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Short- *versus* long-term effects of elevated CO_2 on night-time respiration of needles of Scots pine (*Pinus sylvestris* L.)

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Abstract

Dark respiration rate in the night (R_D) was measured in five-year-old Scots pine (*Pinus sylvestris* L.) trees grown for two years under ambient (AC) and elevated (AC + 400 μmol mol^{-1} = EC) CO_2 concentrations in open top chambers. Two needle age classes (*i.e.*, current-year and one-year-old) were measured at AC and EC in both AC- and EC-grown pines. Additionally different chemical characteristics were determined on the needles, such as nitrogen (N), carbon (C), starch, and soluble sugar concentrations as well as specific leaf area. The direct, short-term and indirect, long-term effects of EC on R_D for the two needle age classes were examined. R_D was expressed on a per needle area, needle mass, N, C, and C/N bases. Direct effects were only pronounced in the AC treatment where inhibition of R_D was found at EC in both current- and one-year-old needles. Indirect effects were only significant in one-year-old needles where a decrease was found in the EC grown trees as compared with AC ones when R_D was expressed per unit needle mass, C, or C/N. R_D per unit needle area and needle N were not sensitive to long-term EC, in any needle age class. Long-term EC treatment also influenced the response of the two needle age classes. One-year-old needles from the EC treatment had significantly lower R_D than current-year needles, but no such response was observed in the AC treatment. Our experiment re-emphasised the importance of expressing R_D on different bases for a correct interpretation of the responses to EC. Moreover, we showed that different needle age classes can respond differently to a CO_2 enrichment.

Additional key words: carbon; C/N; direct and indirect effects; nitrogen; soluble sugars; starch; specific leaf area.

Introduction

Respiration in trees may consume half or more of the carbon fixed in photosynthesis (Farrar 1985, Amthor 1995). Moreover, leaf R_D in trees may constitute more than half of the total whole-plant respiration (Hagihara and Hozumi 1991) and changes in this component in response to atmospheric CO_2 enrichment could have a significant effect on forest carbon budgets. On a global scale, about 10 times more CO_2 is released by plant respiration as is released by fossil fuel burning; therefore any effects of increasing CO_2 on the amount of CO_2 released to the atmosphere by plant respiration are important to the global C cycling (Amthor 1995). Given the importance of plant respiration, it is important to improve our knowledge of the effects of rising atmospheric CO_2 concentration on respiratory carbon release.

While the effects of atmospheric CO_2 on photosynthesis and photorespiration are well documented (Gunderson and Wullschleger 1994, Norby *et al.* 1999, Saralabai *et al.* 1997), much less is known about the response of dark respiration. The measurements of R_D are, however, essential to extrapolate the gas exchange measurements to biomass production (Mousseau and Saugier 1992). It is useful to distinguish direct and indirect effects in identifying mechanisms by which EC might affect respiration (Amthor 1991, 1995, Ryan

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1991, Wullschleger *et al.* 1994). Only few studies, however, have examined both direct and indirect effects of EC on R_D so far (Bunce 1990, Thomas and Griffin 1994, Wullschleger *et al.* 1994, Griffin *et al.* 1996, Amthor 1997, Marek *et al.* 1997, Will and Ceulemans 1997, Urban and Marek 1999).

Mechanisms underlying short-term and long-term reductions in respiratory CO_2 are believed to be different and are still the subject of discussion. Leaf R_D's may be directly and reversibly inhibited by EC (Bunce and Caulfield 1991, El Kohen *et al.* 1991, Bunce 1994, 1995, Wullschleger *et al.* 1994, Ziska and Bunce 1994, Griffin *et al.* 1996). Direct effects of CO_2 on R_D are short-term, resulting in a depression of the apparent R_D of leaf tissue with instantaneous changes in CO_2 concentration (Drake *et al.* 1999). Direct effects might involve specific interactions of CO_2 inside a leaf with regulatory enzymes of the respiratory pathway (Amthor 1997). The direct effect of CO_2 on R_D is in particular important in the case of forest canopies, where daily and seasonal fluctuations can be large at the forest floor, depending on soil activity and canopy structure (Bazzaz and Williams 1991).

