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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

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Abstract

The long-term impact of elevated CO_2 concentration on photosynthetic activity of sun-exposed (E) *versus* shaded (S) foliage was investigated in a *Picea abies* stand (age 12 years) after three years of cultivation in adjustable-lamella-domes (ALD). One ALD is supplied with either ambient air [*ca.* 350 µmol(CO_2) mol^{-1}; AC-variant) and the second with elevated CO_2 concentration [ambient plus 350 µmol(CO_2) mol^{-1}; EC-variant). The pronounced vertical profile of the photosynthetically active radiation (PAR) led to the typical differentiation of the photosynthetic apparatus between the S- and E-needles in the AC-variant estimated from the irradiance-responses of various parameters of the room temperature chlorophyll (Chl) *a* fluorescence parameters. Namely, electron transport rate (ETR), photochemical efficiency of photosystem 2, PS2 (Φ_{PS2}), irradiance-saturated values of non-photochemical quenching of minimum (SV_0) and maximum (NPQ) fluorescence levels, and photochemical fluorescence quenching (q_P) at higher irradiances were all significantly higher for E-needles as compared with the S-ones. The prolonged exposure to EC did not cause any stimulation of ETR for the E-needles but a strongly positive effect of EC on ETR was observed for the S-needles resulting in more than doubled ETR capacity in comparison with S-needles from the AC-variant. For the E-needles in EC-variant a slightly steeper reduction of the Φ_{PS2} and q_P occurred with the increasing irradiance as compared to the E-needles of AC-variant. On the contrary, the S-needles in EC variant revealed a significantly greater capacity to maintain a high Φ_{PS2} at irradiances lower than 200 µmol m^{-2} s^{-1} and to prevent the over-reduction of the PS2 reaction centres. Moreover, compared to the AC-variant the relation between SV_0 and NPQ exhibited a strong decrease (up to 72 %) of the SV_0-NPQ slope for the E-needles and an increase (up to 76 %) of this value for the S-needles. Hence the E- and S-foliage responded differently to the long-term impact of EC. Moreover, this exposure was responsible for the smoothing of the PAR utilisation vertical gradient in PS2 photochemical and non-photochemical reactions within the canopy.

Additional key words: *Picea abies*; shade/sunny needles.

Introduction

Understanding the rọle of forests in the global carbon cycling is an important question when considering the possible role of forests as a significant carbon sink (Denning *et al.* 1995, Norby *et al.* 1999). The problem is very complicated because we still do not know whether the presumed forest ecosystems' sink capacity for carbon will continue as the forest stands grow in the next future. To be able to answer these questions, special experiments must be carried out on the whole-stand scale as well as for a long-term period. The special and not frequently investigated relation of the stand to elevated CO_2 (EC) is a stand development stage named "canopy closure". The structure of forest stand canopy is responsible for differences in solar irradiance within the canopy space resul-

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Abbreviations: Chl – chlorophyll; ERD_{sat} – PAR-saturated rate of radiation energy dissipation [µmol(quantum) m^{-2} s^{-1}]; ETR – electron transport rate [µmol m^{-2} s^{-1}]; ETR_{sat} – PAR-saturated value of ETR [µmol m^{-2} s^{-1}]; NPQ – non-photochemical quenching of F_M; NPQ_{sat} – PAR-saturated NPQ; PAR – photosynthetically active radiation; q_P – photochemical quenching of chlorophyll *a* fluorescence; q_{Psat} – PAR-saturated q_P; ΔSV_0-NPQ – slope of the linear relation between SV_0 and NPQ; SV_0-NPQ – linear regression of the relations between SV_0 and NPQ; Φ_{PS2} – actual yield of chlorophyll *a* fluorescence; Φ_{PS2sat} – PAR-saturated Φ_{PS2}.

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ting in a gradual formation of the different types of foliage, simply divided into two basic classes, *i.e.*, sunny and shade foliage (Woodman 1971, Zhang *et al.* 1995). This phenomenon is of great importance especially in dense coniferous stands.

The canopy response to the long-term impact of EC can be regarded as a demonstration of two types of processes: (*1*) processes related to the different responses of the sunny/shade foliage to EC, where significant stimulation of photosynthesis and growth in the shaded leaves by EC may occur (Würth *et al.* 1998); (*2*) processes that reflect the effect of source-sink relations connected to the biomass allocation (Wolfe *et al.* 1998). The above-mentioned specificity of the whole canopy response to EC can strongly determine an occurrence of photosynthetic acclimation depression (Kramer 1981), *i.e.,* a decrease in the photosynthetic rate observed over a long-term EC-exposure. The depression of photosynthetic acclimation results from different changes in the individual steps involved in the photosynthetic chain. For example: accumulation of phosphorylated sugar intermediates and chloroplast starch (DeLucia *et al.* 1985, Sage and Reid 1994), shortage of inorganic phosphate (Stitt 1993), inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Sage *et al.* 1989), and repression of genes associated with the production of Calvin cycle enzymes (Koch 1996). Moreover, the recent studies show that the long-term influence of EC is responsible for the decreased capacity of photosynthetic electron transport (Pammenter *et al.* 1993, Marek *et al.* 1997) and for an increase in sensitivity of the assimilatory apparatus to excess of solar irradiance (Špunda *et al.* 1998).

