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Light compensation points in shade-grown seedlings of deciduous broadleaf tree species with different successional traits raised under elevated CO₂

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Running head: Shade tolerance in shade-grown seedlings enhanced by elevated CO₂

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ABBREVIATIONS

A, net photosynthetic rate; A_{growth}, light-saturated net-photosynthetic rate measured at the growth CO₂ concentration; J_{max}, maximum rate of electron transport; LAI, leaf mass per area; LCP, light compensation point; R_n, dark respiration rate; V_{cmax}, maximum rate of Rubisco carboxylation; Φ_a, apparent quantum yield

ABSTRACT

We measured leaf photosynthetic traits in shade-grown seedlings of four tree species native to northern Japan raised under an elevated CO₂ condition to investigate the effects of elevated CO₂ on shade tolerance of deciduous broadleaf tree species with different successional traits. We considered *Betula platyphylla* var. *japonica* and *Betula maximowicziana* as pioneer species, *Quercus mongolica* var. *crispula* as a mid-successional species, and *Acer mono* as a climax species. The plants were grown under a shade condition (10% of full sunlight) in a CO₂-regulated phytotron. Light compensation points (LCPs) decreased in all tree species when they were grown under elevated CO₂ (720 μmol mol⁻¹), which were accompanied by higher apparent quantum yields but no photosynthetic downregulation. LCPs in *Q. mongolica* and *A. mono* grown under elevated CO₂ were lower than those in the two pioneer birch species. The LCP in *Q. mongolica* seedlings was not different from that of *A. mono* in each CO₂ treatment. However, lower dark respiration rates were observed in *A. mono* than those in *Q. mongolica*, suggesting greater shade tolerance in *A. mono* as a climax species in relation to carbon loss during nighttime. Thus, elevated CO₂ may have enhanced shade tolerance by lowering the LCPs in all species but the ranking of shade tolerance related to successional traits did not change among species under elevated CO₂, i.e., the highest shade tolerance was observed in the climax species (*A. mono*), followed by a gap-dependent species (*Q. mongolica*), and lower shade tolerance was observed in the pioneer species (*B. platyphylla* and *B. maximowicziana*).

INTRODUCTION

Atmospheric CO₂ concentration is predicted to double during the next century (IPCC, 2001, 2007). Although elevated CO₂ has been linked to increased plant growth via enhanced photosynthetic carbon assimilation (Tissue et al. 1997; Ainsworth & Long 2005; Norby & Zak 2011), few studies have investigated light-limited photosynthesis under elevated CO₂ conditions despite the importance of light-limited photosynthesis in seedlings growing in the forest understory (Hättenschwiler 2001; Norby & Zak 2011).

The deciduous broadleaf forests of northern Japan consist of various tree species with different successional traits. The Japanese white birch (*Betula platyphylla* var. *japonica*) and monarch birch (*Betula maximowicziana*) are typical pioneer species broadly distributed across northern Japan (Koike & Sakagami 1985). Japanese oak (*Quercus mongolica* var. *crispula*) is a gap dependent mid-successional species (Hayashi 1985; Higo 1987, 1994). Japanese maple (*Acer mono*) is a typical shade-tolerant species in northern Japan. It is relatively more shade-tolerant than *Q. mongolica*, as indicated by higher survival rates in intact forest understory (Higo 1994).

Elevated CO₂ increases leaf area index (LAI) after canopy closure in tree stands with a relatively low LAI (Norby & Zak 2011), as simulated by an empirical model (Oikawa 1986). An increase in the LAI results in decreased transmittance of incident light flux through the forest canopy to the forest floor. Shade tolerance is important for the survival of forest understory when growing under elevated CO₂.

