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Stem CO_2 efflux of a *Populus nigra* stand: effects of elevated CO_2, fertilization, and shoot size

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Abstract

To determine whether long-term growth in elevated atmospheric CO_2 concentration [CO_2] and nitrogen fertilization affects woody tissue CO_2 efflux, we measured stem CO_2 efflux as a function of temperature in three different size classes of shoots of *Populus nigra* L. (clone Jean Pourtet) on two occasions in 2004. Trees were growing in a short rotation coppice in ambient (370 µmol mol^{-1}) and elevated (550 µmol mol^{-1}, realised by a Free Air Carbon dioxide Enrichment system) [CO_2], and measurements were performed during the third growing season of the second rotation. Elevated CO_2 did not affect Q_{10} or specific stem CO_2 efflux (E_{10}) of overall poplar shoots. The lack of any effect of N on stem CO_2 efflux indicated that nutrients were sufficient. Specific stem CO_2 efflux differed significantly between shoot sizes, emphasizing the importance of tree size when scaling-up respiration measurements to the stand level. Variation in stem CO_2 efflux could not be satisfactorily explained by temperature as the only driving variable. We hypothesize that transport of CO_2 with the sapflow might have confounded our results and could explain the high Q_{10} values reported here. Predicting the respiratory carbon loss in a future elevated [CO_2] world must therefore move beyond the single-factor temperature dependent respiration model and involve multiple factors affecting stem CO_2 efflux rate.

Additional key words: EUROFACE, growth rate, nitrogen, respiration, sap flow, size class, temperature function, woody tissue.

Introduction

The impact of future high CO_2 concentrations [CO_2] on the carbon balance of ecosystems is of high importance, as an increased terrestrial carbon stock could partly offset increases in atmospheric [CO_2]. Autotrophic respiration is estimated to consume half of the carbon fixed in photosynthesis (Amthor 1989) and forms a large component of the carbon budget of higher plants. In woody perennials, growth and maintenance respiratory costs of long-lived structures such as stems and branches may comprise a sizable portion of the whole plant carbon balance (Korol *et al.* 1991). In studies on coniferous and deciduous trees (Goulden *et al.* 1996, Lavigne *et al.* 1997, Law *et al.* 1999), woody tissue respiration contributed 5 - 15 % to ecosystem respiration, compared to 18 - 43 % from foliage and 48 - 76 % from soil fluxes. Even though respiration fluxes nearly equal ecosystem carbon input

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Abbreviations: AGR - absolute growth rate; E_{10} - stem CO_2 efflux at 10 °C; E_s - stem CO_2 efflux; FACE - free-air CO_2 enrichment; Q_{10} - respiratory temperature coefficient; R_s - predicted stem CO_2 efflux; R_g - growth respiration; R_m - maintenance respiration; T - temperature in °C.

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from photosynthesis (Law *et al.* 1999), it has been historically neglected (Sprugel and Benecke 1991), and only recently detailed studies have addressed the effects of elevated [CO_2] on woody tissue respiration (Edwards *et al.* 2002).

Respiration depends on temperature, and variation in respiration vs. temperature has been frequently modelled with an exponential function, defining a basal respiration rate and a Q_{10} (Van't Hoff 1898, Lavigne 1987, Sprugel and Benecke 1991). Woody tissue respiration is commonly divided into two functional components: maintenance (R_m) and growth respiration (R_g) (McCree 1970). Growth respiration (R_g) refers to the CO_2 evolving from the production of new tissues and consequently varies through the year and with environmental factors that control growth. Maintenance respiration primarily varies with changes in temperatures and is related to maintenance processes in the cell such as the turnover of proteins, maintenance of ion gradients and is, in contrast to R_g, temperature dependent (Penning de Vries 1975).

The response of woody tissue respiration to future high atmospheric [CO_2] is reported to be equivocal (for a review, see Amthor 1991). A larger stem wood volume and subsequent higher maintenance costs, or larger growth rates in elevated [CO_2], might increase woody tissue respiration (Carey *et al.* 1996, Edwards *et al.* 2002, Zha *et al.* 2005). On the other hand, lower N content in elevated [CO_2] may cause maintenance respiration to decline (Ryan 1991, Griffin *et al.* 1996), knowing that most of the organic nitrogen in plants is contained in proteins, and protein repair comprises 60 % of the maintenance respiration (Penning de Vries 1975). In addition, studies on the effects of elevated [CO_2] on plant physiological processes have shown the importance of nutrient availability (Kubiske *et al.* 1998, Murray *et al.* 2000, Oren *et al.* 2001, Sigurdsson 2001).

