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REGULAR ARTICLE

Seasonal and daily time course of the ^{13}C composition in soil CO_2 efflux recorded with a tunable diode laser spectrophotometer (TDLS)

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Abstract Temporal variations of carbon isotope composition of soil CO_2 efflux (F_S and $\delta^{13}C_{FS}$) at different time scales should reflect both temporal variations of the climate conditions that affect canopy functioning and temporal changes in the relative contribution of autotrophic respiration to total F_S. A tunable diode laser spectrophotometer (TDLS) was installed in the Hesse forest (northeast of France) early during the 2007 growing season to determine the seasonal and daily variability in $\delta^{13}C_{FS}$. This method, based on the measurement of the absorption of an infrared laser emission at specific wave lengths of the $^{13}CO_2$ and $^{12}CO_2$, allows the continuous monitoring of the two isotopologues. The concentrations of the two isotopologues in F_S were continuously monitored from June to November 2007 using chamber method and Keeling plots drawn from nocturnal accumulation of CO_2 below the canopy. These TDLS measurements and isotope ratio mass spectrometer based Keeling plots gave very similar values of $\delta^{13}C_{FS}$, showing the reliability of the TDLS system in this context. Results were analysed with regard to seasonal and daily changes in climatic and edaphic variables and compared with the $\delta^{13}C$ of CO_2 respired by roots, litter and soil incubated under controlled conditions. Pronounced daily as well as seasonal variations in $\delta^{13}C_{FS}$ were recorded (up to 1.5‰). The range of variation of $\delta^{13}C_{FS}$ was of the same order of magnitude at both diurnal and seasonal scales. $\delta^{13}C_{FS}$ observed in the field fluctuated between values of litter and of root respiration recorded during incubation, suggesting that temporal (and probably spatial) variations were associated with changes in the relative contribution of the two compartments during the day and during the season.

Keywords $\delta^{13}C$ · Soil CO_2 efflux · Root and litter respiration · TDLS · Daily and seasonal time scales · Beech forest

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Introduction

At an ecosystem scale, soil CO_2 efflux (F_S) is the largest respiratory flux (60% to 80% of the total forest ecosystem respiration) and the second largest carbon flux after the gross primary productivity under temperate latitudes (Janssens et al. 2001). Uncertainty remains about whether or not climate changes will lead to an enhanced decomposition of the large

carbon pool stored in soils, and whether forest ecosystems will be net carbon sinks or sources (Davidson et al. 2000; Ekblad et al. 2005).

Measurements of the natural abundance of carbon 13 (^{13}C) in the different ecosystem compartments have previously been used to improve our knowledge of carbon exchanges in the soil-plant-atmosphere system when the compartments present different carbon isotope signatures (Rochette and Flanagan 1997). The natural abundance in ^{13}C (and by extension the carbon isotope composition, $\delta^{13}C$) in photosynthates is variable and changes rapidly with environmental parameters. Notably, stresses such as drought, high vapour pressure deficit, low irradiance or cold temperature alter CO_2 assimilation rate and stomatal conductance causing variable carbon fractionation during photosynthesis. The temporal variation in photosynthate $\delta^{13}C$ is transferred along the plant-soil pathway and retrieved in each plant compartment as well as in ecosystem respiration (Bowling et al. 2002, 2008; Alstad et al. 2007; Kodama et al. 2008). Thus changes in photosynthetic carbon isotope discrimination are predictably reflected in $\delta^{13}C$ when the assimilated carbon is respired (O'Leary 1981; Farquhar et al. 1989). However, post-photosynthetic and respiratory carbon isotope fractionation as well as their temporal variation might be of importance and might have the potential to partially uncouple the respiratory isotope signal from photosynthetic carbon isotope discrimination (Badeck et al. 2005; Gessler et al. 2007, 2008; Kodama et al. 2008). The study of the pathways and rates of carbon transfer within plants and to the soil from photosynthetic fixation to respiratory losses can thus be attempted using the $\delta^{13}C$ signatures of the different compartments.

Until recently, methods used to estimate $\delta^{13}C$ of the different ecosystem compartments and fluxes, and notably $\delta^{13}C$ of F_S ($\delta^{13}C_{FS}$), did not allow the capture of the short-term temporal variations (hourly, daily). Consequently, these potential variations (1) have not been yet integrated in the models and in the result analyses of prior experiments and (2) could improve the accuracy of the partitioning of net ecosystem exchange (measured by eddy covariance techniques) between gross primary productivity and ecosystem respiration (Bowling et al. 2003). Many studies in the last decade have examined the carbon isotope composition of CO_2 respired by terrestrial ecosystems using the two-component gas mixing model introduced by Keeling (1958). Such measurements give insights about the temporal variability of global ecosystem carbon isotope composition but no information about the signature of individual respiration components. This is considered as a critical issue for our understanding of the total carbon budget and for our ability to model carbon fluxes (Lloyd and Farquhar 1994; Buchmann et al. 1997). Tunable diode laser spectroscopy (TDLS) is a relatively new method for online measurements of the stable isotope composition of atmospheric CO_2 and has already provided valuable information on ecosystem scale studies on an hourly basis (Bowling et al. 2003). This method, based on the measurement of the absorption of an infrared laser emission at the specific wave lengths of $^{13}CO_2$ and $^{12}CO_2$, allows monitoring the two isotopologues at a 5 min temporal resolution.

At an ecosystem level, this new technique may help to answer the following fundamental questions: (1) what is the range of variation in $\delta^{13}C_{FS}$ at hourly, daily and seasonal time scales, (2) what are the relationships between the fine temporal variation of $\delta^{13}C_{FS}$ and the climate conditions that affect canopy functioning, and (3) are these relationships the same whatever the considered temporal scale, *i.e.* day versus season. In this context, the objectives of our study were: (i) to validate *in situ* high frequency measurements of $^{13}CO_2$ and $^{12}CO_2$ fluxes using a TDLS as a promising tool to monitor carbon isotope composition of F_S, (ii) to examine short term and seasonal variations in isotopic signature of F_S in relation with the climate conditions that affect canopy functioning. To answer these objectives, a TDLS was installed in the Hesse forest (northeast of France) early during the 2007 growing season in order to record the temporal variability of ^{13}C composition of CO_2 efflux released by a forest soil at different time scales.

Materials and methods

Experimental site

The experiment was set up in the Hesse state forest (48°40'N, 7°05'E, 305 m a.s.l.). This site is included in the CarboEurope flux monitoring and to the French network of forest ecosystem (ORE "fonctionnement

des écosystèmes forestiers"). The site is located in the middle of a homogenous 65 ha area of 40-year-old forest composed of 90% European beech (*Fagus sylvatica* L.). During the field campaign, maximum LAI averaged 7.5 m^2 m^{-2}. The soil is a stagnic luvisol (FAO classification) covered by oligo-mull humus with a high biological activity. It is an acidic soil (pH_{H2O} and pH_{KCl} reaching 4.5 and 3.8 for the 0–10 cm layer, respectively) that does not contain carbonate (Quentin et al. 2001). In May 2007, a homogenous area, in terms of vegetation and soil type, of about 150 m^2 was delimited in close vicinity of a fully equipped eddy covariance flux tower (see Granier et al. 2000 and Longdoz et al. 2008 for a detailed description).

