Oecologia (2007) 151:268–279
DOI 10.1007/s00442-006-0592-z

ECOSYSTEM ECOLOGY

Seasonal, daily and diurnal variations in the stable carbon isotope composition of carbon dioxide respired by tree trunks in a deciduous oak forest

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Received: 22 June 2005 / Accepted: 12 October 2006 / Published online: 18 November 2006

Abstract The stable C isotope composition ($\delta^{13}C$) of CO_2 respired by trunks was examined in a mature temperate deciduous oak forest (*Quercus petraea*). Month-to-month, day-to-day and diurnal, measurements were made to determine the range of variations at different temporal scales. Trunk growth and respiration rates were assessed. Phloem tissue was sampled and was analysed for total organic matter and soluble sugar ^{13}C composition. The CO_2 respired by trunk was always enriched in ^{13}C relative to the total organic matter, sometimes by as much as 5‰. The $\delta^{13}C$ of respired CO_2 exhibited a large seasonal variation (3.3‰), with a relative maximum at the beginning of the growth period. The lowest values occurred in summer when the respiration rates were maximal. After the cessation of radial trunk growth, the respired CO_2 $\delta^{13}C$ values showed a progressive increase, which was linked to a parallel increase in soluble sugar content in the phloem tissue ($R = 0.95$; $P < 0.01$). At the same time, the respiration rates declined. This limited use of the substrate pool might allow the discrimination during respiration to be more strongly expressed. The late-season increase in CO_2 $\delta^{13}C$ might also be linked to a shift from recently assimilated C to reserves. At the seasonal scale, CO_2 $\delta^{13}C$ was negatively correlated with air temperature ($R = -0.80$; $P < 0.01$). The diurnal variation sometimes reached 3‰, but the range and the pattern depended on the period within the growing season. Contrary to expectations, diurnal variations were maximal in winter and spring when the leaves were missing or not totally functional. By contrast to the seasonal scale, these diurnal variations were not related to air temperature or sugar content. Our study shows that seasonal and diurnal variations of respired ^{13}C exhibited a similar large range but were probably explained by different mechanisms.

Keywords Carbon isotope composition · *Quercus petraea* · Soluble sugars · Stem respiration

Communicated by Nina Buchmann.

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Introduction

Since the nineteenth century, large quantities of CO_2 have been released into the atmosphere by human activities. About 8 Gt of C is emitted every year by fossil C combustion and deforestation (Saugier et al. 2001). As only 3.2 Gt is actually found in the atmosphere, 4.8 Gt of the annual C release is stored in terrestrial and oceanic ecosystems. Currently, it is estimated that 50% of this C is trapped by the continental biosphere. Forest ecosystems, which represent the largest terrestrial C reservoir (540 Gt, i.e. more than 80% of the 650 Gt of continental biomass), play an essential role in this storage (Saugier et al. 2001). Badeau et al. (1996) and Dhôte and Hervé (2000) showed that, in France, forest ecosystem production increased by 1% per year during the previous decades.

This increase in production is probably the consequence of several changes: increases in CO_2 concentration, in temperature, in N deposits and improvement of forest management techniques (Thimonier et al. 1994).

To quantify, clarify and predict the C uptake by forests, studies at the ecosystem level are needed. The purpose of many projects (CarboEuroflux, Ameriflux) is to determine to what extent and under which conditions forests are C sinks. The global network of tower flux sites covers different types of forests. Recently, measurements of the C isotope composition of ecosystem respiration have been developed in order to determine the contribution of the different forest compartments to the ecosystem respiration. Using a Keeling plot approach (Keeling 1958), several projects have highlighted seasonal (Bowling et al. 2002; Baldocchi and Bowling 2003; Knohl et al. 2005) and diurnal (Knohl et al. 2005) variations in the C isotope signature of forests. At the soil level, a large seasonal variation has also been shown (Ekblad and Högberg 2001; Bhupinderpal-Singh et al. 2003; Ekblad et al. 2005). These variations were linked to environmental conditions measured 3–5 days prior, indicating that recently fixed assimilates from the canopy played a role (Ekblad and Högberg 2001). Very recent papers have shown diurnal variations of the stable C isotope composition ($\delta^{13}C$) of CO_2 respired by two *Quercus* forest canopies (Hymus et al. 2005) and by *Pine elliottii* needles (Prater et al. 2005). Nevertheless, to our knowledge, the $\delta^{13}C$ of CO_2 respired by tree trunks has never been examined. Yet, the respiration of the woody compartment is of quantitative importance in the forest C balance (e.g. Ryan et al. 1996; Lavigne and Ryan 1997; Damesin et al. 2002).

In addition to providing information for the interpretation of the isotope signature at the ecosystem level, measurements of the respired CO_2 at the tree level, especially from trunks, could improve analysis of the tree ring signal. At present, functional interpretations of $\delta^{13}C$ rely on Farquhar's discrimination model (Farquhar et al. 1982, 1989). This model integrates the discrimination during leaf photosynthesis and links leaf isotope signature to water use efficiency (= assimilation/transpiration) (Farquhar and Richards 1984). This model was validated at the leaf scale for many species (e.g. von Caemmerer and Evans 1991; Poorter and Farquhar 1994), but the link between the isotope signature of the leaf and that of the stem has not been extensively studied. Some studies, such as that of Berninger et al. (2000), have tried to connect this ''leaf''model with ring $\delta^{13}C$ but the approach did not incorporate any experimental data on the potential discrimination steps between the leaf and the ring. To understand the ring isotope signature in terms of tree functioning, different steps should be examined: (1) the transport of sugars assimilated by leaves (see Keitel et al. 2003; Gessler et al. 2004); (2) the use of reserves; and (3) trunk respiration. Recent works suggest the existence of post-photosynthetic fractionations (Damesin and Lelarge 2003; Badeck et al. 2005). Differences between the $\delta^{13}C$ of respired CO_2 and that of the organic components have been shown in different tissues: herbaceous leaves, shoots and roots (Duranceau et al. 1999; Ghashghaie et al. 2001; Klumpp et al. 2005), and twigs and leaves of woody species (Damesin and Lelarge 2003; Xu et al. 2004).

