PHOTOSYNTHETICA 40 (2): 259-267, 2002

Photosynthetic assimilation of sun *versus* shade Norway spruce [*Picea abies* (L.) Karst] needles under the long-term impact of elevated CO_2 concentration

M.V. MAREK[*,***], O. URBAN[*], M. ŠPRTOVÁ[*], R. POKORNÝ[*], Z. ROSOVÁ[*], and J. KULHAVÝ[**]

Laboratory of Ecological Physiology of Forest Trees, Institute of Landscape Ecology of the Academy of Sciences, Poříčí 3b, CZ-603 00 Brno, Czech Republic[*]
Institute of Forest Ecology, Faculty of Forestry and Wood Technology, Mendel Agricultural and Forestry University, Zemědělská 3, CZ-613 00 Brno, Czech Republic[**]

Abstract

The long-term impact of elevated concentration of CO_2 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(CO_2) mol^{-1}) and the second with elevated CO_2 concentration [EC = AC plus 350 μmol(CO_2) 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 (P_{Nmax}) 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; CO_2 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 CO_2 (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 CO_2 (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 CO_2 concentration can partly compensate the low irradiance on the level of photosynthetic assimilation.

Received 2 April 2002, *accepted* 17 June 2002.
[***]Author for correspondence; phone/fax: +420-5043242017; e-mail: emarek@brno.cas.cz
Acknowledgements: The grants No. LN00A141 (Ministry of Education of the Czech Rep.), No. S6087005 (Grant Agency of the Academy of Sciences), and by the Governmental Research Intention of the Institute of Landscape Ecology No. AVOZ6087904 financially supported this research.

These relative CO_2 effects in shade may be particularly large because higher CO_2 concentrations reduce photorespiration and thus decrease the CO_2 compensation concentration of photosynthesis (Long and Drake 1991, Osborne *et al.* 1997). Suppressed photorespiration resulted in the increased carbon uptake rate (P_N), which is comparable to P_N obtained at high photosynthetic photon flux density (PPFD). In accordance with this statement, the relative CO_2 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 (shade 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 Bílý Kříž in the Beskydy Mts. (North-East part of the Czech Republic, 49°30′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(CO_2) mol^{-1}] while the second DAW was permanently supplied with AC plus 350 μmol(CO_2) mol^{-1} (EC-variant). Each DAW enclosed area of 81 m^2. 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 CO_2 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 CO_2 costs and to the maintenance of the acceptable internal environment within the DAW. A tank containing 15 000 kg of liquid CO_2 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(CO_2) mol^{-1} into the air stream formed the internal CO_2 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).

