Oecologia (2008) 158:1–10
DOI 10.1007/s00442-008-1118-7

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

The effect of carbon dioxide enrichment on apparent stem respiration from *Pinus taeda* L. is confounded by high levels of soil carbon dioxide

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Received: 5 March 2007 / Accepted: 13 July 2008 / Published online: 5 August 2008

Abstract Respiration supports growth and maintenance processes and returns a substantial portion of the CO_2 fixed by photosynthesis to the atmosphere each year. Investigating stem respiration using CO_2 flux measurements is complicated by uncertainty surrounding the source of CO_2 diffusing from tree stems. Over 2 years we measured the stem efflux from 24 trees exposed to ambient or elevated CO_2. The rate of stem CO_2 efflux increased with annual tree diameter increment and the estimated uptake of dissolved CO_2 from the soil. To determine the source of CO_2 diffusing from tree stems, we used the fumigation gas at the Duke Forest Atmosphere Carbon Transfer and Storage-1 elevated-CO_2 experiment as a ^{13}C tracer and measured the presence of soil CO_2 in stem efflux on a subset of these trees. The isotopic composition of soil CO_2 explained a considerable portion of the variation in the composition of CO_2 in stem efflux. We also found that direct measurements of the isotopic composition of phloem-respired CO_2, unlike the CO_2 found in stem efflux, was less variable and distinct from the isotopic composition of soil CO_2. Tree growth rates and soil CO_2 concentrations found at the site together explained 56% of the variance in stem CO_2 efflux among trees. These results suggest that the uptake of CO_2 dissolved in soil water and transported through the vascular system can potentially confound efforts to interpret stem efflux measurements in trees exposed to elevated CO_2 and that previous studies may have overestimated the effects of elevated CO_2 on autotrophic respiration in tree stems.

Keywords Carbon gain · Carbon isotope · Climate change · Forests · Woody respiration

Communicated by Rowan Sage.

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Introduction

Respiration by terrestrial vegetation releases ~60 Gt C to the atmosphere annually, nearly 10 times the amount released by anthropogenic emissions (Prentice et al. 2001), and changes in ecosystem respiration significantly affect the concentration of CO_2 in the atmosphere (Valentini et al. 2000). Forests are key drivers of the global C cycle (Goodale et al. 2002; House et al. 2003; DeLucia et al. 2005); woody respiration accounts for 5–30% of annual ecosystem respiration (Lavigne et al. 1997; Law et al. 1999; Hamilton et al. 2002). While wood respiration is a major component of the C budget of forests, its response to elevated CO_2 and other elements of global change is not well understood.

Human activities are dramatically increasing the concentration of CO_2 in the atmosphere. While elevated CO_2 often causes an increase in photosynthesis, its affect on stem respiration in forests is variable (Wullschleger et al. 1995; Carey et al. 1996; Janous et al. 2000; Ceschia 2002; Edwards et al. 2002; Gielen et al. 2003). Stem CO_2 efflux and carbohydrate content were higher in *Liquidambar styraciflua* trees grown at elevated CO_2 compared to those grown in ambient air (Edwards et al. 2002). Similarly, growth under elevated CO_2 stimulated stem efflux in *Quercus alba* (Wullschleger et al. 1995), *Pinus ponderosa* (Carey et al. 1996) and *Pinus taeda* L. (Hamilton et al. 2002). No consistent changes were found in *Populus* species after exposure to CO_2 enrichment for 3 years (Gielen et al. 2003), and growth at elevated CO_2 decreased stem CO_2 efflux in *Fagus sylvatica* (Ceschia 2002) and *Picea abies* (Janous et al. 2000).

Stem CO_2 efflux is regulated by factors in addition to tissue-specific respiration rates, potentially contributing to the variable responses to CO_2 enrichment. Recent evidence indicates that the rate of efflux is influenced strongly by variation in the concentration of CO_2 within the stem (e.g., Teskey and McGuire 2002; Teskey et al. 2005, 2007; Maier and Clinton 2006; Saveyn et al. 2008), which is in turn affected by height above ground, time of day, respiration of local tissues and the transport of CO_2 into and out of the section of stem (Teskey et al. 2008). The concentration of CO_2 within conifer stems ranges from 0.1 to 13.5% (Teskey et al. 2008) and was as high as 8% in *P. taeda* (Maier and Clinton 2006).

Stem efflux may increase in response to prolonged growth at elevated CO_2 because of greater respiration caused by increased substrate availability and plant growth rate (Hamilton et al. 2002; Davey et al. 2004; Gonzalez-Meler et al. 2004; Gonzalez-Meler and Taneva 2005). However, increases in fine root growth (Norby et al. 2004; Pritchard et al. 2008) and corresponding increases in CO_2 concentration below ground, may contribute to greater uptake of CO_2 from the soil in the transpiration stream and ultimately an increase in stem efflux.

The objective of this study was to examine the effect of growth under elevated CO_2 on the rate of stem efflux, and to characterize the potential contribution of soil-derived CO_2 to that diffusing from stems of *P. taeda* trees. The fumigation gas at the Forest Atmosphere Carbon Transport and Storage-1 (FACTS-1) research site is depleted in the heavier stable isotope of C (^{13}C; Andrews et al. 1999; Allen et al. 2000) and provides an opportunity to determine if soil CO_2 contributes directly to measured rates of stem efflux.