In addition to direct effects, R_D values may be altered through a longer-term acclimation response to EC (Bunce 1994, Wullschleger *et al.* 1994, Norby *et al.* 1999). Indirect effects of CO_2 on R_D are due to long-term EC specific changes in tissue quality and construction costs (Poorter *et al.* 1992, Curtis 1996, Poorter *et al.* 1997, Wullschleger *et al.* 1997). EC generally enhances photosynthesis (Gunderson and Wullschleger 1994, Ceulemans and Mousseau 1994, Saxe *et al.* 1998) stimulating the levels of soluble saccharides (Barnes *et al.* 1995). Enhanced levels of soluble saccharides can potentially increase respiration by stimulating growth and different processes consuming respiratory products. In contrast, decreased concentrations of soluble protein (Ziska and Bunce 1994) may affect the specific maintenance respiration in a negative way. Maintenance respiration seems to be more sensitive to CO_2 concentration than growth respiration (Ryan 1991). Moreover, since maintenance respiration is strongly related to N concentration of the tissue (Ryan 1991), it is expected to decline with EC, because N concentration is generally lower than in AC leaves. To better explain the results, construction and maintenance respiration components of respiration are frequently reported (Ryan 1991, Sprugel and Benecke 1991).

There is a variability in the way R_D is determined: from response to photosynthetically active radiation (PAR) (Nijs *et al.* 1988, Wang *et al.* 1995, Kubiske and Pregitzer 1996), from CO_2 response, or by darkening leaves during the day (Ziska *et al.* 1990, Teskey 1995). However, the best estimate of R_D is obtained when measured at night because the impact of sugar synthesis and transport is then minimised (Amthor 1989, Will and Ceulemans 1997). This is of particular relevance in EC experiments, where C assimilation and consequently sugar production are higher. At night, however, stomatal closure and low night temperatures may restrict the CO_2 evolution from needles (Sprugel *et al.* 1995).

Studies on the effects of EC on respiration have yielded contrasting results. The response of respiration may differ among species, among studies, and likely among plant tissues. Leaf or needle R_D per dry mass may either increase (Gifford *et al.* 1985, Hrubec *et al.* 1985, Williams *et al.* 1992, Thomas *et al.* 1993, Thomas and Griffin 1994), or decrease (Bunce and Caulfield 1991, Wullschleger and Norby 1992, Bunce 1992, 1995, Williams *et al.* 1992, Wullschleger *et al.* 1992, Ziska and Bunce 1993, Azcón-Bieto *et al.* 1994, Teskey 1995, Wang *et al.* 1995, Kubiske and Pregitzer 1996), or remain constant (Gifford *et al.* 1985, Baker *et al.* 1992, Hrubec *et al.* 1985, Ceulemans and Mousseau 1994) under EC.

The objectives of this investigation were to examine the direct and indirect effects of a doubled CO_2 concentration on the night-time respiration of Scots pine needles. We examined whether needle R_D is altered on the short- or long-term by EC, and in which direction. Explanations of the results have been sought by measuring needle chemical characteristics such as N, C, soluble sugar, and starch concentrations, and morphological characteristics such as specific leaf area (SLA). R_D was expressed on the bases of needle area, needle mass, N, C, and C/N, so that comparisons could be made.

Materials and methods

Plants and growth conditions: Three-years-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 (OTC) situated on the campus of the University of Antwerp (UIA), on 21 March 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 the depth of 0.5 m and replaced by forest soil (approx. 0.12 % N on a dry mass basis). No nutrients or water were applied during the course of experiment.

Each decagonal OTC (diameter 3 m, height 4 m) was made of 1-m wide *Perspex* acrylic sheets, and had a usable ground area of 7.1 m^2. The four OTC's were divided into ambient, AC (*ca.* 350 µmol mol^{-1} or the current background level) and elevated, EC (AC + 400 µmol mol^{-1}). The CO_2 enrichment started on 1 April, 1996, and the treatment was continuously applied since then on a 24 h basis, also in wintertime. For a more detailed description of the experimental conditions see Jach and Ceulemans (1999). Results of the second year (1997) of treatment are reported.

Night-time R_D measurements: Steady-state R_D measurements were made in the night using an open system infrared gas analyser (*CIRAS 1, PP Systems*, Herts., UK) and a standard cuvette of 2.5 cm^2 in diameter. Air temperature in the cuvette was measured using *BETATHERM* thermistors (type *200K6*), and leaf temperature was calculated from the energy balance. Input air for the gas exchange system was supplied by passing building air through a *Drierite* column and a soda lime column attached to the *CIRAS* unit, to remove water vapour and CO_2, respectively. Air of the desired CO_2 concentration was supplied by pressurired *Sparklets* CO_2 bulbs (*Hemisyphon*, Solingen, Germany) attached to an automatic CO_2 flow regulation system. A fan inside the cuvette stirred the air to maintain boundary layer resistance to H_2O diffusion less than 0.2 m^2 s^{-1} mol^{-1}. The relative humidity of the air in the cuvette was set at 80 % and the flow rate at 3.33 cm^3 s^{-1}.

