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Control mechanisms of photosynthetic capacity under elevated CO_2 concentration: evidence from three experiments with Norway spruce trees

O. URBAN[*,+], R. POKORNÝ[*], J. KALINA[**,***], and M.V. MAREK[*,***]

Laboratory of Ecological Physiology of Forest Trees, Institute of Landscape Ecology,
Academy of Sciences of the Czech Republic, Poříčí 3b, CZ-603 00 Brno, Czech Republic[*]
Ostrava University, Faculty of Science, Department of Physics, 30. dubna 22, CZ-701 03 Ostrava, Czech Republic[**]
National Research Centrum "Mechanisms, Ecophysiology and Biotechnology of Photosynthesis",
Zámek 136, CZ-373 33 Nové Hrady, Czech Republic[***]

Abstract

Twelve-year-old Norway spruce (*Picea abies* [L.] Karst.) were exposed to ambient (AC) or elevated (EC) [ambient + 350 µmol(CO_2) mol^{-1}] CO_2 concentration [CO_2] using the facilities of open-top-chambers (OTCs) and glass domes (GDs). A combination of gas exchange measurements and application of a biochemical model of photosynthesis were used for the evaluation of CO_2 assimilation characteristics. Morphological change was assessed on the base of specific leaf area (SLA). Nitrogen (N) content in the assimilation apparatus was considered a main factor influencing the biochemical capacity. Three experiments confirm the hypothesis that an adjustment of photosynthetic capacity under EC is controlled by the combination of biochemical, morphological, and physiological feedback mechanisms. We observed periodicity of down-regulation of photosynthetic capacity (Experiment No. *1*) during the vegetation seasons. In the spring months (May–June), *i.e.* during the occurrence of active carbon sink associated with the formation of new foliage, up-regulation (10–35 %) of photosynthetic capacity (P_{Nsat}) was observed. On the contrary, in the autumn months (September–October) down-regulation (25-35 %) of P_{Nsat} was recorded that was mainly associated with reduced carbon sink strength and biochemical change, *i.e.* decrease of N status (up to 32 %) and accumulation of saccharides (up to 72 %) in leaves. Different adjustments of photosynthetic activities were observed in current (C) and one-year-old (C-1) needles exposed to EC (Experiment No. *2*). Strong down-regulation of P_{Nsat} and the diminution of the initial stimulation of photosynthetic rate (P_{Nmax}) was associated with decreases of both ribulose-1,5-bisphosphate carboxylase/oxygenase carboxylation activity (by 32 %) and RuBP regeneration (by 40 %). This performance was tightly correlated with the absence of active carbon sinks, decrease of N content, and starch accumulation in C-1 needles. Finally, different responses of sun- and shade-adapted needles to EC (Experiment No. *3*) were associated with the balance between morphological and biochemical changes. Observed P_{Nsat} down-regulation (by 22 %) of exposed needles in EC was predominantly caused by effects of both higher assimilate accumulation and stronger N dilution, resulting from higher absolute photosynthetic rates and incident irradiances in the upper canopy.

Additional key words: down-regulation; nitrogen; *Picea abies*; sink strength; sun and shade needles.

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[+] Author for correspondence; fax: +420 543 211 560, e-mail: otmar@brno.cas.cz

Abbreviations: AC – ambient [CO_2]; [CO_2] – atmospheric CO_2 concentration; C, C-1 – current and one-year-old needles; C_i – intercellular CO_2 concentration; EC – enhanced [CO_2]; Ex, Sh – exposed and shaded needle variants; GD – glass domes with adjustable windows; I – photosynthetically active irradiance; J_{max} – maximal electron transport; OTC – open-top chamber; P_i – inorganic phosphate; $P_{N(area)}$, $P_{N(mass)}$ – net photosynthetic rate per needle area and needle dry mass, respectively; P_{Nmax}, P_{Nsat} – net photosynthetic rate at saturating irradiance and photosynthetic rate at saturating C_i and saturating irradiance, respectively; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose-1,5-bisphosphate; SLA – specific leaf area; V_{Cmax} – maximal carboxylation rate; α – apparent quantum efficiency; τ – carboxylation efficiency.