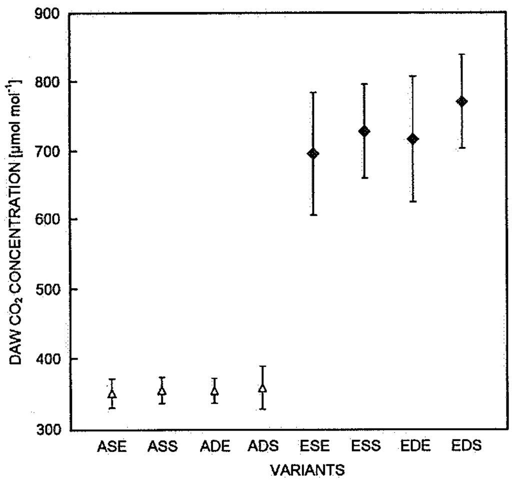

Fig. 1. Seasonal course of CO_2 concentration in the atmosphere of the adjustable windows domes (DAW). The growing season 2000. Error bars indicate SE of the mean. CO_2 measurement each 20 min in the time interval April–November 2000. ASE – ambient DAW, sparse stand, exposed crown layer; ASS – ambient 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 measurement: 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 P_N and PPFD and between P_N and C_i (internal CO_2 concentration) were estimated using an open gas exchange system (*CIRAS-1*, *PP-Systems*, UK). A standard assimilation chamber (Parkinson conifer chamber) was used. A special laboratory-made artificial light source (two 100 W lamps with two thermal filters and fans, maximum PPFD of 1 300 µmol m^{-2} s^{-1}) was mounted perpendicular to the top of the assimilation chamber. The light source provided homogenous irradiation (±20 %) onto the 5×3 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 P_N-PPFD response curve was measured under a predetermined set of PPFD (0, 20, 50, 100, 200, 500, 800, and 1 200 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 P_N-PPFD to [CO_2] was based on measurements at the growth and reversed [CO_2] in DAW. The P_N-C_i response curve was measured under a predetermined set of C_i [20, 60, 100, 200, 500, 800, and 1 200 µmol(CO_2) mol^{-1}]. The individual concentrations of C_i were adjusted using a special valve, which is a part of the *CIRAS-1* device. All P_N observations at each PPFD or C_i 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 LECO 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 layer. 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 CO_2 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. 2*A,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 CO_2 fumigation) differences in the saturated net photosynthetic rate (P_{Nsat}) between AC- and EC-variants were observed. EC fumigation was responsible for the decrease of P_{Nsat} (Table 1). This was more pronounced for E-needles, *i.e.* 25 % in the D-part and 32 % in the Sp-part (Fig. 3*A*), compared to the S-needles, where the P_{Nsat} 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_{Nsat} values between E/S needles (Table 1). However, these differences in P_{Nsat} 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 (Γ_C) significantly only for the S-needles in the D-part (Table 1). Differences in Γ_C values between the EC- and AC-variants for S-needles amounted to 13.5 %.

The carboxylation efficiency, τ 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 τ-value decrease was more pronounced for the Sp-part (up to 34 %) compared to the D-one (Fig. 3*A*). The same trend was observed for the calculated value of the

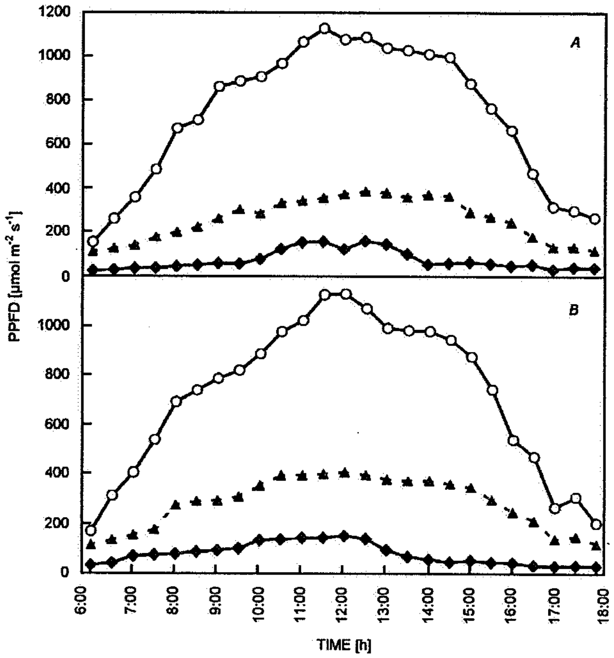

Fig. 2. Daily course of photosynthetically active radiation (PPFD) during a sequence of sunny summer days (end of July 2000) taken as an example, in ambient (*A*) and elevated (*B*) [CO_2] adjustable windows domes. *Open circles* – incident PPFD at the level of sunny (exposed) foliage; *closed diamonds* – PPFD at the level of shaded foliage in the dense part of the stand; *closed triangles* – PPFD at the level of shaded foliage in the sparse part of the stand.

Table 1. Values of selected parameters of P_N-C_I response curve. P_{Nsat} – PPFD and CO_2 saturated P_N [µmol(CO_2) m^{-2} s^{-1}]; τ – carboxylation efficiency [mol(CO_2) m^{-2} s^{-1}] Γ_C – CO_2 compensation concentration [µmol mol^{-1}]; R_S – rate of dark CO_2 evolution into CO_2 free air [µmol(CO_2) m^{-2} s^{-1}]; V_{Cmax} – maximal rate of RuBPCO carboxylation [µmol(CO_2) m^{-2} s^{-1}]; J_{max} – maximal electron transport rate [µmol m^{2-} s^{-1}]; J_m/V_C – ratio of the electron transport rate to the rate of carboxylation. 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.