The aim of this paper is to report an analysis of the selected chlorophyll (Chl) *a* fluorescence parameters in the sunny *versus* shade Norway spruce shoots exposed to ambient CO_2 concentration (AC) and EC for three years. The analysed shoots were developed within a dense part of the experimental stand, which was in the stage of first canopy closure formation. These conditions were responsible for the distinctive formation of sun/shade form of foliage.

Materials and methods

Plants: The long-term impact of EC on the growth of a Norway spruce stand (age 12 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 specially prepared and replanted older saplings (age at the planting date 9 years). The preparation of the saplings for re-planting was based on the repeated (twice, in two followed growing seasons) formation of root bale. These bales make the planting of sapling more easy and protect the roots. The trees for planting were selected on the bases of the same phenotype and phenology of bud break and were obtained from the natural saplings 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. Three replications of this artificial stand were used. Two stands were enclosed into the special experimental facility named adjustable-lamella-dome (ALD) and one stand was planted in the open-air. 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 simulates the stand density of 2 500 trees per ha (mean projected LAI 3.7) and the sparse one (S) represents 1 280 trees per ha with a LAI value of 1.5. The original reason for the establishment of two different densities of the stand was the idea of the possible effects of growth sink strength on the response to EC. Because the main goal of this research was an evaluation of the long-term effect of EC on really shaded and exposed shoots, only the dense parts of the experimental stands were investigated.

Two adjustable-lamella-domes (ALD) were used for the experimental spruce stand long-term cultivation. The dimensions of ALD were 9×9×7 m. One ALD contained AC [*ca.* 350 $\mu mol(CO_2)\ mol^{-1}$] while the second ALD was permanently supplied with AC plus 350 $\mu mol(CO_2)\ mol^{-1}$ (EC-variant). Each ALD contained 56 Norway spruce individuals planted on 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 ALDs were anchored on a concrete base (depth 0.9 m) and include a massive iron frame (approximate mass 17 000 kg) with adjustable lamella-windows. These lamella-windows are opened/closed according to the monitored internal conditions within ALD. It means required atmospheric CO_2 concentration in the EC-variant, air temperature, and soil moisture. The lamella-windows are also opened/closed on the individual walls of ALD according to the wind speed and wind direction (to exclude wind incursions into the internal ALD space). The construction of lamella-windows system significantly attributed to the reduction of CO_2 costs and to the maintenance of the acceptable internal environment within the ALDs. The source of CO_2 was a tank containing 15 000 kg of liquid carbon dioxide. Each ALD was supplied with air using four ventilators. These ventilators were connected to the special ventilation tubes preparing the homogenous air profile in ALD internal space. The construction of ALD with adjustable-lamella windows and the system of air distribution contributed to the acceptable conditions in ALD interior comparable to the outside control stand conditions and significantly reduced CO_2

costs. The control stand outside the ALDs served as a comparison for the evaluation of the "chamber" effect of ALDs. The only solar radiation reduction in ALDs was due to the iron frames. For the detailed description of the ALDs' construction and function see Urban *et al.* (2001).

Canopy PAR profiles within the exposed (sunny) and shaded crown layers were estimated using a set of laboratory-made sensors (composed of 2 photocells separated by 3 cm) based on a photocell (*BPW-21*, 400-700 nm, *ELFA AB*, Solna, Sweden). The spatial distribution of the sensors within each ALD 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*, USA). The measurements of incident and penetrating PAR 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.

Modulated Chl *a* fluorescence was measured using a portable Chl fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany). The measurement of Chl *a* fluorescence was performed on current shoots located in the upper, sunny (exposed), and in the lower, shaded parts of the measured trees. The measured shoots were removed and then transported to the field laboratory for 30-min of pre-darkening. The base of the removed shoot was re-cut under water and then placed into a small bottle containing water. Each variant was measured under the pre-determined air temperature (20±2 °C), relative air humidity (55±5 %), and under cultivating (AC/EC) CO_2 concentration. In a study on spruce published by Špunda *et al.* (1998), 30 min of darkness was adequate for adaptation of Norway spruce needles before the Chl fluorescence measurement.

The rate of regulated thermal energy dissipation (ERD) was determined at saturating PAR. The PAR relation of electron transport rate (ETR), actual photon yield of PS2 (Φ_{PS2}), photochemical (q_P) and non-photochemical quenching of F_M (NPQ), and F_0 (SV_0), were obtained under an interval of the pre-defined individual PAR levels. An external halogen lamp provided these levels, which is the part of the used fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany). The measurements of PAR relations were carried out at the steady state conditions after 15 min of acclimation to each PAR. The F_0' value was determined after the re-oxidation of PS2 during 5 s of the far-red radiation treatment.