Materials and methods

We hypothesized that CO_2 efflux from *P. taeda* stems (1.4 m above the soil surface) was composed of a mixture of locally produced stem respiration and CO_2 transported by mass flow from the soil through the stem. Two approaches were used to test this hypothesis: we partitioned variation in the rate of CO_2 efflux from stems growing in ambient and elevated CO_2 by multiple regression and by analysis of covariance (ANCOVA), with tree growth rate and the CO_2 concentration in the soil air spaces as covariates; and we compared the isotopic composition of CO_2 diffusing from stems with that derived from autotrophic respiration and soil CO_2.

Site description

This research was conducted in 2003 and 2004 at the FACTS-1 research site, managed by Brookhaven National Laboratories and located in the Blackwood Division of Duke Forest in Orange County, North Carolina (35°58′N 79°05′W). Using free air carbon dioxide enrichment (FACE) technology, three of six experimental plots within a continuous unmanaged *P. taeda* plantation were exposed to CO_2 concentrations of ~200 μmol mol^{-1} above ambient levels, with a fumigation gas depleted in ^{13}C (Hendrey et al. 1999). Either ambient or CO_2-enriched air was forced through 32 vertical pipes that surround each 30-m-diameter plot. The pipes contained adjustable ports at 50-cm intervals along their length from the forest floor to above the 18-m-tall forest canopy, which allowed control of atmospheric CO_2 through the entire plot. In 2003 and 2004, fumigation resulted in an average daytime CO_2 concentration of ~577 μmol mol^{-1} in the elevated plots and ~383 μmol mol^{-1} in the ambient plots. Plots were not fumigated at night and the CO_2 concentrations at this time were similar in both treatment and control plots (~410 μmol mol^{-1}; K. Lewin, personal communication).

Measurements of stem CO_2 efflux and tree growth rate

The rate of CO_2 diffusing from the stems of 12 trees (four trees per plot) growing in ambient air and an equal number growing in +200 μmol mol^{-1} CO_2 was measured 2 and 3 times during the growing season of 2003 and 2004, respectively. To minimize the potential effects of CO_2 movement by sap flow, measurements were made at night when transpiration was at a minimum (Edwards et al. 2002; Maguire and Teskey 2004; Bowman et al. 2005). Collars (10-cm-diameter by 5-cm-deep PVC pipe) were installed in June 2003. Prior to installation a small portion of bark was removed from each tree without damaging the underlying cambium. Collars were secured to stems using non-hardening, gas-tight putty (Permagum; Virginia KMP, Dallas, Tex.), and were leak tested by sealing the front of each collar with a PVC cap and filling them with water. The amount of water was used to determine the volume of each collar.

The bark inside each collar was sterilized with a 2% $CuSO_4$ solution prior to each measurement. The rate of stem efflux was measured with a closed chamber (LI6400-09; LiCor, Lincoln, Neb.) and an infrared gas analyzer (LI6400; LiCor). To minimize diffusion between the chamber and the atmosphere, measurements were initiated at a CO_2 concentration near that of ambient nighttime air (~410 μmol mol^{-1}). Variation in stem temperature at night was low (<4°C) and we could not detect significant variation in the rates of stem CO_2 efflux associated with temperature.

The annual growth rate of trees ~1.4 m above ground was measured with a stainless steel dendrometer bands as in Naidu and DeLucia (1999) and Moore et al. (2006). Growth rate was expressed as basal area increment (cm^{-2} $year^{-1}$).

Sap flow measurements

The rate of sap flow was measured on each tree used for stem efflux measurements as in Schäfer et al. (2002). Granier-type sap flow sensors, each consisting of two probes (20 mm long × 2 mm diameter), were inserted in the outer xylem on the north side of each tree. One of the probes was heated and the other was placed 10 cm lower on the tree as a reference. Heat flux density was logged every 30 s and the 30-min averages were converted to sap flux density (Js; g_{water} m^{-2} sapwood) according to (Granier 1987). The volume of water transported by each tree in a day was calculated by multiplying the sap flux density for sensors at multiple depths by the cross-sectional area of functional sapwood as in Oren et al. (1998).

Soil CO_2 concentration

To determine the potential contribution of soil CO_2 to the rate of stem efflux, the concentration of CO_2 in air sampled from gas wells was compared to measured efflux rates. Wells were 15 cm and 30 cm below the soil surface; these depths were chosen because 90% of the fine roots of *P. taeda* in this forest are above 30 cm (Matamala and Schlesinger 2000). There were four gas wells at each depth in each experimental plot; the wells consisted of 5-cm-diameter PVC pipes open at the bottom and closed at the top. Two sealable 0.6-cm-diameter plastic tubes (Kynar; Arkema, Philadelphia, Pa.) projected from the top of each well. Air samples from each well were measured monthly with a portable infra-red gas analyzer (model EGM-1; PP Systems, Stotfold, UK) modified to read CO_2 concentrations between 0 and 100,000 μmol mol^{-1}. No tree was more than 3 m from a gas well. Dissolved CO_2 in soil water was assumed to be in equilibrium with soil air according to Henry's law. To calculate the amount of CO_2 transported from the soil by tree sap flow, the rate of water transport (l day^{-1}) for each tree was multiplied by the concentration of CO_2 dissolved in soil water measured at the nearest gas well. This estimate of CO_2 transported from the soil in the sap flow does not include CO_2 added by root and stem respiration.

