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Fifty years of crop residue management have a limited impact on soil heterotrophic respiration

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ABSTRACT

The impacts of crop residue management on soil microbial biomass, labile carbon and heterotrophic respiration (HR) were assessed at a long-term experimental site in the Hesbaye region in Belgium. Three treatments, residue export (RE), farmyard manure addition (FYM) and residue restitution after harvest (RR), have been applied continuously since 1959. The soil is a Eutric Cambisol with, in 2010, significantly different total soil organic carbon contents of 4.4, 5.1 and 5.9 kg C m^{-2} under the RE, RR and FYM treatments, respectively. Manual field HR measurements were carried out during the 2010 and 2012 crop seasons using a dynamic closed chamber system. Microbial biomass, labile C content and metabolic diversity of soil bacteria were assessed in spring 2012.

Fifty-one years after the beginning of the treatments, residue management had a limited impact on HR. Based on daily averaged values, the treatment had a significant impact ($\alpha = 10\%$) in 2012 but not in 2010. Based on the individual measurement dates, the treatment impact was less obvious in 2012; with the observation of a significant impact ($\alpha = 10\%$) on HR in only 7% and 36.8% of the measurement dates in 2010 and 2012, respectively. Labile C and microbial biomass were significantly lower in the RE treatment than in FYM and RR. Residue management had no significant effect on cold-water extracted carbon and metabolic diversity of heterotrophic soil bacteria. The limited impact of residue management on HR could be explained by (i) the relatively low amounts of recent above-ground crop inputs, (ii) the large proportion of below-ground residues and other non-exportable above-ground residues reducing the potential differences between treatments and (iii) the relatively large spatial variability of HR.

In conclusion, carbon losses due to heterotrophic respiration did not differ between RE, FYM and RR treatments in the studied soil. This contrasts with the different soil organic carbon contents observed in these three treatments after 50 years of experiment. Further investigations regarding the reduction of spatial variability and the potential roles played by organic matter protection within aggregates and biochemical composition of inputs are needed.

1. Introduction

Agricultural soils have frequently been reported to lose large amounts of carbon (C) (Goidts and van Wesemael, 2007; Janssens et al., 2003; Smith, 2004). Soil C loss negatively affects soil stability (Lal, 2004), which is harmful to agricultural productivity. It also results, through the process of heterotrophic respiration (HR) releasing carbon dioxide (CO_2) into the atmosphere, in atmospheric CO_2 concentration level increase, which accelerates global warming. CO_2 emissions due to HR could become more important with increasing temperatures related to climate change (Davidson and Janssens, 2006). Crop management has a high potential to mitigate soil C loss. According to Smith (2012), crop management techniques could actually constitute more important drivers of SOC stock changes and CO_2 emissions in croplands than climatic effects. Future management practices should maintain good soil properties (e.g., fertility, stability, water-holding capacity) and help mitigate climate change (Smith, 2012).

Long-term field experiments have been initiated around the world to assess the impact of crop management, comprising residue and soil management techniques, on SOC stocks and greenhouse gas emissions. Although long-term residue management has been reported to have a large impact on SOC stocks (e.g. Smith et al., 1997 and, specifically about the site investigated in this study, Buysse et al., 2013), its influence on HR (more particularly the relationship between the amounts of crop residue and the HR rates) is not clear

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(Chirinda et al., 2010; Duiker and Lal, 2000; Jacinthe et al., 2002; Mu et al., 2008). HR is driven by many interacting factors, such as substrate quantity and quality, microbial biomass, soil texture, temperature and water content. The large spatio-temporal variability of each HR driving variable, and particularly the amount of available substrate, contributes to the difficulty to predict HR under different crop management scenarios. In order to take this variability into account, the question of crop management influence on HR at the ecosystem spatial scale needs be addressed using field measurements (Chirinda et al., 2010; Duiker and Lal, 2000; Mu et al., 2008; Vinther et al., 2004). This preserves soil structure irregularities, organic matter protection micro- and macro-sites and wind effects, all potentially driving spatial variability (Gesch et al., 2007). According to de Campos et al. (2011) the representativeness of management practices could indeed be affected by sample handling in laboratory measurements.

In temperate ecosystems, temperature is generally considered as the main driving variable of HR (Davidson and Janssens, 2006; Kätterer et al., 1998; Lloyd and Taylor, 1994). To a lesser extent in these ecosystems, soil water content can also affect the process, particularly when dry conditions make substrate less available to micro-organisms (Luo and Zhou, 2006). Besides, both input quantity and quality are important characteristics driving HR fluxes. It might be expected that higher carbon inputs to the soil results in higher HR (Jacinthe et al., 2002). Furthermore, residue quality and accessibility affect the ability of micro-organisms to decompose substrates. Residue input quantity and/or quality can also influence the microbial populations, through changes in microbial biomass or adaptive changes within the metabolic diversity of the microbial community (Calbrix et al., 2007; Govaerts et al., 2007). In the present study, three residue management treatments were selected, characterized by different input quantities and qualities: residue export, residue restitution and farm yard manure input. The most important particularity of this study is the connection between the HR rates measured at the crop season scale and SOC changes observed in the long-term.

This study was implemented on the basis of a long-term residue management experiment where a previous study (Buysse et al., 2013) showed that the treatment had a significant impact on SOC stocks in the long-term (51 years). The objectives of the present study were (i) to assess the impacts of crop residue management (including farmyard manure input) on HR and (ii) to test if more labile soil organic C fractions are affected by long-term residue management and (iii) to try to link the HR rates measured at the crop season scale and SOC changes observed in the long-term. For this purpose, HR was measured in the field over two crop seasons and laboratory measurements (microbial biomass, labile C and microbial diversity) were carried out to add complementary data to HR measurement campaigns.

2. Materials and methods

2.1. Site description

The Longs Tours site is situated in the Hesbaye region in Belgium (50°33′28″ N, 4°43′39″ E, 170 m asl). The climate is temperate maritime with, over the 1959–2012 period, a mean annual air temperature of 9.4 °C and an average annual rainfall of 798 mm. The study field is a fairly flat rectangular area (360 m × 120 m). The soil is classified as a Eutric Cambisol (IUSS Working Group WRB, 2006); the clay, silt and sand proportions are 12, 85 and 3%, respectively. The site is situated at 2 km from the Lonzée Terrestrial Observatory (LTO), fully equipped for measuring CO_2, water vapor and energy fluxes, as well as micrometeorological variables (Moureaux et al., 2006).

The long-term experiment was initiated in 1959 to investigate the influence of residue management on crop yield, SOC content and soil physical properties. The site was part of the Global Change and Terrestrial Ecosystems Soil Organic Matter Network (GCTE SOMNET), established in 1995, assessing the effects of land use changes on soil organic matter, agricultural practice and climate. Six residue management treatments were applied to a total of 36 rectangular plots (70 m × 10 m; 6 replicates per treatment) (Fig. 1). All plots were ploughed every year to a depth of 25 cm. Between 1959 and 1974, the whole field was cultivated following a 4-year rotation cycle (sugar beet–cereal [oats or winter wheat]–legume [horsebean]–cereal [winter wheat or barley]). From 1975 onwards, a 3-year rotation cycle (sugar beet–winter wheat–winter barley) was followed.

