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Effects of season, needle age, and elevated atmospheric CO_2 on chlorophyll fluorescence parameters and needle nitrogen concentration in Scots pine (*Pinus sylvestris*)

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Abstract

Six-year-old Scots pine (*Pinus sylvestris* L.) seedlings were grown in open top chambers (OTCs) at ambient (AC) or elevated (ambient + 400 µmol mol^{-1}; EC) CO_2 concentration for three years (1996-1998). Chlorophyll (Chl) *a* fluorescence of current and one-year-old needles was measured in the field at two-weekly intervals in the period July-October 1998. In addition, Chl, carbon (C), and nitrogen (N) concentrations in both needle age classes were determined monthly during the same period. Chl fluorescence parameters were not significantly affected by EC, suggesting there was no response of the light reactions and the photochemical efficiency of photosystem 2. Chl concentrations were not significantly different but a reduced N concentration was observed in needles of EC treatment. Significant differences between needle age classes were observed for all parameters, but were most apparent under EC and toward the end of the growing season, possibly due to an acclimation process. As a result, significant interactions between CO_2 treatment, needle age class, and season were found. This study emphasizes the importance of repeated measures including different leaf/needle age classes to assess the photosynthetic response of trees under EC.

Additional key words: carbon; C/N ratio; fluorescence quenching; open top chambers; photosystem 2; seasonal response.

Introduction

In the present atmosphere, CO_2 does not saturate C_3 photosynthesis, therefore a doubling of the present CO_2 concentration should more than halve photorespiration (Bowes 1996), and an enhancement of the quantum yield of CO_2 assimilation is predicted (Long 1991). This results in a photosynthesis stimulation of 50-60 % when conifers and deciduous trees are grown under elevated CO_2 concentrations (EC) for more than one season (Saxe *et al.* 1998). However, the magnitude of this response varies widely from 0-216 % (Janssens *et al.* 2000). A number of studies have investigated the photosynthetic performance of trees (Marek *et al.* 1997) and shrubs (Csintalan *et al.* 1996) under EC.

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Abbreviations: Chl = chlorophyll; $\Delta F/F_m'$ = effective photochemical efficiency of photosystem 2; F_0 = minimal Chl fluorescence (dark adapted); F_0'= minimal Chl fluorescence (light adapted); F_m = maximal Chl fluorescence (dark adapted); F_m' = maximal Chl fluorescence (light adapted); $F_v = F_m - F_0$ = variable Chl fluorescence (dark adapted); F_v/F_m = optimal photochemical efficiency of photosystem 2; OTC = open top chamber; PS = photosystem; Q_A = quinone; q_N = non-photochemical quenching coefficient; q_P = photochemical quenching coefficient; SLA = specific leaf area.

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Detailed discussions of the expected effects of EC on the growth, physiology, and phenology of trees are in recent reviews (Ceulemans 1997, Saxe *et al.* 1998, Norby *et al.* 1999, Janssens *et al.* 2000).

To examine whether plants respond to EC at the level of photosynthetic light reactions, in particular photochemical efficiency of photosystem 2 (PS2), Chl *a* fluorescence can be measured. Because of the inverse relationship between Chl fluorescence and photosynthetic carbon assimilation, Chl *a* fluorescence is used as a sensitive *in vivo* probe of photosynthesis (Bolhàr-Nordenkampf and Öquist 1993, Schreiber *et al.* 1994). Genty *et al.* (1989) proposed a linear relation between one component of the Chl fluorescence signal [effective quantum efficiency of PS2, $(F_m' - F_t)/F_m'$] and the quantum yield of photosynthesis at high and moderate irradiances. The relationship was confirmed by Seaton and Walker (1990). Kellomäki and Wang (1997) showed the relation to be curvilinear under photorespiratory conditions. The relation between photosynthesis and Chl fluorescence is, however, very complex (Owens 1994). The rapid induction kinetics of Chl fluorescence can be related to primary photochemistry of PS2, whereas the slow phase of the induction curve is mainly related to interactions between processes in the thylakoid membranes and metabolic processes in the stroma, primarily carbon metabolism (Krause and Weis 1984, Bolhàr-Nordenkampf and Öquist 1993).