In previous studies (Liberloo *et al.* 2005, 2006), we showed that poplars growing in a CO_2 enriched atmosphere (EUROFACE) increased their aboveground biomass production up to 29 %, whereby elevated CO_2 and fertilization modified the diameter frequency distribution and mortality of the poplar shoots. To our knowledge, the combined effects of elevated [CO_2] and fertilization on woody tissue respiration of different size classes of poplar shoots have not been investigated before.

Here we report results of stem CO_2 efflux measurements during the third growing season (2004) of *Populus nigra* growing in elevated [CO_2] and fertilization in a short rotation coppice culture (EUROFACE, Central Italy). We examined the effects of elevated [CO_2], fertilization and shoot size on the stem CO_2 efflux of a poplar stand with the commonly used single-factor temperature model.

Materials and methods

Plantation lay out: Stem CO_2 efflux of shoots of *Populus nigra* (black poplar, clone Jean Pourtet), growing in the EUROFACE plantation (Viterbo, Central Italy), was measured during the dormant season (March) of 2004 and measurements were repeated during the growing season (August - September). Trees were managed in a short rotation coppice culture. Poplars were planted in 1999, coppiced in 2001 (first rotation), after which many shoots resprouted from each stool (second rotation, 2002 - 2004). Concentrations of CO_2 were ambient (3 plots, 370 μmol mol^{-1}) and elevated (3 plots, 550 μmol mol^{-1}). The elevated CO_2 concentrations, measured at 1-min intervals, were within 20 % deviation from the pre-set target concentration for 70 % of time (F. Miglietta, IBIMET-CNR, Florence, Italy, unpublished results). During the growing season, fertilization was applied weekly to half of each plot in amounts proportional to tree growth rate. Further details on the EUROFACE plantation and FACE system are provided by Scarascia-Mugnozza *et al.* (2000), Miglietta *et al.* (2001), Liberloo *et al.* (2005).

Stem CO_2 efflux measurements: In the winter of 2004, prior to the first measurements, we distinguished three shoot diameter classes at 20 cm above ground. These represented the diameter distribution of the entire population and the average diameter for each class was: class A (15 mm), class B (25 mm), and class C (45 mm). Because of time constraints, stem CO_2 efflux measurements were taken only in plot 1 to 4 (two replicas per CO_2 treatment). In each plot half, two shoots were labelled from each diameter class (yielding four shoots per treatment combination). We used the *Li-Cor-6400* (*Li-Cor*, Lincoln, NE, USA) gas analyzer as an open system to measure stem CO_2 effluxes, and a plexiglass cuvette (length 10 cm, diameter 10 cm) was designed to fit on the sensor head. The cuvette was mounted on collars (with different internal diameters), using terostat (Henkel KGaA, Germany) and closed cell foam to ensure perfect sealing from the surrounding air. The whole cuvette was covered with aluminium foil to minimize heating and prevent refixation of respired CO_2 by photosynthetically active bark. Temperature was measured with a thermocouple inserted just under the bark at the north side of the shoots. Before measurements on a new shoot started, the cuvette was left to stabilize and mix internal air for 10 min. Sample and reference cells were matched before each measurement and a leak test was performed by blowing CO_2-rich air around the cuvette. An average of three successive measurements was used in further calculations. After the measurement of shoot stem CO_2 efflux was completed, stem diameters of the shoot at the point of cuvette attachment were determined in two perpendicular directions with a digital calliper (*Mitutoyo*, type *CD-DC*, Telford, UK).

During two weeks, measurements were made from the morning (08:00) until the evening (18:00 in March and 22:00 in August - September) under daylight, and shoots were randomly revisited at different temperatures to maximize the temperature range over which data were collected. The dependence of stem CO_2 efflux to stem temperature was expressed by the linear equation: $\ln(E_s) = \ln(E_{10}) + \ln Q_{10} \times [(T-10)/10]$, where E_s = stem CO_2 efflux, E_{10} = stem CO_2 efflux at a standard temperature of 10 °C, T = temperature and Q_{10} = the change in the rate of stem CO_2 efflux with a 10 °C change in stem temperature.

