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ORIGINAL PAPER

Carbon isotopic signature of CO_2 emitted by plant compartments and soil in two temperate deciduous forests

Florence Maunoury-Danger · Nicolas Chemidlin Prevost Boure · Jérôme Ngao · Daniel Berveiller · Claude Brechet · Eric Dufrene · Daniel Epron · Jean-Christophe Lata · Bernard Longdoz · Caroline Lelarge-Trouverie · Jean-Yves Pontailler · Kamel Soudani · Claire Damesin

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Abstract
• ***Context*** The carbon isotope composition of the CO_2 efflux ($\delta^{13}C_E$) from ecosystem components is widely used to investigate carbon cycles and budgets at different ecosystem scales. $\delta^{13}C_E$, was considered constant but is now known to vary along seasons. The seasonal variations have rarely been compared among different ecosystem components.
• ***Aims*** We aimed to characterise simultaneously the seasonal dynamics of $\delta^{13}C_E$ in different compartments of two temperate broadleaved forest ecosystems.

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Contribution of the co-authors Florence Maunoury-Danger Nicolas Chemidlin Prevost Boure, Jérôme Ngao, Daniel Berveiller, Claude Brechet, Eric Dufrene, Daniel Epron, Jean-Christophe Lata, Bernard Longdoz, Caroline Lelarge Trouverie, Jean-Yves Pontailler, Kamel Soudani, Claire Damesin: performing practical work, field sampling, phenological and climatic measurements and isotopic analysis from Barbeau and Hesse sites.
Florence Maunoury-Danger Nicolas Chemidlin Prevost Boure, Jérôme Ngao, Bernard Longdoz, Daniel Epron, Claire Damesin: data analyses
Claire Damesin: designing the experiment and coordinating the research projects 'Ministère délégué à la recherche-ACI Jeunes Chercheurs' (no. JC10009) and 'Programme National ACI/FNS ECCO PNBC' (convention no. 0429 FNS)
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• ***Methods*** Using manual chambers and isotope ratio mass spectrometry, we recorded simultaneously $\delta^{13}C_E$ and $\delta^{13}C$ of organic matter in sun leaves, current-year twigs, trunk bases and soil in an oak and a beech forest during 1 year.
• ***Results*** In the two forests, $\delta^{13}C_E$ displayed a larger variability in the tree components than in the soil. During the leafy period, a pronounced vertical zonation of $\delta^{13}C_E$ was observed between the top (sun leaves and twigs with higher values) and bottom (trunk and soil with lower values) of the ecosystem. No correlation was found between $\delta^{13}C_E$ and $\delta^{13}C$ of organic matter. Causes for these seasonal variations and the vertical zonation in isotope signature are discussed.
• ***Conclusion*** Our study shows clear differences in values as well as seasonal dynamics of $\delta^{13}C_E$ among different components in the two ecosystems. The temporal and local variation of $\delta^{13}C_E$ cannot be inferred from organic matter signature or CO_2 emission rates.

Keywords Carbon isotopic composition · CO_2 efflux · Oak forest · Beech forest

Abbreviations

E_T	Trunk CO_2 efflux
E_S	Soil CO_2 efflux
E_{ECO}	Ecosystem CO_2 efflux
$\delta^{13}C$	Carbon isotope composition
$\delta^{13}C_E$	Carbon isotope composition of CO_2 efflux
$\delta^{13}C_{EECO}$	$\delta^{13}C_E$ of ecosystem
$\delta^{13}C_{EL}$	$\delta^{13}C_E$ of leaves
$\delta^{13}C_{ETG}$	$\delta^{13}C_E$ of twigs
$\delta^{13}C_{ET}$	$\delta^{13}C_E$ of trunk
$\delta^{13}C_{ES}$	$\delta^{13}C_E$ of soil
$\delta^{13}C_{OM}$	$\delta^{13}C$ of total organic matter
$\delta^{13}C_{OM}$	$\delta^{13}C_{OM}$ of leaf
$\delta^{13}C_{OMT}$	$\delta^{13}C_{OM}$ of twig
$\delta^{13}C_{OMT}$	$\delta^{13}C_{OM}$ of trunk
$\delta^{13}C_{OMS}$	$\delta^{13}C$ of soil total organic matter
Doy	Day of year

1 Introduction

The carbon isotope composition ($\delta^{13}C$) of CO_2 is now commonly used at the ecosystem level as a natural tracer to investigate carbon processes and their responses to environmental conditions. Approaches coupling isotopic and mass balance are used to partition ecosystem CO_2 efflux (E_{ECO}) and photosynthetic fluxes (Zobitz et al. 2007). The $\delta^{13}C$ of E_{ECO} ($\delta^{13}C_{EECO}$) is used to infer ecosystem or regional C sink strength by inversion modelling. Large uncertainties are remaining about the interpretation of $\delta^{13}C_{EECO}$, mainly due to the multi-source nature of E_{ECO}, and the temporal variability of $\delta^{13}C$ values of E_{ECO} components and their contributions to E_{ECO} (Bowling et al. 2002; Hemming et al. 2005). Until recently, $\delta^{13}C_E$ values used to interpret $\delta^{13}C_{EECO}$ were considered to be similar among different ecosystem components (i.e., soil, roots, trunk, twigs and leaves) (Kodama et al. 2008).

So far, few studies have examined $\delta^{13}C_E$ of several components concurrently: between leaf and soil (Mortazavi et al. 2005), trunk and soil (Kodama et al. 2008) or different plant organs (Eglin et al. 2009; Kuptz et al. 2011); they showed significant differences in $\delta^{13}C_E$ among the targeted components. Moreover, the $\delta^{13}C_E$ for a given component can exhibit a high seasonal or diurnal variability, up to 10‰ for leaves (Hymus et al. 2005; Prater et al. 2005), 4‰ for twigs (Damesin and Lelarge 2003), 3–5.5‰ for trunks (Maunoury et al. 2007; Ubierna et al. 2009) and 4.2‰ for soil (Ngao et al. 2005, Marron et al. 2009). However, only a few of these studies included wintertime measurements (Damesin and Lelarge 2003; Maunoury et al. 2007; Kuptz et al. 2011).