The following climate data were used to interpret the variations of the isotopic signature of F_S: incident PPFD (Photosynthetic Photon Flux Density) with a PAR (Photosynthetically Active Radiation) sensor (Delta T-BF2, Cambridge, UK), air temperature and relative humidity (Vaisala HPM45, Helsinki, Finland) above the stand at 22 m height (top of the tower), soil temperature at 5 cm depth with home-made copper-constantan thermocouples and soil water content at 10 cm depth with TDR probes (Trase, SoilMoisture Equipment. Corp., Goleta, CA, USA). Data acquisition was made with a datalogger (CR7, Campbell, UK) at a 10 s pace and 30 min averages were calculated and stored.

Chamber design for TDLS measurements of $\delta^{13}C_{FS}$

An open soil chamber was specifically designed for the experiment to continuously monitor F_S and its isotopic composition ($\delta^{13}C_{FS}$). The chamber was made of stainless steel and allows the enclosure of 314 cm^2 of soil (20-cm diameter, Fig. 1). The chamber was composed of a 12.5-cm high collar, 2.5 cm of which was pushed into the soil, covered with a mobile lid allowing the alternative measurement of several collars without removing them. The chamber design respects the recommendations of Rayment and Jarvis (1997), *i.e.* (i) steady state for chamber CO_2 concentration can be easily achieved with a value relatively close to the atmosphere, (ii) the turbulent conditions at the soil surface must be closest to the outside conditions, and (iii) the pressure difference between the outside and the inside of the chamber must be minimal. The order of magnitude of this difference should be inferior to 0.1 Pa as it has already been shown (Longdoz et al. 2000). This pressure difference was measured *in situ* before the beginning of the monitoring using a low-pressure

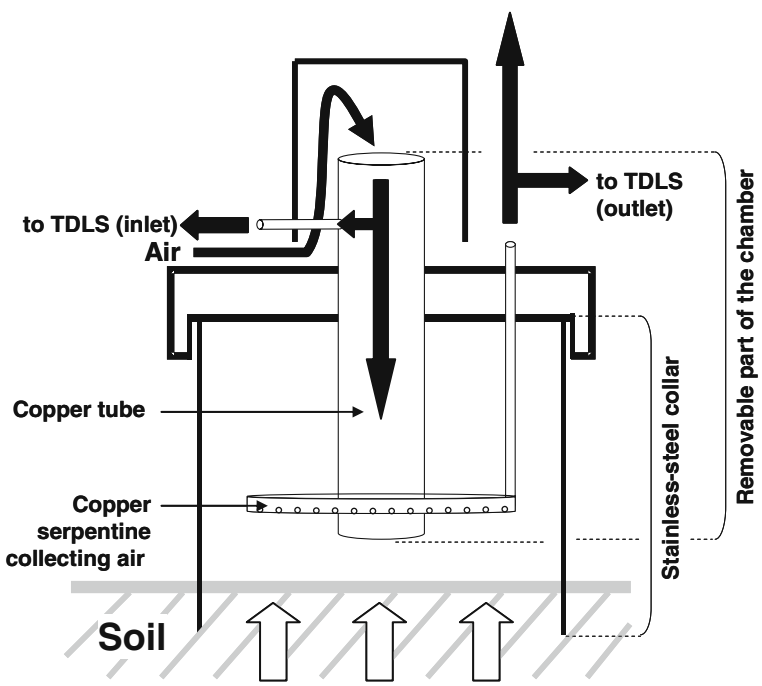

Fig. 1 Schematic representation of the soil respiration chamber design

transducer (FCO42 Furness Controls Ltd) able to measure pressure differences of 0.05 Pa. To achieve the requested value, an inlet diameter aperture of 52 mm (2120 mm^2) was needed for a 2 L min^{-1} air flow rate. On the 7th of May 2007, three collars were positioned in the experiment area with a distance between them of 9.8, 8.5 and 3.5 m. Measurements were alternatively made on each of the three collars during 3–4 consecutive days. The monitoring of the isotope composition of F_S started June 28th, 2007 and ended November 9th, 2007. Twelve sets (3 days to 4 days each) of continuous measurements were made.

TDLS measurements of F_S and $\delta^{13}C_{FS}$

TDLS system setup-The ratio of ^{13}C to ^{12}C of CO_2 entering and leaving the chamber was determined using tunable diode laser absorption spectroscopy (TGA 100A; Campbell Scientific Inc.) located in a mobile lab in the field. This instrument records the concentration of target gases using infrared absorption. The diode laser produces linear wavelength scans centered on selected absorption lines of the target gases. The laser radiation is absorbed proportionally to the concentration of this gas in the sample cell. The dual isotopologue mode was used, which allows the measurement of two isotopologues by alternating the spectral scan wavelength 500 times per second between two nearby absorption lines, in our case one for $^{13}CO_2$ and the other one for $^{12}CO_2$. The absorption lines at 2291.680 cm^{-1} ($^{13}CO_2$) and 2291.542 cm^{-1} ($^{12}CO_2$) were chosen. We recalculated the values as isotope ratios in the delta notation relative to the Vienna Pee Dee Belemnite (VPDB) standard. Three working standard tanks were used for calibration (Air Product, 0.5% certified for CO_2 concentrations). Their $\delta^{13}C$ were measured by IRMS (Delta S, ThermoFinnigan, Bremen, Germany). The CO_2 concentrations and $\delta^{13}C$ of the standards were respectively 318.5 µmol mol^{-1} and −39.4‰, 477.6 µmol mol^{-1} and −40.2‰, and 558.0 µmol mol^{-1} and −40.2‰. Total CO_2 concentration ($[CO_2]_t$) were calculated from the concentrations of individual isotopologues by: $[CO_2]_t=([^{12}CO_2]+[^{13}CO_2])$ / (1 - f_{other}), where f_{other} is the fraction of CO_2 containing all isotopologues other than $^{12}C^{16}O^{16}O$ and $^{13}C^{16}O^{16}O$, and assumed to be 0.00474 (Griffis et al. 2004). For the current application, a manifold was used to switch between each of the three working standards and the soil chamber inlet (reference) and outlet (sample) lines. A mean concentration was measured over 15 s for each working standard or over 25 s for both the reference and sample air streams. A 30 s purge was used in each case. Five minutes were necessary for the complete measurement of the sequence (three working standards - reference - sample - reference). All air streams passed through a low-flow Nafion® counterflow water trap (PD625 dual configurations, PermaPure, Inc.) housed in a rugged shell manufactured by Campbell Scientific Inc., prior to entry into the instruments optical cell at a flow rate of 200 mL min^{-1} (controlled by a mass flow controller; Alicat Scientific, Serie MC-500SCCM-D).

Stability over time- Preliminary experiments showed that the TGA 100 requires frequent calibration to achieve the accuracy required for isotope ratio measurements (Bowling et al. 2003). In the field, instrumental tests were performed by measuring mole fractions of each isotopologue from a 400 ppm CO_2 calibration gas tank every 100 ms during 13 h (Fig. 2a). The coefficient of variation of measured $^{12}CO_2$ and $^{13}CO_2$ concentrations over this time were 0.05% in both cases which induces $\delta^{13}C$ variation ranging from -39 to -43‰ (Fig. 2b). This level of noise is acceptable as the errors in $^{12}CO_2$ and $^{13}CO_2$ concentrations were highly correlated.