A key factor for the survival of seedlings grown under limiting light conditions is the light compensation point (LCP). A lower LCP promises a positive carbon gain, particularly in a limited light environment. The LCP is determined by apparent quantum yield based on incident light (Φ_a) and leaf dark respiration (R_n) (Larcher 2003). Φ_a in C₃ plants increases with increasing CO₂ concentration due to an increase in the partitioning of electron flow to photosynthetic carbon assimilation (Long & Bernacchi 2003). If the dark respiration rate in tree

seedlings is unaffected by elevated CO₂ (Gonzalez-Meler et al. 2004), enhanced Φ_a results in a lower LCP under elevated CO₂.

Elevated CO₂ temporally enhances the photosynthetic rate but eventually results in “photosynthetic acclimation” in many cases due to photosynthetic downregulation, which is typically indicated by a decrease in the maximum Rubisco carboxylation rate ($V_{c,max}$) and a decrease in the maximum electron transport rate (J_{max}) (Rogers & Ellsworth 2002; Ainsworth & Long 2005). If elevated CO₂ induces photosynthetic acclimation in shade-grown seedlings, less of a decrease in the LCP would be expected even under elevated CO₂, which would be a disadvantage for shade acclimation under the higher LAI environment in the future forest understory (Norby & Zak 2011). In contrast, photosynthetic acclimation is mediated by changes in the source: sink balance under elevated CO₂; for example, photosynthetic acclimation is observed significantly when the sink capacity is minimal during flowering (Lewis et al. 2002).

We hypothesized that photosynthetic acclimation would not occur in seedlings grown in the forest understory even under elevated CO₂ because of the low source strength relative to sink capacity under the limited light condition, leading to enhanced shade tolerance with a lower LCP in shade-grown seedlings raised under elevated CO₂. To test this hypothesis, we measured photosynthetic traits in shade-grown seedlings of four deciduous broadleaf tree species native to northern Japan and raised under elevated CO₂. We also investigated the differences in shade tolerance enhanced by elevated CO₂ among deciduous broadleaf tree species across wide ecological groups with different successional traits (cf. Hättenschwiler 2001; within late-successional species).

MATERIALS AND METHODS

Plant materials

Two-year-old seedlings were obtained from a commercial nursery (Oji Forest & Products Co.,

Kuriyama, Japan). We used four deciduous broadleaf tree species; two early-successional (*Betula platyphylla* Sukatchev var. *japonica* Hara and *B. maximowicziana*), one mid-successional [*Quercus mongolica* Fisch. ex Turcz. var. *crispula* (Blume) Ohashi], and one late-successional species [*Acer mono* Maxim. var. *glabrum* (Lév. et Van't.) Hara]. The seedlings were transplanted to vinyl pots filled with a 1: 1 (v/v) mixture of Kanuma pumice soil and clay soil in May 2005. The pots were large enough (volume, 5 l) to allow unrestricted root growth during the experiment, as seedling growth was limited under the shade condition. The seedlings were placed in a phytotron containing six natural-daylight growth cabinets (KG type, Koito, Yokohama, Japan) at the Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo, Japan (42°59'N, 141°23'E). Each of the two CO₂ treatments, i.e., ambient CO₂ treatment (ca. 380 μmol mol⁻¹) and the elevated CO₂ treatment (regulated to 720 μmol mol⁻¹), was replicated in three chambers. CO₂ concentrations in the three chambers for each CO₂ treatment were monitored at 6-min intervals during the growing season. The means of averaged daytime CO₂ concentrations (6:00 – 18:00) during the period across chambers were 379 ± 16 and 710 ± 0.8 μmol mol⁻¹ (mean ± standard deviation, n = 3) in the ambient and elevated CO₂ treatments, respectively. Day/night temperatures were maintained at optimal values (26/16°C) (Koike et al. 1995; Koike et al. 2000). The seedlings were grown under a shade condition; relative light intensity at 10% of full sunlight through shade cloth was used in the ambient and elevated CO₂ treatments. Liquid fertilizer (Hyponex 5: 10: 5, N: P: K; O.M. Scott and Sons, Marysville, OH, USA) was applied once monthly (200 ml of 0.1% liquid fertilizer). The treatments commenced in May 2005 and finished in October 2006, for a total of 513 days. The seedlings were placed outside under natural conditions during winter from leaf shedding to budbreak the next spring.