Variant	P_{Nsat}	τ	Γ_c	R_S	V_{Cmax}	J_{max}	J_m/V_C
ASE	25.1±2.2 a	0.060±0.017 e,3	51.0±2.6	2.20±0.64	41.0±6.6 k	144.0±5.3 o	3.51
ASS	11.0±0.6 a,1	0.024±0.008 e,4	56.0±2.7	1.60±0.32	19.0±2.8 k	56.0±2.1 o,9	2.94
ESE	20.0±1.9 b	0.039±0.013 f,3	49.0±3.2	1.80±0.30	24.0±1.8 l	106.0±1.1 p,10	4.41
ESS	7.6±2.1 b,1	0.018±0.005 f,4	53.0±3.5	1.20±0.18	14.0±2.4 l	47.0±1.6 p,11	3.28
ADE	23.2±2.0 c	0.058±0.006 g,5	50.0±2.2 i,6	2.00±0.79	38.0±7.1m	139.0±5.4 q	3.13
ADS	7.3±0.6 c,2	0.026±0.005 g	67.0±3.8 i,7	1.50±0.13 -,8	20.0±1.4 m	37.0±2.9 q,9	1.76
EDE	19.0±1.8 d	0.042±0.007 h,5	47.0±2.9 j,6	1.40±0.32	25.0±0.3 n	112.0±4.7 r,10	4.48
EDS	6.6±0.3 d,2	0.023±0.002 h	59.0±3.1 j,7	0.60±0.13 -,8	17.0±0.8 n	32.0±2.5 r,11	2.47

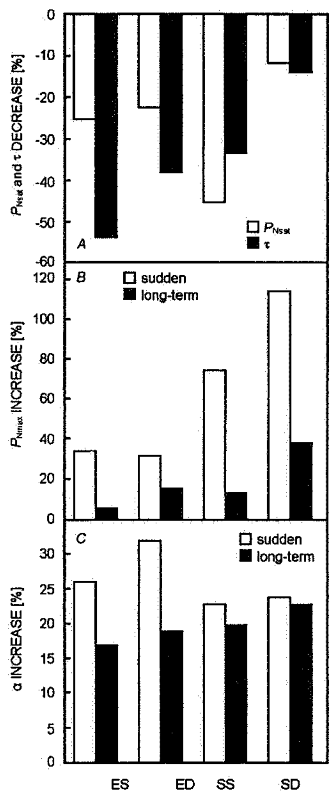

Fig. 3. Depression of the PPFD and CO_2-saturated rate of net photosynthetic rate (P_{Nsat}) and carboxylation efficiency, α (*A*), and stimulation of the PPFD-saturated (maximal) net photosynthetic rate, P_{Nmax} (*B*) or the apparent quantum yield, α (*C*) under long-term or sudden change from AC to EC, expressed as % compared to the ambient (100 %) variant. ES – exposed crown layer, sparse stand; ED – exposed crown layer, dense stand; SS – shaded crown layer, sparse stand; SD – shaded crown layer, dense stand.

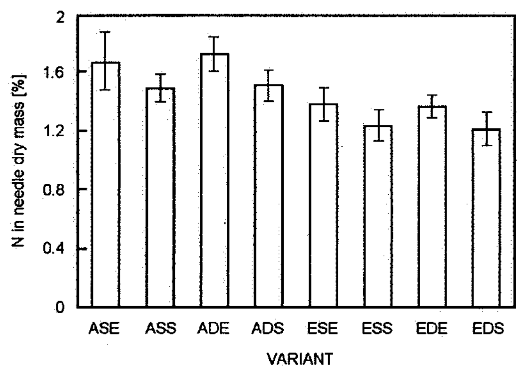

Fig. 4. Foliar content of nitrogen expressed as % of the needle dry mass. Critical value of N % in DM for the Norway spruce is 1.3 %. ASE – ambient DAW, sparse stand, exposed crown layer; ASS – ambient DAW, sparse stand, shaded crown layer; ADE – ambient DAW, dense stand, exposed crown layer; ADS – ambient 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.

