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Changes in soil carbon stocks under perennial and annual bioenergy crops

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Abstract

Bioenergy crops are expected to provide biomass to replace fossil resources and reduce greenhouse gas emissions. In this context, changes in soil organic carbon (SOC) stocks are of primary importance. The aim of this study was to measure changes in SOC stocks in bioenergy cropping systems comparing perennial (*Miscanthus* × *giganteus* and switchgrass), semi-perennial (fescue and alfalfa), and annual (sorghum and triticale) crops, all established after arable crops. The soil was sampled at the start of the experiment and 5 or 6 years later. SOC stocks were calculated at equivalent soil mass, and $\delta^{13}C$ measurements were used to calculate changes in new and old SOC stocks. Crop residues found in soil at the time of SOC measurements represented 3.5–7.2 t C ha^{-1} under perennial crops vs. 0.1–0.6 t C ha^{-1} for the other crops. During the 5-year period, SOC concentrations under perennial crops increased in the surface layer (0–5 cm) and slightly declined in the lower layers. Changes in $\delta^{13}C$ showed that C inputs were mainly located in the 0–18 cm layer. In contrast, SOC concentrations increased over time under semi-perennial crops throughout the old ploughed layer (ca. 0–33 cm). SOC stocks in the old ploughed layer increased significantly over time under semi-perennials with a mean increase of 0.93 ± 0.28 t C ha^{-1} yr^{-1}, whereas no change occurred under perennial or annual crops. New SOC accumulation was higher for semi-perennial than for perennial crops (1.50 vs. 0.58 t C ha^{-1} yr^{-1}, respectively), indicating that the SOC change was due to a variation in C input rather than a change in mineralization rate. Nitrogen fertilization rate had no significant effect on SOC stocks. This study highlights the interest of comparing SOC changes over time for various cropping systems.

Keywords: ^{13}C abundance, bioenergy crops, carbon sequestration, isotope, *Miscanthus*, SOC, soil organic carbon, switchgrass

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Introduction

Biomass can contribute to the energy transition towards low-carbon economies in response to the challenges of climate change and depletion of fossil resources (IPCC, 2011). The use of dedicated bioenergy crops is therefore expected to increase significantly (Chum *et al.*, 2011; Bentsen & Felby, 2012). The development of new conversion technologies and biorefineries allows considering a wide range of candidate crops (Ragauskas *et al.*, 2006; Somerville *et al.*, 2010). However, these crops will have to fulfil several requirements, including high productivity, low greenhouse gas (GHG) emissions, and low environmental impacts (Tilman *et al.*, 2009; Karp & Richter, 2011). Different crop types such as short rotation coppices, perennial grasses, semi-perennial forage, and annual crops are being investigated (Lewandowski *et al.*, 2003; Karp & Shield, 2008; Sanderson & Adler, 2008; Zegada-Lizarazu & Monti, 2011; Van Der Weijde *et al.*, 2013). Among them, perennial C4 crops such as *Miscanthus* and switchgrass are viewed as promising bioenergy crops because of their high biomass production, low nutrient requirements, and low GHG emissions (Don *et al.*, 2011; Cadoux *et al.*, 2014).

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Among the environmental impacts of bioenergy crops, changes in soil organic carbon (SOC) stocks are of particular interest because they result in either carbon dioxide emissions or sequestration. Variation in SOC stock is a key term when calculating the GHG balance in bioenergy production (Don *et al.*, 2011). SOC changes occur as a result of modifications in land use, crop type, and management practices, yielding a new equilibrium. Therefore, changes in SOC stocks due to bioenergy crops will depend not only on crop type and management, but also on the former land-use history. The conversion of forest or grassland to annual bioenergy crops leads to high SOC losses, creating a carbon debt that for several decades negates any reduction in GHG emissions as a result of the move away from fossil fuels to biofuel (Fargione *et al.*, 2008). On arable land, it is generally considered that SOC stocks decrease when crop residues are harvested rather than returned to the soil (Saffih-Hdadi & Mary, 2008; Powlson *et al.*, 2011). The same consequences can be expected with the introduction in crop successions of annual bioenergy crops, the whole aboveground biomass of which is harvested. In contrast, the conversion of arable land to grassland generally increases SOC stocks (Post & Kwon, 2000; Conant *et al*., 2001; Soussana *et al*., 2004). This increase can be explained by higher belowground inputs under grassland through root turnover and rhizodeposition which favour C storage, and slower SOC mineralization due to the absence of soil tillage (Soussana *et al*., 2004) although the actual effect of soil tillage on SOC stocks is questioned (Powlson *et al*., 2014). Grassland management (mowing, grazing intensity, nitrogen fertilization) is known to affect SOC balance (Conant *et al*., 2001; Soussana *et al*., 2004, 2007), but SOC stock changes under forage crops such as fescue or alfalfa managed for bioenergy production have never been investigated. It is expected that the shift from annual cropping systems to perennial grasses such as *Miscanthus* or switchgrass will increase SOC stocks for three reasons: (i) these crops allocate large amounts of C in belowground organs, either rhizomes (Garten *et al*., 2010; Strullu *et al*., 2011) or roots (Neukirchen *et al*., 1999; Ma *et al*., 2000), (ii) significant losses of aboveground biomass prior to harvest have been recorded when crops are harvested in winter, particularly for *Miscanthus* (Amougou *et al*., 2012), and (iii) SOC mineralization might be reduced by the absence of soil tillage (Anderson-Teixeira *et al*., 2013). Although there is an increasing body of work concerning the effect of perennial bioenergy crops on SOC stocks, C sequestration remains very uncertain as shown by the wide variability in experimental results (Anderson-Teixeira *et al*., 2009; Don *et al*., 2011). In their review, Poeplau & Don (2014) reported SOC change rates under *Miscanthus* established on arable land ranging from −6.85 to +7.70 t ha^{-1} yr^{-1}. They could not identify possible explanatory variables (age of the crop, mean temperature, etc.). Furthermore, very few studies have analysed the effects on SOC stocks of management practices of perennial crops, such as nitrogen fertilization or harvest management (e.g. Follett *et al*., 2012).

Some of the uncertainties regarding the effect of bioenergy crops on SOC stocks are probably due to methodological difficulties in measuring SOC stock changes. First of all, most of the published studies use a synchronic approach with paired plots: the soil in which a bioenergy crop has grown is sampled once, simultaneously with an adjacent reference plot. This approach can create a significant bias if the initial soil conditions are heterogeneous among the two plots. Using ^{13}C abundance, Poeplau & Don (2014) showed that the high variability reported in SOC stock changes after *Miscanthus* plantation is probably due to this methodological bias. Secondly, the large spatial heterogeneity of perennial crops such as *Miscanthus* makes it difficult to obtain representative soil samples (Zatta *et al*., 2014). Thirdly, SOC stocks are most often calculated at an equal soil depth between treatments rather than at an equal soil mass, with a few exceptions such as Schmer *et al*. (2011). This may lead to bias in assessing SOC sequestration (Lee *et al*., 2009). Finally, SOC stock changes are likely to vary with time. There is a need for long-term synchronic studies following SOC stock evolution over time and an opportunity to use ^{13}C signatures during the transition between C3 and C4 crops in order to quantify the contribution of 'new' organic carbon to SOC stock changes.

In this study, we aimed at (i) comparing changes in SOC stocks of various bioenergy cropping systems with perennial, semi-perennial, or annual crops established on arable land, and (ii) studying the interaction with crop management, that is nitrogen fertilization rate and harvest date of perennial crops. We analysed the first 6 years of an experiment which began in 2006 (Cadoux *et al*., 2014). The originality of our approach consisted in comparing bioenergy crops at the same site, combining (i) a diachronic approach with the initial spatial variability fully characterized, (ii) a calculation of SOC stocks on an equivalent soil mass (ESM) basis, and (iii) the use of ^{13}C abundance to distinguish 'new' and 'old' SOC stock changes.