Gas exchange measurements

All gas exchange experiments were conducted using an open system with infrared gas analyzers

(LI-6400; Li-Cor, Lincoln, NE, USA). The temperature in the leaf chamber was maintained at 25°C, and the water vapor deficit in the leaf chamber was about 1.2 kPa. Measurements were conducted from July 24 to August 10, 2006. Third or fourth fully expanded mature leaves from the top were used for the measurements in the successive leaf development species, *B. platyphylla* and *B. maximowicziana*. The other two species flushed all leaves once in the spring. To assess the light compensation point (LCP) and apparent quantum yield based on incident light (Φ_a), the dark-respiration rate (R_n) and net photosynthetic rate (A) were measured stepwise at each growth CO_2 concentration with increasing incident photon flux density (PFD) from 0 to $120 \mu\text{mol m}^{-2} \text{s}^{-1}$. CO_2 leak between the leaf chamber gasket and the leaf surface results in false R_n values (Jahnke 2001), which increase with an increase in the difference between the CO_2 concentration inside and outside the leaf chamber (Amthor 2000). The measurements were done inside the growth cabinets to reduce the difference in CO_2 concentration between the leaf chamber and outside to minimize such errors. In contrast, light-saturated net photosynthetic rates were measured at a light intensity of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD and various CO_2 concentrations in the laboratory (ambient CO_2 was approximately $400 \mu\text{mol mol}^{-1}$) for both the ambient- and elevated- CO_2 -grown seedlings transferred from the growth cabinets. The CO_2 concentration inside the chamber was reduced in five steps from 720 to $0 \mu\text{mol mol}^{-1}$. The maximum Rubisco carboxylation rate ($V_{c,\text{max}}$) was calculated based on the light-saturated net photosynthetic rates measured at CO_2 concentrations below $300 \mu\text{mol mol}^{-1}$ (Farquhar et al. 1980; Long & Bernacchi 2003). The maximum electron transport rate was determined after Willson et al. (2000). Leaf chlorophyll content was assessed by the indexed chlorophyll content reading (SPAD reading) using a SPAD chlorophyll meter (SPAD 502; Minolta, Osaka, Japan) (Uddling et al. 2007).

Statistical analysis

All statistics were based on the mean values of the individual chambers (two-sample replicates

for each species) as the sample unit for each CO₂ treatment (n = 3). These values were averaged to provide the sample estimate for that replicate. A split-plot analysis of variance (CO₂ × species) was used to examine the effects of CO₂, species, and their interaction on total dry mass, shoot to root (S:R) ratio, light-saturated net photosynthetic rate measured at each growing CO₂ concentration (A_{growth}), $V_{c,\text{max}}$, J_{max} LCP, Φ_a , R_n , and SPAD reading (R Development Core Team 2014). We conducted post-hoc multiple comparisons to investigate the species ranking for each variable. As main single effects are misleading when the interaction is significant (Sokal & Rohlf 1995), the differences in means among the combinations of CO₂ and species were tested using Holm's pairwise comparisons if the interaction between CO₂ and species was significant. The differences in their means across the CO₂ treatments for each species were tested when the effect of species was significant but the interaction between CO₂ and species was not significant.

RESULTS

No significant differences were detected for total dry mass or the S:R ratio of the shade-grown seedlings between the CO₂ treatments, although differences among species were significant (Table 1, Fig. 1). The ranking of total dry mass averaged across the CO₂ treatments for each species was *B. platyphylla*: 9.7^A > *B. maximowicziana*: 9.7^A > *Q. mongolica*: 9.5^A > *A. mono*: 2.6^B g; the ranking of S:R ratio was *B. platyphylla*: 2.3^A > *B. maximowicziana*: 2.3^A > *A. mono*: 1.1^B > *Q. mongolica*: 0.54^C (different letters indicate significant differences at P < 0.05, Table 1, Fig. 1).