CO_2 saturated carboxylation rate (V_{Cmax}).

The maximum rate of the electron transport (J_{max}) was depressed by the long-term cultivation in EC (Table 1). The degree of the J_{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_{max}/V_{Cmax} ratio between AC- and EC-variants. Greater J_{max}/V_{Cmax} values were found in the EC-variant and for E-needles compared with AC-variant and S-needles (Table 1).

The rate of CO_2 evolution into CO_2-free air at saturated PPFD (R_S) 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_S value is supposed, then the observed lower R_S 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_{Nmax}) was ever stimulated by EC in both types of foliage at the end of the third season of CO_2 fumigation 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. 3*B*). Apparent quantum yield of assimilation, *i.e.* the photochemical efficiency (α), positively reacts to the EC impact. In both investigated types of foliage and stand densities the increase of α was found (Table 2). The S-needles responded more positively compared to the E-needles. Moreover, this positive reaction of α was most obvious in S-needles of the dense part of the stand (Fig. 3*C*).

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_{Nmax} and α 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 CO_2 treatments, stand density, and foliage type (Fig. 4). The critical N content of Norway spruce needles of 1.3 % (Huttl 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_{Cmax}) and τ was found (Fig. 5*A*,*B*). Long-term cultivation in the EC atmosphere was reflected by the steeper slope of relation between V_{Cmax} and % of N in the needle DM.

Table 2. Values of selected parameters of P_N-PPFD response curve. P_{Nmax} – PPFD saturated net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; α – apparent quantum yield of shoot [mol(CO_2) mol(quantum)$^{-1}$] ; Γ_I – compensation PPFD [μmol(quantum) m^{-2} s^{-1}]; R_D – rate of dark respiration [μmol(CO_2) m^{-2} s^{-1}]; $(1 - P_{Na}/P_{Np})$ – 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.

Variant	P_{Nmax}	α	Γ_I	R_D	$(1 - P_{Na}/P_{Np})$
ASE	8.7±0.7	0.023±0.001 f	14.0±1.6 ,6	0.68±0.08 l,10	0.76
ASS	7.1±0.8	0.034±0.002 f,3	18.0±2.0 ,7	0.49±0.03 l,11	0.88
ESE	9.2±0.7 a	0.027±0.003 g	10.0±1.8 ,6	0.51±0.03 m,10	0.72
ESS	8.1±0.5 a	0.038±0.001 g,3	11.0±2.1 ,7	0.29±0.07 m,11	0.84
ASE3/7	11.7±0.8 b	0.029±0.002 h	14.0±1.6	0.85± 0.07 n	0.69
ASS3/7	9.4±0.9 b	0.049±0.002 h	13.0±2.0	0.49±0.03 n	0.77
ADE	8.0±0.1 c,1	0.028±0.003 I,4	12.0±1.0 ,8	0.45±0.02 o,12	0.86
ADS	4.6±0.3 c,2	0.037±0.001 I,5	11.0±1.1 ,9	0.33±0.01 o,13	0.89
EDE	9.4±0.9 d,1	0.034±0.003 j,4	18.0±2.2 ,8	1.02±0.08 p,12	0.87
EDS	6.4±0.3 d,2	0.042±0.002 j,5	16.0±1.1 ,9	0.46±0.06 p,13	0.91
ADE3/7	14.0±1.1 e	0.037±0.006 k	10.0±1.3	0.85±0.09 q	0.82
ADS3/7	9.9±0.08 e	0.046±0.004 k	9.5±2.2	0.46±0.02 q	0.80

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 31st, 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. 2*A,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_N-C_i 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. 3*A*). The values of τ and V_{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_{Cmax} and τ on the needle N compared to the AC-variant (Fig. 5*A,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_{Cmax} slope between AC- and EC-variants amounted to 1.92, the same ratio for τ 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.

