Photosynthetic assimilation of sun versus shade Norway spruce [Picea abies (L.) Karst] needles under the long-term impact of elevated CO₂ concentration

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Abstract

The long-term impact of elevated concentration of CO₂ on assimilation activity of sun-exposed (E) versus shaded (S) foliage was investigated in a Norway spruce stand [Picea abies (L.) Karst, age 14 years] after three years of cultivation in two domes with adjustable windows (DAW). One DAW was supplied with ambient air [AC, ca. 350 μmol(CO₂) mol⁻¹] and the second with elevated CO₂ concentration [EC = AC plus 350 μmol(CO₂) mol⁻¹]. The pronounced vertical profile of the photosynthetic photon flux density (PPFD) led to the typical differentiation of the photosynthetic apparatus between the shaded and sun needles. Namely, photon-saturated values of maximal net photosynthetic rate (Pₙ₉₉₉₉) and apparent quantum yield (α) were significantly higher/lower for E-needles as compared with the S-ones. The prolonged exposure to EC was responsible for the apparent assimilatory activity stimulation observed mainly in deeply shaded needles. The degree of this stimulation decreases in the order: S-needles dense part > S-needles sparse part > E-needles dense part > E-needles sparse part. In exposed needles some signals on a manifestation of the acclimation depression of the photosynthetic activity were found. The long-term effect of EC was responsible for the decrease of nitrogen content of needles and for its smoother gradient between E- and S-needles. The obtained results indicate that the E- and S-foliage respond differently to the long-term impact of EC.

Additional key words: carboxylation efficiency and rate; CO₂ compensation concentration; dark respiration; dense/sparse parts; electron transport rate; quantum yield of assimilation; stand density; sun/shade.

Introduction

Generally, one expects that increasing atmospheric CO₂ (AC) could be responsible for the mitigation of C limitation and for the stimulation of photosynthesis of variety of tree species (Norby et al. 1999). Because of the longevity and significant spatial arrangement of forest trees, it is possible to expect both temporal and spatial variation in effect of elevated CO₂ (EC) concentration on photosynthesis and biomass production.

The spatial arrangement of forest stand canopy, especially in a coniferous stand canopy, is responsible for a specific solar radiation regime in this space leading to a distinctive vertical/horizontal differentiation of morphological and physiological parameters of foliage (Woodman 1971, Lewandowska et al. 1977, Leverenz 1996, Špunda et al. 1998a). This phenomenon is an important feature of the forest tree stand canopy, especially of the dense coniferous stand, where the photosynthetic characteristics vary between shade and sunny needles in the canopy (Marek et al. 1999). Shade acclimated needles in the lower parts of the stand canopy have limited ability to fix C because of the low irradiance which is similar to the situation of the under-storey seedlings (Walters and Reich 1996). Assimilatory apparatus of shaded foliage operates mainly in the quantum yield region of the irradiance response curve, thus EC may be a benefit for it (Bowes 1993). Moreover, increased CO₂ concentration can partly compensate the low irradiance on the level of photosynthetic assimilation.

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These relative CO₂ effects in shade may be particularly large because higher CO₂ concentrations reduce photosynthesis and thus decrease the CO₂ compensation concentration of photosynthesis (Long and Drake 1991, Osborne et al. 1997). Suppressed photosynthesis resulted in the increased carbon uptake rate (Pₘ), which is comparable to Pₙ obtained at high photosynthetic photon flux density (PPFD). In accordance with this statement, the relative CO₂ stimulation of biomass production in temperate forest tree seedlings increased at low PPFD compared to high one (Bazzaz and Miao 1993). Thus, the vertical differentiation of the assimilatory activity of leaves within the canopy is an important consequence of the whole-stand response to the long-term impact of EC.

The effect of EC is strongly modified by the sink strength (Stitt and Quick 1989, Stitt 1991, Bryant et al. 1998, Grimmer and Komor 1999) and thus any environmental factor that can modify sink strength indirectly affects tree responses to EC. Stand density is a significant factor strongly affecting the growth sink activity. Between tree individuals the competition for crown/root growth space strongly affects sink strength and thus the response to EC. Moreover, the role of the sink strength in the realisation of the stimulation effects of EC has distinctive seasonal character (Urban and Marek 1999).