All measurements were made in July 1997. Night-time measurements started 2 h after sunset, at 00:00 h, and ended at about 04:30 h. Measurements were made *in situ* in the OTC's on current-year and one-year-old shoots, all from the third whorl, situated on the south side of the tree. Two fully expanded and attached fascicles (four needles) per tree were enclosed in the cuvette for each R_D measurement. Needles were stretched completely across the cuvette. For each CO_2 treatment, measurements were made at actual night temperatures and at two CO_2 partial pressures (350 and 700 µmol mol^{-1}), beginning with the growth concentration. The record was taken only after CO_2 efflux had reached a steady-state, usually requiring 10 to 15 min. Needle projected area was estimated prior to enclosure in the cuvette, from total needle width and cuvette width measured with a digital micrometer (*Mitutoyo*, Germany). After R_D measurements the fascicles were harvested, dried, and SLA, N and C concentrations were determined as described below.

Measurements of N, C, soluble sugar, and starch concentrations: Immediately after R_D measurements, needles were destructively sampled for measurements of needle area, dry mass (DM), and foliar N and C concentrations. Needle area was calculated from average needle width and needle length. Each set of four needles was freeze-dried and SLA was determined as the ratio of projected needle area to dry mass [cm^2 kg^{-1}], in order to calculate N and C concentrations on an area basis ($(N, C)_{area} = [(N, C)_{mass}/SLA]$. Following drying, needles were ground in a mill (*Cyclotec 1093* Sample Mill, Sweden) to fine powder and analysed for N and C by a Dynamic Flush Combustion Method with a *NC 2100* Soil Analyser (*Carlo Erba*, Rodano, Italy).

Following R_D measurements, additional needles were collected for analysis of starch and soluble sugars. For this purpose, needles growing close to those used for R_D measurements and N and C analyses were sampled. Needles for saccharide analysis (a batch of approx. 30 needles) were harvested in the morning and put in a freeze-drier within 30 min. After drying, the needles were ground to fine powder and shipped to the University of Edinburgh, Scotland where they were analysed for sugars (inositol, sorbitol, galactase, glucose, fructose, sucrose) with a *Dionex DX-500 HPLC* (Sunnyvale, CA, USA) fitted with *GP40* gradient pump, *PA-1 CarboPac,* and *ED40* electrochemical detector. On a sub-sample of the needle powder, starch concentration was determined at the University of Antwerp enzymatically based on the formation of NADPH which is stoichiometric to the amount of D-glucose formed by the hydrolysis of starch (Boehringer 1997).

Statistical methods: A two-way ANOVA was used to investigate the long-term (indirect) effect of CO_2 treatment (*Treat.C*) and needle age (*Age*) on R_D expressed on various bases, and on chemical needle characteristics. A three-way ANOVA was used to investigate the short-term effects of measurement CO_2 concentration in the cuvette (*Cuv.C*), effects of CO_2 treatment (*Treat.C*) and needle age (*Age*) on R_D expressed on different bases. 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 adjust the analysis for its repeated measures design. In this way a compound symmetry correlation structure was assumed. Analyses were performed in *SAS* (version *6.12*) using the *MIXED* procedure (Littell *et al.* 1996).

Results

Direct effects of short-term CO_2 enrichment on R_D were significant only in the AC for both needle age classes (Table 1). Short-term EC decreased R_D whether expressed per unit needle area, mass, N, C, or C/N. The EC caused changes in the direct response of R_D to short-term EC in both needle age classes and independent of the reference basis of expression. Area-based R_D of trees grown in AC compared with EC-trees was much more responsive to short-term changes in cuvette CO_2 as R_D decreased by 65 % in current-year and by 79 % in one-year-old needles when CO_2 was increased from 350 to 700 μmol mol^{-1}, compared with reductions of 31 % in current-year and of 32 % in one-year-old needles in the EC treatment.

One-year-old needles had significantly lower R_D than current-year needles, irrespective of the treatment and cuvette CO_2 concentrations, when R_D was expressed per unit mass or per unit N (Table 1). When measured at AC, in both treatments R_D did not differ between the treatments (Table 1). However, when measured at EC, in both treatments R_D was higher in the EC-trees than AC-trees.

Indirect effects of long-term CO_2 treatment: A summary of the indirect effects of EC on R_D (measurements made at the growth CO_2 concentration) for two needle age classes, expressed per units of needle area, needle mass, N, C, and C/N, is presented in Table 2. Long-term EC treatment resulted in overall reductions in R_D. Differences were, however, only significant in one-year-old needles and only in mass-, C-, and C/N-based measurements (Table 2).