The Allan variance (*i.e.* two-sample variance) procedure was used to examine temporal changes in instrument response and to define a calibration strategy (Allan 1966; Werle et al. 1993; Bowling et al. 2003). The Allan variances (σ^2_A) of the time series in Fig. 2a are shown in Fig. 2b. For averaging times below 30 s, increase of the averaging time decreased the variance as predicted, following the theoretical slope. The variance began to increase for averaging times above 50 s as other factors influence the instrument noise. This provides an estimate of the optimal averaging time. This analysis shows that, to minimize the error in measured mole fractions of each isotope, calibrations have to be performed approximately every minute. Practically, each intake is sampled during 55 s (variance of 10^{-4} for $^{12}CO_2$ and 1.6×10^{-8} for $^{13}CO_2$, Fig. 2b) and calibration is redone after 165 s of inlet - outlet - inlet measurements as described above. This duration is a compromise between the ideal 60 s and our need to measure consecutively the three intakes (inlet - outlet - inlet). Comparable conclusions were obtained by Bowling and coworkers (2003) who have selected a 2 min

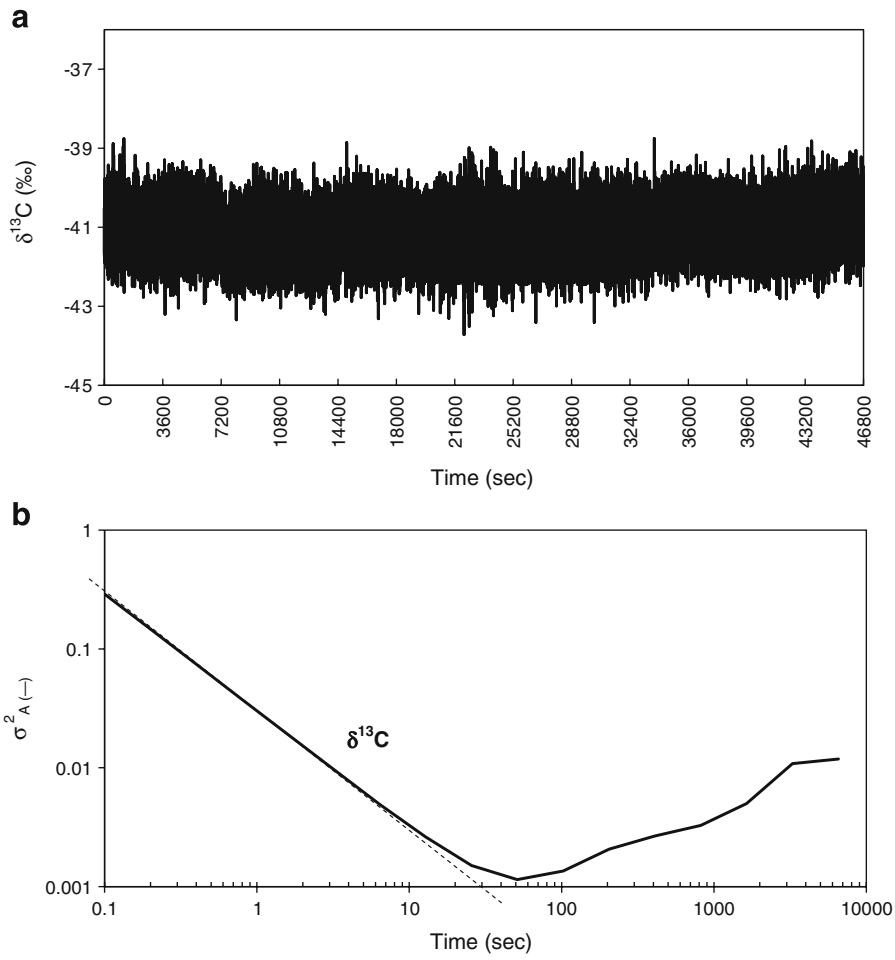

Fig. 2 **a**. Time series of the carbon isotope composition ($\delta^{13}C$, ‰) measured every 100 ms from the 400 ppm CO_2 calibration gas with the TDLS. The data were not calibrated. **b**. Allan variances (σ^2_A) of the time series of $\delta^{13}C$ presented in a. The dashed lines represent the theoretical slopes associated with a stationary random process

calibration scheme based on similar considerations. CO_2 effluxes and its $\delta^{13}C$ estimated by the TDLS have been assessed using two different methods:

-Method1: Soil CO_2 efflux (F_S) and its $\delta^{13}C$ ($\delta^{13}C_{FS}$) were calculated as:

$$Fs = \frac{(CO_{2\,outlet} - CO_{2\,inlet0})\,x\,P\,x\,F}{8.314\,x\,S\,x\,T} \quad and$$

$$\delta^{13}C_{FS} = \frac{\frac{^{13}CO_{2\,outlet} - ^{13}CO_{2\,inlet}}{^{12}CO_{2\,outlet} - ^{12}CO_{2\,inlet}}}{R} - 1$$

where P is the atmospheric pressure (Pa), F is the flow ($m^3\ s^{-1}$), S is the soil surface inside the chamber (m^2), T is the temperature (°K), 8.314 J mol^{-1} K is the ideal gas constant, and R is isotopic ratio of VPDB (0.01118526).

-Method 2: Ecosystem CO_2 efflux and its $\delta^{13}C$ by means of Keeling plots realized overnight with the aboveground accumulation of $^{12}CO_2$ and $^{13}CO_2$ measured at the inlet of the chamber with the TDLS (Bowling et al. 2005). The isotopic composition of F_S was estimated as the intercept of Keeling-plot relationships (Keeling 1958) under the classical form $\delta^{13}C_{sample} = \delta + a$ x $(1/[CO_2]_{sample})$. Least square linear regressions as well as geometric mean linear regressions were performed (Sokal and Rohlf 1995). As only very minor differences were observed between both methods, results obtained from the least square linear regressions only are presented.

IRMS measurements of $\delta^{13}C_{FS}$

One week prior beginning of air sampling, six 7-cm high and 10.3-cm diameter plastic collars were pushed into the soil to a depth of 3 cm and placed in the vicinity of the TDLS stainless-steel collars to

measure F_S signature following a standard method (Ngao et al. 2005). An accumulation chamber was connected to a portable infrared gas analyzer (LiCor Li-6250, Lincoln, NE, USA) and joined to the plastic collar. The CO_2 concentration rose in this closed system because of F_S and after a certain lapse of time, air samples contained a mixture of F_S and atmospheric CO_2 that was initially in the chamber. A home-made air sampling device, consisting of a customized PMMA (PolyMethylMethAcrylate) body, was included in the closed system with a by-pass connection to flow the air through a vial. With this device, five air samples with CO_2 concentration typically ranging between 390 and 650 μmol mol^{-1} were collected from the closed system in 12 mL Exetainer glass-vials This sampling procedure was performed for the six plastic collars during three pairs of consecutive days during 2007: days of year 183-184 (July 2–3, 2007), 220–221 (August 8–9, 2007), and 297–298 (October 24–25, 2007).

