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Research article

# Interactive effects of ultraviolet radiation and elevated $CO_2$ concentration on photosynthetic characteristics of European beech saplings during the vegetation season☆

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ABSTRACT

To test the hypothesis that ultraviolet radiation (UV) modulates photosynthetic responses to elevated $CO_2$ concentration ($[CO_2]$) in plants, saplings of European beech were grown for two vegetation seasons under ambient (400 ppm) and elevated (700 ppm) atmospheric $[CO_2]$. From April to November the saplings were exposed to (i) ambient UV radiation, (ii) excluded and (iii) enhanced UV (150% of ambient). Gas-exchange and chlorophyll fluorescence techniques were used throughout the second vegetation season together with biochemical analyses of the amount and activity of the Rubisco enzyme. We found support for the hypothesis that an impact of elevated $[CO_2]$ on photosynthesis is substantially modulated by UV radiation. Moreover, we found that the $[CO_2]$ × UV interaction is changing along the vegetation season: an enhanced UV radiation stimulated a positive effect of elevated $[CO_2]$ on plant photosynthesis at the beginning of the vegetation season (short-term effect), whilst long-term cultivation reduced the stimulatory effect of elevated $[CO_2]$ (a clear down-regulation of photosynthesis). Down-regulation was, however, not found in plants grown under the conditions of excluded UV radiation. We found evidence that the down-regulation of photosynthesis is associated with a complex acclimation at different hierarchical and functional levels, including an acclimation of primary photochemical reactions, carboxylation activity of Rubisco enzyme, and stomatal conductance.

## 1. Introduction

There is clear evidence that anthropogenic activities lead to an increase of $CO_2$ concentration ($[CO_2]$) in the atmosphere. Depending on emission scenario, $[CO_2]$ is expected to reach 538–936 μmol $CO_2$ $mol^{-1}$ at the end of the century (IPCC, 2013). Together with increased temperature and altered precipitation patterns, elevated $[CO_2]$ is considered to be the most crucial factor affecting terrestrial ecosystems in future. However, plant responses to these factors may be further modulated by other environmental drivers, including ultraviolet (UV) radiation. Whereas recent measurements of stratospheric ozone indicate that the ozone layer has stopped recovering (Ball et al., 2018), short-term variations in UV intensity resulting particularly from changes in cloudiness, aerosol concentrations and seasonal fluctuations of ozone layer thickness are expected to have more important consequences on terrestrial ecosystems (Bornman et al., 2015).

$[CO_2]$ and UV radiation (particularly UV-B) have both direct and indirect effects on photosynthetic processes. $CO_2$ is the activator of Rubisco enzyme (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity (carbamylation), and it is the substrate of the Calvin cycle, when reaction of the active Rubisco-$CO_2$ complex with primary acceptor RuBP (ribulose-1,5-bisphosphate) leads to the formation of triose phosphates (carboxylation). Elevated $[CO_2]$, although reduces stomatal conductance, leads to an increase of photosynthetic $CO_2$ uptake in C3 plants under conditions of sufficient light intensity. Such up-regulation is mediated particularly by an increase in intercellular $[CO_2]$ and $[CO_2]$ in the chloroplasts, reduction of Rubisco oxygenase activity (suppressed photorespiration rate), and insufficiency of the current atmospheric $[CO_2]$ to saturate carboxylation activity of Rubisco (reviewed in Bowes, 1991; Ceulemans and Mousseau, 1994; Leakey et al., 2009; Urban, 2003). However, the degree of photosynthetic response to elevated $[CO_2]$ is very variable. Stimulation of the photosynthesis rate may

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range from several percentages up to several tens depending on plants species, duration of $CO_2$ enrichment, and growing conditions, i.e. interactions with other environmental factors. These interactions can reduce a positive effect of elevated [$CO_2$] as in the case of high [$O_3$] or, conversely, stimulate the response, as in the case of sufficient nitrogen supply (Ainsworth and Long, 2005). For example, Urban et al. (2014) have shown that complex sky conditions, associated with altered light intensity and spectral composition, temperature and vapour pressure deficit, substantially influence relative impact of elevated [$CO_2$] on photosynthesis and stomatal conductance and have thus an effect on the light use efficiency and carbon gain. Moreover, meta-analytical studies have shown more pronounced photosynthetic responses to elevated [$CO_2$] in experiments with a substantial reduction of UV intensity, while a [$CO_2$]-enhanced photosynthesis was relatively low under the natural light conditions (Körner et al., 2005; Leakey et al., 2009; Luo and Mooney, 1999; Nowak et al., 2004). Such findings indicate the need to explore interactive effects of elevated [$CO_2$] with other environmental drivers, including an intensity of UV radiation.

It is usually assumed that high intensities of UV, and particularly UV-B, reduce the positive effects of [$CO_2$] on photosynthesis (Kakani et al., 2004; Rozema et al., 1997; Teramura et al., 1990; Zhao et al., 2004). It has been shown that UV-B may influence the composition and functionality of thylakoid membranes, including the modification of membrane lipids, photosystem II (PSII) components as well as other electron transport carriers (Jordan et al., 2016). Such injuries lead to a reduced ATP and NADPH production, reduced rate of RuBP regeneration and subsequently to a reduced $CO_2$ assimilation rate in Calvin cycle (Bernacchi et al., 2001; Farquhar et al., 1980). Moreover, reduced activities or amounts of sedulohептulose 1,7-bisphosphatase (Allen et al., 1998) and/or Rubisco (Jordan et al., 1992) discovered under supplementary UV-B radiation may result in a decreased photosynthesis rate irrespective of impairments of primary photochemical reactions. Moreover, the UV-B effect on photosynthesis can be mediated through stomata controlling $CO_2$ diffusion into the leaves. However, contradictory effects of UV-B on stomatal conductance were reported including both negative as well as positive effects (reviewed in Hideg and Strid, 2017; Kakani et al., 2003). On the other hand, there is an evidence that an increased penetration of UV-A might increase photosynthesis, particularly at times of the day when plants/leaves are light-limited (Turnbull et al., 2013).

Prediction of [$CO_2$] × UV interactions on photosynthesis is likely to be a more complex phenomenon, since effects of both elevated [$CO_2$] as well as UV on plants vary in time. Over periods of months and years substantial reductions in [$CO_2$]-enhanced photosynthesis relative to the initial [$CO_2$]-stimulated rate (down-regulation) may occur (reviewed in Ceulemans and Mousseau, 1994; Leakey et al., 2009; Luo and Mooney, 1999; Urban, 2003). Photosynthetic down-regulation is particularly associated with a redistribution of inorganic phosphate in chloroplasts, reduced activity and/or amount of Rubisco, reduced contents of chlorophylls and carotenoids, dilution and/or redistribution of nitrogen and altered C:N stoichiometry, and insufficient carbon sink strength, i.e. an ability to effectively translocate assimilates from chloroplasts and to use these assimilates for growth and development (Bowes, 1991; Ceulemans and Mousseau, 1994; Finzi et al., 2006; Leakey et al., 2009; Lemoine et al., 2013; Urban, 2003).

Accordingly, we tested the hypothesis that ultraviolet radiation (UV) modulates photosynthesis responses to elevated [$CO_2$] on European beech seedlings. Since the photosynthetic $CO_2$ uptake is primarily limited by Rubisco activity, RuBP regeneration, and diffusion of $CO_2$ into leaf mesophyll, interactive effects were explored on the level of primary photochemical reactions, secondary reactions of Calvin cycle, and stomatal conductivity. Due to the assumed development of photosynthetic down-regulation during the vegetation season under elevated [$CO_2$], we postulated that UV supports the stimulatory effect of elevated [$CO_2$] on photosynthesis at the beginning of the vegetation season, while UV amplifies down-regulation of photosynthesis at the end of the vegetation season due to a reduced carbon sink strength.