Materials and methods

Study site and experimental design

The study is based on an ongoing long-term experiment established at the INRA experimental station in Estrées-Mons, northern France (49.872°N, 3.013°E) called 'Biomass & Environment' (B&E). The soil is a Haplic Luvisol (IUSS Working Group WRB, 2006). Soil characteristics are given in Table S1. Over the period 2006–2011, the mean annual temperature was 10.6 °C and annual rainfall and potential evapotranspiration were 673 and 737 mm, respectively. Before 2006, the field had been cultivated for many years with annual crops, winter wheat (*Triticum aestivum*) being the most common crop. The soil was mouldboard ploughed annually, straw was not harvested, and there was no organic fertilization.

The B&E experiment was initiated to study biomass production and the environmental impacts of a wide range of bioenergy crops. It compares eight 'rotations': four with C4 perennial crops (monocultures), two with C3 semi-perennial forage crops, and two with C3/C4 annual crops (Table 1). The perennial crops are *Miscanthus* (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* cv. Kanlow). They are harvested either early in October (E) or late in February (L). The semi-perennial crops are fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*). Annual crops are fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (*Triticosecale* Wittmack). The experiment also includes two

Table 1 Treatments of the B&E long-term experiment combining rotation and fertilizer-N rate [Mis = *Miscanthus*, Swi = switchgrass, Fes = fescue, Alf = alfalfa, Sor = fibre sorghum, Tri = triticale, CC = catch crop, E = early harvest (October), L = late harvest (February), and n.h. = not harvested]

		Crop and fertilizer-N rate (kg ha^{-1})						
Rotation	N rate	2006	2007	2008	2009	2010	2011	2012
Mis E		Mis n.h.	Mis E	Mis E	Mis E	Mis E	Mis E	Mis E
	N−	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Mis L		Mis n.h.	Mis L	Mis L	Mis L	Mis L	Mis L	Mis L
	N−	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Swi E		Swi n.h.	Swi E	Swi E	Swi E	Swi E	Swi E	Swi E
	N−	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Swi L		Swi n.h.	Swi L	Swi L	Swi L	Swi L	Swi L	Swi L
	N−	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Fes-Alf		CC/Fes	Fes	Fes	Luz	Luz	Fes	Fes
	N−	0	120	80	0	0	0	120
	N+	0	240	160	0	0	0	240
Alf-Fes		Alf	Alf	Alf	Fes	Fes	Alf	Alf
	N−	0	0	0	40	120	0	0
	N+	0	0	0	80	240	0	0
Sor-Tri*		CC	Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC
	N−	0	0	60	0	60	0	60
	N+	0	120	120	120	120	120	120
Tri-Sor*		Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC	Sor
	N−	0	60	0	60	0	60	0
	N+	0	120	120	120	120	120	120

*Rotations with catch crops (oat in 2006, rye in 2007, mustard in 2008, oat-vetch mixture in 2009, and mustard-clover mixture from 2010 to 2012) which were sown every year in late August or early September after triticale.

nitrogen treatments (N− and N+) with fertilizer-N rates depending on the crops (Table 1).

The 2.7 ha field was divided into two parts to facilitate cultural operations and limit competition between plants due to differences in canopy height (Fig. S1): (i) a split-block design in the west part for perennial crops with rotations in the main plots (*Miscanthus* E, *Miscanthus* L, switchgrass E, switchgrass L) and N fertilization rates in the subplots (N− and N+), and (ii) a split-plot design in the east part for the other crops with rotations in the main plots (fescue-alfalfa, alfalfa-fescue, sorghum-triticale and triticale-sorghum) and N fertilization rates in the subplots (N− and N+). Both parts include three replicate blocks and 24 subplots of 360 m^2. Soil analyses performed in 2006 revealed a slightly higher clay content in the west than in the east part (180 ± 27 vs. 148 ± 19 g kg^{-1} in the 0–30 cm layer).

At the start of the experiment, the field was mouldboard ploughed at a depth of ca. 25 cm. After seedbed preparation with a cultivator, *Miscanthus* was planted in April 2006 (1.5 rhizome m^{-2}) and switchgrass sown in June 2006 (seed rate = 15 kg ha^{-1}). Semi-perennial crops were sown in 2006, 2009, and 2011, usually in April. Before sowing, the previous crop (alfalfa or fescue) was destroyed in late autumn with a cultivator and a disc harrow (15 cm deep) in 2009 and mouldboard ploughed (ca. 22 cm deep) in 2011. Annual crops were cultivated under superficial tillage (12–15 cm deep) with a cultivator and a disc harrow. Fescue and alfalfa were harvested in two or three cuttings depending on years, with the last cut in October. Sorghum was harvested in late September and triticale in late July or early August. Further details about crop management are given by Cadoux *et al.* (2014).

Crop yields

Crop yields were measured every year from 2006 to 2011. On each harvest date, the aboveground biomass was collected manually, weighed, dried, and ground before C content analysis. Details about sampling methodologies and analysis are given by Cadoux *et al.* (2014).

Soil sampling and analysis

The soil was sampled on two dates: May 2006 for the whole experiment, March 2011 for the perennial crops (west part of the field trial), and March 2012 for the other crops (east part of the field trial). Soil cores of 8 cm diameter were extracted with depth increments of 20 cm and inserted into plastic tubes using a powered soil corer (Humax soil sampler, Switzerland). In

2006, two soil cores were taken in each plot (one north and one south of the plot) down to 40 cm depth. In 2011 and 2012, six soil cores were taken in each plot down to 60 cm. All cores were located north of the plots inside a 2.6-m^2 micro-plot and taken in intrarow and inter-row zones. A specific coring strategy was developed for *Miscanthus* to ensure a fully representative sampling scheme (Fig. S2). In each plot, two soil cores were taken in the rhizome area and four outside this area, which corresponded to the estimated fraction of the total field area covered by plant rhizomes (ca. 33%).

From 2005, the ploughing depth was reduced from ca. 30–35 cm to <25 cm in all treatments. The old ploughing depth (referred to below as Y) was identified in the soil cores on each sampling date by detecting changes in soil colour and structure. Soil cores removed from the plastic tubes in the laboratory were divided into three layers (0–20, 20–Y and Y–40 cm) in 2006 and into five layers (0–5, 5–20, 20–Y, Y–40 and 40–60 cm) in 2011 and 2012. Coarse residues (>2 mm), roots, and rhizomes were then carefully removed from the soil by handpicking. The very fine roots could not be removed because it was a very time-consuming operation, especially for perennial crops, which showed a large amount of roots in the cores. However, we could estimate that the C contained in very fine roots represented only 0.04 g C kg^{-1} soil on average and <0.20 g C kg^{-1} in all samples. Soil samples were dried at 38 °C for 96 h, crushed through a 2-mm sieve, subsampled, and finely ground with a ball mill (PM 400, Retsch, Germany) before carbon analysis. 1368 soil samples were analysed for carbon concentration and ^{13}C abundance using an elemental analyser (EURO EA, Eurovector, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta Plus Advantage, Thermo Electron, Germany).

Bulk densities were determined at each sampling date by two methods. The first was used for the 0–5 cm layer and consisted in pushing a steel cylinder (98 cm^3) into the soil and weighing the sample after oven drying for 48 h at 105 °C. The second method consisted of using a dual gamma probe (LPC-INRA, Angers, France) on the 5–10, 10–15, 15–20, 20–25, 25–30, 30–35, and 35–40 cm layers (see for example Pires & Pereira, 2014). In 2006, initial bulk densities were measured in six areas spread over the whole field with five replicates. The old ploughing depth was measured at the same locations in soil trenches of 3 m length with 30 measurements per trench. In 2011 and 2012, bulk densities were measured in each micro-plot used for soil sampling, with four replicates for the 0–5 cm layer and two replicates for the other layers. For the 40–60 cm layer, we assumed that bulk density did not vary with time and used measurements that were taken on six trench walls in 2007 with steel cylinders.

