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Does long-term cultivation of saplings under elevated CO_2 concentration influence their photosynthetic response to temperature?

Ladislav Šigut[1,2], Petra Holišová[1], Karel Klem[1], Mirka Šprtová[1], Carlo Calfapietra[1,3], Michal V. Marek[1], Vladimír Špunda[1,2] and Otmar Urban[1,*]

[1]Global Change Research Centre AS CR, v.v.i., Bělidla 986/4a, 603 00, Brno, Czech Republic, [2]Faculty of Science, Ostrava University, 30. dubna 22, 701 03, Ostrava 1, Czech Republic and [3]National Research Council, Institute of Agro-Environmental & Forest Biology, Via Marconi 2, 05010, Porano, Italy
** For correspondence. E-mail urban.o@czechglobe.cz*

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• **Background and Aims** Plants growing under elevated atmospheric CO_2 concentrations often have reduced stomatal conductance and subsequently increased leaf temperature. This study therefore tested the hypothesis that under long-term elevated CO_2 the temperature optima of photosynthetic processes will shift towards higher temperatures and the thermostability of the photosynthetic apparatus will increase.
• **Methods** The hypothesis was tested for saplings of broadleaved *Fagus sylvatica* and coniferous *Picea abies* exposed for 4–5 years to either ambient (AC; 385 μmol mol^{-1}) or elevated (EC; 700 μmol mol^{-1}) CO_2 concentrations. Temperature response curves of photosynthetic processes were determined by gas-exchange and chlorophyll fluorescence techniques.
• **Key Results** Initial assumptions of reduced light-saturated stomatal conductance and increased leaf temperatures for EC plants were confirmed. Temperature response curves revealed stimulation of light-saturated rates of CO_2 assimilation (A_{max}) and a decline in photorespiration (R_L) as a result of EC within a wide temperature range. However, these effects were negligible or reduced at low and high temperatures. Higher temperature optima (T_{opt}) of A_{max}, Rubisco carboxylation rates (V_{Cmax}) and R_L were found for EC saplings compared with AC saplings. However, the shifts in T_{opt} of A_{max} were instantaneous, and disappeared when measured at identical CO_2 concentrations. Higher values of T_{opt} at elevated CO_2 were attributed particularly to reduced photorespiration and prevailing limitation of photosynthesis by ribulose-1,5-bisphosphate (RuBP) regeneration. Temperature response curves of fluorescence parameters suggested a negligible effect of EC on enhancement of thermostability of photosystem II photochemistry.
• **Conclusions** Elevated CO_2 instantaneously increases temperature optima of A_{max} due to reduced photorespiration and limitation of photosynthesis by RuBP regeneration. However, this increase disappears when plants are exposed to identical CO_2 concentrations. In addition, increased heat-stress tolerance of primary photochemistry in plants grown at elevated CO_2 is unlikely. The hypothesis that long-term cultivation at elevated CO_2 leads to acclimation of photosynthesis to higher temperatures is therefore rejected. Nevertheless, incorporating acclimation mechanisms into models simulating carbon flux between the atmosphere and vegetation is necessary.

Key words: Climate change, CO_2 assimilation, elevated CO_2 acclimation, European beech, *Fagus sylvatica*, Norway spruce, photorespiration, photosystem II photochemistry, *Picea abies*, Rubisco carboxylation, thermotolerance.

INTRODUCTION

Global climate models predict a gradual increase in atmospheric CO_2 concentration and global temperature by as much as 700 μmol CO_2 mol^{-1} and 2·2 °C, respectively, by 2100 (RCP6.0 scenario; IPCC, 2013). In addition, more intense, more frequent and longer lasting heat waves are predicted in the 21st century by a global coupled climate model (Meehl and Tebaldi, 2004). It is therefore important to assess the effect on plants of elevated atmospheric CO_2 concentrations (EC), elevated temperatures and their interactions; in particular, whether EC will cause changes in the temperature sensitivity of plants' metabolism, growth and development and whether EC will increase plants' heat stress tolerance remain open questions.

It is well documented that EC enhances photosynthetic CO_2 uptake under sufficient light intensities in C_3 plants, while photorespiration rate and stomatal conductance are usually reduced (Long, 1991; Jarvis, 1998; Ainsworth and Rogers, 2007; Urban *et al*., 2014). Long-term, field-based studies on European forest tree species have indicated a significant decrease in stomatal conductance (by 21 % on average) for trees grown under EC. The decrease was stronger in young trees than in old trees, in deciduous trees than in coniferous trees, and in water-stressed trees than in nutrient-stressed trees (Medlyn *et al*., 2001). In addition, stomatal density (i.e. the number of stomata per unit of leaf area) may decrease with increasing CO_2 concentration (Woodward and Bazzaz, 1988; Tricker *et al*., 2005), which is

related to an overexpression of the *HIC* (high carbon dioxide) gene encoding an enzyme involved in a negative regulation of stomatal development (Gray *et al*., 2000). These restrictions lead to a reduced dissipation of latent heat via transpiration followed by an increase in leaf temperature that is often observed in plants grown under EC (Siebke *et al*., 2002; Barker *et al*., 2005; Leuzinger and Körner, 2007).

Temperature affects photosynthetic processes influencing the composition of thylakoid membranes and the reaction kinetics of related biochemical processes. Plants grown at low temperatures have lower temperature optima for CO_2 assimilation (Berry and Björkman, 1980; Sage and Kubien, 2007), as well as a lower electron transport rate (June *et al*., 2004) and other photosynthesis-related processes (Hikosaka *et al*., 2006; Sage and Kubien, 2007) than do plants grown at higher temperatures. Plants that are native to or grown in warm environments usually have increased heat stress tolerance, i.e. injuries to plant tissues and metabolism occur at higher temperatures than they do for plants from cool environments (Ghouil *et al*., 2003; Hikosaka *et al*., 2006; Wahid *et al*., 2007; Crous *et al*., 2013). High temperatures can, among other things, impair quantum yield of photosystem II (Taub *et al*., 2000; June *et al*., 2004; Wang *et al*., 2012) and Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) enzymatic activity (Crafts-Brandner and Salvucci, 2000; Ainsworth and Rogers, 2007; Urban *et al*., 2012). Whether an EC expected in the future has a potential, through a reduced transpiration and increased leaf temperature, to induce temperature acclimation of photosynthesis and increase a tolerance of plants to heat waves remains, however, unclear.

It has been shown that the rate and temperature optima (T_{opt}) of light-saturated photosynthesis are considerably higher at saturating CO_2 concentration than ambient CO_2 concentration irrespective of the temperature at which a plant was grown (cf. Berry and Björkman, 1980). Temperature optima instantaneously increase with CO_2 concentration due to a shift of photosynthesis limitation by Rubisco carboxylation activity to a limitation by RuBP (ribulose-1,5-bisphosphate) regeneration capacity, Rubisco kinetics and reduction of photorespiration (reviewed by Hikosaka *et al*., 2006). Therefore, the relative increase in CO_2 assimilation rate under elevated CO_2 concentration is greater at high temperatures than it is at low temperatures (Ainsworth and Ort, 2010). Under heat stress, the reported effects of EC on plant photosynthesis and growth are, however, very variable and differ among functional groups of plants (reviewed by Wang *et al*., 2012).