When expressed on a needle mass basis, R_D was 27 % lower in the EC treatment in current-year needles and 33 % lower in one-year-old needles as compared with the AC treatment. When expressed per unit C, the relative difference in R_D between the EC and AC treat-ments was higher in one-year-old needles (by 32 %) than in current-year needles (by 26 %). And when expressed on a unit C/N basis, R_D was 29 % lower in the EC treatment in current-year needles and 48 % lower in one-year-old needles as compared with the AC treatment.

Age effects were only significant in the EC-treatment, with higher R_D in current-year needles than in one-year-old needles when expressed on a needle mass or C and C/N bases.

Chemical and morphological needle characteristics: N concentration expressed on a mass basis (N_{mass}) was significantly lower in needles from EC as compared with AC, but only in one-year-old needles (Fig. 1). The opposite was found when N was expressed per unit needle area (N_{area}), namely EC resulted in significantly higher N_{area} in the current-year needles, in contrast to one-year-old needles having lower, although not significantly, N_{area} concentrations (Fig. 1). Age effects were only significant for area-based N, with current-year needles having lower N_{area} concentrations as compared with one-year-old needles, independent of the CO_2 treatment (Fig. 1).

No significant differences in C between the treatments were found when C was expressed on a dry mass basis (C_{mass}) (Fig. 1). Also needle age effects on C_{mass} were not significant (Fig. 1). When expressed per unit needle projected area, C concentration (C_{area}) was significantly higher in EC treatment as compared with AC, but the difference was only significant in current-year needles. In both treatments, current-year needles had significantly less C_{area} than one-year-old needles (Fig. 1).

The response of both C and N concentrations expressed on a needle area basis resulted from the response of SLA to the EC treatment (Fig. 1). SLA was lower in the EC treatment as compared with AC in both needle age classes, but the difference was only significant in current-year needles. Age effects on SLA were significant in both treatments, one-year-old needles having lower SLA than current-year needles. Since no differences in C_{mass} were found in either needle age class, significantly higher C_{area} in current-year needles resulted from lower SLA. Similarly, in current-year needles higher N_{area} in EC resulted exclusively from lower SLA (Fig. 1). The significant reduction in N_{mass} in EC in one-year-old needles as compared with AC was partly counterbalanced by an increase in mesophyll tissue (SLA higher, although not significantly). This resulted in similar values of N_{area} of one-year-old needles for both treatments.

EC resulted in a significantly higher C/N ratio as compared with the AC treatment, but only in one-year-old needles (Fig. 1). The age effect was significant only in the AC treatment, where one-year-old needles had a lower C/N ratio as compared with current-year needles (Fig. 1).

The concentration of total soluble sugars was not affected by the CO_2 treatments (Table 3). When different soluble sugars were considered separately, the EC treatment resulted in significantly higher (more than doubled) glucose concentrations in both needle age classes as compared with AC. Also sorbitol concentrations were significantly enhanced by EC, but only in current-year needles (Table 3). One-year-old needles had generally higher concentrations of soluble sugars and this in both treatments, but the differences

Table 1. Direct (short-term) effects of CO_2 partial pressure (cuvette CO_2) on dark respiration rate (R_D) of current-year and one-year-old Scots pine needles grown for two years in either ambient, AC (360 μmol mol^{-1}) or elevated, EC (700 μmol mol^{-1}) atmospheric CO_2 concentrations (growth CO_2). Results are expressed on a projected needle area [μmol m^{-2} s^{-1}], needle dry mass [mol kg^{-1} s^{-1}], needle nitrogen (N) [mmol kg^{-1} s^{-1}], needle carbon (C) [mol kg^{-1} s^{-1}], and needle C/N [nmol s^{-1}] bases. Values shown are means ±SE for each treatment where n = 8. Statistical significance of the single and interactive effects of treatment growth CO_2 concentration (*Treat.C*), needle age (*Age*), and measurement CO_2 concentration in the cuvette (*Cuv.C*) (three-factor ANOVA) is shown as: *** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns = p 0.05. R_D was measured at ambient temperature (T ≈ 17.6 °C) during the night.