Crop residues and belowground biomass

Crop residues (>2 mm) remaining in soil were measured in 2011–2012 at the time of SOC measurements and in the same micro-plots. In 2011, residues present at soil surface after harvest were collected just before soil sampling. Stem bases and fragments (>10 mm) as well as fallen leaves (mulch) of *Miscanthus* L were sampled in one micro-plot per plot. Small stem fragments (2–10 mm) and leaf debris (for *Miscanthus* L) present at soil surface were collected in six areas of 27 × 27 cm within each micro-plot, corresponding to the location of the soil cores. Stem fragments below soil surface (>2 mm) were collected in the 8 cm diameter cores. Residues from the six areas were pooled together, as well as residues from the six soil cores. The residues from semi-perennial and annual crops, buried by soil tillage, were collected in the soil cores in 2012. All residues were dried at 65 °C for 96 h, weighed, and ground before analysis.

Belowground biomass (rhizomes and roots) of perennial crops was also measured in 2011. Rhizomes and roots collected in the soil cores were separated, weighed, and washed. Samples from the six cores of each micro-plot and each layer were pooled, dried at 65 °C for 96 h, weighed, and ground before analysis. For *Miscanthus*, given the very large spatial variability of the rhizome biomass, a second method was used to quantify it more precisely. It consisted in extracting the entire rhizome of a median plant per plot, selected by the number of stems [see Strullu *et al.* (2011) for details]. The C concentration of residues and belowground samples was determined using an elemental analyser (FLASH EA 1112 series, Thermo Electron).

Calculation of soil mass, SOC stocks, and $\delta^{13}C$

SOC stocks were calculated on an ESM basis (Ellert & Bettany, 1995). Soil mass in the 0–z layer was calculated as follows:

$$M(z) = 10 \sum_{j=1}^{z} \rho\,(j) \tag{1}$$

where $M(z)$ is the mass of dry soil (t ha^{-1}), j the soil depth (mm), z the calculation depth (mm), and $\rho(j)$ the bulk density (g cm^{-3}) at depth j.

The bulk density ρ, the depth of the old ploughed layer Y, and the soil mass over the depth 0–Y measured in 2006 did not differ significantly between the west and east parts of the experiment. Their mean values were, respectively, $\rho = 1.37 \pm 0.05$ g cm^{-3}, $Y = 342 \pm 17$ mm, and $M_R = 4669 \pm 135$ t ha^{-1}. The latter value is called 'reference' soil mass. Assuming that there was no erosion (due to the very slight slope and moderate rainfall), the soil mass over the depth 0–Y should remain constant in time and equal to M_R.

In 2011 and 2012, Y was identified in the soil cores to divide the layer 20–40 cm into 20–Y and Y–40, but a more precise estimate of Y was made using bulk density measurements: M was calculated from 0 to 60 cm depth by 1 mm increments using Eqn (1), and Y was determined as the depth at which M equalled M_R.

Five soil layers (0–5, 5–20, 20–Y, Y–40, and 40–60 cm) were analysed separately. The cumulative SOC stock (t ha^{-1}) measured down to the layer n (n = 1–5) is as follows:

$$\mathrm{SOC_m}(n) = 0.001 \sum_{i=1}^{n} M(i) \times C_m(i) \tag{2}$$

where n is the soil layer, $M(i)$ the soil mass (t ha^{-1}) and $C_m(i)$ the SOC concentration measured in layer i (g kg^{-1} dry soil).

The ^{13}C signature of SOC was expressed as $\delta^{13}C$ (‰) relative to the international PDB (Pee Dee Belemnite) standard according to the equation:

$$\delta^{13}C_m(n) = 1000\left(\frac{R(n)}{R_{PDB}} - 1\right) \quad (3)$$

where $R(n)$ is the $^{13}C/^{12}C$ ratio measured in layer n, and R_{PDB} is the $^{13}C/^{12}C$ ratio of the PDB standard. The mean weighted $\delta^{13}C$ of the SOC measured down to the layer n was calculated as follows:

$$\delta^{13}C_{mw}(n) = \frac{0.001}{SOC_m(n)} \sum_{i=1}^{n} M(i) \times C_m(i) \times \delta^{13}C_m(i) \quad (4)$$

where $\delta^{13}C_m(i)$ is the $\delta^{13}C$ measured in layer i (‰).

In 2006, SOC concentrations and $\delta^{13}C$ in each layer were measured at 96 soil sample points spread across the experimental field. This sampling strategy allowed to estimate these variables in the whole field taking a geostatistical approach, using the *gstat* package in R (Pebesma, 2004). A spherical semivariogram model was fitted for each variable and soil layer. Spatial interpolation was achieved on a grid of 4-m^2 cells using ordinary kriging and its quality evaluated by the cross-validation method. The data showed a clear spatial structure, and cross-validation provided good results with a root mean square error of 0.61 g kg^{-1} for SOC concentration and 0.4‰ for $\delta^{13}C$ in the 0–Y layer. Predicted values at the sampling sites in 2011 and 2012 were used rather than the average measured values to calculate initial SOC stocks and $\delta^{13}C$ of the different treatments.

In 2011 and 2012, the SOC concentration in each plot was calculated as the mean of the SOC concentrations of the different soil samples for a given layer. The $\delta^{13}C$ was also calculated as the mean of the different $\delta^{13}C$ analyses weighted by the SOC concentration. For *Miscanthus* and switchgrass, the large belowground biomass found in some soil cores for the 0–5 and 5–20 cm layers resulted in a variable soil mass from one core to another. The SOC concentration and $\delta^{13}C$ were therefore weighted by the soil mass of the cores.

Calculations on an ESM basis were performed in all layers (Fig. 1) using the reference soil masses calculated in 2006 (M_R = 667, 2667, 5553 and 8690 t ha^{-1} for 0–5, 0–20, 0–40, and 0–60 cm respectively). SOC stocks on an ESM basis down to layer n were calculated as follows:

$$SOC(n) = SOC_m(n) - 0.001\,(M(n) - M_R) \times C_m(n) \quad \text{if } M(n) \geq M_R, \quad (5)$$

$$SOC(n) = SOC_m(n) + 0.001\,(M_R - M(n)) \times C_m(n+1) \quad \text{if } M(n) < M_R. \quad (6)$$

Similarly, the mean weighted $\delta^{13}C$ down to layer n on an ESM basis was calculated as follows:

$$\delta^{13}C_w(n) = \frac{\delta^{13}C_{mw}(n) \times SOC_m(n) - 0.001\,(M(n) - M_R) \times C_m(n) \times \delta^{13}C_m(n)}{SOC(n)} \quad \text{if } M(n) \geq M_R, \quad (7)$$

$$\delta^{13}C_w(n) = \frac{\delta^{13}C_{mw}(n) \times SOC_m(n) + 0.001\,(M_R - M(n)) \times C_m(n+1) \times \delta^{13}C_m(n+1)}{SOC(n)} \quad \text{if } M(n) < M_R. \quad (8)$$

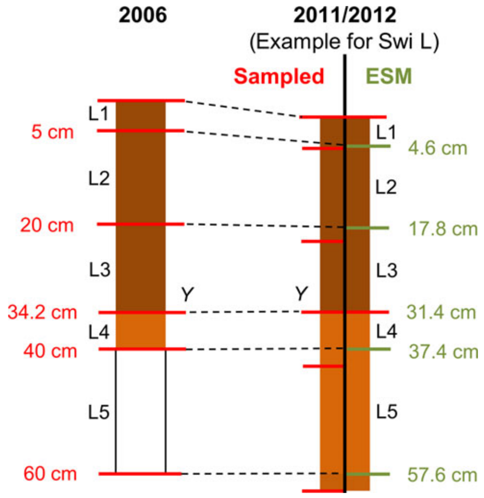

Fig. 1 Soil layers (L) sampled in 2006 and 2011/2012 and used for soil organic carbon stocks calculations on equivalent soil mass (ESM) basis with corresponding soil depths. L5 was not sampled in 2006. L1 = 667 t ha^{-1}, L2 = 2000 t ha^{-1}, L3 = 2002 t ha^{-1}, L4 = 884 t ha^{-1}, and L5 = 3137 t ha^{-1}. Y is the old ploughing depth.