Seasonal variations in $\delta^{13}C_E$ have been related to several factors such as environmental conditions several days before measurements (Bowling et al. 2002; Ekblad et al. 2005), canopy stomatal conductance (Mc Dowell et al. 2004), the nature and/or $\delta^{13}C$ of respiratory substrates (Damesin and Lelarge 2003; Kuptz et al. 2011) or to other metabolic processes, such as variations in respiratory pathways (Tcherkez et al. 2003; Kuptz et al. 2011). Recently, a comprehensive study concluded that seasonal $\delta^{13}C_E$ patterns in one ecosystem were similar for the different components of beech and spruce and between both species (Kuptz et al. 2011), also showing maximal differences for the trunk between summer and winter. The question now arising is how recurrent are seasonal patterns (1) for a given species at different environmental conditions and (2) between closely related species depending on leaf phenology.

In this context, we focused on the spatio-temporal $\delta^{13}C_E$ dynamics of the main tree components (leaves, twigs and trunks) and soil in two temperate broadleaved forest ecosystems. The main objectives of this study were (1) to quantify differences in $\delta^{13}C_E$ among components and (2) to characterize their seasonal variation, in particular between the two main phenological periods (i.e. leafy and winter period), and their potential link with environmental parameters (air and soil temperature, relative humidity, vapour pressure deficit), CO_2 efflux rates and the respective total organic matter $\delta^{13}C$. Synchronous in situ measurements of CO_2 efflux rates and $\delta^{13}C_E$ were performed throughout 1 year in an oak (*Quercus petraea* L.) and a beech (*Fagus sylvatica* L.) forest using isotope mass spectrometry measurements. In order to validate CO_2 field-sampling methods adapted to leaf and twig components (Prater et al. 2005; Werner et al. 2007), we compared two methods of tissue incubation, with CO_2-free air or N_2 flushes usable under field conditions.

2 Materials and methods

2.1 Study sites and experimental setup

The study was conducted in two French forest sites belonging to the CARBOEUROPE IP network (http://www.carboeurope.org/). The Barbeau forest (cluster_FR1, FR–Fon site, 48°29′ N, 02°47′ E, Table 1) is a managed mature oak-dominated (*Q. petraea*) stand with an understory of *Carpinus betulus* L. Soil is a gleyic luvisol [World Reference Base (WRB) classification] of 80 cm depth, on millstone bedrock and covered with an oligomull humus type, named "Barbeau" in the following. The Hesse forest (cluster_FR1, FR–He site, 48°40′ N, 7°05′ E, Table 1), is a young beech-dominated (*F. sylvatica*) stand with a dystric cambisol (WRB classification) of 120 cm depth and an oligomull humus type. This site is subsequently named "Hesse".

The study was conducted from March 2005 (before budburst) to January 2006 during nine field campaigns in Barbeau (06/04, 18/04, 02/05, 01/06, 21/06, 11/07, 09/09, 23/11, and 12/01) and four field campaigns in Hesse (16/03, 18/05, 05/07, and 14/09). Gas exchange measurements, CO_2, and organic matter sampling were always performed between 10:00 and 13:00 UT to avoid diurnal variations in $\delta^{13}C_E$ as previously observed (Maunoury et al. 2007). Each four dominant oak and beech trees were randomly selected for the whole campaign. Soil measurements were performed around the sampled trees, within about 20 m^2.

2.2 Environmental and phenological parameters

At both sites and for each campaign, soil temperature was measured at 10 cm depth using a temperature probe (LM35CZ) in Barbeau and five copper–constantan thermocouples (Faculty of Agronomy of Gembloux, Belgium) in Hesse. Soil surface moisture (in the 0–6 cm layer) was measured using a capacitive ML2x Thetaprobe (Delta-T Device, Cambridge, UK) in Barbeau. Mean air temperature at 2 m height (sensor LM35CZ), rainfall and air relative humidity (to calculate water vapour pressure deficit, VPD) were determined every 30 min by meteorological stations installed on-site.

Budburst dates (Table 1) were determined by field observations as either 50 % of trees showing 50 % of bursted buds in Barbeau, or as the beginning increase in the leaf area index (LAI), measured at regular intervals before and during the leafy period (LI-COR LAI 2000) in Hesse. The leaf fall period was also recorded at both sites. The trunk growth period was determined by tape measurements of the radius at 1.30 m height every week in Barbeau and during each field campaign in Hesse (Table 1). The growing period is defined as the time of trunk growth, while the leafy period includes the time span between bud burst and leaf fall.

2.3 Trunk and soil CO_2 efflux rates

Trunk CO_2 efflux rate (E_T) was measured using a closed chamber system (see Damesin et al. 2005 for a detailed

Table 1 Stand characteristics for Barbeau and Hesse in 2005

	Barbeau (oak forest)	Hesse (beech forest)
Location	48°29′ N, 02°47′ E	48°40′ N, 7°05′ E
Elevation (m above sea level)	90	300
Climate	Modified temperate maritime climate	Continental climate
Mean annual temperature (°C)	10.5	9.2
Annual rainfall (mm)	690	820
Soil type (WRB classification)	Gleyic luvisol	Stagnic luvisol
Humus type	Oligomull	Oligomull
Stand age in 2005 (years)	100–150	40
Ground area ($m^2 ha^{-1}$)	20.7	25.3
Tree density (stems ha^{-1})	3480	1134
Max tree height (m)	30	20
Dominant species	*Quercus petraea* L.	*Fagus sylvatica* L.
Understorey	Dense	Sparse
Represented tree species	*Carpinus betulus* L.	*Quercus petraea* L. *Betula pendula* L. *Carpinus betulus* L.
Budburst (dominant species)	Doy 110	Doy 119
Leaf fall	From doy 287 to 337	From doy 297 to 310
Trunk growth[a]	Between doy 115 and 239	Between doy 115 to 214
Trunk diameter at 1.30 m[a] (cm)	25–39	24–27