## 2. Material and methods

### 2.1. Plants and experimental design

The experiment, described in detail in Uchytilová et al. (2018), was carried out at the experimental research site Bílý Kříž in the Beskydy Mountains, Czech Republic (49°30′N, 18°32′E, 908 m a.s.l.). The site forms part of several national and international research networks and infrastructures including CzeCOS (Czech Carbon Observation System), and AnaEE (Analysis and Experimentation on Ecosystems).

The study was performed on European beech (*Fagus sylvatica* L.) saplings (3-year-old and approximately 0.4 m high at the beginning of the experiment). The saplings were grown in a native soil for two years within the experimental lamellar domes. The lamellas are made from UVT Solar acrylic material (Quinn Plastics, Enniskillen, UK) transmitting more than 90% of incident UV-A and UV-B radiation. The domes enabled continuous growth of saplings at ambient (400 μmol $CO_2$ $mol^{-1}$; hereafter AC) or elevated (700 μmol $CO_2$ $mol^{-1}$; hereafter EC) atmospheric [$CO_2$] from April to November each year (Urban et al., 2001). In brief, an Li-840A infrared gas analyser (LI-COR Biosciences, Lincoln, NE, USA) was used to continuously monitor [$CO_2$] inside the domes. The determined [$CO_2$] was then used for feedback regulation of a mixing module to achieve a requested EC.

Both the AC and EC dome were split into three blocks (replications) within which the UV treatment plots were randomized (see details in Uchytilová et al. (2018)). Three plots representing UV exclusion [UV–], three plots representing ambient UV [UVamb], and three plots representing enhanced (150% of UVamb) UV radiation [UV+] were thus established within each [$CO_2$] treatment. Above each plot (1.0 × 0.75 m) a lamp-bank (approximately 1.20 m height) was built; it was covered by plastic filters. [UV–] plots were covered with clear plastic Lee U.V. 226 filters (Lee Filters, UK) to exclude UV-A and UV-B radiation. [UVamb] and [UV+] plots were covered with diacetate foil. The filters covered the top and also upper third of lamp-bank walls to avoid penetration of direct solar radiation and to avoid cross contamination between individual UV treatments. A modulated UV lamp system earlier described in Klem et al. (2015) was used to achieve enhanced UV intensities. The system consists of three UV-A (TL 20 W/10 SLV; Philips) and four UV-B (TL 20 W/12 RS SLV; Philips) fluorescent lamps. Incident UV-A and UV-B irradiance was monitored under the lamp-bank using SKU 420 and SKU 430 sensors (Skye Instruments, Powys, UK), and lamp output was adjusted to a specified dose using a feedback and amplification circuit. To avoid transmission of UV-C radiation (< 280 nm), the UV fluorescent lamps were wrapped in presolarised (8 h) 0.13 mm thick cellulose diacetate film.

Daily biologically effective UV-B doses ($UV\text{-}B_{BE}$) were close to zero under [UV–] treatments, while $UV\text{-}B_{BE}$ amounted to 11.6–20.5 kJ $m^{-2}$ $day^{-1}$ under [UV+] treatment depending on actual sky conditions and on the season. $UV\text{-}B_{BE}$ was calculated from the emission spectrum of the UV lamps in the range 200–980 nm measured by a spectroradiometer SM 9000 (PSI, Brno, CZ) and from an action spectrum for plant growth inhibition (Flint and Caldwell, 2003). Monthly sums of ambient photosynthetically active radiation (ΣPAR), UV-A (ΣUV-A), and UV-B (ΣUV-B) radiation, together with other microclimatic conditions during the growing season, are shown in Supplementary Table S1. Average monthly air temperatures during the entire experiment were close to long-term averages for a given locality. Generally, the experimental site has a cool and humid climate with high precipitation during the vegetation season amounting up to 110 mm per month. All plots were uniformly irrigated to keep the same soil moisture inside the domes and on the open area.

**Table 1**
The effects of $CO_2$ concentration ($[CO_2]$), ultraviolet radiation (UV), vegetation season (S) and their interactions ( × ) on photosynthetic parameters: $F_v/F_m$ – potential efficiency of photosystem II photochemistry, $\Phi_{PSII}$ – actual yield of photosystem II photochemistry under light saturating conditions, $D$ – thermal energy dissipation under light saturating conditions, $A_{max}$ – light-saturated rate of $CO_2$ assimilation under growth $[CO_2]$, $G_{Smax}$ – light-saturated stomatal conductance under growth $[CO_2]$, $A_{sat}$ – $CO_2$ assimilation rate at saturating light intensity and saturating $[CO_2]$, $V_{Cmax}$ – light-saturated rate of *in vivo* Rubisco carboxylation, $V_{Cmax}$ (25) – light-saturated rate of *in vivo* Rubisco carboxylation normalized to 25 °C, SA – *in vitro* Rubisco specific activity, LSU – amount of large Rubisco sub-unit, SSU – amount of Rubisco small sub-unit, LSU/SSU – ratio of LSU to SSU. Results of three-way ANOVA (F-test, df) analysis are shown (n.s. - non significant,$^{*}P < 0.05$,$^{**}P < 0.01$,$^{***}P < 0.001$).

| Effect | $[CO_2]$ | UV | S | $[CO_2]$ × UV | $[CO_2]$ × S | UV × S | $[CO_2]$ × UV × S |
|---|---|---|---|---|---|---|---|
| *df* | 1 | 2 | 2 | 2 | 2 | 4 | 4 |
| $F_v/F_m$ | $24.3^{***}$ | $8.0^{***}$ | $12.8^{***}$ | $0.5^{n.s.}$ | $4.3^{*}$ | $2.5^{n.s.}$ | $1.1^{n.s.}$ |
| $\Phi_{PSII}$ | $83.6^{***}$ | $51.6^{***}$ | $46.9^{***}$ | $17.8^{***}$ | $0.9^{n.s.}$ | $1.5^{n.s.}$ | $2.0^{n.s.}$ |
| $D$ | $27.3^{***}$ | $6.5^{**}$ | $2.1^{n.s.}$ | $5.6^{**}$ | $1.4^{n.s.}$ | $0.5^{n.s.}$ | $0.2^{n.s.}$ |
| $A_{max}$ | $32.8^{***}$ | $9.6^{***}$ | $24.4^{***}$ | $2.8^{n.s.}$ | $7.5^{**}$ | $9.7^{***}$ | $3.0^{*}$ |
| $G_{Smax}$ | $41.0^{***}$ | $13.0^{***}$ | $4.9^{*}$ | $0.3^{n.s.}$ | $1.8^{n.s.}$ | $3.5^{*}$ | $3.0^{*}$ |
| $A_{sat}$ | $2.9^{n.s.}$ | $5.7^{**}$ | $15.4^{***}$ | $0.9^{n.s.}$ | $2.8^{n.s.}$ | $7.9^{***}$ | $0.3^{n.s.}$ |
| $V_{Cmax}$ | $0.8^{n.s.}$ | $21.7^{***}$ | $77.5^{***}$ | $2.1^{n.s.}$ | $41.1^{***}$ | $13.9^{***}$ | $1.4^{n.s.}$ |
| $V_{Cmax}$ (25) | $11.8^{**}$ | $28.7^{***}$ | $37.6^{***}$ | $0.9^{n.s.}$ | $25.8^{***}$ | $11.3^{***}$ | $0.8^{n.s.}$ |
| SA | $10.4^{**}$ | $53.9^{***}$ | $383.2^{***}$ | $0.4^{n.s.}$ | $6.3^{**}$ | $40.6^{***}$ | $13.5^{***}$ |
| LSU | $25.3^{***}$ | $0.1^{n.s.}$ | $128.1^{***}$ | $8.5^{***}$ | $12.4^{***}$ | $2.2^{n.s.}$ | $3.2^{*}$ |
| SSU | $12.6^{***}$ | $0.4^{n.s.}$ | $49.8^{***}$ | $1.2^{n.s.}$ | $0.1^{n.s.}$ | $1.6^{n.s.}$ | $2.6^{n.s.}$ |
| LSU/SSU | $0.8^{n.s.}$ | $0.2^{n.s.}$ | $2.5^{n.s.}$ | $4.7^{*}$ | $11.9^{***}$ | $2.1^{n.s.}$ | $2.1^{n.s.}$ |

### 2.2. Physiological measurements

The plants were investigated during the second vegetation season (2016) under the controlled conditions. Saplings for investigation were selected from 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). *In situ* physiological measurements were carried out on fully developed beech leaves located in the upper part of the canopy. All measurements were made during the extended noon (10:00–14:00) hours of sunny days, and the examined leaves had no signs of damage. Three saplings per plot were evaluated and the average from these three measurements was used for further statistical analyses. To investigate seasonal effects on physiological traits, the measurements were done at the beginning of July, in August, and at the end of September representing beginning, mid, and end of the vegetation season.