R_D per		Current-year needles 350	700	One-year-old needles 350	700	ANOVA statistics
needle area	AC	1.69 (0.19)	0.48 (0.13)	2.08 (0.15)	0.38 (0.07)	*Treat.C**
	EC	2.09 (0.28)	1.56 (0.07)	2.30 (0.11)	1.55 (0.19)	*Age* ns
						Cuv.C***
						Age×Treat.C ns
						Age×Cuv.C ns
						TreatC×Cuv.C***
						Treat.C×Age×Cuv.C ns
dry mass	AC	11.74 (1.60)	3.20 (0.82)	9.79 (0.70)	1.78 (0.33)	*Treat.C**
	EC	11.42 (1.40)	8.59 (0.40)	9.98 (1.04)	6.52 (0.79)	*Age**
						Cuv.C***
						Age×Treat.C ns
						Age×Cuv.C ns
						Treat.C×Cuv.C***
						Treat.C×Age×Cuv.C ns
N	AC	1.50 (0.25)	0.41 (0.12)	1.00 (0.09)	0.18 (0.03)	*Treat.C**
	EC	1.44 (0.19)	1.09 (0.07)	1.23 (0.13)	0.82 (0.12)	*Age***
						Cuv.C***
						Age×Treat.C ns
						Age×Cuv.C ns
						Treat.C×Cuv.C**
						Treat.C×Age×Cuv.C ns
C	AC	25.50 (3.44)	7.01 (1.79)	20.86 (1.52)	3.77 (0.69)	*Treat.C**
	EC	25.07 (3.10)	18.84 (0.87)	21.32 (2.10)	14.12 (1.73)	*Age* ns
						Cuv.C**
						Age×Treat.C ns
						Age×Cuv.C ns
						Treat.C×Cuv.C*
						Treat.C×Age×Cuv.C ns
C/N	AC	0.21 (0.03)	0.06 (0.01)	0.21 (0.02)	0.04 (0.01)	*Treat.C**
	EC	0.20 (0.03)	0.15 (0.01)	0.17 (0.02)	0.11 (0.01)	*Age* ns
						Cuv.C**
						Age×Treat.C ns
						Age×Cuv.C ns
						Treat.C×Cuv.C***
						Treat.C×Age×Cuv.C ns

were more pronounced in EC, except for inositol (Table 3). Thus, in the EC treatment, concentrations of total soluble sugars, inositol, glucose, and fructose were significantly higher in one-year-old than current-year needles. Starch concentrations were significantly higher under EC as compared to AC, but only in current-year needles. Consequently, starch accumulation in current-year needles under EC resulted in significantly lower SLA as described above.

Table 2. Indirect (long-term) effects of the growth CO_2 concentration on the rate of dark respiration (R_D) of current-year and one-year-old Scots pine needles grown for two years in either ambient, AC (360 μmol mol^{-1}) or elevated, EC (700 μmol mol^{-1}) atmospheric CO_2 concentrations and measured at the growth CO_2. Results are expressed per unit of projected needle area [μmol m^{-2} s^{-1}], needle dry mass [μmol kg^{-1} s^{-1}], needle nitrogen (N) [mmol kg^{-1} s^{-1}], needle carbon (C) [μmol kg^{-1} s^{-1}], and needle C/N [nmol s^{-1}]. Values shown are means ±SE for each treatment where n = 8. Statistical significance of the single and interactive effects of treatment growth CO_2 concentration (*Treat.C*) and needle age (*Age*) (two-factor ANOVA) is shown as: *** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns = $p\geq0.05$. R_D was measured at ambient temperature (T ≈ 17.6 °C) during the night.

R_D per	Current-year needles Growth CO_2			One-year-old needles Growth CO_2			ANOVA statistics
	AC	EC	EC/AC	AC	EC	EC/AC	
leaf area	1.69 (0.19)	1.56 (0.07)	0.92	2.08 (0.15)	1.55 (0.19)	0.74	*Treat.C* ns *Age* ns *Treat.C*×*Age* ns
dry mass	11.74 (1.60)	8.59 (0.40)	0.73	9.79 (0.70)	6.52 (0.79)	0.66	*Treat.C*** *Age** *Treat.C*×*Age***
N	1.50 (0.25)	1.09 (0.07)	0.72	1.00 (0.09)	0.82 (0.12)	0.82	*Treat C* ns *Age* ns *Treat.C*×*Age* ns
C	25.50 (3.44)	18.84 (0.87)	0.74	20.86 (1.52)	14.12 (1.73)	0.67	*Treat.C** *Age** *Treat.C*×*Age***
C/N	0.21 (0.03)	0.15 (0.01)	0.71	0.21 (0.02)	0.11 (0.01)	0.52	*Treat C**** *Age** *Treat.C*×*Age***

Discussion

In this study on Scots pine, we demonstrated evidence not only for a direct, short-term inhibition of needle R_D by EC, but also for some indirect, long-term effects of EC. Short-term exposure to different CO_2 concentrations was sufficient to lower or enhance R_D, and this response was fully reversible. So, leaf R_D of AC-trees decreased when measured in EC, while R_D of EC-trees was enhanced when measured in AC, similarly to the results of El Kohen *et al.* (1991) on sweet chestnut and Will and Ceulemans (1997) on poplar. The magnitude of this change was, however, affected by the growth CO_2 concentration. Short-term effect was only significant in the AC treatment, irrespective of needle age and of the way the results were expressed. A significant interaction between the direct and indirect effects is in agreement with the findings of Bunce (1990) and Thomas and Griffin (1994). In contrast, no differences between the AC and EC growth treatments in the relative reduction of R_D in response to an instantaneous increase in CO_2 concentration were found by Griffin *et al.* (1996).

