



Effects of elevated [CO₂] and soil temperature on photosynthetic responses of mountain maple (*Acer spicatum* L.) seedlings to light



Gabriel Danyagri*, Qing-Lai Dang

Faculty of Natural Resources Management, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B 6 5E1, Canada

ARTICLE INFO

Article history:

Received 30 January 2014

Received in revised form 21 May 2014

Accepted 23 May 2014

Keywords:

Elevated CO₂

Elevated T_{soil}

Photosynthesis

Instantaneous water-use efficiency

Boreal forest

Global change

ABSTRACT

Predicting the future distribution of plants requires a mechanistic understanding of how plants cope with their new environmental conditions under a changed climate. We examined the effects of elevated carbon concentration ([CO₂]) and elevated T_{soil} on the physiological responses to light of a shade-tolerant woody species, mountain maple (*Acer spicatum* L.). Seedlings were exposed to ambient and elevated [CO₂] (392 μmol mol⁻¹ vs. 784 μmol mol⁻¹), current and elevated soil temperatures (T_{soil} 17 vs. 22 °C) at high and low (100% vs. 30%) light conditions for one growing season. It was found that elevated T_{soil} stimulated net photosynthesis (A) by 18% in the low-light treatment but tended to reduce A in the high-light treatment. Elevated [CO₂] increased A by 100% under the elevated T_{soil} and by 48% at the current T_{soil}. The effect of elevated T_{soil} also varied with [CO₂]: it increased A by 13% under the elevated [CO₂] but reduced A by 17% under the ambient [CO₂]. We observed a significant increase in the instantaneous water-use efficiency of photosynthesis (IWUE) in response to the high-light treatment under the elevated [CO₂] but not under the ambient [CO₂]. Our data indicate that the elevated [CO₂] markedly increased nitrogen (N) allocation to the photochemical apparatus of A in seedlings grown under the high-light condition. These results suggest that the predicted increases in atmospheric [CO₂] will likely enhance the growth of mountain maple in canopy gaps in the forest, while the predicted elevated T_{soil} will negate the benefit of high-light conditions.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The ability of shade-tolerant species to utilize increased light supply for net photosynthesis (A) is an important determinant of regeneration (Kubiske and Pregitzer, 1997). However, the physiological responses of shade-tolerant tree seedlings to increased light levels may be limited by other stress factors, e.g., the mid-day depression of A associated with stomatal closure (Krause et al., 2006; Zotz et al., 1995). Leaf-water deficit and the photoinhibition of A have also been observed under high-light conditions (Bazzaz and Wayne, 1994; Krause et al., 2012; Osmond et al., 1999; Pearcy, 1990). However, some shade tolerant species can compensate for the costs associated with continuous high-light exposures (Krause et al., 2006). While the prevailing light regime is often thought to be the major determinant of plant physiological responses to changes in light conditions, the responses may vary with changes in other

factors, such as carbon dioxide concentration ([CO₂]) and soil temperature (T_{soil}). However, there are currently little quantitative data on how elevated [CO₂] and elevated T_{soil} may interactively affect the physiological responses of shade-tolerant species to changes in light conditions. This makes it difficult to include these factors in models for predicting the physiological adaptation and acclimation of shade tolerant species to climate changes.

Increases in the atmospheric [CO₂] can substantially enhance A in C₃ plants (Ellsworth et al., 2004; Norby et al., 1999; Nowak et al., 2004; Onoda et al., 2007, 2009; Sefcik et al., 2006; Takeuchi et al., 2001) because the present [CO₂] does not saturate the major photosynthetic enzyme, Rubisco (Drake et al., 1997; Lambers et al., 2008). Furthermore, elevated [CO₂] reduces the rate of photorespiration, resulting in increased quantum yield and decreased light compensation point of A (Osborne et al., 1997; Saxe et al., 1998). Therefore, elevated [CO₂] can potentially enhance the carbon acquisition under both low and high-light conditions. However, the majority of studies on CO₂ and light typically focussed on the effects of elevated [CO₂] on plants grown under low-light environments (Hättenschwiler and Körner, 2000; Liang et al., 2001; Takeuchi et al., 2001). Hence, there is a general lack of understanding on how

* Corresponding author. Tel.: +1 8073438519.
E-mail address: gdery@lakeheadu.ca (G. Danyagri).

elevated $[\text{CO}_2]$ and high-light interact to affect the physiological traits of shade-tolerant plants.

It is predicted that the increase in the atmospheric $[\text{CO}_2]$ will lead to increases in air and soil temperatures (IPCC, 2007). Elevated T_{soil} can induce changes in leaf physiology and morphology (Ambebe et al., 2010; King et al., 1999; Stoneman and Dell, 1993), root growth and biomass allocation (Boucher et al., 2001). Elevated T_{soil} enhances A through improving nutrients and water uptake (Fitter et al., 1998; Long and Woodward, 1998; Wan et al., 1999) and increasing sink strength (Boucher et al., 2001; Lyr and Garbe, 1995). Elevated T_{soil} can also influence the physiological responses of plant to other environmental factors. For example, Ambebe et al. (2010) demonstrate that elevated T_{soil} enhances the stimulatory effects of elevated $[\text{CO}_2]$ on A in *Betula papyrifera* M. seedlings. Boucher et al. (2001) also report that the physiological processes of *Pinus strobus* L. seedlings are more responsive to light conditions in elevated soils. The influence of elevated T_{soil} on photosynthetic responses to elevated $[\text{CO}_2]$ and increased light availability has been ascribed to changes in sink strength or nutrients uptake. Increased sink strength or carbohydrates utilization (TPU) at elevated $[\text{CO}_2]$ and warmer soils should help maintain higher photosynthetic rates in high-light conditions. Thus, we hypothesize that elevated $[\text{CO}_2]$ and elevated T_{soil} would increase A under high-light conditions.