To improve the analysis of both the C isotope signature of ecosystem respiration and that of the ring, we examined, in situ, the variations of the $\delta^{13}C$ of CO_2 respired by trunks of *Quercus petraea*, a deciduous oak. Measurements were performed at different temporal scales: over the course of the day (diurnal), on a day-to-day basis (daily), and over a year (seasonal), including different phenological periods (budburst, growth and leafless period). Our hypothesis is that there must be a high temporal variability of tree-respired $^{13}CO_2$—in particular, during the day because of the dynamic of stomatal conductance, and also throughout the year (especially between leafy and non-leafy periods) because reserves are the only C source in winter. Thus, the objectives of this study were: (1) to determine at which temporal scale are the maximum variations of the $\delta^{13}C$ values of CO_2 respired by trunks, (2) to compare the $\delta^{13}C$ values of respired CO_2 to that of the total organic matter and soluble sugars that are thought to be the main respiratory substrates, and (3) to analyse the relationship between the $\delta^{13}C$ of CO_2 respired by trunks and both environmental parameters and respiration rates.

Materials and methods

Site description

The experiment was conducted in the Barbeau national forest (48°29′N, 02°47′E), which is located 60 km southeast of Paris (France) at an elevation of 90 m. The average temperature and annual rainfall in this temperate location are 10.5°C and 690 mm, respectively. The soil is 80–90 cm deep, hydromorphic with gley and millstone grit. The trees are rarely subjected to water stress. The site is a managed mature oak forest with a dense understorey of coppiced hornbeam

(*Carpinus betulus*). The dominant species is sessile oak (*Q. petraea*). The oaks are 100–140 years old and the average height of the dominant trees is 29 m. The canopy leaf area index is 5, on average (K. Soudani and E. Dufrêne, personal communication). For the sessile oak, budburst occurred around 23 April in 2004 (E. Dufrêne, personal communication).

Experimental design

The measurements were made on four dominant oaks. Tree diameter at a height of 1.3 m ranged from 28 to 42 cm. Data collection in the field was planned to document seasonal, daily and diurnal variation in $\delta^{13}C$ of trunk-respired CO_2, respiration rates, concentration of total soluble sugars (sucrose, glucose and fructose) and their C isotope signature in the phloem. Measurements were distributed among 15 field campaigns, starting in March 2004 (before the budburst period) and ending in December 2004. For each campaign, the respiration rate measurement, respired CO_2 sampling and phloem sampling were performed between 1000 and 1300 hours (Universal Time). At 4 times during the year (in April, May, June and December), the same protocol was followed for 3 (or 2 in December) consecutive days. Moreover, 5 times during the year, respiration rates and $\delta^{13}C$ of CO_2 respired by trunks were determined 5 (on Julian days 111, 139, 167) or 4 (on Julian days 308, 337) times during the day. Measurements began at sunrise and ended at sunset. To avoid excessive damage of trunks during these experiments, phloem was only collected 3 times a day (early morning, late evening and in the middle of the day).

Throughout the entire text, ''diurnal'' corresponds to measurements achieved one day between sunrise and sunset. ''Daily'' is used for measurements occurring over 3 (or 2) consecutive days.

Phenological and environmental parameters

Rainfall and relative humidity were collected from the nearest (5 km) meteorological station (Météo-France, Le Chatelet en Brie).

Photosynthetic photon flux density and temperature

In order to link daily variation of respired CO_2 $\delta^{13}C$ to environmental factor, photosynthetic photon flux density (PPFD) was measured, for daily measurements, on 3 days in April, May and June. A custom-made PPFD sensor, using a gallium arsenide detector (Pontailler 1990), inter-calibrated with a Li-190sb quantum sensor (Li-Cor, Lincoln, Neb.), was placed 4 m above ground in a clearing close to the experimental site. PPFD was measured every minute and recorded by a data logger (CR10X; Campbell Scientific, Logan, Utah). Below-canopy (2 m height) air temperature was measured automatically every 15 min from March 2004 to December 2004, by a sensor (LM35CZ) that was sheltered from solar radiation and connected to another CR10X data logger.

Leaf mass per area and trunk diameter

Two parameters were considered as indicators of tree phenology: leaf mass per area (LMA) and trunk diameter. LMA was measured every month on each tree, from budburst to leaf fall. For each tree, sun leaves were sampled at the top of the canopy using a rifle. In the laboratory, leaf area was measured using an area meter (Delta-T Devices, UK). Leaf dry weight was determined after 48 h in an oven at 60°C. The growth beginning was determined with an automatic microdendrometer (stainless steel band associated with a movement sensor (MM30; Megatron, Allinges, France; resolution <0.01 mm, stroke 30 mm) and a drawspring. These data were not available later in the season. Therefore, the tree diameter at a height of 1.30 m, that was manually measured during each field visit, was used to follow the growth rate and pattern.

Respiration

Gas exchange measurements

The CO_2 efflux of the trunks was measured using Plexiglas chambers that were applied temporarily. We called this efflux ''trunk respiration'', keeping in mind that the CO_2 released in the chamber could also come from CO_2 transported by the xylem, as suggested in several studies (Hari et al. 1991; Martin et al. 1994; Meir et al. 1999; Teskey and McGuire 2002). At breast height, the chambers (described in Damesin et al. 2005) were fixed on the trunk with a malleable rubber sealant (Terostat-7; Teroson, Ludwisburg, Germany) and straps. The installation was considered to be satisfactory if blowing along the seals caused no increase in the CO_2 level inside the chamber. Before installing the chambers, the trunk was gently scrubbed in order to remove algae and lichens present on the bark.