The SOC concentration and $\delta^{13}C$ in each soil layer n on an ESM basis are as follows:

$$C(n) = 1000\,\frac{SOC(n) - SOC(n-1)}{M(n) - M(n-1)} \quad (9)$$

and

$$\delta^{13}C(n) = \frac{SOC(n) \times \delta^{13}C_w(n) - SOC(n-1) \times \delta^{13}C_w(n-1)}{SOC(n) - SOC(n-1)} \quad (10)$$

In the following, SOC concentrations, $\delta^{13}C$, mean weighted $\delta^{13}C$, and SOC stocks are presented on an ESM basis for all depths. Soil layers are called L1 to L5 (corresponding to 0–5, 5–20, 20–Y, Y–40, and 40–60 cm) and pooled soil layers for cumulative SOC stocks are L1-2 to L1-5.

Calculation of new/old SOC stocks

At the start of the experiment, SOC was derived from a mix of C3 (wheat, sugar beet, etc.) and C4 (maize) crops, with a majority of C3 crops. In rotations which included only C4 (perennials) or C3 (semi-perennials) crops since 2006, it was possible to calculate the proportion of the final SOC stock derived from crop residues applied since the start of the experiment, that is the 'new' SOC stock. According to Andriulo *et al.* (1999), the proportion α of new SOC in the total SOC is as follows:

$$\alpha = \frac{\delta - \delta_0}{\delta_1 - \delta_0} \quad (11)$$

where δ is the final $\delta^{13}C$ measured in 2011 or 2012, δ_0 is the initial $\delta^{13}C$ measured in 2006, and δ_1 the $\delta^{13}C$ of the new crop. δ_1 was assessed as the average of all analyses of aboveground and belowground plant organs. The $\delta^{13}C$ values obtained were −12.7‰, −13.0‰, −28.5‰, and −30.2‰ for *Miscanthus*, switchgrass, fescue, and alfalfa, respectively. For semi-perennial crops, a mean value for each rotation was calculated, taking into account the number of years for each crop in the rotation. The change in new SOC (ΔSOC_{New} in t ha^{-1}) was calculated as follows:

$$\Delta SOC_{New} = \alpha \times SOC \tag{12}$$

where SOC is the SOC stock in 2011 or 2012. The change in old SOC (ΔSOC_{Old} in t ha^{-1}) is as follows:

$$\Delta SOC_{old} = SOC - SOC_0 - \Delta SOC_{new} \tag{13}$$

where SOC_0 is the SOC stock in 2006.

Statistical analysis

All statistical analyses were performed using R (R Core Team, 2014). For the harvested C content over the experimental period and the C content in crop residues and belowground biomass in 2011 or 2012, the effects of rotation, nitrogen, and their interaction were evaluated by analysis of variance (ANOVA). ANOVA was also performed to assess the effects of rotation, nitrogen, and their interaction on bulk densities in each layer in 2011 and 2012.

Rotation, nitrogen, and soil layer effects on SOC concentrations and $\delta^{13}C$ were first tested in 2006, 2011, and 2012, and a second ANOVA was performed to evaluate rotation, nitrogen, and year effects in each layer. Similarly, the effects of rotation, nitrogen, and their interaction on SOC stocks and $\delta^{13}C$ signature were assessed each year using a first ANOVA, while year effects were assessed using a second ANOVA. The effects of rotation, nitrogen, and their interaction on the change in new and old SOC stocks were evaluated using a third ANOVA.

Two linear mixed-effect models were used: the first one, adapted to a split-block design (with blocks, rotation × blocks, and nitrogen × blocks interactions as random factors), was used for perennial crops and the second, adapted to a split-plot design (with blocks and rotation × blocks interaction as random factors), was used for the other crops. The *lme* function from the *nlme* package was used to fit the models (Pinheiro *et al.*, 2014). Significant differences ($P < 0.05$) between treatments were found with the *lsmeans* function (Lenth, 2014). The assumptions of ANOVA were checked by visually examining the residuals against predicted values and using the Shapiro–Wilk and Levene's tests. Log-transformed data or Box–Cox transformation were used if necessary to satisfy these assumptions.

Results

Crop yields

The biomass production of the different crops was presented in an earlier paper (Cadoux *et al.*, 2014). The mean harvested biomass was 15.6 t DM ha^{-1} yr^{-1} for perennial crops in 2006–2010 and 9.5 t DM ha^{-1} yr^{-1} for the other crops in 2006–2011; the mean harvested C content was 7.31 and 4.16 t ha^{-1} yr^{-1}, respectively (Table S2). Yields were higher in N+ than in N−, except for *Miscanthus* L.

Crop residues and belowground biomass

The amount of crop residues found in soil at the time of SOC measurements was much higher in perennial than in other crops: 4.74 vs. 0.35 t C ha^{-1} on average, respectively (Table 2). It was significantly affected by rotations, but not by N fertilizer rate. The residues of perennial crops were mainly located at soil surface, and residues below 5 cm depth were negligible. The soil cropped with *Miscanthus* L (harvested in February) contained many more residues (7.20 t C ha^{-1}) than the soils with other perennial crops (3.47–4.32 t C ha^{-1}). This is due to the presence of senescent leaves accumulated in mulch on the soil surface (2.86 t C ha^{-1}), whereas no significant leaf fall had been recorded in fields of switchgrass or *Miscanthus* E.

Perennial belowground organs of *Miscanthus* and switchgrass represented a large C pool in 2011 (Table 3). Total belowground biomass in L1-5 was 21.7 and 15.3 t DM ha^{-1}, corresponding to 9.90 and 6.78 t C ha^{-1} for *Miscanthus* and switchgrass, respectively. The larger part of this C was located in rhizomes for *Miscanthus* and in roots for switchgrass. There was no significant effect of fertilization on the belowground C content. Most of this carbon was located above 20 cm depth (in L1-2): 96% for *Miscanthus* and 79% for switchgrass.

Table 2 Carbon content (t C ha^{-1}) in crop residues found at soil surface or in soil layers in 2011 for perennial crops and in 2012 for semi-perennial and annual crops (mean of treatments N− and N+) (see Table 1 and Fig. 1 for abbreviations)

	Mis E	Mis L	Swi E	Swi L	Fes-Alf	Alf-Fes	Sor-Tri	Tri-Sor
Soil surface	2.44 (0.25) b	5.83 (0.16) a	2.50 (0.93) b	2.66 (0.12) b	0 (0) A	0 (0) A	0 (0) A	0 (0) A
L1	1.48 (0.59) a	1.35 (0.40) a	0.96 (0.13) a	1.66 (0.94) a	0.23 (0.03) B	0.07 (0.04) B	0.07 (0.15) B	0.46 (0.01) A
L2	0.06 (0.06) a	0.02 (0.04) a	0 (0) a	0 (0) a	0.15 (0.08) AB	0.04 (0.02) C	0.04 (0.03) BC	0.17 (0.02) A
L3	0 (0) a	0 (0) a	0 (0) a	0 (0) a	0.14 (0.01) A	0.03 (0.03) B	0 (0) B	0 (0) B
Total	3.98 (0.69) b	7.20 (0.34) a	3.47 (0.84) b	4.32 (1.06) b	0.51 (0.07) A	0.14 (0.03) B	0.12 (0.02) B	0.63 (0.18) A

Values in brackets are SDs. Letters indicate significant differences ($P < 0.05$) between rotations (lower case: perennial crops; upper case: semi-perennial/annual crops).