In this study, we investigated the effects of elevated $[\text{CO}_2]$ and elevated T_{soil} on the physiological responses of mountain maple seedlings to light supply. Mountain maple is an aggressive understorey shrub species in mixed-species boreal forests (Archambault et al., 1998). It colonizes the understorey of dense multi-layered forest stands but gains dominance upon release from shading (Aubin et al., 2005; Rook, 2002; Sullivan, 1993). Once established, mountain maple often forms a dense canopy that reduces the amount of light reaching the forest floor (Aubin et al., 2000, 2005; Lei and Lechowicz, 1990). Therefore, mountain maple generally impedes the recruitment of commercial tree species and the growth of advanced regeneration once it dominates following disturbance (Archambault et al., 1998; Aubin et al., 2005; Post, 1970). How mountain maple will respond to changes in light conditions associated with canopy gaps under changed climate conditions may have important ecological implications on the species composition and distribution of boreal forests in the future. We tested the hypotheses that: (i) elevated $[\text{CO}_2]$ would lead to a greater photosynthetic enhancement in mountain maple seedlings at elevated T_{soil} and (ii) the beneficial effect of elevated T_{soil} on A would be greater at a higher light level.

2. Materials and methods

2.1. Plant material

Mountain maple seeds were collected from the Lakehead University Jack Haggerty Forest. The forest is located approximately 37 km north of Thunder Bay, ON (48°22'56" N, 89°14'46" W). The seeds were soaked in 1000 $\mu\text{mol mol}^{-1}$ gibberellic acid (GA) solution for 24 h and then stratified at 4 °C for 2 months in germination trays covered with moist paper towels. Following the stratification, seed coats were cracked open to facilitate germination. The seeds were planted in a mixture of peat moss and vermiculite (2:1, v/v). Three weeks after the completion of germination, 160 seedlings (10 per treatment combination) with relatively uniform height were transplanted into plastic containers (31.5 cm tall, 11 cm top diameter and 9.5 cm bottom diameter) for treatments.

2.2. Experimental design

The experiment was setup as a split-split-plot design with $[\text{CO}_2]$ as the whole-plot (392 vs. 784 $\mu\text{mol mol}^{-1}$ $[\text{CO}_2]$), two replications

for each), T_{soil} as the split plot nested within $[\text{CO}_2]$ (17 vs. 22 °C) and light as the split-split-plot nested within T_{soil} (30% vs. 100%). The CO_2 control was achieved using Argus CO_2 generators (Argus systems Ltd, Vancouver, BC, Canada). The T_{soil} of 17 °C represents the mean growing season T_{soil} in the boreal forest zone (Domisch et al., 2001) while the 22 °C T_{soil} reflects the prediction that the average T_{soil} for the region would increase by about 5 °C by 2100 (Christensen et al., 2007; IPCC, 2007). T_{soil} was regulated by circulating temperature-controlled water around the pots in a control box (see Cheng et al., 2000 for a detailed description). Half of the seedlings in each T_{soil} treatment were shaded using neutral density shading material to reduce the photosynthetic photon flux density (PAR) by 70%. We used high-pressure sodium lamps (Model LR48877, P.L. Systems, Grimsby, ON, Canada) to supplement the natural light in the greenhouse on cloudy days, and to lengthen the photoperiod to 16 h (maximum summer photoperiod for the Thunder Bay region according to Environment Canada Weather Report, 2010).

Other environmental conditions in each greenhouse were 22/16 °C day/night air temperature and 50% relative humidity. All the environmental conditions were controlled and monitored using an Argus environmental control system (Argus Systems Ltd., Vancouver, BC, Canada). Nutrients were added to the irrigation water twice a week at concentration 100, 15, 57, 6, 6 and 11 mg/L concentrations for N, P, K, Ca, Mg and S, respectively (Canham et al., 1996; Ingestad, 1981). The seedlings were watered daily.

2.3. Photosynthetic light response

After 4 months of treatments, five seedlings per treatment combination in each CO_2 replicate were randomly selected for the measurement. The measurement was done between 10 and 15 h with an open gas exchange system (LI-6400, LI-COR, Inc., Lincoln, NE, USA) at 1100, 800, 400, 100, 60, 10 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (supplied from red LED) on a mature leaf randomly selected from the top 4th–6th leaf. The measurements were taken at 400 $\mu\text{mol mol}^{-1}$ CO_2 for the ambient CO_2 treatment and at 800 $\mu\text{mol mol}^{-1}$ CO_2 for the elevated $[\text{CO}_2]$ treatment. Leaf temperature and relative humidity in the leaf chamber were set at 22 °C and 50%, respectively. The light compensation points (LCP) and apparent quantum efficiency of photosynthesis were determined using the Photosyn Assistant software (Dundee Scientific, Scotland, UK).

2.4. Photosynthetic CO_2 response

Photosynthetic responses to $[\text{CO}_2]$ (A/C_i curves) were measured on the same seedlings and leaves used for the light response measurement. The measurements were taken at seven $[\text{CO}_2]$ levels: 50, 100, 200, 400, 800, 1000 and 1500 $\mu\text{mol mol}^{-1}$. The PAR, leaf temperature and relative humidity in the leaf chamber were set at 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 22 °C and 50%, respectively. The instantaneous water use efficiency of photosynthesis (IWUE) was determined by dividing A by transpiration rate (E). The ratio of intercellular $[\text{CO}_2]$ (C_i) to external $[\text{CO}_2]$ (C_a) was calculated. C_i/C_a indicates the relative limitation by stomatal and non-stomatal factors to photosynthetic CO_2 assimilation.

The A/C_i curves were analyzed using the A/C_i curve fitting utility version 1.1 developed by Sharkey et al. (2007). The maximum rate of ribulose-1,5-bisphosphate (RuBP) carboxylation (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), maximum rate of photosynthetic electron transport (J , $\mu\text{mol m}^{-2} \text{s}^{-1}$), the rate of triose phosphate utilization (TPU, $\mu\text{mol m}^{-2} \text{s}^{-1}$) and dark respiration (R_d , $\mu\text{mol m}^{-2} \text{s}^{-1}$) were obtained from the analyses. V_{cmax} , J and TPU values were adjusted to the set leaf temperature, to correct for temperature variations during the measurement (Sharkey et al., 2007). V_{cmax} , J and TPU

Table 1
Summary of ANOVA (P values) on net photosynthesis (A), stomatal conductance (g_s), instantaneous water-use efficiency (IWUE), the ratio of internal to external partial CO_2 pressure (C_i/C_a), maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J), J/V_{cmax} ratio, triose phosphate utilization (TPU), day respiration (R_d), photosynthetic light compensation point (LCP) and apparent quantum efficiency in mountain maple seedlings. Seedlings were grown at ambient ($392 \mu\text{mol mol}^{-1}$) or elevated ($784 \mu\text{mol mol}^{-1}$) $[\text{CO}_2]$, current (17°C) or elevated (22°C) T_{soil} and high (100%) or low (30%) light (L) for one growing season. Significant ($P \leq 0.05$) treatment effects are highlighted in bold.