[a]Deduced from the four dominant trees selected at both sites

description). Briefly, a cylindrical polymethyl methacrylate (PMMA) chamber was temporarily fixed on the trunk, cleaned of mosses and lichens, with a rubber sealant (Terosta-7, Teroson, Ludwigsburg, Germany) and connected to an infrared gas analyser (IRGA, EGM4, PPSystems, Hitchin, UK). The installation was considered to be leak-free if blowing air along the seals caused no increase in the CO_2 level inside the chamber. A fan provided air mixing within the chamber. Once tightly fixed to the trunk, the chamber was purged from accumulated CO_2 by opening a 5-cm diameter lid. Once back to ambient CO_2 concentrations, the lid was closed to allow CO_2 accumulation. Three to four E_T measurements of 2 min during linear CO_2 increase were performed each time. The E_T values were determined from the slope of CO_2 concentration increase and expressed per unit volume of living tissue (i.e., phloem and sapwood; in $\mu mol\,m^{-3}$ living tissues^{-1}). For beech, the whole trunk volume was considered because living cells occur all along the trunk radius (Ceschia et al. 2002). For each oak tree, the living tissue cross-section was determined from a trunk core collected near the chamber at the end of the campaign. The chambers were reinstalled at the same place during each campaign.

Soil CO_2 efflux (E_S) was measured using two different closed dynamic systems (Ngao et al. 2006; Chemidlin Prévost-Bouré et al. 2009): In Barbeau, an EGM4 was connected to a homemade PMMA chamber (25.4 L, 12 cm height), while in Hesse, a Li-6200 (LI-COR Inc., Lincoln, NE, USA) IRGA was used with the Li-6000-9 chamber. In both cases, the soil chamber was put on collars previously inserted into the soil under the canopy (500 and 110 mm diameter in Barbeau and Hesse respectively, inserted 2–3 cm deep) at the beginning of the year, allowing measurements at the same place during field sessions. Two collars were installed at Barbeau and three at Hesse. E_S values were calculated from the slope of CO_2 increase and expressed per surface area (in $\mu mol\,m^{-2}$ soil$\,s^{-1}$).

2.4 CO_2 sampling for isotopic analysis

2.4.1 Incubation tests

The gaseous medium (N_2 vs. CO_2-free air) of an incubation setup may have an immediate impact on the CO_2 efflux rate by shortening the oxygen supply to living tissues and potentially influencing the isotope composition of emitted CO_2. We tested this effect using a system close to that described in Werner et al. (2007). Entire mature sun leaves and twigs were sampled from the top of the canopy of three oaks and three beeches at the end of summer. Each sample was immediately inserted into a 50-ml flask previously purged with either pure N_2 or CO_2-free air. Leaves (n=44) and twigs (n=20) were incubated in the dark under ambient temperature. In preliminary CO_2 efflux rate measurements (data not shown), we determined that an incubation time between 10 and 25 min was required to collect enough emitted CO_2 (800–900 ppm). The $\delta^{13}C$ in air from the incubations was analysed by isotope ratio mass spectrometry (IRMS) as described below. These tests revealed no significant effect of the gaseous medium used, neither for beech or oak leaves nor for twigs (p=0.72 for leaves and p=0.53 for twigs, Fig. 1).

2.4.2 Sampling of CO_2 emitted by ecosystem components

For CO_2 emissions at the leaf and twig levels, these components were incubated as described above. Sun leaves and twigs from the tree canopy were sampled using a rifle. Leaves and twigs were immediately inserted into 50-ml airtight syringes with valves (SGE, Australia) prepurged with pure N_2. Air from the incubation syringe was then transferred into an empty syringe in Barbeau or into a 12-ml Exetainer vial (Labco Ltd, High Wycombe, UK) in Hesse, according to the equipment available (see IRMS analyses below).

For trunk CO_2 emissions, the trunk chamber was purged after each E_T measurement with N_2 for 15 min until the CO_2 concentration dropped to near 0 ppm. Then, outlet and inlet tubes of the chamber were connected to allow the

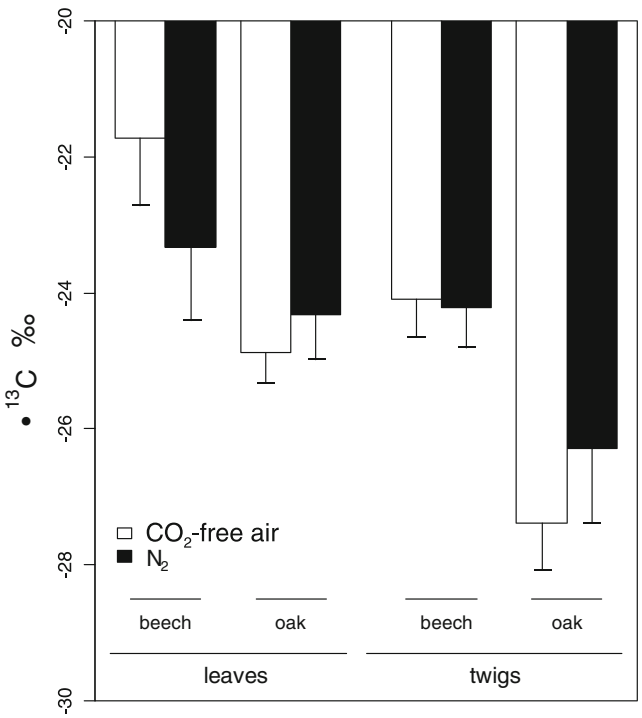

Fig. 1 Mean values of $\delta^{13}C_E$ measured for leaves and twigs of oak and beech incubated after flushing with CO_2-free air (*white*) or N_2 (*black*). Error bars represent standard errors of the mean. n (leaf)=27 for oak and 17 for beech, and n (twig)=10 for oak and 10 for beech

accumulation of CO_2 emitted by the trunk. After an increase of 700–800 ppm (during approximately 10 min in summer and 90 min in winter), the air in the chamber headspace, containing CO_2 originating only from E_T, was sampled using a 50-ml syringe and analysed by IRMS. Again, this N_2 flushing approach gave the same results as that using a CO_2 free-air flush or estimating the $\delta^{13}C$ of emitted CO_2 with the Keeling plot method (Damesin et al. 2005).

