

The CO_2 elevation significantly reduced the light-saturated rate of electron transport (J_{max}) after 2.5 months of treatment and the effect was the least at the moderate light among the three light treatments and greater in black spruce (−39%) than in white spruce (−12%) (Table 1; Fig. 2c). The elevated CO_2 also significantly decreased J_{max} in white spruce at 100% and 50% light after 4.5 months of treatment and the magnitude of the effect decreased with decreasing light levels (−48% and −18%, respectively, at 100% and 50% light), but had no significant effect at 30% light (Fig. 2d; Table 2). Elevated CO_2 also did not significantly affect J_{max} in black spruce at 30% but increased it at 100% and 50% light (Fig. 2d). J_{max} generally decreased with decreasing light in both measurements, both species and both CO_2 treatments (Fig. 2c and d).

DISCUSSION

CO_2 elevation significantly increased the carbohydrate production capacity of black spruce and white spruce and the response was influenced by light conditions. The CO_2 elevation in this study significantly affected all the physiological parameters in such a way that favours higher carbohydrate production. For example, it increased net photosynthetic rate (P_n) and photosynthetic water use efficiency (IWUE), and decreased stomatal conductance (g_s) and transpiration (E). However, the effects of increased IWUE and reduced transpiration on carbohydrate production were indirect, i.e. by reducing the water cost per unit of carbohydrate produced and alleviating the negative impact of low water supply and/or reducing the severity of water stress that the foliage experiences. While the effects observed in this study are generally consistent with the literature (Drake and Leadley 1991; Gunderson *et al.* 2002; Körner 2000; Naumburg *et al.* 2003; Rogers and Ellsworth 2002; Zhang and Dang 2005, 2006), the responses varied with light conditions and tree species, e.g. the CO_2 stimulation was greatest at the modest light level (50%) and least at the highest light (100%) among the three light treatments (Table 3). The net CO_2 stimulations of photosynthesis at 100%, 50% and 30% light were 29%, 81% and 65%, respectively, for black spruce,