In this study, we tested two hypotheses: (1) plants grown under EC conditions experience higher leaf temperatures than AC plants; and (2) higher leaf temperatures subsequently lead to temperature acclimation of carbon assimilation. Such temperature acclimation is defined here as an increase in temperature optima that does not disappear after exposing EC plants to ambient CO_2 concentrations. The hypotheses were tested in two distinctive temperate-zone tree species (broadleaved *Fagus sylvatica* and coniferous *Picea abies*) under the growth CO_2 concentrations (385 versus 700 μmol CO_2 mol^{-1}) as well as those concentrations in reverse. The study's specific objectives were to investigate (1) temperature response curves of CO_2 assimilation rate, (2) temperature response curves of photorespiration rate and (3) thermal stability of photosystem II photochemistry in plants exposed to ambient and elevated atmospheric CO_2 concentrations.

MATERIAL AND METHODS

Plants and experimental design

The experiment was carried out at the Bílý Kříž experimental research site in the Beskydy Mountains, Czech Republic (49°30′N, 18°32′E, 908 m a.s.l.). The area has a cool (annual mean air temperature 6·8 °C) and humid (annual mean relative humidity 84 %) climate with high annual precipitation (average for 1998–2011 was 1293 mm). In the present study, we compared photosynthetic activity in two widespread temperate-zone tree species: broadleaved European beech (*Fagus sylvatica*) and coniferous Norway spruce (*Picea abies*). Three-year-old saplings were planted and thereafter grown at ambient (385 μmol CO_2 mol^{-1}; hereafter AC) and elevated (700 μmol CO_2 mol^{-1}; hereafter EC) atmospheric CO_2 concentrations for 4–5 years using glass dome facilities (Urban *et al*., 2001). Carbon dioxide enrichment under the glass dome was continuous from April to November each year. The saplings' mean height (± s.d.) was 2·6 ± 0·5 m (*F. sylvatica*) and 1·5 ± 0·3 m (*P. abies*) at the start of measurements in 2010. Saplings were grown in their native soil. The geological bedrock was formed of Mesozoic Godula sandstone (flysch type) and is overlain by ferric podzols. Environmental air conditions inside the domes were maintained by an air-conditioning device together with an adjustable window system enabling also throughput of incident rainfall. In case of differences between soil moisture outside and inside the domes, irrigation was provided by an automatic system. A detailed description of the glass dome microclimate can be found in Urban *et al*. (2014).

Monthly climatic conditions for AC and EC saplings are given in Table 1. Growth conditions 7 d prior to individual measuring campaigns are given in Supplementary Data Table S1. To assess leaf surface temperature (T_{leaf}) and its spatial distribution, images from a Ti55FT thermal camera (Fluke, Mississauga, Ontario, Canada) were collected during a sunny day at 1-h intervals (Fig. 1). At least 100 points (individual beech leaves or spruce shoots) were manually selected from each thermal image for temperature analysis. Only evenly sunlit leaves and shoots were selected.

Physiological measurements

Thirty-six spruce and 63 beech saplings per treatment were planted in the layout of an equilateral triangle (side length 1·20 m). Saplings within each dome (AC and EC domes) were split into three plots (each with an area of 33 m^2) per dome in a south–north orientation. Each dome plot consisted of 12 spruce and 21 beech saplings and was considered as a separate replication.

In situ physiological measurements were carried out during mid-season (July and August) in two consecutive years (2010, 2011) on sun-exposed and fully developed beech leaves and current-year spruce shoots. Two saplings per plot were evaluated and the average from these two measurements was used for statistical analyses. Investigated saplings were selected among those of average height and stem diameter with similar leaf chlorophyll content estimated *in vivo* using an SPAD-502 Chlorophyll Meter (Konica Minolta, Osaka, Japan).

TABLE 1. *Daily mean (s.d.) and minimum–maximum air temperatures (T_{air}; °C) and relative humidities (RH; %) for individual months of the 2010 and 2011 growing seasons inside glass domes with ambient (AC) and elevated (EC) CO_2 concentrations. Sums of precipitation (P_{sum}; mm) originate from a nearby meteorological station. Measurements were made automatically at 10-min frequency*

	May	June	July	August	September	October
2010						
T_{air} AC	10·0 (3·37)	15·1 (4·26)	18·5 (4·24)	16·3 (3·56)	10·4 (2·97)	5·1 (3·4)
	0·9–22·4	6·6–32·9	7·9–35·1	6·5–29·4	1·6–21·9	–2·2 to 22·9
T_{air} EC	10·3 (3·48)	15·7 (4·43)	18·9 (4·33)	16·5 (3·60)	10·7 (3·05)	5·4 (3·46)
	1·1–23·5	6·8–33·4	8·4–35·5	6·6–30·8	1·8–23·3	–1·9 to 21·5
RH AC	92 (7·6)	80 (13·0)	76 (15·5)	84 (9·1)	89 (8·6)	86 (9·4)
	44·1–99·8	32·8–99·9	39·6–99·8	39·6–99·8	38·7–99·8	28·4–99·7
RH EC	92 (7·6)	80 (13·0)	77 (15·51)	84·1 (9·2)	89 (8·6)	86 (9·4)
	44·4–99·9	33·1–99·9	28·8–99·9	39·9–99·9	39·0–99.	28·6–99·9
P_{sum}	394·2	115·8	155·6	221·4	203·2	29·8
2011						
T_{air} AC	12·7 (5·04)	15·5 (2·56)	15·2 (4·0)	17·4 (3·39)	14·4 (2·79)	7·0 (4·11)
	–1·9 to 29·6	7·8–27·3	6·0–29·0	6·4–31·1	5·3–28·2	–2·4 to 23·8
T_{air} EC	12·8 (5·14)	15·6 (2·66)	15·4 (4·12)	17·9 (3·54)	14·8 (2·89)	7·2 (4·48)
	–2·2 to 29·2	7·6–28·4	5·9–29·9	6·3–32·7	5·5–29·7	–2·6 to 28·4
RH AC	69 (13·6)	82 (8·3)	86 (10·6)	80 (8·4)	79 (9·5)	85 (10·23)
	24·2–99·7	42·8–99·7	39·6–99·8	38·1–99·8	34·7–99·3	32·4–99·7
RH EC	68 (14·3)	81 (9·4)	85 (18·8)	79 (9·6)	77 (11·3)	84 (12·4)
	24·4–99·9	37·6–99·9	37·7–99·9	21·2–99·9	29·7–99·9	27·0–99·9
P_{sum}	122.6	145·8	256·6	84·4	41·0	57·4

Gas-exchange measurements

Temperature response curves of basic photosynthetic characteristics (CO_2 assimilation rate A, stomatal conductance G_S and intercellular CO_2 concentration C_i) were measured on intact leaves using the Li-6400 gas-exchange system (Li-Cor, Lincoln, NB, USA) within a T_{leaf} range from 10 to 45 °C. Target T_{leaf} was controlled using an integrated Peltier thermoelectric module. Vapour pressure deficit (VPD) values varied naturally along with temperature from 0·6 kPa at 10 °C to 4·2 kPa at 45 °C. These changes in VPD were, however, identical for both CO_2 concentration treatments and both species studied (Supplementary Data Fig. S1).