For CO_2 emitted by soil, the Keeling plot method (Keeling 1958) was applied to determine the $\delta^{13}C_E$ of E_S (Chemidlin Prévost-Bouré et al. 2009). The sampling setup consisted of 50-ml airtight syringes in Barbeau, or a home-made sampling device by-passing the Li-6200 air circuit (see Ngao et al. 2005 for details) in Hesse. After each E_S measurement, CO_2 concentration was allowed to increase again within the closed system. During this increase, five air samples were taken at steps of 50–100 ppm within a 400–1000 ppm range inside Exetainer vials or 50-ml syringe and analysed by IRMS. From the Keeling plots, the $\delta^{13}C_{ES}$ for each collar was estimated using the ordinary least square regression model (Zobitz et al. 2007). $\delta^{13}C_{ES}$ was determined as the intercept of the linear regression between the inverse of the CO_2 concentration and the $\delta^{13}C$ of the air samples. $\delta^{13}C_{ES}$ values having a standard error >5 % of the estimated value were discarded.

2.5 Sampling of plant and soil material for isotopic analysis

At both sites and for each campaign, trunk phloem samples of the four trees were taken using a core borer (0.5 cm diameter) at the chamber level or up to 30 cm above. Leaves and twigs used for the incubations and phloem samples were lyophilised and powdered using a ball mill (Type MM200, Retsch, Haan, Germany). Four soil cores (0–15 cm depth and 1.2 cm diameter) were sampled about 15 cm away from each collar. Soil samples did not include litter or roots.

2.6 IRMS analyses

To maintain airtight conditions, gas-filled syringes were processed within 12 h by laboratories close to the sites. Gas samples from the Barbeau were analysed with a NA-1500 elemental analyser (Carlo Erba, Milan, Italy) coupled to a VG Optima IRMS (Fison, Villeurbanne, France), as described by Maunoury et al. (2007). Those from Hesse were injected into a gas purification device (Gas–Bench II, ThermoFinnigan, Bremen, Germany) coupled to a Delta S IRMS (ThermoFinnigan, Bremen, Germany). All solid samples were analysed with the NA-1500/IRMS setup.

All $\delta^{13}C$ values were expressed relative to the Vienna Pee Dee Belemnite international standard. Different laboratory working standards (glutamic acid, –28.06‰ for organic matter samples; air with 500 $\mu mol\,mol^{-1}$ of CO_2, –53.10‰ for air samples) were measured after each group of 12 samples to correct for any offset of the IRMS. The precision for isotopic measurements was ±0.2‰, based on repeated measurements of the laboratory working standards. Both IRMS systems were inter-calibrated for gas analyses using the same reference gas as above, revealing a discrepancy of 0.7‰ that was removed to the values measured at Hesse to have comparable values between both sites.

2.7 Statistical analysis

Pearson's correlation coefficients were calculated between $\delta^{13}C_E$ of each component and climatic data or CO_2 efflux rates solely at Barbeau where measurements were more frequent. All climatic variables from the measurement day and the day before were tested.

Pearson correlations were also established between $\delta^{13}C_E$ of different components. Non-parametric Kruskal–Wallis rank sum tests should reveal differences among components at each site, and during the two main phenological periods, followed by Mann–Whitney tests to compare one component to another.

A one-way ANOVA was applied to compare $\delta^{13}C_E$ measurements in the incubation tests with N_2 or CO_2-free air flushings.

All analyses were performed using Statistica (version 7, Statsoft Inc., USA) and R 2.11.1 (R development core team 2010).

3 Results

3.1 CO_2 efflux rates

At both sites, the trunk CO_2 efflux E_T showed a pronounced seasonal evolution and ranged from 10 (April) to 130 (June) $\mu mol\ CO_2\,m^{-3}$ of living $tissues^{-1}$ in Barbeau (Fig. 2a), and from 10 (March) to 88 (July) $\mu mol\ CO_2\,m^{-3}$ of living $tissues^{-1}$ in Hesse (Fig. 2b). Variations of soil CO_2 efflux were also marked, especially in Barbeau where E_S ranged from 0.7 (November) to 5.1 (July) $\mu mol\ CO_2\,m^{-2}s^{-1}$. In Hesse, it ranged from 0.7 (March) to 1.8 (September) $\mu mol\ CO_2\,m^{-2}s^{-1}$ but summer efflux data, which are assumed to be highest, are missing. The maximum observed values of trunk and soil CO_2 efflux at Barbeau (Fig. 2a) occurred during the trunk growth period when air and soil temperatures were high (Fig. 3). The lowest CO_2 efflux rates occurred during winter.

3.2 Carbon isotope composition of emitted CO_2

In oak forest, the carbon isotope composition of leaves ($\delta^{13}C_{EL}$) showed the largest seasonal variations (4.6‰,

