In the context of climate change, the amount of carbon allocated to soil, particularly fresh litter, is predicted to increase with terrestrial ecosystem productivity, and may alter soil carbon storage capacities. In this study we performed a 1-year litter-manipulation experiment to examine how soil CO₂ efflux was altered by the amount of fresh litter. Three treatments were applied: litter exclusion (E), control (C, natural amount: 486 g m⁻²) and litter addition (A, twice the natural amount: 972 g m⁻²).

Litter decomposition rate was not affected by fresh litter amount. However, the addition or exclusion of fresh litter quickly increased or decreased total soil CO₂ efflux (Fₛ) significantly, but the relative contribution of fresh litter to total soil respiration remained unchanged between the C and A treatments, as determined by laboratory measurements. Variation in Fₛ among treatments was not related to modification of its temperature sensitivity which was not affected by fresh litter amount (Q₁₀: 3.5 for E, 3.2 for C, 3.6 for A). While litter exclusion was the main cause of the Fₛ decrease in the E treatment, only 68% of Fₛ was directly attributable to litter addition in the A treatment. The remaining 32% of Fₛ in the A treatment was related to a real priming effect that appeared to be a long-lasting phenomenon. This priming effect lasting over 1 year may be related to a continuous release of organic compounds from litter to soil because of the progressive decomposition of leaf litter. Q₁₀ estimates and isotopic data lead to the hypothesis that the priming effect corresponded to the activation of the whole soil system.

As a consequence, the increase in ecosystem productivity may lead, via an increase in the amount of litter, to an increase in carbon turnover in soil. Further labelling experiments involving high-frequency carbon stable isotope measurements of CO₂ efflux would help to clarify the relative importance of bulk soil and rhizosphere in the priming effect.

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(Bingeman et al., 1953; Kuzyakov et al., 2000; Nottingham et al., 2009); and via changes in diversity of organisms involved in soil functioning (Fontaine et al., 2004; Nottingham et al., 2009). These studies suggest that a priming effect commonly occurs in most plant–soil systems as frequent fresh organic residue inputs appear through litterfall or fine root death. Quantitative variations in fresh organic carbon inputs to soil (determined by climate change) are likely to rapidly affect the whole soil organic carbon mineralization process under natural conditions (Fontaine et al., 2004, 2007; Sulzman et al., 2005; Crow et al., 2009). Understanding how soil carbon cycling may be modified needs consideration of the response of heterotrophic respiration components (litter respiration and bulk soil respiration; referred to in the following as \( F_1 \) and \( F_m \), respectively) to quantitative variations of fresh organic carbon inputs.

In parallel with CO2 efflux, measurements of the natural abundance of \(^{13}\text{C}\) have been used to improve our knowledge of the origins of carbon pools used for soil respiration. In forest soils, clear temporal variation in CO2 efflux \(^{13}\text{C}\) values (up to 3.5\%) has been observed (Ekblad et al., 2005; Hörgberg et al., 2005; Mortazavi et al., 2005; Marron et al., 2009) and correlated with variation in the contribution of the different respiration sources to total soil respiration and to shifts in the \(^{13}\text{C}\) values for respiration substrates (Ethlinger et al., 2000; Blasi et al., 2005; Hörgberg et al., 2005; Tu and Dawson, 2005; Kuzyakov, 2006). As a result, \(^{13}\text{C}\) measurements would help to examine whether the amount of litter induces a significant change in the respective contributions of the different soil components and/or respiration substrates mobilised by some "priming effect" process (Fontaine et al., 2007).

In this study, we performed an in situ litter-manipulation experiment over 1 year to determine to what extent an increase in the amount of fresh litter affects the heterotrophic mineralization of organic carbon in a temperate deciduous forest. Litter manipulation lasted 1 year and consisted in the allocation of fresh litter to experimental plots according to three treatments: litter exclusion (E); natural fresh litter amount, i.e. control (C); and litter addition at twice the natural amount (A). Every month, measurements of the rate and carbon isotopic composition \(^{13}\text{C}\) of total soil CO2 efflux were performed in situ (field measurements) and measurements of the rate and carbon isotopic composition \(^{13}\text{C}\) of heterotrophic CO2 efflux in litter and soil (without roots) were performed in vitro (short-term incubations, laboratory measurements). In parallel, soil and litter bacterial community structure were monitored by means of molecular fingerprinting. "Functional" data (CO2 efflux and carbon isotope composition measurements) are presented in this manuscript. Data concerning the dynamics of microbial communities are presented by Chemidlin Prévost-Bouré et al. (in press) where they are related to "functional" data to explore the link between bacterial community structure and soil functioning.