$\delta^{13}C$ of CO_2 in the glass vials was measured with a mass spectrometer (Delta S, ThermoFinnigan, Bremen, Germany). Analyses were performed within 48 h after each sampling episode. Isotopic composition was expressed relative to the international Vienna Pee Dee Belemnite (VPDB) standard. The isotopic composition of F_S (Method 3) was estimated as the intercept of Keeling-plot relationships (Keeling 1958) as described above for Method 2. Keeling plots were established independently for each collar, and a weighted average of the regression coefficients was calculated for the six collars (n=6), with weights proportional to the reciprocals of the squared standard errors from the individual fits, (Murtaugh 2007). Means are given with their respective confidence intervals at P<0.05.

Incubations

Litter and soil cores (20 cm in depth) were collected from the experimental area a few days prior the beginning of the measuring campaign. Samples were stored at 5°C before measurement. Roots were removed from the cores, rapidly washed, superficially dried by using filter paper, and incubated. The $\delta^{13}C$ of CO_2 respired by litter (59.1±13.4 g of fresh weight), soil including roots (319.6±46.8 g_{FW}) and roots (8.3±2.8 g_{FW}) was determined for samples incubated and subjected to temperature variations. Temperature was controlled using Peltier effect cells. Samples were subjected to seven one-hour steps from 5 to 35°C, with a 5°C increase between each step, followed by three reverse steps from 35°C to 5°C with a 10°C decrease between the steps, to check for the reversibility of the potential variations in $\delta^{13}C$ of respired CO_2 with temperature. The 1-L incubation chamber was made of stainless steal and PTFE (PolyTetraFluoroEthylene). The $\delta^{13}C$ of respired CO_2 was monitored with a TDLS as described above. $\delta^{13}C$ of respired CO_2 was not affected by temperature variations in any of the samples (data not shown). For this reason, mean values are presented. Samples were then dried at 75°C during 48 h, ground to powder, and $\delta^{13}C$ of the organic matter was determined by mass spectrometry (Delta S, ThermoFinnigan, Bremen, Germany).

Statistical analyses

Data analyses were performed with the statistical package SPSS (SPSS, Chicago, IL). Means were calculated with their confidence intervals at P<0.05 (±CI). Differences among incubation results were tested for significance by using one-way ANOVA followed by a Scheffé test. Linear correlations between $\delta^{13}C_{FS}$ and climatic data were tested for significance using Pearson's correlation coefficients. Maximal diurnal values of photosynthetic photon flux density (PPFD), air temperature (T_{air}) and relative humidity (RH) and 24-h means of $\delta^{13}C_{FS}$, soil temperature (T_{soil}) and soil water content (SWC) were used for the establishment of the correlations. Pearson correlations were also established with an increasing time lag between $\delta^{13}C_{FS}$ and climatic data, $\delta^{13}C_{FS}$ being correlated with the climatic data measured one to eight days earlier. Only linear correlations were tested as no other relationship between $\delta^{13}C_{FS}$ and climate has been described in the literature, and the range of the climatic conditions was too narrow to test any alternative regression.

Results

Isotope signature of litter, roots and soil respiration under controlled conditions

The comparison between the results of the incubation experiments (litter, roots and soil incubated separately)

and the organic matter analyses show no significant difference between the isotopic signature of an organic matter type (litter, roots or soil) and the signature of its respiration. However, the $\delta^{13}C$ of CO_2 respired by litter (−30.2‰) was significantly different ($P<0.05$) from the $\delta^{13}C$ of CO_2 respired by roots or by soil (−26.8‰ and−27.3‰, respectively) (Table 1). Due to the fact that the incubated soil cores included the roots, the $\delta^{13}C$ of the microbial component of soil respiration was even more negative than the values measured for the bulk soil.

Comparison of $\delta^{13}C$ obtained by three different methods

The carbon isotope composition of F_S ($\delta^{13}C_{FS}$) was estimated using three different methods: (1) via the difference between chamber outlet and inlet in $^{12}CO_2$ and $^{13}CO_2$ concentrations measured with the TDLS, knowing the flow of air through the chamber, (2) by means of Keeling plots realized overnight with the aboveground accumulation of $^{12}CO_2$ and $^{13}CO_2$ measured at the inlet of the chamber with the TDLS (Bowling et al. 2005), and (3) with Keeling plots established from gas samples collected at increasing CO_2 concentrations in a closed chamber and analyzed by mass spectrometry (Keeling 1958; Ngao et al. 2005). The results obtained with the three methods are presented in Table 2 for the six common dates and compared in Fig. 3. The seasonal course of $\delta^{13}C$ obtained with the nocturnal Keeling plots (nocturnal Keeling intercepts; method 2) is presented in Fig. 4a in parallel with chamber values presented in Fig. 4c (method 1). Overall, the $\delta^{13}C$ estimated via nocturnal Keeling intercepts (method 2) were slightly more negative than the values obtained from the chamber during the same period (0.6‰ more negative on an average).

Table 1 Carbon isotope composition ($\delta^{13}C$) of CO_2 respired by litter, roots and soil (including roots) determined by incubation and $\delta^{13}C$ values of the corresponding organic matter

	$\delta^{13}C$ respiration		$\delta^{13}C$ organic matter	
Litter	−30.21±0.18	A	−29.39±0.76	A
Roots	−26.82±0.92	B	−27.78±0.20	B
Soil	−27.33±0.94	B	−26.75±0.74	C

Means (± CI) of 4 values. Significant differences among litter, roots, and soil ($P<0.05$) are indicated by different letters.

Time course of $\delta^{13}C_{FS}$ and relationships with microclimate

Soil CO_2 efflux (F_S) and its carbon isotope composition ($\delta^{13}C_{FS}$) were variable at seasonal (Figs. 4b and c) as well as at daily time scales (Fig. 5). Results were slightly dependent on the collar on which the measurements were done. Values of soil CO_2 efflux and $\delta^{13}C_{FS}$ were indeed systematically higher for collar C as compared to the two other collars. However, the differences among the three collars were declined from mid-September (around day 250) and up to the end of the growing season.

Daily variation- Three representative examples of daily cycles of F_S and $\delta^{13}C_{FS}$, one for each of the three collars, are shown on Fig. 5. During the chosen periods (days 193 to 198, mid-July; 263 to 267, mid-September, and 284 to 288, mid-October), the range of daily variation was 1.0 to 1.5 μmol m^{-2} s^{-1} for F_S and 0.5 to 1.5‰ for $\delta^{13}C_{FS}$ (Fig. 5). Clear trends were observed at daily time scales for F_S and its $\delta^{13}C$. Maximal values of both variables were observed in the middle of the night while minimal values were reached in the early afternoon. F_S and its $\delta^{13}C$ were significantly correlated between them (r=0.73, 0.57, and 0.78, respectively for the three periods; $P\leq0.001$; Fig. 5). However, for two out of the three collars, a delay in the correspondence between F_S and $\delta^{13}C_{FS}$ was observed (Fig. 5). The coefficients of correlation between the two variables indeed increased when F_S was associated to $\delta^{13}C_{FS}$ measured up to 3 h later for collar B (r increasing from 0.57 to 0.68) and up to 1.5 h later for collar C (from 0.78 to 0.85), while a decrease of the coefficient was observed for collar A when a time lag was included (data not shown).