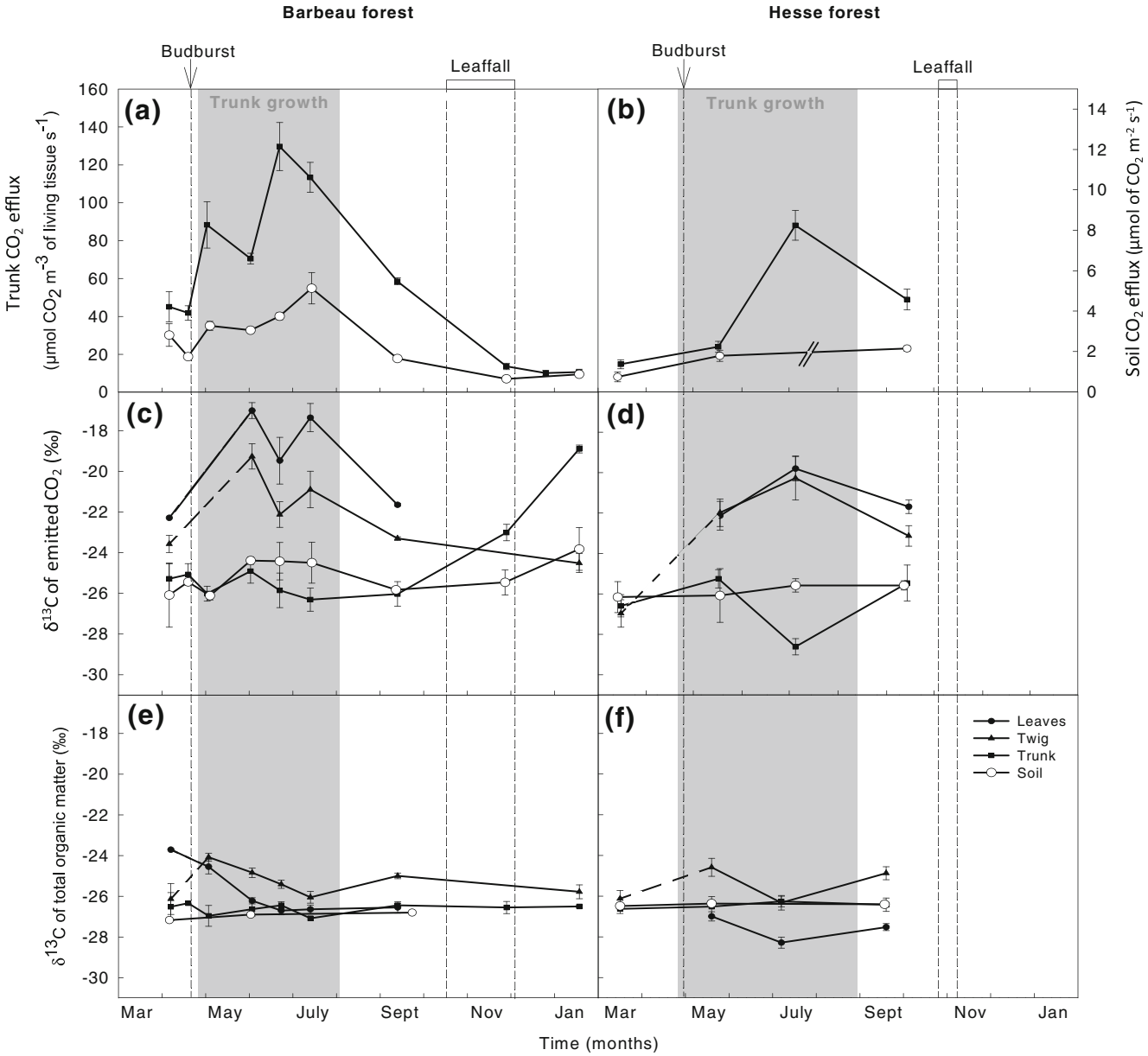

Fig. 2 Seasonal changes in trunk and soil respiration rate in Barbeau (oak forest) (**a**) and Hesse (beech forest) (**b**), in $\delta^{13}C$ of emitted CO_2 in Barbeau (**c**) and in Hesse (**d**), and in $\delta^{13}C$ of total organic matter in Barbeau (**e**) and in Hesse (**f**), of leaf (*filled circles*), twig (*filled triangles*), trunk (*filled squares*) and total soil (*open circles*). *Vertical dashed lines* delimitate the budburst date and the leaf fall period. Before budburst, $\delta^{13}C_E$ and $\delta^{13}C_{OM}$ of leaves and twigs were measured on buds and previous year-twig. The trunk growth period is indicated in *gray*. Error bars represent standard errors of the mean

Fig. 2c). It was always higher than $\delta^{13}C$ of CO_2 emitted by buds in spring, i.e. the first measurement before budburst (−22.3‰). It increased during the whole growing season, with a small decrease in June and then decreased from September onwards. $\delta^{13}C_{EL}$ showed a similar pattern in Hesse albeit at a smaller observed range (2.3‰) and lower values (Fig. 2d).

At both sites, $\delta^{13}C_E$ of twigs ($\delta^{13}C_{ETG}$) clearly increased between budburst and May (Fig. 2c, d). In September, the values were near those before budburst for oak or those in May for beech. During the trunk growth period, the $\delta^{13}C_{ET}$ was lower than the $\delta^{13}C_E$ of the two canopy components at both sites (Fig. 2c, d). In Barbeau, $\delta^{13}C_{ET}$ increased during winter and reached a maximum of −18.9‰ in January 2006 (Fig. 2c).

Soil $\delta^{13}C_{ES}$ was rather similar at both sites, and seasonal variations were low (Fig. 2c, d). E_S and $\delta^{13}C_{ES}$ were not correlated (Table 2).

In Barbeau, $\delta^{13}C_{ETG}$ was not related to any climatic variable, whereas $\delta^{13}C_{EL}$ was related to soil moisture