2. Materials and methods

2.1. Site description

The experiment took place in Barbeau National Forest (CAR-BOEUROPE IP network site [Cluster_FRI, Site FR-Fon: 48 29′N, 02 47′E; 60 km southeast of Paris, France; 90 m elevation). This site is a managed mature oak forest (Quercus petraea L., 100–150 years old) with a dense understorey of coppiced hornbeam (Carpinus betulus L.). The mean annual temperature was 10.7 °C and the annual rainfall was 880 mm (1980–1996).

The soil was a gleyic luvisol (IUSS Working Group WRB, 2006) of 0.8 m depth on millstone bedrock covered with an oligomull humus. The soil texture was loam in the top soil (USDA referential; Sand: 41.5%; Clay: 19.3%; Silt: 38.8%) and clay loam at the bottom of the soil profile (USDA referential; Sand: 35.2%; Clay: 32.8%; Silt: 32.0%). The main soil characteristics were determined by the INRA Soil Analysis Laboratory at Arras (France). The granulometric fractions were determined according to the French norm NF X 31-107 by the dispersion of mineral particles after destruction of the organic matter by hydrogen peroxide and separation of the particles into different classes by sedimentation (particles <50 μm; Gee and Bauder, 1986). Gleyic colour patterns were observed from 30 cm depth down to the bottom of the soil profile. The soil pH measured in water (NF ISO 10390, in a 1:5 (v/v) ratio of soil and water suspension) and the soil carbon content (in a 1 g sample calcinated at 550 °C and crushed to 250 μm) both decreased with depth. The soil carbon content was 14.5% of soil dry mass at the soil surface and 0.2% of soil dry mass at 10 cm depth.

2.2. Experimental design

A 17.6 m\(^2\) experimental area (two 1.2 m \(\times\) 7.3 m rows) representative of the stand was chosen. From mid-October to the end of December 2005, fresh litter was collected from the experimental area with a net (0.5 mm mesh) installed 50 cm above the soil surface. Lateral litter inputs were prevented by surrounding the experimental area with another net (0.5 mm mesh). The collected fresh litter was air-dried at ambient temperature and the species composition was determined as oak (70% of total dry mass) and hornbeam (30% of total dry mass).

The experimental area was divided into 12 adjoining plots (each 1.2 m \(\times\) 1.2 m) delimited by a PVC fence of 20 cm height. Treatments were applied in the field following a randomised-complete-block design with one replicate of each treatment per block. Before starting the experiment, the homogeneity of the plots was checked once for: environmental conditions (soil water content, soil temperature at 10 cm depth); carbon content and isotopic composition of total soil organic carbon and “old” litter (naturally deposited during autumn 2005); \( F_s \), \( F_m \), \( \delta^{13}\text{C}_{F_s} \), and \( \delta^{13}\text{C}_{F_m} \). The carbon content and the isotopic composition of total soil organic carbon and remaining litter were determined using the GC-C-IRMS system described in Section 2.7. “Old” litter mass was estimated by sampling litter in the four corners of the plots (15 cm \(\times\) 15 cm), drying at 50 °C and weighing.

The three treatments were: litter exclusion (E), natural conditions, i.e. control (C, 486 g m\(^{-2}\) litter dry mass) and litter addition (A, 972 g m\(^{-2}\) litter dry mass). Litter was added to treatments C and A on 24 March 2006 in the observed 7:3 oak:hornbeam ratio. During leaf fall in autumn 2006 new litter inputs were avoided by replacing the collecting nets upon and around the experimental area.

Every measurement described in the following was performed monthly from May 2006 to March 2007, except for litter mass loss which was measured every 2 months. Carbon isotopic composition is expressed in ‰ relative to the Pee Dee Belemnite standard.

2.3. Fresh litter degradation measurements

During litter deposition, 12 litter bags (mesh size: 1 mm) containing 5 g of litter ground by hand were placed in each plot of the C and A treatments. Three litter bags were collected every 2 months; their contents were dried at 50 °C for 48 h and weighed. Fresh litter mass loss was estimated by calculating the difference between the initial litter dry mass and the remaining litter dry mass. The fresh litter degradation coefficient \((k)\) was estimated according to Granier et al. (2000) as the exponent of an exponential decay relationship between remaining litter dry mass \((M_t)\) and time since deposition \((t, \text{days})\).

\[
M_t = M_0 \times e^{-k \cdot t}
\]
2.4. Field measurement of total soil CO$_2$ efflux intensity: $F_S$

