Photosynthetic downregulation in leaves of the Japanese white birch grown under elevated CO$_2$ concentration does not change their temperature-dependent susceptibility to photoinhibition

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To determine the effects of elevated CO$_2$ concentration ([CO$_2$]) on the temperature-dependent photosynthetic properties, we measured gas exchange and chlorophyll fluorescence at various leaf temperatures (15, 20, 25, 30, 35 and 40°C) in 1-year-old seedlings of the Japanese white birch (Betula platyphylla var. japonica), grown in a phytotron under natural daylight at two [CO$_2$] levels (ambient: 400 μmol mol$^{-1}$ and elevated: 800 μmol mol$^{-1}$) and limited N availability (90 mg N plant$^{-1}$). Plants grown under elevated [CO$_2$] exhibited photosynthetic downregulation, indicated by a decrease in the carboxylation capacity of Rubisco. At temperatures above 30°C, the net photosynthetic rates of elevated-CO$_2$-grown plants exceeded those grown under ambient [CO$_2$] when compared at their growth [CO$_2$]. Electron transport rates were significantly lower in elevated-CO$_2$-grown plants than ambient-CO$_2$-grown ones at temperatures below 25°C. However, no significant difference was observed in the fraction of excess light energy ([1 − qP] × F$'$/F$_{m'}$) between CO$_2$ treatments across the temperature range. The quantum yield of regulated non-photochemical energy loss was significantly higher in elevated-CO$_2$-grown plants than ambient, when compared at their respective growth [CO$_2$] below 25°C. These results suggest that elevated-CO$_2$-induced downregulation might not exacerbate the temperature-dependent susceptibility to photoinhibition, because reduced energy consumption by electron transport was compensated for by increased thermal energy dissipation at low temperatures.

Introduction

Long-term exposure to an elevated atmospheric CO$_2$ concentration ([CO$_2$]) results in photosynthetic downregulation, typically a decrease in the carboxylation capacity of Rubisco ($V_{c,\text{max}}$), accompanied by a reduction in area-based leaf nitrogen content ($N_{\text{area}}$) and an accumulation of carbohydrate, especially under insufficient nitrogen supply or restricted rooting volumes (Sage 1994, Drake et al. 1997, Sims et al. 1998, Stitt and Krapp 1999). Elevated-[CO$_2$]-induced photosynthetic downregulation can occur even in the absence

Abbreviations – ETR, electron transport rate; FACE, free-air CO$_2$ enrichment; LED, light emitting diode; NPQ, non-photochemical quenching; PFD, photon flux density; PSII, photosystem II; RuBP, ribulose-1,5-bisphosphate; Y(NO), quantum yield of non-regulated non-photochemical energy loss in PSII; Y(NPQ), quantum yield of regulated non-photochemical energy loss in PSII.
of any rooting restriction in free-air CO2 enrichment (FACE) experiments for wheat (Nie et al. 1995), loblolly pine (Rogers and Ellsworth 2002), poplars (Bernacchi et al. 2003a) and even for a nitrogen-fixing species, soybean (Bernacchi et al. 2005). A decrease in the maximum rate of ribulose-1,5-bisphosphate (RuBP) regeneration (Jmax) is also observed in plants grown under elevated [CO2], but generally to a lesser extent than that in Vc,max (Long et al. 2004).

The Japanese white birch (Betula platyphylla Sukatchev var. japonica Hara) is a variety of white birch (B. platyphylla), which is broadly distributed across the Eurasian Continent. As the Japanese white birch has a high sink strength related to an indeterminate growth pattern (Kikuzawa 1983) and a high growth rate (Koike 1988), which is characteristic of the white birch, sustained photosynthetic stimulation under elevated [CO2] may be expected under fertile conditions, as has been reported in the ecologically similar Populus (Gielen and Ceulemans 2001). On the other hand, as a pioneer species, the Japanese white birch is also often found in infertile habitats such as those with volcanicogenous regosols, which are widely distributed in northern Japan. Under such nutrient-limiting conditions, photosynthetic downregulation to elevated [CO2] in the Japanese white birch could occur (Kitao et al. 2005, 2007, Eguchi et al. 2008).