The light-saturated net photosynthetic rate measured at the respective growth under CO₂ condition (A_{growth}) was significantly higher in the elevated-CO₂-grown seedlings than in the ambient-CO₂-grown seedlings (Table 1, Fig. 2). A_{growth} of *Q. mongolica* was higher than that of *B. platyphylla* and *B. mongolica*, regardless of CO₂ treatment. The CO₂ treatments showed no

significant effects on $V_{c,max}$ or J_{max} (Table 1, Fig. 2). The $V_{c,max}$ ranking averaged across the CO₂ treatments was *Q. mongolica*: 38.7^A > *A. mono*: 31.9^B > *B. platyphylla*: 21.2^C > *B. maximowicziana*: 17.9^C $\mu\text{mol m}^{-2} \text{s}^{-1}$; the J_{max} ranking was *Q. mongolica*: 82.7^A > *A. mono*: 69.3^B > *B. platyphylla*: 46.8^C > *B. maximowicziana*: 38.4^D $\mu\text{mol m}^{-2} \text{s}^{-1}$ (different letters indicate significant differences at $P < 0.05$, Fig. 2).

Significant interactions between CO₂ and species were observed for the LCP and Φ_a (Table 1). Based on Holm's pairwise comparisons, the LCP decreased in the elevated CO₂ treatment, and lower LCPs were observed in *Q. mongolica* and *A. mono* than those in *B. platyphylla* and *B. maximowicziana*, regardless of CO₂ treatment (Fig. 3). Among seedlings grown under ambient CO₂, Φ_a of *Q. mongolica* was significantly higher than those of *B. platyphylla* and *B. maximowicziana*, whereas Φ_a of *Q. mongolica* was higher than those of the other three species grown under elevated CO₂ (Fig. 3). Elevated CO₂ significantly decreased R_n (Table 1, Fig. 3). The extent of the decrease in R_n at elevated CO₂ for each species was as follows: *B. platyphylla*: -1.8% (actual increase), *B. maximowicziana*: 8.3%, *Q. mongolica*: 0.1%, and *A. mono*: 7.1%, respectively. Assuming that R_n was unaffected by elevated CO₂, the LCPs at elevated CO₂ estimated from Φ_a at elevated CO₂ and R_n at ambient CO₂ increased by 2.3 and 1.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *B. maximowicziana* and *A. mono*, respectively from the actually measured LCP at elevated CO₂. Thus, the decrease in R_n at elevated CO₂ appeared to have only a minor contribution to lowering the LCP. The ranking of R_n averaged across the CO₂ treatments was *B. maximowicziana*: 0.96^A > *B. platyphylla*: 0.89^B > *Q. mongolica*: 0.71^C > *A. mono*: 0.62^D $\mu\text{mol m}^{-2} \text{s}^{-1}$ (different letters indicate significant differences at $P < 0.05$, Fig. 3). The SPAD reading was not different between the CO₂ treatments (Table 1); the ranking was *Q. mongolica*: 47.3^A > *A. mono*: 42.0^B > *B. platyphylla*: 23.4^C > *B. maximowicziana*: 23.3^C (different letters indicate significant differences at $P < 0.05$, Fig. 3).

DISCUSSION

Elevated CO₂ has been reported to enhance growth when plants are grown under natural daylight (Tissue et al. 1997; Ainsworth & Long 2005; Norby & Zak 2011). No significant difference was found in total dry mass between seedlings grown under ambient and elevated CO₂ (Fig. 1), although photosynthetic capacity was significantly enhanced by elevated CO₂, as indicated by the increases in A_{growth} and Φ_a (Figs. 2 and 3). The lack of a significant difference in total dry mass may be attributed to an increase in root exudates under the elevated CO₂ condition (Phillips et al. 2006; Phillips et al. 2009, 2011). Furthermore, the increase in photosynthetic carbon gain under elevated CO₂ may be too small to detect using total dry mass in seedlings grown under a limited light condition (10% of full sunlight) during our experimental period.