$F_S$ was measured in the field on circular collars (12 cm diameter, 7 cm height, 4 collars per plot) buried 2 cm into the soil before litter addition. In each collar the litter mass per unit area was equivalent to that in the relevant plot. Four measurements were performed per plot (one per collar) and averaged. Manual $F_S$ measurements were conducted on these collars monthly with a CIRAS analyser (PP Systems International Inc., Amsbury, MA) configured in closed system with SRC-1 soil respiration chamber (wind speed = 0.1 m s$^{-1}$, as recommended by Le Dante et al., 1999). Measurements lasted 2 min or the time needed for an increase in CO$_2$ concentration of 100 ppm in the chamber. Prior to measurements, the stability of fluxes was tested on two collars per plot by three repeated measurements. Mean $F_S$ values were calculated for each plot, expressed in $\mu$mol m$^{-2}$ s$^{-1}$. Measurements were converted to gC m$^{-2}$ for comparison with fresh litter mass loss derived from the fresh litter degradation model.

During $F_S$ measurements, the soil temperature was measured at 10 cm depth around each collar using a linear precision resistance probe (STP-1, PP Systems). The soil water content (SWC, %) was measured for the 0–10 cm layer by drying soil at 105°C and relating the mass loss to soil dry mass.

2.5. The role of litter in the modification of total soil CO$_2$ efflux intensity

To determine the role of litter in the modification of total soil CO$_2$ efflux intensity, the CO$_2$ efflux intensity measured in situ was compared to the expected CO$_2$ efflux intensity calculated as follows:

$$F_{S,E}^{\text{EXPECTED}} = F_{S,C} - \text{LMMC} \quad (2)$$

$$F_{S,A}^{\text{EXPECTED}} = F_{S,C} + \text{LMMC} \quad (3)$$

where, in Eq. (2), $F_{S,E}^{\text{EXPECTED}}$ is the expected flux in the E treatment calculated from the measured efflux in the control ($F_{S,C}$) minus the decay of litter (LMMC) in the control, which corresponds to the litter excluded in the E treatment; and where, in Eq. (3), $F_{S,A}^{\text{EXPECTED}}$ is the expected flux in the A treatment calculated from the measured efflux in the control ($F_{S,C}$) plus the decay of litter (LMMC) in the control, which corresponds to the litter added in the A treatment. Every efflux was calculated in gC m$^{-2}$. These equations were previously described by Crow et al. (2009). If the expected efflux intensity is equivalent or higher than the measured efflux intensity, the difference in the efflux intensity between the treatment and the control may be attributed to the decomposition of litter. If the expected efflux intensity is lower than the measured efflux intensity, it would be assumed that litter decay cannot be the only source explaining the increase of efflux intensity and that this increase corresponds to soil priming according to Crow et al. (2009). In these conditions, soil priming (PE) would be determined as the percent of total soil CO$_2$ efflux measured in situ according to the equation described by Crow et al. (2009):

$$\text{PE} = 100 \times \left( \frac{F_S - F_S^{\text{EXPECTED}}}{F_S} \right) \quad (4)$$

where, for a given treatment, $F_S$ corresponds to soil respiration measured in situ and $F_S^{\text{EXPECTED}}$ is the expected soil respiration determined according to the equations described above (Eq. (2) or (3)).

2.6. Laboratory measurement of heterotrophic CO$_2$ efflux in litter and soil without roots: $F_I$ and $F_m$

2.6.1. Sampling

Soil and litter samples were collected monthly in every plot according to the treatment. The soil sample consisted of three randomly taken soil cores (0–10 cm depth, 1.2 cm diameter) which were combined and sieved at 2 mm in the field (ca. 50 g equivalent dry mass of soil).

Litter samples were also composite samples constituted of three samples randomly taken in each C and A plots. Litter samples per plot corresponded to ca. 2.5 g equivalent dry mass of litter.

2.6.2. CO$_2$ efflux measurement

About 1.5 h after sampling, soil and litter samples were incubated in the laboratory using flasks sealed with a Teflon® septum. Measurements were carried out on three 5 g replicates per plot for soil and one replicate for litter because of limited material. At the beginning of the incubation, flask air was decarboxylated by pushing air several times through a soda-lime column. Then samples were incubated for 13–20 h to avoid strong variations in terms of microbial biomass and diversity, and to limit shifts in metabolic pathways or mineralized carbon sources (Andrews et al., 1999). Incubations were performed in a water bath regulated at the soil temperature previously recorded in the field. At the end of the incubation, 25 mL of flask air was sampled with a 50 mL valved syringe (SGE) and injected into new flasks flushed with pure nitrogen. Air samples were analysed for their CO$_2$ concentration and $\delta^{13}$C$_{CO_2}$ (see Section 2.7 for details).