Based on literature, leaf R_D is on average reduced by 15 to 20 % when CO_2 is increased from 350 to 700 μmol mol^{-1} (González-Meler and Siedow 1999). The metabolic explanation of the direct response remains hypothetical and the precise manner in which CO_2 affects R_D is still unknown. The inhibition appears, however, to be a classic case of feed-back control by an end-product (Amthor *et al.* 1992, González-Meler *et al.* 1996, González-Meler and Siedow 1999).

In response to the long-term EC, there was a considerable difference in R_D between the two needle age classes (for the effects of leaf age on photosynthetic characteristics see Šesták 1985). The indirect response of R_D to EC was larger in one-year-old needles, since they experienced a longer exposition to the treatment. Similar results were found in poplar clone Beaupré where the CO_2 effect was minimal in very young leaves and increased with leaf age (Will and Ceulemans 1997, Jarvis 1998). In one-year old needles R_D significantly decreased under EC as compared with AC, whether expressed on a mass, C, or C/N basis.

Similar to other studies (Poorter *et al.* 1992, Thomas *et al.* 1993, Azcón-Bieto *et al.* 1994, Thomas and Griffin 1994, Mitchell *et al.* 1995, Griffin *et al.* 1996) the

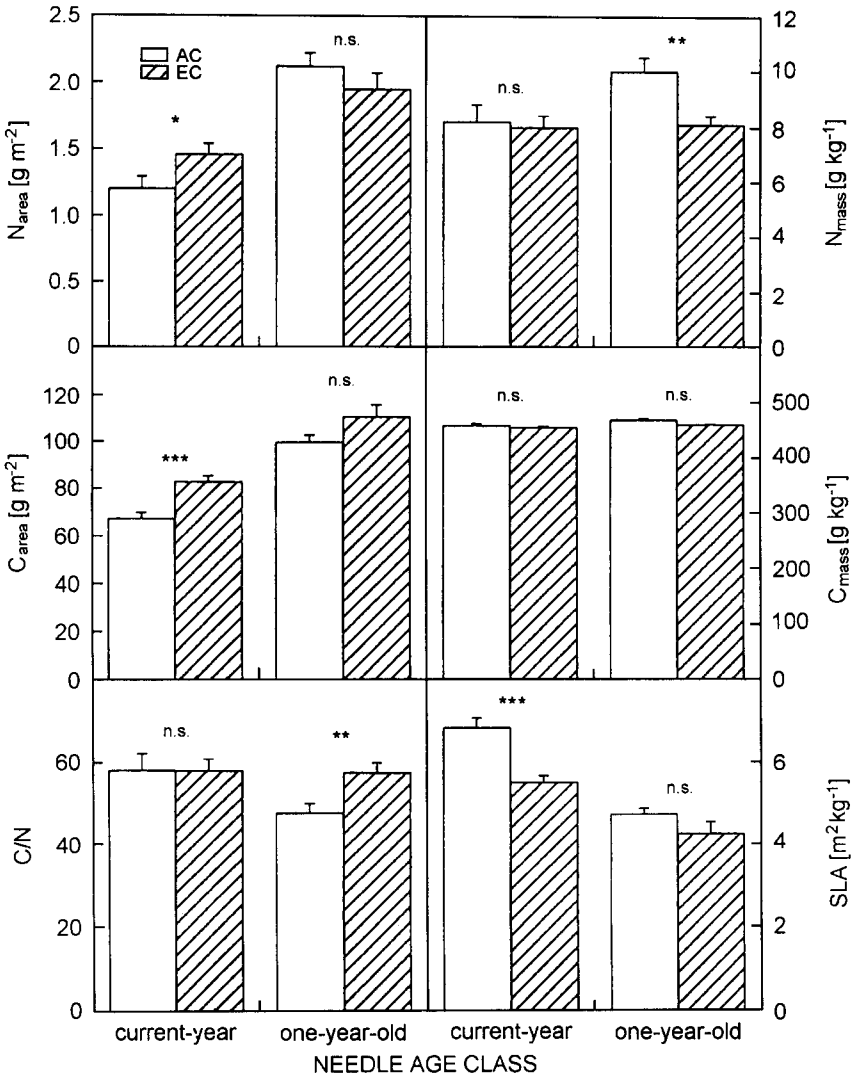

Fig. 1. Needle nitrogen (N) and carbon (C) concentrations, both on an area and a mass basis, carbon to nitrogen ratio (C/N), and specific leaf area (SLA) for current-year and one-year-old needles during the second growing season in ambient (AC) and elevated (EC) CO_2. Mean values ± SE are shown, with n = 8. Only treatment differences within the needle age class are shown; differences between the needle age classes within the treatments are described in the text. Levels of significance are indicated as: *** p<0.001, ** p<0.01, * p<0.05, ns p 0.05.