The effects of elevated CO₂ on biomass allocation are relatively unresponsive but dependent on growth conditions; for example, elevated CO₂ suppresses shoot growth relative to that of roots by the nitrogen depletion in a whole plant due to enhanced plant growth under limited nitrogen availability (Rogers et al. 1996; Poorter & Nagal 2000). Elevated CO₂ had no significant effect on the S:R ratio of the four deciduous broadleaf tree species, suggesting that nutrient status in the elevated-CO₂-grown seedlings was not different from that in the ambient-grown seedlings. This observation is supported by the finding that no photosynthetic downregulation, as indicated by decreases in V_{c,max} and J_{max}, was observed in the seedlings grown under elevated CO₂ (Fig. 2), as elevated CO₂-induced photosynthetic downregulation is frequently observed under limited nitrogen availability (Rogers & Ellsworth 2002; Ainsworth & Long 2005; Bernacchi et al. 2005; Kitao et al. 2005).

As the electron flow partitioned into photosynthesis increases under elevated CO₂ (Long & Bernacchi 2003), shade tolerance of tree species is expected to be enhanced under elevated CO₂ without photosynthetic downregulation (Hättenschwiler 2001). In fact, all tree species in the present study showed enhanced shade tolerance, as indicated by the lower LCPs

(Fig. 3). The mid- and late-successional species *Q. mongolica* and *A. mono* showed higher apparent quantum yield (Φ_a) among species, despite the CO₂ treatment, which may have resulted from higher leaf absorbance with higher leaf chlorophyll content shown by the SPAD readings (Fig. 3, Bauerle et al. 2004; Uddling et al. 2007). Although we observed a decrease in the dark respiration rate (R_n) in the seedlings grown under elevated CO₂ (Table 1, Fig. 3, cf. Gonzalez-Meler et al. 2004), the contribution of decreased R_n to lowering the LCP was minor.

Among the species, the LCPs in *Q. mongolica* and *A. mono* grown under elevated CO₂ were lower than those in the two pioneer birch species (Fig. 3), suggesting that mid- and late-successional species with an inherent ability to survive in the forest understory attain further shade-tolerance under elevated CO₂ (Hättenschwiler 2001; Norby & Zak 2011). Although no difference in the LCP was observed between *Q. mongolica* and *A. mono* when compared under each CO₂ treatment (Fig. 3), the lower dark respiration rate observed in *A. mono* than in *Q. mongolica* suggests greater shade tolerance in *A. mono* as a climax species in relation to carbon loss during nighttime (Higo 1994; Kitao et al. 2006). Thus, elevated CO₂ may enhance shade tolerance in tree seedlings by lowering the LCP but the ranking related to successional traits did not change among species in response to the rising atmospheric CO₂ concentrations (Fig. 4).

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Table 1. Summary of the split-plot ANOVA to test the effects of CO₂ treatment ($F_{1,4}$), species ($F_{3,12}$) and their interaction ($F_{3,12}$) on total dry mass, shoot to root (S:R) ratio, light-saturated net photosynthetic rate at each growing CO₂ concentration (A_{growth}), the maximum Rubisco carboxylation rate ($V_{\text{c,max}}$), the maximum electron transport rate (J_{max}), light compensate point (LCP), apparent quantum yield (Φ_a), dark respiration rate (R_n) and SPAD reading in shade-grown seedlings of the four deciduous broadleaf tree species. *Denotes significance at $P < 0.05$, ** $P < .01$ and *** $P < 0.001$.