Light-saturated rates of CO_2 assimilation (A_{max}) and gross CO_2 assimilation rate (A_{gross}) were measured after 10 min exposure to a saturating irradiance (1400 μmol photons m^{-2} s^{-1}) and growth CO_2 concentration (i.e. 385 μmol CO_2 mol^{-1} for AC plants and 700 μmol CO_2 mol^{-1} for EC plants). A_{gross} was measured using air supply with 2 % O_2. In 2011, measurement of the temperature response curve of A_{max} at growth CO_2 concentrations was coupled with an estimation of A_{max} at the opposite CO_2 concentration (i.e. EC plants were exposed to 385 μmol CO_2 mol^{-1} and AC plants to 700 μmol CO_2 mol^{-1}). The aim was to assess the instantaneous effect of CO_2 concentration on temperature responses of CO_2 assimilation rate and to assess the sensitivity of Rubisco-limited and RuBP-limited rates of CO_2 assimilation to temperature.

The photorespiration rate at saturating irradiance (R_L) was quantified as the difference between the CO_2 assimilation rate measured under 2 % oxygen (A_{gross}) and that measured under normal 21 % oxygen (A_{max}). Due to an inhibition of oxidative phosphorylation in photosynthesizing cells by competition for available ADP, mitochondrial respiration (the Krebs cycle activity) in light was assumed to be negligible compared with A_{max} and R_L (Sharkey, 1988).

To estimate the rate of *in vivo* Rubisco carboxylation (V_{Cmax}), the initial linear phase of the A/C_i response curves was measured at low C_i (50–250 μmol CO_2 mol^{-1}) and saturating irradiance (1400 μmol photons m^{-2} s^{-1}). V_{Cmax} values were subsequently calculated according to the equations of Farquhar *et al.* (1980). The temperature dependence of the Michaelis–Menten constants of Rubisco for carboxylation (K_c) and oxygenation (K_o), which are key parameters of Farquhar's photosynthetic model, were calculated as $Parameter = \exp(c - \Delta H_a/RT_{leaf})$, where R is the molar gas constant (8·314 J mol^{-1} K^{-1}), c represents a scaling constant (38·05 for K_c and 20·30 for K_o) and ΔH_a is activation energy (79·43 for K_c and 36·38 kJ mol^{-1} for K_o) as suggested by Bernacchi *et al.* (2001).

Chlorophyll fluorescence measurement

Temperature responses of chlorophyll *a* fluorescence (Chl-F) parameters were estimated on the dark-adapted detached needles or leaf discs using a pulse amplitude-modulated fluorometer (PAM 101/103; Heinz Walz, Effeltrich, Germany). The system for linear heating of needle or leaf samples consisted of a temperature-controlled chamber (LD2/2 leaf-disc oxygen electrode chamber; Hansatech Instruments, King's Lynn, UK) equipped with an optical lid for the PAM fibre-optic guide and connected to an ME-4 programmable temperature-controlled water bath (Julabo, Seelbach, Germany) adjusted to produce approximately 1 °C min^{-1} heating of the needle or leaf surface within the temperature range 20–48 °C. Needle or leaf disc surface temperature was continuously monitored using a thermocouple during the heating regime. The Chl-F measurements were carried out in darkness to estimate potential efficiency of photosystem (PS) II photochemistry [$F_V/F_M = (F_M - F_0)/F_M$] or under a moderate actinic light intensity (250 μmol photons m^{-2} s^{-1}) to assess the allocation of light absorbed by PS II to photochemical reactions: actual yield of PS II photochemistry [$P = (F_M' - F_T)/F_M'$] and thermal energy dissipation [$D = 1 - (F_M' - F_0')/F_M'$] according to Demmig-Adams *et al.* (1996). Samples were inserted into the measuring chamber on

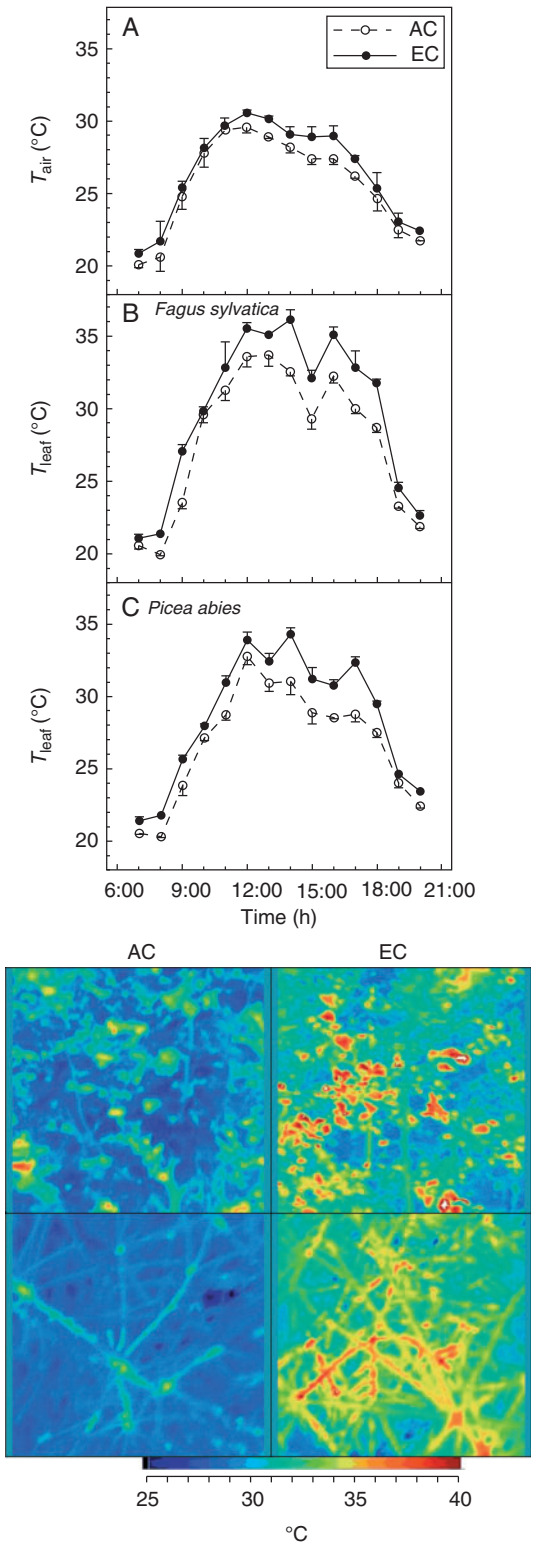

FIG. 1. Diurnal courses of (A) air temperature (T_{air}) and leaf temperature (T_{leaf}) of (B) *Fagus sylvatica* and (C) *Picea abies* in glass domes with ambient (AC) and elevated (EC) atmospheric CO_2 concentrations. Points indicate hourly averages (with error bars for standard deviation) from 15-s readings of T_{air} and T_{leaf} estimates from thermal camera images. The scans were taken during two consecutive days (23–24 August 2011). Example thermal images (bird's-eye view) acquired at the time of greatest differences between leaf temperature of trees from the AC and EC conditions (14:00 local mean time) are shown.

water-soaked foam (to avoid desiccation during measurement) and pre-acclimated at 20 °C for 10 min prior to the start of heating, either in darkness (only at a weak measuring light; <0·1 μmol photons m^{-2} s^{-1}) or at the given actinic illumination (to reach steady-state Chl-F signal). Saturation pulses (duration 0·8 s; intensity approx. 5000 μmol photons m^{-2} s^{-1}) were applied at 20 °C and then approx. every 2 min (immediately following a 2 °C increase in leaf surface temperature) to determine a maximum Chl-F in dark-adapted (F_M) and/or light-adapted (F_M') states. Before application of the saturation pulse, readings of the minimum Chl-F in the dark-adapted state (F_0) and/or actual Chl-F in the light-adapted state (F_T) were taken. In light-adapted samples, the actinic light was switched off 10–15 s after a saturation pulse for 5 s and the minimum Chl-F in the light-adapted state (F_0') was estimated as the lowest Chl-F intensity during that period.

