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Research article

Effect of season, needle age and elevated CO_2 concentration on photosynthesis and Rubisco acclimation in *Picea abies*

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ABSTRACT

While downward photosynthetic acclimation in response to elevated CO_2 (EC) is frequently accompanied by reduction in Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), the exact mechanism behind this decrease and its dynamics are not well understood. We comprehensively studied Rubisco adjustment to EC in coniferous *Picea abies* using an electrophoretic (protein content), spectrophotometric (initial ($RA_{initial}$) and total (RA_{total}) in vitro Rubisco activities), and gas-exchange (maximum carboxylation activity in vivo (V_{Cmax})) techniques. With respect to differing carbon sink strength and nitrogen remobilization, we hypothesized greater acclimation of photosynthesis in one-year-old as compared to current-year needles and at the end than at the beginning of the vegetation season. EC treatment led to a decrease in V_{Cmax} values in current-year needles, but the ribulose-1,5-bisphosphate (RuBP)-limited rate of photosynthesis (J_{max}) remained unaffected. Indeed, both V_{Cmax} and J_{max} were reduced by the EC treatment in one-year-old needles. The extent of photosynthetic acclimation in EC plants did not increase, however, during the vegetation season. EC decreased the activation state of Rubisco ($RA_{initial}$/RA_{total}) by 16% and 5% in current-year and one-year-old needles, respectively (averaged over the growing season). While during spring (short-term effect) EC treatment did not influence the Rubisco content per unit leaf area and decreased its specific activity (activity per unit Rubisco mass) in both needle age classes studied, exposure to EC during the entire vegetation season tended to reduce the Rubisco content while increasing its specific activity. Irrespective of CO_2 treatment and needle age, a hyperbolic-decay relationship was observed between Rubisco-specific activity and its content.

1. Introduction

Global climate models predict that atmospheric CO_2 concentration ([CO_2]) may increase up to 800 μmol (CO_2) mol^{-1} by 2100 [1]. Whether rising [CO_2] will cause faster growth and higher photosynthetic carbon storage in C3 plants remains an open question [2,3].

Theoretically, elevated [CO_2] should lead, under sufficient light intensities [4], to enhanced C3 photosynthetic CO_2 uptake for at least two reasons: (1) photorespiration is expected to be depressed, and (2) substrate binding of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase; EC 4.1.1.39), the primary carbon-fixing enzyme in C3 plants, is expected to be enhanced [5,6].

Although initial exposure to elevated [CO_2] indeed leads to increase of the CO_2 assimilation rate in C3 plants, substantial reductions in [CO_2]-enhanced photosynthesis (termed acclimation or down-regulation) may occur over long time periods of CO_2 treatment [2,7,8]. Acclimation is usually manifested by lower rates of CO_2 assimilation in high-[CO_2]-grown plants than in their ambient-[CO_2]-grown counterparts when measured at ambient [CO_2], i.e. a reduction of Rubisco-limited photosynthesis. One may

Abbreviations: A_{sat}, light-saturated rate of CO_2 assimilation at growth CO_2 concentration per unit leaf area; AC (EC), ambient (elevated) atmospheric CO_2 concentration ([CO_2]); AS, Rubisco activation state; J_{max} ($J_{max,25}$), light-saturated electron transport rate at actual leaf temperature (normalized at 25 °C); N_{area}, nitrogen content per unit leaf area; $R_{content}$, Rubisco protein content in leaves; $RA_{initial}$, initial in vitro Rubisco activity; $RA_{specific}$, specific in vitro Rubisco activity; SLA, specific leaf area; T (T_{Leaf}), (leaf) temperature; V_{Cmax} ($V_{Cmax,25}$), in vivo light-saturated rate of Rubisco carboxylation at actual leaf temperature (normalized at 25 °C).

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also observe a reduction of ribulose-1,5-bisphosphate (RuBP)-limited photosynthesis, i.e. a photosynthesis rate under saturating [CO_2] and saturating light intensity. Accordingly, three types of acclimation have been found [6,7]: (i) the Rubisco-limited rate of carboxylation (V_{Cmax}) is unaffected by elevated [CO_2] treatment, but the RuBP-limited rate of carboxylation (J_{max}) decreases; (ii) V_{Cmax} decreases whereas J_{max} remains unaffected; and (iii) both V_{Cmax} and J_{max} are reduced. The degree of responses is variable, depending upon, among other things, plant species [9,10], duration of CO_2 enrichment [2,11,12], nutrient (nitrogen) supply [8,13], and sink strength for carbohydrates [6,7,14].