Seasonal variation- Overall, during the whole growing season, the range of variation of F_S was 6 μmol m^{-2} s^{-1}, varying between 1 and 7 μmol m^{-2} s^{-1} (Fig. 4), well within the range of values commonly observed for this site (Epron et al. 2004). $\delta^{13}C_{FS}$ was between −26.5 and −30‰. A slow decrease of both soil CO_2 efflux and $\delta^{13}C_{FS}$ was observed from the end of August (around day 240) to the end of the growing season.

Pearson correlations were calculated between 24 h means of $\delta^{13}C_{FS}$ and climatic (PPFD, T_{air}, and RH; maximal daily values) and edaphic data (T_{soil} and SWR; 24 h means) (Table 3 and Fig. 6). Correlations were also calculated with an increasing time lag, from

Table 2 Carbon isotope composition of CO_2 effluxes ($\delta^{13}C$, ‰ vs. VPDB) obtained via the difference between the outlet and inlet of the chamber measured with the Tunable Diode Laser Spectrophotometer (1), and via Keeling plot intercepts on overnight above ground accumulation measured with the TDLS (2) via traditional Keeling plot established with gas samples analyzed by mass spectrometry (3), at 6 dates during the growing season

Method	Period	Days of year					
		183	184	220	221	297	298
1. Chamber Inlet / Outlet (TDLS)	12 AM-5 PM	−28.7 ±0.13	−28.3 ±0.09	−27.8 ±0.05	−27.2 ±0.05	−28.1 ±0.40	−29.0 ±0.08
	8 PM-6 AM	−28.4 ±0.03	−28.4 ±0.06	−27.7 ±0.11	−27.5 ±0.03	−28.6 ±0.06	−29.0 ±0.07
2. Nighttime Keeling plot intercepts at chamber inlet (TDLS)	8 PM-6 AM	−29.0 ±0.37	−28.7 ±0.42	−28.1 ±0.24	−28.0 ±0.83	−29.2 ±1.24	−29.6 ±0.48
3. Traditional Keeling plot intercepts (IRMS)	12 AM-5 PM	−28.4 ±0.65	−28.1 ±0.56	−28.8 ±0.66	−28.6 ±0.52	−29.0 ±0.68	−28.4 ±1.04

Means (± Confidence Intervals) of 6 repetitions for (3) and from 5 min. values for (1).

1 to 8 days, in order to estimate whether climatic conditions of the previous days affects the isotopic signature of F_S of the current day (time lag=0). For the three collars, the strongest correlations were observed between $\delta^{13}C_{FS}$ and soil and air temperatures of the same day. The correlations among $\delta^{13}C_{FS}$ and climatic conditions were dependent on the collars. For collars A and B, $\delta^{13}C_{FS}$ was strongly positively correlated to PPFD of the current day. For collar A, $\delta^{13}C_{FS}$ was negatively correlated to relative humidity of the current day, while they were positively correlated for collar B. For collar C, the tightest correlations between $\delta^{13}C_{FS}$ and PPFD, RH, and T_{air} were observed with climatic data measured 3 days to 4 days earlier.

Discussion

Cross validation of the experimental setup for field measurements

The slightly more negative results obtained from the chamber as compared to the traditional Keeling plots might be due to the difference of footprint of the two methods: the TDLS chamber enclosed 314 cm^2 of soil (method 1) while the values measured at the inlet of the chamber are probably representative of a much larger area. Nevertheless, it cannot explain the systematically more negative $\delta^{13}C$ obtained with method 2. This is probably caused by the fact that the CO_2 measured at the inlet of the chamber does not

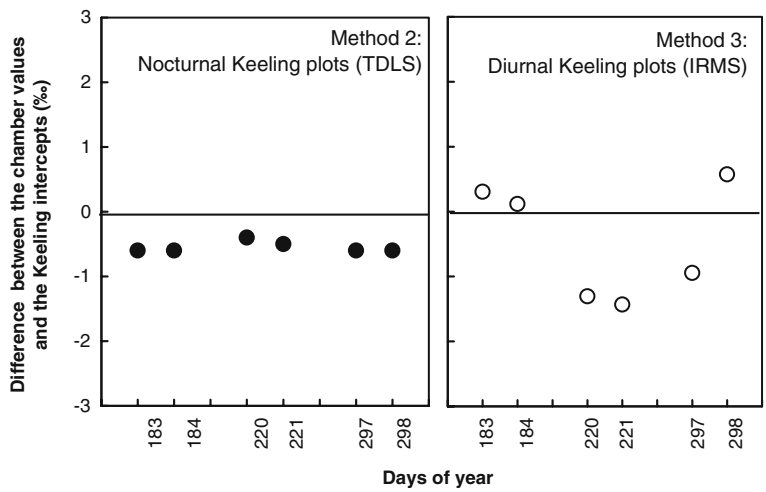

Fig. 3 Absolute differences between the Keeling plot intercept values of $\delta^{13}C$ assessed with Methods 3 and 2 and the chamber values (Method 1) (± CI). See the Materials and methods section for the description of the three methods