Table 3 Carbon content (t C ha^{-1}) in roots and rhizomes of perennial crops measured in 2011 (mean of treatments N− and N+)

	Rhizome				Roots			
Soil layer	Mis E	Mis L	Swi E	Swi L	Mis E	Mis L	Swi E	Swi L
L1	1.79 (0.37) A	2.10 (0.19) A	1.75 (0.13) A	2.06 (0.88) A	0.52 (0.10) c	0.62 (0.11) bc	1.21 (0.07) ab	1.23 (0.37) a
L2	5.36 (1.11) A	6.31 (0.56) A	0 (0) B	0 (0) B	1.26 (0.13) b	1.14 (0.31) b	2.28 (0.53) a	2.21 (0.56) a
L3	0 (0)	0 (0)	0 (0)	0 (0)	0.22 (0.04) b	0.21 (0.03) b	0.98 (0.16) a	0.73 (0.20) a
L4	0 (0)	0 (0)	0 (0)	0 (0)	0.04 (0.00) b	0.04 (0.02) b	0.16 (0.03) a	0.17 (0.04) a
L5	0 (0)	0 (0)	0 (0)	0 (0)	0.10 (0.04) b	0.10 (0.05) b	0.41 (0.04) a	0.38 (0.13) a
L1-5	7.15 (1.48) A	8.41 (0.75) A	1.75 (0.13) B	2.06 (0.88) B	2.14 (0.20) b	2.11 (0.38) b	5.03 (0.73) a	4.72 (1.12) a

Values in brackets are SDs. Letters indicate significant differences ($P < 0.05$) between rotations (upper case: rhizome; lower case: roots).

Soil bulk densities

Bulk densities measured in spring 2006 were 1.39 g cm^{-3} on average in the 0–40 cm layer (Table S3). In 2011 and 2012, bulk densities varied significantly with rotation, but not with nitrogen fertilization. The bulk density in the 0–40 cm layer was higher for perennial than for other crops (1.49 vs. 1.44 g cm^{-3}, respectively), probably due to soil compaction occurring during the harvest of perennial crops (particularly for late harvest) and absence of soil tillage. No significant difference between treatments was found below 30 cm. In the 40–60 cm layer, the mean bulk density was 1.57 g cm^{-3}.

SOC concentration in the vicinity of belowground organs

It was hypothesized that perennial crops, and particularly *Miscanthus*, would increase the spatial heterogeneity of SOC because of their heterogeneous plant cover and belowground organs. This hypothesis was evaluated by testing the correlations between the biomass of rhizomes and roots found in individual soil cores (BG, in g DM kg^{-1} of dry soil) and three variables measured in the same cores: the SOC concentration (g kg^{-1}), its $\delta^{13}C$ composition (‰), and the SOC derived from the new crop (g kg^{-1}). Significant positive correlations were found between BG and each of the three variables in layers L1 and L2 for *Miscanthus* and in L1 for switchgrass (Table S4). The correlation with $\delta^{13}C$ was also significant for switchgrass in L2. The highest correlations were found for *Miscanthus* in L2, which contained the highest amount of BG (154 g kg^{-1}): the regression equation for SOC concentration was $C = 0.023\ BG + 10.96$ ($r = 0.54$, $P < 0.001$, $n = 72$). This shows that SOC concentration is higher (up to 32%) in the vicinity of belowground organs than in the rest of the soil and that the increase is due to the supply of C4 plant material to the soil organic matter. These findings show the importance of a proper sampling protocol for perennial crops to make representative SOC stock calculations at plot scale.

Initial SOC concentrations and $\delta^{13}C$

In 2006, SOC concentrations and $\delta^{13}C$ in the different soil layers were mapped across the entire experimental field using ordinary kriging. Both variables showed a clear spatial structure (Figs S3 and S4). In the old ploughed layer (L1, L2, L3), SOC concentrations were higher in the west than in the east part of the field, which was consistent with the differences observed for the clay content. Initial SOC concentrations in the 0–20 cm (L1-2) layer were on average 11.4 ± 0.5 and 10.5 ± 0.2 g kg^{-1} for perennial and other crops, respectively (Fig. 2). The spatial structure of $\delta^{13}C$ was more north–south oriented, so there was less difference between the mean initial values of the treatments (Fig. 3). The statistical analysis performed for each part of the experiment showed an effect of the soil layer (Table S5). SOC concentrations in layer L3 were 6% lower on average than in layers L1 and L2. SOC concentrations below the old ploughed depth (L4) were about 50% lower than above it with a mean value of 5.4 g kg^{-1}. The mean $\delta^{13}C$ was −25.8‰ in L1 and L2 layers and slightly increased with depth to reach −25.4‰ in L4.

Changes in SOC concentrations

Soil organic carbon concentrations under perennial crops in 2011 varied significantly among soil layers, but not between experimental treatments (Table S5). They were much more stratified within the profile than in 2006 (Fig. 2), with the highest concentrations in L1 (14.0 g kg^{-1} on average). The change between 2006 and 2011 was also tested for each layer. A significant increase in SOC concentration was observed between 2006 and 2011 for all treatments in L1. Conversely, there was a tendency to a slight decrease in SOC concentra-

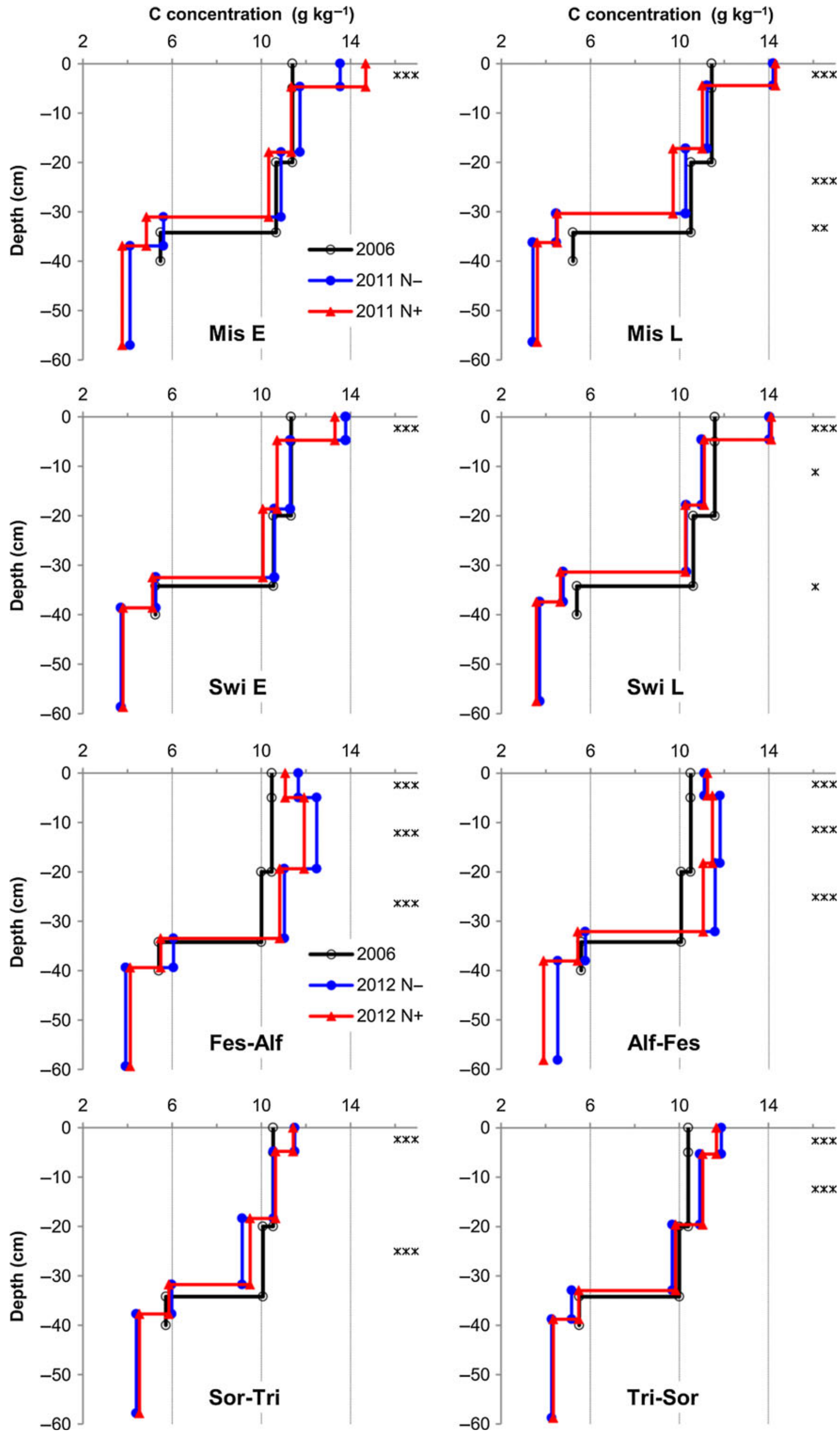

Fig. 2 Profile of soil organic carbon (SOC) concentration (g kg^{-1}) on equivalent soil mass (ESM) basis measured in each treatment in 2006 and 2011 (perennial crops) or 2012 (other crops). The 2006 data are averaged between N− and N+ (see Table 1 for abbreviations). Asterisks indicate significant changes between the two dates (*P < 0.05; **P < 0.01; and ***P < 0.001).