During the measurement period in March, trees were still dormant and leafless, though bud set occurred one week after measurements were taken. During the summer measurements, tree growth was insignificant, as growth peaked in early spring. We pooled the two data sets together to examine the effects of elevated CO_2 and fertilization on the stem CO_2 efflux, considering that the measured stem CO_2 efflux approached the CO_2 loss by maintenance respiration. The temperature range during each measurement period was too small to fit the data separately.

We tested the single-factor temperature dependent model on two days during summer, when stem CO_2 efflux of a C-class shoot was independently monitored on an hourly base in an ambient and elevated $[CO_2]$ plot.

Growth of shoots was monitored monthly by measuring the diameter at 140 and 153 cm aboveground (the outer edges of the cuvette plus two attached collars), in two perpendicular directions. From these growth measurements, relative (RGR) and absolute (AGR) growth rates were calculated during the period of summer measurements (August - September) and as a yearly average (RGR_{year} and AGR_{year}).

Data analysis: The main effects of elevated CO_2, fertilization, size class and their interactions on stem CO_2 efflux were evaluated by analysis of variance (*ANOVA*). The design was a randomized complete-block with CO_2, fertilization, size-class and their interactions as fixed factors, and block as a random factor. Statistical tests were performed with the software package *SAS* system *9.1* (SAS Institute, Cary, NC, USA). When the *ANOVA* F-test showed significant interactions, *a posteriori* comparison of means was performed. *P*-values of these multiple comparisons were Tukey corrected, to reduce the chance on type I errors. Plots were considered to be replicates, data were tested for normality with the Shapiro-Wilk statistics, and differences between means were considered significant when the *P*-value of the *ANOVA F*-test was < 0.05.

Results

Effects of CO_2, fertilization and shoot size on stem CO_2 efflux: Linear fittings of data of stem CO_2 efflux (E_s) as a function of stem temperature were all significant ($P < 0.05$), with r^2 values ranging between 0.42 and 0.96. In our model, based on the linear fittings of pooled CO_2 efflux data, 66 % of variation in CO_2 efflux was explained by stem temperature ($P < 0.0001$). E_{10} values of different size classes ranged for smallest shoots (A-class) between 25.9 and 66.1 μmol $m^{-3}s^{-1}$ for middle class shoots (B-class) between 2.2 and 8.9 μmol m^{-3} $s^{-1,}$ and for the largest shoot size class (C-class) between 1.3 and 5.6 μmol $m^{-3}s^{-1}$, depending on the treatment combination. Values of Q_{10} were highly variable and varied between 2.2 and 4.6 for A-class shoots, between 3.7 and 11.1 for B-class shoots and between 4.8 and 22.6 for C-class shoots.

Neither elevated CO_2 nor fertilization changed the specific CO_2 efflux measured on different size classes; overall values of E_{10} and Q_{10} were unchanged (Fig. 1*A*,*B* $P > 0.05$). Specific CO_2 efflux differed between size classes (Fig. 1*C*): the smallest shoots (A class) exhibited, next to a high variability between replicates, significantly higher E_{10} values ($P < 0.0001$, Fig. 1*C*). The largest shoots (C class) had remarkably higher Q_{10} values ($P = 0.0059$, Fig. 1*C*) than the two other shoot size classes. In particular for these largest shoots, Q_{10} was not significantly reduced ($P > 0.05$) in elevated $[CO_2]$ (Fig. 2).

The single-factor temperature model: On a representative day in August 2004 when sapwood temperatures ranged between 18 and 29 °C (Fig. 3), we tested the single-factor temperature model. Stem CO_2 loss at breast height was smaller in the elevated $[CO_2]$ plots compared to the ambient $[CO_2]$ plots. Modelled rates of E_s (*i.e.* R_s) in elevated $[CO_2]$ were up to 76 % lower compared to ambient $[CO_2]$. This trend was confirmed by a decrease down to 55 % of independently measured CO_2 efflux rates (E_s) in elevated $[CO_2]$ as compared to ambient $[CO_2]$. In elevated $[CO_2]$, the effective CO_2 efflux was underestimated by our model by on average 31 %, whereas in ambient $[CO_2]$, the model overestimated the measured CO_2 efflux with on average 15 %. This might have increased the chance to find a significant decrease of Q_{10} and E_{10} in elevated $[CO_2]$, without actually being an effect (type I error). However, the *ANOVA* test did not indicate any significant CO_2 effect.