Photoinhibition, defined as a long-term decline in photosynthetic efficiency, is a relevant factor to determine plant survival and distribution. For example, photoinhibition at low temperatures at the alpine timber line contributes to limit the habitat of tree species (Germino and Smith 1999, 2000, Kitao et al. 2004). Conversely, photoinhibition at high temperatures would also be a relevant factor for tree regeneration in tropical regions (Königer et al. 1998, Kitao et al. 2003). An electron flow consumed by photosynthesis and photorespiration (Cornic and Briantais 1991, Cornic and Fresneau 2002, Kitao et al. 2003) is an effective pathway of energy dissipation to mitigate photoinhibition as well as thermal energy dissipation in the photosystem II (PSII) antennae (Demmig-Adams et al. 1996, Park et al. 1996, Baroli and Melis 1998, Kitao et al. 2012). In many cases, thermal energy dissipation appears to play the role of compensating less electron flow capacity, i.e. a higher electron flow is accompanied by less thermal energy dissipation, as indicated by the lower non-photochemical quenching (NPQ), whereas a lower electron flow is compensated by a higher NPQ (Kitao et al. 2000, 2003, 2006, Rosenqvist 2001, Einhorn et al. 2004).

Whether photosynthesis is regulated by Rubisco carboxylase or RuBP regeneration depends on temperature as well as intercellular CO2 concentration (Ci) (Bernacchi et al. 2001, 2002, 2003b). With short-term elevated [CO2], photosynthesis is stimulated at a given temperature and the optimal temperature rises, because of the limited factor of photosynthesis shifting from RuBP carboxylase to RuBP regeneration under elevated [CO2] (Hikosaka et al. 2006). As the rate of electron transport is also limited by Rubisco carboxylase/oxygenase and RuBP regeneration (Long and Bernacchi 2003), changes in the temperature dependency of electron transport with photosynthetic downregulation in plants grown under elevated [CO2] would change the temperature-dependent susceptibility to photoinhibition.

Plants grown under elevated [CO2] have been reported as changing their susceptibility to photoinhibition at high and low temperatures. In winter, elevated [CO2] exacerbated photoinhibition in photosynthetically downregulated leaves of the loblolly pine (Pinus taeda) (Hymus et al. 1999) and snow gum (Eucalyptus pauciflora) (Roden et al. 1999). Conversely, photochemistry of PSII in the downregulated leaves of the cork oak (Quercus suber L.) was more tolerant to heat stress (Faria et al. 1996), whereas those of two species of eucalyptus (Eucalyptus macrorhyncha and Eucalyptus rossii) were more sensitive when grown under elevated [CO2] (Roden and Ball 1996). Taub et al. (2000) also reported the enhanced heat tolerance of PSII in various plant species grown under elevated [CO2]. However, few studies exist investigating the susceptibility to photoinhibition in photosynthetically downregulated leaves grown under elevated [CO2], related to the temperature-dependent rates of photosynthesis, electron transport and thermal energy dissipation at a wide range of both high and low temperatures.

We hypothesized that photosynthetic downregulation on RuBP carboxylation and RuBP regeneration under elevated [CO2] at N limitation would change the temperature dependency of electron transport and the susceptibility to photoinhibition. To test this hypothesis, we evaluated temperature-dependent photosynthetic characteristics in leaves of the Japanese white birch developed under ambient and elevated [CO2] treatments under limited N availability in glasshouses by simultaneously measuring gas exchange and chlorophyll fluorescence.

Materials and methods

Plant growth conditions

One-year-old Japanese white birch (B. platyphylla var. japonica) seedlings about 30 cm in height were transplanted in free draining plastic pots (diameter: 11 cm,
depth: 20 cm and volume: approximately 2 l) filled with clay loam soil mixed with Kanuma pumice soil (1:1 volume). These seedlings were grown in two natural daylight phytotron chambers (Koitotron K30-1602-G; Koito Industries, Yokohama, Japan) in the Forestry and Forest Products Research Institute (Tsukuba, Japan, 36°01′N, 140°08′E) from mid-June 2010. Twenty seedlings were grown under each CO2 treatment – about 400 (ambient [CO2] treatment) and 800 μmol mol−1 (elevated [CO2] treatment). The CO2 concentration in the chambers was monitored by an infrared CO2 analyzer (ZFP9; Fuji Electric, Tokyo, Japan). Liquid CO2 was used as the source of elevated [CO2] treatment. Ambient [CO2] was 392 ± 28 μmol mol−1 (mean ± SD). Elevated [CO2] was regulated to within ±1% of the target level. The day/night temperature was maintained at 26/20 ± 1°C during the experiment, where the temperature was programmed to increase linearly from 20 to 26°C from 6:00 to 12:00 h, remain at 26°C until 15:00 h and then linearly decrease again to 20°C by 18:00 h. The relative humidity was controlled at 70 ± 3% with a humidifier (RV-160XE; MIURA, Matsuyama, Japan) and a dehumidifier (B-600-2EA; Munters, Tokyo, Japan), and the maximum light intensity was approximately 1700 μmol m−2 s−1, which was measured with a quantum sensor (Li-190SA; Li-Cor, Lincoln, NE). To reduce the chamber effects, the treatments and seedlings were switched between the chambers about every 10 days. To induce photosynthetic downregulation, nitrogen supply was limited to 100 ml of liquid fertilizer (Hyponex 6-10-5, HYPONeX JAPAN, Osaka, Japan, 1000 times dilution) applied once weekly, for a total of 90 mg N plant−1 (Kitao et al. 2005, 2007), and pots were placed on trays to prevent nutrient drainage.