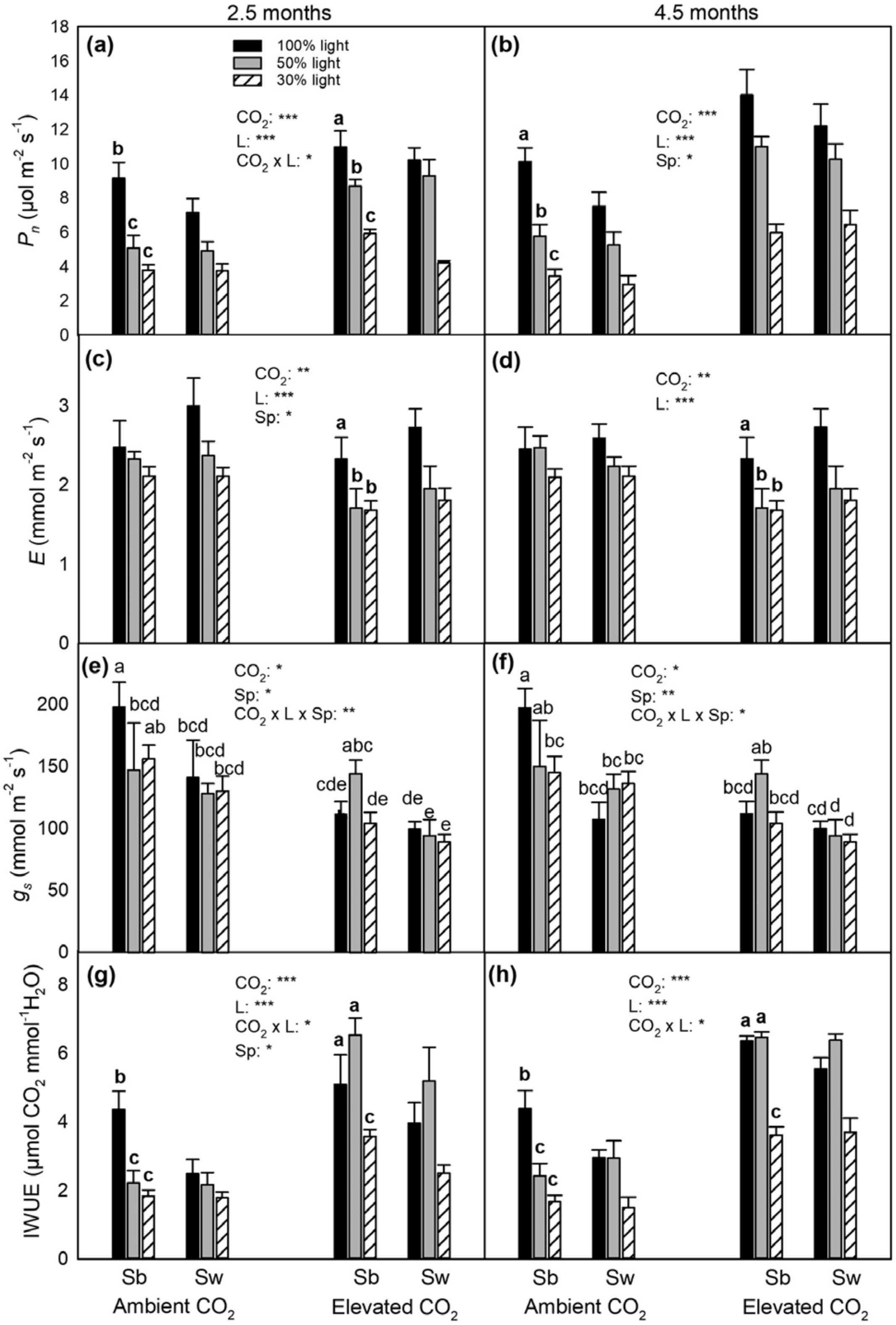

Figure 1: Net photosynthesis (P_n, **a** and **b**), transpiration rate (E, **c** and **d**), stomatal conductance (g_s, **e** and **f**) and photosynthetic water use efficiency (IWUE, **g** and **h**) of black spruce (Sb) and white spruce (Sw). One-year-old seedlings were exposed to two CO_2 concentrations (360 μmol mol^{-1} and 720 μmol mol^{-1}) and three light levels (100%, 50% and 30% of the light in the greenhouse) for one growing season in the greenhouse. A/C_i curves were measured after 2.5-month treatment and again after a further 2-month treatment). Note: ***$P \le 0.001$, **$P \le 0.01$, *$P \le 0.05$. Means with different letters are significantly different from each other at $P \le 0.05$. P_n and E under treatment CO_2 concentration were estimated from the A/C_i measurements.

Table 3: Net effects of CO_2 elevation ((elevated-ambient)/ambient, %) on P_n (net photosynthesis), E (transpiration rate), g_s (stomatal conductance), IWUE (instantaneous water use efficiency), V_{cmax} (maximum carboxylation rate of Rubisco), J_{max} (light-saturated electron transport rate), TM (total biomass), SM (shoot biomass) and SMR (shoot mass ratio) in black (Sb) and white (Sw) spruce

		Light level			
Parameter	**Species**	**100%**	**50%**	**30%**	**Mean**
Net effect (%) on physiology					
P_n	Sb	29	81	65	58
	Sw	53	92	65	70
E	Sb	−5	−29	−20	−18
	Sw	−2	−15	−14	−10
IWUE	Sb	31	181	106	106
	Sw	74	128	94	98
V_{cmax}	Sb	−30	−24	−33	−29
	Sw	−22	−8	−30	−20
J_{max}	Sb	−11	8	−18	−7
	Sw	−24	2	−28	−17
Net effect (%) on biomass					
TM	Sb	74	74	252	133
	Sw	14	45	406	155
SM	Sb	70	73	289	144
	Sw	13	47	497	186
SMR	Sb	−1	−1	4	1
	Sw	−1	1	8	3

Growth responses are calculated from Marfo and Dang (2009) for the purpose of discussion only. The values were averaged of measurements taken after 2.5 and 4.5 months of treatment. See Tables 1 and 2 for other explanations.