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Introduction

The role of forests in the global carbon budget and global changes of climate is extremely important because of the longevity of forest trees and the importance of forest stands to fix atmospheric carbon dioxide into biomass. The biomass production responds through the mediation of the photosynthetic, respiration, and transpiration processes to the atmospheric [CO_2] (Watson *et al.* 2000). During the last two decades, many papers describing responses of plants and terrestrial ecosystems to elevated [CO_2] (EC) have been published (for reviews, see Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Saralabai *et al.* 1997, Luo *et al.* 1999, Urban 2003). The initial assessments (Farquhar *et al.* 1980, Sage 1990) supposed up-regulation of photosynthetic rate (P_{Nmax}; maximal value of P_N to irradiance response), and down-regulation of photosynthetic capacity (P_{Nsat}; maximal value of P_N to intercellular CO_2 response).

Wide range of experimental data in 80's and 90's (reviewed by Eamus and Jarvis 1989, Sage *et al.* 1989, Ceulemans and Mousseau 1994, Ceulemans 1997, Luo *et al.* 1999) confirmed increase of P_{Nmax} but rejected the speculation that P_{Nsat} is always decreased. The degree of photosynthetic response to EC depends on species, growing conditions, mineral nutrition status, time duration of CO_2 enrichment, and/or synergetic influence of other stresses. The primary stimulation (short-term effect) of CO_2 enrichment is mediated by increase in CO_2 diffusion gradient and reduction in the oxygenase component of RuBPCO, *i.e.* suppression of photorespiration.

Both the down-regulation of P_{Nsat} and relatively lower stimulation of P_{Nmax} after long-term influence of EC may be explained by (*1*) a decrease of RuBPCO amount and/or activity (Sage *et al.* 1989), (*2*) dilution/redistribution of nitrogen and phosphorus mineral status of assimilatory apparatus (Marek *et al.* 1995, Stitt and Krapp 1999), (*3*) starch accumulation potentially connected with the chloroplast disruption and intercellular shading (DeLucia *et al.* 1985), (*4*) decrease of content of photosynthetically active pigments (chlorophylls and carotenoids) and diminution of light-harvesting complexes (Wullschleger *et al.* 1992, Špunda *et al.* 1998), (*5*) inhibition of photosynthetic genes (*e.g.* mRNA, RuBPCO, carbonic anhydrase) by end-product (hexoses; reviewed by Webber *et al.* 1994), and (*6*) differences in the new sinks-source status of the plant (Wolfe *et al.* 1998).

Following these alternative hypotheses, Luo *et al.* (1994) proposed a general model for responses of photosynthesis to EC to generalise inconsistent results. The model reflects a combination of adjustments in biochemical capacity and changes in leaf morphology on the leaf-level CO_2 assimilation. Changes in biochemistry include CO_2 fixation by RuBPCO, RuBP regeneration, and P_i regeneration (Farquhar *et al.* 1980, Sage 1990, Webber *et al.* 1994). Morphological changes involve assimilate accumulation, increases in leaf thickness and mesophyll cell number per unit of leaf area (Vu *et al.* 1989), or new additional sink formation on whole plant level (Ceulemans 1997, Wolfe *et al.* 1998). Treatment in EC leads to increased carboxylation efficiency and subsequently to greater production of saccharides that have three possible fates: (*1*) Translocation out of leaves (increase of root/shoot ratio). (*2*) Biochemical (ΔB) down-regulation (storing of saccharides as starch and/or sugars in leaves). (*3*) Morphological (ΔM) up-regulation, *i.e.* utilisation of saccharides in leaf structural growth (Luo *et al.* 1994).