The ETR and Φ_{PS2} were calculated as follows: ETR = $\Delta F/F_{M'}$ 0.5 PAR α; $\Phi_{PS2} = \Delta F/F_{M'}$ (Genty *et al.* 1989). The absorption coefficient $\alpha = 0.87$ was estimated especially for Norway spruce using a spectrophotometer (*LI-1800*, *LI-COR*, USA). The ERD was measured at saturating PAR (1 200 μmol m^{-2} s^{-1}) and was determined as: $[(F_V/F_M - F'_V/F'_M)\ PAR_{sat}]$ (Demmig-Adams *et al.* 1996). The q_P was obtained according to Bilger and Schreiber (1986) as: $q_P = (F'_M - F'_S)/(F'_M - F'_0)$. NPQ and SV_0 were calculated using Stern-Volmer formalism (Härtel and Lokstein 1995) as $NPQ = F_M/F'_M - 1$ and $SV_0 = F_0/F'_0 - 1$, respectively.

Statistics: Each treatment (AC, EC, E, S) was represented by a sample of 10 individual trees. Two shoots from the investigated crown layer (sunny/shaded) on each sampled tree were measured The statistical significance of the differences between the S- and E-needles was tested on the basis of the *F*- and *t*-tests of mean values, respectively. The zero assumption was the equality of the mean values. The analysis was carried out with an analytical tool of the *Statistica* (*Statprof*, Czech Republic) programme package. The correlation coefficient (*r*) of a linear regression analysis was used to determine the solution acceptability.

Results

The real shade conditions were obtained only in the dense parts of the experimental stand enclosed in the adjustable-lamella domes (ALD). Thus, the considerations on the differences between E/S-needles under the long-term EC are based only on results obtained in these parts of the stands. After three years of cultivation in the ALDs, the radiation conditions of shaded crown layers were strictly different from those occurring in the sun-exposed ones in both CO_2 cultivation regimes. The observed radiation conditions are presented as an example for a sunny summer day, July 28th, 1999 (Fig. 1*A*,*B*). The PAR receipt of S-shoots amounted to 10 and 11 % of the E-shoots receipt in the AC and EC variants, respectively.

The exposed needles differed from the shaded ones in relation between electron transport rate (ETR) and PAR (Fig. 2*A*,*B*) and the irradiance-saturated values of ETR (ETR_{sat}) varied in the investigated foliage types (Table 1). For both AC and EC variants the shaded conditions were responsible for a significant decrease ($p = 0.01$) of ETR_{sat} for the S- compared to the E-needles. However, a significantly reduced difference in ETR_{sat} between S- and E-needles was observed for the EC variant. Whereas for the AC variant the ETR_{sat} was by 71 % lower for the S-needles as compared to the E-ones, for the EC variant the shaded conditions resulted in only 40 % reduction of the ETR_{sat}. The effect of long-term exposure to EC on the ETR was not evident for the E-needles (Fig. 2*A*,*B*). On the contrary, for the S-needles the ETR at higher irradiances was significantly stimulated in the EC variant (Fig. 2*A*,*B*) and ETR_{sat} was by 110 % higher as compared to the AC-one.

Φ_{PS2} was not significantly affected by the long-term cultivation under EC (Fig. 2C,*D*). For S-needles the initial decrease of Φ_{PS2} values at low PAR was steeper and PAR-saturated Φ_{PS2} was lower compared to the E-needles (Table 1). The vertical gradient of Φ_{PS2} within the canopy, which is probably the result of vertical differentiation of PAR after the canopy closure, was more distinct in the AC variant. EC was responsible for smoothing of this gradient.