Source of variation	C	T	C × T	L	C × L	T × L	C × T × L
A	0.0065	0.8071	0.0114	0.0004	0.6714	0.0143	0.9382
g_s	0.7402	0.2021	0.5550	0.2525	0.9746	0.0694	0.2424
IWUE	0.0121	0.1924	0.4097	0.0794	0.0345	0.8345	0.9473
C_i/C_a	0.0403	0.1465	0.3492	0.1207	0.6408	0.7688	0.3681
V_{cmax}	0.0088	0.0808	0.9589	0.7091	0.0078	0.4507	0.4737
J	0.2312	0.4288	0.4622	0.2529	0.0331	0.0180	0.4658
J/V_{cmax}	0.0563	0.1814	0.4841	0.0515	0.0274	0.8490	0.6140
TPU	0.112	0.3119	0.9186	0.0134	0.1854	0.0744	0.4364
R_d	0.1177	0.9318	0.5950	0.0022	0.4190	0.9431	0.9800
LCP	0.0239	0.9425	0.9227	≤ 0.0001	0.6894	0.7512	0.5888
ϕ	0.0045	0.9652	0.6799	0.8678	0.2262	0.5753	0.3722

indicate the rate-limiting reactions of photosynthesis. V_{cmax} and J limit A at low and high $[\text{CO}_2]$, respectively, while TPU limitation occurs when there is a higher rate of production than the capacity to use RuBP (Lambers et al., 2008; Onoda et al., 2005; Sharkey et al., 2007). Nitrogen partitioning between rubisco carboxylation and ribulose biphosphate (RuBP) regeneration (Hikosaka, 2005; Onoda et al., 2009) was estimated using the J/V_{cmax} ratio.

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to test the main and interactive effects of $[\text{CO}_2]$, T_{soil} and light. The normality of distribution and homogeneity of variance were examined graphically using probability plots and histograms, respectively. All the data met those two assumptions of ANOVA. The Scheffé's post hoc test was performed when ANOVA showed a significant interaction ($P \leq 0.05$). The Data Desk 6.01 statistical software (Data Description 1996) was used for the analyses.

3. Results

There were significant interactive effects between T_{soil} and light, and between $[\text{CO}_2]$ and light on A (Table 1). The high-light treatment increased A by 41% at the current T_{soil} but had no significant effect on A at the elevated T_{soil} (Fig. 1A). In the low-light treatment, elevated T_{soil} increased A by 18% (Fig. 1A). No significant effect of elevated T_{soil} on A was found in the high-light treatment although A appeared to have decreased (Fig. 1A). Under the ambient $[\text{CO}_2]$, the elevated T_{soil} reduced A by 17% (Fig. 1B). In contrast, elevated T_{soil} increased A by 13% under the elevated $[\text{CO}_2]$ (Fig. 1B). Furthermore, the elevated $[\text{CO}_2]$ increased A by 48% and 100% at the current and elevated T_{soil} , respectively (Fig. 1B).

There was a significant interactive effect of $[\text{CO}_2]$ and light on IWUE (Table 1). While the light levels had no significant effect on IWUE under the ambient $[\text{CO}_2]$, the high-light treatment increased IWUE by 18% under the elevated $[\text{CO}_2]$ (Fig. 1C). The elevated $[\text{CO}_2]$ increased IWUE by 79% and 125% in the low- and high-light treatment, respectively (Fig. 1C). No interactions among light, T_{soil} and $[\text{CO}_2]$ or between T_{soil} and light or $[\text{CO}_2]$ were significant (Table 1). The elevated $[\text{CO}_2]$ significantly reduced C_i/C_a ratio (0.65 at ambient vs. 0.63 at the elevated $[\text{CO}_2]$) but no other factors or their interactions significantly affected C_i/C_a (Table 1).

The $[\text{CO}_2]$ -light interaction significantly influenced the maximum rate of carboxylation (V_{cmax}), maximum rate of photosynthetic electron transport and J/V_{cmax} ratio (Table 1). In the high-light treatment, V_{cmax} and J were significantly lower, while J/V_{cmax} was significantly greater, in seedlings grown in the elevated $[\text{CO}_2]$ than those grown in the ambient $[\text{CO}_2]$ (Fig. 2A–C). In contrast, $[\text{CO}_2]$ did

not significantly affect V_{cmax} , J or J/V_{cmax} in the low-light treatment. Under the ambient $[\text{CO}_2]$, V_{cmax} and J were significantly greater in the high-light treatment than the low-light treatment, but light had no significant effect on the J/V_{cmax} ratio (Fig. 2A–C). Under the elevated $[\text{CO}_2]$, the high-light treatment significantly decreased V_{cmax} but increased J/V_{cmax} while it had no significant effect on J (Fig. 2A–C).

The maximum rate of photosynthetic electron transport (J) was significantly affected by the light- T_{soil} interaction (Table 1). In the elevated T_{soil} treatment, light levels did not affect J significantly. In the current T_{soil} , however, J was 16% greater in the high than the low-light treatment (Fig. 2D). At the low-light level, J was significantly greater in the elevated T_{soil} than in the current T_{soil} treatment (Fig. 2D). In the high-light treatment, J did not significantly differ between the current and elevated T_{soil} treatments, although J appeared to be lower in seedlings grown at the elevated T_{soil} (Fig. 2D). TPU was significantly greater in the high than the low-light treatment (13.08 vs. 11.82 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Table 1). $[\text{CO}_2]$ and T_{soil} had no significant effects on TPU (Table 1).

Light levels significantly affected the rate of dark respiration (R_d) (Table 1). R_d was 24% higher in the high than in the low-light treatment (1.36 at the low-light vs. 1.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the high-light). The elevated $[\text{CO}_2]$ reduced the LCP of A by 21% (14.9 at the ambient vs. 11.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the elevated $[\text{CO}_2]$, Table 1). Furthermore, the high-light treatment increased LCP by 133% (8.0 at low-light vs. 18.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at high-light) (Table 1). The apparent quantum efficiency increased significantly in response to the elevated $[\text{CO}_2]$ (0.045 at the ambient vs. 0.050 $\text{mol CO}_2 \text{ mol}^{-1}$ at the elevated $[\text{CO}_2]$) (Table 1).