Among the six initial treatments, we selected the three management practices most contrasted in carbon input and residue type. For residue export (RE), most aerial residues (except stubble which may represent about 30% of above-ground residues, the exact proportion being not measured) were removed after harvest and no other organic C was added to the soil. Farmyard manure (FYM) consisted in the application of fresh manure (30–60 t ha^{-1}) to the soil once every 4 (until 1975) or 3 (from 1975 onwards) years, at the beginning of each sugar beet crop season. In the residue restitution treatment (RR), all crop residues were returned to the soil at the end of the cropping seasons. Catch crops (vetch or mustard) were grown only in the RR treatment, during the fallow periods preceding the sugar beet crops, every 3 years from 1970. Average total C input amounted 315 ± 76, 472 ± 82 and 487 ± 93 gC m^{-2} $year^{-1}$ in the RE, FYM and RR treatments, respectively (Table 1). In May 2010, 51 years after the start of the long-term experiment, SOC was significantly higher under FYM, compared to RE and RR (sampled in the horizon 0–25 cm). More details about crop details and the long-term soil carbon budget at the Longs Tours site can be found in Buysse et al. (2013).

2.2. Field HR flux measurements

Heterotrophic respiration was measured in three replicate plots of the three selected treatments during the 2010 and 2012 crop seasons (Table 2, Fig. 1). These two measurement campaigns corresponded to periods before and after the farmyard manure spreading to the field (10 August 2011). All plots were situated in a well-drained part of the field. HR measurements were performed within one square area (9 m^2) in each plot. In 2010, glyphosate was applied to the young winter wheat shoots 2 weeks before the measurements, a delay allowing the microbial population to recover its original activity (Haney et al., 2002). Glyphosate application may cause a small extra CO_2 flux emission due to the decomposition of the young winter wheat shoots and roots. However it is important to state that this was applied similarly in all three treatments, which then makes possible to compare the crop seasonal CO_2 emissions from the three treatments on a same basis. In 2012, the young sugar beet shoots were removed manually. The weeded areas differed in 2010 and 2012 in order to prevent a long-term bias due to the experimental set-up.

Additionally, the short-term impact of FYM addition on HR was assessed from August to October 2011, about 2 weeks after farmyard manure spreading (Table 2). For this purpose, two 9-m^2-areas were prepared in each of the three investigated FYM plots. One square in each plot was protected with a PVC sheet during manure application.

In order to measure HR, PVC rings (9.6 cm diameter) were inserted into the soil (3 cm) in each weeded area. In 2010 and 2012, four PVC rings were placed within a 1 m-side square centered on each weeded area. In 2011, six PVC rings were positioned in three staggered rows 50 cm apart, also centered on each weeded square.

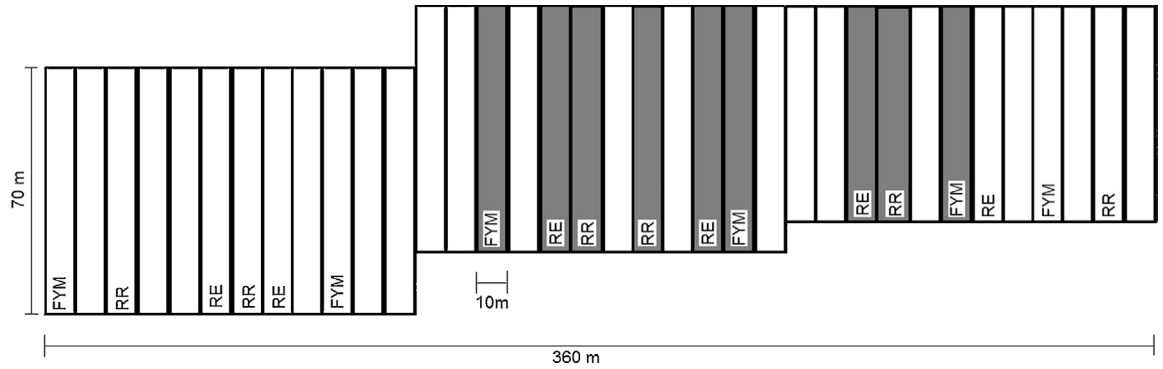

Fig. 1. Layout of the Longs Tours long-term experimental site. The plots selected for heterotrophic respiration measurements in the three treatments residue export (RE), farmyard manure addition (FYM) and residue restitution (RR) management are shown in dark gray.

Table 1
Average annual C input, total SOC content and long term average yields in the three treatments investigated at the Longs tours site: residue export (RE), farm yard manure (FYM) addition and residue restitution (RR). Details about how the average annual above-ground and left-over residue amounts were evaluated, on the basis of information compiled since the beginning of the long-term experiment at the Longs Tours site and collected in the literature, can be found in Buysse et al. (2013). The error values represent the 95% confidence intervals.

Crop and soil C characteristics	RE	FYM	RR
Average annual above-ground C input ($gC\,m^{-2}\,year^{-1}$) (Buysse et al., 2013)	–	157 ± 31	172 ± 53
Average annual left-over residues[a] ($gC\,m^{-2}\,year^{-1}$) (Buysse et al., 2013)	315 ± 76	315 ± 76	315 ± 76
Total average annual C input ($gC\,m^{-2}\,year^{-1}$)	315 ± 76	472 ± 82	487 ± 93
Total SOC content in May 2010 ($kg\,C\,m^{-2}$)	4.4 ± 0.2 [a]	5.9 ± 0.2 [b]	5.1 ± 0.3 [c]
Long-term average crop yields	RE	FYM	RR
Horse bean ($cwt\,ha^{-1}$)	34.3 ± 12.5	34.1 ± 12.5	34.4 ± 12.3
Winter wheat ($cwt\,ha^{-1}$)	65.1 ± 5.9	67.0 ± 5.3	66.6 ± 6.1
Sugar beet ($kg\,sugar\,ha^{-1}$)	8361.8 ± 1463.9	8580.4 ± 1637.3	8510.2 ± 1436.7
Oats ($cwt\,ha^{-1}$)	57.8 ± 9.9	59.6 ± 8.4	59.4 ± 10.7
Barley ($cwt\,ha^{-1}$)	58 ± 14.1	62 ± 16.2	64 ± 14.8

[a] This term comprises below-ground C inputs, weeds, and above-ground crop residues that cannot be exported at harvest due to technical constraints.

HR measurements were taken using the dynamic closed chamber system (Norman et al., 1997), most of the time (95%) with a Li-COR Li-6400XT equipped with a 6400-09 Soil Chamber (Li-COR Biosciences Inc., USA) and, during a Li-COR maintenance period in 2012, with a PP Systems EGM-4 IRGA analyzer connected to a SRC-1 chamber (PP Systems, Haverhill, MA). On each of the 36 PVC rings, two HR measurements were carried out. Between these two measurements, the CO_2 concentration in the soil chamber

Table 2
Main characteristics of the crop types and of the three heterotrophic respiration measurement campaigns performed at the Longs Tours site.