Absolute and relative growth rates: Poplar shoots grew fast in early spring (Fig. 4), but aboveground growth declined afterwards. During the third growing season, there was no significant stimulating effect of elevated $[CO_2]$ or fertilization on overall aboveground poplar growth. In contrast, the relative (RGR) and absolute (AGR) growth rates of the largest shoot (class C) significantly decreased in elevated $[CO_2]$ (interaction CO_2 × Class: $P < 0.05$ for RGR_{year} and AGR; $P < 0.0001$ for AGR_{year}; Fig. 4). Effects of fertilization were small and restricted to an increase of AGR_{year} of only the largest shoot class (significant Class × F interaction, $P < 0.01$). The specific CO_2 efflux (E_{10}) and values of Q_{10} were not significantly correlated with AGR and RGR ($P > 0.05$); variation in E_{10} and Q_{10} was mainly explained by shoot size. During the two measurement periods (March and end of summer, indicated with two arrows in Fig. 4), growth of poplar shoots was insignificant.

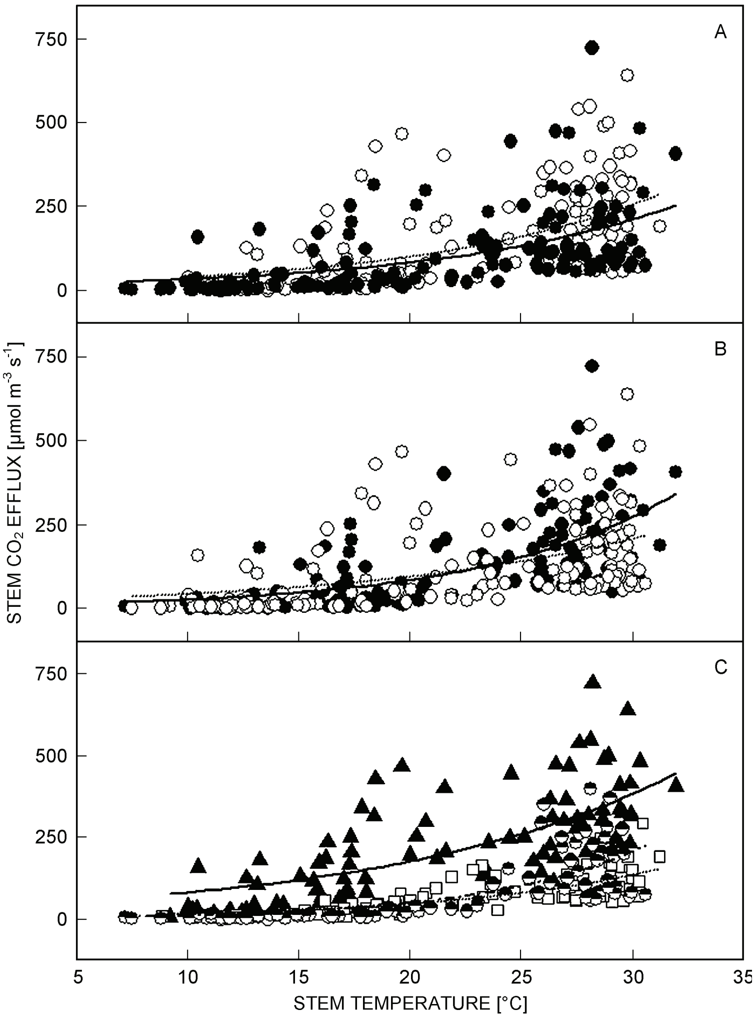

Fig. 1. CO_2 efflux as a function of stem temperature measured on shoots of *P. nigra* belonging to three different size classes (A, B, C; A - smallest size, C - largest size). CO_2 efflux was expressed on a volume base, as the young shoots consisted mainly of living, respiring sapwood. Raw data were separated by CO_2 treatment (*A*, ambient [CO_2]- *open circles*, elevated [CO_2] - *closed circles*), fertilization (*B*, unfertilized - *open circles*, fertilized - *closed circles*) and size class (*C*, A class - *triangles*, B class - *squares*, C class - *circles*). Lines indicate exponential fittings ($E_s = E_{10} \times Q_{10}^{[(T-10)/10]}$ of raw data; *full line* - elevated [CO_2] (*A*), fertilized treatment (*B*), or A-class (*C*), *dotted line* - ambient [CO_2] (*A*), unfertilized treatment (*B*) or B-class (*C*), *dash-dotted line*: C-class (*C*).