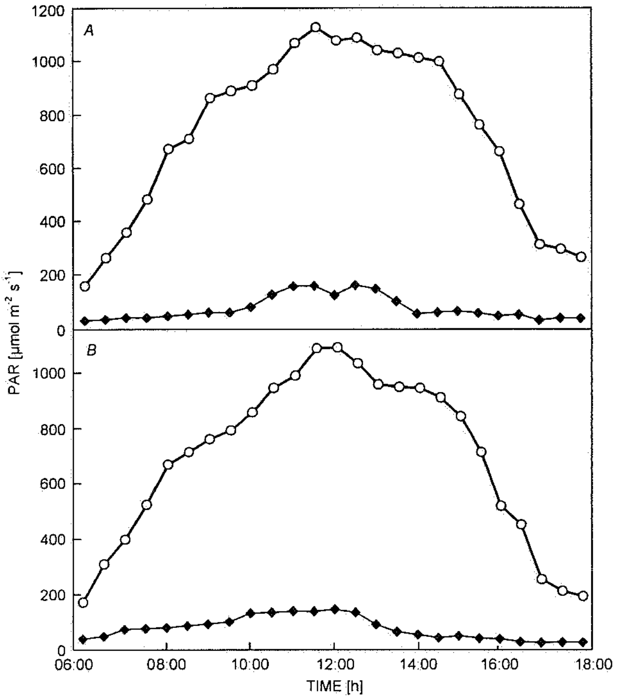

Fig. 1. Daily course of photosynthetically active radiation (PAR) during a sunny summer day (28 July, 1999) taken as an example, in ambient (*A*) and elevated CO_2 (*B*) adjustable-lamella-domes. *Open circles* – incident PAR at the level of sunny (exposed) foliage; *closed diamonds* – PAR at the level of shaded foliage in the dense part of the stand.

The values of PAR-saturated rate of regulated thermal energy dissipation (ERD_{sat}) were not significantly different between E- and S-needles in the EC variant (Table 1). The significantly higher ($p = 0.01$) value of ERD_{sat} up to 1.4 of that in the S-needles was obtained for the E-needles of AC variant only. However, for S-needles the ERD_{sat} values differed significantly ($p = 0.01$) between the AC and EC variants.

The overall shape of relationship between q_P and PAR was the same for both CO_2 variants and investigated types of shoots (Fig. 3*A*,*B*). The ability to maintain the PS2 reaction centres open, that was estimated from the q_P dependence on irradiance, was similar for the E-needles in both AC and EC variants (Fig. 3*A*,*B*). Only the trend line indicated that q_P was slightly more sensitive to the high irradiances in the EC variant as compared to the AC one. The S-needles in both variants were characterised by steeper decrease of q_P upon increasing irradiance as compared to the E-needles, but a significant difference was observed between EC and AC variants. The q_P values below 0.4 indicating over-reduction of PS2 were reached only for the S-needles of AC variant at irradiances exceeding 500 μmol m^{-2} s^{-1}. The shaded needles in the EC variant exhibited lower values of q_P in comparison with the EC-needles, but the over-reduction state was not induced within the whole range of applied irradiances.

The dependencies of NPQ on irradiance indicated a typical sun/shade foliage differentiation for the AC variant only (Fig. 3*D*). The NPQ-PAR relation in the S-needles of AC-variant revealed a steep increase of NPQ at low irradiances, whereas an initial slope of this dependence was significantly lower for E-needles (Fig. 3*C*,*D*). On the contrary, at high irradiances the S-needles exhibited lower NPQ as compared with the E-ones, indicating a reduced capacity of non-radiative dissipative processes under the shaded conditions. The irradiance-saturated NPQ (NPQ_{sat}) values of the E-needles were 1.6 times that of the S-needles in the AC variant. EC was responsible for the higher values of NPQ_{sat} in E-needles compared to the AC variant (Fig. 3*D*). For the S-needles

Table 1. Selected parameters of chlorophyll *a* fluorescence. ETR_{sat} – PAR-saturated value of ETR [μmol m^{-2} s^{-1}]; q_{Psat} – PAR-saturated value of q_P [relative]; Φ_{PS2sat} – PAR-saturated value of Φ_{PS2} [relative]; NPQ_{sat} – PAR-saturated NPQ [relative]; $\Delta SV_0/NPQ$ – slope of the linear relation between SV_0 and NPQ; ERD_{sat} – PAR-saturated rate of radiation energy dissipation PAR [μmol(quantum) m^{-2} s^{-1}]. *Identical letters* – level of significance at 95 %. Only values in the same rows are compared. N – number of investigated leaves; AC-E/AC-S – exposed/shaded needles from dense part of the stand in ambient CO_2 adjustable-lamella-dome; EC-E/EC-S – exposed/shaded needles from dense part of the stand in elevated CO_2 adjustable-lamella-dome. Means ± SE of 20 determinations.

Parameter	Variant AC-E	AC-S	EC-E	EC-S
ETR_{sat}	120.7±11.2 a	38.8±9.6 a,c	124.4±11.1 b	73.8±8.4 b,c
Φ_{PS2sat}	0.399±0.044 d	0.046±0.019 d,f	0.323±0.061 e	0.064±0.041 e,f
q_{Psat}	0.753±0.035 g	0.319±0.068 g,i	0.718±0.038 h	0.469±0.045 h,i
NPQ_{sat}	3.39±0.84 j	2.15±0.70 j	3.86±0.38 k	2.87±0.51 k
ERD_{sat}	566.2±49.4 l	399.4±31.4 l,m	536±32.8	517.1±32.2 m
$\Delta SV_0/NPQ$	0.129	0.086	0.093	0.152
r	0.91*	0.90*	0.89*	0.95*

approximately the same NPQ_{sat} values were estimated for the EC and AC variants. However, the characteristics of the NPQ-PAR relation typical for the shaded foliage disappeared for the samples collected from the trees cultivated at EC and an initial slope of this relation was the lowest among all the measured variants.