	Measurement campaigns		
	2010	2012	Complementary (2011)
Studied treatments	RE, FYM, RR	RE, FYM, RR	FYM, 3 plots receiving manure and the other 3 not
Crop characteristics			
Crop type during the previous year	Sugar beet	Barley	Winter wheat
Organic carbon amount brought to the soil	RE: left-over residues (exact amount unknown) FYM: left-over residues (exact amount unknown) + manure ($500\,gC\,m^{-2}$) RR: left-over residues (exact amount unknown) + sugar beet shoots ($160\,gC\,m^{-2}$)	RE: left-over residues (exact amount unknown) FYM: left-over residues (exact amount unknown) + manure ($350\,gC\,m^{-2}$) RR: left-over residues (exact amount unknown) + barley above-ground crop residues ($55\,gC\,m^{-2}$) + green manure ($200\,gC\,m^{-2}$)	FYM: left-over residues (exact amount unknown) + manure ($500\,gC\,m^{-2}$)
Period at which organic carbon was added	November 2009	August 2011 (green manure in October 2011)	August 2010
Measurement characteristics			
Measurement period	2 April–30 July	6 March–27 July	25 August–24 October
Weeding date	15 March	Manual removal of young beet shoots	/
Number of measurement dates	14	21	8
Number of points/treatment/date	12	12	18
Total number of measurement points/date	36	36	36

decreased to the initial atmospheric concentration. One single measurement lasted for 90 s. CO_2 concentration in the soil chamber was recorded every 2 s.

Soil temperature (T_s, digital thermometer; 5 cm depth) and SWC (ThethaProbe Soil Moisture Sensor ML2X, Delta-t Devices, Cambridge, UK; 0–5 cm depth) were measured manually next to each PVC ring. In addition, rainfall was estimated from the automatic measurements taken with a pluviometer at the LTO. Given that the heterotrophic respiration measurements were measured on bare soil surfaces, where T_s and SWC changes thus occur more rapidly, the relatively shallow T_s and SWC measurement depths were dedicated to follow these fluctuations as closely as possible.

2.3. Soil carbon and microbial diversity

2.3.1. Soil sampling

Soils were sampled on 18 May 2012 in all plots of the RE, FYM and RR treatments ($n = 6$, Fig. 1). In each plot, ten subsamples (auger sampling at 20 cm depth, 2 cm diameter) were sieved (4 mm diameter) and mixed to obtain six composite samples for each treatment. This sampling depth was considered to be sufficient to detect potential effects of residue management on microbial diversity, whose main part occurs very close to the soil surface. Soil moisture was determined after drying at 105 °C for 3 h (Allen, 1986).

2.3.2. Microbial biomass

A 30 g soil portion was used to assess microbial biomass (MB) using the fumigation-extraction method (Jenkinson and Powlson, 1976; Vance et al., 1987). Half of each soil portion was fumigated with ethanol-free chloroform for 3 days at 25 °C in the dark. Non-fumigated and fumigated soils were extracted with 50 ml 0.5 M K_2SO_4 and filtered (diameter 4.4 μm, filter 595 1/2, Whatman, Germany). Dissolved Organic Carbon (DOC) in fumigated and non-fumigated extracts was measured with a TOC analyzer (Labtoc, Pollution and Process Monitoring Limited, UK). MB was calculated as the difference between the DOC extracted from fumigated and non-fumigated samples, divided by a coefficient representing the efficiency of microbial C extraction (0.45; Joergensen, 1996).

2.3.3. Cold- and hot-water extractable carbon

Fresh soil (5 g) was used for cold-water soluble C (CWC) and hot-water extractable C (HWC) measurements, using the extraction method described by Ghani et al. (2003). The soil was shaken (120 rpm) with deionized water (30 ml) for 30 min and the soil solutions were filtered (diameter 0.45 μm, Pall Corporation, MI, USA). The remaining wet soils were then mixed again with deionized water (30 ml) and placed in an oven for 16 h at 80 °C. These soil solutions were filtered (diameter 0.45 μm, Pall Corporation, MI, USA). The filtrates collected after both filtrations were kept at 4 °C before analysis with the TOC analyzer.

2.3.4. Community-level physiological profiles (CLPP)

The metabolic diversity of heterotroph soil bacteria was measured using Biolog Eco-plates (BIOLOG™, California). Ecoplates contain 31 carbon sources potentially used by soil microorganisms and control wells containing only water. Each well contained an oxidized tetrazolium dye, changing from colorless to purple when reduced through bacterial respiration of the carbon source provided in the well. One gram fresh soil was extracted with sodium cholate (0.1%, 9 ml) after shaking for 1 min with a vortex (Super-Mixer, Lab-Line Instruments, Inc., Melrose Park, USA). These extracts were then brought to 10^{-4} final dilution with NaCl (0.85%, w/v) solutions, corresponding to 1000–2000 CFU (colony forming unit). A 100 μl aliquot of these 10^{-4} dilutions was then inoculated into each of the 32 microplate wells. The plates were incubated at 20 °C and visually analyzed after 72 h. The metabolic diversity was measured for each of the 18 samples, and expressed as the ratio of the number of wells where a reaction had occurred to the total number of wells in the relevant category of substrates (amino acids, carbohydrates, carboxylic acids, amines and polymers).

2.4. Data processing and statistics

2.4.1. HR data analysis

The increase with time of CO_2 concentration in the chamber was calculated by mean least squares regression and expressed as HR flux (μmol CO_2 m^{-2} s^{-1}), taking measurement volume, atmospheric pressure, soil temperature, gas constant and measurement area into account. Data were checked and selected according to two criteria related to (i) the quality of the linear increase of the CO_2 concentration in the chamber and (ii) the measurement repeatability. The first criterion varied with the magnitude of the flux: for small fluxes, the 95% confidence interval for the slope could not exceed an absolute error of 0.005 μmol mol^{-1} s^{-1}; for large fluxes, the ratio between the 95% confidence interval for the slope and the slope value itself could not be larger than 10%. The limit between small and large fluxes was set at the point where the small and large flux errors were equal (0.05 μmol mol^{-1} s^{-1}). To meet the second criterion, the two measurements on the same PVC ring had to be within 10–20% of the mean value of these two fluxes. With these criteria, about 75% of all data were retained.

Through an iterative process for each of the 2010 and 2012 campaigns (all treatments), the temperature-standardized HR fluxes (Section 2.4.2) greater than the [median + 1.5 × inter-quartile] were considered as outliers and the corresponding raw fluxes were removed from the dataset. Standardized fluxes were chosen rather than raw fluxes in order to take the flux temperature dependency into account in the selection.

For each measurement date and each PVC ring, both measurements were averaged. These values were also averaged over each plot, and over all PVC rings of each treatment to obtain daily mean values for each plot and each treatment.