The contributions of annual tree diameter increment and soil CO_2 concentration to stem efflux were estimated with one- and two-factor regression analysis (Proc Reg, SAS version 9.0; SAS, Cary, N.C.). In addition, the effect of growth in elevated CO_2 on the rate of efflux was estimated with a randomized complete block ANCOVA (Proc mixed, SAS) with diameter increment and the amount of CO_2 transported from the soil as covariates.

Ecosystem ^{13}C tracer

We used the isotopic label of the fumigation gas at the Duke FACE experiment to differentiate the contribution of soil CO_2 to stem efflux from that derived from local autotrophic respiration originating from the underlying cambium and ray parenchyma, plus CO_2 derived from root respiration and microorganisms in the rhizosphere consuming recently fixed C. The elevated-CO_2 plots are fumigated with CO_2 derived from natural gas that is strongly depleted in ^{13}C; consequently, the C isotope composition of CO_2-derived microbial respiration of old soil C was distinct from that derived from recently fixed CO_2, including CO_2 derived from stem respiration, root respiration or microbial respiration from root exudates.

The isotopic composition of the CO_2 diffusing from the stem ($\delta^{13}C_{efflux}$) was estimated from gas samples collected from stem collars installed on four or five *P. taeda* trees in two elevated-CO_2 plots in 2003 and 2004. Gas was sampled from 11 a.m. to 3 p.m. in June and August 2003, and July and August 2004. To test for differences in the isotopic composition of efflux between night and day, stem-respired CO_2 also was sampled between 11 pm and 3 am in August 2003 and July 2004. Gas samples were collected with 150-ml evacuated glass flasks as in Trueman and Gonzalez-Meler (2005). Samples were drawn through a water trap connected to the sealed chamber (LI6400-09; LiCor) and an infrared gas analyzer (LI6400; LiCor) at previously installed stem collars, and aliquots were collected at ~80 μmol mol^{-1} increments as CO_2 accumulated in the cuvette. An air-filled balloon inside the gas-exchange chamber was used to replace the air volume removed by sample collection to avoid pressure changes.

The Keeling plot approach was used to determine the $\delta^{13}C_{efflux}$ (Keeling 1958; Pataki et al. 2003). The isotopic composition of the efflux was estimated as the intercept of the regression of $\delta^{13}C$ against the reciprocal of the CO_2 concentration in the chamber (Keeling 1958). The intercepts of linear regressions between the $\delta^{13}C$ and the reciprocal of the CO_2 concentration of gas samples were

estimated by geometric mean regression (model II, Matlab 7.1 for Windows; MathWorks, Natick, Mass.). Each Keeling plot spanned a range of CO_2 concentration of ≥320 μmol mol^{-1}, and data with a coefficient of determination lower than 0.9 were omitted from analysis.

The isotopic composition of soil CO_2 ($\delta^{13}C_{soil}$) was measured from gas collected from wells near each tree as described above (Andrews et al. 1999). Gas samples were collected approximately 1–3 h before stem efflux measurements using evacuated (10^{-5} Pa) 75-cm^3 stainless steel gas cylinders (Whitey; Whitey, Highland Heights, Ohio) sealed with Nupro bellows valves equipped with Kel-F stem tips (Nupro, Willoughby, Ohio).

To determine the proportion of stem CO_2 efflux originating from local stem respiration, it would have been desirable to measure isotopic composition of CO_2 derived from the phloem and cambium (hereafter referred to as "phloem"), which presumably has the isotopic signature of recent photosynthate. Destructive sampling of phloem was not possible at the FACE experiment so the isotopic composition of CO_2 derived from autotrophic respiration ($\delta^{13}C_{auto}$) was determined by measuring gas respired by needles sampled from the upper crown. Fifteen times over two summers, needles were harvested 1–3 h before sampling stem efflux and incubated in a sealed non-porous PVC chamber connected to an infrared gas analyzer (LI-6252; LiCor) (Trueman and Gonzalez-Meler 2005; Hymus et al. 2005). The incubation chamber was flushed with CO_2-free air until the respiration rate became constant. The chamber was sealed and needle-respired CO_2 was allowed to accumulate. After 10–30 min, gas from needle respiration was collected in a glass flask connected to the chamber.

To confirm that needles were reasonable proxies for phloem we compared the isotopic composition of phloem-respired CO_2 and needle-respired CO_2 from trees outside the experimental plots. Needles were removed from the upper crown and phloem tissue was removed from the same tree no more than 3 h later. A total of six trees were sampled between June and October 2004. Each tree was sampled twice, allowing 2–3 weeks recovery time between samples. To estimate the $\delta^{13}C$ of CO_2 respired by needles and phloem, tissues were incubated and gases sampled as described above.

Gas samples from foliage, phloem, stem efflux and soil were purified by cryogenic extraction as in Trueman and Gonzalez-Meler (2005) and the isotopic composition of CO_2 was measured with an isotope ratio mass spectrometer (Finnegan MAT Delta plus XL; Finnegan MAT, Bremen, Germany) at the University of Illinois at Chicago. By convention, all values of $\delta^{13}C$ (‰) were expressed relative to the reference ratio of ^{13}C to ^{12}C in Pee Dee Belemnite as follows: $\delta^{13}C = [(R_{sample} - R_{reference})/R_{reference}] \times 1{,}000$, where $R = {}^{13}C/{}^{12}C$.