$F_I$ and $F_m$ were calculated as the ratio between CO$_2$ concentrations accumulated during the incubation over incubation time. To scale to field measurements, laboratory measurements of $F_I$ were converted from $\mu$mol g$^{-1}$ dry mass s$^{-1}$ to $\mu$mol m$^{-2}$ s$^{-1}$ using litter dry mass present on the field plots. Litter dry mass on the field plots was determined as the difference between the initial litter dry mass and the litter mass lost during the elapsed time between the start of the experiment and the date of sampling as estimated via the exponential decay function derived from fresh litter degradation measurements. Then, $F_I$ was used to estimate the contribution of fresh litter to total soil CO$_2$ efflux. $F_m$ was also expressed in $\mu$mol m$^{-2}$ s$^{-1}$ using the apparent density of soil (1.35) and soil core volume (1.13 × 10$^{-3}$ m$^3$). Conversely to $F_I$ measurements, $F_m$ measurements were only used to test the impact of the treatment on heterotrophic soil CO$_2$ efflux intensity. Because sieving modified the conditions of diffusion, these measurements did not allow the estimation of the contribution of $F_m$ to total soil CO$_2$ efflux.

2.7. Measurement of the isotopic composition of respired CO$_2$: $\delta^{13}$C$_{F_I}$, $\delta^{13}$C$_{F_F}$ and $\delta^{13}$C$_{F_m}$

The isotopic ratio of total soil-respired CO$_2$ ($\delta^{13}$C$_F$) was determined in the field for each plot by the Keeling plot method (Keeling, 1958). Soil air was sampled in the field using a pump-equipped EGM-1 analyser (PP Systems) and a custom cylindrical closed chamber (25.4 L, 12 cm height) made of transparent acrylic resin and equipped with a fan. The chamber was laid on a collar inserted 2 cm into the soil. For each Keeling plot, five air samples were taken directly from the closed circuit using 50 mL valved syringes (SGE, Australia). Air samples were taken every 100–250 ppm CO$_2$ increase in the range 500–1500 ppm. Air samples were analysed for their $\delta^{13}$C$_{CO_2}$ with an elemental analyser (Model NA-1500, Carlo Erba, Milan, Italy) coupled to an isotopic ratio mass spectrometer (VG Optima, Fison, Villeurbanne, France; measurement standard error = 0.2‰). Linearity and stability of the analysis system were tested during measurements by analysing standard air samples of
Fig. 1. Temporal variation in soil temperature at 10 cm depth (T_{10 cm}, diamonds, dotted line) and soil water content (SWC, %, bars) in the three treatments: litter exclusion (E, white bars), control (C, grey bars) and litter addition (A, black bars). Error bars represent standard error of the mean (n = 3–4).

different CO2 concentration and with the same δ^{13}C. δ^{13}C_F was estimated as the y-intercept of the linear regression between the inverse of CO2 concentration (x-axis) and δ^{13}CO_{2} (y-axis). Linear regression was performed using the Ordinary Least Square linear regression model. Estimates having a standard error higher than 5% of the estimated value were excluded (Chemidlin Prévost-Bouré et al., 2009).

The same system was used to measure δ^{13}C_F and δ^{13}C_{m} on air samples taken from the laboratory incubation assays. A standard curve was established from the CO2 concentration of standard air samples and the corresponding peak height, and used to determine the CO2 concentration in the samples.

2.8. Statistical analysis

For each field session, measured variables were averaged per treatment as arithmetical means, excepted δ^{13}C_F. Mean δ^{13}C_F (δ^{13}C_{F,i}) was calculated by weighting each estimate by its standard error (Murtaugh, 2007). Equations are:

\[ \delta^{13}C_{F,i} = \frac{1}{n} \sum_{i=1}^{n} w_i \times \delta^{13}C_{F,i} \] 

with

\[ w_i = \frac{1}{\text{SE}(\delta^{13}C_{F,i})^2} \left( \frac{1}{\sum_{i=1}^{n} (1/\text{SE}(\delta^{13}C_{F,i})^2)} \right) \]

SE(δ^{13}C_{F,i}) is the standard error of the ith estimation of δ^{13}C_F, and n the total number of observations.

The standard error of the mean was calculated as follows:

\[ \text{SE}(\delta^{13}C_F) = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^{n} (w_i \times (\delta^{13}C_{F,i} - \delta^{13}C_F)^2)} \]
Non parametric paired tests (Friedman repeated measures one-way analysis of variance on ranks, cited as RMANOVA; and Wilcoxon’s test for paired samples on ranks) were used to check for differences between the three treatments. Significance level was set at 5%. All statistical analyses were performed using Statistica software (Statsoft Inc., Tulsa, USA).