The objective of present study is to assess the effects of EC on the photosynthetic characteristics of Norway spruce needles differing in their position within the canopy (shady and sun-exposed needles) in relation to stand density. We hypothesise that: (1) the short- and long-term response of shaded foliage to EC is different compared to the response of exposed one; (2) acclimation depression of photosynthesis as the result of the long-term impact of EC is not so distinctive in shade foliage as in sunny foliage; (3) spatial differentiation of photosynthetic activity within crown space is affected by the stand density.

Materials and methods

Plants: The long-term impact of EC on the growth of a Norway spruce stand (age 14 years, average height 2.7 m) was investigated at the research site Bílé Kříž in the Beskydy MtS. (North-East part of the Czech Republic, 49°12’N, 18°32’E, 908 m a.s.l.). The experimental stand was planted in 1997 using special prepared and replanted older saplings (age at the planting date: 10–12 years). The preparation of the saplings for replanting was based on the repeated (twice, in two followed growing seasons) formation of a massive root-bales. These bales made the planting of sapling more easy and protected the roots. The trees for planting were selected on the base of the same phenotype and phenology of bud break. The saplings were obtained from advance growth located close to the experimental station. Details of sapling preparation and evaluation of planting success were described by Marek et al. (2000).

The saplings were used for planting of an artificial stand in three replications. Two stands, each of them composed of 56 individuals, were enclosed into the special experimental facilities. The same stand was planted in the open-air conditions. Each stand was planted according to the pre-defined stand structure to achieve the differences between the considered sparse and dense parts of the stand. The dense part (D) simulates the stand density of 1 tree m⁻² (10 000 trees ha⁻¹, mean projected LAI of 3.7, spring 2000) and the sparse one (S) represents 5 000 trees ha⁻¹ (mean projected LAI of 1.5, spring 2000). Thus, 34 trees represent the D-part and 22 trees represent the S-part. The replication of each simulated stand structure is based on the rows of investigated individuals. The S/D density is represented by 3/2 internal rows surrounded by 2 protective ones. The reason for the establishment of two different densities of the stand was the basic idea on possible effects of stand density on the Norway spruce response to EC.

Two glass domes with adjustable windows (DAW) were used for the experimental spruce stand long-term cultivation under the artificial atmosphere. The dimensions of DAW are 9×9×6 m. One DAW contained AC [ca. 350 μmol(CO₂) mol⁻¹] while the second DAW was permanently supplied with AC plus 350 μmol(CO₂) mol⁻¹ (EC-variant). Each DAW enclosed area of 81 m². Because of windy, heavy snow cover and ice loading at the mountain conditions of the experimental research site, the DAWs were anchored on a concrete base (depth: 0.9 m) and included a massive iron frame (approximate mass of 17 000 kg) with adjustable lamella-windows. These windows are opened/closed according to the monitored internal conditions within DAW versus external ones. Monitored conditions were (1) mean required atmospheric CO₂ concentration in the EC-variant, (2) air temperature, and (3) soil moisture. The adjustable windows were also opened/closed on the individual walls of DAW according to the wind speed and wind direction (to exclude wind incursions into the internal DAW space). The construction of the lamella-windows system significantly attributed to the reduction of CO₂ costs and to the maintenance of the acceptable internal environment within the DAW. A tank containing 15 000 kg of liquid CO₂ served as its source. Each DAW was supplied by air using four ventilators that were connected to the special ventilation tubes preparing the homogenous air profile in DAW internal space. Continuously added 350 μmol(CO₂) mol⁻¹ into the air stream formed the internal CO₂ concentration in the EC-variant (Fig. 1). The construction of DAW and the system of air distribution were responsible for the acceptable conditions in DAW interior comparable to the outside control stand conditions. The control stand outside the DAW served as a comparison for the evaluation of the "chamber" effect of DAW. Only solar radiation was reduced in DAW (up to 30 %) because of the iron frames.
The detailed description of the DAW construction and function is given by Urban et al. (2001).

Fig. 1. Seasonal course of CO₂ concentration in the atmosphere of the adjustable windows domes (DAW). The growing season 2000. Error bars indicate SE of the mean. CO₂ measurement each 20 min in the time interval April–November 2000. ASE – ambient DAW, sparse stand, exposed crown layer; ASS – ambient DAW, sparse stand, shaded crown layer; ADE – ambient DAW, dense stand, exposed crown layer; ADS – ambient DAW, dense stand, shaded crown layer; ESE – elevated DAW, sparse stand, exposed crown layer; ESS – elevated DAW, sparse stand, shaded crown layer; EDE – elevated DAW, dense stand, exposed crown layer; EDS – elevated DAW, dense stand, shaded crown layer.