According to the proposed model, increased photosynthetic capacity (P_{Nsat}) is maintained when the additional leaf mesophyll grows (sink strength) more than it is compensated by biochemical changes (especially nitrogen dilution), *i.e.* $\Delta M > \Delta B$. Both, modellers and experimentalists, were urged to design experiments to examine this holistic concept (Luo *et al.* 1999).

A major objective of this study is to determine whether down-regulation of P_{Nsat} in field-grown Norway spruce (*Picea abies* [L.] Karst.) trees in EC can be explained by the above mentioned conceptual model (Luo *et al.* 1994). Here we assess the results of three experiments supporting the model's assumption that a balance between biochemical and morphological adjustments controls photosynthetic capacity.

Materials and methods

All experiments were carried out at the experimental research site Bílý Kříž in the Beskydy Mts. (Czech Republic, 49°30'N, 18°32'E, 908 m a.s.l.). A detailed description of the experimental locality was given by Urban *et al.* (2001). In Experiment No. *1*, eight individual Norway spruce (*Picea abies* [L.] Karst.) trees (age 12 years, average height 2.5 m) were treated in open-top-chambers (OTCs; Janouš *et al.* 1996) supplied with ambient or ambient plus 350 µmol(CO_2) mol^{-1} from spring 1992 till autumn 1995. All analyses presented here were done on one-year-old intact shoots situated in the upper third of the south-western part of the crown during three subsequent growing seasons 1993, 1994, and 1995. In Experiments No. *2* and *3*, small populations of Norway spruce were grown in semi-open glass domes (GDs) with adjustable windows (Urban *et al.* 2001) so that there were mutual interactions (leaf area index *ca.* 1.7) between individual trees (age 12, average height 1.8 m). The domes contain an ambient (treatment AC) or ambient plus 350 µmol(CO_2) mol^{-1} (treatment EC) atmosphere.

The trees (56 in each treatment) were exposed to AC or EC from spring 1997 till autumn 2001, except during the winter dormancy period. Current (C) and one-year-old (C-1) shoots from the middle of the S/W part of the tree crown were used in Experiment No. *2*. Gas exchange measurements were carried out at the beginning of September when specific leaf area of current needles was fully developed (Urban *et al.* 2000).

An open portable gas-exchange system with infrared gas analyser *CIRAS-1* (*PP-Systems*, U.K.) was used for measurements of P_N/C_i and P_N/I responses. Photosynthetic capacity (P_{Nsat}) was estimated at saturating I (1 300 μmol m^{-2} s^{-1}) and saturating [CO$_2$] [1 500 μmol(CO$_2$) mol^{-1}]. Maximal photosynthetic rate (P_{Nmax}) was estimated at saturating irradiance (1 300 μmol m^{-2} s^{-1}) and cultivation [CO$_2$], *i.e.* 350 or 700 μmol(CO$_2$) mol^{-1}. Relatively constant microclimatic conditions were kept inside the assimilation chamber during all the measurements (needle temperature 20±2 °C, relative air humidity 55±3 %). Following a biochemical based model of photosynthesis (Farquhar *et al.* 1980), maximal rates of carboxylation (V_{Cmax}) and electron transport (J_{max}) were estimated (see Urban and Marek 1999 for details).

Specific leaf area (SLA; the ratio of projected fresh leaf area to its dry mass) considered as useful measure of leaf structure, thickness, and amount of mechanical tissues in leaves (*e.g.* Gilmore *et al.* 1995) was used for the estimation of morphological changes. Portable Area Meter *LI-3000A* (*LI-COR*, USA) was used to estimate one-side projected fresh needle area. Needles were oven-dried at 80 °C for 48 h, and their masses determined by analytical balance *Sartorius* (Japan). Nitrogen content was determined on the identical needles using a method of dry combustion at 1 100 °C and measured with an auto-analyser *CNS-2000* (*LECO*, USA).