The CO_2 concentration in the chamber was measured using an infrared gas analyser (IRGA) that was installed in a closed system (EGM1; PPSystems, Hitchin, UK). The EGM1 was modified to work in a

closed circuit: an air outlet was arranged at the exit to the analysis cell. The internal software was also changed to avoid automatic zeros that would have caused CO_2-free air to be introduced into the circuit. The chamber was connected to the IRGA by two independent flexible tubes of approximately 80 cm in length (Excelon BEV-A LINE IV, a polycarbonate tube with inert liner; Thermoplastic Processes, Stirling, N.J.). A constant air flow (10^{-4} m^3 min^{-1}) was maintained in the system by the IRGA's pump, and a fan located in the chamber allowed a good mixing of the air. Respiration measurements lasted for 2 min. The CO_2 increase was checked for linearity during the measurement period by taking an intermediary value of CO_2 inside the chamber at the end of the first minute. Between measurements, a removable stopper (5 cm in diameter), located at the fan's level, was opened to purge stored CO_2 and to allow the CO_2 content of the chamber to decrease to a value close to ambient. Three repetitions were performed each time (if values were too variable, a fourth repetition was done) and the average value was used. Chambers were removed after each series of measurements and reinstalled at the same place for each field campaign.

From these measurements, respiration rates were calculated and expressed on the basis of trunk area or per unit volume of living tissue (i.e. phloem and sapwood). The depth of the living tissue was determined for each tree from a trunk core collected near the chamber. The volume of the living tissue was calculated using this depth and taking into account the cylindrical shape of the trunk.

CO_2 sampling

After respiration rate measurements, the chamber was purged with N for 15 min until the CO_2 concentration inside the chamber dropped to 20–30 p.p.m. Following this, the outlet and inlet pipes were connected together to make a completely closed system allowing the respired CO_2 by the trunk to accumulate in the chamber. After accumulating 700–800 p.p.m. (this took approximately 10 min in summer and 90 min in winter), the chamber air (containing CO_2 coming only from trunk efflux) was sampled after being mixed by the internal fan, using a 50-ml valved syringe (SGE, Australia) that was previously fixed to the chamber. On the same day or the following day, the contents of the syringes were analysed in the laboratory with a mass spectrometer to determine the isotope signature of trunk-respired CO_2. This method has been tested by Damesin et al. (2005) and gave the same results as a Keeling plot method applied to the same chambers. Ambient air was also collected monthly in syringes at a height of 2 m near the measured trees in the forest.

Sugar extraction and contents

Sugar content and C isotope signature were determined in the phloem that is, the tissue in which sugar transport from the leaves occurs. Phloem of the four trees was sampled at the chamber level, or up to 30 cm above, using a punch and a hammer. The piece of phloem was plunged directly into liquid nitrogen before being lyophilised and finely ground in a ball mill (type MM200; Retsch, Haan, Germany).

From 50 mg powdered tissue, sugars were extracted, at 4°C, with 1 ml fresh water in a 2-ml Eppendorf. The soluble fraction was heated to 100°C for 3 min to denature all proteins present in the extract. After centrifugation (5 min, 5°C, 12,000 *g*), the sample was frozen. Prior to analysis, the extract was filtered through 0.45-μm filters (HV; Nihon Millipore Kogyo, Japan).

A total of 25 μl of this solution was injected into a high-pressure liquid chromatography apparatus. Sucrose, glucose and fructose were separated by a carbohydrate precolumn (7.5 mm× 4.6 mm; Alltech, Templemars, France) and then by a Sugar Pak1 column (300 mm× 6.5 mm; Waters, Guyancourt, France). The flow was fixed at a constant value of 0.5 ml min^{-1}, pressure was maintained around 40 bars and the temperature of the column was 90°C.

Peaks for the three sugars were detected using a refractometer (refractive index detector 133; Gilson, Villiers-le-Bel, France). Sugar concentrations were determined by the surface areas of the peaks, compared with a range of standards prepared with sugar-known solutions. The same method was used to prepare and isolate pure fractions of sugars to determine their isotope signature. To obtain sufficient material (to be detectable by the mass spectrometer), 80–200 μl was injected into the column. Soluble sugars were collected and were freeze-dried. They were dissolved in distilled water and installed in tin capsules for the isotopic analyses. Duranceau et al. (1999) verified that no isotopic discrimination occurs at the time of elution from the column.

Isotopic analysis

For the respired CO_2, we used the system derived by Tcherkez et al. (2003). Gas samples were injected into the elementary analyser (model NA-1500; Carlo Erba,

Milan) through a 15-ml loop. The different gases were separated (mainly N_2/O_2 and CO_2) and the connection valve between the elemental analyser and the isotope ratio mass spectrometer (VG Optima; Fison, Villeurbanne, France) was opened when the CO_2 peak emerged from the elemental analyser.

The solid samples of sugars and organic matter were conducted in the same elemental analyser and isotope ratio mass spectrometer. Gases resulting from combustion were separated and the CO_2 peak was sent to the isotope ratio mass spectrometer.

Isotope composition was calculated from the sample isotopic ratio $^{13}C/^{12}C$ (R_s) measured by the IRMS and was expressed using the conventional delta notation (in ‰) according to the relationship:

$$\delta^{13}C = \frac{(R_s - R_{PDB})}{R_{PDB}} \times 10^3,$$

where PDB (Pee Dee Belemnite) is the international standard.

Precision for isotopic measurements was ±0.2‰ based on repeated measurements of a laboratory working standard (i.e. glutamic acid).

Statistical analyses

For all statistical analysis described below, the number of replicates was $n = 4$, corresponding to the four trees. ANOVA was used to test for an effect of time of season and of day on $\delta^{13}C$ of CO_2 respired by the trunk, $\delta^{13}C$ of phloem total organic matter, $\delta^{13}C$ and concentration of phloem sugars, and respiration rate. Whenever the interaction was statistically significant, post hoc Scheffé tests were performed to identify differences between individual means. Results were described as significant when $P < 0.05$. Correlations between variables were quantified by Pearson's correlation coefficient. Statistical analyses were performed using a statistical software package (Superanova; Abacus Concepts, Piscataway, N.J.).