4. Discussion

The elevated $[\text{CO}_2]$ enhanced the elevated T_{soil} stimulation of A but reduced the responsiveness of A , J and TPU to light. We found that the elevated $[\text{CO}_2]$ increased A of mountain maple by 100% at the elevated T_{soil} but only 48% at the current T_{soil} . Because CO_2 is a substrate for A , an increase in $[\text{CO}_2]$ stimulates A in C_3 plants (Norby et al., 1999; Sefcik et al., 2006). Elevated $[\text{CO}_2]$ also increases the photosynthetic enzyme activity in well-fertilized seedlings of other boreal woody species (Zhang and Dang, 2006). The stimulation of A by the elevated T_{soil} could be attributed to increased sink capacity for A . Furthermore, water viscosity decreases while root growth increases with elevated T_{soil} , leading to enhanced water and nutrient uptake by the roots (Boucher et al., 2001; King et al., 1999). Several other studies have reported that elevated T_{soil} up to a threshold T_{soil} increases water and nutrients absorption (Dawes et al., 2011; Weih and Karlsson, 2002), which in turn increases A . Efficient nutrients absorption coupled with increased substrate

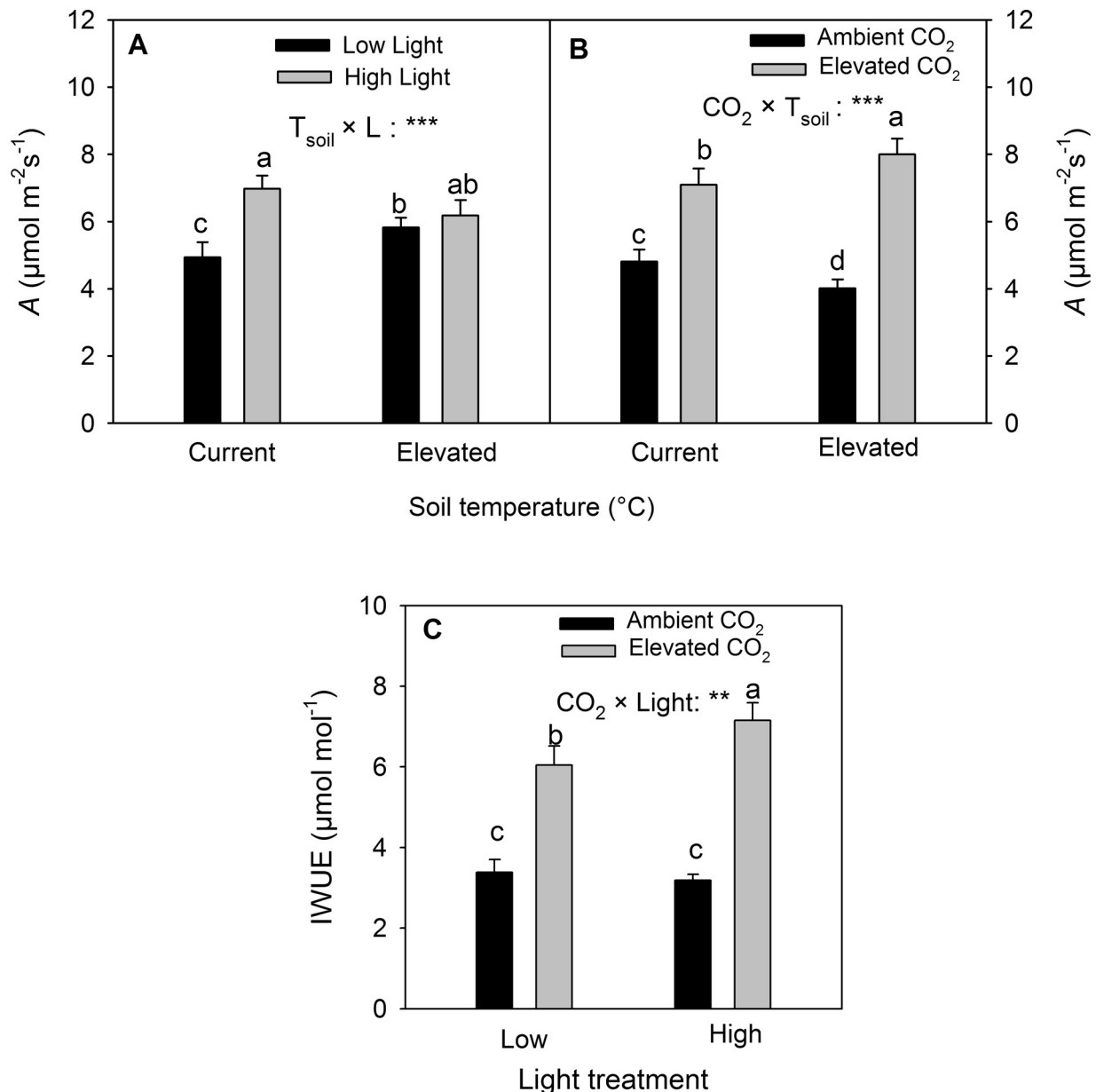


Fig. 1. Net photosynthesis (A) (A and B) and instantaneous water-use efficiency (IWUE) (C) of mountain maple (*Acer spicatum* L.) seedlings grown at high-light or low-light, ambient ($392 \mu\text{mol mol}^{-1}$) or elevated ($784 \mu\text{mol mol}^{-1}$) [CO_2], and current (17°C) or elevated (22°C) T_{soil} treatments. Measurements were taken after one growing season. Data are pooled across [CO_2] treatments for Fig. 1A, light treatments for Fig. 1B and T_{soil} for Fig. 1C because of the lack of a significant effect ($P > 0.05$). Different letters depict significant differences between treatments ($P \leq 0.05$) determined by ANOVA. Significant effects are marked as: *** $P \leq 0.01$, ** $P \leq 0.05$.