Down-regulation of Rubisco-limited photosynthesis under elevated [CO_2] is often accompanied by reduced Rubisco activity [5,7,15–18]. Although Rubisco is present in six different forms in higher plants, only two of these are photosynthetically active [5,19,20]. Decreased Rubisco activity can thus be caused by (1) a decline in Rubisco protein content, (2) inhibition of the carbamylated enzyme, or (3) an increased proportion of inactive non-carbamylated Rubisco forms. However, studies investigating Rubisco content in parallel with its activity as well as studies reflecting seasonal courses in Rubisco activity and/or content in relation to CO_2 treatment are not frequent.

With few exceptions [10,11,13,21–24], acclimation studies on shoots of different age classes have received only limited attention. In this paper, we systematically examine acclimation of photosynthesis to elevated [CO_2] mediated by changes in Rubisco (in vivo and in vitro activities, activation state, and content) in coniferous Norway spruce (*Picea abies* L. Karsten), a widespread forest tree species of the temperate zone. Two hypotheses were tested using sun-acclimated current-year and one-year-old shoots that contribute most significantly to the photosynthetic production of juvenile coniferous trees [25,26]: (1) Since old needles are characterized by the absence of carbon sinks [7,14], resulting from the lack of active basal meristems, we suggest more pronounced photosynthetic acclimation to elevated [CO_2] in one-year-old as compared to current needles. (2) Due to enhanced accumulation of assimilates, lower carbon sink strength and remobilization of nitrogen away from photosynthetic proteins through the vegetation season [7,8,11,13], we expect different extents and mechanisms of photosynthetic acclimation in spring and autumn.

2. Results

Climate characteristics during the studied vegetation season and the leaf temperatures (T_{Leaf}) during gas exchange measurements are summarized in Table 1. The nitrogen content in current-year needles increased during the vegetation season, while it gradually declined in one-year needles (Table 2). Similar changes were registered also for the leaf mass per area (LMA) (Table 2) of current-year needles, but not in the old ones. Although EC treatment led to a decrease in nitrogen content per unit leaf area (N_{area}) in both current-year (22–26%) and one-year-old needles (14–16%), these differences were mostly statistically non-significant regardless of sampling date (Table 2).

Growth in EC increased the light-saturated CO_2 assimilation rates per unit leaf area (A_{sat}) by 57% and 44% (seasonal average) in current-year needles and one-year-old needles, respectively (Fig. 1). This stimulation was null in one-year-old needles under EC treatment in July (Fig. 1), at which time leaf temperatures measured were at their highest (Table 1).

Despite EC-induced stimulation of A_{sat}, there was evidence of photosynthetic acclimation. While significant decrease in V_{Cmax} was found in both needle age classes (Table 3), EC treatment led to a significant decrease in J_{max} in one-year-old needles only (Table 3) under actual as well as normalized (25 °C) temperatures. Representative A/Ci curves for each treatment/needle age are presented in Fig. 2. Detailed post-hoc analysis of seasonal changes revealed that the effect of EC on Rubisco-limited carboxylation was significant in one-year-old needles only in July (Fig. 3a,b), whereas RuBP-limited carboxylation was significantly influenced in the first half of the vegetation season (May–July; Fig. 3c,d).

Such photosynthetic acclimation to EC was accompanied by an increase (24–30%) in the J_{max}/V_{Cmax} ratio in current-year needles, whereas the ratio slightly decreased (albeit without being

Table 1
Climate parameters recorded at the Bílý Kříž experimental site during the 2008 vegetation season and actual leaf temperatures during the gas exchange measurements. Mean values ± standard deviations are presented. T_{min} – mean daily minimum temperature, T_{max} – mean daily maximum temperature, T_{avg} – mean daily temperature, T_{Leaf} – leaf temperature adjusted during the gas-exchange measurements.