Results

Phenology and environmental conditions

Budburst occurred at the end of April and leaf fall occurred in November. LMA increased from May to July, with a maximum at 115 g dry matter (DM) m^{-2} (Fig. 1) and started to decrease in October. The trunk growth began just before budburst, during the first week of April (6–7 April 2004; data not shown) and lasted to the end of July (Fig. 1). There was no clear difference between trees when growth started. In 2004, air relative humidity varied between 53 and 99%, with

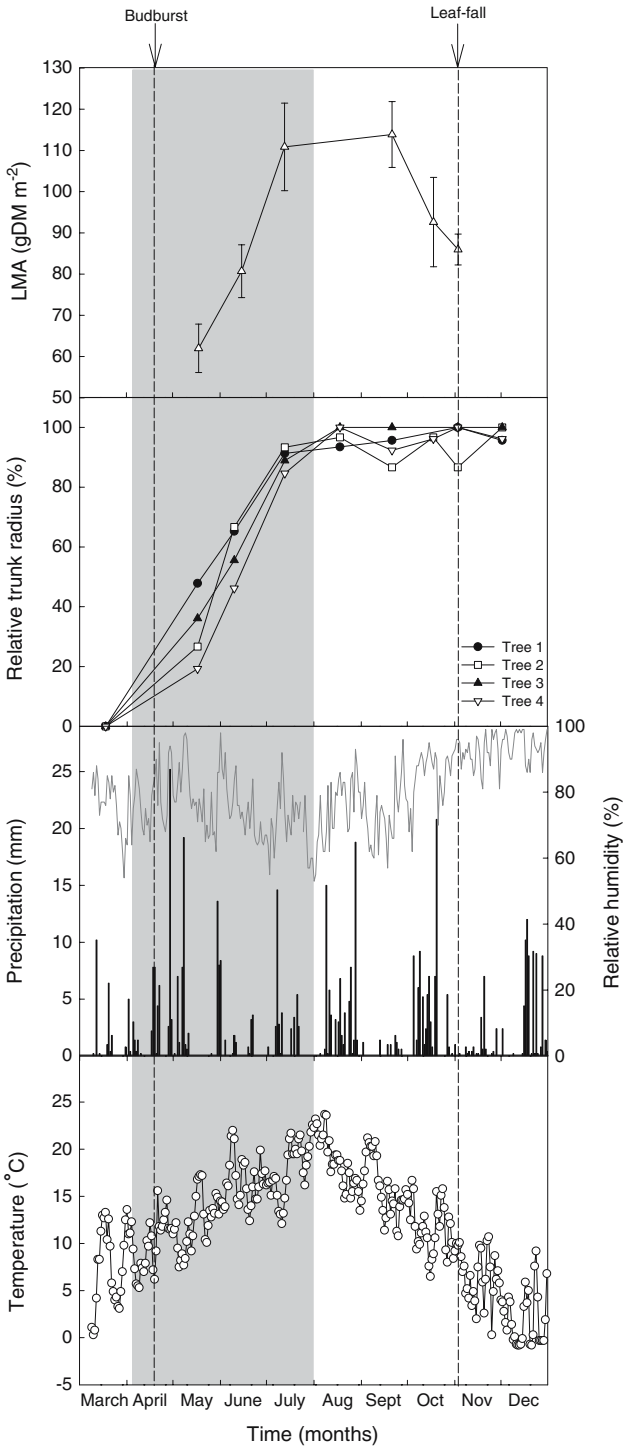

Fig. 1 Seasonal changes in leaf mass area (*LMA*), individual trunk radial increment, average daily relative humidity of air (*grey line*) and daily rainfall (*histogram*), and average daily air temperature (below canopy). Trunk growth period is indicated by *shading*. *Error bars* represent ±1 SE (mean ± SE). *DM* Dry matter

an annual average of 81% ± 11 (Fig. 1), rainfall reached 602 mm (Fig. 1) and the average air temperature was 10.2°C (Fig. 1). During trunk growth (April–July), the average temperature was 14.5°C and rainfall was 182 mm. The C isotope signature of the forest ambient air (ambient atmosphere at a height of 2 m) oscillated between –8 and –11‰ (data not shown).

Seasonal variation

$\delta^{13}C$ of respired CO_2 and total organic matter

The isotope signature of the total organic matter measured for phloem varied throughout the year (Fig. 2). Its range of variations was small (from –26.3 to –27.2‰, with an annual mean of –26.8‰). Among trees, the average $\delta^{13}C$ of respired CO_2 varied significantly over the year between –22.6 and –25.9‰ (Fig. 2). A relative maximum occurred during the beginning of the growth period, in the first days of May. After this time, the isotope signature stayed low throughout the growth period. After the end of growth and a little before leaf fall, the respired CO_2 isotope composition began to increase progressively during autumn, reaching a second relative maximum (–23.0‰) in early winter (beginning of December). $\delta^{13}C$ of respired CO_2 was negatively correlated with air temperature (see inset Fig. 2). On the whole, the isotope composition of CO_2 respired by the trunk was higher (from +1 to +4–5‰) than that of phloem organic matter.

Respiration rates

The respiration rate, expressed per unit volume of living tissue, increased from 35 to 102 μmol CO_2 m^{-3} s^{-1} from May to July (Fig. 2). It decreased progressively, after growth stopped, to 11 μmol CO_2 m^{-3} s^{-1} in December. The relative maximum of the respired CO_2 $\delta^{13}C$ occurred in May. At this point, the trunk respiration had not yet begun to increase. The maximum and minimum values of $\delta^{13}C$ occurred when respiration rates were low and high, respectively; in addition, there was a linear relationship ($R = –0.89$; $n = 10$; $P < 0.01$) between these two variables from March to December.

Concentrations and $\delta^{13}C$ of phloem sugars

Both concentration and $\delta^{13}C$ of phloem sugars significantly varied during the season (Fig. 2). From March to May, the sugar pool in the phloem decreased and reached a minimum when the $\delta^{13}C$ of respired CO_2 showed a maximum. After this minimum, the sugar content increased, especially once growth had stopped. Considering the values from July to December, the $\delta^{13}C$ of respired CO_2 was correlated to the phloem sugar content ($R = 0.95$; $n = 6$; $P < 0.01$).