Few measurements of Chl fluorescence under long term CO_2 exposure have been made thus far, leading to conflicting results. Kellomäki and Wang (1997) found a 3 % higher (although not significant) optimal photochemical efficiency of PS2 (F_v/F_m) of one-year-old needles of *Pinus sylvestris* in the EC after three years of growth in OTCs. In a similar study of *Picea abies*, F_v/F_m was reduced under EC (Marek and Kalina 1996, Kalina *et al.* 1997). In *Eucalyptus cladocalyx*, F_0 was lower under EC than AC, but the differences in F_m, F_v, and their ratio were small and insignificant (Palanisamy 1999).

The specific objectives of the present study were to examine the interactive effects of season (time of the year), needle age, and elevated atmospheric CO_2 concentration on various Chl fluorescence parameters of young *P. sylvestris* plants in their third year of treatment in open top chambers. Because of the complex interpretation of Chl fluorescence signals, additional information on the nitrogen and Chl concentrations was simultaneously collected.

Materials and methods

Plants and growth conditions: Three-year-old, pot-grown and dormant Scots pine (*Pinus sylvestris* L.) seedlings, obtained from the Institute for Forestry and Game Management in Groenendaal (Belgium), were planted in the ground in four open top fumigation chambers (OTCs) situated on the campus of the University of Antwerp (UIA), on March 21, 1996. Mean annual temperature and rainfall at the site are 11.98 °C and 769 mm, respectively. All seedlings were from the same Belgian provenance (south from Samber and Maas) and were about 0.4 m tall at the time of planting. Eleven trees per chamber were planted in a circular planting pattern, 70 cm apart from each other and from the walls. To reduce the boundary effect, each OTC was surrounded by seedlings of the same seed lot. The original heavy loam soil was excavated to a depth of 0.5 m and replaced by forest soil (about 0.12 % N on a dry mass basis). No nutrients or water were applied during the experiment.

Each decagonal OTC (diameter 3 m, height 4 m) was made of 1 m wide *Perspex* (plexiglas) acrylic sheets, and had a usable ground area of 7.1 m^2. Incoming air was supplied by large axial fans (type *VM 40-10*) at a rate of about 1.40 $m\ s^{-1}$ (5000 $m^3\ h^{-1}$), resulting in nearly two air volume changes per min. Air distribution within each OTC was effected through a flexible spiroid pipe supplying a perforated, fiber-reinforced polythene annulus positioned 1 m above the ground. The four OTCs were divided into two CO_2 treatments: ambient (AC, *ca.* 350 $\mu mol\ mol^{-1}$ or the current background level) and elevated (AC, ambient + 400 $\mu mol\ mol^{-1}$). The actual CO_2 concentration that the plants in the elevated OTCs experienced was about 750 $\mu mol\ mol^{-1}$. Carbon dioxide input was regulated by a mass flowmeter/controller (*Brooks* type *5851E*, Rosemount, The Netherlands). The CO_2 treatments started on April 1, 1996 and have been applied continuously since then on a 24 h basis, also during the winter. Results for the third growing season in the OTCs (1998) are reported.

Chl *a* fluorescence measurements were performed *in situ* in the OTCs during the period from July to October, 1998 (at approximately 15-d-intervals) using a portable modulated fluorometer (*PAM-2000, Heinz Walz*, Effeltrich, Germany). The parameter values were those given by standard settings of the instrument. Measurements were made on current-year and one-year-old shoots, from the fourth whorl, situated on the south side of the tree to obtain needles with comparable exposure. Fluorescence was measured between 08:30 h and late afternoon, and on warm days a ventilator was used to prevent overheating of the measured needles. Trees were randomly selected between the two chambers of each treatment. Following a dark adaptation of 30 min, a sample of four fascicles per tree and per needle age class was mounted in the leaf clip of the instrument for determination of the initial Chl fluorescence (F_0). Immediately afterwards, maximal Chl fluorescence (F_m) was determined by applying a saturating radiation pulse. Optimal photochemical efficiency of PS2 [$F_v/F_m = (F_m - F_0)/F_m$] according to Butler (1978) was calculated on line. After 7 min adaptation to actinic irradiance (±900 μmol m^{-2} s^{-1}) provided by an external halogen lamp, Chl fluorescence at steady-state (F_t) and at the delivery of a saturating pulse (F_m') was determined. To correct for F_0 quenching, also F_0' was measured during the application of far-red radiation. Effective photochemical efficiency of PS2 [$\Delta F/F_m' = (F_m' - F_t)/F_m'$ according to Genty *et al.* 1989], photochemical [$q_P = (F_m'-F_t)/(F_m' - F_0')$], and non-photochemical [$q_N = (F_m - F_m')/(F_m - F_0')$] quenching coefficients were calculated.