Discussion

The effects of elevated [CO_2], fertilization and shoot size on stem CO_2 efflux: After six years of growth in elevated [CO_2], no significant overall effect of CO_2 on specific stem CO_2 efflux rates of poplar shoots could be detected: Q_{10} and E_{10} did not vary between CO_2 treatments when averaged over three shoot size classes. This observation confirmed results of the first rotation of the EUROFACE (POPFACE) plantation: Gielen *et al.* (2003) did not find any significant effect of elevated [CO_2] on total respiration, supported by the absence of a CO_2 effect on stem growth and wood density during the first rotation. In a study on stem respiration rate of *Quercus alba* growing in elevated [CO_2], neither growth nor maintenance respiration of stems were affected by elevated [CO_2], but respiration was consistent with faster stem growth and larger stem volume in elevated [CO_2] (Wullschleger *et al.* 1995). However, in a study on Norway spruce (Janouš *et al.* 2000), specific stem respiration decreased under CO_2 enrichment, possibly by a nitrogen deficiency of the wood combined with a lower portion of living parenchyma cells and a decreased stomatal conductance, decreasing CO_2 release from the sap flow. In contrast, Edwards *et al.* (2002) found in their study on *Liquidambar styraciflua* that elevated CO_2 increased both growth and maintenance respiration. The authors concluded that this increase was driven by an increased supply of substrates rather than by a mechanistic change in the processes involved in respiration. This diversity in responses of CO_2 efflux to elevated [CO_2] should be interpreted considering the physiological difference between deciduous and evergreen trees.

Growth rate of poplar trees was negligible during the measurement periods (Fig. 4). Therefore, we postulate that the CO_2 efflux from the stem resulted primarily from the maintenance of the wood. However, it is known that respiration can lag behind variations in growth by 20 to 40 d, as wood synthesis occurs after cell expansion (Edwards and Hanson 1996). During the third growing season, largest shoots grew slower in elevated [CO_2] compared to ambient [CO_2] (Fig. 4), although a study on leaf photosynthesis in the same year indicated that the CO_2 uptake of poplars was still enhanced in elevated [CO_2] (Liberloo *et al.* 2007). Probably ambient [CO_2] trees were catching up slightly with elevated [CO_2] trees in terms of exploring the larger available space left in the canopy, and were the increased amounts of taken-up sugars in elevated [CO_2] trees transported to the roots.

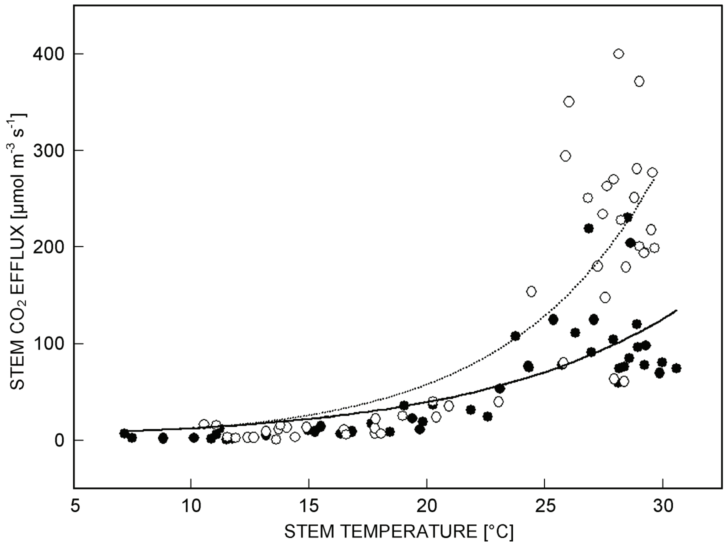

Fig. 2. Stem CO_2 efflux as a function of temperature of large C-class shoots of *P. nigra* growing in ambient (*open circles*) and elevated [CO_2] (*closed circles*) treatments. Data are pooled over fertilization treatments and were taken in March and August - September of the third growing season of the second rotation.