3. Results

3.1. Homogeneity of treatment plots before litter manipulation

The results of assessments carried out on the plots prior to the start of the experiment are reported in Table 1. No significant differences were observed for soil water content or soil temperature. Soil organic C content and its isotopic composition was similar in each treatment, as were “old” litter mass and isotopic composition. The treatments were also homogeneous in terms of $F_5$, $F_m$ and their isotopic composition.

Additionally, the isotopic composition of soil organic C was significantly different from that of the fresh litter used for the experiment ($P < 0.05$, $\Delta$ fresh litter–soil = −1.8%).

3.2. Soil temperature and moisture variations

Soil temperature ($T_{10cm}$) ranged between 2.7 and 18.5 $^\circ$C with an annual mean of 11.4 $^\circ$C (Fig. 1). The highest values were observed in summer. In March 2007, $T_{10cm}$ was 4.5 $^\circ$C higher than values observed at the same period 1 year earlier because winter 2007 was particularly warm (Piao et al., 2008). Soil water content (SWC, 0–10 cm) was not affected by the quantity of fresh litter and ranged from 7.6% to 26.0% during the study period in the E, C and A plots. SWC variations were negatively correlated to $T_{10cm}$ variations ($|r| > 0.85$). SWC was lower than the “theoretical” wilting point threshold (estimated using the model of Saxon et al. (1986)) in July 2006, indicating a drought-constraint. This corresponds to a 15-day period (27 June–12 July 2006) during which only 10 mm of rain was recorded.

3.3. Fresh litter decomposition

In both C and A plots, an exponential decay function well fitted the data for litter dry mass loss with time ($r^2 > 0.7$). Litter degradation rate was not significantly different for the C and A treatments as the decay constants ($k$ in Eq. (1)) in these treatments were not different: $2.01 \times 10^{-3}$ (standard error: $6.47 \times 10^{-5}$) and $1.93 \times 10^{-3}$ (standard error: $5.84 \times 10^{-5}$), respectively.

3.4. Response of total soil CO2 efflux to variations in fresh litter amount

Fresh litter amount had a significant impact on $F_5$ measured in the field (RMANOVA, $\chi^2 = 22; P < 0.0001$), as presented in Fig. 2. $F_5$ was significantly reduced in the E treatment (by 25–45%) compared to the control (C, Wilcoxon’s test, $P < 0.001$) while $F_5$ was significantly increased in the A treatment (by 60–120% depending on the period considered) compared to the control (Wilcoxon’s test, $P < 0.004$). No block effect was detected (Kruskal–Wallis test, $P > 0.5$). However, fresh litter addition or exclusion did not modify the seasonal trends of $F_5$. The maximum values occurred in June 2006 (2.2, 3.0 and 5.6 $\mu$mol m$^{-2}$ s$^{-1}$ for E, C and A treatments, respectively) and the minimum in March 2007 (0.4, 0.5 and 0.9 $\mu$mol m$^{-2}$ s$^{-1}$ for E, C and A treatments, respectively). In July 2006, $F_5$ was slightly reduced compared to June 2006 despite a higher soil temperature. In each treatment, the seasonal variations in $F_5$ were well explained by a $Q_{10}$ relationship to temperature ($r^2 > 0.8$). In this relationship, $Q_{10}$ values (3.5, 3.2, 3.6 in the E, C and A treatments, respectively) were not affected by the treatment, whereas basal respiration at $10^\circ$C (0.8, 1.1, 2.0 $\mu$mol m$^{-2}$ s$^{-1}$ in the E, C and A treatments, respectively) was significantly ($P < 0.001$) changed by the fresh litter amount.

3.5. Role of litter in the modification of total soil CO2 efflux intensity

To determine whether the significant variation in total soil CO2 efflux intensity ($F_5$) could be attributed to the addition or the exclusion of fresh litter, the field measurements of $F_5$ in the E and A treatments were compared to the expected total soil CO2 efflux intensity ($F_{5,\text{EXPECTED}}$) in these treatments. In the A treatment, field measurements of $F_5$ ($F_{5A}$) were significantly higher ($P < 0.001$) than $F_{5,\text{EXPECTED}}$ (Fig. 3). Under these conditions, soil priming corre-