At budburst, the $\delta^{13}C$ of phloem sugar was similar to that of CO_2 respired by the trunk (Fig. 2). Just after the early spring peak, the $\delta^{13}C$ of respired CO_2 was lower than that of phloem sugars. At the beginning of winter, the $\delta^{13}C$ of phloem sugars reached their

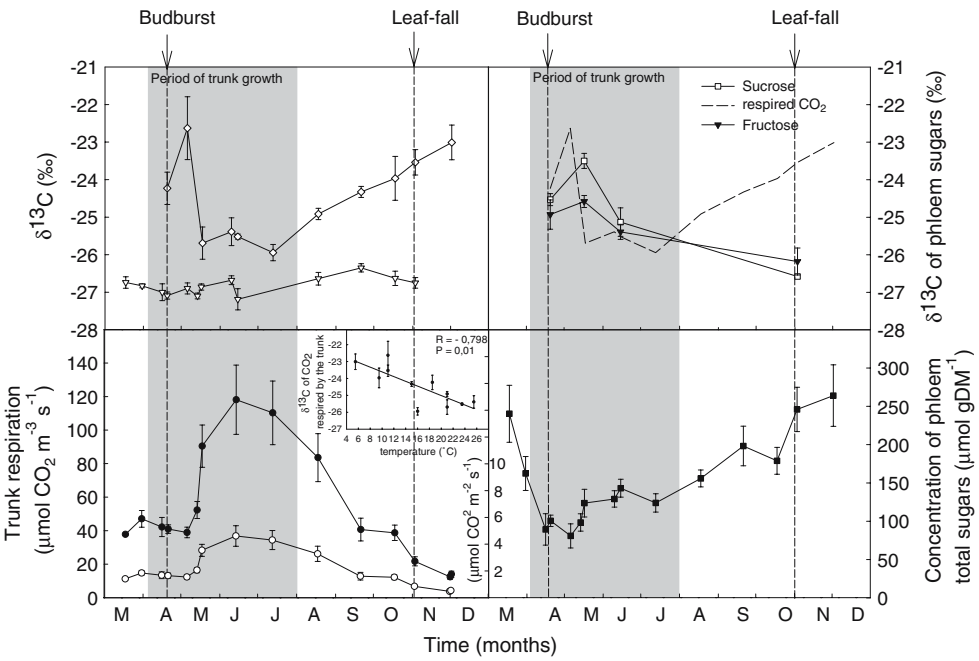

Fig. 2 Seasonal changes in C isotope composition of CO_2 respired by trunks (*open diamonds*) and $\delta^{13}C$ of phloem total organic matter (*open triangles*), trunk respiration rate expressed on the basis of living tissue volume (*filled circles*) and expressed on the basis of trunk area (*open circles*), $\delta^{13}C$ of phloem sugars (sucrose, fructose) (respired CO_2 $\delta^{13}C$ is shown by the *dashed line*), and concentrations of total phloem sugars (sucrose, glucose and fructose). *Inset* Correlation between $\delta^{13}C$ of CO_2 respired by the trunk and air temperature. Trunk growth period is indicated by *shading*. *Error bars* represent ±1 SE (mean ± SE)

minimum value (–26.6‰), whereas the $\delta^{13}C$ of respired CO_2 was near its maximal value (–23.5‰).

Daily variation

Environmental parameters (temperatures, PPFD, precipitation, relative humidity and weather) were generally not different between days (Table 1). In April only, weather conditions varied clearly between days. Regardless of the season, the respiration rates and the $\delta^{13}C$ of respired CO_2 were fairly stable between consecutives days (Fig. 3). The maximum variation of the $\delta^{13}C$ obtained over 3 days was small (0.64‰ in June).

Diurnal variation

The diurnal variation in $\delta^{13}C$ of CO_2 respired by trunks showed different patterns depending on the measurement period. At budburst (April), the diurnal respired CO_2 $\delta^{13}C$ significantly varied between –21.2 and –24.2‰, i.e., within an amplitude of 3‰ (Fig. 4). The maximum value occurred around 1200 hours (Universal Time). After this midday peak, the $\delta^{13}C$ decreased until night-time. The $\delta^{13}C$ measured in the evening (–23.5‰) was similar to that measured in the morning (–23.8‰). These diurnal variations were not correlated with respiration rate (Fig. 4), nor with the concentration or C isotope signature of the sugars (Fig. 5, April). At the beginning of the day, there was no difference between $\delta^{13}C$ of CO_2 and that of sugars. CO_2 was ^{13}C enriched compared to sugars, at midday (+3.31‰) and in the evening (+1.13‰).

During the growth period (May and June), the $\delta^{13}C$ of respired CO_2 was lower than in April (as shown previously), and only showed a slight but not significant decrease during the day. No trend in the diurnal variation of phloem sucrose $\delta^{13}C$ was observed (Fig. 5, May and June). However, the gap between $\delta^{13}C$ of CO_2 and that of sucrose was not the same between the 2 days (around –1.2 to –2‰ in May, and –0.4 to 0.2‰ in June). Variations in respiration rate were small, even though temperature showed large variations during these 2 months (Fig. 4; May and June). In June, the sucrose content showed a diurnal decrease (from 70.3 to 23.7 μmol g DM^{-1}), like the $\delta^{13}C$ of respired CO_2 (Fig. 5, June).

During the leaf-fall period (November and December), the C isotope composition of the CO_2 respired by trunks showed a decrease (significant only in November) during the day (Fig. 4), whereas the respiration rate remained stable (Fig. 4). The isotope signature of respired CO_2 was higher than that of sucrose (Fig. 5, November). The difference ranged between –1 and –4‰. Sucrose content in the phloem ranged between 99 and 124 μmol g DM^{-1} and showed the highest values within all dates (Fig. 5, November).