Inferring the source of stem CO_2 by isotope analysis

Typically, a two end-member mixing model would be used to determine the relative contribution of two sources of isotopically distinct CO_2 to a given flux (Ehleringer et al. 1993). In this case, uncertainties about potential fractionation of C isotopes during transport in the transpiration stream from the soil, into the roots and through the xylem precluded the use of a mixing model. Instead, a regression approach was used to estimate the sources of CO_2 diffusing from the stem. $\delta^{13}C_{efflux}$ was regressed against $\delta^{13}C_{auto}$ and $\delta^{13}C_{soil}$ individually and in a combined regression model. Given that $\delta^{13}C_{soil}$ was unrelated to $\delta^{13}C_{auto}$ ($P = 0.73$, $R^2 = 0.01$) the amount of variation explained by each of these factors indicates the relative contribution of each source to stem CO_2 efflux. The relationship between the C isotopic composition of stem efflux, needle respiration and soil CO_2 was determined by one- and two-factor regression analysis (Proc Reg, SAS version 9.0).

Results

When only the CO_2 treatment was considered in the statistical analysis, the rate of stem CO_2 efflux was greater for trees grown under elevated CO_2 (mean value for all trees and both years: 4.2 ± 1.8 SD μmol m^{-2} s^{-1}, $n = 56$) than ambient air (3.4 ± 1.6 SD μmol m^{-2} s^{-1}, $n = 58$). However, ANCOVA revealed that stem diameter increment ($F = 119.69$, $P < 0.001$) and soil CO_2 concentration ($F = 8.50$, $P = 0.004$) were significant covariates and when they were included in the analysis, there no longer was a significant difference in stem efflux between trees growing in ambient air and elevated CO_2. A multiple linear regression model indicated that stem diameter increment and CO_2 transported in sap flow together accounted for 61% of the variation in stem efflux (Table 1).

Instantaneous rates of stem CO_2 efflux were correlated with annual tree diameter increment (Fig. 1), and when measurements were pooled over both years, mean stem efflux of trees grown in ambient and elevated CO_2 was linearly correlated with the mean soil CO_2 concentration [efflux (μmol m^{-2} s^{-1}) = 2.47 + 4.15 × 10^{-5} × soil CO_2 (μmol mol^{-1}); $P < 0.05$, $R^2 = 0.54$]. The concentration of CO_2 in the soil air spaces was higher in elevated-CO_2 plots (combined depths averaged over both years: 19.2 mmol mol^{-1} ambient plots; 26.0 mmol mol^{-1} elevated plots; one tailed t-test, $t = 1.98$, $P = 0.06$). By multiplying the rate of water transport by the concentration of CO_2 dissolved in soil water, it was estimated that the amount of soil CO_2 transported by sap flow explained between 12 and 50% of the variation in stem efflux.

Table 1 Factors contributing to the variation in the rate of stem CO_2 efflux (μmol m^{-2} s^{-1}) measured at night from 22 to 24 *Pinus taeda* in 2003 and 2004 at the Forest Atmosphere Carbon Transport and Storage-1 (FACTS-1) research site. A multiple linear regression was used to estimate the effects of both stem diameter increment and CO_2 transported by sap flow from the soil air space on stem efflux. The model explained 61% of the variation in stem CO_2 efflux

		Sum of squares	Mean square	*F*-value	*P*-value
Overall model	2	203.04	101.52	86.21	<0.0001
Error	109	128.36	1.12	–	–

Variable	df	Estimate	SE	*t*-value	*P*-value
Intercept	1	0.82	0.25	3.29	0.0013
Diameter increment (cm $year^{-1}$)	1	5.74	0.44	13.07	<0.0001
CO_2 in sap flow (g CO_2 day^{-1})	1	0.28	0.13	2.21	0.0295

The $\delta^{13}C_{efflux}$ estimated from Keeling plots varied from values near the composition of autotrophic respiration (-45.16 ± 0.18‰) to values close to the composition of soil CO_2 (-33.22 ± 1.85‰; Table 2). The Keeling plots had coefficients of determination close to 1 and SEs of $\delta^{13}C_{efflux}$ were lower than 2‰ for most trees (Table 2). No difference in $\delta^{13}C_{efflux}$ was detected between samples collected during day or night.

The C isotopic composition of CO_2 derived from needle respiration explained 25% of the variation in $\delta^{13}C$ of CO_2 derived from the stem during the daytime ($P < 0.05$) and the isotopic composition of soil CO_2 explained 41% of the residual variation ($P < 0.05$; Fig. 2). A regression model including both factors explained 56% of the observed variation in the isotopic composition of efflux, indicating factors unaccounted for by this model had a considerable influence on the $\delta^{13}C$ of stem CO_2 efflux. The residual variation in this estimate was not explained by tree size, time of day or by the magnitude of the efflux.