Because of relatively small data sets (number of replications up to 12), normal distributions of the data were assumed. The statistical significance of differences between the treatments was based on the F- and *t*-tests of the mean values. The analysis was carried out using the analytical tools in the *EXCEL* programme package.

Results

Experiment No. *1* – Seasonal periodicity of down-regulation: In spring (May–June) months, up-regulation by 10-35 % of P_{Nsat} and stimulation of P_{Nmax} up to 130 % (Fig. 1*A,B*) owing to inhibited photorespiration by 45 % (Urban and Marek 1999) were observed. At this time of the vegetation season, spruce trees are characterised by a rapid growth and formation of new foliage, as demonstrated by an increase of leaf area index by *ca.* 20 %, that represents a strong active carbon sink. However, down-regulation by 25-35 % of P_{Nsat} in the autumn months (September–October) was recorded, as well as relatively lower stimulation of P_{Nmax} by EC (Marek *et al.* 1997) in three subsequent seasons (Fig. 1*A,B*).

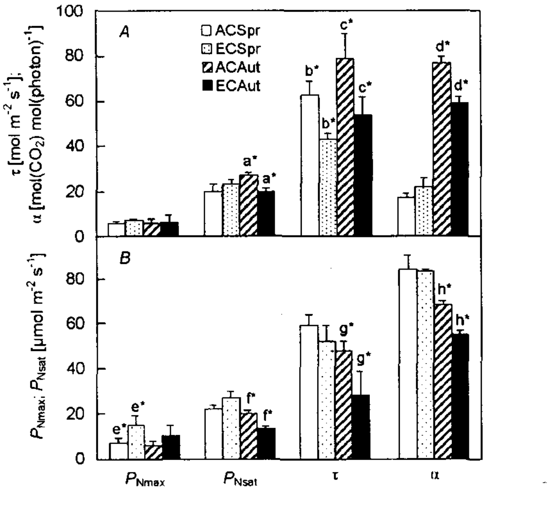

Fig. 1*A,B*. Acclimation of the maximal photosynthetic rate (P_{Nmax}), photosynthetic capacity (P_{Nsat}), carboxylation efficiency (τ), and apparent quantum efficiency (α) to elevated [CO$_2$]. The data were collected at the beginnings (May; Spr) and ends (October; Aut) of vegetation seasons 1993 (*A*) and 1995 (*B*). Ambient (elevated) [CO$_2$] – AC (EC) variants. *Columns* represent mean values, *bars* represent standard deviations. *Letters* (p<0.05) and *asterisks* (p<0.01) denote statistical differences; n = 12.

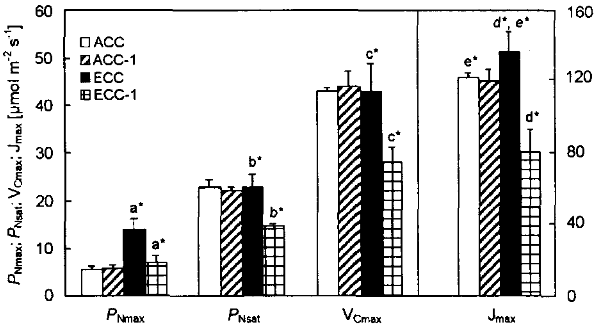

Fig. 2. Means (*columns*) and standard deviations (*bars*) of the maximal net photosynthetic rate (P_{Nmax}), photosynthetic capacity (P_{Nsat}), maximal carboxylation rate (V_{Cmax}), and maximal electron transport rate (J_{max}) in current (C) and one-year-old (C-1) needles exposed to ambient (AC) and elevated (EC) CO$_2$ concentration. *Letters* (p<0.05) and *asterisks* (p<0.01) denote statistical differences; n = 7.