Modelling of temperature response curves

The instantaneous rates (k; μmol CO_2 m^{-2} s^{-1}) of A_{max} and A_{gross} were modelled as a general parabolic function of actual leaf temperature (T; °C) according to Säll and Pettersson (1994):

$$k = k_{opt} - b\left(T - T_{opt}\right)^2 \quad (1)$$

where T_{opt} is optimal leaf temperature at which k achieves its highest value, k_{opt} is the assimilation rate at T_{opt} and parameter b defines the spread of the parabola.

The asymmetric temperature responses of V_{Cmax} and R_L were fitted by a modified Arrhenius function including a component to take account of the deactivation processes that occur above the optimum temperature (Dreyer *et al.*, 2001):

$$k = \frac{k_r e^{\left[\frac{\Delta H_a}{RT_r}\left(1-\frac{T_r}{T}\right)\right]}}{1 + e^{\left[\frac{\Delta ST - \Delta H_d}{RT}\right]}}\left(1 + e^{\left[\frac{\Delta ST_r - \Delta H_d}{RT_r}\right]}\right) \quad (2)$$

where k_r is the parameter value at reference temperature T_r (25 °C, 298·15 K), ΔH_a is the activation energy (J mol^{-1}), ΔH_d is the deactivation energy of the given parameter (J mol^{-1}), ΔS is entropy (J K^{-1} mol^{-1}), R is the molar gas constant (8·314 J mol^{-1} K^{-1}) and T (K) is leaf temperature.

The optimal temperature T_{opt} (°C) of either V_{Cmax} or R_L can be computed from eqn (2) as:

$$T_{opt} = \frac{-\Delta H_d}{R\ln\left(\frac{-\Delta H_a}{\Delta H_a - \Delta H_d}\right) - \Delta S} - 273.15 \quad (3)$$

Statistical data analysis

The aforementioned models were fitted (using Microsoft Office Excel 2010 with the Solver add-in and R statistical programming language) to the data points of individual samples ($n = 6$) to allow for statistical testing between treatments. Estimated parameters were tested for normal distribution

TABLE 2. *Mean values (±s.d.) of stomatal conductance at saturating irradiation (G_{smax}) in mol H_2O m^{-2} s^{-1} estimated across the entire temperature range (10–45 °C) for* Fagus sylvatica *and* Picea abies *grown under ambient (AC) and elevated (EC) CO_2 concentrations*

	AC 2010	EC 2010	AC 2011	EC 2011
F. sylvatica	0·24 ± 0·11	0·18 ± 0·09*	0·12 ± 0·04	0·11 ± 0·06
P. abies	0·13 ± 0·05	0·11 ± 0·04	0·11 ± 0·07	0·08 ± 0·05

Measurements were performed during campaigns in 2010 (July) and 2011 (August) at growth CO_2 concentration.

*Statistically significant differences between AC and EC treatments at $P < 0·05$, $n = 35$.

(Shapiro–Wilk test) within treatments and for equality of variances between the pair of treatments (F-test). Testing for statistical differences between means was performed using R software (R Development Core Team, 2014) with the Wilcoxon test for non-normal data and a two-sample t-test or Welch's two-sample t-test for normal data with equal or unequal variances, respectively.

RESULTS

Mean values of stomatal conductance under saturating irradiance (G_{Smax}) were obtained from temperature response measurements during campaigns in 2010 and 2011 as the average G_{Smax} for the entire measured temperature range. We found a decrease in G_{Smax} for EC-treated plants compared with AC plants of 13–30 %, although these differences were only significant ($P < 0·05$) for beech in 2010 (Table 2).

Analysis of thermal camera images (Fig. 1) revealed differences in T_{leaf} between AC and EC plants. On average, EC plants recorded 2 °C higher T_{leaf} compared with AC plants, with the greatest difference (3·5 °C) coming in the afternoon of a sunny day (Fig. 1B, C). These differences can be attributed only in part to an increase in air temperature (T_{air}) under EC, because T_{air} observed in the EC dome was higher by 0·9 °C on average as compared with that for the AC condition (Fig. 1A).

The EC condition led to the stimulation of A_{max} in both tree species (Fig. 2A–D). For example, significant ($P < 0·05$) stimulations of A_{max} at optimal temperature (A_{opt}) were found for beech (53–74 %) and spruce (22–47 %) saplings cultivated under EC conditions (Table 3). This stimulatory effect was, however, negligible or reduced at low and high temperatures (approx. below 15 °C and above 40 °C, respectively). During the two consecutive seasons, we observed significantly higher temperature optima for A_{max} [$T_{opt}(A_{max})$] in EC beech (by 2·9–3·5 °C) and spruce (by 3·3–6·0 °C) in comparison with their AC counterparts (Fig. 2A–D; Table 3). However, an estimation of both A_{opt} and $T_{opt}(A_{max})$ for saplings from both CO_2 concentration treatments revealed no significant differences ($P > 0·05$) between AC and EC saplings when the saplings were measured at the same CO_2 concentration (Fig. 2E–H; Table 3).

Temperature response curves of V_{Cmax} (Fig. 3) showed a significant ($P < 0·05$) shift of temperature optima for Rubisco carboxylation rate by 3·4–3·7 °C for EC saplings of both species compared with their AC counterparts. The values of V_{Cmax} at reference leaf temperature 25 °C were lower for EC plants compared with AC plants by 12–13 % for both species (Table 4). These differences were not, however, statistically significant.

The values of A_{gross} showed a similar temperature curve pattern as those for A_{max} (Fig. 4A, B). In both species, EC stimulated A_{gross} only at high temperatures, while differences in A_{gross} between AC and EC saplings were negligible at temperatures below 25 °C. Accordingly, significant ($P < 0·05$) stimulations of A_{gross} at optimal temperature (A_{opt}) were found for beech (30–41 %) and spruce (29–64 %) saplings cultivated under EC conditions (Table 3). A significant increase in $T_{opt}(A_{gross})$ due to the EC treatment was confirmed for beech (by 3·5–3·6 °C; $P < 0·05$) as well as spruce (3·1–4·5 °C; $P < 0·01$) in the two consecutive years studied (Table 3). Suppression of photorespiration at low temperatures led to a slight increase in $T_{opt}(A_{gross})$ compared with $T_{opt}(A_{max})$, particularly for spruce (Tables 3 and 4).

Values of R_L were estimated as the difference between A_{gross} and A_{max} rates. EC had a similar effect on the pattern of temperature response curves of photorespiration in both species (Fig. 4C, D). While the maximum rates of R_L were the same for both CO_2 treatments, EC saplings achieved maximum R_L at temperatures 5·5–5·6 °C higher than those for AC saplings (Table 4). Therefore, significantly lower R_L values in EC plants as compared with AC saplings were observed only in a relatively narrow temperature range of 15–30 °C. At low and high temperatures (approx. below 15 °C and above 30 °C, respectively), the R_L values of AC and EC saplings tended to converge. In contrast to R_L, dark mitochondrial respiration (R_D) and its temperature response curve of R_D were not influenced by CO_2 treatment (see Supplementary Data Fig. S2).