#### 2.2.1. Gas-exchange measurements

Basic photosynthetic characteristics ($CO_2$ assimilation rate $A$, stomatal conductance $G_S$ and intercellular $CO_2$ concentration $Ci$) were measured on intact leaves using a Li-6400 gas-exchange system (LI-COR Biosciences, USA). Leaf temperature ($T_{leaf}$) and vapour pressure deficit (VPD) were kept constant for the measurements in the given season following natural seasonal variability. $T_{leaf}$ and VPD thus ranged from 20 to 28 °C and from 1.1 to 1.9 kPa, respectively.

Light-saturated rates of $CO_2$ assimilation ($A_{max}$) and stomatal conductance ($G_{Smax}$) were measured after 10 min exposure to a saturating irradiance (1400 µmol photons $m^{-2}$ $s^{-1}$) and growth $[CO_2]$ (i.e. 400 µmol $CO_2$ $mol^{-1}$ for AC plants and 700 µmol $CO_2$ $mol^{-1}$ for EC plants). In addition, photosynthetic capacity ($A_{sat}$), $CO_2$ assimilation rate at saturating light intensity and saturating $[CO_2]$ (1500 µmol $CO_2$ $mol^{-1}$), were measured.

To estimate the rate of *in vivo* Rubisco carboxylation ($V_{Cmax}$), the initial linear phase of the $A/Ci$ response curves was measured at low $Ci$ (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). In addition, temperature functions proposed by Bernacchi et al. (2001) for Rubisco-limited photosynthesis were applied to normalize $V_{Cmax}$ rates to uniform leaf temperature (25 °C; $V_{Cmax}$ (25)). See Šigut et al. (2015) for detailed parameterization of photosynthesis model.

#### 2.2.2. Chlorophyll fluorescence measurements

Chlorophyll *a* fluorescence (Chl-F) parameters were estimated on the dark-adapted intact leaves during the night and leaves adapted to saturating irradiance (1400 µmol photons $m^{-2}$ $s^{-1}$) using a pulse amplitude-modulated fluorometer PAM 2500 (H.Walz, Effeltrich, Germany). Chlorophyll fluorescence signal at the red band (near 690 nm) was measured using short measuring flashes (10 ms pulses with intensity of approximately 0.003 µmol photons $m^{-2}$ $s^{-1}$) 800 ms apart.

Night-time Chl-F measurements were carried out to estimate potential efficiency of photosystem (PS) II photochemistry [$F_v/F_m = (F_m - F_0)/F_m$], while daily measurements of light-adapted leaves were performed to assess an actual yield of PS II photochemistry [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] and thermal energy dissipation [$D = 1 - (F_m' - F_0')/F_m'$] according to Demmig-Adams et al. (1996). $F_m$ and $F_m'$ is the maximum fluorescence level observed during 1 s application of saturating pulse (intensity approx. 5000 µmol photons $m^{-2}$ $s^{-1}$) in fully dark-adapted leaves and light-adapted leaves, respectively. 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 ($F_s$) were taken. In light-adapted samples, the actinic light was switched off 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.

#### 2.2.3. Determination of Rubisco amount and activity in vitro

Approximately 0.6 g of leaf material was sampled between 10:00 and 14:00 under natural saturating irradiances (≥1100 µmol photons $m^{-2}$ $s^{-1}$). After determining the planar area of leaves using a portable leaf area meter (Li-3000A, LI-COR Biosciences, USA), the samples were immediately frozen in liquid nitrogen and transported to the laboratory.

Rubisco protein was extracted from the leaves using the procedure described in Hrstka et al. (2008). Total *in vitro* Rubisco activity was assayed spectrophotometrically by the continuous measurement of 3-phosphoglycerate-dependent NADH oxidation in a coupled enzyme system. The changes in absorbance were measured at 340 nm using a spectrophotometer Helios γ (Spectronic Unicam, UK).

The contents of large (LSU) and small Rubisco sub-unit (SSU) were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a Mini-PROTEAN 3 system (Bio-Rad, Hercules, CA, USA), as described by Hrstka et al. (2008), using purified Rubisco protein (Sigma Aldrich) as a standard. The quantification of individual bands was performed on an HP Scanjet 5590P running the