2.4.2. HR responses to soil temperature and soil water content

To analyze the HR response to T_s, we hypothesized that the treatment did not affect HR sensitivity to temperature. This appeared realistic because the soil texture in the three treatments was the same and all treatments were ploughed in the same way, leading to similar soil aggregation and organic matter protection. The HR response to temperature was analyzed independently for the 2010 and 2012 campaigns by applying a Q_{10} relationship (Eq. (1), based on Kätterer et al., 1998) to raw HR fluxes:

$$HR = HR_{15} \cdot Q_{10}^{(T_s-15)/10} \quad (1)$$

where HR_{15} is the basal HR flux at the reference temperature of 15 °C [μmol CO_2 m^{-2} s^{-1}], Q_{10} is the HR temperature sensitivity [−], and T_s is the soil temperature [°C]. Curve fitting was performed using the Levenberg–Marquardt algorithm with MATLAB software (R2010b version, *The Mathworks*, Natick, USA). In 2010, the fluxes measured after two very important rain events on 28 May and 16 July were not used in assessing the HR responses to T_s and SWC because they were unlikely to be representative of usual soil conditions.

To study the global HR response to SWC in each measurement campaign, the HR fluxes were first standardized by dividing them by the Q_{10} factor ($Q_{10}^{(T_s-15)/10}$), taking account in each case of the Q_{10} value obtained from the previous fitting step. The standardized fluxes were then grouped into classes based on 15, 20, 25, 30, 35 and 40% vol.

2.4.3. Statistical analyses

The impact of residue management on temperature-standardized HR (HR_s) was investigated for cumulative HR fluxes over each cropping season and for HR fluxes at each sampling date. Cumulative HR_s fluxes were calculated over the measurement dates for each PVC ring. Averages for each plot were calculated within each treatment and a one-way ANOVA (factor = treatment, $n = 3$) was applied separately for the 2010 and 2012 campaigns. When unavailable, due to the data selection procedure (Section 2.4.1), fluxes were replaced by mean HR_s at the considered plot. When all fluxes from a given plot had been excluded from the dataset, they were replaced by mean HR_s calculated from the two other plots of the same treatment at the same date. HR_s fluxes at each measurement date were averaged over each plot and analyzed by one-way ANOVA (factor = treatment, $n = 3$). Multiple comparisons of means were performed in both analyses using Tukey's test (MATLAB R2010b version, The Mathworks, Natick, USA).

The impact of recent farmyard manure addition on HR was analyzed in the 2011 complementary measurement campaign for each sampling date, with one-way ANOVA (factor = treatment, $n = 3$). In this case, raw HR fluxes were used instead of standardized fluxes given the very poor quality of Q_{10} model fitting to these data, probably due to the narrower temperature range of this campaign and to the huge spatial variability of FYM after spreading.

The global influence of SWC on HR (all treatments and dates together: total $N = 144$ in 2010 and total $N = 252$ in 2012) was assessed with an unbalanced one-way ANOVA (factor = SWC class) using MATLAB (R2010b version, The Mathworks, Natick, USA).

The spatial variability of HR within the treatments for the 2010 and 2012 measurement campaigns was evaluated from the coefficients of variation calculated for each treatment based on HR_s. These percentages were then multiplied by the global fitted HR_{15} parameter determined for each measurement campaign (Section 2.4.2 – Eq. (1)) in order to obtain estimates of HR spatial variability within treatments. The importance of spatial variability in the results was then assessed by comparing these HR values with the HR flux differences between treatments estimated from the establishment of the long-term SOC budget in the three treatments (Buysse et al., 2013).

Differences in MB, CWC and HWC between treatments were analyzed with one-way ANOVA (factor = treatment, $n = 6$). Multiple comparisons of means were performed in both analyses using Tukey's test (MATLAB R2010b version, The Mathworks, Natick, USA).

In the CLPP measurements, color response data (Section 2.3.4) were then further explored under principal component analysis (PCA) (MATLAB R2010b version, The Mathworks, Natick, USA) through which the number of independent variables could be reduced and problems of multicollinearity solved. All meaningful loadings (i.e., loadings > 0.40) were included in the interpretation of principal components (PC). Graphic interpretation of PCA is done by constructing biplots, with the original variables drawn as vectors that summarize the correlation between the variable and both illustrated axes.

These biplots are a convenient way of mapping the original variables into PC space because the angles between variables express their correlation. Sample values are then projected into the new PC space by computing principal component scores for each sample. These scores are the new coordinates in PC space; one can interpret the biplot by noting the position in PC space of particular known samples compared to the new coordinates. For example, if two treatment samples appeared at one end of a PC axis while all the others were at the opposite end of the axis, this would clearly suggest that the first two treatments have unique characteristics.

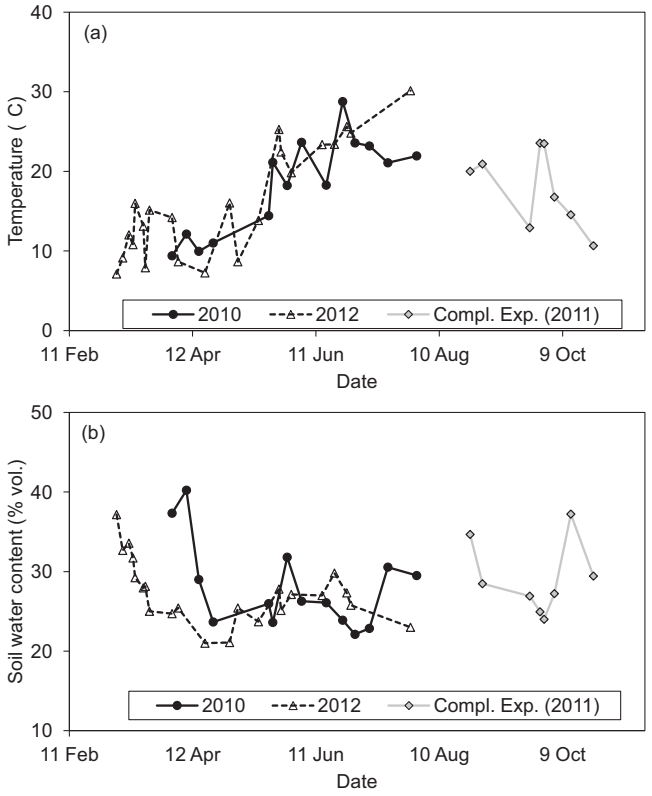

Fig. 2. Temporal evolution of the average soil temperature (T_s) and soil water content (SWC) at the Longs Tours site during the three heterotrophic respiration measurement campaigns (2010, 2012 and the complementary measurement campaign in 2011).

Differences in metabolic diversity between the three treatments were analyzed with two-way ANOVA (factors = treatments and principal components, $n = 6$) using the scores obtained for PC1 and PC2 for each replicate.