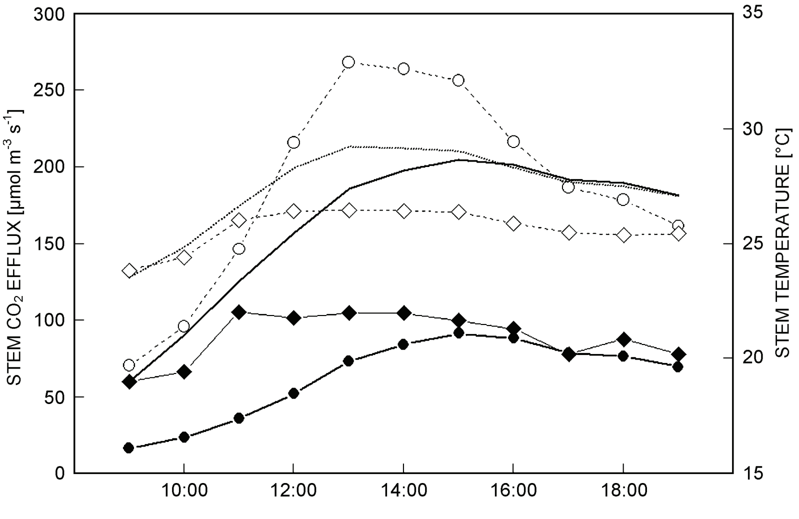

Fig. 3. Stem CO_2 efflux (E_s) during the course of a representative day in August 2004 of a C-class shoot growing in an unfertilized ambient (*open diamonds*) and elevated [CO_2] (*closed diamonds*) plot. Temperature of the sapwood under the bark is given (*full line* - elevated [CO_2], *dotted line* - ambient [CO_2]). Modelled stem CO_2 efflux (R_s), calculated with fitted E_{10} and Q_{10} values, under unfertilized, ambient (*open circles*) and elevated [CO_2] (*closed circles*) conditions are indicated.

If this is true, then the decreased aboveground growth in elevated [CO_2] in the period before the measurements (June - July; Fig. 4) could possibly explain the lower CO_2 efflux we found for the largest shoots in elevated [CO_2].

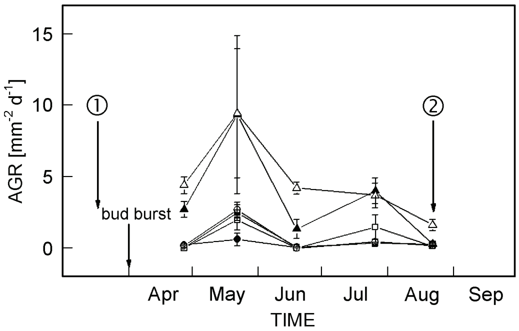

Fig. 4. Course of absolute growth rate (AGR) of three size classes of shoots (A-class - *squares*, B-class - *dots*, C-class - *triangles*), of *Populus nigra*, growing in ambient (*open symbols*) and elevated (*closed symbols*) [CO_2] under fertilized and unfertilized conditions during the growing season 2004. Values are pooled across fertility levels as fertilization did not affect AGR. The two measurement periods are indicated with *arrows* (1, 2), and the moment of bud burst is presented.

Nevertheless, if growth did not affect the stem CO_2 efflux during the measurement periods, any change in the tissue N content could still have altered the rate of maintenance respiration (R_m), as R_m and tissue nitrogen content are strongly correlated (Ryan 1991, Maier 2001). After all, nitrogen shortages induced by accelerated growth in elevated [CO_2] could cause lower N concentrations in plant tissues (Norby *et al.* 1999). It was not possible to make destructive harvests to determine the carbohydrate and nitrogen concentrations of the poplar stems at the time of measurements. However, in our study elevated [CO_2] did not decrease the CO_2 efflux in the unfertilized plots. Several other studies in the EUROFACE project could not detect any effect of fertilization on poplar trees growing in elevated [CO_2] (Calfapietra *et al.* 2005, Liberloo *et al.* 2005, 2006, 2007). Soil analysis indicated that the nitrogen availability in the EUROFACE plantation did not decrease, even after six years of growth in elevated [CO_2] (Hoosbeek *et al.* 2004, Liberloo *et al.* 2006). We attributed this to the high rates of fertilization during the previous agricultural land use (Liberloo *et al.* 2006).