The EC treatment led to slightly higher F_V/F_M values at 46 and 48 °C in spruce as compared with the AC treatment (Fig. 5B), but that was not the case in beech (Fig. 5A). Irrespective of treatment, spruce had higher F_V/F_M than did beech at such high temperatures. Similarly, slightly higher temperature optima for actual yield of PS II photochemistry, P (Fig. 5C, D), and thermal energy dissipation, D (Fig. 5E, F), were observed in spruce than in beech. Long-term acclimation to EC, however, had no effect on the temperature response curve of P and D in either tree species across the entire temperature range studied.

DISCUSSION

In accordance with previous studies (Siebke *et al.*, 2002; Barker *et al.*, 2005; Leuzinger and Körner, 2007), we have confirmed the hypothesis that growth under EC increases foliage temperature in both broadleaved and coniferous trees species as compared with AC conditions by decreasing stomatal conductance (Fig. 1). We therefore further hypothesized parallel acclimation of photosynthesis to EC and temperature and that growth of plants under EC would lead to a shift of temperature optima for CO_2 assimilation rate to higher temperatures and to enhanced heat stress tolerance. Transitory (more intense and frequent heat waves) or continually (increase in global air

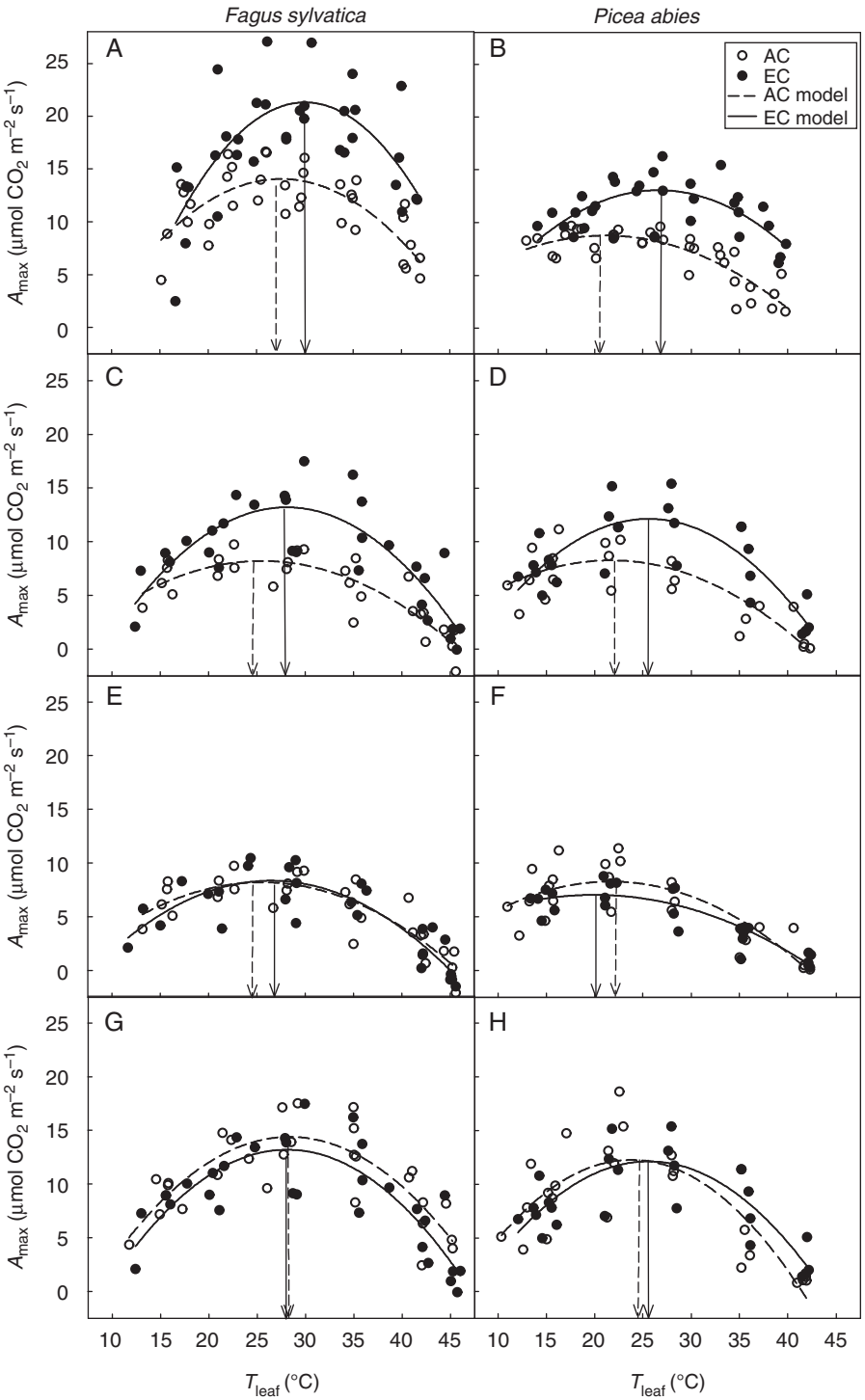

FIG. 2. Temperature response of net CO_2 assimilation rate at saturating irradiance (A_{max}) in *Fagus sylvatica* and *Picea abies* grown at ambient (AC) and elevated (EC) atmospheric CO_2 concentrations. Measurements were performed during campaigns in 2010 (A, B) and 2011 (C, H) at growth (A–D), ambient (E, F) and elevated (G, H) CO_2 concentrations. Parabolic fits for all samples of individual treatments and respective estimated temperature optima are displayed for AC and EC. The individual measured values are shown; R^2 ranged from 0·43 to 0·79 ($P < 0·01$). The vertical line indicates the optimum temperature of the fit.

temperature) high temperatures may cause an array of injuries at different hierarchical levels of plants (Sage and Kubien, 2007; Wahid *et al*., 2007; Way and Sage, 2008), which may subsequently affect plant growth, development, economic yield and/or geographical distribution. It remains an open question whether long-term growth under EC induces temperature acclimation of photosynthesis and increases the heat stress tolerance of plants.

TABLE 3. *Parameters (mean ± s.d.) of the temperature response curves of light-saturated CO_2 assimilation rate (A_{max}) and gross CO_2 assimilation rate under 2 % oxygen (A_{gross}) calculated for individual leaves of* Fagus sylvatica *and* Picea abies *grown at ambient (AC) and elevated (EC) CO_2 concentrations*