3. Results

3.1. HR responses to T_s and SWC

HR ranged from 0.67 to 3.28 μmol CO_2 m^{-2} s^{-1} in 2010 and from 0.09 to 2.71 μmol CO_2 m^{-2} s^{-1} in 2012 across all treatments, with no marked difference between the two cropping seasons (Figs. 2 and 3). Only two sharp HR increases on 28 May and 16 July 2010 corresponded to SWC increases after important rain events (4.4 mm in 10 h and 25 mm in 5 h, respectively; LTO data). The widest temperature range (7.1–30.2 °C) was observed in the 2012 campaign.

In the 2011 complementary campaign, HR decreased sharply from the beginning of the measurement campaign till the end, and varied from 1.07 and 5.22 μmol CO_2 m^{-2} s^{-1} across both treatments (Fig. 4).

Applying the Q_{10} model to the HR fluxes in 2010 and 2012 resulted in HR_{15} values of 1.25 ± 0.09 ($R^2 = 0.21$, $p < 0.05$) and 0.83 ± 0.08 μmol CO_2 m^{-2} s^{-1} ($R^2 = 0.48$, $p < 0.05$) in 2010 and 2012, respectively (Fig. 5). The Q_{10} values were 1.37 ± 0.10 ($R^2 = 0.21$, $p < 0.05$) and 2.48 ± 0.23 ($R^2 = 0.48$, $p < 0.05$) in 2010 and 2012, respectively.

After standardizing the HR fluxes to 15 °C and sorting the fluxes by SWC classes, no significant effect of SWC on HR could be found in the 2010 dataset (one-way ANOVA, $p = 0.44$, Fig. 6a) nor in the 2012 dataset, with the only exception that HR was significantly greater (one-way ANOVA, $p = 0.03$, Fig. 6b) at an SWC of 25 vol.%

Table 3
Results of the one-way ANOVA applied to the daily averaged standardized HR fluxes for both the 2010 and 2012 measurement campaign (SS = sum of squares, df = degrees of freedom, MS = mean square).

	Statistics									
	SS		df		MS		*F*-value		$p > F$	
	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012
Treatments	0.06	1.08	2	2	0.03	0.54	0.18	5	0.84	0.01
Error	6.74	5.85	39	54	0.17	0.11				
Total	6.80	6.93	41	56						

than 35 vol.%. It has to be noted that this absence of significant impact of SWC on HR likely relates to the small depth of measuring SWC (0–5 cm depth), where SWC is more responsive to evaporation conditions than over the whole depth of soil microbial activity (0–30 cm).

3.2. Residue management effect on HR

In 2010, the daily averaged HR_s fluxes were 1.33 ± 0.19, 1.43 ± 0.16 and $1.38 \pm 0.20\ \mu mol\ CO_2\ m^{-2}$ in the RE, FYM and RR treatments, respectively. In 2012, they amounted 0.74 ± 0.10, 0.79 ± 0.11 and $1.05 \pm 0.15\ \mu mol\ CO_2\ m^{-2}$ in the RE, FYM and RR treatments, respectively (the error terms represent the 90% confidence interval). There was no significant difference between averaged HR_s in the three treatments in 2010, while the HR_s fluxes were significantly different ($p < 0.10$) between the three treatments in 2012 (Table 3). The HR_s fluxes of the RR treatment were higher than in the other two treatments in this measurement campaign.

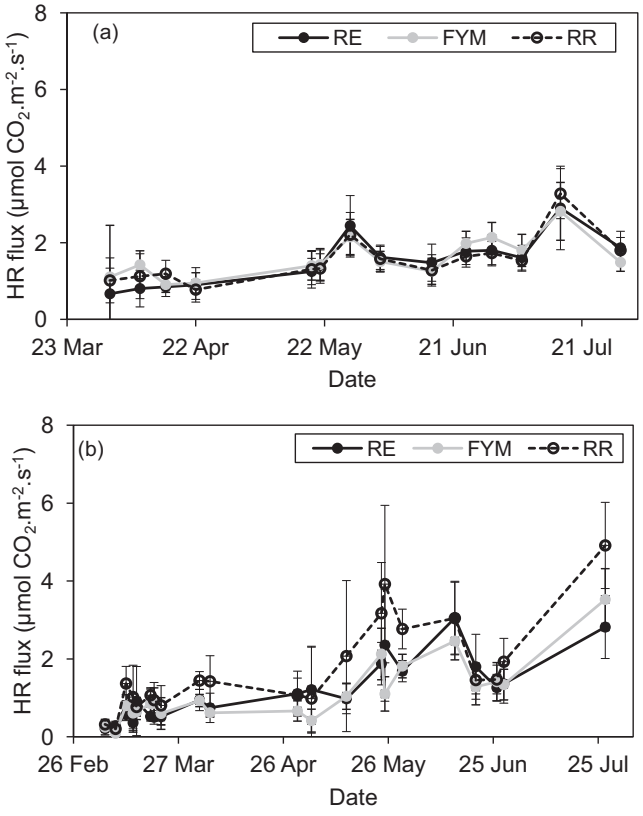

Fig. 3. Temporal evolutions of daily averaged heterotrophic respiration in the three treatments (residue export [RE]: closed black dots, farmyard manure [FYM]: gray dots, residue restitution [RR]: open black dots] during both heterotrophic respiration measurement campaigns at the Longs Tours site in 2010 (a) and 2012 (b). The error bars represent the 95% confidence intervals.

Table 4
Amounts of microbial biomass (MB), cold-water (CWC) and hot-water (HWC) carbon in the three treatments (residue export [RE], farmyard manure addition [FYM] and residue restitution [RR]) in 2012. All values are expressed in mg C (kg dry soil)$^{-1}$. Statistically significant differences between treatments (letters a and b) were determined with one-way ANOVA (factor = treatment, $n = 6$). The values between brackets represent the 90% confidence intervals.

	MB	CWC	HWC
RE	91.3 (62.9; 119.7) a	46.62 (23.8; 69.4) a	165.1 (152.1; 178.11) a
FYM	144.3 (115.9; 172.7) b	48.09 (25.3; 70.9) a	224.1 (211.1; 237.11) b
RR	155.0 (126.6; 183.5) b	45.24 (22.5; 68.0) a	206.6 (193.6; 219.6) b

For individual sampling dates, HR_s was not significantly affected by the treatment in 2010 (one-way ANOVA, $p > 0.1$) except for one date (15 April 2010, $p = 0.0859$). In 2012, there was a treatment impact (one-way ANOVA, $p < 0.10$) in 7 out of 19 cases (9 March, 12 March, 19 March, 2 April, 5 April, 25 May and 30 May, therefore in 36.8% of the measurement dates in 2012) with RR providing the highest HR_s for 5 of these 7 dates.

In the complementary measurement campaign in 2011, recent farmyard manure addition did not affect HR significantly, whatever the measurement date (one-way ANOVA, $p > 0.05$ at all dates).

In terms of spatial variability, the coefficients of variation were 40.2, 37.5 and 40.5% (about 0.5 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) in the RE, FYM and RR treatments, respectively, in 2010, and 56.6, 50.6 and 52.2% (about 0.4–0.5 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) in the RE, FYM and RR treatments, respectively, in 2012. These high values, demonstrating high spatial variability in the HR measurements, can partly explain the difficulties in finding out differences in HR between the studied treatments.