The presented relations between SV_0 and NPQ revealed a linear character for all investigated variants (Fig. 4*A,B*). The E-needles in the AC variant exhibited a higher slope of SV_0-NPQ relation (ΔSV_0/NPQ), *i.e.*, 1.47 times that of the S-needles (Table 1). Compared to the AC variant, the long-term cultivation under EC caused a remarkable decrease (up to 78 %) of the ΔSV_0/NPQ for the E-needles and an increase (up to 76 %) of this value for the S-needles. Surprisingly, the slope of SV_0-NPQ relation was steeper for the S-needles than for the E-ones in the EC variant.

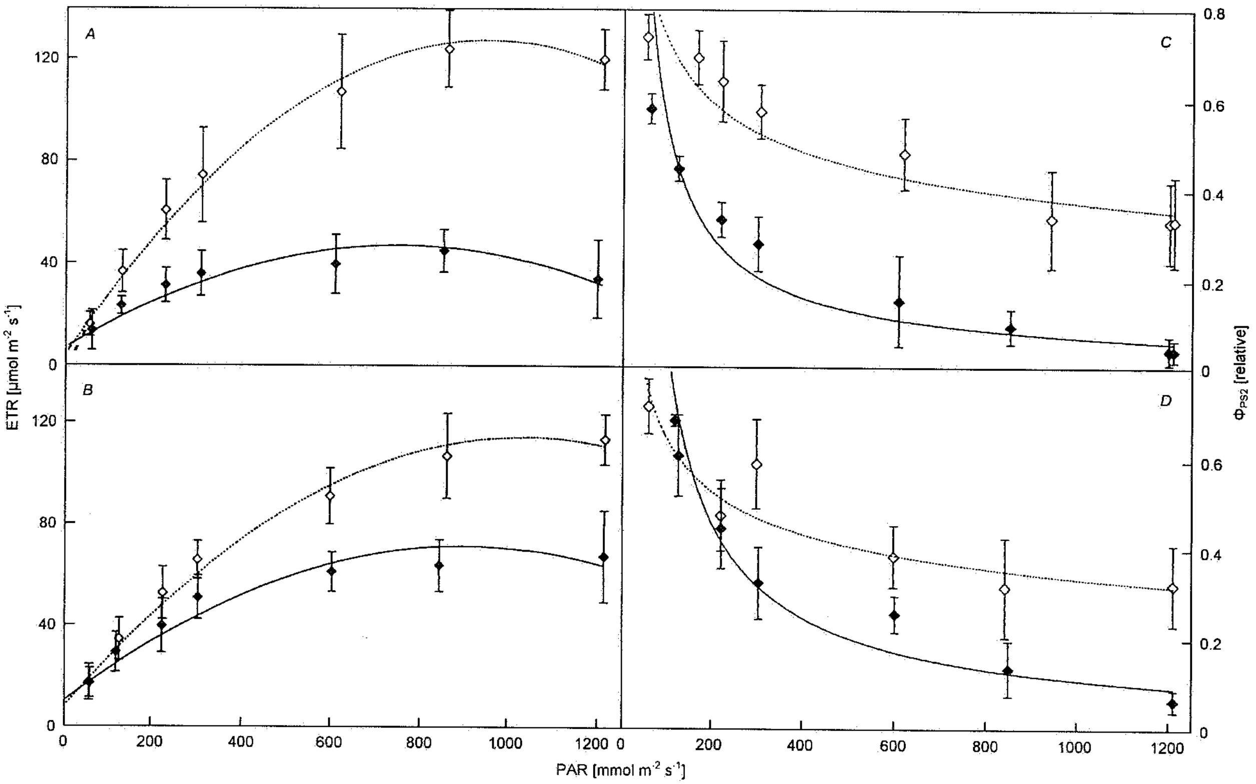

Fig. 2. Irradiance response of the electron transport rate (ETR) (A, B) and the actual yield of chlorophyll *a* fluorescence, Φ_{PS2} (C, D) in exposed and shaded needles from ambient, AC (A, C) and elevated, EC (B, D) CO_2 variants. The trend fitted lines represent the relationship. *Open diamonds and dotted line*: exposed needles; *closed diamonds and full line*: shaded needles. No. of exposed/shaded shoots in AC/EC variant: 20. *Vertical bars*: standard error.

Discussion

Three years of cultivation of the Norway spruce in the experimental stand in ALD were responsible for a significant differentiation of PAR conditions between E- and S-foliage of the investigated parts of the stands. Under the presented sunny summer day the exposure time of S-needles to PAR of 100 μmol m^{-2} s^{-1} took only 3 h of the light part of the day (Fig. 1*A,B*). Thus, the canopy closure, which was already achieved in the dense parts of the experimental stands in the AC/EC ALDs, was responsible for the formation of some real shaded conditions.