![Fig. 2. Temporal variation in total soil CO2 efflux ($F_5$) in the litter exclusion (E, dotted line), control (C, grey) and litter addition (A, black) treatments, respectively. Error bars correspond to one standard error of the mean. Inserted graphic: response of $F_5$ to soil temperature at 10 cm depth ($T_{10cm}$) in the litter exclusion (E, black filled circles), control (C, grey filled circles) and litter addition (A, open circles) treatments, respectively. A $Q_{10}$ model: $F_5 = R_{SOH} \times Q_{10}(T_{10cm} - 10)/10$; well fitted $F_5$ temperature response. $Q_{10}$ estimates: 3.5, 3.2, 3.6 in the E, C and A treatment, respectively. $R_{SOH}$ estimates: 0.8, 1.1, 2.0 $\mu$mol m$^{-2}$ s$^{-1}$ in the E, C and A treatments, respectively.](image1)

![Fig. 3. Measured (in situ) and expected total soil CO2 efflux over time in the litter exclusion (E) and litter addition (A) treatments across the experiment. Solid lines: measured total soil CO2 efflux in the E (grey) and A (black) treatments. Dashed lines: expected total soil CO2 efflux in the E (grey) and A (black) treatments. Errors bars are standard errors of the mean (n = 4).](image2)
sponded to +32% of \( F_S \) measured in the A treatment. In the E treatment, \( F_S \) measured in the field (\( F_{SE} \)) was not significantly different from \( F_{SE}^{\text{EXPECTED}} \) (\( P > 0.2 \)), showing that litter decomposition in the control accounted for the difference in soil respiration between control and E treatment.

3.6. Response of heterotrophic CO2 efflux in litter and soil to variations in fresh litter amount

Laboratory measurements of fresh litter CO2 efflux (\( F_l \)) in the A treatment were twice as high as in the control (Fig. 4; Wilcoxon’s test, \( P < 0.01 \)), but differences were constrained by season (no differences in September 2006 and February 2007). Mean \( F_l \) was 0.13 and 0.22 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in the C and A treatments, respectively. This difference was determined by fresh litter amount, since fresh litter respiration per g of dry mass was similar in the control and A treatments (\( P < 0.60 \)). Fresh litter contribution to total soil CO2 efflux ranged from 2% to 18% for both control and treatment A, and the higher litter contribution was observed during the period of leaf fall.

Heterotrophic CO2 efflux in soil (\( F_m \)), measured in the laboratory, was not affected by litter addition (treatment A) or exclusion (treatment E) (Fig. 4b, RMANOVA, \( \chi^2 = 4.9, P < 0.10 \)). Similar ranges of \( F_m \) were observed in all treatments: ca. 0.5–4 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The seasonal variations of \( F_m \) were also similar among the three litter treatments and were not related to incubation temperature.

3.7. The isotopic composition of total soil-respired CO2 and heterotrophically respired CO2 from litter and soil

Litter treatment did not significantly affect \( \delta^{13}\text{C}_{F_l} \) measured in the field (RMANOVA, \( \chi^2 = 3.8, P < 0.15 \)), which ranged from –27.9‰ to –22.5‰, in all treatments (Fig. 5a). Nevertheless, a trend was observed for \( \delta^{13}\text{C}_{F_l} \) with CO2 from treatment E being generally slightly \( ^{13}\text{C} \)-enriched relative to other treatments.

Exclusion or addition of fresh litter did not affect \( \delta^{13}\text{C}_{F_m} \) (\( \chi^2 = 4.9, P < 0.09 \), Fig. 5b). Ranges of variation of \( \delta^{13}\text{C}_{F_m} \) were between –21.7‰ and –28.7‰ through the measurement period. \( \delta^{13}\text{C}_{F_m} \) exhibited a clear increase in summer and a decrease during winter. CO2 derived from heterotrophic respiration in soil was always \( ^{13}\text{C} \)-enriched relative to total soil organic matter (mean apparent discrimination: 2.2‰).

Like \( \delta^{13}\text{C}_{F_m} \), \( \delta^{13}\text{C}_{F_l} \) was not affected by fresh litter addition (\( P < 0.60 \)). Mean annual \( \delta^{13}\text{C}_{F_l} \) was –25.9‰ and –26.3‰ in the control and A treatments, respectively (Fig. 5c). \( \delta^{13}\text{C}_{F_l} \) was not different from \( \delta^{13}\text{C}_{F_m} \) and most of the time was \( ^{13}\text{C} \)-enriched relative to litter organic matter (mean apparent discrimination: 1.3‰ estimated per date of sampling, data not shown). The range and the temporal dynamics of \( \delta^{13}\text{C}_{F_l} \) were similar to those of the isotopic composition of root respired CO2 (root excision method, data not shown).