Experiment No. *2* – Down-regulation relates to needle age: We observed stimulation of the photosynthetic rate in the current needles (100 %; P_{Nmax}) and no effect on P_{Nsat} by the EC treatment (Fig. 2). Nevertheless, C and C-1 needles growing in AC did not show any difference in P_{Nmax} and P_{Nsat}. In contrast, we observed strong down-regulation of the P_{Nsat} and diminution of the initial positive effect on P_{Nmax} of one-year-old needles from EC. The acclimation of CO_2 assimilation processes in C-1 needles was associated with decreases of both carboxylation activity of RuBPCO (V_{Cmax}; decrease by 32 %) and maximal electron transport rate (J_{max}; decrease by 40 %).

Experiment No. 3 – Differences of exposed/shaded foliage responses: We observed that needle position in the canopy significantly affected the photosynthetic response of *Picea abies* to EC, and moreover, we have characterised the variability of this acclimation (*i.e.* down-regulation of the photosynthetic capacity) over time (Fig. 3*A,B*). While stimulation of photosynthetic capacity (P_{Nsat}) by EC for the ECEx (by 37 %) was observed, the ECSh needles showed loss of the initial stimulation or slight down-regulation (by 10 %) of P_{Nsat} after three years of CO_2 enrichment. Because the ACEx and ACSh needles have similar ($p>0.05$) photosynthetic capacity per dry mass (*ca.* 8 %; $P_{Nsat(mass)}$), anatomical structure of the ACSh needles may be considered as the main reason of decreased P_{Nmax} value (by 59 %) in the AC treatment (Fig. 3*A*). In contrast, persistent difference of $P_{Nsat(mass)}$ (by 47 %; $p>0.01$) in the EC treatment is consistent with biochemical changes (Kubiske and Pregitzer 1997).

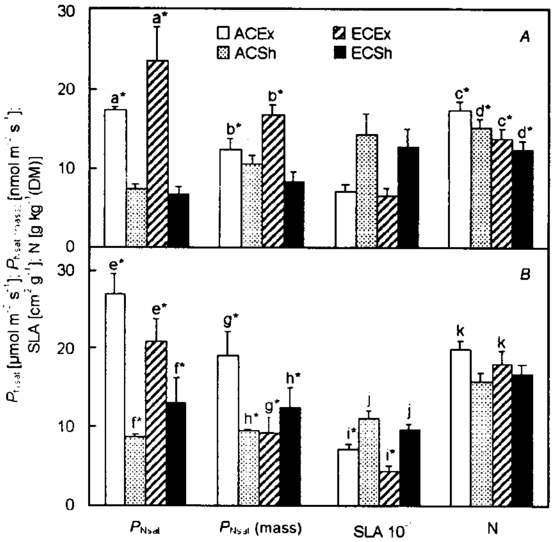

Fig. 3*A,B*. Means ± standard deviations of the photosynthetic capacity (P_{Nsat}), photosynthetic capacity per mass unit ($P_{Nsat(mass)}$), specific leaf area (SLA), and nitrogen (N) content in exposed (Ex) and shaded (Sh) needles treated in the ambient (AC) and elevated (EC) CO_2 concentration. Situations in October 1999 (*upper panel*) and October 2001 (*lower panel*). *The same letter* indicates statistically significant difference ($p<0.05$), * high statistically significant difference ($p<0.01$). Differences between the Ex and Sh needles are significant at $p<0.01$ level, except the ACEx and ACSh for the $P_{Nsat(mass)}$ value (see the text); $n = 7$.

Nevertheless, the relative changes in specific nitrogen concentration (n) and leaf mass per unit area (h = 1/SLA) at EC were calculated as 0.86 for sun leaves and 0.97 for shade-adapted needles. This result predicts future up-regulation, *e.g.* increase of photosynthetic capacity, for ECSh needles (Luo *et al.* 1994) and its down-regulation for ECEx needles in comparison with AC.