3.3. Residue management effect on microbial biomass, labile carbon and metabolic diversity

There was no significant difference between the three treatments for CWC (one-way ANOVA, $p > 0.1$, Table 4). HWC and MB were significantly lower in the RE treatment, compared to FYM and RR (one-way ANOVA, $p < 0.1$, Table 4).

Residue management had no significant impact on the CLPP (Table 5); there is no significant difference between the scores of PC1 and PC2 among treatments. PC1 and PC2 are mostly built from the "amines" and from the "carbohydrates" and the "carbonic acids" variables, respectively (Table 6). The distribution of the points in the biplot graphic (Fig. 7) (i) does not show any specific substrate use pattern for any treatment and (ii) indicates that the

Table 5
Results of the two-way ANOVA ($n = 6$) performed on the scores of the first and second principal components in each treatment replicate (SS = sum of squares, df = degrees of freedom, MS = mean square).

	SS	df	MS	*F*	$p > F$
Principal components	0	1	0	0	1
Treatments	25.70	2	12.85	0.02	0.98
Interaction	355.60	2	177.82	0.29	0.75
Error	18381.50	30	612.717		
Total	18762.90				

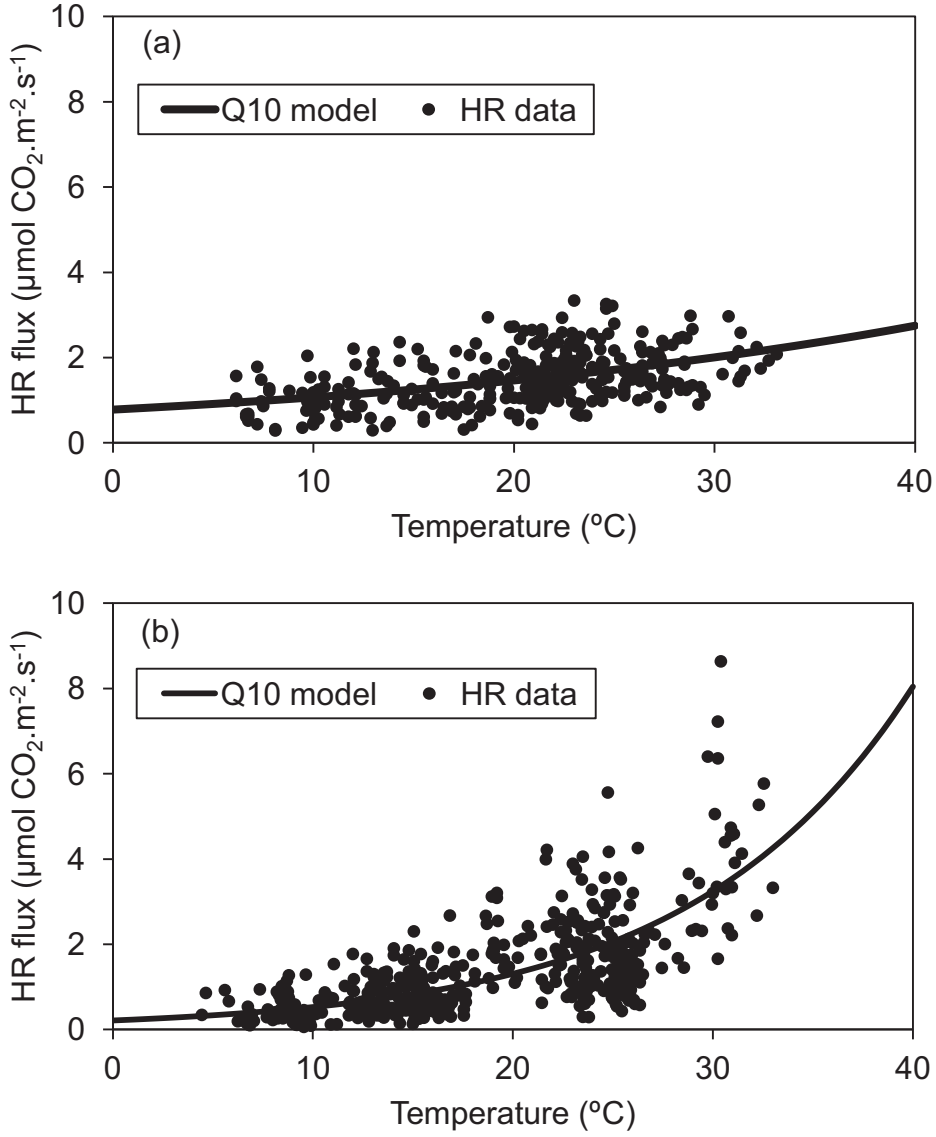

Fig. 4. Temporal evolution of daily averaged heterotrophic respiration in the FYM treatment, with manure input (closed black dots) or without manure input (open black dots) during the complementary measurement campaign in 2011.

substrate use variability within each treatment appears larger than the one potentially existing between treatments. It has to be noted that some treatment replicates had the same scores than others (RE1 = RR3 and FYM4 = RE2 = RR1).

4. Discussion

4.1. HR response to soil temperature and soil water content

Average HR measured in the RE, FYM and RR treatments during the 2010 and 2012 campaigns varied between 0.09 and 3.28 µmol CO_2 m^{-2} s^{-1}, which is slightly higher than HR measured in bare plots established in an agricultural field at the nearby LTO (0.4–2.6 µmol CO_2 m^{-2} s^{-1}, Suleau et al., 2011). Our measurements are within the lower range of soil CO_2 flux measurements performed on bare plots established in agricultural fields of comparable soil texture in Denmark (1.26–5.37 µmol CO_2 m^{-2} s^{-1}, Vinther et al., 2004) and in Japan (0.002–5.42 µmol CO_2 m^{-2} s^{-1}, Mu et al., 2008). Lower HR at the Longs Tours site than at these two sites could be explained by the fact that the flux measurements started earlier in the season (early March) at our site, a period characterized by lower temperatures. Also, differences in HR for similar high temperatures (about 30 °C) between these different studies can result from spatial variability of soil water content, substrate availability and/or soil structure. Maximum HR fluxes up to 5.22 µmol CO_2 m^{-2} s^{-1} at the beginning of the complementary campaign of 2011 in this study might be due to the release of extra carbon through the rupture of soil aggregates during a tillage operation, two weeks before start of the measurements. Temperature (20.0 °C on 25 August 2011, the first date of the measurement campaign) could indeed not explain this much larger HR flux if we consider our Q_{10}-model applications that give an approximate flux of 1.5 µmol CO_2 m^{-2} s^{-1} for a temperature of 20 °C.

Table 6
Loadings on the two retained principal components of substrate utilization for the studied treatments.