tions in the L2, L3, and L4 layers, although it was significant only for *Miscanthus* L (L3 and L4) and switchgrass L (L2 and L4). SOC concentrations under semi-perennial and annual crops in 2012 also depended on soil layer. A stratified SOC distribution was observed in the old ploughed layer for annual crops, but not for semi-

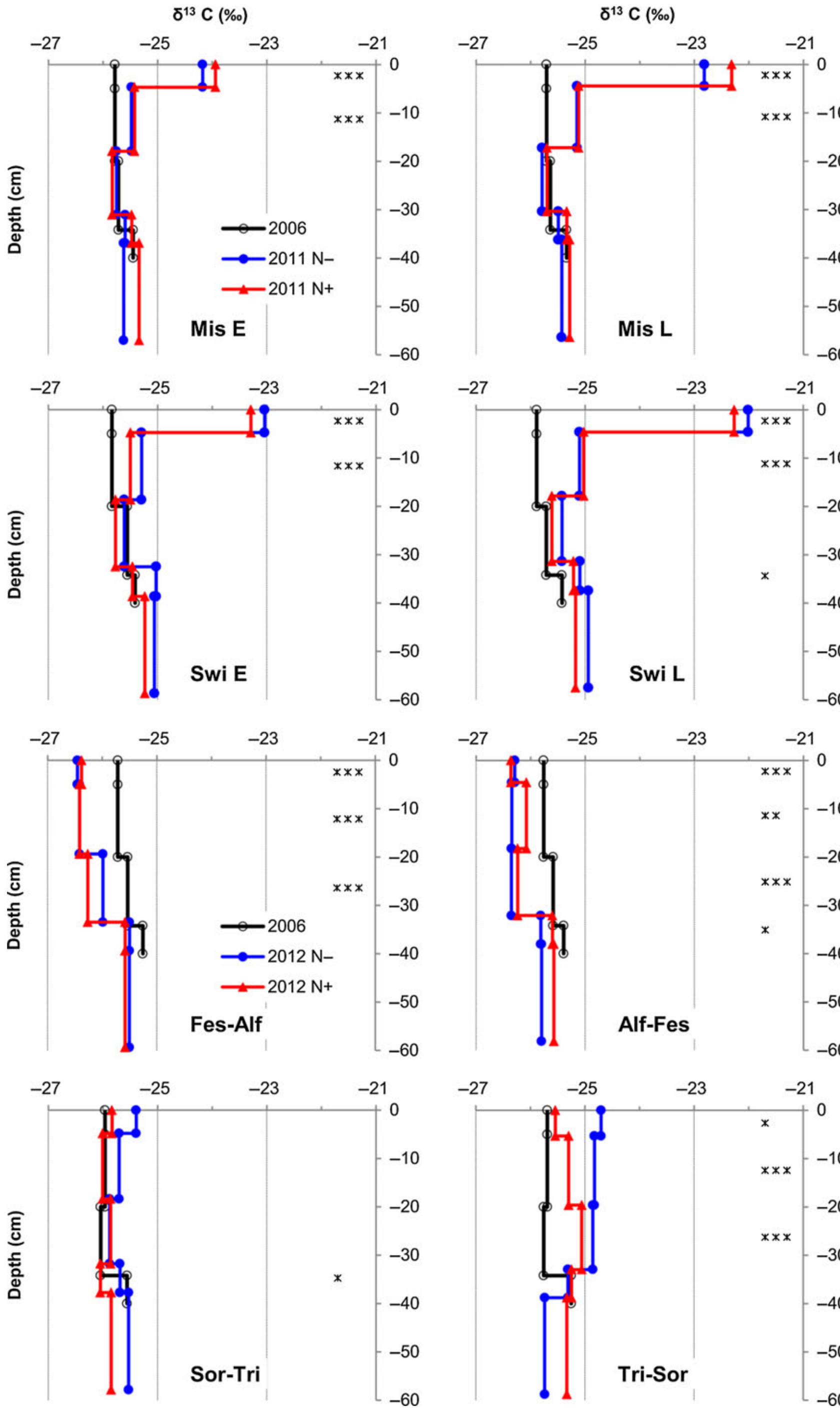

Fig. 3 Profile of $\delta^{13}C$ (‰) of soil organic carbon (SOC) on equivalent soil mass (ESM) basis measured in each treatment in 2006 and 2011 (perennial crops) or 2012 (other crops). The 2006 data are averaged between N− and N+. Asterisks indicate significant changes between the two dates (*$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$).

perennial crops. SOC concentrations significantly increased between 2006 and 2012 in L1 for the two annual rotations. An increase was also observed in L2 for the triticale-sorghum rotation. In L3, there was a tendency for a decrease, but it was only significant for the sorghum-triticale rotation. Under semi-perennial crops, SOC concentrations in layers L1, L2, and L3 had significantly increased between 2006 and 2012, from 10.3 to 11.5 g kg^{-1}. Finally, SOC concentrations in L5 in 2011 and 2012 were small and homogeneous

(4.0 ± 0.4 g kg^{-1}), with no significant differences between treatments.

Changes in δ^{13}C

The δ^{13}C composition of SOC measured in 2011 under perennial crops varied markedly with depth (Table S5): it was much higher in L1 (−23.0‰ on average) than in the other layers (Fig. 3). It significantly increased between 2006 and 2011 in L1 and L2 for all treatments, indicating C inputs from C4 plants, but did not change significantly below ca. 18 cm except for switchgrass L. Soils under semi-perennial crops had a significantly lower δ^{13}C composition than soils under annual crops, which is consistent with the signature of C inputs (pure C3 for semi-perennial and mixed C3/C4 for annual crops). The effect of N fertilization was complex: there was no effect on semi-perennials, but a tendency for annual rotations to have lower δ^{13}C in more fertilized treatments, which may result from a greater response of yield to N fertilization in triticale than in sorghum. δ^{13}C did not differ significantly between soil layers for annual crops, but was significantly lower in the old ploughed layer (L1-L3) than below (L4-L5) for semi-perennials. The change in δ^{13}C between 2006 and 2012 in each layer depended on the rotation. δ^{13}C significantly decreased in the old ploughed layer for semi-perennial crops and significantly increased in the same layers for the triticale-sorghum rotation.

SOC stocks and mean weighted δ^{13}C

The spatial distribution of SOC stocks and mean weighted δ^{13}C measured in 2006 in the old ploughed layer (L1-3) was heterogeneous, but well structured throughout the experimental field (Fig. 4). SOC stocks varied from 45.9 to 54.4 t ha^{-1} and δ^{13}C from −26.4‰ to −24.8‰. The main gradient of SOC stocks was oriented west-east, consistently with SOC concentrations. As a result, the initial SOC stocks in L1-3 were significantly higher for perennial crops than for other crops (51.6 vs. 48.0 t ha^{-1}) (Table 4). In contrast, the ^{13}C signatures did not differ significantly between the two parts of the field. In each part, there was no difference between treatments except for the semi-perennial and annual crops for which a slight significant difference in initial SOC stocks (0.3 t C ha^{-1} in L1-3) was detected between N rates (Table S6).