Gas exchange and chlorophyll fluorescence measurements

In August 2010, measurements of photosynthesis at various CO2 concentrations were conducted for leaves of eight individual seedlings per CO2 treatment, using a portable photosynthesis system (Li-6400; Li-Cor) combined with a leaf chamber fluorometer (Li-6400-40; Li-Cor). Thereafter, temperature-dependent gas exchange and chlorophyll fluorescence were measured for the same leaves. A 50-day-old fully expanded leaf was selected per seedling. The measurements were conducted in an artificial light chamber (Koitotron K30-1602-GC; Koito Industries) adjacent to those of natural daylight where the seedlings were grown. The seedlings were transferred into an artificial light chamber for the measurements. The chamber temperature was controlled to within 5°C of the target leaf temperatures; the light intensity provided by metal halide lamps was set to about 300 μmol m−2 s−1 at the leaf surface, and the relative humidity was controlled to about 70% by a humidifier (RV-160XE; MIURA) and a dehumidifier (B-600-2EA; Munters). Furthermore, the leaf temperature was regulated to remain within 0.5°C of target temperatures, using the Li-6400 leaf chamber. Before measurements, seedlings were acclimated to the chamber temperature for at least 30 min.

The maximum rates of RuBP carboxylation (Vc,max) and RuBP regeneration were derived from photosynthetic responses to various Ci at a leaf temperature of 25°C and saturating photon flux density (PFD) of 1000 μmol m−2 s−1, which was provided by a red/blue light emitting diode (LED) array (Li-6400-40; Li-Cor) with blue light comprising 10% of total PFD (Long and Bernacchi 2003). The temperature-dependent net photosynthetic rate (Pn), quantum yield of PSII electron transport (ΦPSII), photochemical quenching (qP), photochemical efficiency of the open PSII (Fv′/Fm′) and quantum yield of non-regulated [Y(NO)] and regulated non-photochemical energy loss in PSII [Y(NPQ)] were measured at a photosynthetic steady state, at various temperatures (15, 20, 25, 30, 35 and 40°C), ambient CO2 concentrations of 400 and 800 μmol mol−1 and saturating PFD of 1000 μmol m−2 s−1. The minimum (Fo) and maximum fluorescence (Fm) were determined after an overnight dark adaptation with saturating light of 7000 μmol m−2 s−1 PFD for 1 s. The fluorescence yield under the photosynthetic steady state, (Fs) and the maximum fluorescence, (Fm′) (determined at the saturating light of 7000 μmol m−2 s−1 PFD for 1 s) were used to calculate ΦPSII = (Fm′− Fo)/Fm′ (Genty et al. 1989). Fo was determined by turning off the actinic light and applying far red light immediately after a saturating light pulse. This parameter was used to derive qP and Fv′/Fm′ = (Fm′− Fo)/Fm′, calculated according to Schreiber et al. (1994). Y(NO) and Y(NPQ) were determined as Fv/Fm′ and (Fm′− Fo)/Fm′, respectively (Klughammer and Schreiber 2008). The fraction of excess energy (Excess) was also calculated as (1 − qP) Fv/Fm′ (Demmig-Adams et al. 1996). Electron transport rate (ETR) was calculated as ETR = ΦPSII × leaf absorbance × light intensity × 0.5 (Schreiber et al. 1994). Leaf absorbance was calculated based on a calibration curve between SPAD readings (measured using a SPAD chlorophyll meter, SPAD 502; Minolta, Osaka, Japan) and leaf absorbance. Leaf absorbance (at 400–700 nm with a halogen light source) was measured with a spectroradiometer (Li-1800C; Li-Cor) and an external integrating sphere (Li-1800-12S; Li-Cor). The calibration curve is described as: leaf absorbance with the halogen light = 1.14 × SPAD reading/(12.3 + SPAD reading) − 0.01, n = 24, r² = 0.99 and P < 0.0001. Furthermore, to account
for the difference in the spectral composition between the halogen light source and the red/blue LED, the actual absorptance with the LED was 0.974 × leaf absorptance with the halogen light + 0.053 (n = 16, r² = 0.99 and P < 0.0001) (Kitao et al. 2007).