53%, 92% and 65%, respectively, for white spruce. The photosynthesis data support the hypothesis that CO_2 elevations will be more beneficial to trees growing under lower light conditions only when the data at 100% and 50% light are compared. A critical implication of the results is that light effect on CO_2 stimulation of photosynthesis depends on the specific light conditions. For instance, the comparison of the results at 100% and 50% would lead to the conclusion that the CO_2 stimulation of photosynthesis increased with decreasing light whereas the comparison of results between 50% and 30% light would lead to an opposite conclusion that CO_2 stimulation to photosynthesis decreased with decreasing light. These results suggest that the primary response mechanism is determined by the primary limiting factor. For instance, light was presumably the primary limiting factor to photosynthesis in the 30% light treatment in this study and the improved photosynthetic carboxylation efficiency by elevated CO_2 permitted the trees to increase the total leaf area (Marfo and Dang 2009) so that more light could be captured and the morphological acclimation was the primary response mechanism. The shift in acclimation strategy in response to elevated CO_2 at different light conditions represents a key finding of this study for understanding the ecophysiological responses of trees to climate change. However, our findings also point to the danger of drawing general conclusions from experiments with limited levels of treatment without qualifying the conclusions. We need to be extremely careful when comparing the results of different studies, particularly where different light levels are used in different studies. We recommend that multiple levels of treatment be used and the interpretation of results should be focussed on the pattern of the response or response curve unless we are only interested in some specific levels of the treatment. Furthermore, our results indicate that the continued increases in atmospheric CO_2 will likely be more beneficial to seedlings and saplings of the two species growing under the partially shaded conditions under the forest canopy than those growing in the open field, effectively increasing the shade tolerance of the two species and promoting their understorey regeneration. Currently, tree planting following the clear-cutting of forest stands is a common practice in forest management. Our results suggest that regenerating the two species prior to the harvesting of the existing forest stands and allow the young trees to grow under the canopy of the existing trees will likely improve the growth of the new generation. Such a practice will effectively shorten the rotation of the forest and provide more favourable environmental conditions than the traditional clear-cutting and tree planting because the two generations of trees will share the growing space before the older trees are harvested.

The trend of light effect on CO_2 stimulation to photosynthesis differs from the trend of light effect on seedling biomass. In a previous study, Marfo and Dang (2009) observe that the increase of biomass by CO_2 elevation is more than 3-folds greater at 30% light than at 50% and 100% light (Table 3). Danyagri and Dang (2014a) also observe that mountain

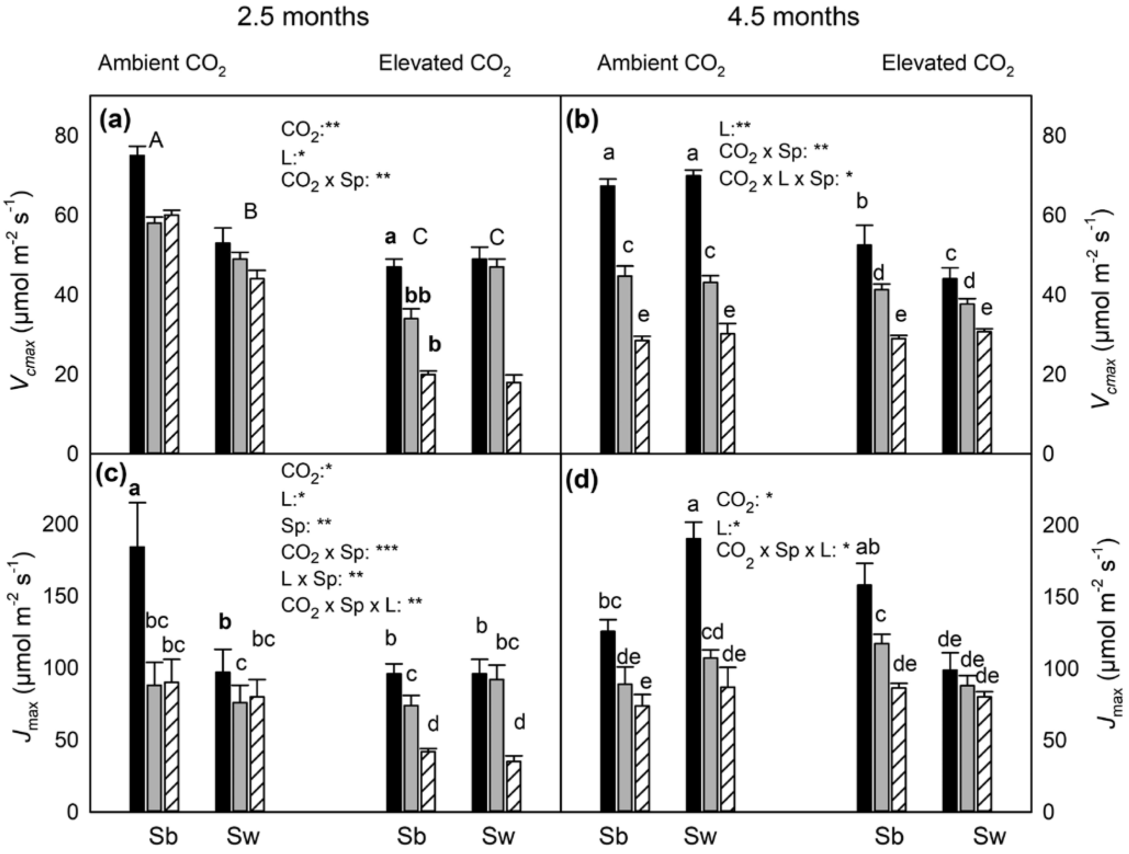

Figure 2: Maximum rate of Rubisco carboxylation (V_{cmax}, **a** and **b**) and light-saturated electron transport rate (J_{max}, **c** and **d**) of black spruce (Sb) and white spruce (Sw). See Fig. 1 for more explanations.