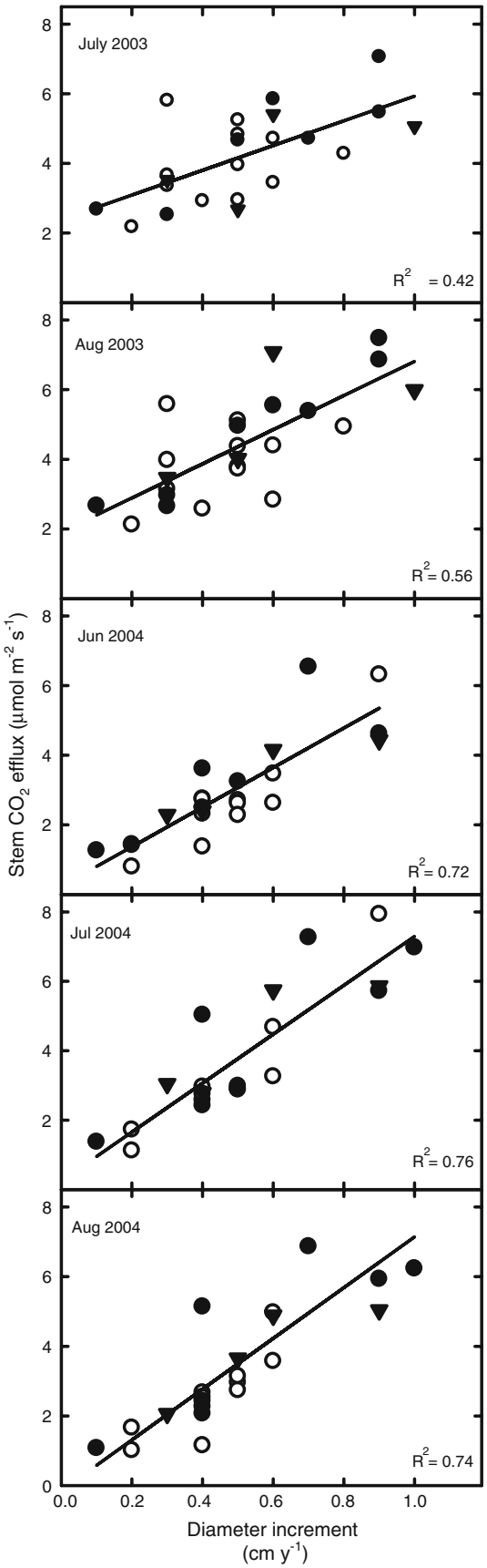
The mean value of $\delta^{13}C$ from needle (-28.39 ± 2.55‰) and phloem respiration (-28.38 ± 1.2‰) sampled outside the fumigated plots was the same (Fig. 3), which demonstrated that the isotope values for needle-respired CO_2 provided a reasonable estimate of the isotopic composition of C originating from phloem respiration ($\delta^{13}C_{auto}$).

The $\delta^{13}C$ values for CO_2 derived from excised phloem were less variable than for needle-respired CO_2 (Fig. 3), indicating that extreme values of either enriched or depleted photosynthate were buffered when mixed with older photosynthate and transported down the stem. While the range of values for the $\delta^{13}C$ composition of needle-respired CO_2 was almost identical in the fumigated and non-fumigated trees (~12‰, Fig. 3; Taneva et al. 2006), the range of efflux values was more than twice that observed in the excised phloem tissue and was skewed toward the isotopic value of soil CO_2, indicating that a source of CO_2 other than that provided by current photosynthesis was present in some of the efflux samples (Fig. 3). In contrast to the $\delta^{13}C$ of efflux measurements within the fumigated plots, approximately 47% of the variation in the isotopic composition of phloem-respired CO_2 was explained by the composition of needle-respired CO_2 from the same trees ($P < 0.05$). The signature of the CO_2 in stem efflux appears to be a mixture of both needle-respired CO_2 and soil CO_2 (Fig. 3).

Discussion

The rate of CO_2 diffusing from tree stems is a poor measure of wood respiration because efflux is strongly influenced by stem CO_2 concentration (Teskey and McGuire 2002, 2005; Maier and Clinton 2006; Teskey et al. 2007, 2008; Saveyn et al. 2008). In some cases, the rate of stem CO_2 efflux is entirely independent of the actual rate of wood respiration (Teskey et al. 2007). The average rate of CO_2 efflux from *P. taeda* stems was higher in trees grown in elevated compared to ambient CO_2. However, tree growth rate and soil CO_2 concentration contributed substantially to the variation in the rate of efflux (Table 1) and the isotopic composition of the CO_2 diffusing from the stem suggests that a portion of the CO_2 diffusing from *P. taeda* stems originated from soil-derived CO_2 (Fig 2). For *P. taeda* as well as other tree species (Teskey et al. 2008), the uptake of CO_2 dissolved in soil water and transported through the vascular system can potentially confound efforts to accurately measure woody respiration under field conditions.

By increasing photosynthesis (Crous and Ellsworth 2004), exposure to elevated CO_2 increased the rate of diameter growth in *P. taeda* (Moore et al. 2006; Finzi et al. 2006; Norby et al. 2005), and rates of stem CO_2 efflux were correlated with faster rates of wood production. Annual stem diameter increment explained between 42 and 74% of the variation in stem CO_2 efflux (Fig. 1), which is consistent with Damesin et al. (2002). However, it is unlikely that tissue-specific rates of respiration were affected by growth under elevated CO_2. Respiration associated with the construction of new biomass is the same (Hamilton et al. 2002), and N concentration, a proxy for maintenance respiration, is similar or lower for *P. taeda* stems growing in ambient and elevated CO_2 (Finzi et al. 2002, 2006). Previous research concluded that respiration per unit growth either increases (Carey et al. 1996; Edwards et al. 2002) or remains constant (Wullschleger et al. 1995; Gielen et al. 2003) with elevated CO_2, but these studies did not consider



◀ **Fig. 1** The rate of stem CO_2 efflux (µmol m^{-2} s^{-1}) measured at night from 22 to 24 *Pinus taeda* trees with respect to annual stem diameter increment (cm y^{-1}). Trees were grown in either ambient (*open symbols*) or elevated CO_2 (*closed symbols*) and *triangles* refer to trees from which isotopic gas samples were drawn. Fumigation began in 1997 and stem CO_2 efflux was measured during the growing season of 2003 and 2004. Efflux rates, predicted using linear regression, are plotted as *lines* and the R^2 for each regression (where $P < 0.05$) is shown. *Jun* June, *Jul* July, *Aug* August

the potential contribution of CO_2 originating from the soil in their estimates of stem efflux.