**Leaf N, sugar and starch concentration**

Leaf N, sugar and starch contents were determined for the leaves used for the gas exchange and chlorophyll fluorescence measurements. After leaf area measurements, the leaves were dried at 70°C and weighed to calculate the leaf mass per area. The dried leaves were then ground using a micro beads smasher (MS-100; Tomy Seiko, Tokyo, Japan) at 4000 g for 60 s. The leaf N concentration was determined by a combustion method (Bremner 1996) with an NC analysis system comprising a nitrogen/carbon determination unit (Sumigraph, NC-900; Sumika Chem. Anal. Service, Osaka, Japan) and a gas chromatograph (GC-8A; Shimadzu, Kyoto, Japan) and a data processor (Chromatopac, C-R6A, Shimadzu). Sugars were extracted with 80% ethanol and determined by the phenol–sulfuric acid method (Dubois et al. 1956). Starch in the residue was solubilized by potassium hydroxide and digested to glucose with amyloglucosidase (A9228; Sigma, St. Louis, MO) solution (Kabeya et al. 2003). The digested glucose was determined with Wako Autokit Glucose (439-90901; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Statistical analysis**

Photosynthetic parameters (Vc,max and Jmax), leaf contents (leaf N, soluble sugar and starch) and Fv/Fm were compared with growth [CO₂] condition by Student’s t-test, respectively. Moreover, the correlation between each photosynthetic parameter and leaf content was analyzed. The effects of leaf temperature, measurement [CO₂] and growth [CO₂] on photosynthetic traits [Pn, ETR, ΦPSII, qP, Fv/Fm′, Excess, Y(NO) and Y(NPQ)] were analyzed by three-way ANOVA with repeated measures on the first two factors (R Development Core Team 2005). Furthermore, the differences in terms of photosynthetic traits between growth [CO₂] were also analyzed with Student’s t-test at each leaf temperature compared at the respective growth [CO₂].

**Results**

**Photosynthetic properties and leaf N and carbohydrate contents**

The responses of the Pn to the intercellular CO₂ concentration (Ci) are shown in Fig. 1. At any given Ci, white birch seedlings grown at elevated [CO₂] displayed less Pn than ambient. The maximum rates of RuBP carboxylation (Vc,max) and regeneration (Jmax) obtained from the Pn–Ci curves were also significantly less under elevated [CO₂] (Table 1). Elevated [CO₂] decreased leaf N and increased the soluble sugar contents per leaf area (Table 2), whereas the starch contents showed no significant difference between CO₂ treatments. The leaf N contents positively correlated with Vc,max and Jmax, soluble sugar negatively correlated with only Vc,max and no correlation was observed for starch contents. The switching treatments in the present study seemed efficient enough to reduce the chamber effects because Ci-dependent photosynthesis and photosynthetic downregulation, as indicated by a decrease in Vc,max, were well reproduced compared with the earlier study using three replicate chambers per treatment (Kitao et al. 2007).