Treatment		A_{max}			A_{gross}		
		A_{opt}	T_{opt}	b	A_{opt}	T_{opt}	b
2010							
F. sylvatica	AC 385	13·8 ± 2·1	27·0 ± 1·0	0·03 ± 0·01	20·2 ± 1·9	27·8 ± 0·3	0·06 ± 0·01
	EC 700	21·1 ± 1·7**	29·9 ± 0·9*	0·06 ± 0·02*	26·2 ± 2·9*	31·4 ± 0·8**	0·06 ± 0·01
P. abies	AC 385	8·9 ± 1·0	20·8 ± 1·5	0·02 ± 0·01	11·7 ± 0·8	24·0 ± 0·7	0·04 ± 0·01
	EC 700	13·2 ± 1·4*	26·8 ± 0·3**	0·03 ± 0·01	15·1 ± 2·0*	28·5 ± 0·7**	0·04 ± 0·02
2011							
F. sylvatica	AC 385	7·6 ± 1·9	24·6 ± 1·1	0·02 ± 0·01	10·7 ± 1·3	26·6 ± 0·4	0·03 ± 0·01
	EC 700	13·2 ± 1·3*	27·9 ± 0·8*	0·03 ± 0·01	15·1 ± 1·9*	30·5 ± 1·8*	0·04 ± 0·01
	AC 385	7·6 ± 1·9	24·6 ± 1·1	0·02 ± 0·01	10·7 ± 1·3	26·6 ± 0·4	0·03 ± 0·01
	EC 385	7·5 ± 2·0	26·8 ± 0·9	0·02 ± 0·01	10·1 ± 1·1	27·4 ± 1·2	0·03 ± 0·01
	AC 700	14·1 ± 1·3	28·1 ± 0·3	0·03 ± 0·01	17·1 ± 2·5	29·0 ± 0·9	0·05 ± 0·01
	EC 700	13·2 ± 1·3	27·9 ± 0·8	0·03 ± 0·01	15·1 ± 1·9	30·5 ± 1·8	0·04 ± 0·01
P. abies	AC 385	8·5 ± 1·4	22·2 ± 1·0	0·03 ± 0·01	8·8 ± 1·9	22·5 ± 0·7	0·03 ± 0·01
	EC 700	12·5 ± 2·0*	25·6 ± 0·5**	0·04 ± 0·01	14·4 ± 0·4**	25·6 ± 0·6**	0·04 ± 0·01
	AC 385	8·5 ± 1·4	22·2 ± 1·0	0·03 ± 0·01	8·8 ± 1·9	22·5 ± 0·7	0·03 ± 0·01
	EC 385	7·1 ± 1·0	20·0 ± 1·3	0·02 ± 0·01*	8·9 ± 1·3	23·4 ± 1·8	0·02 ± 0·01
	AC 700	13·0 ± 2·2	24·6 ± 0·5	0·04 ± 0·01	13·5 ± 1·7	24·3 ± 0·6	0·05 ± 0·01
	EC 700	12·5 ± 2·0	25·6 ± 0·5	0·04 ± 0·01	14·4 ± 0·4	25·6 ± 0·6	0·04 ± 0·01

A general parabolic function (eqn 1) was fitted to A_{max} and A_{gross} data: A_{opt}, assimilation rate at optimal temperature (μmol CO_2 m^{-2} s^{-1}); T_{opt}, optimal leaf temperature (°C). Measurements were performed under 385 μmol CO_2 mol^{-1} (385; corresponding to AC) and/or 700 μmol CO_2 mol^{-1} (700; corresponding to EC) CO_2 concentrations. Statistically significant differences between AC and EC treatments are indicated as follows: *0·01 < P < 0·05, **P < 0·01; n = 3.

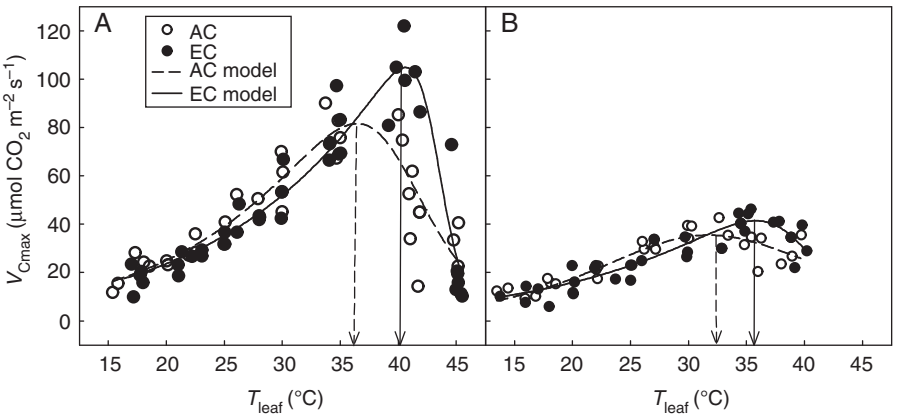

FIG. 3. Temperature dependences of light-saturated Rubisco carboxylation rate (V_{Cmax}) in *Fagus sylvatica* (A) and *Picea abies* (B) grown at ambient (AC) and elevated (EC) atmospheric CO_2 concentration. The modified Arrhenius function (eqn 2) was fitted to the data. The individual measured values are shown; R^2 ranged from 0·80 to 0·89 (P < 0·01). The vertical lines indicate modelled temperature optima of V_{Cmax} for AC and EC trees.

In addition, it has been shown that simulated carbon flux between the atmosphere and vegetation can dramatically differ between versions of models that do and do not include acclimation (Smith and Dukes, 2013). In contrast to instantaneous responses, mechanisms of acclimation to increasing atmospheric CO_2 concentration and temperature are, however, rarely presented and incorporated into the models.

To test this hypothesis, we investigated sensitivities of photosynthesis-related processes within the temperature range 10–45 °C. Although it was impossible to maintain low VPD values at high temperatures, this change was identical for both the AC and the EC conditions (Supplementary Data Fig. S1). High VPD values may lead to increased stomatal limitation of photosynthesis. However, it has been shown in many coniferous and broadleaved tree species that the sensitivity of stomata to VPD is not affected by growth under EC conditions (Jarvis, 1998). The presented differences between the AC and EC treatments are therefore not affected by changing VPD. In addition, the relatively slow response of stomata to different environmental parameters, particularly in coniferous *P. abies* (Košvancová *et al.*, 2009), leads to the presumption that the effect of VPD on the photosynthetic temperature response curve is relatively small.

Temperature responses of photosynthetic processes

Similarly to previous studies (e.g. Berry and Björkman, 1980; Long, 1991; Hikosaka *et al.*, 2006), considerably higher