(CO_2) availability and enhanced photosynthetic enzyme activity could have led to the greater stimulation of A by elevated [CO_2] at the elevated T_{soil} in this study. Ambebe et al. (2010) have reported similar results for white birch (*B. papyrifera* M.).

A surprising observation in this study was the reduction of A in response to elevated T_{soil} in seedlings grown under the ambient [CO_2]. A and T_{soil} are generally positively correlated (Boucher et al., 2001; Day et al., 1991; King et al., 1999). It is possible that the carbohydrate production under the ambient [CO_2] could not satisfy the increased demand by the stimulated root respiration under the elevated T_{soil} , leading to reduced water and nutrient uptake by roots and subsequent stresses in the leaves (Atkin et al., 2000; Chmura et al., 2011; Pregitzer et al., 2000). This argument is supported by the greater stimulation of A by the elevated [CO_2] at the elevated than at the current T_{soil} . Similar findings are reported by other researchers (Chmura et al., 2011; Long, 1991; Saxe et al., 1998).

For example, Ambebe and Dang (2009) find that A of *B. papyrifera* M. seedlings increases with an increase in T_{soil} under elevated [CO_2] but not ambient [CO_2]. Our observation indicates that as the atmospheric [CO_2] increases, A of mountain maple will respond strongly to elevated T_{soil} across a range of light conditions.

In this study, the A response to elevated T_{soil} was similar in seedlings in the low-light and high-light treatments. This is in contrast to our prediction that elevated T_{soil} would stimulate A to a greater extent under the high-light than the low-light treatment. The lack of significant difference in the response between the two light treatments suggests that the light conditions did not influence the ability of the species to respond to elevated T_{soil} . In other words, Elevated T_{soil} would be equally beneficial to mountain maple growing under different light conditions. Therefore, the predicted increases in T_{soil} will not likely influence the colonization of mountain maple in forest canopy gaps.

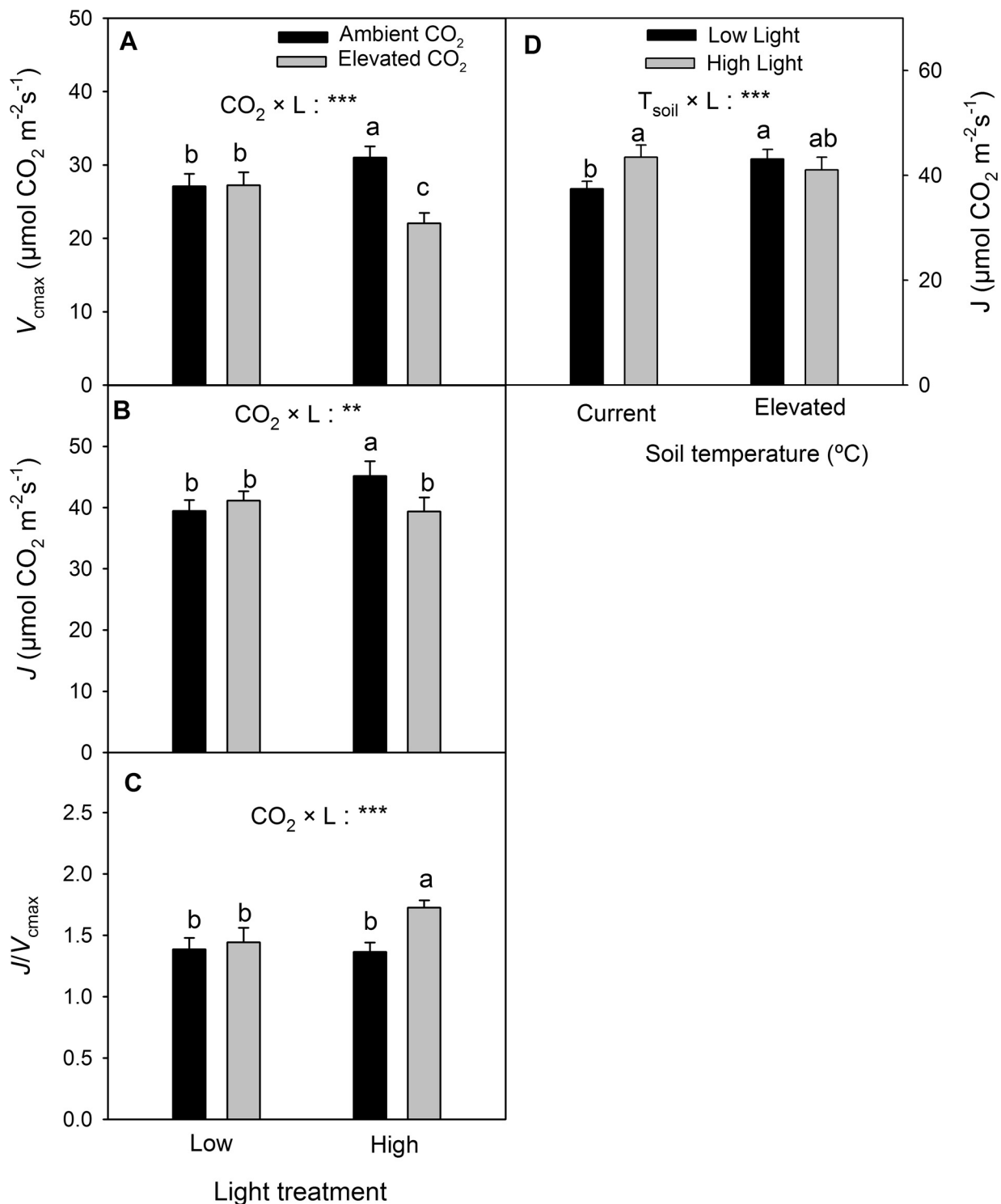


Fig. 2. Maximum carboxylation rate (V_{cmax}) (A), maximum electron transport rate (J) (B and D) and J/V_{cmax} ratio (C) of mountain maple seedlings. Data are pooled across T_{soil} treatments for Figs. 2A–2C and $[\text{CO}_2]$ treatments for Fig. 2D because of the lack of a significant effect ($P > 0.05$). Refer to Fig. 1 for other descriptions.