	Rotated loadings	
Substrates	PC1	PC2
Amino-acids	0	0
Carbohydrates	0.05	0.52
Carbonic acids	0.01	0.83
Amines	0.99	−0.05
Polymers	0.06	0.18

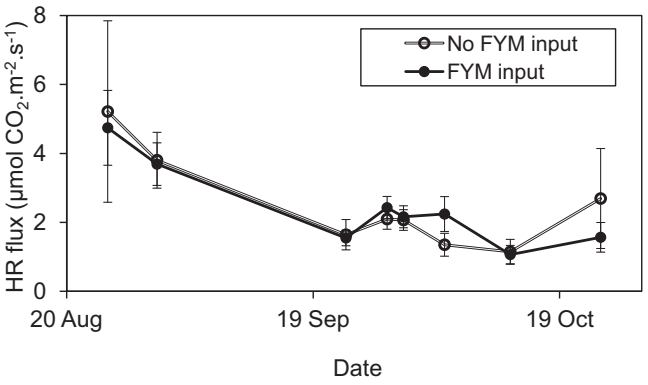

Fig. 5. Relationships between heterotrophic respiration and soil temperature across all treatments (residue export [RE], farmyard manure addition [FYM], residue restitution [RR]) investigated using data from the 2010 and (a) 2012 (b) measurement campaigns.

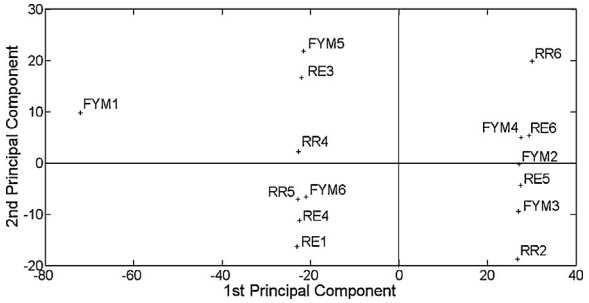

Fig. 7. Biplot of the principal component analysis of substrate utilization patterns using BIOLOG™ ecoplate microplates for the six repetition plots (1–6) in each of the three treatments (residue export [RE], farmyard manure addition [FYM] and residue restitution [RR]) in 2012.

In both 2010 and 2012 measurement campaigns, HR response to temperature was positive, but the parameters of the applied Q_{10} model differed between the two years. At the highest temperatures, HR was higher in 2012 than in 2010, but the flux variability was also larger in 2012. The basal respiration rate (HR_{15}) was higher and the temperature sensitivity (Q_{10}) lower in 2010 than in 2012. The Q_{10} values observed in 2010 (1.37 ± 0.10) and 2012 (2.48 ± 0.23) were at the lower and higher extremes, respectively, of those usually reported in the literature for cropland soils (Davidson and Janssens, 2006; Kätterer et al., 1998). These values, however, need to be interpreted jointly with the HR_{15} values. The higher basal HR rates in 2010 could be explained by the decomposition of fine roots after glyphosate application in March 2010, coinciding with the period of low temperatures. In 2012, manual weeding and whole plant removal did not supply extra substrate for HR. This does not, however, influence the comparison between treatments within each measurement campaign, as these techniques were similarly applied in all three treatments. The important difference between 2010 and 2012 in terms of HR flux response to temperature could also simply be due to the nature, timing and amounts of previous crop residues brought to the soil (Table 1).

Our hypothesis that temperature sensitivity was the same in the three treatments can be considered as relevant because when we compared temperature sensitivity between treatments within each measurement campaign (data not shown), we observed that the inter-annual differences were much larger than the differences between treatments (that were actually not detected).

The very limited HR flux response to SWC we observed in this study can be explained (i) by the fact that SWC was measured close to the bare soil surface (0–5 cm depth), possibly leading to mismatches in SWC range and HR range, the latter probably resulting from microbial activity over the first 25–30 cm depth and (ii) to a lesser extent by the nature of the precipitation regime that occurs at the measurement site, which was regular and induced neither drought nor flooding in the field, SWC therefore remaining between wilting point and field capacity, a range where SWC is not always seen to have an important influence on HR (Luo and Zhou, 2006). This matter is however controversed, as several studies (e.g. Curtin et al., 2012) show a significant impact of SWC on HR in that soil moisture range.

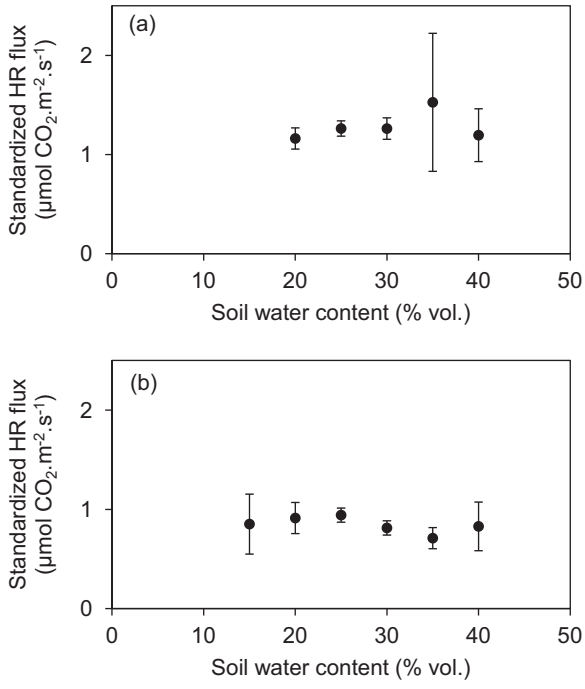

Fig. 6. Temperature-standardized heterotrophic respiration for different soil water content (SWC) classes in the treatments (residue export [RE], farmyard manure addition [FYM], residue restitution [RR] and with/without FYM input) investigated in the 2010 (a) and 2012 (b) measurement campaigns.

4.2. Limited impact of residue management on HR

Assuming that the total SOC content had reached an equilibrium in each of the three treatments 50 years after the start of the long-term experiment (Buysse et al., 2013) and considering the differences of above-ground C inputs between the three treatments, higher HR could have been expected in the FYM and RR treatments than in the RE treatment. Some authors indeed found a direct relationship between soil substrate quantity and HR (Jacinthe et al., 2002; Liu et al., 2006; Vinther et al., 2004). This study showed that the treatments had a limited impact on HR. On the basis of the daily averaged fluxes, a significant ($\alpha = 10\%$) treatment impact on HR was only observed in 2012 but not in 2010. The treatment impact in 2012 was however less clear when analyzed on the basis of the individual measurement dates as the RR treatment caused the highest fluxes in only about 30% of the measurement dates. In addition, the complementary measurement campaign performed on amended/non-amended plots in the FYM treatment did not show any impact of recent farm yard manure input on HR. All these results therefore indicate that there is no simple and obvious relationship between the amounts of residue/manure input to the soil and HR, as also observed by Duiker and Lal (2000) and Mu et al. (2008).