In August measurements of Chl fluorescence were also made at different actinic irradiances. Response of $\Delta F/F_m$' to irradiance was determined after 5 min adaptation to each irradiance.

Biochemical and morphological needle characteristics: Monthly, eight current-year and one-year-old needles, used for the Chl fluorescence measurements, were sampled and needle area was calculated from the average needle length and needle radius. After drying (80 °C, 48 h), specific leaf area (SLA) was determined as the ratio of projected needle area to dry mass, in order to express N and C concentrations on an area basis. Needles were ground in a mill (*Cyclotec 1093* Sample Mill, Sweden) and analysed for N and C by a Dynamic Flush Combustion Method with a *NC 2100* Soil Analyser (*Carlo Erba Strumentazione*, Rodano, Italy).

For determination of Chl concentrations, samples of three fascicles, growing close to those used for Chl fluorescence measurements, were collected monthly. Two pieces of 10 mm were cut from the centre of each needle, giving four pieces per fascicle, or twelve in total. Projected needle area was determined by measuring the radius of each needle with a digital micrometer (*Mitutoyo*, type *CD-15DC*, U.K.) and multiplying by a known constant length (10 mm). Consequently, pieces were fresh weighted and put in vials containing 9 cm^3 (or 18 cm^3 if dilution was necessary) N,N-dimethylformamide. Chls were extracted at 4 °C for about 48 h and Chl *a* and Chl *b* concentrations were determined spectrophotometrically according to Porra *et al.* (1989) using an UV/VIS spectrophotometer (*Shimadzu UV-160*, Japan).

Statistical analysis: A nested ANOVA was used to investigate the effects of CO_2 treatment (Treat), needle age (Age), and time (Time) or PAR (photosynthetic active radiation, for the response of fluorescence parameters to irradiance) on different dependent variables. All analyses were performed in SAS (*SAS Institute*, Cary, NC, USA) using the mixed procedure (Littell *et al.* 1996). In each analysis also chamber (nested within treatment) and individual plant (nested within chamber) were added as random effects to the model. Adding individual as random effect to the model and the use of Satterthwaite's procedure to obtain the denominator degrees of freedom, adjusts the analysis for its repeated measures design. By doing so a compound symmetry correlation structure was assumed. Effects of the CO_2 treatment, needle age, time (or PAR), and their interactions were considered significant when $p \leq 0.05$ and are presented in Tables 1 and 2.

Levels of significance for differences between the treatments and needle age classes on one particular measuring date are derived from parameter estimates as given by SAS for an analysis split into measuring periods. When $p \leq 0.05$ after Bonferroni correction, differences were considered significant.

Results

Chl fluorescence: The ratio F_v/F_m was not significantly affected by the CO_2 treatment (significance for the effect of CO_2 treatment, *p*-Treat = 0.1234). One-year-old needles showed lower values compared with current-year ones (*p*-value for the effect of needle age, *p*-Age = 0.0001), especially under EC (*p*-value for the interaction between CO_2 treatment and needle age, *p*-Treat×Age = 0.0859). Differences were only significant in September and October (Fig. 1), resulting in a significant interaction between measuring period and needle age (Table 1). There was considerable variation with time, and F_v/F_m of one-year-old needles decreased during the growing season (Fig. 1).