The elevated $[\text{CO}_2]$ enhanced the light stimulation of IWUE. The high-light treatment increased IWUE by 18% under the elevated $[\text{CO}_2]$ but appeared to have decreased IWUE under the ambient $[\text{CO}_2]$ (not significant statistically). It is interesting to note that neither A nor g_s was significantly affected by the $[\text{CO}_2] \times$ light interaction in this study. Generally, an increase in IWUE under elevated $[\text{CO}_2]$ is the result of increased A and/or decreased g_s (Onoda et al., 2009; Saxe et al., 1998). Whole-plant morphological acclimation to elevated $[\text{CO}_2]$ may also have contributed to the increase in IWUE

in seedlings in the high-light treatment. Elevated $[\text{CO}_2]$ tends to decrease the leaf area to root mass ratio (LARMAR) so that plants growing under elevated $[\text{CO}_2]$ have greater IWUE than those under ambient $[\text{CO}_2]$ (Norby and O'Neill, 1991). Poorter (1999) has also reported that decreases in LARMAR enhance IWUE in the seedlings of 15 rain-forest tree species grown in high-light conditions. The LARMAR also decreased under the elevated $[\text{CO}_2]$ in the high-light treatment in this study (Danyagri and Dang, 2013). Thus, it is reasonable to conclude that increased biomass investment in the root

system under the elevated $[\text{CO}_2]$ had a greater influence on IWUE than did gas exchange parameters in mountain maple. The elevated CO_2 -mediated increase in IWUE may be particularly important for improving the drought tolerance of mountain maple seedlings growing in canopy gaps under future climate conditions.

We found a strong down-regulation of A in mountain maple seedlings in responses to the elevated $[\text{CO}_2]$ and high-light treatment. Our results indicate that the elevated $[\text{CO}_2]$ significantly reduced V_{cmax} and J in seedlings in the high-light treatment. Consequently, A in seedlings under the elevated $[\text{CO}_2]$ was not significantly different from that under the ambient $[\text{CO}_2]$ across the light treatments. Reductions in V_{cmax} and J under elevated $[\text{CO}_2]$ are generally accompanied by a decline in foliar N concentration (Ellsworth et al., 2004; Medlyn et al., 1999; Nowak et al., 2004). Increased sink strength due to the close proximity of upper canopy leaves to rapidly growing tissues can lead a lack of photosynthetic down-regulation in the upper canopy (Kubiske et al., 1997; Takeuchi et al., 2001). Although seedlings in this study were regularly fertilized, the faster growth rates under the elevated $[\text{CO}_2]$ in the high-light treatment could have diluted foliar N concentration (Coleman et al., 1993; Kubiske and Pregitzer, 1997), leading to the reductions in V_{cmax} and J . Because upper canopy leaves were used for the gas exchange measurements, reduced foliar N rather than sink limitation may be the primary cause of the declines in V_{cmax} and J in this study.

Elevated $[\text{CO}_2]$ increased stomatal limitation to A but positively affected light response parameters of A . Both C_i/C_a ratio and LCP decreased while the apparent quantum yield increased in response to the elevated $[\text{CO}_2]$. A reduction in the C_i/C_a ratio is generally observed when A is more limited by the stomata than by non-stomata factors (Ambebe and Dang, 2009; Flexas et al., 2004). The accumulation of carbohydrates in leaves due to higher photosynthetic rate under elevated $[\text{CO}_2]$ has been found to restrict CO_2 diffusion (Stitt, 1991). Although there was no significant reduction of g_s in response to the elevated $[\text{CO}_2]$ in this study, our result indicates that stomatal limitation to A still reduced the potential stimulation of A by the elevated $[\text{CO}_2]$. Increased quantum yield and lower LCP resulting from higher RuBP carboxylation (inhibition of photosynthetic carbon oxidation, PCO) and lower respiration rates have been reported in C_3 plants grown under elevated $[\text{CO}_2]$ (Kubiske and Pregitzer, 1996; Osborne et al., 1997). More efficient photosynthetic light use and decreased respiratory carbon loss increase A in elevated $[\text{CO}_2]$ (Drake et al., 1997; Kubiske and Pregitzer, 1996). The elevated CO_2 -induced decreases in LCP and increases in quantum yield may compensate for the increased stomatal limitation to A , and improve plant carbon gain (Kubiske and Pregitzer, 1996; Liang et al., 2001).

We acknowledge the limitations in the model used to estimating the V_{cmax} and J in this study. However, since our biochemical parameters are within the range of published data estimated from other photosynthetic models (Ambebe et al., 2010; Takeuchi et al., 2001), we believe that our values of these parameters are reasonable. This study was restricted to short-term responses of mountain maple seedlings that may differ from mature trees growing in the field (Cavender-Bares and Bazzaz, 2000; Zotz et al., 2005). Therefore, considerable precautions should be taken in extrapolating the physiological response of mountain maple seedlings to light, elevated $[\text{CO}_2]$ and elevated T_{soil} . Further long-term experiments with mature mountain maple trees are needed to determine future response patterns.

In conclusion, we found that A responded positively to the combination of elevated $[\text{CO}_2]$ and high-light regardless of T_{soil} . The study demonstrated that elevated $[\text{CO}_2]$ alleviated the elevated T_{soil} -induced stress on photosynthesis. These results suggest that, for this species and potentially other light-foraging shade-tolerant species, increases in $[\text{CO}_2]$ may stimulate A , whereas elevated

T_{soil} may reduce A in high-light environments under the current $[\text{CO}_2]$. The results of this study also demonstrate the importance of whole-plant morphological acclimation in influencing the physiological responses of plants, such as IWUE, to environmental factors, e.g. light and $[\text{CO}_2]$. A balance between water-losing and water-absorbing organs is likely to be the deciding factor influencing IWUE in mountain maple seedlings under elevated $[\text{CO}_2]$ and high-light environments.

Acknowledgements

We appreciate the help of Ms. Joan Lee in setting up the experiment. We thank Dr. Jian Wang for permission to use the gas exchange system. This study was funded by Natural Sciences and Engineering Research Council of Canada Discovery grant to Q.-L.D.