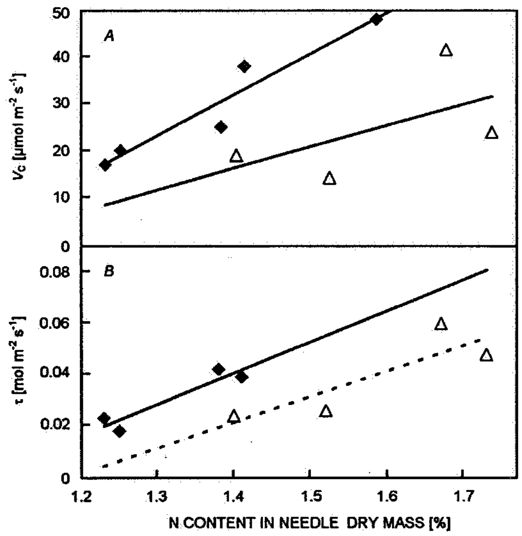

Fig. 5. Linear relationship between (*A*) the maximal carboxylation rate (V_{Cmax}) and (*B*) carboxylation efficiency, τ and foliar nitrogen content in elevated (♦) and ambient (Δ) CO_2 variants.

The obtained acclimation depression of photosynthesis differed between the foliage types. Generally, the degree of this depression was smaller in shade needles (Fig. 3*A*) 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. 3*A*). These results indicate the possible positive compensation effect of EC on typical shade foliage assimilatory activity. EC can compensate the PPFD limitation 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 limitations of electron transport *versus* carboxylase activity (Table 1). A higher J_{max}/V_C indicates that P_N is significantly co-limited by lower carboxylation rather than by lower RuBP regeneration associated with electron transport. A higher J_{max}/V_C ratio was observed for the E-needles of both [CO_2] variants and stand densities compared to the S-needles. The J_{max}/V_C ratio in EC-variant was higher than in the AC-one in both types of investigated 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 content 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 treatment 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_{Nmax} (Table 2) and τ (Table 1), represents RuBPCO activity limited assimilation and the saturated part of the P_N-C_i, *i.e.* P_{Nsat} (Table 1) response curve and α (Table 2) represent the RuBP regeneration limitation of assimilation (Long and Hällgren 1993). Obtained E- *versus* S-needle differences were greater and distinctive especially for the P_{Nsat} and τ (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. Moreover, 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 photorespiration (Stitt 1991, Long and Drake 1992). The rate of photorespiration was evaluated indirectly *via* the rate of CO_2 evolution into CO_2-free air at saturating PPFD (R_S). Thus, the obtained depression of R_S values in the EC-variant (Table 1) confirmed this general phenomenon of the effect of depression by EC on the rate of photorespiration. The more distinctive R_S depression observed in S-needles of the EC-variant was related to low irradiance (Fig. 2*B*) and low leaf N content (Fig. 4). In an experiment with EC and oxygen concentration decreased to 1 %, Osborne *et al.* (1997) found that α was not altered between AC- and EC-variants. They also described a decreased RuBPCO and light-harvesting chlorophyll protein content of leaves (up to 30 %), which was associated with a decreased P_{Nmax} but not with decreased α. Indeed, for E- and S-needles from Sp- and D-parts of EC-variant the long-term CO_2 stimulation of P_{Nmax} was lower compared to α (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 photorespiration. 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 $1 - P_{Na}/P_{Np}$ (Table 2). The term P_{Na} represents the actual assimilation rate, *i.e.* observed rate of CO_2 uptake at given PPFD. The corresponding potential rate, P_{Np} at the same PPFD is the rate taken from the initial slope of the P_N-PPFD curve, assuming 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}. Generally, 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 decrease was described as the result of induced changes or acclimation of photosystem 2 function (Špunda *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 $1 - P_{Na}/P_{Np}$ ratio. 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. 3*B*).