	F^2 -statistics		
	CO ₂ treatment	Species	CO ₂ x Species
Total dry mass	0.34	13.1***	1.48
S:R ratio	1.24	59.1***	2.18
A_{growth}	53.1***	32.7***	3.03
$V_{\text{c,max}}$	5.54	145***	2.51
J_{max}	1.57	116***	0.41
LCP	148***	228***	4,52*
Φ_a	159***	76.9***	13.1***
R_n	11.9*	87.2***	1.75
SPAD reading	2.68	728***	0.26

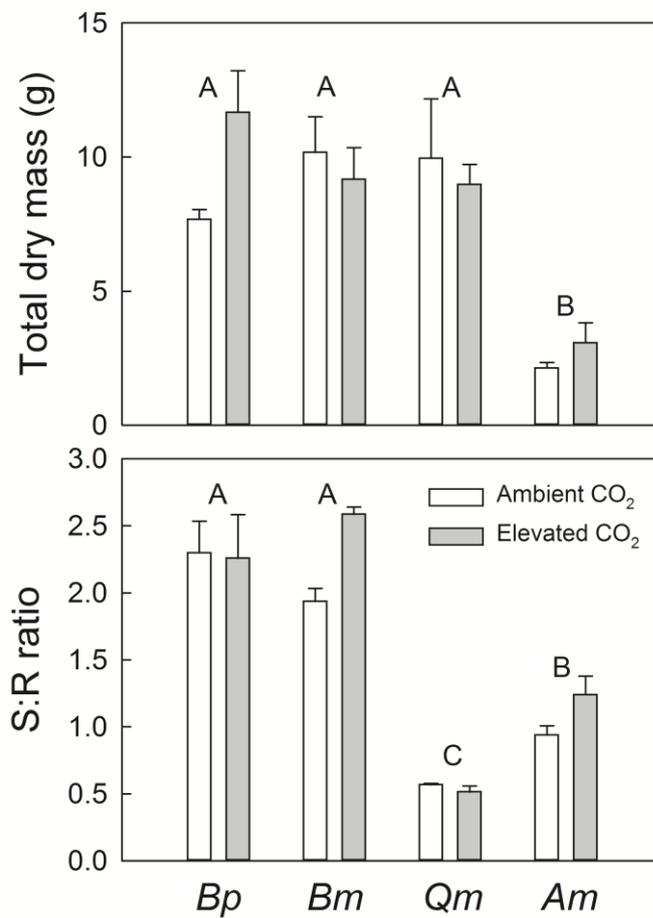


Figure 1. Total dry mass and shoot to root (S: R) ratio in shade-grown seedlings of *Betula platyphylla* (*Bp*), *B. maximowicziana* (*Bm*), *Quercus mongolica* (*Qm*), and *Acer mono* (*Am*) under ambient (white bars) and elevated CO₂ (gray bars). Values are means + standard errors (n = 3). Different capital letters indicate significant differences in means across CO₂ treatments among species (P < 0.05).