Indeed, after five years of EC treatment we observed up-regulation of P_{Nsat} by 51 % (Fig. 3*B*) and significant increase of maximal photosynthetic rate (Marek *et al.* 2002) for shade-adapted needles grown in EC (ECSh). These changes are associated with changes in N redistribution between upper and lower part of the canopy (Fig. 3*B*). While in AC a typical decline of N content in the canopy (20 % from top to bottom) was observed, a uniform N distribution (gradient of 7 %) between Ex and Sh needles was formed in EC. Generally, foliage of trees planted in EC is characterised by lower N content (10-12 %).

Because the relative changes in N concentration and leaf mass per unit area in EC were > 1 in both sun- (1.32) and shade-adapted (1.14) needles, we can predict an up-regulation of P_{Nsat} and P_{Nmax} comparing to the AC treatment.

Discussion

Experiment No. *1* – Seasonal periodicity of down-regulation: As earlier reported, identical trends of P_N/C_i and P_N/I relationships were observed during three subsequent seasons from 1993 till 1995 (Marek *et al.* 1995, 1997, Urban and Marek 1999). The P_N/C_i response curves observed after the long-term CO_2 fumigation at the end of vegetation season (Urban and Marek 1999) show that P_N was decreased over the whole C_i interval. This means that both the RuBPCO carboxylation efficiency (τ) and capacity for RuBP regeneration or apparent quantum efficiency (α) were decreased (see Fig. 1*A,B*). Limitations resulting from inhibition of photosynthetic metabolism by end-products (increase up to *ca.* 170 %) together with P_i (decrease up to *ca.* 32 %), N (decrease up to *ca.* 14 %), and decrease in RuBPCO activity (up to *ca.* 70 %) (Urban and Marek 1999) may indicate an excess of some components of the photosynthetic apparatus (Sage 1990). This allows investment of the assimilates into non-photosynthetic plant organs, *i.e.* root and stem wood production, or the formation of new alternative sinks, *e.g.* internodal branches, and thus an increase in the sink capacity (Opluštilová and Dvořák 1997). Thus, the pattern of photosynthesis acclimation supports the concept of the tight correlation of photosynthesis with the carbon sink capacity.

There is evidence that so-called "sink limited plants" are unable to use additional saccharides in the processes of respiration, growth, or carbon partitioning (Ceulemans and Mousseau 1994). On the base of the previous findings (Marek *et al.* 1995, 1997, Opluštilová and Dvořák 1997, Špunda *et al.* 1998) and the results presented here, *P. abies* may be classified as typical sink-limited species.

Similar trends of seasonal effects have been shown to influence the photosynthetic responses to EC in deciduous species (*e.g. Betula pendula*; Rey and Jarvis 1998) and other coniferous species (*e.g. Pinus ponderosa*; Tissue *et al.* 1999). Increased down-regulation of P_{Nsat} as the growing season progresses reflects (*1*) the strong seasonal effect of temperature (Overdieck *et al.* 1998) and (*2*) the inherent seasonal growth of new wood (Tissue *et al.* 1999).

Experiment No. *2* **– Down-regulation relates to needle age**: Older needles grown in AC are characterised by the absence of carbon sinks resulting from the lack of an active basal meristem and prior starch accumulation (Šesták 1981, Gilmore *et al.* 1995). The different degree of the activity in C and C-1 needles could be demonstrated by the differences in seasonal dynamics (morphological change) of specific leaf area (SLA). We noticed strong decrease of SLA by 56 % from July to September for C needles, while one-year-old needles manifested practically constant SLA in this period (Urban *et al.* 2000). Moreover, Loach and Little (1973) presented a hypothesis of decrease in SLA with age resulting from the absence of active carbon sinks. The needle area remains almost constant and accumulation of saccharides leads to limitation of photosynthetic carbon fixation by feedback inhibition (Gilmore *et al.* 1995). These facts again support the concept of the tight correlation between the stronger photosynthetic down-regulation in EC (Fig. 2) and limited carbon sink capacity.