present study re-emphasizes the importance of a multiple expression of values for the interpretation of the indirect effects of CO_2 on R_D. Changes in R_D in response to EC have often been described in terms of changes in sugar accumulation in the leaves and of a limited transport or sink activity. This could lead to excessive saccharide accumulation and consequently to a decrease in SLA, resulting in a decreased R_D on a mass basis, but increase area-based R_D (Thomas and Griffin 1994).

Nevertheless, no long-term CO_2 treatment effect was found when R_D was expressed on a needle area or N basis. Azcón-Bieto *et al.* (1983) suggested that respiration is limited by a supply of glucose. Since glucose concentration on an area basis was significantly higher in both needle age classes in the EC treatment as compared with AC (Table 3) we could expect that this contributed to an absence of treatment differences in the area-based R_D. On an area basis, most studies on the long-term effects of EC on R_D report decreases, although also an increase or no change has been found (Jarvis 1998). R_D per unit projected needle area was similar to those reported in the literature for Scots pine needles measured at comparable ambient temperatures (Leverenz 1988).

Table 3. Soluble sugar and starch concentrations expressed on a projected needle area [g m^{-2}] basis in current-year (CY) and one-year-old (OY) needles of Scots pine trees sampled in July of the second growing season under ambient (AC) and elevated (EC) CO_2 conditions. Mean values of 22 plants per treatment ± SE are shown. Δ% = relative difference between the treatments calculated as 100 [(EC – AC)/AC]. Statistical significance is shown as: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ns $p > 0.05$.

	Needle age	AC	EC	Sign. diff.	Δ%
Inositol	CY	1.562 (0.074)	1.779 (0.088)	ns	+ 14
	OY	2.027 (0.087)	2.383 (0.141)	ns	+ 18
	Sign. diff.	***	**		
Sorbitol	CY	0.931 (0.386)	0.105 (0.047)	*	- 88
	OY	0.910 (0.415)	0.454 (0.212)	ns	- 50
	Sign. diff.	ns	ns		
Glucose	CY	1.900 (0.233)	3.874 (0.265)	***	+104
	OY	2.067 (0.309)	4.684 (0.230)	***	+127
	Sign. diff.	ns	*		
Fructose	CY	4.330 (0.456)	4.371 (0.382)	ns	+ 1
	OY	4.724 (0.576)	5.832 (0.479)	ns	+ 23
	Sign. diff.	ns	*		
Sucrose	CY	1.543 (0.571)	1.277 (0.194)	ns	- 17
	OY	3.427 (1.127)	1.099 (0.149)	ns	- 68
	Sign. diff.	ns	ns		
Total sugars	CY	10.270 (1.000)	11.410 (0.640)	ns	+ 11
	OY	13.160 (1.900)	14.650 (0.850)	ns	+ 11
	Sign. diff.	ns	**		
Starch	CY	1.09 (0.19)	5.28 (0.82)	*	+384
	OY	2.20 (0.37)	2.84 (0.52)	ns	+ 29
	Sign. diff.	ns	ns		

In one-year-old needles, the lower R_D per unit mass under EC resulted from a combination of both a lower (although not significantly) SLA and area-based R_D. In current-year needles, the significant decrease in SLA under EC as compared with AC did not counterbalance the absence of treatment differences in area-based R_D, with consequentially no differences in mass-based R_D. The significantly lower SLA in current-year needles in EC resulted from a significant starch accumulation in those needles. Indirect effects on needle R_D on a mass basis were also found in *Pinus palustris* (Mitchel *et al.* 1995) and *Pinus ponderosa* (Griffin *et al.* 1996).