The effective quantum efficiency of PS2 [$\Delta F/F_m' = (F_m' - F_t)/F_m'$], measured at different irradiances, was significantly higher under EC than AC (*p*-Treat = 0.0011). There was, however, an interaction between CO_2 treatment and needle age (Table 1) because only current-year needles had a higher $\Delta F/F_m$' under EC compared to the same needle age under AC (Fig. 2). The

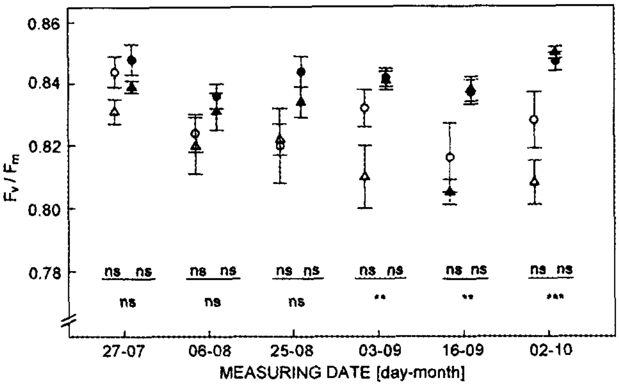

Fig. 1. Optimal quantum efficiency of photosystem 2 (PS2) (F_v/F_m) of current-year (*full symbols*) and one-year-old (*empty symbols*) needles of Scots pine for six measuring periods during the third growing season in open top chambers under ambient (*circles*) and elevated CO_2 (*triangles*) concentrations. Symbols are the average ± SE of 10 measurements. At each measuring period the differences between the treatments are shown separately for each needle age class above the line; the differences between needle age classes from the same treatment are shown under the line. Levels of significance are indicated as: ns (no significant difference) $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

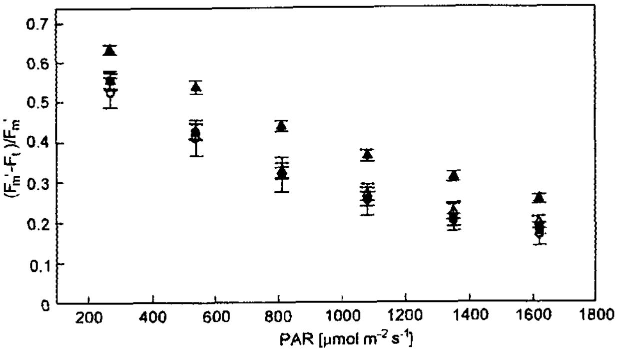

Fig. 2. Irradiance (PAR) response curve of the effective quantum efficiency of photosystem 2 (PS2) [$\Delta F/F_m' = (F_m' - F_t)/F_m'$] of current-year (*full symbols*) and one-year-old (*empty symbols*) needles of Scots pine in the third growing season in open top chambers under ambient (*circles*) and elevated CO_2 (*triangles*) treatments as a function of photosynthetically active radiation (PAR). Measurements were made in August and symbols are the average ± SE of six measurements. Levels of significance of the effects of irradiance, CO_2 treatment, needle age, and their interactions are shown in Table 1.

difference was most prominent at about 1300 μmol m^{-2} s^{-1} when $\Delta F/F_m'$ of current-year needles was 53 % higher under EC than AC. During the two-weekly measurements $\Delta F/F_m'$ was not significantly affected by CO_2 treatment (Table 1). Consequently, there was also no significant CO_2 effect on the Chl fluorescence quenching coefficients (Table 1) although q_P of one-year-old needles was lower and q_N higher under EC than AC (Fig. 3). At the end of the growing season, in September and October, there was a marked difference between needle age classes of the EC treatment with one-year-old needles having a considerable lower q_P and a higher q_N compared to current-year needles (Fig. 3). This difference for q_P was only significant at the beginning of September.

Biochemical and morphological needle characteristics: On a dry mass basis, needle N concentration was reduced under EC, although not significantly (p-Treat = 0.0829) (Table 2). Needles showed a 33 % higher N concentration on a projected needle area basis (N_{area}) under AC when measuring periods were pooled together. Nevertheless, no overall treatment effect was observed (p-Treat = 0.1093) (Table 2). In both treatments current-year needles had less N_{area} as compared to one-year-old needles and this difference decreased toward the end of the growing season (Fig. 4). Therefore not only an overall age effect but also a significant interaction between needle age and time was found (Table 2). During the growing season there was a decrease of foliar N_{area} in one-year-old needles and an increase in current-year needles (Fig. 4). There were no changes under EC in C concentration on a mass or area basis (Table 2). Again the needle age effect was significant; the relative difference between current-year and one-year-old needles was +37 % (AC) and +33 % (EC) in July and decreased afterwards. As a result, treatment differences in C/N ratio were caused by a decreased N concentration under EC (p-Treat = 0.0662, Table 2 and Fig. 4). Overall effects of needle age, time, and interactions of measuring period, respectively, with needle age and treatment were significant for the C/N ratio (Table 2). The separate statistical analyses showed only a significantly higher C/N ratio of one-year-old needles compared with current-year needles in September and October (Fig. 4). In July a slightly higher C/N ratio was observed in current-year needles. During the growing season there was an increase of the C/N ratio between July and August followed by a decrease in current-year needles, potentially due to reallocation of N from one-year-old to current-year needles.