Measurements of the canopy PPFD profiles: To estimate PPFD within the exposed (sunny) and shaded crown layers, a set of laboratory-made sensors (composed of two photocells separated by 3 cm) based on a photocell (BPW-21, 400-700 nm, Elfa AB, Solna, Sweden) was used. The spatial distribution of the sensors within each DAW followed a random scheme suitable for each of the investigated plots (sparse/dense). The sensors were calibrated against a standard sensor (LI-180, LI-COR, Lincoln, USA). The measurements of PPFD, incident and penetrating into the exposed and shaded crown layers, were made at 30-min intervals; the data were logged into a data logger (Delta-T, England) during the whole vegetation season, i.e. April-October 2000.

Gas exchange measurements: The photosynthetic parameters were estimated on one-year-old needles situated in the exposed and shaded parts (E- and S-needles) at the end of July 2000. After the gas-exchange measurements, the shoots were removed and their projected area of needles was estimated using the LI-3000A leaf area meter (LI-COR, Lincoln, USA). The relationships between PN and PPFD and between PN and Ci (internal CO₂ concentration) were estimated using an open gas exchange system (CIRAS-1, PP-Systems, UK). A standard assimilation chamber (Pankinson conifer chamber) was used. A special laboratory-made artificial light source (two 100 W lamps with two thermal filters and fans, maximum PPFD of 1300 μmol m⁻² s⁻¹) was mounted perpendicular to the top of the assimilation chamber. The light source provided homogenous irradiation (±20%) onto the 5 x 3 cm plane within the assimilation chamber. Measurements in the assimilation chamber were made under standardised conditions (leaf temperature 20±2 °C; water vapour pressure deficit 15 HPa). The PN-PPFD response curve was measured under a predetermined set of PPFD (0, 20, 50, 100, 200, 500, 800, and 1200 μmol m⁻² s⁻¹). The PPFD was adjusted using a potentiometer, which is a part of the light source. The estimation of the short-term response of PN-PPFD to [CO₂] was based on measurements at the growth and reversed [CO₂] in DAW. The PN-Ci response curve was measured under a predetermined set of Ci (20, 60, 100, 200, 500, 800, and 1200 μmol(CO₂) mol⁻¹). The individual concentrations of Ci were adjusted using a special valve, which is a part of the CIRAS-1 device. All PN observations at each PPFD or Ci were recorded within a ca. 3-min time interval.

Nutrient analysis: Nitrogen content in the dry mass of needles was detected by an automatic analyser (CNS-2000, LECO Corporation, St. Joseph, MI, USA) in 200 mg uncrushed sub-samples of needles (i.e. per AC and EC-variants, Sp – sparse and D – dense sub-variants, and E – exposed and S – shaded needle types). The analyser was calibrated on Sulfamethazine LECO 502-298 and Alfa LEO 502-273. Before analysis, each sub-sample was dried to a constant mass in an oven (105 °C) during two days.

Statistical processing of data: Presented results are based on eight shoot variants, i.e. exposed/shaded shoots; sparse/dense part of the stand; AC/EC DAW. Six individuals represented each variant. These individuals were located in the 3/2 rows of trees enclosed inside the sparse/dense parts. Thus, in the sparse part two individuals represented each row. In the dense part three individuals represented each row. Three shoots were measured on each of tree selected individuals. Three replicated gas exchange measurements were made on each shoot.
Results

The temporal development of the experimental stands enclosed in DAW was responsible for the formation of two distinctive crown layers according to the solar radiation conditions, i.e., the sunny (exposed) and shaded crown layers. After three years of cultivation in the DAW, the radiation conditions of shaded crown layers were different from those occurring in the sun-exposed layers in both CO₂ cultivation regimes. This differentiation was strongly dependent on the stand density. Observed irradiations are presented as an example for a sunny summer day, July 28th, 2000 (Fig. 2A, B). The noon PPFD receipt of S-shoots was 10 and 11 % in the D-parts or 32 and 38 % in the Sp-parts of the E-shoots receipt in the AC- and EC-variants, respectively.