The acclimation to shade was reflected by the ETR-PAR relation especially under high PAR, as was confirmed by the trend lines fitted to values (Fig. 2*A,B*). A typical degree of reduction of PAR utilisation in ETR was observed for the shaded needles of the AC-grown spruces. The significantly (by 70 %) lower ETR_{sat} values indicate some limitations of electron transport kinetics, a lesion in PS2 (Larkum and Wood 1993) and/or a change in the capacity of the dissipation processes of excitation energy (Bornman 1989). The prolonged exposure to EC did not cause any stimulation of ETR for the E-needles (Fig. 2*B*). As shown recently, an exposure of Norway spruce to EC is accompanied with initial stimulation of the ETR, but this stimulation may disappear within several months of prolonged CO_2 treatment (Kalina *et al.* 2000). On the contrary, a strongly stimulating effect of

EC on ETR was observed for the S-needles. The more than doubled ETR_{sat} in the S-needles of EC variant compared to the S-needles from AC variant indicated a permanent stimulation of photosynthesis in the shaded foliage even after three years of EC impact on the tested spruce stand.

The high ETR_{sat} values obtained in the S-needles of the EC- compared to the AC-variant suggest an effect of some important sink strength (Stitt 1991, Long and Drake 1992, Wolfe *et al.* 1998) probably caused by growth competition between individuals and branches. This phenomenon is strongly connected to the PAR limitation. EC could compensate the PAR limitation of photosynthesis by a stimulation of assimilatory activity of the shaded needles (Lawlor and Mitchell 1991).

Thus, we suggest that for the trees grown under EC the selective stimulation of photosynthetic activity of the S-foliage should disturb the differentiation of the Chl *a* fluorescence characteristics typically observed between the S- and E-needles. In agreement with the above-mentioned results of ETR, a clear differentiation of the dependencies of Φ_{PS2} and q_P was observed between the S- and E-needles of the AC variant (Figs. 2 and 3). The influence of growth under EC was of opposite direction for the E- and S-needles, respectively. For the E-needles a slightly steeper reduction of both Φ_{PS2} and q_P occurred with the increasing irradiance as compared to the E-needles of AC-variant. This can be interpreted as a manifestation of moderate acclimation depression of photosynthetic activity of E-needles, resembling that observed before for the Norway spruce individuals closed in the open-top chambers for the same cultivation period at EC (Kalina *et al.* 1997). On the contrary, the S-needles of the EC variant revealed a significantly greater capacity to maintain a high PS2 photochemical efficiency at lower irradiances (up to 200 $\mu\text{mol m}^{-2}\text{ s}^{-1}$) and to prevent the over-reduction of the PS2 reaction centres (Fig. 3). This result is closely related to the stimulated capacity of PAR utilisation in photosynthetic reactions exceeding that usually observed for the strongly shaded needles.

The light-induced NPQ reflects the concentration of quenching complexes using Stern-Volmer formalism (Gilmore *et al.* 1995) and represents the relative efficiency of non-radiative dissipation (Demmig-Adams and Adams 1996, Brugnoli *et al.* 1998). The NPQ is probably correlated with the content of de-epoxidised components of the xanthophyll cycle (Demmig-Adams and Adams 1996, Brugnoli *et al.* 1998). The NPQ_{sat} obtained at irradiances saturating the F_M quenching were significantly higher in the E-needles than in the S-ones of the both CO_2 treatments (Fig. 3). This observation confirmed the photoprotective role of radiant energy dissipation for the E-