Discussion

Discrimination during respiration

This study revealed an enrichment in ^{13}C of the CO_2 respired by trunks relatively to the one of the bulk biomass. The phloem $\delta^{13}C$ was rather stable and the weak variations observed could probably be attributed to sampling, rather than to a real seasonal dynamic. Indeed, as for leaves when the major structural material is fixed, there are only minor temporal changes in the C isotope composition of the tissue (see Damesin et al. 1998; Fessenden and Ehleringer 2003). For *Q. petraea*, the phloem is depleted in ^{13}C by about 1‰ relative to the sapwood (see Damesin et al. 2005). As a consequence, the ^{13}C enrichment of respired CO_2

Table 1 Environmental characteristics during the four series of measurements shown in Fig. 3. *PPFD* Photosynthetic photon flux density

	April			May			June			December	
Date	19	20	21	17	18	19	14	15	16	1	2
Minimum temperature (°C)	3.3	5.8	10.6	9.8	10.5	10.3	7.9	12.9	14.5	1.3	0.7
Maximum temperature (°C)	10.4	13.8	22.1	23	23.1	24	22	25.7	22.3	5.2	5.5
Mean temperature (°C)	6.2	9.2	15.6	16.8	17.1	17.3	15.1	18.9	18.3	4	3.8
PPFD maximum (μmol phot m^{-2} s^{-1})	1,689	1,278	1,778	1,828	1,836	1,830	1,894	1,938	2,141	No data	
Rainfall (mm)	7.8	0.2	4.4	0	0	0	0	0	0	0	2.4
Relative humidity (%)	84	90	76	71	78	75	72	74	71	93	96
Weather	Cloudy, rainy	Cloudy	Cloudy, rainy	Sunny	Sunny	Sunny	Sunny	Sunny	Sunny	Sunny	Cloudy, rainy

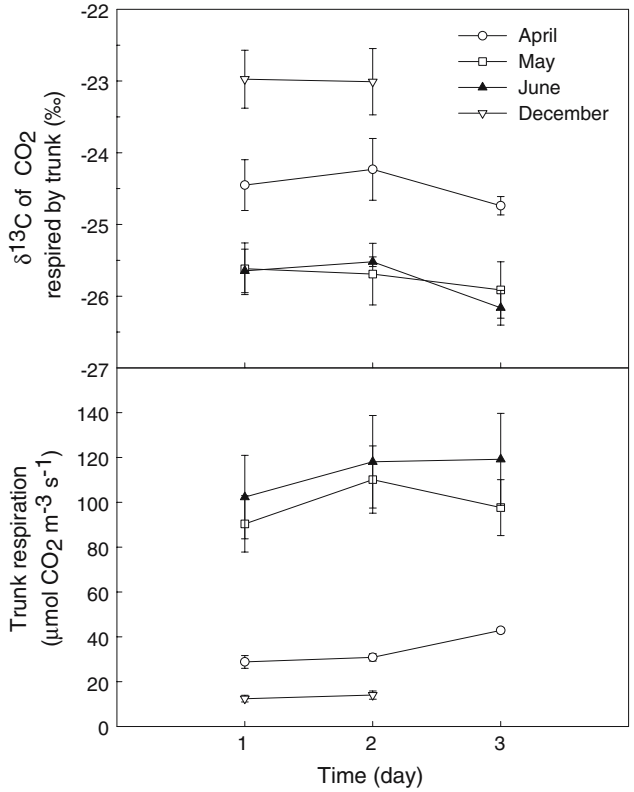

Fig. 3 Changes over 3 consecutive days (2 days for December) in $\delta^{13}C$ of CO_2 respired by the trunk, and trunk respiration rate (expressed on the basis of living tissue volume). The field days were 19–21 April, 17–19 May, 14–16 June and 1–2 December 2004. *Error bars* represent ±1 SE (mean ± SE)

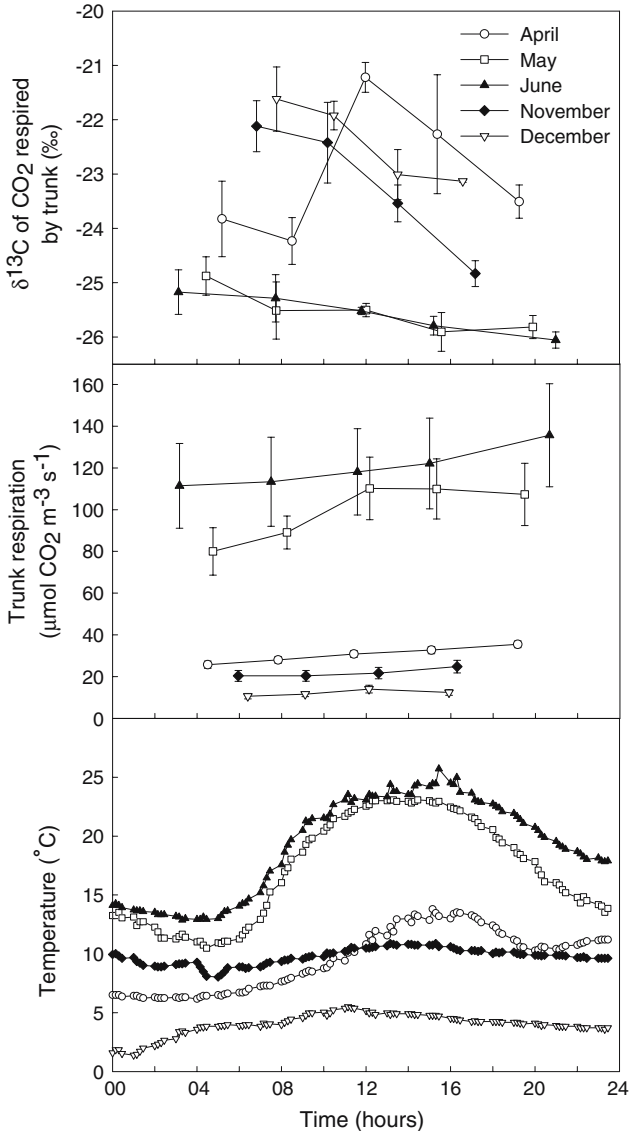

Fig. 4 Diurnal variation in $\delta^{13}C$ of respired CO_2 by trunks, trunk respiration rate (expressed on the basis of living tissue volume), and air temperature. *Error bars* represent ±1 SE (mean ± SE)

observed relatively to phloem (1–5‰) remains valid when considering the xylem. This ^{13}C enrichment is consistent with results previously obtained when CO_2 $\delta^{13}C$ is compared to $\delta^{13}C$ of total organic matter, for leaves of different herbaceous species (Duranceau et al. 1999, 2001; Ghashghaie et al. 2001; Xu et al. 2004), for shoots (Klumpp et al. 2005), and for young branches of beech (Damesin and Lelarge 2003). However, the values in this study differ from those recently obtained for roots of sunflower and alfalfa, for which the respired CO_2 was ^{13}C-depleted compared with bulk biomass (Klumpp et al. 2005).