Our results demonstrated that specific rates of stem CO_2 efflux (E_{10}) declined with increasing plant size in agreement with prior studies (Carey *et al.* 1997, Levy and Jarvis 1998, Tjoelker *et al.* 1999, Ceschia *et al.* 2002). The amount of living cells is one of the main causes of spatial variation in woody tissue respiration (Sprugel *et al.* 1995). The periderm contains the largest amount of living cells, and the proportion of the periderm compared to the other tissues decreases when the diameter of the organ increases (Ceschia *et al.* 2002). Additionally, a larger sapwood width in larger trees might increase the resistance to diffusion of CO_2. The fact that CO_2 effects differed between size classes underlines the importance of differentiating size classes when estimating the carbon budget of an entire population.

The single-factor temperature model: Stem temperature, the commonly accepted variable to predict stem respiration, could not satisfactorily explain the observed variation in stem CO_2 efflux. Q_{10} values of the largest shoots were very high when stem temperature was the only explaining variable in the model. When we tested the model (Fig. 3) during the summer, the highest variation was found around midday, decreasing towards the evening. We postulate that the classical approach of analyzing stem CO_2 efflux into its components of maintenance and growth respiration explained only by variations in stem temperature has serious weakness as other processes must have interfered with our measurements.

First, it has recently been shown that CO_2 transport with the sap flow can strongly interfere with the total amount of CO_2 diffusing from out of the stem (Bowman *et al.* 2005, Teskey and McGuire 2005). Levy *et al.* (1999) demonstrated that CO_2 in the sap, originating from the soil, could account for 12 % of peak stem respiration rates. However, Martin *et al.* (1994) found that transport of CO_2 in the sap flow could decrease CO_2 efflux by as much as 6.7 %. In a study on *Dacrydium cupressinum* trees (Bowman *et al.* 2005), a positive correlation between the difference between predicted and measured rates of CO_2 efflux and the maximum sap flux density suggested that larger amounts of CO_2 were transported by the sap flow when high sap flow was observed. As only the largest shoots had a considerable leaf area to drive the sap flow, this effect might have been of minor importance to the other two size classes, and explain why the largest shoots showed such high values of Q_{10}. The highest variation occurred around the warmest part of the day, when sap flow was large (Fig. 3). In addition, a study on the canopy transpiration of the EUROFACE trees (Marco Pecchiari *et al.*, in preparation) showed that the whole tree sap flow increased in elevated [CO_2], as the increased leaf area in elevated [CO_2] prevailed over the CO_2 effect that reduces stomatal conductance. A recent study on Norway spruce growing under ambient and elevated atmospheric [CO_2] (Kupper *et al.* 2006) also indicated an increased daily sap flux of the trees growing under elevated [CO_2], attributed to a higher soil water content and more economical water use under elevated [CO_2]. These findings support the hypothesis that the difference between CO_2 efflux of C-class shoots in ambient and elevated [CO_2] was caused by the transport of CO_2 with the sapflow, rather than by processes of growth or maintenance. After all, the increased sapflow in elevated [CO_2] could have carried up more of the respired CO_2 at breast height (*i.e.* the place were measurements were taken) up to the canopy, were it would diffuse as the higher stem temperatures at the top limit the amount of CO_2 that can dissolve in the sap.

Second, phenological processes might have interfered with our measurements in March. Trees were almost ready for bud set, and the increased transport of sugars to support the development of leaves could have disturbed our respiration measurements.

Third, the calculation of Q_{10} using different trees may have added to the variability in CO_2 efflux (Gielen *et al.* 2003). The use of a constant Q_{10} and E_{10} throughout the season might also have confounded our results (Carey *et al.* 1997, Stockfors and Linder 1998).

Conclusions: We conclude that elevated [CO_2] and fertilization did not significantly affect specific rates of stem CO_2 efflux of *P. nigra* shoots, when data were analysed with the commonly used single-factor temperature model. The stem CO_2 efflux depended strongly on shoot size, and tree size should therefore be taken into account when respiration measurements are used to scale-up to the stand level. Nevertheless, our results clearly show that great caution is necessary when interpreting stem CO_2 efflux measurements as woody tissue respiration. The large difficulties in separating the processes of respiration from the movement, storage and release of CO_2 with the sap flow hampers, apart from several necessary simplifying assumptions, to predict the respiratory carbon loss in a future high CO_2 world from a single-factor temperature dependent model. The development of models which combine the several processes influencing the effective CO_2 efflux from a stem is a challenge for future research, as a way to explore the still largely unknown area of woody tissue respiration.

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