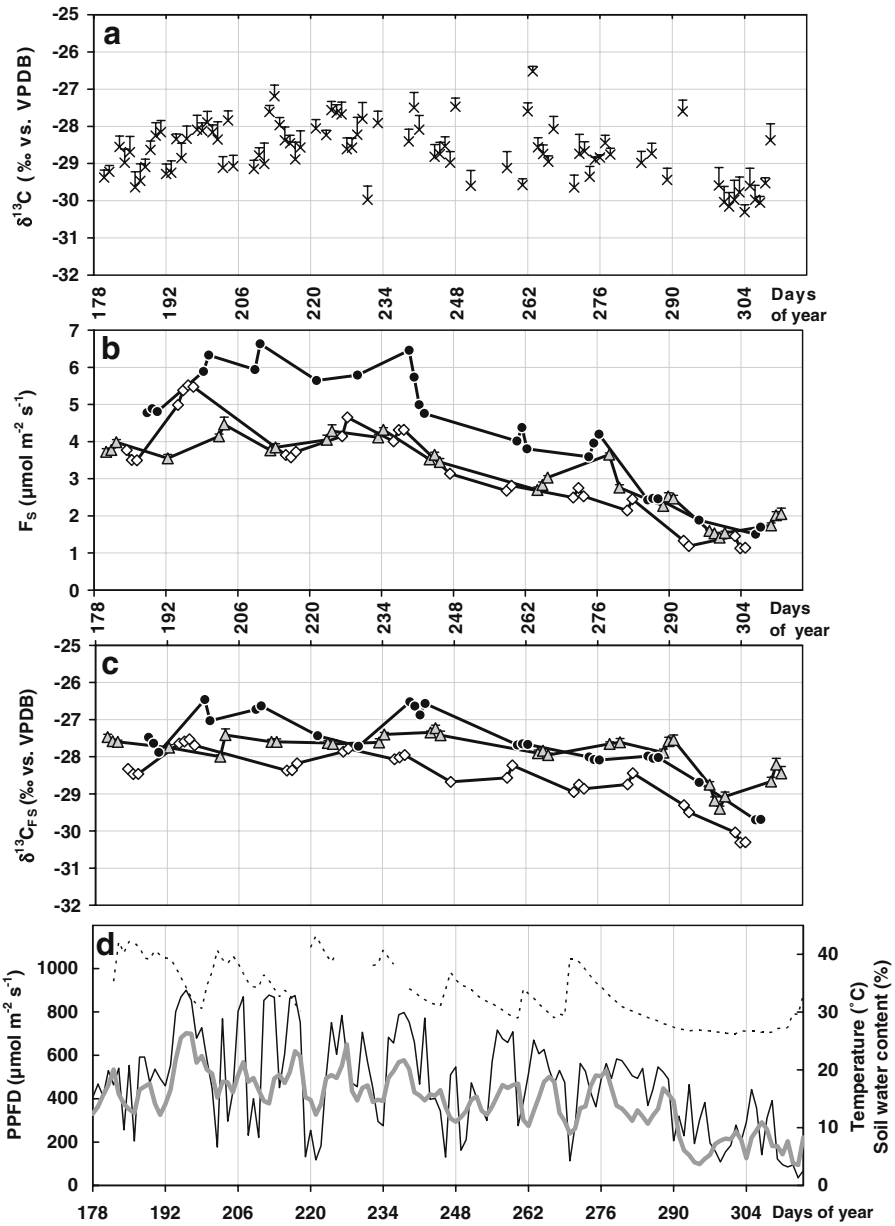

Fig. 4 Time course of soil carbon isotope content ($\delta^{13}C_{FS} \pm CI$) obtained from the nocturnal Keeling plots at chamber inlet (panel **a**), soil CO_2 efflux ($F_S \pm CI$, panel b) and $\delta^{13}C_{FS}$ ($\pm$ CI) obtained from the TDLS method (panel **c**), and climatic conditions (photosynthetic photon flux density, PPFD, thin line; air temperature, thick grey line; soil water content, dashed line; panel **d**) during the period June 29 (day 180) to November 7 (day 311), 2007. Daily means of 48 values per day for panels (**b**) and (**c**) (collar A = diamonds, collar B = triangles, and collar C = circles)

just represent soil effluxes, as trunk respiration may also influence it. It has indeed been observed in the Hesse forest that the $\delta^{13}C$ of CO_2 respired by trunks is generally more negative that for the one of CO_2 released by the soil (unpublished results) and could consequently influence the signature obtained from the nocturnal Keeling plots by lowering the soil signal. On the other hand, the comparison between the traditional Keeling intercepts (method 3) and the chamber ones (method 1) measured between 12 AM and 5 PM revealed an average difference of 0.5‰ between $\delta^{13}C_{FS}$ obtained with the two methods. The highest differences between both methods were observed at days 220 and 221. These days were very rainy which could have altered the gas sampling needed for the establishment of the traditional Keeling plots (method 3). In any case, similar $\delta\ ^{13}C_S$ values were obtained with the three different methods.

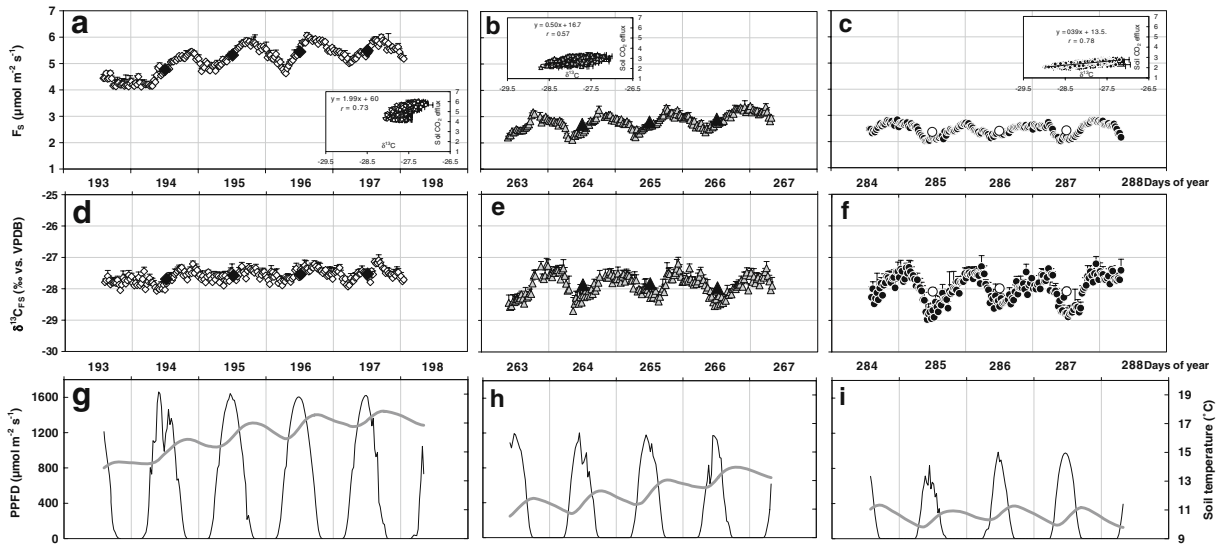

Fig. 5 Time course of F_S (panels a to c), its carbon isotope content ($\delta^{13}C_{FS}$, panels d to e) based on 30 min means (empty diamonds, grey triangles, and full circles) and on 24 h means (full diamonds, full triangles, and empty circles), and photosynthetic photon flux density (PPFD, black line; panels g to i) and air temperature (grey line; panels g to i) during the period July 12 to 18 (days 193 to 198, collar B), September 20 to 24 (days 263 to 267, collar A), and October 11 to 15 (days 284 to 288, collar C), 2007. Means (± CI) of 6 values per 30 min. period or 48 values per day. The relationship between F_S and its carbon isotope content ($\delta^{13}C_{FS}$) is shown as inserts in panels a to c

Table 3 Pearson's correlation coefficients between carbon isotope composition of F_S ($\delta^{13}C_{FS}$) and climate and soil conditions: PPFD = photosynthetic photon flux denstiy, T_{air} = air temperature above the canopy, RH = Vaisala relative humidity, T_{soil} = soil temperature at −5 cm depth, SWC = soil water content at −10 cm depth, for the three collars (A, B, C), with an increasing time lag and based on seasonal daily means calculated for the diurnal period