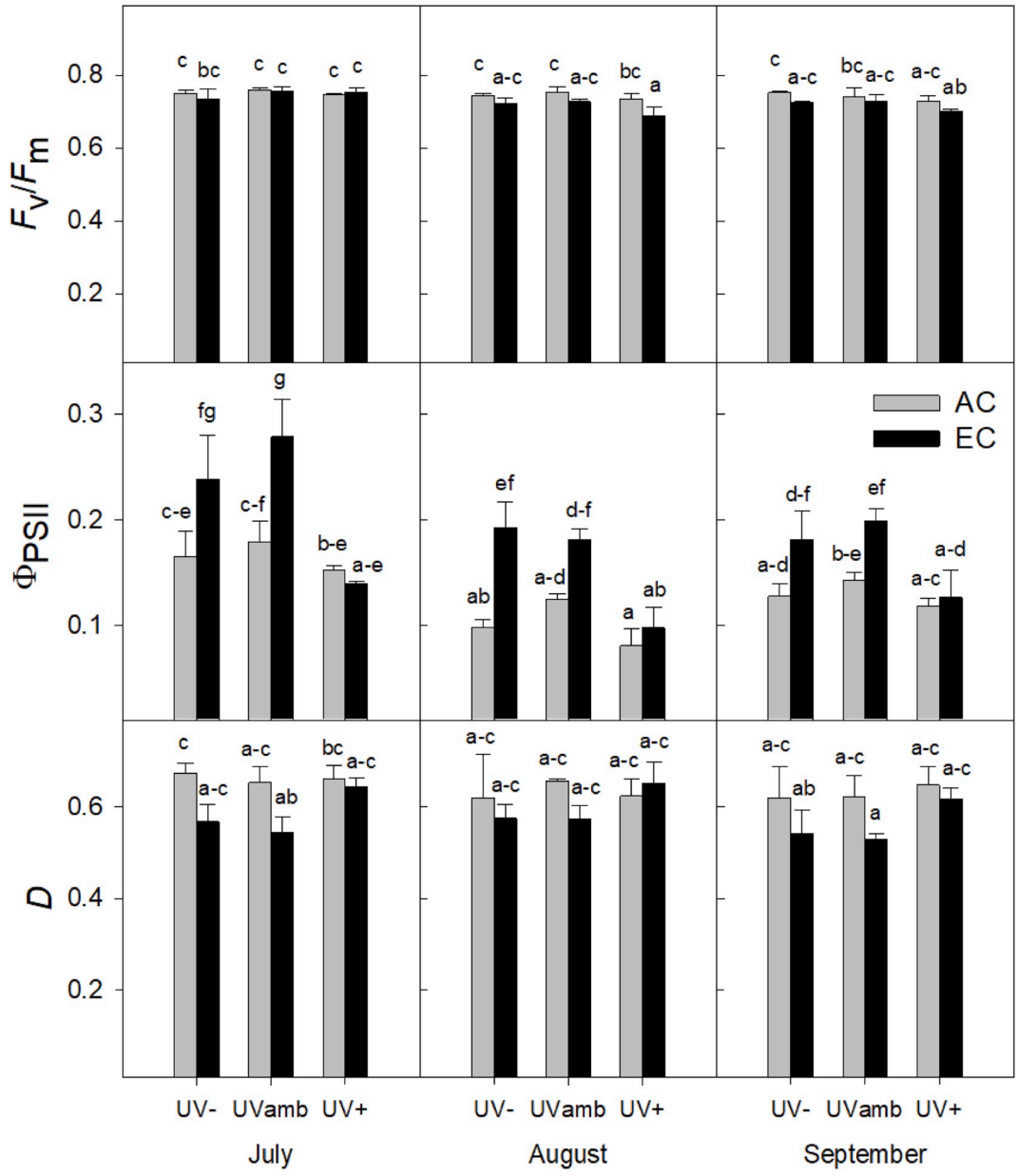


**Fig. 1.** Potential efficiency of photosystem II photochemistry ($F_v/F_m$), actual yield of photosystem II photochemistry under light saturating conditions ($\Phi_{PSII}$), and thermal energy dissipation under light saturating conditions (*D*) estimated during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. AC – ambient (400 μmol $CO_2$ $mol^{-1}$) $CO_2$ concentration, EC – elevated (700 μmol $CO_2$ $mol^{-1}$), UV– – excluded UV, UVamb – ambient UV intensity, UV + – enhanced UV intensity (150% of UVamb). The plants were investigated during the second vegetation season (2016) under the controlled conditions. Means (columns) and standard deviations (error bars) are presented ($n = 3$; experimental plots). Different letters denote statistically significant differences ($P < 0.05$) between acclimation treatments and individual months using Tukey's ANOVA post-hoc test.

program Advanced Image Data Analyser, version 3.23.001 (Raytest, Straubenhardt, Germany). Rubisco activity was further normalized by the Rubisco amount to calculate specific activity (SA) expressed in μmol of $CO_2$ fixed per second and per unit of protein mass.

### 2.3. Statistical analysis

Three-way analysis of variance (ANOVA) was used for the analysis of [$CO_2$], UV radiation and seasonal effects and their mutual interaction. Tukey's HSD post-hoc ($P = 0.05$) test was used to analyse significant differences between means. Statistical analyses were conducted using the software Statistica 12 (StatSoft, Tulsa, CA, USA). The bar graphs representing means with standard deviations and the multiple scatter plots with regression lines were created in the software SigmaPlot 11.0 (Systat Software, San Jose, CA, USA).

## 3. Results

Three-way ANOVA analysis (Table 1) revealed significant effects of [$CO_2$], UV, and vegetation season on most of Chl-F and gas-exchange parameters investigated. While [$CO_2$] and Season had significant effects on Rubisco SA and contents of both LSU and SSU, UV treatment influenced significantly only SA ($P < 0.001$).

Statistically significant ($P < 0.01$) interactions of [$CO_2$] and UV treatments were observed for Chl-F parameters estimated under saturating irradiance ($\Phi_{PSII}$ and *D*), but not in dark adapted leaves ($F_v/F_m$). [$CO_2$] and UV treatments had also a significant ($P < 0.05$) interactive effect on the content of large Rubisco sub-unit (LSU) and LSU/SSU

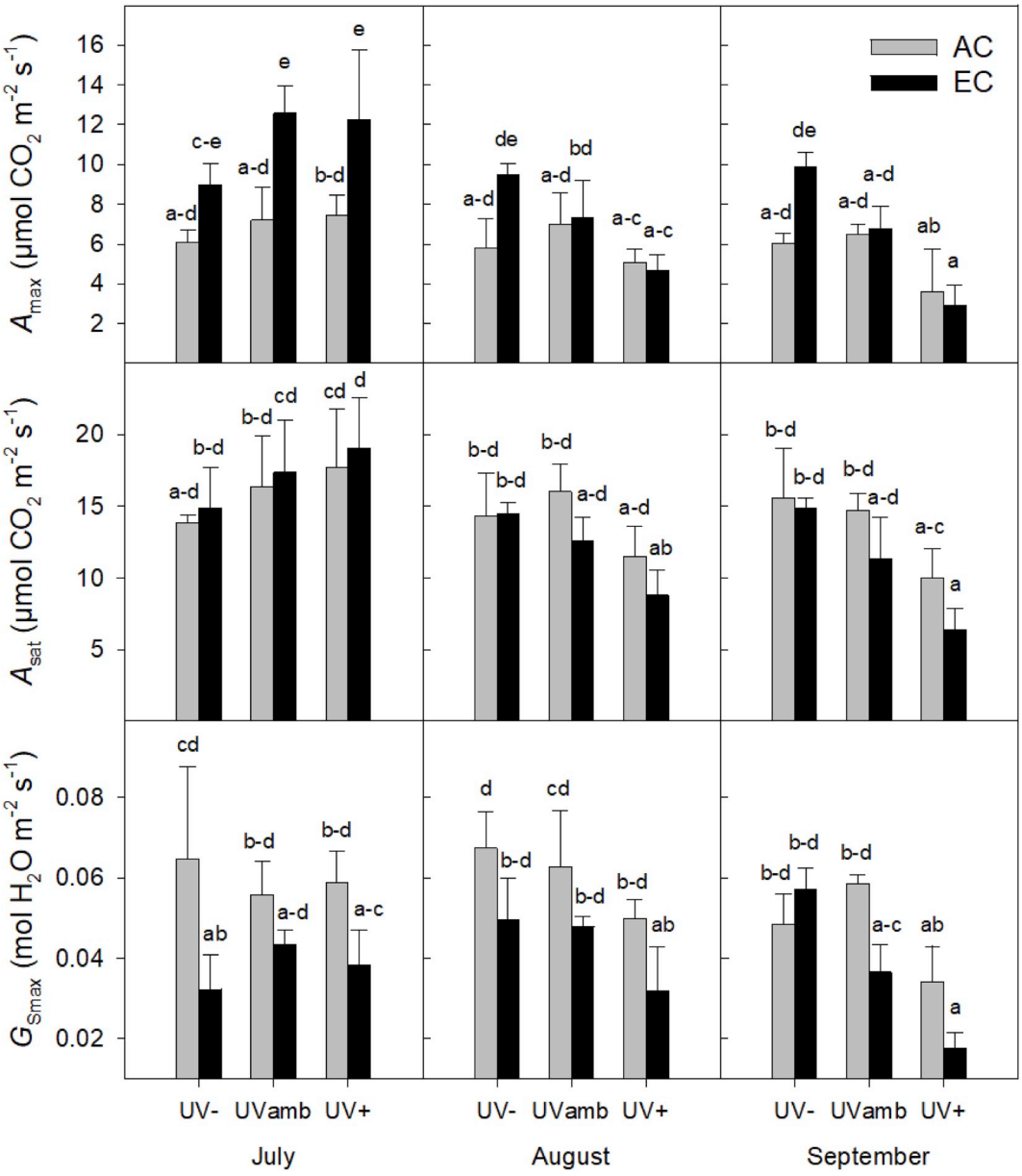


**Fig. 2.** Light-saturated rate of $CO_2$ assimilation at growth $CO_2$ concentration ($A_{max}$), $CO_2$ assimilation capacity under the conditions of saturated light intensity and $CO_2$ concentration ($A_{sat}$), and light-saturated stomatal conductance at growth $CO_2$ concentration ($G_{Smax}$) estimated during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. AC – ambient (400 µmol $CO_2$ $mol^{-1}$) $CO_2$ concentration, EC – elevated (700 µmol $CO_2$ $mol^{-1}$), UV– – excluded UV, UVamb – ambient UV intensity, UV+ – enhanced UV intensity (150% of UVamb). The plants were investigated during the second vegetation season (2016) under the controlled conditions. Means (columns) and standard deviations (error bars) are presented (n = 3; experimental plots). Different letters denote statistically significant differences ($P < 0.05$) between acclimation treatments and individual months using Tukey's ANOVA post-hoc test.

ratio; however, no interactive effect of [$CO_2$] and UV treatments on gas-exchange parameters was observed (Table 1). Statistically significant interactive effects of [$CO_2$], UV and Season on gas-exchange parameters ($A_{max}$, $G_{Smax}$; $P < 0.05$) and Rubisco properties (SA, LSU; $P < 0.05$) were observed (Figs. 6 and 7), while an interactive effect on Chl-F parameters was not found.