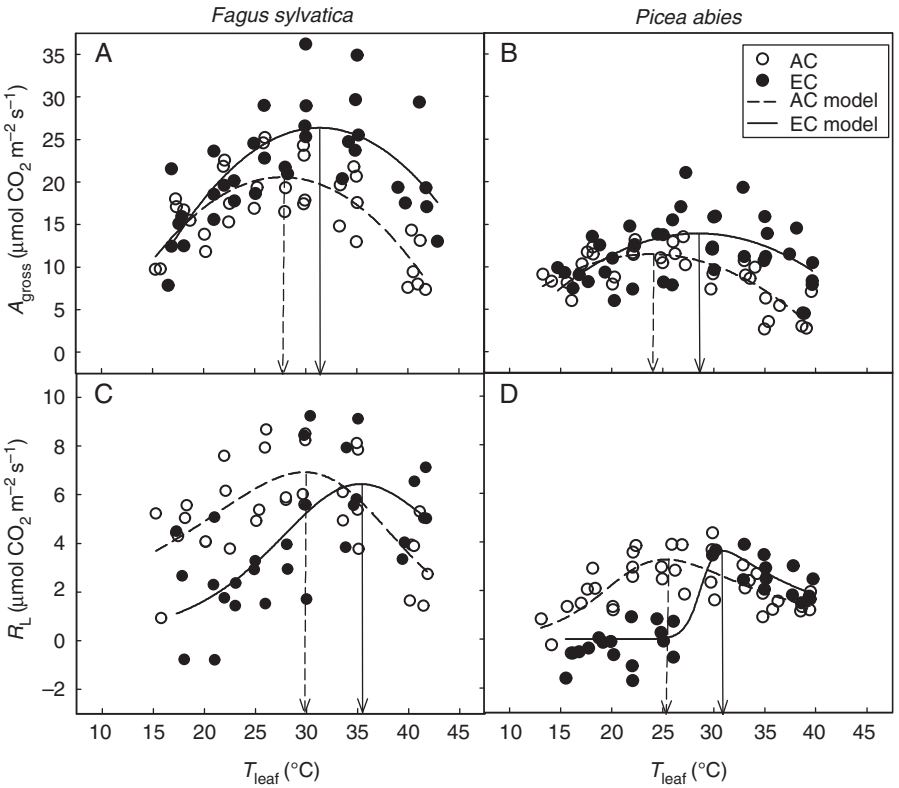

FIG. 4. Temperature response curves of light-saturated rate of gross CO_2 assimilation (A_{gross}; A, B) and photorespiration (R_L; C, D) in *Fagus sylvatica* (A, C) and *Picea abies* (B, D) grown under ambient (AC) and elevated (EC) atmospheric CO_2 concentration. Gas-exchange measurements were made at growth CO_2 concentration and 2 % O_2 in the atmosphere. The parabolic function (eqn 1) and modified Arrhenius function (eqn 2) were fitted to the A_{gross} ($R^2 = 0{\cdot}38$–$0{\cdot}65$; $P < 0{\cdot}01$) and R_L ($R^2 = 0{\cdot}42$–$0{\cdot}84$; $P < 0{\cdot}01$) data, respectively. Individual measured values are shown. The vertical lines indicate modelled temperature optima of V_{Cmax} for AC and EC trees.

TABLE 4. *Selected parameters (mean ± s.d.) of temperature response curves of light-saturated rate of Rubisco carboxylation (V_{Cmax}) and light-saturated rate of photorespiration (R_L) calculated for individual leaves of* Fagus sylvatica *and* Picea abies *grown at ambient (AC) and elevated (EC) CO_2 concentration*

Parameter	Units	*F. sylvatica*		*P. abies*	
		AC	EC	AC	EC
$V_{Cmax,25}$	μmol m^{-2} s^{-1}	40·1 ± 3·9	34·4 ± 2·8	26·5 ± 1·4	22·6 ± 2·6
V_{Cmax} at T_{opt}	μmol m^{-2} s^{-1}	82·6 ± 5·7	105·7 ± 10·6*	37·2 ± 2·8	42·2 ± 3·1
$T_{opt}(V_{Cmax})$	°C	36·3 ± 0·9	40·0 ± 0·9**	32·4 ± 0·3	35·8 ± 0·3**
$R_{L,25}$	μmol m^{-2} s^{-1}	6·3	3·2	3·3	0·1
R_L at T_{opt}	μmol m^{-2} s^{-1}	6·9	6·4	3·3	3·6
$T_{opt}(R_L)$	°C	29·8	35·3	25·3	30·9

$V_{Cmax,25}$ and $R_{L,25}$ are V_{Cmax} and R_L rates at reference leaf temperature 25 °C; $T_{opt}(V_{Cmax})$ and $T_{opt}(R_L)$ are temperature optima of V_{Cmax} and R_L. Measurements were performed at growth CO_2 concentration and 2 % oxygen (R_L) or at intercellular CO_2 concentrations ranging between 50 and 250 μmol CO_2 mol^{-1} (V_{Cmax}). Statistically significant differences between AC and EC treatments are indicated as follows: *$0{\cdot}01 < P < 0{\cdot}05$, **$p < 0{\cdot}01$; $n = 3$. See Supplementary Table S2 for the complete list of parameters of the modified Arrhenius function (eqn 2) fitted to the V_{Cmax} and R_L data.

rates and temperature optima of A_{max} and A_{gross} were found under EC than under AC (Figs 2A–D and 4A, B). The effect of EC on assimilation rate was, however, reduced at high (>40 °C) and particularly low temperatures (<15 °C). As CO_2 concentration increases, photosynthesis is increasingly limited by the capacity for RuBP regeneration, i.e. by photosynthetic electron transport chain capacity (Farquhar *et al.*, 1980). Hikosaka *et al.* (2006) noted that the optimal temperature of the RuBP-limited assimilation rate is higher than that of the Rubisco-limited assimilation rate in many species, thus leading to an increase in $T_{opt}(A_{max})$ at high CO_2 concentrations (Fig. 2). In addition, temperature-stimulated electron flow through PS II (estimated here by *P*; Fig. 5C, D) and reduced photorespiration under EC (Fig. 4C, D) consequently lead to an increase in the