Greater rates of stem CO_2 efflux in rapidly growing trees may be related to greater CO_2 storage in the stems of large trees or increased uptake of dissolved CO_2 in the transpiration stream. In *Platanus occidentalis* approximately half of the CO_2 diffusing from stems originates from non-local sources (Teskey and McGuire 2007). The CO_2 concentration at the base of *P. occidentalis* stems was correlated with the diameter of the stem and the authors concluded that larger stems have larger root systems and would therefore transport more autotrophic CO_2 from their own root systems upwards into the main stem (Teskey and McGuire 2007). It also is possible that larger root systems would accumulate greater quantities of CO_2 dissolved in water taken up by the roots from the soil water.

Greater root production and root biomass, as well as greater aboveground litter inputs (Hamilton et al. 2002; Taneva et al. 2006; Pritchard et al. 2008) contributed to higher CO_2 concentrations in the soil in elevated- compared to ambient-CO_2 plots (Bernhardt et al. 2006). Mean efflux from stems of ambient and elevated-CO_2 trees was positively correlated with mean soil CO_2 concentration, suggesting that a portion of CO_2 diffusing from stems was derived from the soil. In a study of *L. styraciflua* and *P. occidentalis*, stem CO_2 concentration was varied by placing cut tree stems in water of varying CO_2 concentrations, and the rate of stem efflux increased linearly with CO_2 concentration (Teskey and McGuire 2005), demonstrating that CO_2 in solution can be transported through the stem via the vascular system. This suggests that CO_2 in the soil solution could have affected directly the measured rates of efflux in our study.

The CO_2 in the soil airspaces is a mixture of that produced from root respiration and microbial respiration of both new and old C substrates. To evaluate the contribution of soil CO_2 from microbial respiration of old substrates on stem efflux, we relied on the depleted ^{13}C signature of newly fixed C in trees exposed to elevated CO_2. The isotopic composition of respired needle CO_2 ($\delta^{13}C_{auto}$) explained only 25% of the variation in measured efflux; however, 41% of the residual variation was explained by the isotopic composition in soil CO_2 (Fig. 2). The combined regression

Table 2 The isotopic composition of CO_2 diffusing from *P. taeda* stems ($\delta^{13}C_{efflux}$) measured during the 2003 and 2004 growing seasons at the FACTS-1 research site. Measurements were made during the day or at night. The $\delta^{13}C_{efflux}$, its SE, and the coefficient of determination of the Keeling plot used to calculate each value are shown for each sampled tree

Date	Tree	$\delta^{13}C_{efflux}$	Day			Night	
			SE	R^2	$\delta^{13}C_{efflux}$	SE	R^2
June 2003	38,035	−39.35	2.62	0.983	–	–	–
June 2003	33,006	−33.42	1.85	0.976	–	–	–
June 2003	48,013	−35.92	1.44	0.991	–	–	–
June 2003	47,010	−41.98	3.96	0.947	–	–	–
August 2003	38,035	−44.92	4.66	0.936	−40.82	1.39	0.993
August 2003	33,006	−36.04	0.6	0.997	−43.13	1.26	0.995
August 2003	48,013	−45.06	0.18	1	−40.63	2.68	0.975
August 2003	47,010	−40.64	1.11	0.994	−39.82	0.8	0.999
July 2004	33,006	−40.77	0.08	1	−35.54	0.71	0.997
July 2004	37,048	−44.93	1.3	0.991	−41.56	1.1	0.995
July 2004	38,035	−37.98	0.58	0.998	−37.41	0.05	1
July 2004	47,010	−38.14	2.03	0.979	−43.82	1.43	0.997
August 2004	33,006	−42.53	0.1	1	–	–	–
August 2004	47,010	−40.87	1.73	0.986	–	–	–
August 2004	48,013	−43.66	2.56	0.978	–	–	–

model which included both CO_2 sources explained 56% of the observed variation in the isotopic composition of stem efflux (Fig 2). This relatively low coefficient of determination may be explained by fractionation associated with movement of sugars throughout the stem (Damesin and Lelarge 2003) and utilization of C fixed at a previous time (Brandes et al. 2006). Also, the substrate used for respiration in the stem would be a mixture of C fixed by the entire canopy (not just sun needles) under variable weather conditions and C that has been stored for weeks or longer (Körner 2003; Trumbore 2006). That direct measurements of the isotopic composition of phloem-respired CO_2 were less variable than CO_2 derived from needle-respired CO_2 (Fig. 3), supports the contention that respiration in the stem relies on substrate integrated over the entire canopy.

When we compared $\delta^{13}C$ in CO_2 respired directly by phloem with stem efflux, we found that the range of values for $\delta^{13}C_{efflux}$ (11.5‰) was more than twice the range for CO_2 respired from phloem (4.7‰). While the $\delta^{13}C$ of needles and phloem respiration were similar in trees outside the fumigated plots, the $\delta^{13}C$ of stem efflux deviated strongly from the $\delta^{13}C$ of needle respiration in the direction of the $\delta^{13}C$ of soil CO_2 (Fig. 3), consistent with the presence of the more enriched soil CO_2 in stem efflux. Since our method precludes detection of recycled autotrophic CO_2 respired by the root system, we conclude that non-autotrophic soil CO_2 represents a significant contribution to the CO_2 diffusing from the stem.