References

- Ambebe, T.F., 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., Dang, Q.-L., Li, J., 2010. Low soil temperature inhibits the effect of high nutrient supply on photosynthetic response to elevated carbon dioxide concentration in white birch seedlings. *Tree Physiol.* 30, 234–243.
- Archambault, L., Morissette, J., Bernier-Cardou, M., 1998. Forest succession over a 20-year period following clearcutting in balsam fir yellow birch ecosystems of eastern Quebec, Canada. *For. Ecol. Manage.* 102, 61–74.
- Atkin, O.K., Evans, J.R., Ball, M.C., Lambers, H., Pons, T.L., 2000. Leaf respiration of snow gum in the light and dark. Interactions between temperature and irradiance. *Plant Physiol.* 122, 915–923.
- Aubin, I., Beaudet, M., Messier, C., 2000. Light extinction coefficients specific to the understorey vegetation of the southern boreal forest, Quebec. *Can. J. For. Res.* 30, 168–177.
- Aubin, I., Messier, C., Kneeshaw, D., 2005. Population structure and growth acclimation of mountain maple along a successional gradient in the southern boreal forest. *Ecoscience* 12, 540–548.
- Bazzaz, F.A., Wayne, P.M., 1994. Coping with environmental heterogeneity: the physiologia ecology of tree seedlings regeneration across the gap-understorey continuum. In: Caldwell, M.M., Pearcy, R.W. (Eds.), *Exploitation of Environmental Heterogeneity by Plants. Ecophysiological Processes Above- and Belowground*. Academic Press, Inc., USA, pp. 349–390.
- Boucher, J.F., Bernier, P.Y., Munson, A.D., 2001. Radiation and soil temperature interactions on the growth and physiology of eastern white pine (*Pinus strobus* L.) seedlings. *Plant Soil* 236, 165–174.
- Canham, C.D., Berkowitz, A.R., Kelly, V.R., Lovett, G.M., Ollinger, S.V., Schnurr, J., 1996. Biomass allocation and multiple resource limitation in tree seedlings. *Can. J. For. Res.* 26, 1521–1530.
- Cavender-Bares, J., Bazzaz, F.A., 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia* 124, 8–18.
- Cheng, S., Dang, Q.-L., Cai, T.-B., 2000. A soil temperature control system for ecological research in greenhouses. *J. For. Res.* 5, 205–208.
- Chmura, D.J., Anderson, P.D., Howe, G.T., Harrington, C.A., Halofsky, J.E., Peterson, D.L., Shaw, D.C., Brad StClair, J., 2011. Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *For. Ecol. Manage.* 261, 1121–1142.
- Christensen, J.H., Hewitson, B., Busuioic, A., Chen, A., Gao, X., Held, I. e. a., 2007. Regional climate projections. In: *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 University Press, Cambridge.
- Coleman, J.S., McConnaughay, K.D.M., Bazzaz, F.A., 1993. Elevated CO_2 and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? *Oecologia* 93, 195–200.
- Danyagri, G., Dang, Q.-L., 2013. 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.* 6, 1–9.
- Dawes, M.A., Hagedorn, F., Zumbunn, T., Handa, I.T., Hättenschwiler, S., Wipf, S., Rixen, C., 2011. Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. *New Phytol.* 191, 806–818.
- Day, T.A., Heckathorn, S.A., Delucia, E.H., 1991. Limitations of photosynthesis in *Pinus taeda* (loblolly Pine) at low soil temperatures. *Plant Physiol.* 96, 1246–1254.
- Domisch, T., Finér, L., Lehto, T., 2001. Effects of soil temperature on biomass and carbohydrate allocation in Scots pine (*Pinus sylvestris*) seedlings at the beginning of the growing season. *Tree Physiol.* 21, 465–472.
- Drake, B.G., González-Meler, M.A., Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO_2 ? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 609–639.