When values were pooled over the growing season, a reduction by -12.9 % of total Chl concentration (on a projected needle area basis) of current-year needles was observed under EC (Fig. 5). This overall CO_2 effect, however, was not significant (Table 2). There were significant needle, time, and time×needle effects on needle Chl concentration (Table 2). In July the relative difference between one-year-old and current-year needles was -33 and -40 % under AC and EC, respectively (Fig. 5); in the following months this difference was lower. Chl *a/b* ratio was markedly lower, although not

Table 1. Analysis of variance for chlorophyll fluorescence parameters of current-year and one-year-old needles of Scots pine under ambient (AC) and elevated (EC) CO_2 treatment, measured at six periods or irradiances (PAR response) during the third growing season in open top chambers. Statistical significance of the main effects of CO_2 treatment (Treat), needle age (Age), measuring period (Time), or PAR (photosynthetically active radiation), and their respectively two- and three-way interactions are shown as: ns (not significant) $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

Source of variation	F_v/F_m	$\Delta F/F_m'$	q_P	q_N	Source of variation (PAR response)	$\Delta F/F_m'$
Treat	ns	ns	ns	ns	Treat	***
Age	****	****	****	**	Age	****
Time	***	***	****	***	PAR	****
Treat×Time	ns	ns	*	*	Treat×PAR	ns
Treat×Age	ns	**	*	****	Treat×Age	****
Age×Time	*	ns	ns	ns	Age×PAR	ns
Treat×Age×Time	ns	**	ns	**	Treat×Age×PAR	ns

Table 2. Analysis of variance for nitrogen (N) and carbon (C) concentrations on a dry mass and on a projected needle area basis, for C/N ratio, for chlorophyll (Chl) *a*+*b* and Chl *a*/*b* on a projected needle area basis of current-year and one-year-old needles of Scots pine under ambient and elevated CO_2 treatments, measured at four dates during the third growing season in open top chambers. Statistical significance of the main effects of CO_2 treatment (Treat), needle age (Age), measuring date (Time), and their respectively two- and three-way interactions are shown as: ns (not significant) $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

Source of variation	% N	N(area)	% C	C(area)	C/N	Chl *a*+*b*	Chl *a*/*b*
Treat	ns	ns	ns	ns	ns	ns	ns
Age	**	****	***	*****	****	****	****
Time	****	ns	****	ns	****	****	****
Treat×Time	**	ns	**	ns	*	ns	Ns
Treat×Age	ns	ns	ns	ns	ns	ns	**
Age×Time	****	****	ns	ns	****	***	*
Treat×Age×Time	ns	ns	ns	ns	ns	ns	ns

significant, under EC but the differences were only observed in one-year-old needles (Table 2). Pooled over the measuring periods, Chl *a*/*b* was enhanced by 11.4 % in current-year needles under EC relative to one-year-old needles of the same treatment. Both the needle age effect and the interaction of needle age with treatment were significant [Table 2; Chl *a*/*b* in July = 2.72 ± 0.05 (EC, last-year needles), 3.05 ± 0.05 (EC, current-year needles), 2.88 ± 0.07 (AC, last-year needles) and 3.06 ± 0.05 (AC, current-year needles)] because the age effect was highest under EC treatment.