Long-term EC stimulation of assimilation was observed in the value of P_N-PPFD relation (Table 2, Fig. 3*B*). 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_{Nmax} and α between 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-needles in both parts of the stands, *i.e.* Sp and D (Björkman 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 compensate the PPFD limitation of photosynthesis by a stimulation of the assimilatory activity of S-needles. The assimilation activity of S-layers of the stand canopy is important as a long-term active target of the simulative actions of EC.

Our results support the importance of the vertical differentiation 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.

References

Bazzaz, F.A., Miao, S.L.: Successional status, seed size, and responses of tree seedlings to CO_2, light, and nutrients. – Ecology **74**: 104-112, 1993.

Besford, R.T., Mousseau, M., Matteucci, G.: Biochemistry, physiology and biophysics of photosynthesis. – In: Jarvis, P.G. (ed.): European Forest and Global Change: The Likely Impacts of Rising CO_2 and Temperature. Pp. 29-78. Cambridge University Press, Cambridge 1998.

Björkman, O.: Responses to different quantum flux densities. – In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): Physiological Plant Ecology I. Pp. 57-107. Springer-Verlag, Berlin – Heidelberg – New York 1981.

Bowes, G.: Facing the inevitable: plants and increasing atmospheric CO_2. – Annu. Rev. Plant Physiol. Plant mol. Biol. **44**: 309-332, 1993.

Bryant, J., Taylor, G., Frehner, M.: Photosynthetic acclimation to elevated CO_2 is modified by source:sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. – Plant Cell Environ. **21**: 159-168, 1998.

Givnish, T.J.: Adaptation to sun and shade: a whole-plant perspective. – Aust. J. Plant Physiol. **15**: 63-92, 1988.

Grimmer, C., Komor, E.: Assimilate export by leaves of *Ricinus communis* L. growing under normal and elevated carbon dioxide concentrations: the same rate during the day, a different rate at night. – Planta **209**: 275-281, 1999.

Huttl, R.F.: Die Blattanalyse als Monitoring-Instrument in Waldökosystem. – UIFRO Workshop on Monitoring Air Pollution Impact on Permanent Plots, Data Processing and Result Interpretation. Pp. 139-147. Prachatice 1991.

Innes, J.L.: Forest health, its assessment and status. – CAB International, Wallingford 1993.

Kalina, J., Čajánek, M., Kurasová, I., Špunda, V., Vrána, J., Marek, M.V.: Acclimation of photosystem 2 function of Norway spruce induced during first season under elevated CO_2 in lamellar domes. – Photosynthetica **38**: 621-627, 2000.

Kramer, P.J.: Carbon-dioxide concentration, photosynthesis, and dry matter production. – BioScience **31**: 29-33, 1981.

Lawlor, D.W., Mitchell, R.A.C.: The effects of increasing CO_2 on crop photosynthesis and productivity – a review of field studies. – Plant Cell Environ. **14**: 807-818, 1991.

Leverenz, J.W.: Shade-shoot structure, photosynthetic performance in the field, and photosynthetic capacity of evergreen conifers. – Tree Physiol. **16**: 109-114, 1996.

Lewandowska, M., Hart, J.W., Jarvis, P.G.: Photosynthetic electron transport in shoots of Sitka spruce from different levels in a forest canopy. – Physiol. Plant. **41**: 124-128, 1977.

Long, S.P., Drake, B.G.: Effect of the long-term elevation of CO_2 concentration in the field on the quantum yeld of photosynthesis of the C_3 sedge, *Scirpus olneyi*. – Plant Physiol. **96**: 221-226, 1991.

Long, S.P., Drake, B.G.: Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. – In: Baker, N.R., Thomas, H. (ed.): Crop Photosynthesis: Spatial and Temporal Determinants. Pp. 69-103. Elsevier Science Publ., Amsterdam 1992.

Long, S.P., Hallgren, J.-E.: Measurements of CO_2 assimilation by plants in the field and the laboratory. – In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.): Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. Pp. 129-167. Chapman & Hall, London – Glasgow – New York – Tokyo – Melbourne – Madras 1993.