Time lag (days	Climate									Soil					
	PPFD			T_{air}			RH			T_{soil}			SWC		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0.71[†] ***	0.68 ***	ns	0.92[†] ***	0.80 ***	0.58 **	-0.57[a] **	0.61 **	ns	0.92[†] ***	0.84 ***	0.79 ***	ns	0.57 ***	ns
1	0.78 ***	0.56 **	0.54 *	0.90 ***	0.78 ***	0.60 **	-0.54 **	0.70 ***	-0.52 *	0.84 ***	0.81 ***	0.82 ***	0.43 *	0.62 **	ns
2	0.72 **	0.44 *	ns	0.72 ***	0.78 ***	0.65 **	ns	0.57 **	-0.50 *	0.71 ***	0.81 ***	0.84 ***	0.65 ***	0.63 **	ns
3	0.64 ***	0.41	0.67 **	0.42 *	0.76 ***	0.80 ***	ns		-0.49 *	0.58 **	0.82 ***	0.86 ***	0.78 ***	0.61 **	ns
4	ns	0.40 *	0.72 ***	ns	0.80 ***	0.78 ***	ns		-0.47 *	0.56 **	0.81 ***	0.85 ***	0.90[a] ***	0.62 **	ns
5	ns	0.49 *	0.62 **	ns	0.73 ***	0.73 ***		ns		0.59 **	0.79 ***	0.79 ***	0.90 ***	0.62 **	0.48 *
6	ns	0.63 ***	ns	ns	0.71 ***	0.58 **		ns		0.64 ***	0.77 ***	0.71 ***	0.88 ***	0.61 **	0.66 **
7	ns	0.67 ***	ns	ns	0.65 ***	ns		ns		0.68 ***	0.70 ***	0.64 **	0.88 ***	0.63 **	0.76 ***
8	ns	0.55 **	ns	ns	0.51 **	ns	ns		0.52 *	0.62 ***	0.64 ***	0.58 **	0.75 ***	0.70 ***	0.79 ***

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; and ns = non significant. [a] = correlations presented in Fig. 6.

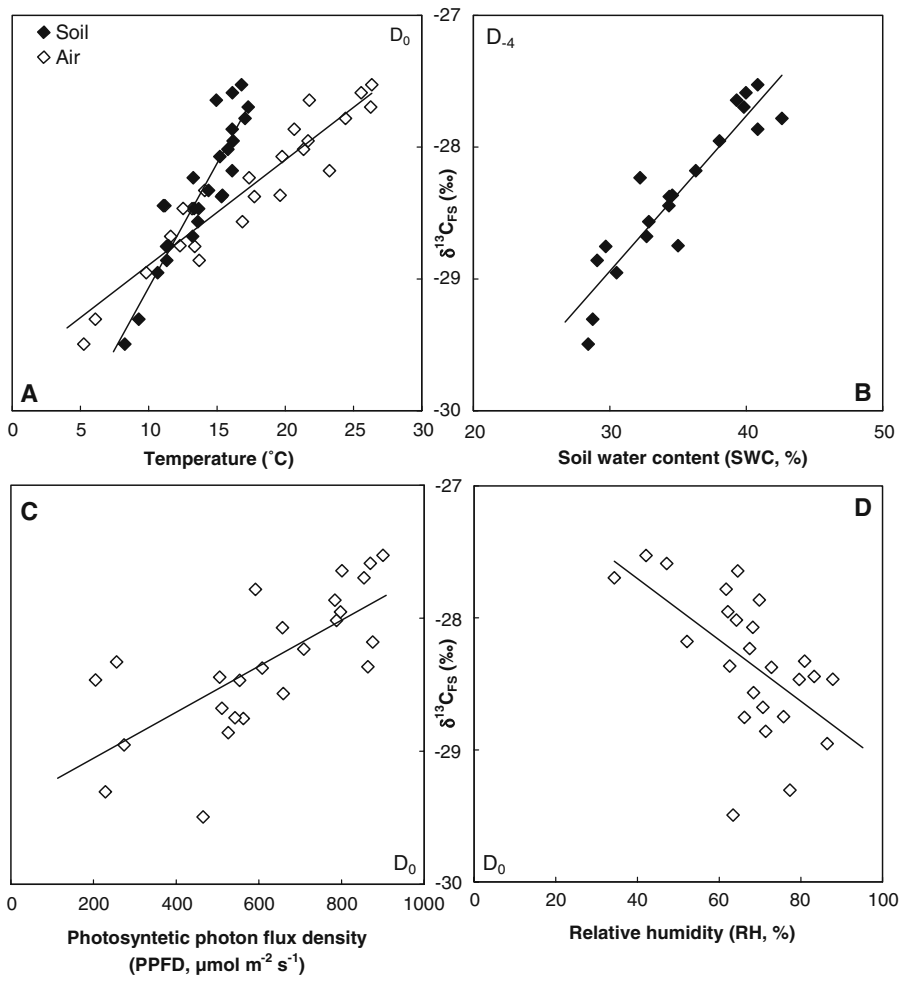

Fig. 6 Correlations between the carbon isotope composition of F_S ($\delta^{13}C_{FS}$) and (A) soil and air temperatures measured at −5 cm depth and above the canopy, respectively, (B) soil water content (SWC), (C) photosynthetic photon flux density (PPFD), and (D) relative humidity (RH), based on diurnal daily means for collar A. Correlations are presented between $\delta^{13}C_{FS}$ and climatic conditions of the current day except for panel B that corresponds to the correlation between $\delta^{13}C_{FS}$ and SWC measured four days earlier. See Table 3 for the corresponding Pearson's correlation coefficients

Middle-term and long-term temporal variation of F_S and $\delta^{13}C_{FS}$

Large daily and seasonal variations were observed for F_S and $\delta^{13}C_{FS}$ in our experiment. The ranges of variation observed at these two time scales were almost of the same order of magnitude, underlying the importance of the period of the day at which efflux is collected to obtain relevant and comparable day to day values of $\delta^{13}C_{FS}$.

Daily variation- The daily time course observed in our study, with a maximum ^{13}C enrichment in the middle of the night, is inverted as compared with the one observed by Kodama et al. (2008) in a pine forest. On the other hand, Betson et al. (2007) showed no significant diel cycle for the ^{13}C signature of F_S in a boreal forest. These two studies are however based on many fewer measurements than the present study. For each of the three collars, different relationships between F_S and $\delta^{13}C_{FS}$ were observed. However, the significant correlations between F_S and $\delta^{13}C_{FS}$ observed in any case show that the dependence of both variables on climatic variations could be connected.

As no significant $\delta^{13}C$ change was observed in response to temperature fluctuation during the time course of the incubations, the daily variations of $\delta^{13}C_{FS}$ observed in the field are probably due to the fluctuation of the relative contribution of the different respiratory sources having different signatures in the soil. However, the values obtained during the incubation experiment have to be interpreted cautiously because only one time point has been sampled at the beginning of the monitoring to avoid soil disturbance during the experiment and Gessler et al. (2007) have

shown that the $\delta^{13}C$ of bulk and soluble root organic matter as well as root-emitted CO_2 can vary by approximately 1.5 to 2‰ within a few days. The $\delta^{13}C$ signature of CO_2 respired by the different sources in the soil probably did not vary significantly at a daily time scale, but the relative intensity of the respiratory fluxes for the different sources might have been influenced to different degrees by climatic variations (PPFD, air and soil temperature, and SWC). It is well-known that the various components of F_S have different temperature sensitivities (Lloyd and Taylor 1994; Kirschbaum 1995; Qi et al. 2002) and notably, that respiration by roots is more temperature-sensitive than the respiration of bulk soil (Boone et al. 1998; Epron et al. 2001). During the day, root respiration could become a larger proportion of total soil respiration in response to the increase in temperature and progressively dilute the more negative $\delta^{13}C_{FS}$ signature observed before sunrise, as the $\delta^{13}C$ signature of CO_2 released by roots was significantly higher (−26.8‰) compared to litter (−30.2‰) under incubation conditions. The root respiration is also influenced by the PPFD through the photosynthate production rate in contrast to the litter decomposition. As an additional factor, changes in soil moisture could also affect the transport time of gas-phase soil CO_2 from deeper soil to surface (Stoy et al. 2007), thus potentially altering the time lag between below-ground respiration and CO_2 emission from the soil surface.