The isotopic analysis suggests that non-autotrophic CO_2 rather than CO_2 from root respiration could explain the difference in stem efflux between ambient and elevated CO_2 (Table 1). The efflux values presented in this manuscript are consistent with previous studies of tissue construction cost and respiration of excised tissues (Hamilton et al. 2001, 2002) in that, despite an apparent effect of elevated CO_2 on stem efflux, there is no evidence for a treatment effect on autotrophic respiration.

The potential contribution of soil CO_2 to the measured efflux can be estimated from the difference in efflux rate attributed to a 1% increase in soil CO_2 (0.277 μmol m^{-2} s^{-1} per 1% for *L. styraciflua*; Teskey and McGuire 2005). Teskey and McGuire (2005) found that the rate of efflux varied linearly with stem CO_2 concentration in trees severed at the base and placed in water with low (0.04%), medium (8.8%) or high (14.1%) concentrations of CO_2. By comparison, soil CO_2 concentration in air spaces in our study ranged from 1.8% to approximately 7.2% with an average value of 2.6% (26,000 μmol mol^{-1}) in the elevated plots.

If we take the average soil CO_2 concentration (2.6%) and the average efflux rate (3.81 μmol m^{-2} s^{-1}) measured in this study and apply the 0.277 μmol m^{-2} s^{-1} per 1% contribution estimated for *L. styraciflua* (Teskey and McGuire 2005), we find that 19% of the average efflux is contributed by CO_2 taken up from the soil and not respired by local woody tissues. This value is based on studies of a different species with different bark and cambium characteristics and therefore it is only an estimate of the contribution of soil CO_2. Given that the soil CO_2 concentration was as high as 7.2% in the middle of the summer in 2004, failure to account for the effect of CO_2 transported from the soil could lead to considerable overestimation of woody respiration based on measurements low on the stem.

Using the daily transpiration rate of *P. taeda* measured in 2003 (R. Oren, unpublished results) and the average soil

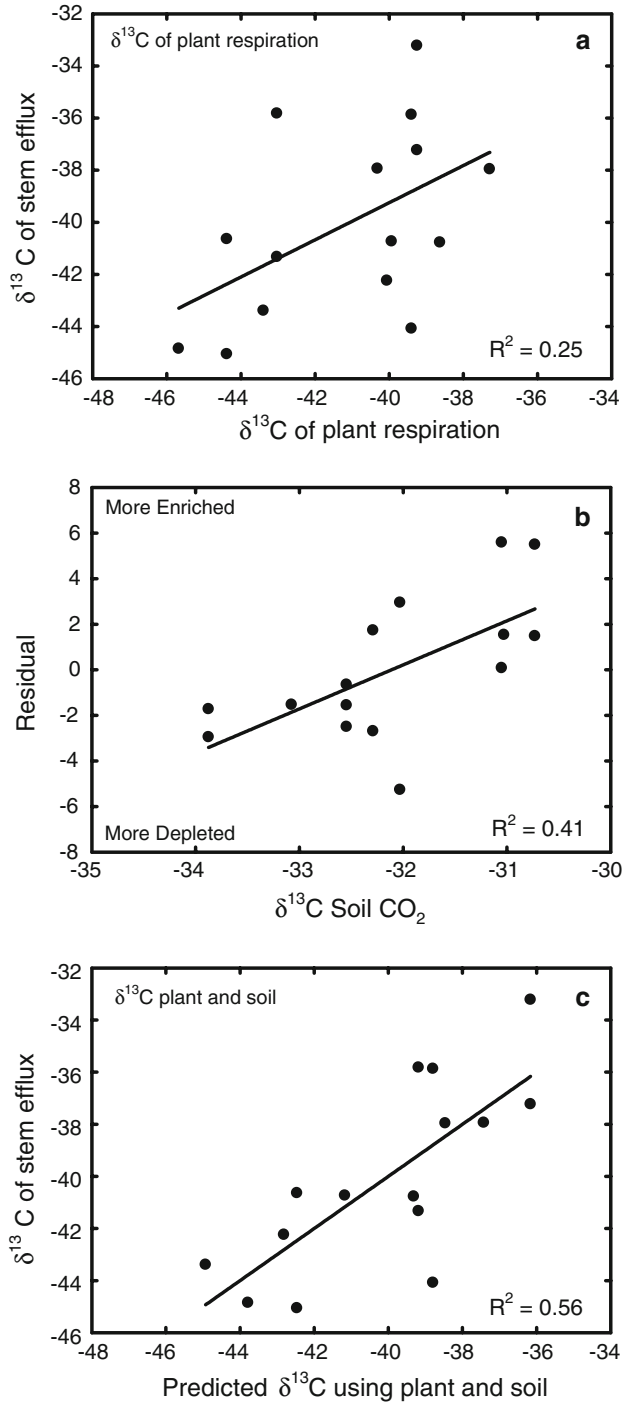


Fig. 2 The contribution of plant respiration and soil CO_2 to the isotopic composition of stem CO_2 efflux in a *P. taeda* at the Forest Atmosphere Carbon Transport and Storage-1 (FACTS-1) research site in 2003 and 2004. The C isotopic composition of CO_2 diffusing from the stem at 1.4 m ($\delta^{13}C_{efflux}$) as a function of the C isotope composition measured in plant respiration ($\delta^{13}C_{auto}$) on the same tree (**a**), the residual variation not explained by $\delta^{13}C_{auto}$ as a function of the $\delta^{13}C$ of soil air at 15 and 30 cm (**b**), and the $\delta^{13}C_{efflux}$ in relation to the predicted $\delta^{13}C_{efflux}$ by a multiple linear regression including both plant respiration and soil CO_2 as sources of variation (**c**). Gas was sampled from 11 a.m. to 3 p.m. in June and August 2003, and July and August 2004 on four or five *P. taeda* trees in two elevated-CO_2 plots