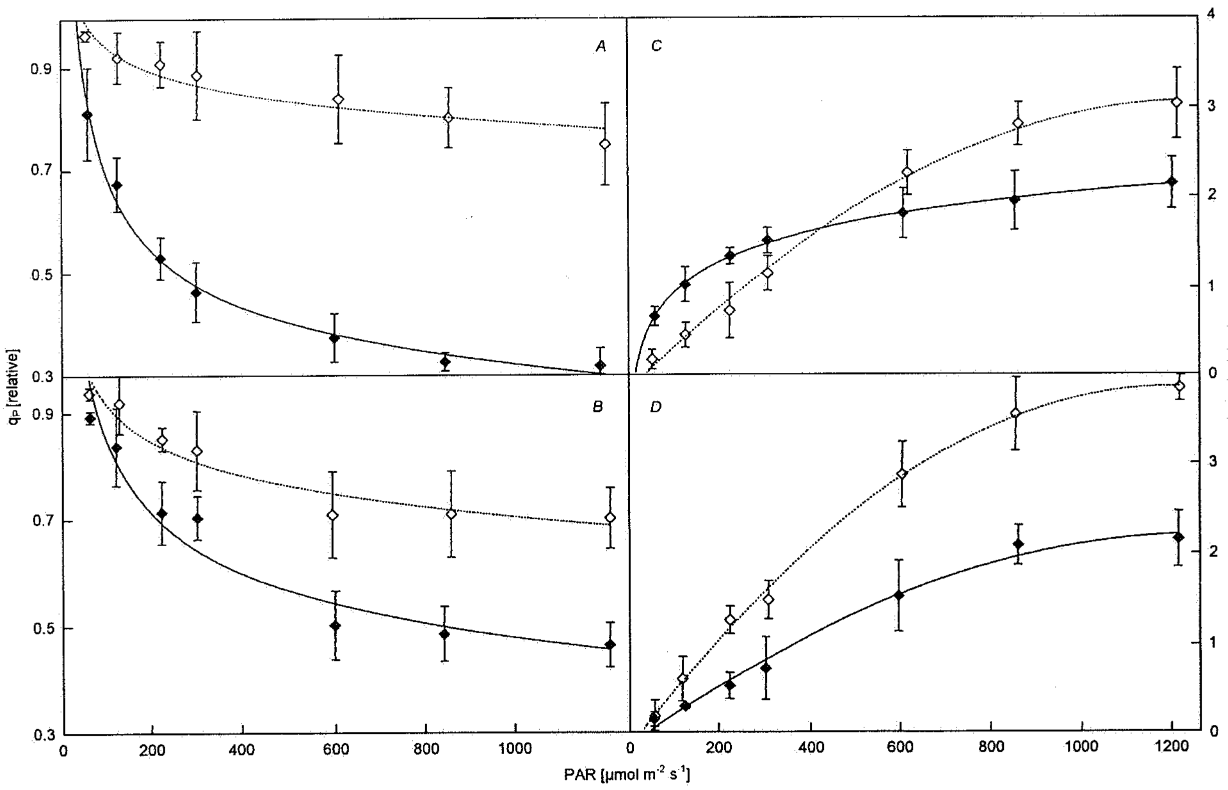

Fig. 3. Irradiance response of the photochemical (q_P) and non-photochemical quenching (NPQ) of chlorophyll *a* fluorescence in exposed and shaded needles from ambient, AC (*A*, *C*) and elevated, EC (*B*, *D*) CO_2 variants. The *trend fitted lines* represent the relationship. *Open diamonds and dotted line*: exposed needles; *closed diamonds and full line*: shaded needles. No. of exposed/shaded needles in AC/EC variant: 20. *Vertical bars*: standard error.

needles eliminating an excess of the absorbed radiant energy to the needs of photosynthesis as shown by Špunda *et al.* (1998). The increased capacity of non-radiative energy dissipation belongs to the main manifestations of the adaptation of numerous C_3 species to high irradiances (Demmig-Adams and Adams 1996, Brugnoli *et al.* 1998, Logan *et al.* 1998). The dependencies of NPQ on irradiance revealed differences typical between the S- and E-needles only for the samples taken from AC trees. The S-needles of AC variant exhibited higher values of NPQ under lower PAR compared to the E-needles (Fig. 3*B*,*D*). The enhanced induction of NPQ already at low irradiances mitigates the over-reduction of PS2 reaction centres resulting from low capacity of radiant energy utilisation in photochemical reactions (Špunda *et al.* 1998). However, the non-radiative energy dissipation was insufficient to prevent an increase of the PS2 reduction state, expressed as $1 - q_p$ already at low PAR ($1 - q_p$ can be obtained from Fig. 3). Hence, the significantly lower Φ_{PS2} of the S-needles in AC variant represented not only the increased heat dissipation, but also an increased PS2 reduction state. The achievement of the PS2 over-reduction is documented by the critical values of q_P up to 0.4 (Demmig and Winter 1988). This value of q_P was reached for the S-needles in the AC variant at irradiances exceeding 500 μmol m^{-2} s^{-1}. Both the steeper increase of NPQ and higher NPQ_{sat} values for the E-needles of EC variant as compared to the AC-one supported importance of the radiant energy dissipation as a signal of acclimation depression attack on the light reactions of photosynthesis (Fig. 3 *B*,*D*).