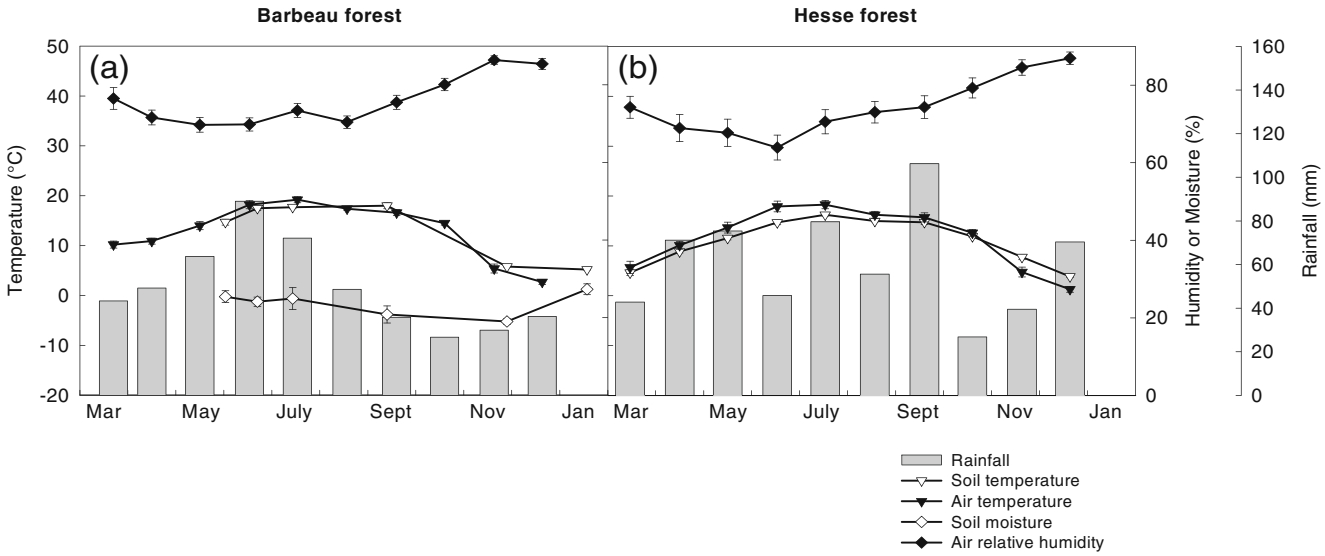

Fig. 3 Seasonal changes in rainfall (*gray histogram*), soil (*open triangles*) and air (*filled triangles*) temperature, soil moisture (*open diamonds*) and air relative humidity (*filled diamonds*), in Barbeau (oak forest) (**a**) and Hesse (beech forest) (**b**). Error bars represent standard errors of the mean

(p=0.041, Table 2) and $\delta^{13}C_{ET}$ was related to air and soil temperature (p=0.011 and p=0.012, Table 2) and vapour pressure deficit (p=0.033). $\delta^{13}C_{ES}$ values were only related to soil moisture (p=0.006). Moreover, $\delta^{13}C_{ET}$ was negatively related to E_T and to E_S (r=−0.715; p=0.030 and r=−0.667; p=0.050, respectively, Table 2). If only the values from the leafy period were kept, correlations between $\delta^{13}C_{EL}$ and $\delta^{13}C_{ETG}$ (r=0.955; p=0.011), $\delta^{13}C_{ES}$ (r=0.917; p=0.029) and soil moisture (r=0.960; p=0.041), and between $\delta^{13}C_{ES}$ and soil moisture (r=0.966; p=0.034) were remaining.

From May to September (leafy period), $\delta^{13}C_E$ exhibited a vertical zonation in both ecosystems, revealing significant differences between components (Kruskal–Wallis ANOVA, p<0.001 for each site, Fig. 4).$\delta^{13}C_{EL}$ values were globally the highest, and $\delta^{13}C_{ETG}$ values were lower in Barbeau (p=0.003) but not in Hesse (p=0.380). $\delta^{13}C_{ETG}$ values were always higher than those of trunk (p=0.001, both systems) and soil (p=0.001 in Barbeau and p=0.002 in Hesse). At both sites, $\delta^{13}C_{ET}$ and $\delta^{13}C_{ES}$ values were not significantly different (p=0.07 in Barbeau and p=0.16 in Hesse) and represented the lowest $\delta^{13}C_E$ values.

During leaf fall (October) and winter (January), the vertical zonation was not maintained (Fig. 4). This period was characterised by an increase in $\delta^{13}C_{ET}$.

3.3 $\delta^{13}C$ of total organic matter

In contrast to $\delta^{13}C_E$, $\delta^{13}C$ of total organic matter ($\delta^{13}C_{OM}$) showed weak temporal variations. At both sites, the most

Table 2 Pearson's correlation coefficient (r) matrix between carbon isotope signatures of emitted CO_2 in leaves, twigs, trunk, soil and soil and climatic conditions in oak forest or $\delta^{13}C_E$ of leaves, twigs, trunk and soil

	Instant T_{air}	VPD	Instant T_{soil}	$H_{v\ soil}$	E_T	E_S	$\delta^{13}C_{EL}$	$\delta^{13}C_{ETG}$	$\delta^{13}C_{ET}$	$\delta^{13}C_{ES}$
$\delta^{13}C_{EL}$	ns	ns	ns	0.960	ns	ns		0.955	ns	0.917
	p=0.853	p=0.611	p=0.316	p=0.041	p=0.334	p=0.220		p=0.011	p=0.953	p=0.029
$\delta^{13}C_{ETG}$	ns	ns	ns	ns	ns	ns	0.955		ns	ns
	p=0.438	p=0.373	p=0.370	p=0.953	p=0.228	p=0.156	p=0.011		p=0.301	p=0.625
$\delta^{13}C_{ET}$	−0.789	−0.708	−0.909	ns	−0.715	−0.667	ns	ns		ns
	p=0.011	p=0.033	p=0.012	p=0.527	p=0.030	p=0.050	p=0.953	p=0.301		p=0.174
$\delta^{13}C_{ES}$	ns	ns	ns	0.934	ns	ns	0.917	ns	ns	
	p=0.817	p=0.940	p=0.702	p=0.006	p=0.755	p=0.759	p=0.029	p=0.625	p=0.174	

Only significant r values are presented (p<0.05)

Instant T_{air} and Instant T_{soil} air and soil instantaneous temperature during measurements (°C); *VPD* daily mean vapour pressure deficit (mBar) during the day of measurement; *$H_{v\ soil}$ (%)* soil volumetric moisture