### 3.1. Chlorophyll fluorescence parameters

While $F_v/F_m$ values of [UV–] and [UVamb] treated plants remained unaffected over the vegetation season under both AC and EC conditions, small but statistically significant ($P < 0.05$) decreases were observed in [EC UV+] plants at the end of the vegetation season (Fig. 1). Also the Chl-F parameters estimated under saturating light conditions, $\Phi_{PSII}$ and $D$, revealed a distinct acclimation of [EC UV+] plants. While EC treatment led to a significant stimulation of $\Phi_{PSII}$ in plants exposed to [UV–] and [UVamb] conditions, such stimulation was not observed at supplementary UV radiation. On the contrary, EC treatment led to a reduction of thermal energy dissipation ($D$) in [UV–] and [UVamb] plants, but not in beech leaves exposed to [UV+].

### 3.2. Gas-exchange parameters

Typical increases of $A_{max}$ and decreases of $G_{Smax}$ in EC as compared to AC conditions were found under all UV treatments in July (Fig. 2). Stimulation of $A_{max}$ by EC in August and September was, however, found only at [UV–] conditions, whereas differences between AC and EC counterparts exposed to [UVamb] and [UV+] conditions were

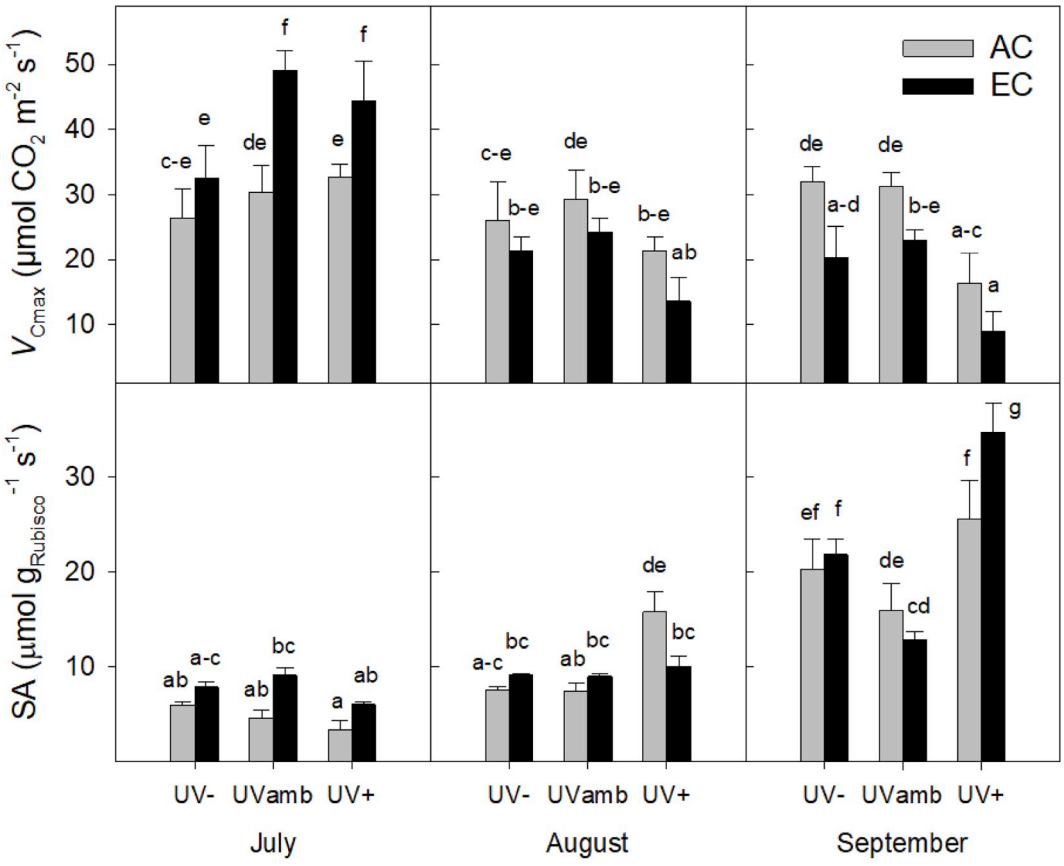


**Fig. 3.** *In vivo* light-saturated rate of Rubisco carboxylation ($V_{Cmax}$) and *in vitro* specific carboxylation activity of Rubisco (SA) determined during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. AC – ambient (400 µmol $CO_2$ $mol^{-1}$) $CO_2$ concentration, EC – elevated (700 µmol $CO_2$ $mol^{-1}$), UV– – excluded UV, UVamb – ambient UV intensity, UV+ – enhanced UV intensity (150% of UVamb). The plants were investigated during the second vegetation season (2016) under the controlled conditions. Means (columns) and standard deviations (error bars) are presented ($n = 3$; experimental plots). Different letters denote statistically significant differences ($P < 0.05$) between acclimation treatments and individual months using Tukey's ANOVA post-hoc test.

negligible and statistically not significant. Although $G_{Smax}$ remained reduced under the EC conditions over the whole vegetation season as compared to AC plants, these differences were statistically not significant ($P > 0.05$).

Assimilation capacity ($A_{sat}$) remained constant in [AC UV–] and [AC UVamb] plants over the whole period investigated. In contrary, there was a substantial decrease of $A_{sat}$ in [AC UV+] plants amounting up to 43.5% when $A_{sat}$ values from July and September are compared. Moreover, $A_{sat}$ values remained unchanged by $CO_2$ treatment under [UV–] conditions over the whole period explored. However, decreases in $A_{sat}$ values were found in [EC UVamb] and [EC UV+] plants amounting up to 22.8 and 36.3%, respectively when compared to their AC counterparts. At the end of vegetation season, $A_{sat}$ values gradually decreased with an increasing UV radiation intensity under both [$CO_2$].

### 3.3. Rubisco activity and content

EC stimulated *in vivo* Rubisco carboxylation rate ($V_{Cmax}$) at the beginning of the vegetation season (up to 61.4% in [EC UVamb] plants as compared to AC counterparts); however, this stimulation diminished during the vegetation season (Fig. 3). Lower $V_{Cmax}$ values were then observed under EC than AC conditions at the end of vegetation season (August, September), irrespective of UV treatment. Although these decreases amounted up to 36.2% (UV–), 26.5% (UVamb), and 45.1% (UV+) in September, the differences were statistically not significant ($P > 0.05$). Similarly to $A_{max}$, there were no statistically significant differences in $V_{Cmax}$ between [UV–] and [UVamb] plants treated under AC conditions. However, a significant ($P < 0.05$) decrease in $V_{Cmax}$ amounting up to 48.7% was found in [AC UV+] plants as compared to [AC UV–] plants in September.

Rubisco *in vitro* specific activity (SA) rose significantly ($P < 0.05$) during the vegetation season under both AC and EC conditions (Fig. 3). Although EC conditions tended to increase SA values under all UV treatment, these differences were mostly statistically not significant. [UV+] treatment led to the highest increase in SA values along the vegetation seasons as compared to [UV–] and particularly [UVamb] treatments.

The contents of both LSU and SSU tended to decrease at the end of the vegetation season as compared to July and August (Fig. 4). We have also observed slight decreases in contents of LSU and SSU under EC as compared to AC conditions, while there were no significant effects of UV treatment (Table 1). Although the effects of Season, [$CO_2$] and UV on the LSU/SSU were statistically not significant (Fig. 4; Table 1), [$CO_2$] × UV and [$CO_2$] × Season had significant interactive effects on this ratio. While EC reduced LSU/SSU ratio at the beginning of the vegetation season (under all UV treatments), higher LSU/SSU ratios were observed in August and September in [EC UVamb] and [EC UV+] plants than in their AC counterparts. In contrary, reduced LSU/SSU ratio persisted during the whole vegetation season in EC than AC plants treated under [UV–] conditions.