Discussion

CO_2 treatment effects: The ratio F_v/F_m, related to the optimal photochemical quantum efficiency of PS2 (Butler 1978), was not significantly affected by the CO_2 treatment in this study and values were similar to those of healthy plants (Björkman and Demmig 1987), indicating that trees did not experience a stress situation. This is in accordance with most studies in the European ECOCRAFT research network (Besford *et al.* 1998), as well as with experiments on *P. sylvestris* (Kellomäki and Wang 1997), *P. abies* (Tausz *et al.* 1996), and *Pinus radiata* (Conroy *et al.* 1986). A reduction after two and three years of treatment has been shown for *P. abies* in OTCs (Marek and Kalina 1996, Kalina *et al.* 1997), but not in a comparable branch bag experiment (Marek and

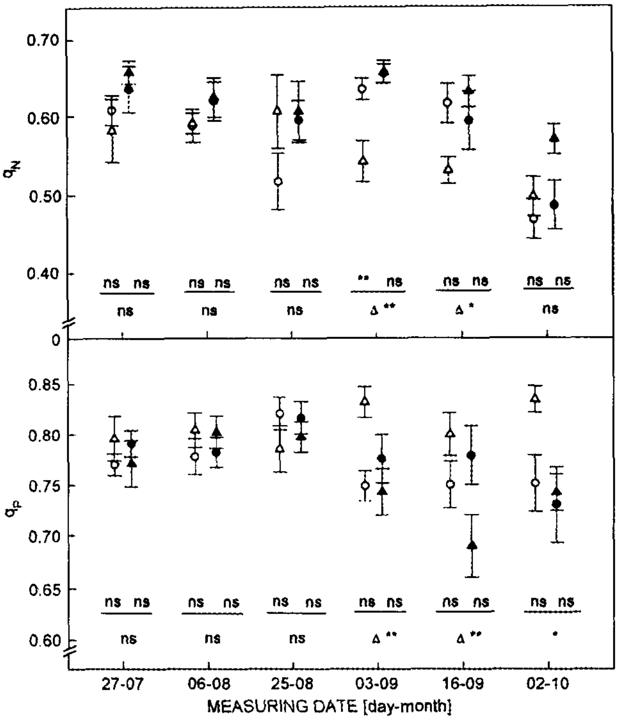

Fig. 3. Photochemical (q_P) and non-photochemical (q_N) quenching coefficients of current-year (*full symbols*) and one-year-old (*empty symbols*) needles of Scots pine under ambient (*circles*) and elevated (*triangles*) CO_2 treatments for six measuring periods during the third growing season in open top chambers. Symbols are the average ± SE of 10 measurements. At each measuring period the differences between the treatments are shown separately for each needle age class above the line; the differences between needle age classes from the same treatment are shown under the line. When the difference between the two needle age classes was significant in the elevated CO_2 treatment and not in the ambient treatment, the symbol Δ is used. Levels of significance are indicated as: ns (no significant difference) $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

Kalina 1996).

Because of the curvilinear relation between the quantum yield of CO_2 assimilation and $\Delta F/F_m'$ (Harbinson *et al.* 1990, Seaton and Walker 1990, Kellomäki and Wang 1997), an enhancement of $\Delta F/F_m'$ could be expected. Indeed a more efficient use of excitation energy was observed in the present experiment (response to irradiance). The increase of $\Delta F/F_m'$ was associated with an increased fraction of oxidated Q_A (q_P) in current-year needles under EC (values not shown). Nevertheless, during the growing season measurements of $\Delta F/F_m'$, q_N and q_P showed no significant effect of CO_2 treatment. Hogan *et al.* (1997) could not prove their hypothesis that *P. radiata* needed less photoprotection mechanisms under EC. A comparable OTC experiment of *P. sylvestris* also showed no significant effect of CO_2 treatment on q_P; q_N of one-year-old needles under high irradiances was enhanced under EC in the same experiment (Wang and Kellomäki 1997). A fast growing *Populus* clone (Beaupré) showed an increase in $\Delta F/F_m'$ and q_P, accompanied by a decrease in q_N (Kalina and Ceulemans 1997). In the same study with a slower growing clone (Robusta), however, opposite results were found. In general, the effect of CO_2 treatment on Chl fluorescence parameters was small but considerable species variation has been reported in literature. In the present experiment no significant change of Chl fluorescence characteristics under EC was found during the growing season and no modification of the shape of the irradiance response curves was observed. Therefore our results suggest that there was no significant modification of the structure and organisation of the light reactions of Scots pine seedlings under EC.