In 2011, SOC stocks did not differ significantly between treatments under perennial crops (Table S7). The average SOC stock over the old ploughed layer was 52.3 t ha^{-1} (Table 5). The only significant effect of N rate was found in the deepest layer (L1-5) where the mean SOC stock was 69.1 t ha^{-1} in N− and 67.5 t ha^{-1} in N+. The δ^{13}C signature was affected by the rotation only in the upper soil layer: *Miscanthus* L and switchgrass L had a significantly higher δ^{13}C value (−22.6‰ and −22.1‰) than *Miscanthus* E (−24.1‰). For *Miscanthus*, the difference could be attributed to the presence of leaf mulch in the L treatment but not in the E treatment. The mean δ^{13}C in the old ploughed layer was -25.0‰. It was not significantly different between N− and N+.

In 2012, SOC stocks under semi-perennial and annual crops were significantly affected by the rotation, as well as δ^{13}C. SOC stocks were greater under semi-perennial than annual crops (53.6 vs. 48.4 t ha^{-1}, respectively, in L1-3), except in the first soil layer. As expected, δ^{13}C sig-

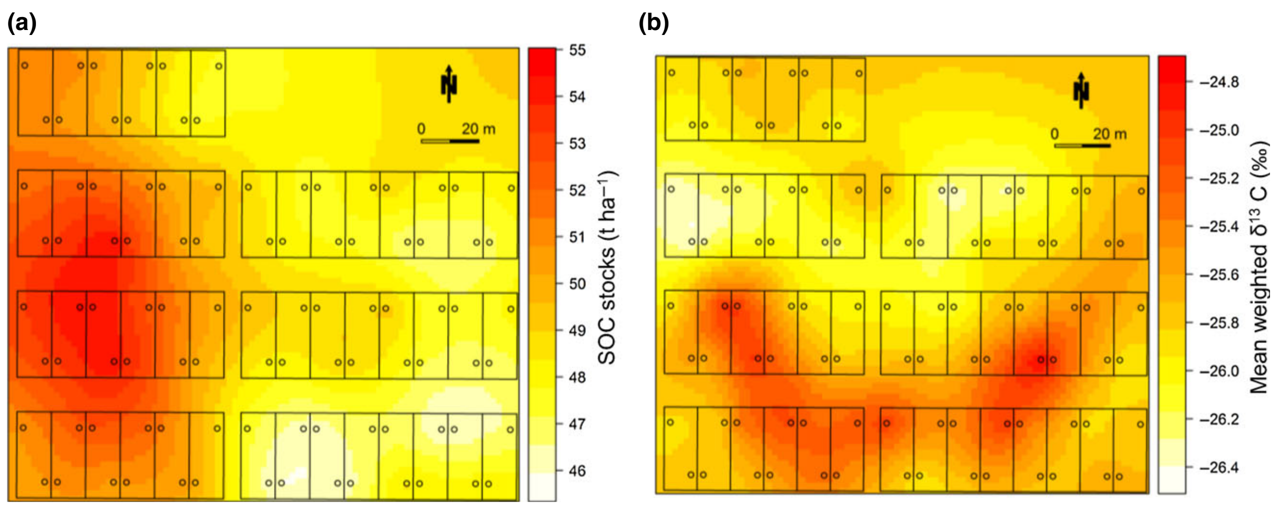

Fig. 4 Map representing the spatial variability of (a) soil organic carbon (SOC) stocks (t C ha^{-1}) and (b) mean weighted δ^{13}C (‰) measured in 2006 in the old ploughed layer, obtained by ordinary kriging from the sampled points (open circles). Lines represent the outlines of the 48 plots.

Table 4 Cumulative soil organic carbon stocks (SOC, t C ha^{-1}) and mean weighted $\delta^{13}C$ ($\delta^{13}C_w$, ‰) measured at the start of the experiment (2006) in the two parts of the field: perennial and semi-perennial/annual crops. Values in brackets are SDs between the 24 plots of each part

			SOC (t C ha^{-1})		$\delta^{13}C_w$ (‰)	
Soil layer	Soil mass (t ha^{-1})	Depth (cm)	Perennial crops	Semi-perennial and annual crops	Perennial crops	Semi-perennial and annual crops
L1	667	5	7.62 (0.30)	6.98 (0.15)***	−25.8 (0.3)	-25.8 (0.4) ns
L1-2	2667	20	30.47 (1.18)	27.90 (0.59)***	−25.8 (0.3)	-25.8 (0.4) ns
L1-3	**4669**	**34.2**	**51.59 (1.71)**	**47.99 (0.98)*** **	**−25.7 (0.3)**	**-25.9 (0.3) ns**
L1-4	5553	40	56.29 (1.84)	52.96 (0.76)***	−25.7 (0.3)	-25.8 (0.3) ns

Asterisks indicate significant differences (***P < 0.001; ns = not significant). Bold values correspond to the old ploughed layer (L1-3).

natures were smaller in semi-perennials (pure C3) than in annual crops (mixed C3/C4).

A statistical analysis of the temporal variation in SOC stocks and mean weighted $\delta^{13}C$ was performed for each part of the experiment (Table S8). Only semi-perennial crops showed a significant change between initial and final SOC stocks in the old ploughed layer (L1-3), with a mean increase of 0.93 ± 0.28 t ha^{-1} yr^{-1} (Fig. 5a). The same conclusions were found for L1-4. However, SOC stocks in L1-2 significantly increased in all rotations, except for sorghum-triticale. No significant effect of N fertilization was found. Changes in $\delta^{13}C$ were significant in all rotations and soil layers, except for the sorghum-triticale rotation (Fig. 5b). $\delta^{13}C$ in the old ploughed layer increased under perennial crops (between 0.08 ± 0.05 and 0.22 ± 0.01‰ yr^{-1}) and triticale-sorghum rotation (0.11 ± 0.04‰ yr^{-1}). It decreased under semi-perennial crops by −0.10 ± 0.02‰ yr^{-1} on average.

Changes in new and old SOC stocks

In 2011, the proportion of SOC in the old ploughed layer derived from the C4 perennial crops established 5 years earlier was 5.6% on average (Table S9). It was significantly higher for switchgrass L (8.6%) than for *Miscanthus* E and switchgrass E (3.1 and 4.6%, respectively), *Miscanthus* L being intermediate (6%). This proportion was much higher in L1 (21.8% on average). In 2012, the SOC derived from the new C3 semi-perennial crops represented on average 16.9% of the SOC stock in the old ploughed layer and did not significantly differ between the two rotations.

Using these data, the rates of change in new and old SOC stocks during the experimental period were calculated (Table 6). The accumulation rate of new SOC in the old ploughed layer was significantly affected by the rotation. It was highest for semi-perennial crops (1.50 ± 0.28 t ha^{-1} yr^{-1} on average). Within the perennial crops, the highest accumulation rate was found under switchgrass L (0.89 ± 0.03 t ha^{-1} yr^{-1}) and the lowest under *Miscanthus* E (0.33 ± 0.18 t ha^{-1} yr^{-1}). Simultaneously, a decrease in old SOC stock was calculated in most situations. The rate of decrease in the old ploughed layer did not differ significantly between perennial and semi-perennial crops. The mean rate of decrease for all crops was −0.50 ± 0.45 t ha^{-1} yr^{-1}.

Discussion

Crop residues and belowground biomass

The amounts of crop residues found on the soil surface or within the soil were much higher for perennial crops than for the other crops. This difference is probably due to the absence of soil tillage and to more recalcitrant residues with perennial crops (Amougou *et al*., 2011). In the case of *Miscanthus* L, there was an additional C input through leaf fall during winter. The amount of mulch derived from fallen leaves (2.86 t C ha^{-1}) was almost identical to that measured by Amougou *et al*. (2012) 1 year earlier in the same experiment. The yearly input of leaves to the soil, estimated at 1.40 t C ha^{-1} by Amougou *et al*. (2012), was probably in equilibrium with the decomposition rate of the leaf mulch.

The belowground biomass of perennial crops represented a large C pool. The rhizome biomass of *Miscanthus* was close to that measured in the same experiment by Strullu *et al*. (2011) in February 2010. For switchgrass, the rhizome biomass was higher than that reported by Garten *et al*. (2010), but the root biomass over 0–60 cm (4.9 t C ha^{-1}) was similar and represented the major part of the belowground C.