At the time of \( P_N \) measurements (end of the third season of CO₂ fumigation) differences in the saturated net photosynthetic rate (\( P_{Net} \)) between AC- and EC-variants were observed. EC fumigation was responsible for the decrease of \( P_{Net} \) (Table 1). This was more pronounced for E-needles, i.e., 25 % in the D-part and 32 % in the Sp-part (Fig. 3A), compared to the S-needles, where the \( P_{Net} \) depression was smaller, i.e., 11 and 21 % in the D- and Sp-parts, respectively. EC was responsible for the smoothing of the vertical gradient of \( P_{Net} \) values between E/S needles (Table 1). However, these differences in \( P_{Net} \) between E- and S-needles were smaller in the Sp-part compared to the D-one.

Long-term cultivation in the EC atmosphere was responsible for the changes in the carbon dioxide compensation concentration (\( \Gamma_C \)) significantly only for the S-needles in the D-part (Table 1). Differences in \( \Gamma_C \) values between the EC- and AC-variants for S-needles amounted to 13.5 %.

The carboxylation efficiency, \( \tau \) was significantly depressed by the long-term cultivation in EC variant (Table 1) compared to the AC variant. This depression was more evident for E-needles of the Sp-part (up to 54 %) than for the D-one (up to 38 %). In S-needles the \( \tau \)-value decrease was more pronounced for the Sp-part (up to 34 %) compared to the D-one (Fig. 3A). The same trend was observed for the calculated value of the
CO₂ saturated carboxylation rate \( (V_{C_{\text{max}}}) \).

The maximum rate of the electron transport \( (J_{\text{max}}) \) was depressed by the long-term cultivation in EC (Table 1). The degree of the \( J_{\text{max}} \) value depression compared to the AC-variant was obtained in the order: E-needles of the Sp-part (36 %); E-needles of the D-part (24 %); S-needles of the Sp-part (19 %); S-needles of the D-part (17 %).

EC induced changes in the \( J_{\text{max}}/V_{C_{\text{max}}} \) ratio between AC- and EC-variants. Greater \( J_{\text{max}}/V_{C_{\text{max}}} \) values were found in the EC-variant and for E-needles compared with AC-variant and S-needles (Table 1).

The rate of CO₂ evolution into CO₂-free air at saturated PPFD \( (R_0) \) indicates (Table 1) some effects of long-term impacted EC on the re-assimilation processes within the needle interior, i.e. depression. Compared to the AC-variant this depression was more distinctive for S-needles of the Sp- and D-parts of the experimental stand. If the important participation of photorespiration on the \( R_0 \) value is supposed, then the observed lower \( R_0 \) values in EC-variant, and especially in S-needles, well support the generally accepted idea of the primary effects of EC, i.e. the depression of photorespiration.

The photon-saturated assimilation rate \( (P_{\text{Nmax}}) \) was ever stimulated by EC in both types of foliage at the end of the third season of CO₂ fertilization compared to the AC-variant (Table 2). However, the degree of this stimulation decreased in the order: S-needle D-part > S-needle Sp-part > E-needle D-part > E-needle Sp-part (Fig. 3B). Apparent quantum yield of assimilation, i.e. the photochemical efficiency (\( \alpha \)), positively reacts to the EC impact. In both investigated types of foliage and stand densities the increase of \( \alpha \) was found (Table 2). The S-needles responded more positively compared to the E-needles. Moreover, this positive reaction of \( \alpha \) was most obvious in S-needles of the dense part of the stand (Fig. 3C).

The long-term impact of EC did not change the vertical foliage stratification within the dense stand canopy as is possible to conclude from \( P_{\text{Nmax}} \) and \( \alpha \) values of E- and S-needles. However, EC was responsible for the smoothing of differences between E- and S-needles, compared to the control AC-variant (Table 2).

Nitrogen content of needles was different for both CO₂ treatments, stand density, and foliage type (Fig. 4). The critical N content of Norway spruce needles of 1.3 % (Huttli 1991, Innes 1993) was found in S-needles of Sp- and D-plots in the EC-variant. The long-term effect of EC was responsible for the decrease of N content in needles and for smoother gradient between E- and S-needles. The linear relation between the needle N content and maximum rate of ribulose-1,5-bisphosphate carboxylase/oxygenase carboxylation, RuBPCO \( (V_{C_{\text{max}}}) \) and \( \tau \) was found (Fig. 5A, B). Long-term cultivation in the EC atmosphere was reflected by the steeper slope of relation between \( V_{C_{\text{max}}} \) and % of N in the needle DM.
Table 2. Values of selected parameters of $P_{\text{N}}$-PPFD response curve. $P_{\text{Nmax}}$ — PPFD saturated net photosynthetic rate [μmol(CO$_2$) m$^{-2}$ s$^{-1}$]; α — apparent quantum yield of shoot [mol(CO$_2$) mol(quantum)$^{-1}$]; $\Gamma_1$ — compensation PPFD [μmol(quantum) m$^{-2}$ s$^{-1}$]; $R_0$ — rate of dark respiration [μmol(CO$_2$) m$^{-2}$ s$^{-1}$]; (1 — $P_{\text{N}}$/$P_{\lim}$) — ability to utilise radiant energy. Mean and standard error values for 18 numbers of observations; the same letters/numerals: level of significance at 95% for the comparison of the exposed/shaded needles and AC/EC variants. This comparison was done only for directly measured values.