**Temperature dependence of photosynthesis and chlorophyll fluorescence**

The temperature-dependent photosynthetic responses of the birch seedlings grown under ambient and elevated [CO₂] are shown in Fig. 2A. Compared under the same measurement [CO₂], i.e. 400 or 800 μmol mol⁻¹, elevated-CO₂-grown plants showed lower Pn than ambient-CO₂-grown ones across the temperature range (Fig. 2A, Table 3). Optimal temperatures to maximize Pn appeared to increase when measured at 800 μmol mol⁻¹ CO₂ for both plants grown under ambient and elevated [CO₂]. Compared at the respective growth [CO₂], i.e. 400 for the ambient-grown plants and 800 μmol mol⁻¹ for the elevated-CO₂-grown ones, higher Pn was observed in elevated-CO₂-grown plants above 30°C.
Table 1. Maximum RuBP carboxylation rate (Vc,max) and RuBP regeneration rate (Jmax) of leaves of Japanese white birch seedlings grown under ambient (ca. 400 μmol mol⁻¹) or elevated (ca. 800 μmol mol⁻¹) [CO2]. Each parameter was obtained by fitting data to the model of Farquhar et al. (1980) and standardized to 25°C. Data were shown as mean ± SE (n = 8). Significant differences in each parameter between growth CO2 conditions were observed (Student’s t-test, *P < 0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ambient</th>
<th>Elevated</th>
<th>t-test</th>
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<tbody>
<tr>
<td>Vc,max (μmol CO2 mol⁻¹ s⁻¹)</td>
<td>39.7 ± 3.4</td>
<td>23.7 ± 2.0</td>
<td>*</td>
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<tr>
<td>Jmax (μmol CO2 mol⁻¹ s⁻¹)</td>
<td>63.0 ± 2.8</td>
<td>50.1 ± 2.9</td>
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</table>

Table 2. Leaf nitrogen (N), soluble sugar and starch contents of Japanese white birch seedlings grown at ambient or elevated [CO2]. Each content was shown as mean ± SE (n = 8). Asterisks indicate significant differences among the growth CO2 regimes (Student’s t-test, *P < 0.05) and significant correlation with Vc,max or Jmax (*P < 0.05). NS, not significant.

<table>
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<tr>
<th>Content (g m⁻²)</th>
<th>Correlation Vc,max</th>
<th>Correlation Jmax</th>
</tr>
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<tr>
<td>Nitrogen (N)</td>
<td>0.69 ± 0.03</td>
<td>0.60 ± 0.02</td>
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<tr>
<td>Soluble sugar</td>
<td>3.23 ± 0.13</td>
<td>3.73 ± 0.14</td>
</tr>
<tr>
<td>Starch</td>
<td>5.29 ± 0.81</td>
<td>6.14 ± 1.08</td>
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</table>

The ETR measured at 800 μmol mol⁻¹ CO2 was higher than that at 400 μmol mol⁻¹ CO2 above 30°C for both ambient- and elevated-CO2-grown plants (Fig. 2B). Compared at the respective growth [CO2], significantly lower ETR was observed in elevated-CO2-grown plants than those grown under ambient [CO2] at temperatures below 25°C.

The quantum yield of non-photochemical energy loss, the non-regulated one [Y(NO)] was identical, regardless of the growth and measurement [CO2], whereas the regulated one [Y(NPQ)] significantly increased in elevated-CO2-grown plants over the ambient when compared at the respective growth [CO2] below 25°C (Fig. 3D, E, respectively). There was no significant difference in the maximum photochemical efficiency of PSII (Fv/Fm) after overnight dark adaptation between ambient and elevated growth CO2 regimes (Table 4).

Fig. 2. The temperature-dependent Pn (A) and ETR (B) in leaves of ambient- (circle) and elevated-CO2-grown plants (triangle) measured at 400 (open) and 800 μmol CO2 mol⁻¹ (closed symbols). Measurements were made at PFD of 1000 μmol m⁻² s⁻¹. Values are mean ± SE (n = 8). An asterisk denotes Student’s t-test significance between growth CO2 regimes measured with their growth CO2 concentration (between A_400 and E_800) at the given leaf temperature (P < 0.05).