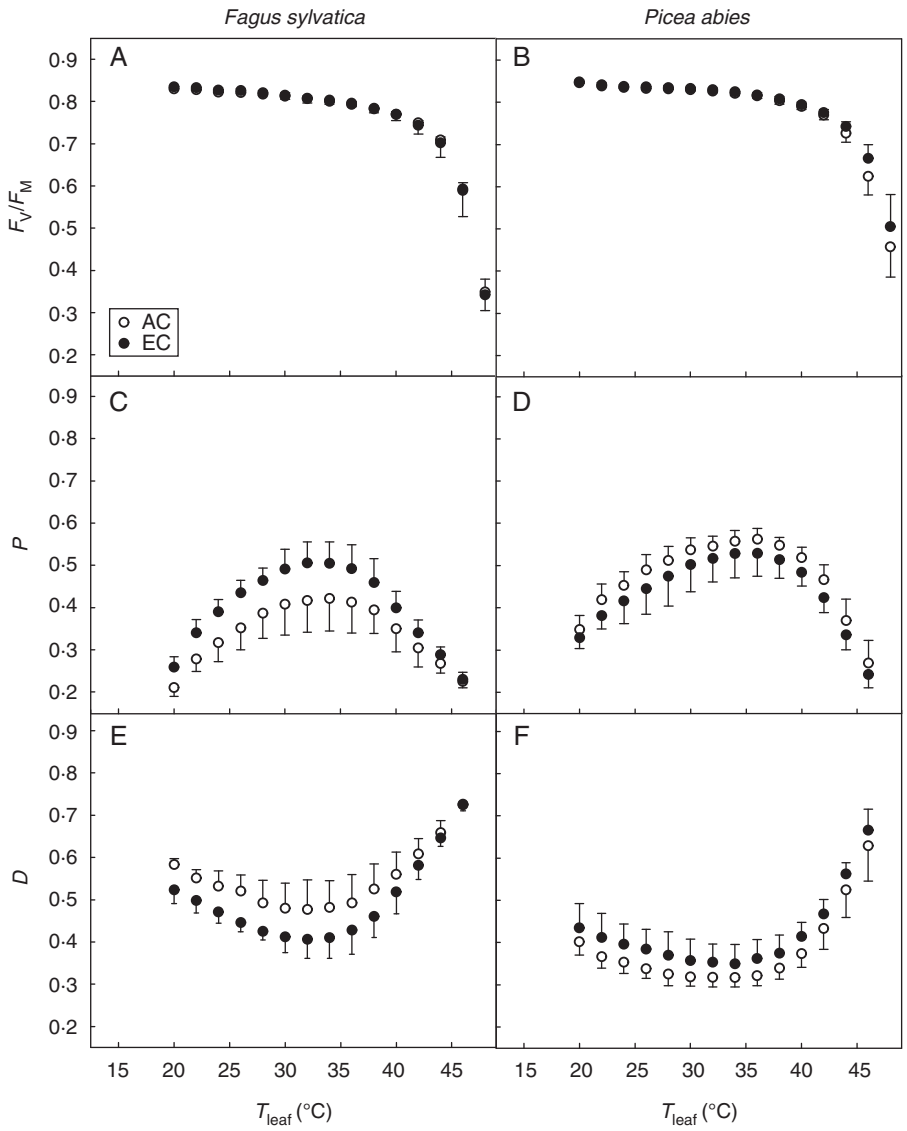

FIG. 5. Temperature response curves of potential efficiency of photosystem (PS) II photochemistry in dark-adapted leaves (F_V/F_M; A, B), actual yield of PS II photochemistry (P; C, D) and thermal energy dissipation (D; E, F) in *Fagus sylvatica* (A, C, E) and *Picea abies* (B, D, F) grown at ambient (AC) and elevated atmospheric CO_2 concentration (EC). Mean values with error bars for standard deviation are shown.

optimal temperature of the Rubisco-limited CO_2 assimilation rate (Franco and Lüttge, 2002; Sage and Kubien, 2007; Yamori *et al*., 2008). In our experiment, the shift in T_{opt} observed for AC versus EC saplings under growth CO_2 concentrations, however, was considerably reduced or completely disappeared when the saplings were exposed to the same CO_2 concentration, i.e. 385 or 700 μmol CO_2 mol^{-1} (Fig. 2E–H; Table 3). Based on these results, we conclude that EC has a significant, instantaneous and reversible effect on T_{opt}, but long-term cultivation under EC does not lead to typical photosynthetic acclimation to elevated temperatures as reported in reviews by Berry and Björkman (1980) and Sage and Kubien (2007).

Nevertheless, the observed shift of the V_{Cmax} optimum to higher temperatures in both species when EC treated as compared with when AC treated (Fig. 3; estimated at low C_i of 50–250 μmol CO_2 mol^{-1}) indicates certain temperature acclimation of Rubisco's kinetic properties. Possible mechanisms contributing to the reduction in Rubisco activity at high temperatures include an increase of mesophyll resistance to CO_2 diffusion followed by Rubisco decarbamylation (Bernacchi *et al*., 2001), reduction of Rubisco activation state due to Rubisco activase constraint (Crafts-Brandner and Salvucci, 2000) and/or increased synthesis of Rubisco inhibitor, xylulose-1,5-bisphosphate (Newman and Gutteridge, 1994), and an insufficiency of Pi in chloroplast stroma followed by a limitation of ATP production (June *et al*., 2004). In our previous studies using the same tree species (Košvancová *et al*., 2009; Urban *et al*., 2012), we have shown that high intercellular CO_2 concentration in EC plants protects Rubisco against decarbamylation and maintains a higher proportion of Rubisco in its active form in comparison with AC plants. In contrast, the often reported inaccessibility of Pi in EC plants may reduce the

ATP/ADP ratio and subsequently lead to reduced Rubisco activase activity (Crafts-Brandner and Salvucci, 2000). Such contrasting CO_2-dependent mechanisms of Rubisco regulation may consequently lead to the reported species-specific and seasonal variability in V_{Cmax} temperature dependence (Ziska, 2000; Urban *et al*., 2012; Crous *et al*., 2013).

Rubisco oxygenase activity, measured here as photorespiration rate (R_L; Fig. 4C, D), was significantly reduced by EC, particularly at temperatures between 20 and 25 °C. Further increase in T_{leaf}, however, resulted in a sharp increase in R_L values to the level of those observed for AC plants. Such increase in R_L is probably due to the reduced solubility of CO_2 compared with O_2 (Ehleringer and Björkman, 1977) and/or reduced activity of carbonic anhydrase (Badger and Price, 1994) at high temperatures. The EC treatment consequently resulted in a significant shift of photorespiration temperature optima in both species studied (Fig. 4C, D; Table 4). Our results thus show, in contrast to earlier predictions (Long, 1991; Bowes, 1996), that the effects of EC-reduced photorespiration on the stimulation of CO_2 uptake are relatively small or even negative at temperatures above 30 °C.

Thermal stability of PS II photochemistry

The steeper decline in F_V/F_M in T_{leaf} above 40 °C demonstrates the lower thermostability of PS II in beech leaves under both CO_2 concentration treatments as compared with spruce needles (Fig. 5A, B). We observed a smaller decline in F_V/F_M for EC spruce needles as compared with AC needles at temperatures above 40 °C, but no such decline for beech leaves. These data suggest that EC's effect on the enhancement of PS II thermal stability is negligible. Growth under EC has been shown, however, to protect *Quercus suber* (Faria *et al*., 1996) as well as *Pinus taeda* and *Quercus rubra* (Ameye *et al*., 2012) from short-term heat shock at the levels of both PS II photochemical efficiency and CO_2 assimilation rate. Also, Taub *et al*. (2000) found enhanced PS II thermotolerance, measured as F_V/F_M decline, in both woody and herbaceous species and in both monocotyledonous and dicotyledonous species cultivated under EC conditions. Although the exact mechanisms responsible for increased PS II thermotolerance in plants grown at elevated CO_2 remain unclear, enhanced thermotolerance probably relates to an increased production of heat shock proteins, chemical composition of the thylakoid membrane and chloroplast stroma, as well as isoprene production and its integration into the thylakoid membrane (reviewed by Taub *et al*., 2000).

A number of authors (Ghouil *et al*., 2003; Daas *et al*., 2008; Way and Sage, 2008; Yamori *et al*., 2008) have observed significant increases in T_{opt} of P and thermotolerance of PS II when growth temperature increased by more than 8 °C. The sensitivity to temperature of Chl-F parameters that we present in this study (Fig. 5C–F) demonstrates that the EC-related increase in T_{leaf} was below the threshold level for inducing acclimatory changes resulting in significantly enhanced PS II thermostability and shift of T_{opt} to higher temperatures.

To conclude, we have confirmed higher temperature optima (T_{opt}) of A_{max} for EC plants than for AC plants when measured at their growth CO_2 concentrations. This is caused mainly by reduced photorespiration and limitation of photosynthesis by RuBP regeneration under EC. However, the instantaneous differences in T_{opt} between AC and EC disappeared when the plants were exposed to identical CO_2 concentrations. Enhanced thermostability of PS II in EC saplings was not confirmed. We therefore rejected the hypothesis that EC conditions led to temperature acclimation of photosynthesis in both species studied.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: relationships between vapour pressure deficit and air temperature inside the assimilation chamber of the gas-exchange system during measurement of the temperature response curve of photosynthesis. Figure S2: temperature response curves of dark mitochondrial respiration (R_D) in *Fagus sylvatica* and *Picea abies* grown under ambient and elevated atmospheric CO_2 concentrations. Table S1: comparison of daily air temperature and relative humidity statistics between glass domes with ambient and elevated CO_2 concentrations for the 7 d preceding individual measurement periods. Table S2: parameters of temperature response curves of light-saturated rate of Rubisco carboxylation and light-saturated rate of photorespiration calculated for individual leaves of *Fagus sylvatica* and *Picea abies* grown at ambient and elevated CO_2 concentrations.

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