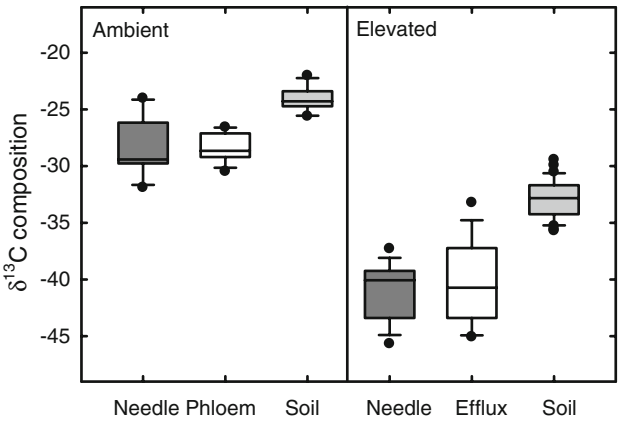


Fig. 3 The range of C isotopic composition in plant respiration, stem efflux, and soil CO_2 in non-fumigated and fumigated portions of a *P. taeda* forest at FACTS-1 in summer 2003 and 2004. The *left panel* shows the C isotopic composition of CO_2 respired ($\delta^{13}C$) by excised *P. taeda* needles, excised phloem tissue, and for soil CO_2 outside the fumigated plots. The *right panel* shows the C isotopic composition of CO_2 respired ($\delta^{13}C$) by excised *P. taeda* needles, diffusing from the bole, and soil CO_2. Tissue was removed from the same trees during the same time period. The *solid line* in each box represents the median, the *error bars* represent the 95th and 5th percentiles and each outlier is plotted as a *filled circle*. Note that phloem could not be harvested from trees in the elevated-CO_2 plots, and Keeling plot were generated only for trees growing in these plots. All $\delta^{13}C$ values were more negative for CO_2 derived from tissues harvested in the elevated-CO_2 plots, reflecting depletion of ^{13}C in the injection gas used to fumigate these plots

CO_2 concentration above depths of 30 cm, we estimate that 100 trees would remove 87 g C as CO_2 from the soil by mass flow each day. Since transpiration occurs most of the year in this forest these calculations suggest that between 24 and 45 g C m^{-2}, or approximately 10% of the amount of C thought to be released by woody respiration (Hamilton et al. 2002), is taken up by roots from the soil annually. This "soil flow" is equivalent to up to 4% of soil respiration. It has been concluded previously that 95–99% of plant C gain is derived from the atmosphere while the remainder is obtained from root uptake (Enoch and Olesen 1993); 45 g C m^{-2} is approximately 2% of gross primary production at this forest (DeLucia et al. 2006).

In absolute terms, CO_2 transported from the soil is a small C flux; however, our results suggest that the effect of this transport on current estimates of autotrophic respiration could be considerable, especially in assessing the difference in forest function under current ambient and future elevated atmospheric CO_2 levels, where soil contributions are likely to be different between the treatment and control. Because efflux measurements made at 1.4 m on the tree typically are scaled up to estimate stand-level woody respiration, errors caused by transport of soil CO_2 would propagate in any scaling calculation.

The efflux of CO_2 from tree stems often is mistakenly reported as woody respiration. Though not true at all times

of the year (Maier and Clinton 2006), it is now evident that the transport and concentration of CO_2 within tree stems exerts a strong influence on the CO_2 efflux from the stem (Negisi 1978; Hari et al. 1991; Martin et al. 1994; Teskey and McGuire 2005). The high, fluctuating CO_2 concentration within tree stems is thought to be associated with transport of plant-respired CO_2 in the xylem sap (Negisi 1978; Hari et al. 1991, Teskey and McGuire 2002). We suggest that when the soil CO_2 concentration is high a substantial portion of the stem CO_2 low in the stem actually is derived from the soil. Given the potential magnitude of the errors this may introduce to estimates of stem respiration, ecosystem-level estimates of woody respiration based on stem efflux rates should be revisited.

Acknowledgements This research was supported by the Office of Science BER Program, US Department of Energy with additional support by grants from TECO-DOE (DE-FG05-95ER62083), NASA (TE/97-20024), and NSF-IOB (0528069). We gratefully acknowledge G. R. Hendrey, K. F. Lewin, J. Nagy, A. Palmiotti, G. Hon, R. LaMorte, A. Mace, and R. Nettles (Brookhaven National Laboratory), and S. Oleynik (UIC) for logistical support. We thank J. Edeburn and the staff of the Duke Forest for operational support at the field site. Statistical advice and comments on earlier drafts of this manuscript by S. Long, F. S. Hu, F. Miguez, A. Leakey, R. Gallery, R. Knepp, M. Prater, O. Dermody, E. Ainsworth, J. Tang, M. Aldea, A. Malmberg, J. Hu and R. Oren were greatly appreciated. This research was carried out in compliance with the laws of the United States of America.

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