It is interesting to note the similarity between the temporal variation pattern observed in the respired CO_2 isotope signature during ring growth and the spatial variation found for the rings by Helle and Schleser (2004). By cutting rings in slices of about 100 µm, these authors showed an intra-annual variation of about 3‰ in the $\delta^{13}C$ of ring cellulose of *Q. petraea*, with a peak in the earlywood. This result suggests that a ^{13}C enrichment of the CO_2 respired by the trunk (our results) and of the organic matter built into the trunk (their results in rings) occurred at the same period of the year. The similarity between $\delta^{13}C$ of CO_2 and organic matter may be explained by the variation in the substrates used for both respiration and building organic matter.

Temporal variations in $\delta^{13}C$ of respired CO_2

Seasonal variations

The range of the isotope composition of respired CO_2 is consistent with those obtained for young beech stems (Damesin and Lelarge 2003), for shoots and roots of alfalfa, ryegrass and sunflower (Klumpp

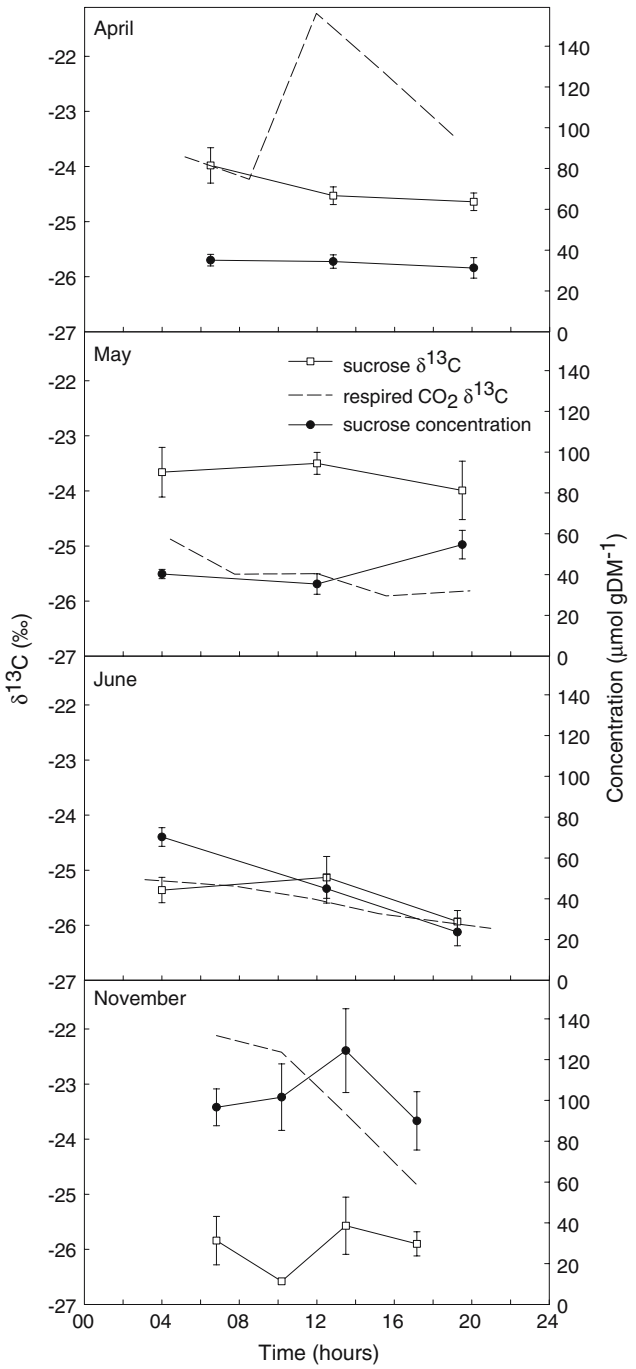

Fig. 5 Diurnal changes in sucrose $\delta^{13}C$ (*open squares*) and concentration (*filled circles*) on 20 April, 18 May, 15 June and 3 November. *Error bars* represent ±1 SE (mean ± SE). Respired CO_2 $\delta^{13}C$ shown by *dashed lines* to clarify the differences with sucrose $\delta^{13}C$

et al. 2005). This seasonal pattern in $\delta^{13}C$ of respired CO_2 is similar to that observed for beech twigs by Damesin and Lelarge (2003). Such seasonal variations in $\delta^{13}C$ of respired CO_2 have been highlighted recently at the forest level using the Keeling plot approach (Ehleringer et al. 2002; Scartazza et al. 2004; Baldocchi and Bowling 2003; Knohl et al. 2005), but these variations are still poorly understood. The isotope signature of soil respiration has also been shown to vary between seasons (Ekblad and Högberg 2001; Ekblad et al. 2005). Our study presents the first evidence of such seasonal variations of the $\delta^{13}C$ of respired CO_2 for the aboveground woody component of deciduous trees in the field. These variations were linearly related to air temperature as previously obtained by Tcherkez et al. (2003) on French beans (*Phaseolus vulgaris*) in laboratory conditions.

The dynamic of the $\delta^{13}C$ of respired CO_2 during growth could be explained by a shift in the respiratory substrates. At the beginning of growth, when the respired CO_2 $\delta^{13}C$ is high, trunks probably use reserves (i.e. starch) as the substrate source for respiration. Starch is often enriched in ^{13}C, with an isotope signature reaching high values, such as –21 and –23‰ (O'Leary 1981; Brugnoli et al. 1988; Damesin and Lelarge 2003; Xu et al. 2004; Tcherkez et al 2005). C. Damesin (unpublished data) found values of –23.5‰ for trunk starch $\delta^{13}C$ of *Q. petraea* at the same site as our study. After budburst, leaves become autotrophic and progressively they become a C source for growth of other tree tissues (Barbaroux and Bréda 2002). They supply trunks with recently assimilated sugars that are ^{13}C-depleted by discriminating steps during CO_2 assimilation (Farquhar et al. 1982), and that lead to $\delta^{13}C$ values around –25 and –26‰ (Fig. 2), i.e. lower than starch ones. At this period, the measured respired CO_2 was sometimes depleted in ^{13}C by sugars (see Fig. 2). This could be explained by the metabolic branching that could result in CO_2 depletion in ^{13}C compared to sugars when at the same time ^{13}C-enriched compounds like starch are produced from the same substrate.