A significant ($P < 0.001$) negative correlation between the content of LSU and Rubisco SA was found (Fig. 5). The observed changes were caused mainly by seasonal effect, while the effects of [$CO_2$] and UV treatments were relatively minor. High LSU contents (up to 37 mg $g^{-1}$)

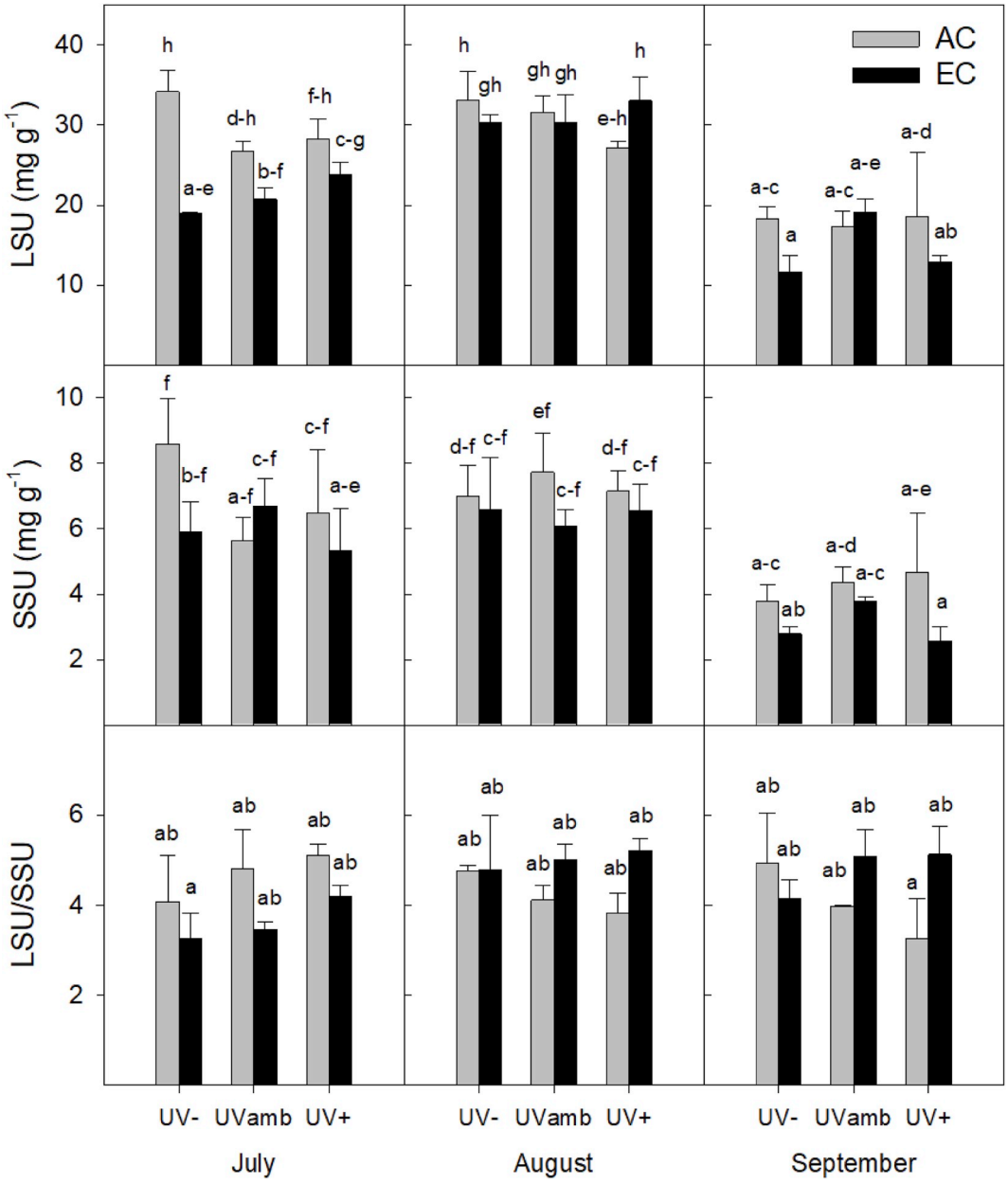


**Fig. 4.** Content of large Rubisco sub-unit (LSU), small Rubisco sub-unit (SSU), and the ratio of large to small Rubisco sub-unit (LSU/SSU) determined during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. AC – ambient (400 μmol $CO_2$ $mol^{-1}$) $CO_2$ concentration, EC – elevated (700 μmol $CO_2$ $mol^{-1}$), UV– – excluded UV, UVamb – ambient UV intensity, UV+ – enhanced UV intensity (150% of UVamb). The plants were investigated during the second vegetation season (2016) under the controlled conditions. Means (columns) and standard deviations (error bars) are presented ($n = 3$; experimental plots). Different letters denote statistically significant differences ($P < 0.05$) between acclimation treatments and individual months using Tukey's ANOVA post-hoc test.

at the beginning of the vegetation season were associated with low SA (3–7 μmol $g_{Rubisco}^{-1}$ $s^{-1}$), while low LSU contents (amounting to 10–15 mg $g^{-1}$) at the end of the vegetation season were associated with high SA values (up to 37 μmol $g_{Rubisco}^{-1}$ $s^{-1}$) (Fig. 5).

Moreover, we have found a significant ($P < 0.001$) positive correlation between $A_{max}$ and $V_{Cmax}$ (Fig. 6). Irrespective of [$CO_2$] treatment, the highest $V_{Cmax}$ values associated with highest $A_{max}$ values were observed in July under [UVamb] and [UV+] treatments, while the lowest $V_{Cmax}$ values were detected in September in plants treated under [UV+] conditions.

## 4. Discussion

In agreement with our prediction, we found that EC leads to the stimulation of $A_{max}$ under [UVamb] conditions at the beginning of the vegetation season. However, there was a diminution of the initially [$CO_2$]-stimulated $A_{max}$ and a lower photosynthetic capacity $A_{sat}$ occurred at the end of the vegetation season (Fig. 2). Such seasonal dynamics in photosynthetic acclimation to EC conditions is often attributed to a reduced carbon sink strength due to a lowered activity of growth meristems in roots and leaves at the end of the vegetation season (Ainsworth and Long, 2005; Lemoine et al., 2013). Similar dynamics of acclimation to EC were observed in plants exposed to [UV+] conditions, while indications of photosynthetic down-regulation in UV– plants were not found. A significant ($P < 0.05$) interactive effect of [$CO_2$] × UV × Season was confirmed by ANOVA (Table 1). Such findings support the hypothesis that enhanced UV radiation stimulates a positive effect of elevated [$CO_2$] on plant photosynthesis at the

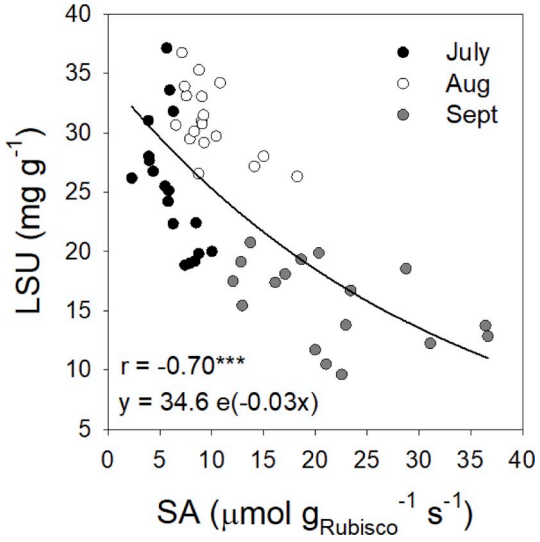


**Fig. 5.** Relationship between the content of Rubisco large sub-unit (LSU) and *in vitro* specific carboxylation activity of Rubisco enzyme (SA) determined during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. An exponential decay function was fitted to the data irrespective of treatment. The data represent the whole dataset obtained during the second vegetation season (2016) of plants exposure to controlled conditions. Coefficient of correlation ($r$) and significance level (***$P < 0.001$) are shown.

beginning of the vegetation season (short-term effect), while long-term exposure reduces this positive effect and even leads to down-regulation of photosynthesis.