Foliage N is a good predictor of R_D, because both growth respiration (Will and Ceulemans 1997) and maintenance respiration (Ryan 1991) are highly correlated with N concentration. A decreased N_{mass} as commonly observed for leaf tissues grown under EC (Curtis 1996, Cotrufo *et al.* 1998, Medlyn *et al.* 1999) may have contributed to the decreased R_D per mass basis (Amthor 1991, Wullschleger *et al.* 1994) since a lower N_{mass} leads to lower construction costs and maintenance requirements (Mitchell *et al.* 1995). The absence of a treatment effect on R_D per unit N may reflect that there was no difference in growth or saccharide transport at a given level of foliar N (Mitchell *et al.* 1995). Indeed, growth rates (Jach and Ceulemans 1999) and soluble saccharide concentrations were similar in both treatments at the time of R_D measurements. In contrast to our results, R_D per unit foliar N were higher in the EC treatment than in AC in *Pinus palustris* (Mitchell *et al.* 1995). However, in some studies a decreased R_D per unit leaf N, following a long-term EC treatment, has been found (Ziska and Bunce 1993, Ceulemans and Mousseau 1994).

There was less N per unit C in the one-year-old EC needles as compared with AC (Fig. 1). EC generally increases C/N ratio (Norby *et al.* 1992, Field *et al.* 1992) and thus may alter the relationship between R_D per unit dry mass and per unit N. An increased C/N ratio tends to reduce tissue specific respiration (R_D per unit of tissue mass) because more saccharides are stored in tissue grown under EC at little cost in maintenance respiration (Saxe *et al.* 1998). The C/N basically represents a balance between the supply of respiratory products (C) and the demand for respiratory products (N) and that is why the C/N ratio may also be a good indicator of

respiration (Griffin *et al.* 1996). In the present experiment, R_D per unit C/N was significantly decreased under EC, but only in one-year-old needles. In contrast to our findings, no indirect effects on R_D per unit C/N basis were found in *Pinus ponderosa* (Griffin *et al.* 1996).

The significantly lower R_D in one-year-old needles grown in EC as compared with current-year needles from the same treatment could possibly be attributed to a reduced SLA, but also to a lower demand for ATP and a reduced respiratory machinery (Sprugel *et al.* 1995). As leaves age, they become increasingly shaded and also more distant from active sinks (Sprugel *et al.* 1995). In the present experiment, shading effects became more pronounced in EC treatment than in AC (Jach and Ceulemans 1999). How R_D changes with leaf age in response to EC has not been, however, addressed in many studies. Young needles have higher R_D because they are still growing and construction respiration is occurring, but with maturation R_D decreases dramatically (Sprugel *et al.* 1995). Generally, conifers have lower R_D than broadleaf plants and R_D of conifers can vary considerably within the vertical canopy profile and with needle age (Sprugel *et al.* 1995). Because age related effects on R_D were only present in the EC treatment, the CO_2 treatment might have enhanced needle ontogeny.

There is some evidence in the literature that R_D varies with time due to leaf and plant ontogeny (Šesták 1985, Poorter *et al.* 1992, Mousseau 1993). In *Castanea sativa* leaf R_D in EC-trees were lower than in the AC ones in spring, but the effect disappeared with the prolongation of growing season (El Kohen *et al.* 1991). In the present study, R_D derived from the PAR response curves during the day was not affected by the EC treatment during the growing season (Jach and Ceulemans 2000), but a large seasonal variation in the response of R_D was found, with highest R_D during the phase of rapid growth. The needle age effect was significant only in mid-summer and disappeared later. So, for a good understanding of the impact of EC on R_D it is essential to measure R_D throughout the growing season and for different leaf age classes.

In conclusion, both direct and indirect effects of EC on R_D were reported. The direct effects were only pronounced in the AC treatment, where a suppression of R_D was found with a short-term CO_2 increase. The indirect effects were only significant in one-year-old needles, where a decreased R_D was found in EC grown needles as compared with the AC ones, when R_D was expressed on a mass, C, or C/N bases. Long-term age-related effects on R_D were only significant in the EC treatment, where current-year needles had higher R_D than one-year-old needles on a mass, C, or C/N bases. Area- and N-based R_D seemed not to be very sensitive to long-term CO_2 exposure. Mass-, C-, and C/N-based R_D mirrored best both treatment- and age- related effects of long-term CO_2 exposure.

Since respiration may consume half of the CO_2 fixed in photosynthesis and since atmospheric CO_2 concentrations are rising in the range of the inhibitory effects, a better understanding of the long-term effects of increasing CO_2 on R_D is necessary to model plant and ecosystem responses to future climate. The highly variable and inconsistent response of R_D to CO_2 reported in the literature may be caused by the different ways of expressing the results, by leaf age, saccharide and nitrogen concentrations as well as by temperature at the time of measurement. These factors have seldom been considered in measurements of leaf R_D at either AC or EC. To resolve uncertainties about the response of leaf R_D to EC, both short-term direct effects and long-term acclimation effects should be considered in future experiments.

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