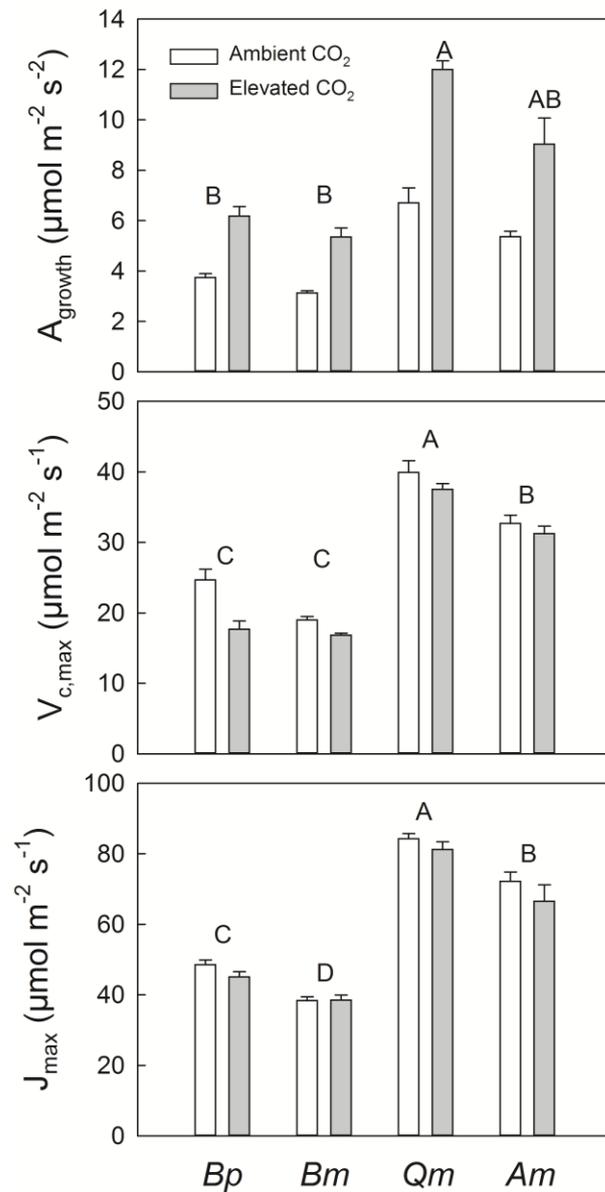


Figure 2. Light-saturated net photosynthetic rate measured at the growth CO₂ concentration (A_{growth}), the maximum Rubisco carboxylation rate ($V_{\text{c,max}}$), and the maximum electron transport rate (J_{max}) in shade-grown seedlings of *Betula platyphylla* (*Bp*), *B. maximowicziana* (*Bm*), *Quercus mongolica* (*Qm*), and *Acer mono* (*Am*) under ambient (white bars) and elevated CO₂ (gray bars). Values are means + standard errors ($n = 3$). Different capital letters indicate significant differences in means across CO₂ treatments among species ($P < 0.05$).