Discussion

White birch seedlings grown at elevated [CO2] under limited N supply showed photosynthetic downregulation, indicated by decreases in the maximum rates of RuBP carboxylation (Vc,max) and RuBP regeneration (Jmax) (Fig. 1, Table 1, Long et al. 2004, Kitao et al. 2005, 2007, Onoda et al. 2005). Temperature-dependent photosynthetic carbon assimilation is determined as a consequence of the temperature dependencies of RuBP carboxylation and RuBP regeneration (Bernacchi et al. 2001, Medlyn et al. 2002, Yamori et al. 2010). In general, photosynthesis is regulated by RuBP regeneration at lower temperatures and by RuBP carboxylation at higher temperatures (Hikosaka et al. 1999, 2006, Han et al. 2003). In the present study, photosynthetic rates appeared to be regulated by RuBP regeneration below 25°C in both ambient- and elevated-CO2-grown plants because ETRs were independent of the measuring [CO2] (Fig. 2B, Hikosaka et al. 1999, 2006). Conversely, as the ETRs were enhanced by elevated [CO2] at gas exchange measurements above 30°C, photosynthetic
rates were limited by RuBP carboxylation at higher temperatures. Photosynthetic carbon assimilation rates were synergistically enhanced above 30°C under elevated [CO₂] because of the higher ETR and suppression of photorespiration under elevated [CO₂] (Fig. 2A, Farquhar et al. 1980). It is noteworthy that based on the temperature-dependent photosynthesis, higher photosynthetic rates were observed above 30°C in elevated-CO₂-grown plants measured under elevated [CO₂] than in ambient-grown ones measured under ambient [CO₂]. This suggests that elevated [CO₂] would boost photosynthetic carbon assimilation at higher temperatures even though photosynthetic downregulation occurred.

Excessive light energy neither utilized by photosynthetic electron transport nor dissipated as heat is assessed by \( (1 - q_P) \frac{F_v'}{F_m'} \) (Excess, Demmig-Adams et al. 1996, Kato et al. 2003, Kornyeyev et al. 2010). Excess is also revealed as \( F_v'/F_m' - \Phi_{PSII} \) (Schreiber et al. 1994, Demmig-Adams et al. 1996, Kato et al. 2003). The decrease in ETR at low temperatures in elevated-CO₂-grown plants (Fig. 2B) might be compensated for by increased thermal energy dissipation, as indicated by the lower photochemical efficiency of open PSII (Fig. 3B, \( F_v'/F_m' \)) as well as the higher Y(NPQ) (Fig. 3E, Demmig-Adams et al. 1996, Hendrickson et al. 2004, Klughammer and Schreiber 2008). Consequently, no difference was observed in Excess between CO₂ treatments at a temperature range of 15–40°C (Fig. 3C).

The degree of photoinhibition is generally evaluated by a decrease in the \( F_v'/F_m' \) (Krause and Weis 1991, Krause et al. 1995), which is determined as a consequence of photoinactivation and repair of PSII (Tsonev and Hikosaka 2003, Kornyeyev et al. 2010, Nishiyama et al. 2011). Excessive light energy (Excess) neither utilized via photosynthesis nor dissipated as heat, assessed by \((1 - q_P) \frac{F_v'}{F_m'}\), is closely related to the rate of photoinactivation of PSII, regardless of the growing light or nutrient condition (Kato et al. 2003) and measurement temperatures (Kornyeyev et al. 2010). Assuming that the same correlation between the rate of photoinactivation and Excess is applicable for the Japanese white birch grown under both CO₂ regimes, the

![Fig. 3](image-url)

**Fig. 3.** The temperature-dependent \( q_P \) (A), \( F_v'/F_m' \) (B), the Excess (C), Y(NO) (D) and Y(NPQ) (E) in leaves of Japanese white birch seedlings grown under ambient (circle) and elevated CO₂ concentration (triangle). Measurements were made at 400 (open) and 800 μmol CO₂ mol⁻¹ (closed symbols) and PFD of 1000 μmol m⁻² s⁻¹. Values are mean ± SE (n = 8). An asterisk denotes Student’s t-test significance between growth CO₂ regimes measured with their growth CO₂ concentration (between A_400 and E_800) at the given leaf temperature (\( P < 0.05 \)).
rate of photoactivation was presumed to be unaffected by the elevated [CO2] treatment across the temperature range from 15 to 40°C (Fig. 3C, Kato et al. 2003, Tsonev and Hikosaka 2003, Kornyeyev et al. 2010, Oguchi et al. 2011). Furthermore, there was no difference in the Y(NO) among the combinations of growth and measurement CO2 conditions across the temperature range (Fig. 3D), the higher values of which indicate an inability to protect against excess illumination (Klughammer and Schreiber 2008).