4. Discussion

During the experiment, soil temperature and soil water content (SWC) were not modified by the treatment. A similar observation had been previously reported for soil temperature (Sulzman et al. 2010).
et al., 2005) but not for SWC. This lack of variations of SWC may be explained by the dense canopy above the experimental area (leaf area index = 5.1; Delpierre et al., 2009) which strongly limited water evaporation from soil, particularly during the vegetative period (Rey et al., 2002). During this period, SWC variations may have mainly been driven by root water uptake. Added to that, the humus layer remained thin despite litter addition. Therefore, litter addition at our site may not have significantly modified the resistance of the humus layer to water infiltration into soil and water evaporation from soil (Baldocchi et al., 2000; Schäfer et al., 2002) throughout the experiment.

Temporal variations in $F_S$ reported in this study for the different treatments (E, C and A) were in the range of those reported in the literature for different types of temperate deciduous forests: 0.5–6.0 $\mu$mol m$^{-2}$ s$^{-1}$ (Davidson et al., 1998; Hibbard et al., 2005; Vincent et al., 2006). The reduction in $F_S$ observed in July 2006, while soil temperature was rising, was attributed to a drought event appearing in every treatment. In accordance with different field studies (Boone et al., 1998; Epron et al., 2004; Sulzman et al., 2005), the addition/exclusion of fresh litter significantly increased/decreased $F_S$, without modifying the contribution of fresh litter to $F_S$. These variations in $F_S$ between treatment E, control and treatment A were not explained by variations in the temperature sensitivity of $F_S$ ($Q_{10}$). Indeed, $Q_{10}$ was not significantly modified by the quantity of fresh litter and differences in $F_S$ between treatments were mainly explained by changes in basal soil respiration at 10 °C ($F_{1010}$). In every treatment, the $F_{10}$ value (3.2–3.6) was in the range of those reported in the literature (Boone et al., 1998; Davidson et al., 1998; Perrin et al., 2004; Gaumont-Guay et al., 2006), and was in the range of those corresponding to recalcitrant material according to Davidson and Janssens (2006) and Davidson et al. (2006). Therefore, the stability of the $Q_{10}$ may be related to a higher consumption of soil organic matter relative to litter organic matter, which would limit the imprint of litter quantity itself on the $F_{10}$. This hypothesis is supported by the relatively small contribution of $F_S$ to $F_m$ (8% on average). Consequently, the variations in $F_S$ and $F_{10}$ between treatments would be related to variations in soil activity mediated by the quantity of fresh litter.

Modifications of soil activity, and then of $F_S$ and $F_{10}$, may be related to changes in microbial biomass, according to the trend observed by Soe and Buchmann (2005), and/or to changes in soil microbial community composition (Nottingham et al., 2009), and/or changes in substrate availability or composition since microbial biomass is carbon-limited in soils (Kuzyakov et al., 2000; and/or changes in substrate availability or composition since microbial biomass is carbon-limited in soils (Kuzyakov et al., 2000; Fontaine et al., 2003; Crow et al., 2009). Therefore, the stability of the $Q_{10}$ may be related to a higher consumption of soil organic matter relative to litter organic matter, which would limit the imprint of litter quantity itself on the $F_{10}$. This hypothesis is supported by the relatively small contribution of $F_S$ to $F_m$ (8% on average). Consequently, the variations in $F_S$ and $F_{10}$ between treatments would be related to variations in soil activity mediated by the quantity of fresh litter.

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In accordance with the definition provided by Bingeman et al. (1953) and refined by Kuzyakov et al. (2000), soil priming detected in the A treatment corresponds to a “real” priming effect, i.e. a priming effect for which the carbon source corresponds to organic matter and not microbial biomass, which remained unchanged by the treatment (Blagodatskaya and Kuzyakov, 2008). The contribution of the priming effect estimated here (32% of $F_S$ measured in the A treatment on average) was in the range of those reported in situ by Sulzman et al. (2005) and Crow et al. (2009). According to Fontaine et al. (2003) and Kuzyakov et al. (2000), the addition of fresh organic matter may have allowed the co-metabolic decomposition of more recalcitrant soil organic matter (SOM). This would be supported by $Q_{10}$ estimates but not by the lack of variation in $\delta^{13}C_S$. Indeed, according to the linear mixing model (Phillips and Gregg, 2001), if only SOM had been mineralized during priming, the contribution of $F_m$ to $F_S$ would have been modified and then $\delta^{13}C_F$ would have been less negative in the A treatment than in the control, especially during the vegetative period when differences between $\delta^{13}C_F$, $\delta^{13}C_S$, and $\delta^{13}C_{fam}$ were maximal (2–3‰), above the limit needed to discriminate between CO2 sources (Phillips and Gregg, 2001). Because this increase was not observed, we may assume that primed respired CO2 was not only derived from SOM but also from the rhizosphere as proposed by Subke et al. (2004) and that litter addition activated the whole soil system, accelerating C turnover processes. Further labelling experiments involving high-frequency carbon stable isotope measurements of CO2 efflux would help to clarify the relative importance of bulk soil and rhizosphere in the priming effect.