- Ellsworth, D.S., Reich, P.B., Naumburg, E.S., Koch, G.W., Kubiske, M.E., Smith, S.D., 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO₂ across four free-air CO₂ enrichment experiments in forest, grassland and desert. *Global Change Biol.* 10, 2121–2138.
- Fitter, A.H., Graves, J.D., Self, G.K., Brown, T.K., Bogie, D.S., Taylor, K., 1998. Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. *Oecologia* 114, 20–30.
- Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D., 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. *Plant Biol.* 6, 269–279.
- Hättenschwiler, S., Körner, C., 2000. Tree seedling responses to in situ CO₂-enrichment differ among species and depend on understorey light availability. *Global Change Biol.* 6, 213–226.
- Hikosaka, K., 2005. Nitrogen partitioning in the photosynthetic apparatus of *Plantago asiatica* leaves grown under different temperature and light conditions: similarities and differences between temperature and light acclimation. *Plant Cell Physiol.* 46, 1283–1290.
- Ingestad, T., 1981. Nutrition and growth of birch and grey alder seedlings in low conductivity solutions and at varied relative rates of nutrient addition. *Physiol. Plant.* 52, 454–466.
- IPCC, 2007. Summary for policymakers. In: Solomon, S. <ET-AL> (Ed.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*.
- King, J.S., Pregitzer, K.S., Zak, D.R., 1999. Clonal variation in above- and below-ground growth responses of *Populus tremuloides* Michaux: influence of soil warming and nutrient availability. *Plant Soil.* 217, 119–130.
- Krause, G.H., Gallé, A., Virgo, A., García, M., Bucic, P., Jahns, P., Winter, K., 2006. High-light stress does not impair biomass accumulation of sun-acclimated tropical tree seedlings (*Calophyllum longifolium* Willd. and *Tectona grandis* L. f.). *Plant Biol.* 8, 31–41.
- Krause, G.H., Winter, K., Matsubara, S., Krause, B., Jahns, P., Virgo, A., Aranda, J., García, M., 2012. Photosynthesis, photoprotection, and growth of shade-tolerant tropical tree seedlings under full sunlight. *Photosynth. Res.* 113, 273–285.
- Kubiske, M.E., Pregitzer, K.S., 1996. Effects of elevated CO₂ and light availability on the photosynthetic light response of trees of contrasting shade tolerance. *Tree Physiol.* 16, 351–358.
- Kubiske, M.E., Pregitzer, K.S., 1997. Ecophysiological responses to simulated canopy gaps of two tree species of contrasting shade tolerance in elevated CO₂. *Funct. Ecol.* 11, 24–32.
- Kubiske, M.E., Pregitzer, K.S., Mikan, C.J., Zak, D.R., Maziasz, J.L., Teeri, A., 1997. *Populus tremuloides* photosynthesis and crown architecture in response to elevated CO₂ and soil N availability. *Oecologia* 110, 328–336.
- Lambers, H., Chapin III, S.F., Pons, T.L., 2008. *Plant Physiological Ecology*. Springer, New York.
- Lei, T.T., Lechowicz, M.J., 1990. Shade adaptation and shade tolerance in saplings of three *Acer* species from eastern north America. *Oecologia* 84, 224–228.
- Liang, N.H., Tang, Y., Okuda, T., 2001. Is elevation of carbon dioxide concentration beneficial to seedling photosynthesis in the understorey of tropical rain forests? *Tree Physiol.* 21, 1047–1055.
- Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: has its importance been underestimated? *Plant Cell Environ.* 14, 729–739.
- Long, S.P., Woodward, F.I., 1998. *Plant and Temperature*. The Company of Biologists Limited, Department of Zoology, University of Cambridge, Cambridge.
- Lyr, H., Garbe, V., 1995. Influence of root temperature on growth of *Pinus sylvestris*, *Fagus sylvatica*, *Tilia cordata* and *Quercus robur*. *Trees – Struct. Funct.* 9, 220–223.
- Medlyn, B.E., Badeck, F.W., De Pury, D.G.G., Barton, C.V.M., Broadmeadow, M., Ceulemans, R., De Angelis, P., Forstreuter, M., Jach, M.E., Kellomäki, S., Laitat, E., Marek, M., Philippot, S., Rey, A., Strassmeyer, J., Laitinen, K., Liozon, R., Portier, B., Robertz, P., Wang, K., Jstbid, P.G., 1999. Effects of elevated [CO₂] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant Cell Environ.* 22, 1475–1495.
- Norby, R.J., O'Neill, E.G., 1991. Leaf area compensation and nutrient interactions in CO₂-enriched seedlings of yellow-poplar (*Liriodendron tulipifera* L.). *New Phytol.* 117, 515–528.
- Norby, R.J., Wullschlegel, S.D., Gunderson, C.A., Johnson, D.W., Ceulemans, R., 1999. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Environ.* 22, 683–714.
- Nowak, R.S., Ellsworth, D.S., Smith, S.D., 2004. Functional responses of plants to elevated atmospheric CO₂: do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol.* 162, 253–280.
- Onoda, Y., Hikosaka, K., Hirose, T., 2005. Seasonal change in the balance between capacities of RuBP carboxylation and RuBP regeneration affects CO₂ response of photosynthesis in *Polygonum cuspidatum*. *J. Exp. Bot.* 56, 755–763.
- Onoda, Y., Hirose, T., Hikosaka, K., 2007. Effect of elevated CO₂ levels on leaf starch, nitrogen and photosynthesis of plants growing at three natural CO₂ springs in Japan. *Ecol. Res.* 22, 475–484.
- Onoda, Y., Hirose, T., Hikosaka, K., 2009. Does leaf photosynthesis adapt to CO₂-enriched environments? An experiment on plants originating from three natural CO₂ springs. *New Phytol.* 182, 698–709.
- Osborne, C.P., Drake, B.G., LaRoche, J., Long, S.P., 1997. Does long-term elevation of CO₂ concentration increase photosynthesis in forest floor vegetation? Indiana strawberry in a Maryland forest. *Plant Physiol.* 114, 337–344.
- Osmond, C.B., Anderson, J.M., Ball, M.C., Egerton, J.J.G., 1999. Compromising efficiency: the molecular ecology of light-resource utilization in plants. In: Press, M.C., Scholes, J.D., Barker, M.G. (Eds.), *Physiological Plant Ecology*. Blackwell Science Ltd., Oxford, UK, pp. 1–24.
- Pearcy, R.W., 1990. Sunflecks and photosynthesis in plant canopies. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41, 421–453.
- Poorter, L., 1999. Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Funct. Ecol.* 13, 396–410.
- Post, L.J., 1970. Dry-matter production of mountain maple and balsam fir in north-western New Brunswick. *Ecology* 51, 548–550.
- Pregitzer, K.S., King, J.S., Burton, A.J., Brown, S.E., 2000. Responses of tree fine roots to temperature. *New Phytol.* 147, 105–115.
- Rook, E.J.S., 2002. *Shrubs of the Northwoods*, <http://www.rook.org/earl/bwca/nature/shrubs/index.html>
- Saxe, H., Ellsworth, D.S., Heath, J., 1998. Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytol.* 139, 395–436.
- Sefcik, L.T., Zak, D.R., Ellsworth, D.S., 2006. Photosynthetic responses to understorey shade and elevated carbon dioxide concentration in four northern hardwood tree species. *Tree Physiol.* 26, 1589–1599.
- Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D., Singaas, E.L., 2007. Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant Cell Environ.* 30, 1035–1040.
- Stitt, M., 1991. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant Cell Environ.* 14, 741–762.
- Stoneman, G.L., Dell, B., 1993. Growth of *Eucalyptus marginata* (Jarrah) seedlings in a greenhouse in response to shade and soil temperature. *Tree Physiol.* 13, 239–252.
- Sullivan, J., 1993. *Acer spicatum*. In: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. Fire Sciences Laboratory. Fire Effects Information System.
- Takeuchi, Y., Kubiske, M.E., Isebrands, J.G., Pregitzer, K.S., Hendrey, G., Karnosky, D.F., 2001. Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO₂ enrichment. *Plant Cell Environ.* 24, 1257–1268.
- Wan, X.C., Landhauser, S.M., Zwiazek, J.J., Lieffers, V.J., 1999. Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. *Tree Physiol.* 19, 879–884.
- Weih, M., Karlsson, P.S., 2002. Low winter soil temperature affects summertime nutrient uptake capacity and growth rate of mountain birch seedlings in the subarctic, Swedish lapland. *Arct. Antarct. Alp. Res.* 34, 434–439.
- Zhang, S., Dang, Q.-L., 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. *Tree Physiol.* 26, 1457–1467.
- Zotz, G., Harris, G., Königer, M., Winter, K., 1995. High rates of photosynthesis in the tropical pioneer tree, *Ficus insipida*. *Willd. Flora* 190, 265–272.
- Zotz, G., Pepin, S., Körner, C., 2005. No down-regulation of leaf photosynthesis in mature forest trees after three years of exposure to elevated CO₂. *Plant Biol.* 7, 369–374.