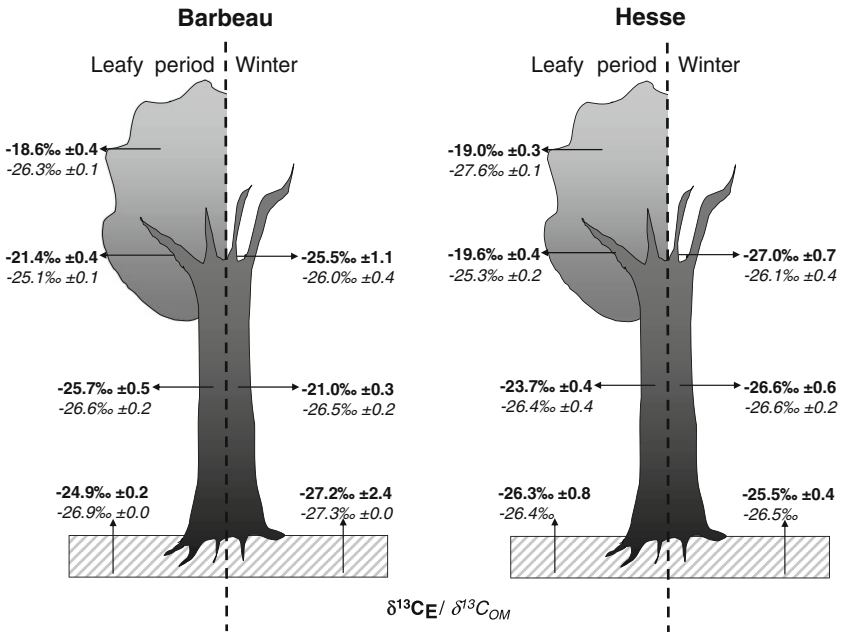

Fig. 4 Mean values (±standard errors of the mean) of $\delta^{13}C_E$ (*bold*) and $\delta^{13}C_{OM}$ (*italic*) observed for each component (leaf, twig, trunk and soil) during the leafy period and winter, in Barbeau (oak forest) (*left*) and Hesse (beech forest) (*right*)

pronounced variations occurred in leaves and twigs with a slight decrease in July and a slight increase towards September (Fig. 2e, f). Variations in $\delta^{13}C_{OMT}$ during the year were very narrow, with the same annual average of −26.5‰ for the two forests (Fig. 2e, f). $\delta^{13}C_{OMS}$ values were stable and averaged −27.01‰±0.06 in Barbeau and −26.21‰±0.08 in Hesse.

When comparing organic matter to CO_2 efflux $\delta^{13}C$, differences between $\delta^{13}C_{EL}$ and $\delta^{13}C_{OML}$ were up to 9.3‰ (Barbeau) and 8.5‰ (Hesse). Differences between $\delta^{13}C_{ETG}$ and $\delta^{13}C_{OMTG}$ for oak and beech were lower, with maxima of 5.6 and 6.1‰, respectively. During spring and summer, the difference between $\delta^{13}C_{ET}$ and $\delta^{13}C_{OMT}$ was maximum 1.7‰ (Barbeau) and ranged between −2.4 and 1.3‰ (Hesse). After leaf fall in Barbeau, this difference reached values as high as 7.6‰ in January. Differences between $\delta^{13}C_{ES}$ and $\delta^{13}C_{OMS}$ were always positive in Barbeau (1.0–2.5‰) but could be negative in Hesse (−1.6–1.0‰.).

Finally, the differences in $\delta^{13}C_{OM}$ between organs were lower compared to those for $\delta^{13}C_E$. Even if $\delta^{13}C_{OMTG}$ was generally less negative than the $\delta^{13}C_{OM}$ of other components, no stable vertical zonation was apparent (Fig. 4).

4 Discussion

4.1 Incubation methods for leaf and twig in the field

Field protocols to collect CO_2 efflux from tissue at the top of the canopy showed that $\delta^{13}C_E$ of attached leaves was similar to that of detached ones (Prater et al. 2005). Furthermore, an in-tube incubation method using CO_2-free air flushes had been validated for $\delta^{13}C_E$ measurements and showed no difference to online gas exchange measurement (Werner et al. 2007). Here, we complete the methods debate on which approaches are compatible to field conditions, by testing two variants of tissue incubation with CO_2-free air or N_2 flushes. Our results clearly demonstrate that the different gases used do not change the measured $\delta^{13}C_E$ of leaves or twigs. Tissue incubation in vials previously flushed with N_2 or CO_2-free air can thus be used in the field to sample the emitted CO_2 from current-year branches.

4.2 Vertical zonation of $\delta^{13}C_E$ from canopy to soil during the leafy period

In both forests, our measured $\delta^{13}C_E$ values were comparable to those obtained for leaves in deciduous (Hymus et al. 2005) or coniferous forests (Prater et al. 2005), twigs (Damesin and Lelarge 2003), trunks (Damesin et al. 2005; Maunoury et al. 2007; Kuptz et al. 2011) and soil of deciduous forests (Ngao et al. 2005), coniferous forests (Ekblad et al. 2005) or rainforests (Buchmann et al. 1997).

We revealed that during the leafy period, $\delta^{13}C_E$ values significantly differed among the ecosystem components, overall decreasing from the top of the canopy to the soil (Fig. 4). We observed this zonation at both sites, two different deciduous tree forests in distinct climatic conditions. Interestingly, no such zonation was found by Kuptz et al. (2011) in spruce and beech forests during the leafy period.

When there was a difference in $\delta^{13}C_E$ between twigs and leaves, it systematically consisted of a ^{13}C impoverishment of the CO_2 emitted by twigs relative to leaves. This difference cannot be explained by differences in substrate $\delta^{13}C$ because leaves have generally a significantly lower $\delta^{13}C$ for starch and soluble sugars than twigs (Damesin and Lelarge 2003;

Eglin et al. 2009). The gap between $\delta^{13}C_{EL}$ and $\delta^{13}C_{ETG}$ might be related to a difference in the balance of CO_2 released by either pyruvate decarboxylation (resulting in ^{13}C-enriched CO_2) or by the Krebs cycle (resulting in ^{13}C-depleted CO_2, Tcherkez et al. 2003; Gessler et al. 2009).

The main hypotheses explaining differences in $\delta^{13}C_E$ between twigs and trunks are (1) an isotope discrimination during the assimilate transport in the phloem along twigs and trunk (Damesin and Lelarge 2003; Gessler et al. 2007), (2) changes in PEPc activity (Gessler et al. 2009; Kuptz et al. 2011), known to discriminate against ^{12}C during carbon fixation (Cernusak et al. 2009), (3) or a substantial contribution of belowground-evolved CO_2 brought by the xylem sap stream to the upper part of the tree, i.e., in the trunk (Aubrey and Teskey 2009; Grossiord et al. 2012). Other processes may contribute to the ^{13}C impoverishment in trunk compared to twigs, especially the progressive mixing along metabolite translocation from sun and shade leaves via twigs to trunk (Eglin et al. 2010). It will be interesting to address in future studies in more detail the reasons for the differential isotope discrimination in twigs and trunk found in our study.