<table>
<thead>
<tr>
<th>Variant</th>
<th>$P_{\text{Nmax}}$</th>
<th>α</th>
<th>$\Gamma_1$</th>
<th>$R_0$</th>
<th>(1 — $P_{\text{N}}$/$P_{\lim}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASE</td>
<td>8.7±0.7</td>
<td>0.023±0.001 f</td>
<td>14.0±1.6,6</td>
<td>0.68±0.08,1,10</td>
<td>0.76</td>
</tr>
<tr>
<td>ASS</td>
<td>7.1±0.8</td>
<td>0.034±0.002 f,3</td>
<td>13.0±2.0,7</td>
<td>0.64±0.03,1,11</td>
<td>0.88</td>
</tr>
<tr>
<td>ESE</td>
<td>9.2±0.7 a</td>
<td>0.027±0.003 g</td>
<td>10.0±1.8,6</td>
<td>0.51±0.03 m,10</td>
<td>0.72</td>
</tr>
<tr>
<td>ESS</td>
<td>8.1±0.5 a</td>
<td>0.038±0.001 g,3</td>
<td>11.0±2.1,7</td>
<td>0.29±0.07 m,11</td>
<td>0.84</td>
</tr>
<tr>
<td>ASE3/7</td>
<td>11.7±0.8 b</td>
<td>0.029±0.002 h</td>
<td>14.0±1.6</td>
<td>0.85±0.07 n</td>
<td>0.69</td>
</tr>
<tr>
<td>ASS3/7</td>
<td>9.4±0.9 b</td>
<td>0.039±0.002 h</td>
<td>13.0±2.0</td>
<td>0.49±0.03 n</td>
<td>0.77</td>
</tr>
<tr>
<td>ADE</td>
<td>8.0±0.1 c,1</td>
<td>0.028±0.003 l,4</td>
<td>12.0±1.0,8</td>
<td>0.45±0.02 o,12</td>
<td>0.86</td>
</tr>
<tr>
<td>ADS</td>
<td>4.6±0.3 c,2</td>
<td>0.037±0.001 l,5</td>
<td>11.0±1.1,9</td>
<td>0.33±0.01 o,13</td>
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<tr>
<td>EDE</td>
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<td>0.034±0.001 j,4</td>
<td>18.0±2.2,8</td>
<td>1.02±0.08 p,12</td>
<td>0.87</td>
</tr>
<tr>
<td>EDS</td>
<td>6.4±0.3 d,2</td>
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<td>16.0±1.1,9</td>
<td>0.46±0.06 p,13</td>
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</tr>
<tr>
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<td>0.80</td>
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</table>

Discussion

CO$_2$ concentration differentiation (Fig. 1) between the AC and EC domes with adjustable windows (DAW), which was reached during the three investigated growing seasons, is acceptable and enables to place DAW among reasonably functioning tools used in the long-term EC experiments (Urban et al. 2001).

Three-year formation of the experimental spruce stands in DAW was connected to the differentiation of PPFD conditions within the stand canopy. Under the presented sunny summer days (July 28th to 31th, 2000) the exposure time of S-needles to the PPFD of 100 μmol m$^{-2}$ s$^{-1}$ amounted to 11 or 3 h of the light-part of the day within sparse or dense parts of the experimental stand (Fig. 2A,B). Thus the canopy closure, which was already achieved in the dense parts of the experimental stands in the AC/EC DAW, was responsible for the formation of real shaded conditions.