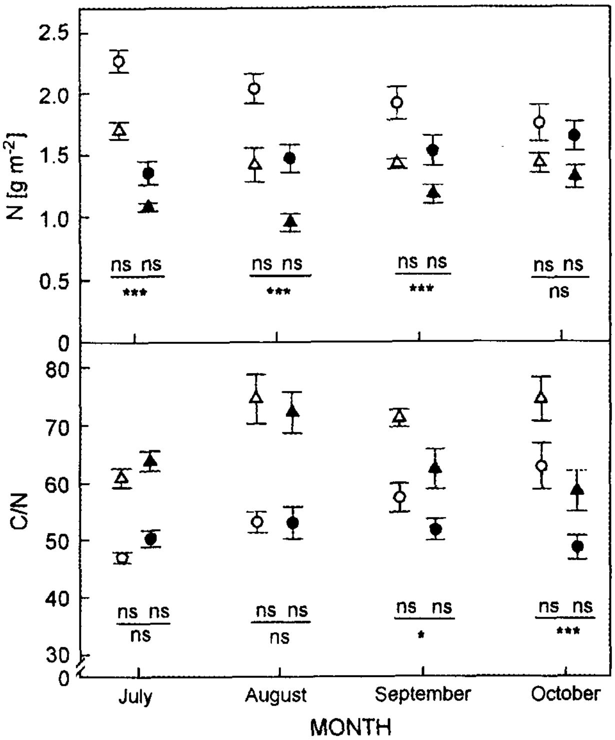

Fig. 4. Nitrogen concentration on a projected needle area basis and C/N ratio of current-year (*full symbols*) and one-year-old (*empty symbols*) needles of Scots pine for four measuring dates during the third growing season in open top chambers under ambient (*circles*) and elevated (*triangles*) CO_2 treatments. Symbols are the average ± SE of 10 measurements. At each measuring period the differences between the treatments are shown separately for each needle age class above the line; the differences between needle age classes from the same treatment are shown under the line. Levels of significance are indicated as: ns (no significant difference) $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

These findings were confirmed by non-significant changes in total Chl concentrations and Chl *a/b* ratios of current-year and one-year-old needles. This observation suggested that there was no adaptation to photon absorption. Long-term CO_2 exposure of *Pinus ponderosa* (Tissue *et al.* 1999), *Pinus taeda* (Lewis *et al.* 1996), and *P. abies* (Tausz *et al.* 1996, Kalina *et al.* 1997) resulted in a reduction of total Chl concentration. Other studies showed no change (Tissue *et al.* 1996, Rey and Jarvis 1998, Turnbull *et al.* 1998). Additionally, Hogan *et al.* (1997) and Rey and Jarvis (1998) found similar Chl *a/b* ratios under two different CO_2 treatments.

Based on many studies of woody plants an average reduction of foliar N on a mass basis by 19 % has been reported (Cotrufo *et al.* 1998). We found a reduction of foliar N under EC, although not significant due to the small sample size. Contrary to observations for *P. ponderosa* (Tissue *et al.* 1999) and *Fagus sylvatica* (Epron *et al.* 1996) a similar reduction was observed when N concentrations were expressed on a projected needle area basis. The same observations were reported for *P. taeda* (Lewis *et al.* 1996, Tissue *et al.* 1997) and *Betula pendula* (Rey and Jarvis 1998). This is a consequence of an unchanged SLA of needles in the present experiment which suggests that there was no needle morphological adaptation or starch accumulation. Also in the previous growing season (1997) no significant starch accumulation was observed (Jach and Ceulemans 2000). In general, a reduction in N concentration on a mass basis is explained by a dilution effect due to accumulation of saccharides as reported by Turnbull *et al.* (1998) for *P. radiata* and by Lewis *et al.* (1996) for *P. taeda*. Long-term experiments with *P. taeda* (Tissue *et al.* 1997) and *P. ponderosa* (Tissue *et al.* 1999) also showed that differences in N concentration persisted after correction for total concentration of non-structural saccharides (dilution effect).

An important fraction of foliar N is associated with the photosynthetic apparatus, namely the photon absorption complexes, enzymes of the electron transport, and the Calvin cycle. As 30-50 % of foliar N is invested in ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO (Woodrow and Berry 1988), our observations suggest a decrease of RuBPCO content (cf. Urban and Marek 1999). This is in line with last years' results and suggests a downward acclimation response after a two- to three-years exposure to EC (Jach and Ceulemans 2000).