Seasonal variation- The strong correlations found between soil and air temperatures on the one hand, and $\delta^{13}C_{FS}$ on the other hand suggest that the origin of this correlation could be similar to the causes of the daily variations explained above (variation of the relative contribution of the sources). In all cases, $\delta^{13}C_{FS}$ was also tightly correlated to soil water content measured 4 days to 7 days prior to $\delta^{13}C_{FS}$ measurements. The correlation between $\delta^{13}C_{FS}$ and soil water content is positive, the opposite of what is generally expected (Alstad et al. 2007). This apparent contradiction is likely due to the fairly narrow SWC range of variation (ranging between 30% and 40% during the entire growing season) and soil water was never a limiting factor for tree growth during the 2007 growing season. The observed positive correlation between $\delta^{13}C_{FS}$ and SWC is probably due to indirect causal links among climate variables: rainy periods are generally less sunny and colder, and so temperature, soil humidity and PPFD are closely linked (data not shown).

The F_S signature reflects the signature of different compartments contributing to the total F_S. Large differences were observed during the incubation experiment between $\delta^{13}C$ of litter and root respirations. The $\delta^{13}C_{FS}$ values observed in the field at the seasonal scale are in-between the $\delta^{13}C$ values of root and litter respiration observed under incubation conditions (Fig. 7). Andrews et al. (2000) have shown under long-term incubation conditions at various temperatures (4, 22, and 40°C) that the $\delta^{13}C$ signature of soil-respired CO_2 by root-free bulk soil was only significantly higher for the lowest temperature. This difference was associated with a significantly lower microbial species richness at 4°C than at 22 and 40°C. However, the soil samples used in Andrews et al. (2000) were incubated for up to 50 days while the incubation duration of our experiment was limited to 7 h. In our case, the duration of the incubation experiment was probably short enough to avoid change in microbial communities that would have added an indirect effect (change in the type of substrate used) to the direct effect of temperature on substrate use. Moreover, the temperature measured during the season varied between 4 and 26°C, while the intermediate temperatures were not tested in the

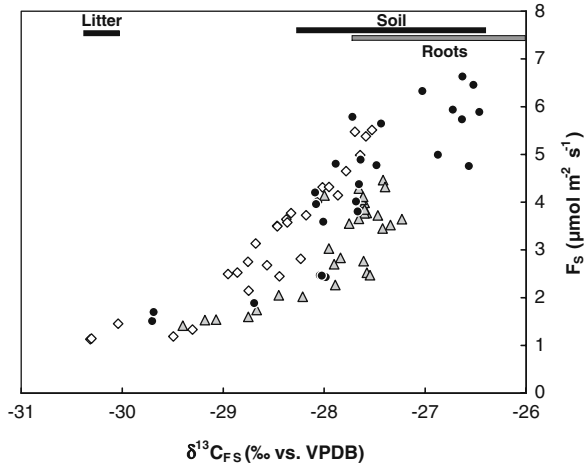

Fig. 7 Relationship between F_S and its carbon isotope content ($\delta^{13}C_{FS}$) based on seasonal 24 h means for the three collars (A = diamonds, B = triangles, and C = circles). The ranges of variation of $\delta^{13}C$ respired by litter, roots and soil are represented with black and grey bars (values are presented in Table 1)

Andrew et al. (2000) study. In the field, at the seasonal scale, the evolution of $\delta^{13}C_{FS}$ could be under the influences of both qualitative changes (changes in the sources themselves) and quantitative changes (changes in the relative contribution of the different respiratory sources). For collar C, root respiration could represent a more important part of the total F_S than for both other collars. This collar indeed showed less negative $\delta^{13}C_{FS}$ values than both other collars during the major part of the growing season. At the end of the growing season, when root respiration is likely to decline, F_S and $\delta^{13}C_{FS}$ measured from collar C approximated the values obtained from both other collars. This hypothesis is supported by the fact that collar C was the only one for which a time lag was observed between $\delta^{13}C_{FS}$ and aboveground climate conditions. Because of transport time in the tree, one might expect a time lag for the autotrophic respiration. Then, the observed time lag should depend on the relative contribution of the autotrophic component of F_S. For a spruce forest, Ekblad et al. (2005) showed that the temporal variation of the $\delta^{13}C_{FS}$ was also explained to a large extent by the variations in aboveground weather conditions 2 days to 6 days before sampling and that both variations in the $\delta^{13}C$ signature of root respiration as well as variations in the relative contribution of root respiration to total F_S can play a role. Similar results were found in various studies, with variable time lags: 1–4 days for a Scots pine forest (Ekblad and Högberg 2001), and 5–10 days for different forests in western Oregon (Bowling et al. 2002). However, the results of the three previously cited studies are based on few seasonal measurements of $\delta^{13}C_{FS}$ as compared with the present study and have to be considered cautiously. Recently, Kodama et al. (2008) showed for a pine forest that direct relations between $\delta^{13}C$ of recent assimilates and respired CO_2 may not be present on a diel time scale, and other factors lead to short-term variations in $\delta^{13}C$ of ecosystem-emitted CO_2. The more negative values of $\delta^{13}C_{FS}$ observed for both other collars (A and B) could have been under the more marked influence of the $\delta^{13}C$ signature of litter CO_2 effluxes as litter respiration is likely to be more influenced by climate conditions (essentially temperature) of the current day than root respiration. The reduction of $\delta^{13}C_{FS}$ observed at the end of the growing season for the three collars is also probably due to the decreasing involvement of root respiration (see black horizontal bars on Fig. 7). It has been shown that variations in F_S through the growing season in a temperate hardwood forest are determined mainly by temperature responses of root respiration and rhizosphere heterotrophs (Boone et al. 1998; Epron et al. 2001) even if the contribution of litter to the total efflux greatly vary during the growing season, peaking in mid-summer (Ngao et al. 2005).

Conclusion

Tunable diode laser spectroscopy appears to be a promising tool to monitor short-term variations in $\delta^{13}C_{FS}$. This technique indeed allows us to highlight pronounced daily variations of $\delta^{13}C_{FS}$, underlying the importance of the time of the day at which $\delta^{13}C_{FS}$ measurements are collected to obtain relevant values. The temporal variation of $\delta^{13}C_{FS}$ appears to arise from changes in the relative contribution of respiration from sources producing CO_2 with different signatures. This exploratory study provides a better understanding of the origins of temporal variation of the respired CO_2 isotope composition required for the development of atmospheric inversion models and their simulation of CO_2 source / sink maps (Santaren et al. 2007). It also emphasizes spatial heterogeneity in the relationship between climatic or edaphic factors and $\delta^{13}C_{FS}$, even in small areas, highlighting again the very complex and interconnected processes determining $\delta^{13}C_{FS}$.

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