A decrease in the overnight dark-adapted Fv/Fm could be a measure of sustained photoinhibition (Krause et al. 1995, Logan et al. 2007). No difference was observed in the overnight dark-adapted Fv/Fm between CO2 treatments in the seedlings having experienced a leaf temperature of about 30°C in daytime (corresponding to 26°C chamber temperature and 1700 μmol m−2 s−1 photosynthetic PFD in the phytotron) (Table 4). Based on a FACE experiment (Eguchi et al. 2008), overnight dark-adapted Fv/Fm in the photosynthetically downregulated leaves of the Japanese white birch grown in the field under elevated [CO2] (500 μmol mol−1) showed no difference from that in ambient-CO2-grown ones from October to November when the mean daily temperature decreased from 15 to 5°C although the values of Fv/Fm declined toward senescence (Watanabe unpublished data). This suggests no difference in the degree of sustained photoinhibition between CO2 regimes at a wide range of growth temperatures (Krause et al. 1995, Logan et al. 2007), which might result from the lack of differences in the rate of photoinactivation (indicated by Excess) and the inability of photoprotection (Y(NO)) observed in the present study, and is probably attributable to the lack of difference in the PSII repair processes overnight. Thus, it is suggested that photosynthetic downregulation in leaves of the Japanese white birch grown under elevated [CO2] might not exacerbate the temperature-dependent susceptibility to photoinhibition.

Table 3. A summary of three-way ANOVA with two repeated measures (Fp-qr design) to test for the effects of growth [CO2] (CO2growth), measurement [CO2] (CO2mes), measurement leaf temperature (Temp) and their interaction on the Pn, ETR, qP, Fv/Fm, Excess, Y(NO) and Y(NPQ). Significant level: *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.

<table>
<thead>
<tr>
<th></th>
<th>Temp</th>
<th>CO2growth</th>
<th>CO2mes</th>
<th>Temp × CO2growth</th>
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<th>Temp × CO2growth × CO2mes</th>
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<tbody>
<tr>
<td>Y(NPQ)</td>
<td>46.0***</td>
<td>25.2***</td>
<td>152.5***</td>
<td>12.0**</td>
<td>0.3</td>
<td>NS</td>
<td>398.8***</td>
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<tr>
<td>Y(NO)</td>
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<td>13.9**</td>
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<td>199.6***</td>
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<td>24.8***</td>
<td>358.9***</td>
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<tr>
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<td>28.9***</td>
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<td>17.8***</td>
<td>376.3***</td>
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<td>0.1 NS</td>
<td>0.1 NS</td>
<td>0.1 NS</td>
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Table 4. The maximum photochemical efficiency of PSII (Fv/Fm) after an overnight dark adaptation in leaves of Japanese white birch grown under ambient and elevated [CO2]. Values are mean ± SE (n = 8). NS, not significant at P > 0.05.

<table>
<thead>
<tr>
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<th>Ambient</th>
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<th>t-test</th>
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<tr>
<td>Fv/Fm</td>
<td>0.80 ± 0.002</td>
<td>0.79 ± 0.009</td>
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</table>

Plants grown under elevated [CO2] have been reported as showing enhanced PSII thermostolerance to high temperature stress (Faria et al. 1996, Taub et al. 2000). Although there was no difference in terms of the susceptibility to photoinhibition, indicated by Excess, Y(NO) and Fv/Fm across the temperature range, less Y(NPQ) observed in ambient-CO2-grown plants temporally exposed to elevated [CO2] (800 μmol mol−1) at higher temperatures compared with that at lower temperatures suggested that they maintained some capacity to invoke regulated thermal energy dissipation at higher temperatures (Hendrickson et al. 2004, Klughammer and Schreiber 2008). Thus, short-term [CO2] elevation might contribute to circumvent photoinhibition at higher temperatures in photosynthetically non-downregulated plants. In contrast to Japanese white birch, leaves of evergreen trees such as loblolly pine (Hymus et al. 1999) and snow gum (Rodin et al. 1999) grown under elevated [CO2] become susceptible to photoinhibition in winter. As the Japanese white birch is a deciduous broad-leaved tree species, mature leaves of the Japanese white birch would not acclimatize the photosynthetic traits to low temperature, which might result in a different response from evergreen trees.

Conclusion

Elevated [CO2] under limited N supply resulted in photosynthetic downregulation in the seedlings of the Japanese white birch. Even though photosynthetic downregulation occurred, elevated [CO2] would enhance
photosynthetic carbon assimilation at higher temperatures. Furthermore, elevated-CO₂-induced downregulation under a limited N supply might not exacerbate the temperature-dependent susceptibility to photoinhibition in the Japanese white birch across the temperature range from 15 to 40°C.

Acknowledgements – We thank Dr Kabeya for his assistance in carbohydrate analysis. This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 22114514).

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Edited by W. S. Chow