maple, a shade tolerant tree species, benefits more from CO_2 elevation under lower light. However, the current study shows that the CO_2 stimulation to photosynthesis was highest at 50% light and lowest at 100% light. While it is intuitive that photosynthesis and growth/biomass should be positively correlated, photosynthetic rate expressed on a per unit leaf area or mass basis may not correlate with growth. Growth and biomass production are more influenced by carbon budget and biomass allocation than by photosynthetic rate (Lambers and Oliveira 2019). Photosynthesis is only one of the multiple physiological and morphological mechanisms that plants can use in responding to changes in the environment and the primary mechanism of response generally varies with the specific environment conditions and species. For instance, morphological adjustment is the primary mechanism of white birch in response to phosphorus availability under (Danyagri and Dang 2014b). Furthermore, growth is generally more closely correlated to the total photosynthesis of the tree than to photosynthetic rate per unit leaf area (Dang *et al.* 2020).

The finding that CO_2 stimulation to photosynthesis was greatest at the moderate light condition is surprising but provides a key piece of evidence that the two spruce species used different mechanisms in responding to different combinations of CO_2 and light conditions. Both elevated CO_2 and lower light can increase photosynthesis by reducing light compensation point and dark respiration (reduction in Rd is less common for elevated CO_2 than for lower light) and increasing light use efficiency (Körner 2006; Kubiske and Pregitzer 1996; Long and Drake 1991; Man and Lieffers 1997; Osborne *et al.* 1997; Walters and Reich 1999; Ward and Strain 1999). The results of this study suggest that the effects of elevated CO_2 and lower light can be synergistic within a certain range. Indeed, the CO_2 stimulation to photosynthesis was much greater at 50% light than at 100% light. However, our data suggest that the 30% light was too low to support a higher photosynthetic capacity and instead triggered the switching of response mechanism from physiology to morphology. Indeed, the specific leaf area of the two species at 30% light was more than double of those at 50% and 100% and the differences are larger under the elevated than ambient CO_2 (Marfo and Dang 2009), indicating the species produced much thinner foliage under the very low light condition. Apparently, the switching from physiological to morphological response was very effective and led to much greater growth response to the CO_2 elevation at 30% light than at 50% and 100% light (Marfo and Dang 2009). The effects of elevated CO_2 and low light were synergistic at both 30% light and 50% light but the synergy was achieved via different response mechanisms (morphological *vs*. physiological).

Our results suggest that elevated CO_2-induced photosynthetic response could vary with light supply, species shade tolerance and time. While the elevated CO_2 reduced both V_{cmax} and J_{max} in both species in mid-growing season in this study, the effect became insignificant in the lowest light treatment in later in the growing season. While the elevated CO_2-induced downregulation of V_{cmax} was greater in the more shade tolerant black spruce than white spruce, the downregulation of J_{max} was greater in white spruce than in black spruce (Table 3). In fact, the black spruce seedlings grown under 100% and 50% and elevated CO_2 increased J_{max} while white spruce decreased it (Fig. 2d). The opposite response of the two species and between V_{cmax} and J_{max} is intriguing. Generally, the photosynthetic mechanisms of more shade tolerant species are more prone to photodamages when they are exposed to high light and their electron transport capacity exceeds the carboxylation capacity (Lambers and Oliviera 2019). Thus, a low electron transport capacity and a higher capacity of other energy dissipation mechanisms should act to protect the photosynthetic machinery. However, when the condition becomes more favourable to carboxylation, such as under elevated CO_2, a higher J_{max} is needed to re-balance the biochemical and photochemical reactions of photosynthesis. While this explanation is plausible for explaining our results, the responses of different energy dissipation mechanisms and the balance and re-balance of photosynthetic processes in the two species to elevated CO_2 need to be investigated. Furthermore, the interactive effects of shade tolerance, light supply, time and CO_2 present new challenges to modellers in their efforts to predict the responses of trees, forest stands and forest ecosystems to climate change. More research in this area should help to improve the reliability and precision of model predictions, particularly process-based models.

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