The high CO_2 $\delta^{13}C$ values occurring in winter were also observed for beech and oak trunks by Damesin et al. (2005), and also at the forest level for hardwood species by Knohl et al. (2005) and Mortazavi et al. (2005). However, high values in winter are not always found (e.g. for a pine forest—Mortazavi et al. 2005; and for soil—Bhupinderpal-Singh et al. 2003; Ekblad et al. 2005; Mortazavi et al. 2005). The highest values we found remained lower than the maximum values observed for leaves, such as –18‰ (Tcherkez et al. 2003) and –16‰ (Duranceau et al. 1999). The winter increase in $\delta^{13}C$ of respired CO_2 could be caused by a progressive shift of substrates from photosynthesis sugars to reserves (starch). Nevertheless, it is surprising to note that phloem sugar $\delta^{13}C$ does not increase. Indeed,

when starch is remobilised, it should not only result in CO_2 ^{13}C enrichment, but should also influence the sugars' isotope signature.

Another explanation for the winter increase is the importance of the respiration rate relatively to the substrate availability. The work of Tcherkez et al. (2003) suggests the existence of a "fractionation" (^{13}C enrichment) during respiration. The expression of this fractionation could be dependent on respiration rate. An elevated temperature leads to a much faster consumption of respiratory carbohydrates and a decrease in the sugar pool. In this condition, the "fractionation" could be hidden and results in more ^{13}C-depleted respired CO_2. This hypothesis is consistent with the correlation obtained between sugar content and the $\delta^{13}C$ of respired CO_2 (from July to December).

Daily variations

The daily variations in the respired CO_2 isotope signature were lower than those observed at the seasonal and diurnal scales. These variations were also lower than those observed at the forest level (3‰) by Knohl et al. (2005) and Barbour et al. (2005). The high degree of stability, observed during consecutive days, could be explained by non-contrasted meteorological data during these days. In April, the increase in temperature on the third day did not have any immediate effect on the $\delta^{13}C$. Indeed, many recent studies have shown a link between climatic conditions (especially air vapour pressure deficit and air temperature) and the C isotope signature of the soil (Ekblad and Högberg 2001; Ekblad et al. 2005) and the ecosystem (Knohl et al. 2005; Mortazavi et al. 2005) with a time lag of 1–4 days. Their results suggest that variations in the $\delta^{13}C$ of CO_2 respired by soil are caused by variations in isotopic discrimination during photosynthesis, and that sugars take several days to be transported from the canopy to the roots or the soil, where they are used as respiration substrates. Studies on the transport of assimilates by the phloem improve the understanding of the time lag between assimilate formation in leaves and their use as a respiration substrate in the various compartments of the ecosystem (Keitel et al. 2003; Gessler et al. 2004).

Diurnal variations

Contrary to what is expected, we note that the largest diurnal variations of $\delta^{13}C$ of CO_2 respired by the trunk occurred when leaves were non-functional or only slightly functional. The peak observed in the middle of the day during the budburst period has been also observed for *Quercus ilex*, a Mediterranean evergreen oak (C. Barbaroux, personal communication), and for needles of *Pinus elliottii*, an evergreen conifer (Prater et al. 2005), at the same phenological stage. It is interesting to note that Knohl et al. (2005) also found a midday increase in the $\delta^{13}C$ measured at the ecosystem level for a temperate deciduous forest in spring. As leaves were not functional (missing or very young), the diurnal variations cannot be linked to leaf functioning. A plausible explanation is the variation in the anabolic pathways of the living trunk cells. We could assume a diurnal change in the dominant metabolic pathways (see Tcherkez et al. 2003). For example, we are suggesting that a high synthesis of ^{13}C-depleted products (such as lignin and lipids) in the middle of the day could result in an increase in respired CO_2 $\delta^{13}C$. Quantitative studies on the dynamic of the main metabolic fluxes are needed, and the C isotope composition values of the products (proteins, secondary metabolites synthesised from acetyl-CoA) should be determined in order to confirm this hypothesis.

In conclusion, the C isotope composition of the CO_2 respired by trunks may exhibit large seasonal and diurnal variations. Our results show that seasonal (but not diurnal) variation of the $\delta^{13}C$ of CO_2 respired by trunks was correlated to air temperature. From an ecophysiological point of view, the seasonal pattern of the CO_2 $\delta^{13}C$ could be explained either by a shift of substrates that are used for respiration or by variations of respiration rate relatively to the substrate pool and may explained the intra-ring $\delta^{13}C$ variations. In contrast, the diurnal variations are probably linked to changes in the metabolic pathways of living trunk cells. Our study confirms that the C isotope composition of respired CO_2 cannot be considered to be equal to that of the total organic matter. The difference should probably be considered in the C cycle model in order to improve the interpretation of the CO_2 $\delta^{13}C$ signal at the ecosystem level. Our work on the trunk compartment complements other studies that have been undertaken on forest soil and foliage and may have implications for understanding the dynamic change in the ^{13}C isotopic exchange between forests and the atmosphere.

Acknowledgements Many thanks to Alice Michelot and Amandine Hansart for their assistance in the field and laboratory work. The authors are grateful to the Office National des Forêts, especially Michel Bénard, for facilitating the experimental work in the Barbeau forest. The meteorological data (precipitation, humidity) of Fig. 1 were provided by Météo-France (Melun, France). We acknowledge Elizabeth Gerson and International Science Editing for correcting the English language of the manuscript. This research was financed by the French projects: Ministère délégué à la recherche-ACI Jeunes Chercheurs

(no. JC10009) and Programme National ACI/FNS ECCO, PNBC (convention no. 0429 FNS), and the ESE laboratory supported by the University Paris XI and CNRS. We are grateful to five anonymous reviewers for useful suggestions, which enabled the authors to improve the clarity of the manuscript. The study complies with current French law.

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