On the level of $P_{\text{N}}$-C$_1$ relation, the obtained occurrence of acclimation depression of photosynthesis (Kramer 1981, Marek et al. 1995, Grimmer and Komor 1999) due to the long-term impact of EC was observed in both types of the foliage and stand densities (Table 1, Fig. 3A). The values of α and $V_{\text{Cmax}}$ and their depression, compared to the same needles in the AC-variant, corresponded to their lower needle N content (Fig. 4). The significant effect of N nutrition on the assimilatory activity of needles exposed to EC is supported by the steeper dependencies of $V_{\text{Cmax}}$ and $\tau$ on the needle N compared to the AC-variant (Fig. 5A,B). Hence the changes in needle tissue N in the EC-variant are accompanied by a large change in the carboxylation rate and its efficiency. Both dependencies were linear. However, whereas the ratio of the $V_{\text{Cmax}}$ slope between AC- and EC-variants amounted to 1.92, the same ratio for $\tau$ was 1.20. The RuBPCO activity can be divided into two components, i.e. amount and efficiency. Thus, the amount of RuBPCO instead of its efficiency is more affected by the lower needle N content in the EC-variant. Besford et al. (1998) and Urban and Marek (1999) reported the importance of changed RuBPCO activity, not divided into amount and efficiency, for a manifestation of the photosynthetic assimilation depression in Norway spruce individuals cultivated for three years in EC.

![Fig. 5. Linear relationship between (A) the maximal carboxylation rate ($V_{\text{Cmax}}$) and (B) carboxylation efficiency, $\tau$ and foliar nitrogen content in elevated (△) and ambient (★) CO$_2$ variants.](image-url)

The obtained acclimation depression of photosynthesis differed between the foliage types. Generally, the degree of this depression was smaller in shade needles (Fig. 3A) and the distinctive shade needles in the dense
stand part show lower degree of depression, at the same
N content, than the S-needles from the Sp-part (Table 1,
Fig. 3A). These results indicate the possible positive
compensation effect of EC on typical shade foliage as-
similatory activity. EC can compensate the PPFD li-
tation of photosynthesis by a stimulation of assimilatory
activity of the shaded needles (Lawlor and Mitchell 1991,
Marek et al. 2001).

Zhang et al. (1995) and Marek et al. (1999) presented
the ratio of $J_{max}/v_{C}$, which indicates the relative limi-
tations of electron transport versus carboxylase activity
(Table 1). A higher $J_{max}/v_{C}$ indicates that $P_{N}$ is signif-
ically co-limited by lower carboxylation rather than by
lower RuBP regeneration associated with electron trans-
port. A higher $J_{max}/v_{C}$ ratio was observed for the E-
needles of both [CO$_2$] variants and stand densities com-
pared to the S-needles. The $J_{max}/v_{C}$ ratio in EC-variant
was higher than in the AC-one in both types of investi-
gated shoot types and stand densities (Table 1). These
findings support an idea on the main assimilation co-
limitation by carboxylation because of the lower N con-
tent of needles in the EC-variant compared to the AC-
one. The generally lower values of the $J_{max}/v_{C}$ ratio in S-
needles compared to the E-needles in both CO$_2$ treat-
ment variants indicate that $P_{N}$ is co-limited mainly by
the low RuBP regeneration. However, the S-needles of
the EC-variant are more co-limited by carboxylation (higher
$J_{max}/v_{C}$ ratio) than the S-needles of the AC-variant.

The saturated part of the $P_{N}$, PPFD response curve, i.e.
$P_{N_{max}}$ (Table 2) and $\tau$ (Table 1), represents RuBPCO
activity limited assimilation and the saturated part of the
$P_{N}$, $C_{i}$, i.e. $P_{N_{sat}}$ (Table 1) response curve and $\alpha$ (Table 2)
represent the RuBP regeneration limitation of assimila-
tion (Long and Hallgren 1993). Obtained $E$ versus $S$
needle differences were greater and distinctive especially
for the $P_{N_{sat}}$ and $\tau$ (Tables 1 and 2). These differences
enable to support the mentioned findings on the lower
RuBP regeneration limitation in the S-needles in the EC-
variant. However, Marek et al. (2001) report on the basis
of the chlorophyll $a$ fluorescence a positive effect of long-term EC on the electron transport rate of S-needles.