Priming effect phenomena have been previously reported in laboratory studies (Hamer and Marschner, 2005; Kuzyakov et al., 2006; Fontaine et al., 2007), but only in a few field studies using complex carbon sources like litter (Subke et al., 2004; Sulzman et al., 2005; Crow et al., 2009). Here, $F_S$ data suggest that the priming effect lasted for over 1 year, compared to a few days to weeks in laboratory studies. This long-lasting priming effect may be related to a continuous release of organic compounds from litter to soil because of the progressive decomposition of leaf litter. This is in agreement with the trends observed in the literature for the duration of a priming effect with increasing substrate complexity: e.g. Hamer and Marschner (2005) reported fast 1-day priming after addition of simple organic compounds (fructose, alanine), whereas Fontaine et al. (2007) and Nottingham et al. (2009) reported a priming effect that lasted 30 days after pure cellulose addition or chopped/ground maize litter, respectively.

Temporal variation in isotopic data can also be informative. The isotopic values were in the range of those reported in the literature for $\delta^{13}C_{fam}$ (e.g. Ekkblad and Hogberg, 2001; Bluhupinderpal-Singh et al., 2003; Ngao et al., 2005) and for $\delta^{13}C_{fam}$ (Andrews et al., 1999, 2000; Tu and Dawson, 2005). Both $\delta^{13}C_{fam}$, $\delta^{13}C_{fam}$ and $\delta^{13}C_{fam}$ showed temporal variations throughout the experiment (amplitude of variations: from 2.4‰ to 6.3‰). Over the whole season, these temporal variations were not related to soil climatic conditions (soil temperature, SWC). Nevertheless, some of them have probably been influenced by climatic conditions: $\delta^{13}C_{fam}$, $\delta^{13}C_{fam}$ and $\delta^{13}C_{fam}$ increased during the drought stress, which is in agreement with the response of photosynthesis to a drought stress (Farquhar et al., 1989) and the link between plant physiology and soil respiration (Högberg et al., 2010) in the determination of $F_m$ (in a range close to that of the confidence interval of the measure) are likely to produce large differences in respiration rate when extrapolated to the soil column. In our study, such small differences may have been masked by the impact of soil sieving on CO2 emission (Kuzyakov, 2006). Therefore, we may conclude that the variations in $F_S$ and $F_{10}$ may have been determined by modification of substrate availability or composition in soil, promoting the use of soil C.

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et al., 2001). Plant physiology may not be the only determinant of δ13C$_{F}$, δ13C$_{Cr}$, or δ13C$_{Cr}$ temporal variations. Indeed, these variations could also be the result of changes in the isotopic composition of the mineralized organic compounds or in isotopic discrimination during organic matter decomposition (Santruckova et al., 2000; Wynn et al., 2006). These changes may be determined by variations in the composition of the soil microbial community that were demonstrated, in our study, to occur with time both in soil and litter (Chemidlin Prévost-Bouré et al., in press). Temporal variation in δ13C$_{Cr}$ was found to be significantly related to temporal variation in the litter bacterial community in the A treatment, but not in the other treatments and not in soil. Considering concomitantly temporal variation in δ13C and microbial community composition may provide valuable clues to better understand organic matter mineralization dynamics and to test mineralization models dealing with microbial community variation (Neill and Gignoux, 2006). This would need “high frequency” isotopic measurements (CO$_2$ and organic matter) and isotopic labelling techniques to distinguish between active and non-active microbial populations.

5. Conclusions

Litter manipulation showed that soil CO$_2$ efflux was stimulated when fresh litter was added and was reduced when fresh litter was excluded. The observed activation was not determined by differences in soil climatic conditions (temperature and moisture) between treatments or by variation in temperature sensitivity ($Q_{10}$) of $F_S$. Indeed, it appears to be the result of a real priming effect, for which the intensity may be affected by seasonal variation in soil climatic conditions. This priming effect represented a large proportion of soil CO$_2$ efflux in the litter addition treatment and probably resulted in the activation of the whole soil system. This study highlights the complexity of direct and indirect interconnections between soil carbon source components in the context of climate change as modifying the size of the young carbon pools (here litter) may lead to the fast remobilization of older soil C stocks through respiration, particularly in temperate forests. In addition, this study highlights the importance of labelling experiments to improve our knowledge of litter–soil interaction and to decipher the mechanisms of organic matter mineralization.

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