In the S-needles the stimulating effect of EC on NPQ was not observed (Fig. 3*B*,*D*). Contrary to the stimulation of ETR values within the whole interval of used PAR (Fig. 2*A*,*B*), NPQ was significantly reduced over the range of low and medium irradiances in comparison to S-needles in the AC variant. The NPQ reflects the quenching processes localised both within the light-harvesting complexes (LHC) and the core of PS2 (Gilmore *et al.* 1995, Färber *et al.* 1997, Čajánek *et al.* 1999). The extent of energy dissipation in LHC has been related selectively to the non-radiative quenching of F_0 characterised by the value of SV_0 (Demmig-Adams and Adams 1994, 1996). The linear relation between SV_0 and NPQ (Fig. 4*A*,*B*) provides the slope of $\Delta SV_0/NPQ$, which is a qualitative indicator of the contribution of quenching localised within LHC to the total non-radiative dissipation. The higher value of $\Delta SV_0/NPQ$ indicates an increased efficiency of the non-radiative energy dissipation in LHC connected either to the direct quenching of excited Chl *a* by zeaxanthin or to the zeaxanthin-mediated structural changes of LHC (Adams *et al.* 1990, Ruban *et al.* 1994). This higher extent of SV_0 was found for E-needles of the AC variant, which is in accordance with results of Špunda *et al.* (1998) obtained for the needles from sunny parts of the Norway spruce canopy. The most striking differentiation of the EC effects on E- and S-needles was found for the relations between SV_0 and NPQ (Fig. 4). The long-term influence of EC was responsible for the substantial decrease of $\Delta SV_0/NPQ$ for the E-needles but for the S-needles almost twofold $\Delta SV_0/NPQ$ was estimated in comparison with the S-needles of AC variant. These changes could be hardly explained assuming a typical manifestation of the adaptation to excess irradiance, which induced an increased capacity of the non-radiative dissipation within LHC. Rather a direct effect of EC should be considered. It is difficult to say if EC directly influenced the extent of zeaxanthin-mediated energy dissipation in opposite manner in the S- and E-needles, as the de-epoxidation state of the xanthophyll cycle pigments was not measured during this experiment. However, we found recently that the short-term exposure of barley to saturating CO_2 concentration selectively affected SV_0 and convertibility of violaxanthin to zeaxanthin at high irradiances, but did not alter the NPQ (Kalina et al., unpublished). We are sure that a detailed analysis of this phenomenon is needed. Nevertheless, the long-term EC exposure completely disturbed the differentiation of SV_0 quenching typically found between shaded and exposed needles.

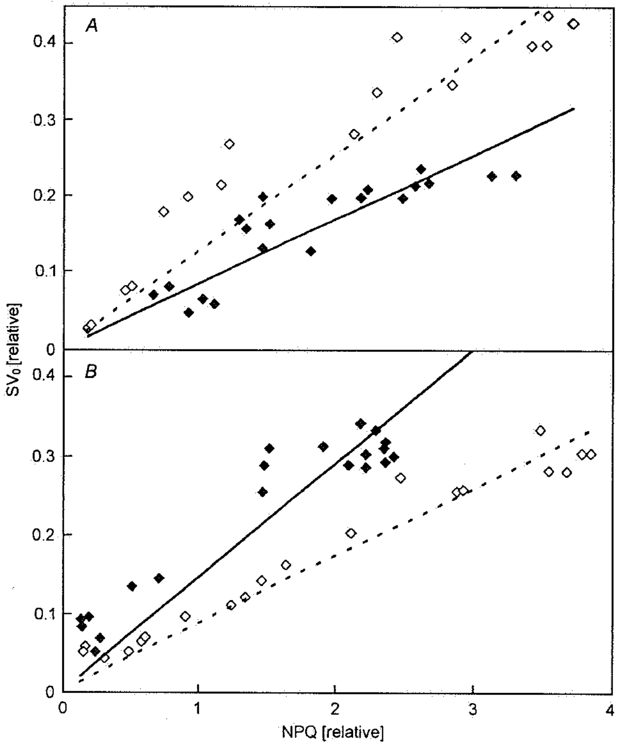

Fig. 4. Linear regression of the relations between the non-photochemical quenching of minimum (SV_0) and maximum chlorophyll *a* fluorescence (NPQ) in exposed and shaded needles from ambient (*A*) and elevated (*B*) CO_2 variants. *Open diamonds and dotted line*: exposed needles; *closed diamonds and full line*: shaded needles.

Our results indicate the canopy-position-dependent reactions of characteristics of Chl *a* fluorescence in Norway spruce needles to the long-term impact of EC. For the exposed needles no sign of stimulation of capacity of the PS2 photochemical reactions was found. Instead of that a moderate down-regulation depression of photosynthetic reactions was observed from the reduced ability to maintain an oxidised state of the PS2 reaction centres at higher irradiances. On the contrary, a strong stimulation of the ETR capacity was estimated for the shaded needles. This stimulation led to the decrease or even cessation of the typical differentiation between the S- and E-needles under prolonged exposure to EC regarding all the measured dependencies of the Chl *a* fluorescence parameters on irradiance. The EC could compensate the PAR limitation of photosynthesis by a stimulation of the shaded needles assimilatory activity (Lawlor and Mitchell 1991). Thus, the assimilation activity of the stand canopy S-layers can be important as the long-term active target of the stimulative actions of EC. Moreover, our results supported the idea of the importance of the vertical differentiation of the assimilatory ability of leaves within the stand canopy. This differentiation must be regarded as a serious phenomenon of the whole-stand response to the long-term impact of EC.

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