A known effect of EC described in the literature is the
depression of photosynthesis (Stitt 1991, Long and
Drake 1992). The rate of photosynthesis was evaluated
indirectly via the rate of CO$_2$ evolution into CO$_2$-free air
at saturating PPFD ($R_{S}$). Thus, the observed depression of
$R_{S}$ values in the EC-variant (Table 1) confirmed this gen-
eral phenomenon of the effect of depression by EC on the
rate of photosynthesis. The more distinctive $R_{S}$ depression
observed in S-needles of the EC-variant was related to
low irradiance (Fig. 2B) and low leaf N content (Fig.
4). In an experiment with EC and oxygen concentration
decreased to 1%, Osborne et al. (1997) found that $\alpha$ was
not altered between AC- and EC-variants. They also de-
scribed a decreased RuBPCO and light-harvesting chlo-
rophyll protein content of leaves (up to 30%), which was
associated with a decreased $P_{N_{max}}$ but not with decreased
$\alpha$. Indeed, for E- and S-needles from Sp- and D-parts of
EC-variant the long-term CO$_2$ stimulation of $P_{N_{max}}$ was
lower compared to $\alpha$ (Figs. 4 and 5). Thus the observed
increase of $P_{N}$ in the tested needle types and stand
densities of EC-variant was mainly a function of
suppressed photosynthesis. This stimulation was larger
for S-needles from the D-part of the stand. These findings
again support our idea on the compensation effects of EC
under low PPFD.

It is possible to evaluate the nature of ability to utilise
radiant energy using the ratio $1 - P_{N_{sat}}/P_{N_{max}}$ (Table 2). The
term $P_{N_{sat}}$ represents the actual assimilation rate, i.e. ob-
served rate of CO$_2$ uptake at given PPFD. The cor-
responding potential rate, $P_{N_{max}}$ at the same PPFD is the rate
taken from the initial slope of the $P_{N_{sat}}$-PPFD curve, as-
suming a constant quantum yield at all irradiances
(Schreiber and Bilger 1987). The comparison is based on
the PPFD saturation value of 1 300 μmol m$^{-2}$ s$^{-1}$. Gen-
erally, the low ratio indicates the higher ability to utilise
radiant energy in increasing PPFD. Thus, as generally
known, this ability was higher for E-needles. The long-
term cultivation in EC led to a decrease of photosynthetic
radiant energy utilisation at saturating PPFD. This de-
crease was described as the result of induced changes or
acclimation of photosystem 2 function (Spunda et al.
1998b, Kalina et al. 2000) and changes in the features of
the electron transport (Marek et al. 2001). The sudden
application of the doubled [CO$_2$] to the needles of AC-
variants remarkably increased the 1 $P_{N_{sat}}/P_{N_{max}}$. The
greatest sensitivity of this sudden change of AC was
found for S-needles, especially for the S-needles from the
D-part of the stand (Fig. 3B).

Long-term EC stimulation of assimilation was ob-
served in the value of $P_{N_{sat}}$-PPFD relation (Table 2,
Fig. 3B). Larger stimulation was found for the S-needles,
especially for those from the dense part of the stand.
However, the comparison of values of $P_{N_{sat}}$ and $\alpha$ be-
tween the AC and EC variants (Table 2) indicates that
the long-term influence of EC did not change the basic
physiological differentiation between the E- and S-need-
les in both parts of the stands, i.e. Sp and D (Byrkjør
1981, Givnish 1988). EC stimulation observed especially
for S-needles of the D-part of the stand supports our idea on
the compensatory effect of EC on the shaded needles
assimilation under limited irradiance (Lawlor and
Mitchell 1991, Osborne et al. 1997). EC could compen-
sate the PPFD limitation of photosynthesis by a stimu-
lation of the assimilatory activity of S-needles. The as-
similatory activity of S-layers of the stand canopy is im-
portant as a long-term active target of the simulative
actions of EC.

Our results support the importance of the vertical dif-
ference of assimilatory activity response for the long-
term effect of [CO$_2$]. Really, the responses of shade and
sunny foliage to EC are different. Shaded needles of the
EC-variant respond more significantly to EC than the
shade needles of the AC-variant. The prolonged exposure
to EC was responsible for the apparent stimulation of assimilatory activity observed mainly in deeply shaded needles. The degree of this stimulation decreases in the order: S-needles D-part > S-needles Sp-part > E-needles D-part > E-needles Sp-part. Manifestation of acclimation depression of the photosynthetic activity was found preferentially for the sunny needles. Thus, the stand canopy is heterogeneous in its reaction to EC. This heterogeneity may be regarded as a serious phenomenon of the whole-stand response to the long-term impact of EC.

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