Previous studies (Caldwell et al., 2007; Kakani et al., 2004; Rozema et al., 1997; Teramura et al., 1990; Zhao et al., 2004) have often shown an antagonistic interaction of [$CO_2$] × UV in different plant species, including interactive effect on $A_{max}$, light-use efficiency, root and total biomass, and/or leaf area. However, these experiments were only short-termed and the plants were exposed to high, ecologically irrelevant, doses of UV radiation. Moreover, these experiments focused particularly on annual crop species, while the seasonal experiments with tree species are still rare (Caldwell et al., 2007).

### 4.1. Acclimation at carboxylation level

Generally, three types of down-regulation associated with reduced carboxylation rate of Rubisco, reduced electron transport rate and subsequently reduced RuBP regeneration, or their combination were described in the literature (Bowes, 1991; Urban et al., 2012). Reduced Rubisco activity can be caused by reduced Rubisco content and/or an increased abundancy of inactive Rubisco forms (Bowes, 1991; Parry et al., 2008). A synergistic interaction of [$CO_2$] and UV radiation on Rubisco is expected. This hypothesis is based on previous findings that both an accumulation of hexoses under the conditions of elevated [$CO_2$] as well as supplementary UV-B radiation may supress transcription of genes encoding Rubisco subunits, and/or enzymes associated with Rubisco activity like Rubisco-activase and carbonic anhydrase (Jordan et al., 1992; Sheen, 1994; Van Oosten and Besford, 1996). The hypothesis is supported by very low $V_{Cmax}$ values observed under [EC UV +] conditions in August and September. Such low *in vivo* activity of Rubisco is in agreement with observed increase in C:N ratio found in the studied beech saplings (for details see Uchytilová et al. (2018)) indicating an establishment of a new sink-source status in plants (Finzi et al., 2006; Lemoine et al., 2013).

Moreover, Jordan et al. (1992) reported that nuclear-encoded genes of Rubisco are more sensitive to UV-B than chloroplast-encoded genes and should thus lead to an increase of LSU/SSU ratio. Indeed, we have found slight increases in LSU/SSU ratio with increasing UV intensity at the beginning of the vegetation season. Such increase was independent on the growth [$CO_2$]. However, this phenomenon persisted during vegetation season only under EC conditions, while decrease of LSU/SSU ratio with increasing UV intensity was found in AC grown plants in August and September (Fig. 4). Low LSU/SSU ratio may indicate transition of Rubisco activity towards photorespiration, whereas photosynthetic $CO_2$ assimilation in Calvin cycle may be reduced.

To properly investigate an amount of photosynthetically active Rubisco, SA (the rate of carboxylation *in vitro* per unit amount of Rubisco) was calculated. EC treatment led to slightly higher SA values as compared to AC treatment. This result may be attributed to a higher amount of Rubisco maintained in the carbamylated state as it was earlier shown in coniferous *Picea abies* and broadleaved *Fagus sylvatica* grown under elevated [$CO_2$] (Košvancová et al., 2009). High intercellular [$CO_2$] associated with EC conditions contributes to the carbamylation process and has also a protective role against binding of daily and nocturnal inhibitors on Rubisco's active catalytic sites (Parry et al., 2008). Our results show that enhanced UV radiation further contributes to an increase in SA (Fig. 3). In accordance with previous studies on coniferous tree species (Sallas et al., 2003; Urban et al., 2012), a substantial increase in SA along the vegetation season was observed and resulted in highly significant ($P < 0.001$) interaction of [$CO_2$] × UV × Season as proved by ANOVA (Table 1). Also our earlier

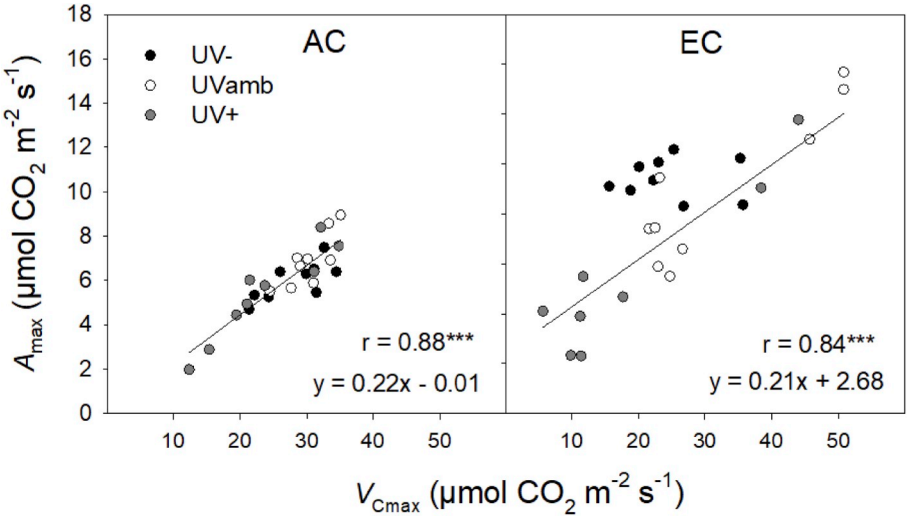


**Fig. 6.** Relationship between light-saturated rate of $CO_2$ assimilation ($A_{max}$) and *in vivo* light-saturated rate of Rubisco carboxylation ($V_{Cmax}$) estimated during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. A linear function was fitted to the data separately for AC plants (left panel) and separately for EC plants (right panel). AC – ambient (400 µmol $CO_2$ $mol^{-1}$) $CO_2$ concentration, EC – elevated (700 µmol $CO_2$ $mol^{-1}$), UV– – excluded UV, UVamb – ambient UV intensity, UV+ – enhanced UV intensity (150% of UVamb). The data represent the whole dataset obtained during the second vegetation season (2016) of plants exposure to controlled conditions. Coefficient of correlation ($r$) and significance level (***$P < 0.001$) are shown.

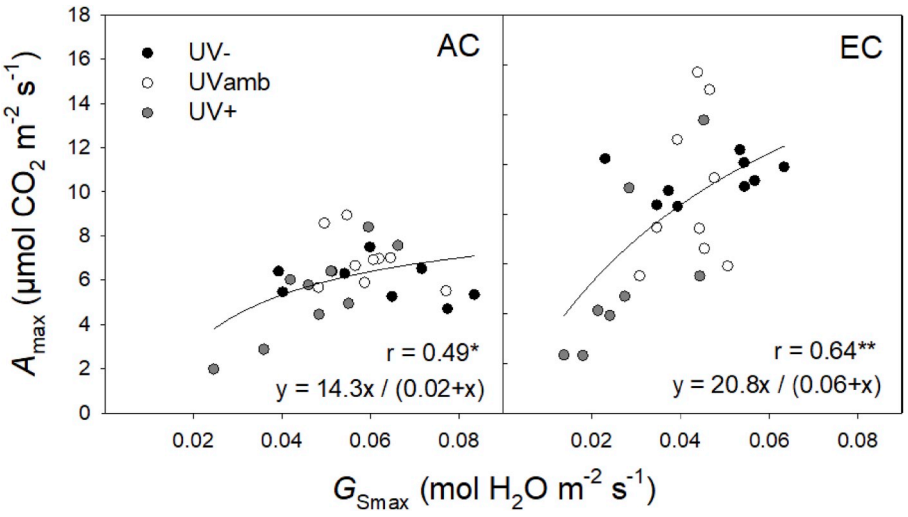


**Fig. 7.** Relationship between light-saturated rate of $CO_2$ assimilation ($A_{max}$) and light-saturated stomatal conductance ($G_{Smax}$) estimated during the vegetation season in leaves of *Fagus sylvatica* acclimated to a combined effect of atmospheric $CO_2$ concentration and UV radiation. A rectangular hyperbola function was fitted to the data separately for AC plants (left panel) and separately for EC plants (right panel). AC – ambient (400 µmol $CO_2$ $mol^{-1}$) $CO_2$ concentration, EC – elevated (700 µmol $CO_2$ $mol^{-1}$), UV– – excluded UV, UVamb – ambient UV intensity, UV+ – enhanced UV intensity (150% of UVamb). The data represent the whole dataset obtained during the second vegetation season (2016) of plants exposure to controlled conditions. Coefficient of correlation ($r$) and significance levels ($^*P < 0.05$, $^{**}P < 0.01$) are shown.

study with *P. abies* (Urban et al., 2012) has shown that Rubisco amount and Rubisco SA are irreversibly proportional, and a gradually reducing Rubisco content along the vegetation season is compensated by higher abundance of Rubisco active forms. Studies supporting this findings in other plant species are, however, missing.