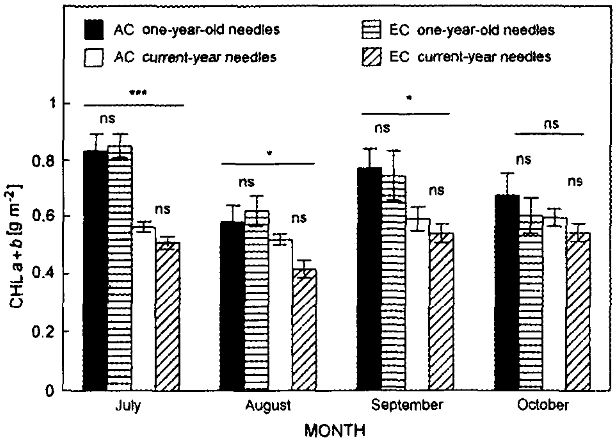

Fig. 5. Chlorophyll (Chl) $a + b$ concentration on a projected needle area basis of current-year and one-year-old needles of Scots pine for four measuring dates during the third growing season in open top chambers under ambient and elevated CO_2 treatments. Symbols are the average ± SE of 10 measurements. At each measuring period the differences between the treatments are shown separately for each needle age class under the line; the differences between needle age classes from the same treatment are shown above the line. Levels of significance are indicated as: ns (no significant difference) $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

Needle age effects: One-year-old needles are generally more shaded than the current-year needles, and shade adapted conifer needles have lower Chl *a/b* ratio (Alberte *et al.* 1976) and quantum yield (Lewandowska and Jarvis 1978). As they are longer exposed to EC (because of being older) a different response to the CO_2 treatment might be expected. Although an important difference between foliage age in response to EC has already been reported (Wang *et al.* 1995, Besford *et al.* 1998, Turnbull *et al.* 1998), few studies have considered different leaf age classes. In the present study, effects of needle age were more significant than those of CO_2 treatment, and interactions between needle age and treatment have been observed since some needle age effects became most apparent under EC. Under EC, one-year-old needles showed lower efficiency of excitation energy transfer, coupled with a lower q_P and higher q_N. Though not significant, a reduced $\Delta F/F_m'$ under EC was detected in the one-year-old needles at the end of growing season. During previous photosynthesis measurements, a reduction of the carboxylation efficiency has been detected (Jach and Ceulemans 2000). Since this acclimation response was observed near the end of the growing season in one-year-old needles, this could be a possible explanation for our results since Chl fluorescence measurements were made under high irradiances in the RuBPCO limited zone of the net photosynthetic rate to intercellular CO_2 concentration (P_N/C_i) curve. An alternative explanation, proposed by Turnbull *et al.* (1998), is a difference in the demand of saccharides between needle age classes or an accelerated senescence of needles under EC. In our study an advanced senescence and needle fall was observed under EC. Possibly also competition for radiant energy was larger in the OTCs with EC due to an earlier closure of the canopy.

Since obvious differences in photosynthesis and response to EC between needle/leaf age classes are observed, one should include different leaf age classes in experiments as already recommended by Šesták (1985), especially when modelling of total photosynthesis is the objective.

Seasonal effects: Coniferous species are photosynthetically active throughout the year under different environments. When measurements are made only during summer under optimal conditions, a considerable overestimation of annual carbon gain can be made (Lewis *et al.* 1996). Frequent measurements are important to study the long-term response of trees under EC as shown in the present experiment where already a significant variation with time of season was observed during a short period.

Since the EC treatment had no significant effect on the efficiency and organisation of the light reactions of photosynthesis, we conclude that the previously observed photosynthetic stimultation of Scots pine needles under EC (Jach and Ceulemans 2000) is the consequence of a photorespiratory reduction. Important differences between the two needle age classes, interactions between treatment and needle age, and interactions with the measuring period emphasize the importance of repeated measures throughout the season including different leaf or needle age classes.

Our measurements were restricted to the needles at only one particular height in the canopy. Many uncertainties about the response of entire canopies after a long-term exposure to EC still exist. Results are influenced by species, environmental conditions, and competition. Detailed studies at the ecosystem level combined with modelling remain necessary to predict the future response of ecosystems to a changing climate.

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