Marek, M.V., Kalina, J., Matoušková, M.: Response of photosynthetic carbon assimilation of Norway spruce exposed to long-term elevation of CO_2 concentration. – Photosynthetica **31**: 209-220, 1995.

Marek, M.V., Pokorný, R., Šprtová, M.: An evaluation of the physiological and growth activity of Norway spruce saplings after planting. – J. Forest Sci. **46**: 91-96, 2000.

Marek, M.V., Šprtová, M., Urban, O., Špunda, V.: Chlorophyll *a* fluorescence response of Norway spruce needles to the long-term effect of elevated CO_2 in relation to their position within the canopy. – Photosynthetica **39**: 437-445, 2001.

Marek, M.V., Šprtová, M., Urban, O., Špunda, V., Kalina, J.: Response of sun versus shade foliage photosynthesis to radiation in Norway spruce. – Phyton **39**: 131-137, 1999.

Norby, R.J., Wullschleger, S.D., Gunderson, C.A., Johnson, D.W., Ceulemans, R.: Tree responses to rising CO_2 in field experiments: implications for the future forest. – Plant Cell Environ. **22**: 683-714, 1999.

Osborne, C.P., Drake, B.G., LaRoche, J., Long, S.P.: Does long-term elevation of CO_2 concentration increase photosynthesis in forest floor vegetation? Indiana strawberry in a Maryland forest. – Plant Physiol. **114**: 337-344, 1997.

Schreiber, U., Bilger, W.: Rapid assessment of stress effect on plant leaves by chlorophyll fluorescence measurement. – In: Tenhunen, J.D., Catarino, F.M., Lange, O.L., Oechel, W.C. (ed.): Plant Responses to Stress. Pp. 27-53. Springer-Verlag, Berlin – Heidelberg – New York – London – Paris – Tokyo 1987.

Špunda, V., Čajánek, M., Kalina, J., Lachetová, I., Šprtová, M., Marek, M.V.: Mechanistic differences in utilisation of absorbed excitation energy within photosynthetic apparatus of Norway spruce induced by the vertical distribution of photosynthetically active radiation through the tree crown. – Plant Sci. **133**: 155-165, 1998a.

Špunda, V., Kalina, J., Čajánek, M., Pavlíčková, H., Marek, M.V.: Long-term exposure of Norway spruce to elevated CO_2 concentration induces changes in photosystem II mimicking an adaptation to increased irradiance. – J. Plant Physiol. **152**: 413-419, 1998b.

Stitt, M.: Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. – Plant Cell Environ. **14**: 741-762, 1991.

Stitt, M., Quick, W.P.: Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. – Physiol. Plant. **77**: 633-641, 1989.

Urban, O., Janouš, D., Pokorný, R., Marková, I., Pavelka, M., Fojtík, Z., Šprtová, M., Kalina, J., Marek, M.V.: Glass domes with adjustable windows: A novel technique for exposing juvenile forest stands to elevated CO_2 concentration. – Photosynthetica **39**: 395-401, 2001.

Urban, O., Marek, M.V.: Seasonal changes of selected parameters of CO_2 fixation biochemistry of Norway spruce under the long-term impact of elevated CO_2. – Photosynthetica **36**: 533-545, 1999.

Walters, M.B., Reich, P.B.: Are shade tolerance, survival, and growth linked? Low light and nitrogen effects on hardwood seedlings. – Ecology **77**: 841-853, 1996.

Woodman, J.N.: Variation of net photosynthesis within the crown of a large forest grown conifer. – Photosynthetica **5**: 50-54, 1971.

Zhang, H.H., Sharifi, M.R., Nobel, P.S.: Photosynthetic characteristics of sun versus shade plants of *Encelia farinosa* as affected by photosynthetic photon flux density, intercellular CO_2 concentration, leaf water potential, and leaf temperature. – Aust. J. Plant Physiol. **22**: 833-841, 1995.