Comparable values of isotope composition of CO_2 efflux in trunk and soil may be explained by the coupling of both components via C assimilates. Carbon substrates are rapidly transferred in broadleaved species from trunk to roots and via root exudates also to soil microorganisms (Dannoura et al. 2011; Epron et al. 2011). The comparable values for trunk and soil also suggest that contrary to substrate translocations from leaves to twigs, there is no apparent isotope discrimination during carbon translocation from trunk to soil.

4.3 Seasonal variations in $\delta^{13}C_E$

Seasonal ranges of emitted CO_2 $\delta^{13}C$ were in agreement with ranges previously observed for beech leaves (Eglin et al. 2009), beech twigs (Damesin and Lelarge 2003), oak trunks (Maunoury et al. 2007) and hardwood forest soil (Mortazavi et al. 2005; Marron et al. 2009). Our maximum $\delta^{13}C_E$ values of leaves and twigs have been obtained during summer, in agreement with $\delta^{13}C_E$ of a coniferous forest ecosystem (Bowling et al. 2002; Mortazavi et al. 2005) but oppositely to $\delta^{13}C_E$ of a deciduous forest ecosystem (Mortazavi et al. 2005).

$\delta^{13}C_E$ seasonal variations in leaves might be related to changes in respiratory substrate $\delta^{13}C$ due to variable ^{13}C discrimination during C assimilation. The latter is itself linked to environmental conditions like soil moisture (Mortazavi et al. 2005). The correlation between $\delta^{13}C_{EL}$ and $\delta^{13}C_{ETG}$ (Table 2) suggests that $\delta^{13}C_{ETG}$ variability is linked to the same mechanisms as that of $\delta^{13}C_{EL}$. The increase in $\delta^{13}C_{ET}$ in winter might be explained by a switch of respiratory substrates from photosynthesis-derived sugars (lower $\delta^{13}C$) during the leafy period to stored carbohydrates, i.e. starch with higher $\delta^{13}C$, in the dormancy period as suggested before (Maunoury et al. 2007; Kuptz et al. 2011). Another explanation for these winter values is that, during winter time, transpiration is null and $\delta^{13}CE_T$ was no more influenced by a possible contribution of belowground-evolved CO_2. Surprisingly, we did not observe any increase in $\delta^{13}C_E$ for twigs, which, like trunks, probably use starch as main respiratory substrate. This unexpected difference between trunk and twig $\delta^{13}C_E$ may be explained by a metabolic discrepancy such as, e.g. differences in PEPc activity, during winter. Correlations between $\delta^{13}C_{ET}$ and E_T or air temperature have already been observed by Maunoury et al. (2007) in the same oak forest, with comparable correlation coefficients, suggesting that the respiration rate, which is influenced by air temperature, affects $\delta^{13}C_{ET}$.

During the leafy period, respiratory substrates are partially derived from C recently assimilated by leaves and transported by the phloem towards the trunk base and roots (Dannoura et al. 2011; Epron et al. 2011) and may finally end up via root exudates as organic matter in the soil. Such a substrate similarity between trunk and soil may result in $\delta^{13}C_{ES}$ values close to those measured for trunk during the leafy period. In contrast, during winter, a lack C assimilation lowers dramatically this recent C source for soil respiration, while trunk stored compounds with higher $\delta^{13}C$ values supply substrates for E_T. Furthermore, carbon supplies to the soil via root exudates are much lower or nil during winter. In addition, the heterotrophic component, i.e. microbial respiration, contributes more to the soil CO_2 efflux and to $\delta^{13}C_{ES}$ than during the leafy period (Epron et al. 2001). This decoupling between trunk and soil respiratory substrate pools therefore may explain the significant differences in $\delta^{13}C_E$ of both components in winter.

At the soil and ecosystem level, temporal variations of $\delta^{13}C_E$ have been explained by environmental conditions with or without time lag (from 1 to 10 days) before measurement (e.g. Bowling et al. 2002; Ekblad et al. 2005; Marron et al. 2009). The present study showed a differential impact of environmental conditions on the $\delta^{13}C_E$ of the different components studied., Specifically, in contrast to other studies, we found no correlation between $\delta^{13}C_{EL}$ or $\delta^{13}C_{ETG}$ and climate (Mc Dowell et al. 2004; Mortazavi et al. 2005), which might be explained by the lower measurement frequency in our study. Yet, we found several correlations of environmental factors with $\delta^{13}C_{ET}$, suggesting that the trunk data integrate the overall climate effect on the tree. This argument can be supported by the facts that (1) a time lag exists for substrate transport in phloem from leaves to soil (Barnard et al. 2007) and (2) a progressive mixing of several different substrates (e.g. recent vs. stored compounds, top vs. bottom of tree canopy) along this C translocation.

Lastly, the general enrichment of $\delta^{13}C_E$ in comparison with $\delta^{13}C_{OM}$ has been classically highlighted in previous studies (Damesin and Lelarge 2003; Klumpp et al. 2005; Maunoury et al. 2007). Our results show a mismatch between the $\delta^{13}C_E$ of each component and $\delta^{13}C_{OM}$ of the mature leaf, but these discrepancies were not stable throughout the year. Thus, our results invalidate the hypothesis of Bowling et al. (2008) that bulk leaf $\delta^{13}C$ can be used as a reference value to predict differences between $\delta^{13}C_E$ and $\delta^{13}C_{OM}$ of plant components and ecosystem.

5 Conclusion

Our study highlights isotopic differences of CO_2 emitted by the top (sun leaves and twigs) and the bottom of the forest (trunk base and soil) both with regard to higher $\delta^{13}C$ values during the leafy period (higher at the top) and of seasonal dynamics (higher at the top). Variations in substrate $\delta^{13}C$ (via the use of stored compounds or a mixing effect) might be the major—but not the only—explanation for these differences. Our study confirmed that $\delta^{13}C$ of CO_2 emitted by the forest components cannot be deduced from the $\delta^{13}C$ of the total organic matter of the component, or from CO_2 efflux intensity. Nowadays, high frequency measurements by tunable diode laser spectroscopy allow the analysis of temporal dynamics in $\delta^{13}C_E$ (Marron et al. 2009), especially during winter time, and offer thus better possibilities to understand inherent variability its link to metabolic processes.

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