Month	T_{min} °C	T_{max} °C	T_{avg} °C	T_{Leaf} °C	Precipitation mm
May	7.0 ± 5.3	22.0 ± 7.2	13.0 ± 7.4	19	85
June	11.0 ± 2.1	25.0 ± 4.5	16.0 ± 5.1	21	156
July	12.0 ± 3.8	23.0 ± 6.2	16.0 ± 6.0	26.5	111
August	11.0 ± 3.1	22.0 ± 4.1	16.0 ± 4.7	–	161
September	6.0 ± 1.8	14.0 ± 4.7	9.0 ± 3.9	13	277
October	3.0 ± 3.2	9.0 ± 5.2	5.0 ± 4.3	11	100

Table 2
Mean ± standard deviation of leaf mass per area (LMA), nitrogen content per unit leaf area (N_{area}), ratio between light-saturated rate of carboxylation and electron transport rate (J_{max}/V_{Cmax}) under actual leaf temperature, and $J_{max,25}/V_{Cmax,25}$ ratio normalized at leaf temperature 25 °C in current (C) and one-year-old (C-1) needles of spruces cultivated under ambient (AC) and elevated (EC) CO_2 concentration. Identical superscripts (a–d) indicate homogeneous groups with statistically non-significant differences ($p > 0.05$); $n = 7$–15.

Age-class	Month	[CO_2]	LMA g m^{-2}	N_{area} g m^{-2}	J_{max}/V_{Cmax} dimensionless	$J_{max,25}/V_{Cmax,25}$ dimensionless
C	06	AC	91 ± 9^{b}	0.9 ± 0.05^{a}	2.7 ± 0.27^{a}	2.4 ± 0.23^{a}
	06	EC	94 ± 11^{b}	0.7 ± 0.11^{a}	3.3 ± 0.20^{ab}	2.7 ± 0.14^{a}
	09	AC	137 ± 38^{a}	2.3 ± 0.60^{c}	4.2 ± 0.56^{b}	2.9 ± 0.33^{a}
	09	EC	141 ± 28^{a}	1.7 ± 0.50^{b}	5.5 ± 0.36^{c}	3.8 ± 0.25^{b}
C-1	05	AC	179 ± 45^{a}	3.8 ± 1.27^{d}	4.2 ± 0.26^{b}	3.7 ± 0.23^{c}
	05	EC	182 ± 40^{a}	3.2 ± 0.85^{cd}	4.1 ± 0.25^{b}	3.5 ± 0.22^{c}
	07	AC	161 ± 26^{a}	2.7 ± 0.47^{bc}	3.5 ± 0.37^{ab}	3.6 ± 0.39^{c}
	07	EC	167 ± 28^{a}	2.3 ± 0.43^{abc}	3.0 ± 0.50^{a}	3.1 ± 0.51^{bc}
	10	AC	147 ± 17^{a}	2.0 ± 0.21^{a}	4.1 ± 0.34^{b}	2.7 ± 0.24^{ab}
	10	EC	154 ± 19^{a}	1.7 ± 0.18^{ab}	3.7 ± 0.26^{ab}	2.4 ± 0.33^{a}

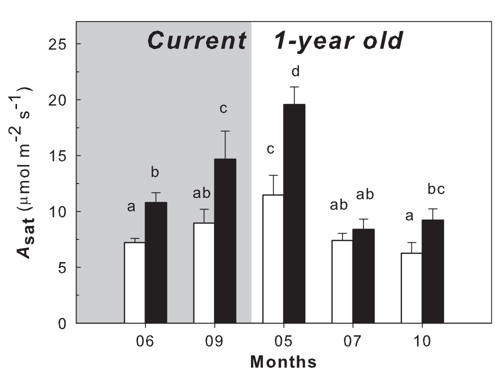

Fig. 1. Seasonal courses of light-saturated (1400 μmol(photons) m^{-2} s^{-1}) rates of CO_2 assimilation measured at growth [CO_2], i.e. 385 or 700 μmol(CO_2) mol^{-1}, and expressed per unit leaf area (A_{sat}) in current-year and one-year-old needles of *Picea abies* cultivated under ambient (AC; white columns) and elevated (EC; black columns) [CO_2]. Means (columns) and standard deviations (bars) are presented ($n = 7$–12). Identical superscripts indicate homogeneous groups with statistically non-significant differences ($p > 0.05$).