Overdieck *et al.* (1998) and Tissue *et al.* (2001) also reported that the ageing of needles significantly affected photosynthetic performance of *Pinus sylvestris* and *P. radiata* shoots grown in EC. Generally, it is suggested that EC accelerates the natural decline in photosynthetic performance associated with leaf ageing and senescence (*e.g.* Sage *et al.* 1989). Enhanced ontogenetic development may result in the remobilisation of nitrogen and other minerals away from photosynthetic proteins (Roberntz and Stockfors 1998, Luo *et al.* 1999). Nevertheless, Tissue *et al.* (2001) demonstrated in *P. radiata* that age-based differences are more likely explained by a difference in sink strength between the needle age classes.

Experiment No. *3* **– Differences of exposed/shaded foliage responses**: Trees cultivated in the GDs are characterised by a high degree of canopy closure (Urban *et al.* 2001) which lead to the strong vertical distribution of photosynthetically active irradiances and formation of sun (Ex) and shade (Sh) adapted foliage with different responses to EC (Kalina *et al.* 2001, Marek *et al.* 2002). The irradiance receipt of Sh amounted to 11 % of the Ex shoots. Although Osborne *et al.* (1998) rejected the null hypothesis that photosynthetic acclimation to EC is identical throughout the canopy, there is a lot of discrepancies in the literature (*e.g.* Kerstiens 1998, Osborne *et al.* 1998, Kalina *et al.* 2001, Tissue *et al.* 2001).

Our results show that these discrepancies may be explained by the model based on the description of relative changes in specific leaf N concentration (n) and leaf mass per unit area (h = 1/SLA) at EC (Luo *et al.* 1994). Whenever $n \times h > 1$, predicted photosynthesis is up-regulated. If $n \times h < 1$, predicted photosynthesis may be either down-regulated or depressed ($n \times h < 0.6$) (Luo *et al.* 1994, 1999).

We suggest that the relatively lower content of N in ECEx needles is the most likely result of the stronger dilution effect (Stitt and Krapp 1999) resulting from the higher absolute assimilation rates in the upper canopy (Marek *et al.* 2002). Also, Pons *et al.* (1993) proposed two mechanisms of the regulation of N allocation in plants, *i.e.* leaf age and the irradiance gradient as a factor associated with the density of a canopy. Both assimilate accumulation (Webber *et al.* 1994) and low N/C ratio (Martin *et al.* 2002) lead to the inhibition of transcription of important photosynthetic genes, predominantly in the upper canopy owing to higher ambient irradiances. This is reflected by the diminution of P_{Nmax} stimulation by EC after the long-term treatment (Kalina *et al.* 2001, Marek *et al.* 2002) and P_{Nsat} down-regulation (by 22 %) in ECEx needles (Fig. 3*B*). In contrast, relatively higher N content in the lower canopy leads to P_{Nsat} up-regulation (by 51 %) as well as stronger stimulation of P_{Nmax} (Marek *et al.* 2002) in ECSh needles. On the base of these findings, the whole-tree response to EC is dependent on the ratio between exposed and shaded leaf area of the canopy.

Conclusion: All above-mentioned experiments demonstrate the hypothesis that the balance between morphological and biochemical changes controls the final response of plants to EC. These experiments indicate that tree response to EC is primarily based on carbon sink strength and not on the duration of exposure to EC. Sink limitation occurs when assimilate production is in excess over utilisation. It leads to the accumulation of photosynthetic assimilates and N depletion and subsequent feedback mechanisms of gene transcription inhibition. Results of all three experiments indicate that spruce tree response to EC is primarily based on (*1*) needle age distribution within the canopy, (*2*) sun to shade adapted leaf area ratio of the canopy, (*3*) progress of the vegetation season, and (*4*) the ability to produce new active carbon sinks.

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