Our observations of absence of marked HR difference between the three treatments raise the question on the fate of the C added to the soil. While larger inputs were brought under the FYM and RR treatments than under RE, soil C loss through HR was similar in the three treatments. This suggests that, if the soil C stocks were really at equilibrium, as suggested by recent SOC stock measurements (Buysse et al., 2013), organic carbon might be lost by the soil through other processes than HR. Our plots were however situated in the same field and soil erosion and leaching processes would have occurred in the same way in all plots. It is possible that crop residue amounts added to the soil at this site are insufficient to induce measurable HR flux differences between treatments.

In addition, the evaluation of the annual SOC budget in the three treatments in a previous study (Buysse et al., 2013) showed that the expected mean HR difference ($0.4\ \mu mol\ CO_2\ m^{-2}\ s^{-1}$) between treatments was small, due to the input of non-exportable residues (roots, small straw, weeds) in the three treatments. This value was calculated considering that the soil is at steady state with known soil C stocks and annual C input amounts (Buysse et al., 2013). In these conditions, the few observed differences between treatments in this study could simply be the result of the observed HR spatial variability. HR is indeed highly influenced by soil CO_2 diffusivity, soil structure, porosity, drainage, faunal activity and wind effects; these influences probably varying in both space and time over the season. Increasing the number of measurement points would constitute a first step to better integrate flux spatial variability.

Our observations agree with the results reported by Duiker and Lal (2000), in which treatments with increasing levels of above-ground crop residues returned to the soil (from 0 up to $670\ gC\ m^{-2}\ year^{-1}$) established on a silt loam soil in Ohio were compared after 8 years of experiment, showing no significant differences in HR between treatments. They attributed this to the presence of undecomposed residue probably not contributing to CO_2 efflux, and to sampling spatial variability. These two explanations can also be considered at our site. In addition, our observation regarding no recent manure input impact on HR agrees with a similar study by Rochette et al. (2004) reporting that pig slurry application was followed by a large peak in CO_2 emission 6 h after its application, but that there was no measurable effect a month later.

Our results, however, contradict several studies reporting increased microbial activity after manure or crop residue input to agricultural fields established on silt or sandy loams (Chirinda et al., 2010; Jacinthe et al., 2002). But in these studies, conducted under similar climatic conditions as those in our study, the difference between residue input amounts between treatments was much larger than in our study. Firstly, above-ground crop C residues were up to four times higher in Jacinthe et al. (2002) and about twice as large in Chirinda et al. (2010) compared to our study. Secondly, no crops were grown on the plots investigated in Jacinthe et al. (2002) and therefore no below-ground residue input was provided to the RE plots, in contrast to our study. This suggests that the potential differences between treatments, in terms of available substrate, were lower in our experiment.

4.3. Linking HR to SOC stocks indicates short- and long-term responses to residue management

The amounts of MB, CWC and HWC measured in the three treatments were in the same range as the values reported in the study by Uchida et al. (2012), also performed on agricultural soils. MB, HWC (both in this study) and total SOC (Buysse et al., 2013) were significantly affected by residue management at the Longs Tours site. Lower values of these three variables were observed in the RE treatment, which can be linked to the lower above-ground crop residue C amounts entering the soil in that treatment compared to the FYM and RR treatments. Differences between the FYM and RR treatments 51 years after the start of the long-term experiment were only observed with respect to total SOC, being significantly higher in the FYM than in the RR treatment (Buysse et al., 2013). No significant differences in terms of CWC were reported in the present study. These results suggest that long-term residue management mainly affects the amounts of refractory carbon and total SOC through slow processes. Refractory carbon (e.g. lignin compounds) is found largely in roots (Kätterer et al., 2011) and also derives from above-ground crop residue decomposition, with farm yard manure being more recalcitrant to microbial degradation than fresh crop residues. Decomposition dynamics are likely to be driven by residue input quantity and quality, potentially leading to different amounts of refractory carbon between the treatments. The proportions of refractory SOC were not evaluated in this study, but this would constitute an interesting perspective.

In our study, CWC was not significantly affected by residue management and was not related to total SOC in each treatment. Uchida et al. (2012) reported similar observations when comparing two soil types with significantly different SOC contents. The absence of significant CWC differences between treatments, conjugated to the very short CWC turn-over in soils (3 days, Jandl and Sollins, 1997), could suggest that readily available soil carbon was probably limiting. The concurrent absences of marked CWC and HR differences between the three treatments in the present study, considering that MB can change rapidly with substrate availability, suggest that HR is linked mainly to readily available substrate and recent inputs, which agrees with the findings reported by Schimel et al. (1994) and Taneva et al. (2006). According to Buysse et al. (2013) and as shown in Table 1, similar amounts of above-ground crop residues (rather available substrate) were left on the ground after harvest because they could not be totally exported (=the so-called "left-over residues"). This could then have led to amounts of labile carbon being close enough to each other in the three treatments to reduce CWC differences and, hence, the potential HR differences between treatments.

Metabolic diversity of soil bacteria was not affected by residue management. This could be related to the relatively low amount of crop residues added to the soil, which probably made the differences between treatments too small to be detected (Calbrix et al., 2007). Although Govaerts et al. (2007) showed significant differences 15 years after the initiation of a long-term tillage and residue management trial, comparable metabolic diversity in the three treatments in our study could be due to the longer time elapsed since the beginning of the long-term experiment (50 years). This probably allowed the microbial populations to develop so that similar ecological functions were represented in each treatment. In addition, as the residue management treatments were maintained for 50 years, no recent important stress could possibly induce large changes within the microbial populations.

Microbial and labile C were determined only once during our study in the 2012 season. Given the potentially large temporal variability of labile C content (Uchida et al., 2012), the absolute amounts could depend on the measurement date. Even if the values we measured for MB, CWC and HWC were in good agreement with the study by Uchida et al. (2012), also performed on agricultural soils, and if HWC and MB could be directly linked to average above-ground residue inputs added to the soil, it would therefore be desirable to repeat the microbial and labile C measurements several times over the season.

5. Conclusion

This study evaluated the impacts on heterotrophic respiration (HR) of residue export (RE), farm yard manure (FYM) amendment

and residue restitution (RR) in a long-term (50 years) experiment. Residue management had a limited impact on HR, metabolic diversity of soil bacteria and soil cold-water extracted carbon. Spatial variability, relatively low amounts of above-ground crop inputs and a large proportion of below-ground residues, common to the three treatments, might have reduced the potential differences between treatments and contributed to minimize HR differences between treatments. Spatial variability could be further reduced by increasing the number of sampling points.

The small differences in carbon losses due to heterotrophic respiration between the RE, FYM and RR treatments in the studied soil contrast with the differences in total soil organic carbon content observed 50 years after the start of the experiment (Buysse et al., 2013) and with the differences in microbial biomass and hot-water carbon observed during the 2012 crop season. These results suggest that both short- and long-term processes are likely to occur concurrently in response to residue management. HR rates would be determined mainly by short-term processes related to readily available carbon, whereas SOC stocks would be influenced by long-term processes, leading to crop residues evolving into stabilized organic matter forms. These hypotheses could be further investigated by multiplying the microbial biomass and labile C measurements over the season, and increasing spatial and temporal frequencies of HR measurements and/or determining the proportions of stabilized carbon in the three treatments.

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