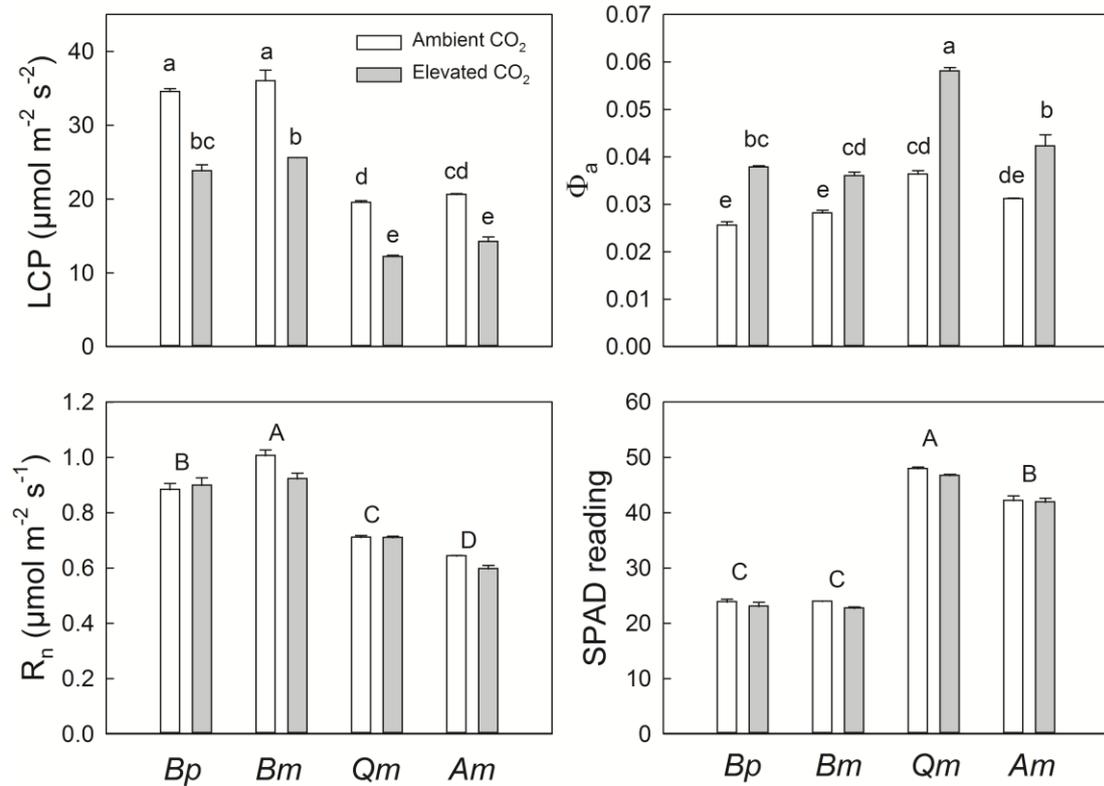


Figure 3. Light compensation point (LCP), apparent quantum yield (Φ_a), dark respiration rate (R_n), and SPAD reading in shade-grown seedlings of *Betula platyphylla* (*Bp*), *B. maximowicziana* (*Bm*), *Quercus mongolica* (*Qm*), and *Acer mono* (*Am*) under ambient (white bars) and elevated CO₂ (gray bars). Values are means + standard errors (n = 3). Different capital letters indicate significant differences in means across CO₂ treatments among species (P < 0.05). Different lower case letters indicate significant differences among the combinations of CO₂ × species (P < 0.05).

Shade tolerance under elevated CO₂

Shade tolerance increased in all species under elevated CO₂ as the LCP decreased.

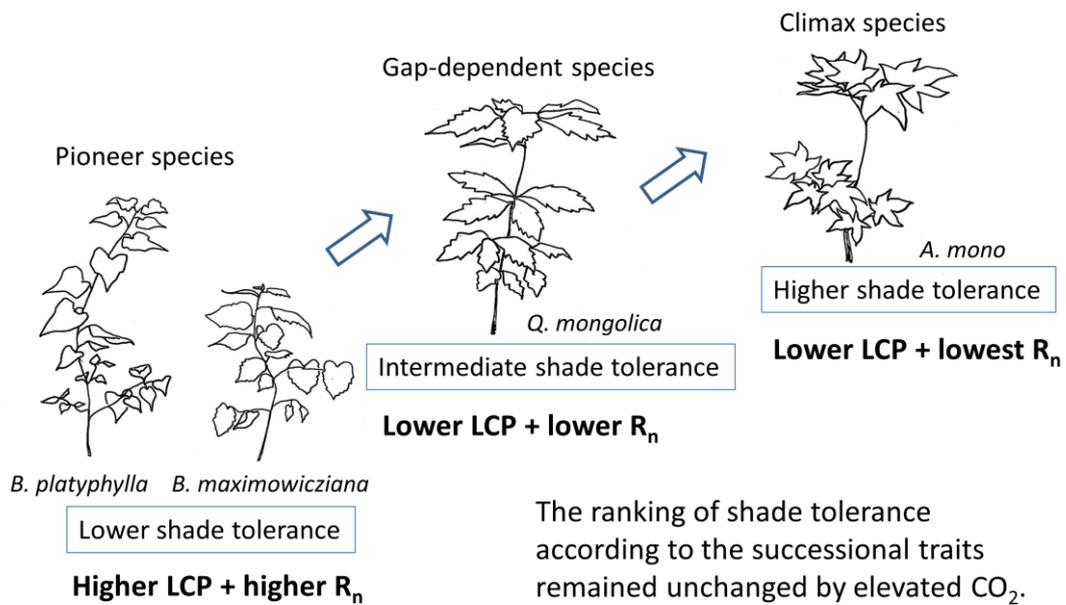


Figure 4. Summary of shade tolerance in seedlings of the four deciduous broadleaf tree species grown under elevated CO₂.