### 4.2. Acclimation at the level of photochemical reactions

In addition to limitation by Rubisco activity, photosynthesis may be limited, particularly under EC conditions (Šigut et al., 2015), by the rate of RuBP regeneration. Regeneration of RuBP in Calvin cycle depends on the rate of electron transport, production of ATP and NADPH (Bernacchi et al., 2001; Farquhar et al., 1980), and activities of phosphatases like sedulopheptulose 1,7-bisphosphatase (Allen et al., 1998). RuBP-limited photosynthesis is particularly reflected by $A_{sat}$ values.

Although the differences in $A_{sat}$ (Fig. 2) are mostly statistically not significant, the different [$CO_2$] × UV interactions were observed at the beginning (July) and at the end of the vegetation season (August, September). While EC stimulated $A_{sat}$ in July with a small additive effect of UV, probably caused by a stimulatory effect of UV-A radiation (Turnbull et al., 2013), long-term EC treatment led to substantial reduction of $A_{sat}$ in plants exposed to UVamb and [UV+] conditions.

Such synergistic interactive effect of [$CO_2$] and UV on $A_{sat}$ reduction at the end of growing season may arise from the fact both elevated [$CO_2$] and enhanced UV reduce the content of chlorophylls (Ceulemans and Mousseau, 1994; Wullschleger et al., 1992), increase the accumulation of inactive or damaged reaction centres of photosystem II (Jordan et al., 2016; Kalina et al., 2001; Šprtová et al., 2003), and/or modify proteins of thylakoid membrane involved in electron transport (reviewed in Hideg and Strid, 2017; Urban, 2003). For example, Sheen (1994) have shown that high concentration of hexoses accumulated under elevated [$CO_2$] negatively influences transcription of genes encoding D1 and D2 proteins of PSII core and cytochrome *f*; i.e. components being also shown as possible targets of UV-B radiation (Jordan et al., 2016). An accumulation of hexoses over the vegetation season in plant leaves thus may contribute to an explanation of positive and negative EC × UV interaction at the beginning and end of the growing season, respectively. Moreover, a reduced activity and abundance of ATP synthase have been shown under enhanced UV-B intensity (Strid et al., 1994). Similarly, a shortage of inorganic phosphate under elevated [$CO_2$], bound to accumulated phosphorylated sugar intermediates of sucrose biosynthesis, can lead to a substrate-limitation of ATP synthase activity (Sage and Reid, 1994; Urban, 2003). Moreover, regeneration of RuBP is tightly controlled by an activity sedulopheptulose 1,7-bisphosphatase, enzyme being early reported as a potential target of UV-B radiation (Allen et al., 1998).

Such acclimation processes may subsequently contribute to a reduced $\Phi_{PSII}$ and increased non-photochemical quenching, including particularly an increased thermal dissipation $D$, of absorbed light energy. Our results, however, show that both $\Phi_{PSII}$ and $D$ remain constant in all UV treatments under AC conditions. EC treatment led to a stimulation of $\Phi_{PSII}$ and simultaneously to suppression of $D$ when the plants are exposed to [UV–] and/or [UVamb] conditions (Fig. 1). An expected synergistic effect of elevated [$CO_2$] and UV on reduced $\Phi_{PSII}$ and increased $D$ was thus observed only under [EC UV+] conditions.

Facilitated non-radiative dissipation of absorbed light energy in [EC UV+] plants may result from an increased total xanthophyll pool and stimulated de-epoxidation of violaxanthin to antheraxanthin and particularly to zeaxanthin observed under enhanced UV (Klem et al., 2015; Šprtová et al., 2003) as well as under elevated [$CO_2$] (Špunda et al., 2005; Urban et al., 2014). Reports on [$CO_2$] and UV synergistic effect on the dynamics of xanthophyll cycle are, however, still missing.

### 4.3. Acclimation at stomatal conductance level

Finally, photosynthetic $CO_2$ uptake is substantially modulated by a stomatal conductance to $CO_2$ diffusion (Lichtenthaler et al., 2007). Generally, stomatal conductance is directly affected by the osmotic potential of the guard cells, the water potential of the guard cells, and/or the water potential of the epidermal cells (Buckley and Mott, 2013). Environmental factors controlling stomatal conductance thus act through these potential. While elevated [$CO_2$] usually leads to a reduced stomatal conductance (Ainsworth and Long, 2005; Ceulemans and Mousseau, 1994; Leakey et al., 2009), effects of enhanced UV are inconsistent including both stimulation and suppression of stomatal conductance. Notwithstanding, UV-induced reduction of stomatal conductance appears to be a more frequent phenomenon (Bornman et al., 2015; Caldwell et al., 2007). For example, Urban et al. (2006) reported a significant reduction of $G_{Smax}$ under saturating light conditions in *Calamagrostis villosa* and *C. arundinacea* exposed to 25% enhanced UV-B radiation.

Accordingly, an interactive, synergistic or additive, effect of [$CO_2$] and UV radiation on $G_{Smax}$ could be thus hypothesized. Indeed, we have found substantially reduced $G_{Smax}$ values in [EC UV+] plants in August and September, but not in July (Fig. 2). A significant ($P < 0.05$) interaction of [$CO_2$] × UV × Season was thus revealed by ANOVA (Table 1). It has been, however, shown that stomatal opening is strongly controlled also by other co-occurring environmental drivers, particularly vapour pressure deficit that may overwhelm UV effects (Jansen and Van Den Noort, 2000) and/or substantially modify stomatal response to elevated [$CO_2$] (Urban et al., 2014). High values of vapour pressure deficit, associated with low air humidity and high air

temperatures, thus represent the main reason of reduced carbon uptake during summer months (Lichtenthaler et al., 2007; Špunda et al., 2005).

To test whether the changes in $G_{Smax}$ constitute the primary reason for the reduced $A_{max}$ under [UV+] conditions the $A_{max}$ values were plotted against $G_{Smax}$ (Fig. 7). It is obvious that low $A_{max}$ values in [UV+] plants at the end of vegetation season are tightly connected with reduced $G_{Smax}$ irrespective of [$CO_2$] treatment. However, the open question remains whether such decrease in $G_{Smax}$ is caused by an effect of enhanced UV on leaf/plant water balance, and/or by an altered transport of abscisic acid from roots.

## 5. Conclusion

We conclude that an impact of elevated [$CO_2$] on photosynthesis is substantially modulated by UV radiation. Moreover, we found evidence supporting the hypothesis that the [$CO_2$] × UV interaction is changing throughout the vegetation season. While an enhanced UV radiation stimulates a positive effect of elevated [$CO_2$] on plant photosynthesis at the beginning of the vegetation season (short-term effect), long-term cultivation reduces the stimulatory effect of elevated [$CO_2$] and lead to the down-regulation of photosynthesis. Down-regulation was, however, not found in plants grown under the conditions of excluded UV radiation. We found evidence that the down-regulation of photosynthesis is associated with a complex acclimation at different hierarchical and functional level, including an acclimation of primary photochemical reactions, carboxylation activity of Rubisco enzyme, and stomatal conductance.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.plaphy.2018.08.026.

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