Changes in SOC concentrations and $\delta^{13}C$

The change in SOC concentration between 2006 and the second sampling date varied between soil layers, except for the semi-perennial crops, which showed a similar change from L1 to L3. This is probably due to differences in soil tillage between treatments. Indeed, in contrast to the other treatments, semi-perennial crops were mouldboard ploughed 18 months before the second sampling. SOC stratification appears in continuous reduced tillage systems, in contrast to conventional tillage systems (e.g. Dimassi *et al.*, 2014; Powlson *et al.*, 2014). The δ^{13}C change observed under perennial crops showed that C inputs were greater in the upper layer. However, it was likely that rhizome and roots' turnover and/or rhizodeposition made a significant contribution to the C inputs under perennial crops. Indeed, a significant change of δ^{13}C signature was observed in the L2 layer, which contained the highest belowground biomass. This hypothesis was also confirmed by the correlation found between the biomass of belowground organs in the soil samples and the SOC derived from the new crops. Zatta *et al.* (2014) found the same kind of relationship for a 6-year-old *Miscanthus* with soil cores taken inside or outside the rhizome area.

Changes in SOC stocks

Soil organic carbon stocks in 2006 displayed a high spatial heterogeneity. Therefore, the synchronic approach (comparison of treatments at a given time without estimating the initial SOC stocks), which is most often used, would have led to very different conclusions. For example, SOC stocks were 4.3 t ha^{-1} higher under *Miscanthus* L in 2011 than under sorghum-triticale in 2012, which might have been interpreted as an important C sequestration with *Miscanthus*, whereas a comparison with the initial values (in 2006) indicated that no significant sequestration occurred in either rotation (Fig. 5a). It was also important to apply the recommendation to compare stocks on an ESM basis. Calculation to a fixed depth of 40 cm rather than an ESM of 5553 t ha^{-1} would have led to an overestimation of SOC change after 5 years by 2.5 t C ha^{-1} under *Miscanthus* L.

No significant change in SOC stocks could be detected under perennial crops (using either 4669 or 5553 t ESM ha^{-1}), despite small SDs in measurements. A significant SOC increase was found in the upper layer (L1), offset by a decrease in the lower layers (L2-4). These results may appear to contradict the meta-analyses of Don *et al.* (2011) and Poeplau & Don (2014), who found a mean SOC increase of 0.66 ± 0.94 t ha^{-1} yr^{-1} and 0.40 ± 0.73 t ha^{-1} yr^{-1}, respectively (mean ± SD), under *Miscanthus* harvested late and grown on former croplands, but these studies show a wide variability. In fact, studies dealing with young *Miscanthus* plantations (<10 years old) often fail to show a significant change in SOC stocks (Zimmermann *et al.*, 2012). Significant increases have generally been observed in older plantations (Hansen *et al.*, 2004; Dondini *et al.*, 2009; Felten & Emmerling, 2012; Dufossé *et al.*, 2014). However, Richter *et al.* (2015) found equal SOC stocks under a 14-year-old *Miscanthus* and an arable reference plot. Published studies with switchgrass refer to relatively young plantations (<9 years old). The observed SOC stock changes under switchgrass are generally positive, but highly variable and often nonsignificant (Liebig *et al.*, 2008; Schmer *et al.*, 2011; Dou *et al.*, 2013; Bonin & Lal, 2014). For example, Liebig *et al.* (2008) found a mean increase of 1.1 ± 1.4 t C ha^{-1} yr^{-1} across ten sites after 5 years (in the 0–30 cm layer), but only four sites showed a significant increase.

In our experiment, no significant change in SOC stocks was observed under annual crops (using 4669 t ESM ha^{-1} or more). The sorghum-triticale rotation showed a tendency to a decrease, but the inversed rotation did not. This result was unexpected because experimental and modelling studies generally show a decrease in SOC stocks when the whole aboveground biomass of annual crops is removed (Saffih-Hdadi & Mary, 2008; Powlson *et al.*, 2011). Other changes in crop management may have compensated for this effect. Indeed, a catch crop has been grown every other year since 2006 and the introduction of catch crops has been shown to increase SOC stocks (Constantin *et al.*, 2010). The difference observed between the two rotations might also be due to the higher yields of the triticale-sorghum rotation over the period 2006–2011 (5.11 vs. 3.87 t C ha^{-1} yr^{-1}), probably leading to higher amounts of crop residues (stubble and roots) returned to the soil. Unlike the sorghum-triticale rotation, this rotation also showed a significant increase in δ^{13}C, which was consistent with the higher sorghum production in this rotation (C4 crop).

The only significant change in SOC stocks in the old ploughed layer was observed for semi-perennial crops. This result was consistent with the meta-analyses on the effect of arable land conversion to grassland (Conant *et al.*, 2001; Soussana *et al.*, 2004). Soussana *et al.* (2004) estimated that the mean increase in SOC stocks after conversion is 0.49 ± 0.26 t ha^{-1} yr^{-1} over 20 years. The net increase rate in the fescue/alfalfa rotations of our experiment was 0.93 ± 0.28 t ha^{-1} yr^{-1} over 6 years, that is approximately two times greater than for grassland.

SOC stocks will have to be monitored over a longer period to confirm the differences between bioenergy crops and test the occurrence of long-term effects.

Changes in new and old SOC stocks

The variations in new and old SOC contents are indicators of C inputs (above and belowground plant materi-

Table 5 Cumulative soil organic carbon stocks (SOC, t C ha^{-1}) and mean weighted $\delta^{13}C$ ($\delta^{13}C_w$, ‰) on equivalent soil mass (ESM) basis measured (a) in 2011 for perennial crops and (b) in 2012 for semi-perennial/annual crops (mean of N− and N+)

		(a) Perennial crops											
		Mis E			Mis L			Swi E			Swi L		
Soil layer	Soil mass (t ha^{-1})	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)
L1	667	4.7	9.41 (0.69) a	−24.1 (0.7) A	4.4	9.51 (0.93) a	−22.6 (0.8) B	4.8	9.03 (0.72) a	−23.2 (0.2) AB	4.6	9.39 (0.72) a	−22.1 (0.9) B
L1-2	2667	17.9	32.49 (1.31) a	−25.1 (0.4) A	17.2	31.76 (2.44) a	−24.4 (0.6) A	18.6	31.04 (1.83) a	−24.7 (0.1) A	17.8	31.50 (1.19) a	−24.2 (0.6) A
L1-3	**4669**	**31.1**	**53.73 (2.21) a**	**−25.3 (0.4) A**	**30.4**	**51.76 (3.10) a**	**−24.9 (0.4) A**	**32.5**	**51.73 (2.65) a**	**−25.1 (0.1) A**	**31.4**	**52.10 (1.49) a**	**−24.7 (0.5) A**
L1-4	5553	36.9	58.36 (3.06) a	−25.4 (0.3) A	36.2	55.73 (3.51) a	−24.9 (0.4) A	38.6	56.32 (2.88) a	−25.1 (0.1) A	37.4	56.26 (1.47) a	−24.7 (0.5) A
L1-5	8690	57.1	70.70 (5.11) a	−25.4 (0.3) A	56.4	66.75 (4.22) a	−25.0 (0.4) A	58.7	68.10 (2.80) a	−25.1 (0.1) A	57.6	67.71 (1.72) a	−24.8 (0.5) A
		(b) Semi-perennial and annual crops											
		Fes-Alf			Alf-Fes			Sor-Tri			Tri-Sor		
Soil layer	Soil mass (t ha^{-1})	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	$\delta^{13}C_w$ (‰)
L1	667	4.9	7.58 (0.39) a	−26.4 (0.3) A	4.6	7.46 (0.05) a	−26.3 (0) A	4.8	7.65 (0.25) a	−25.6 (0.2) B	5.3	7.86 (0.17) a	−25.1 (0.5) B
L1-2	2667	19.4	31.99 (1.49) a	−26.4 (0.5) A	18.2	30.74 (1.03) ab	−26.2 (0.4) A	18.4	28.82 (0.38) b	−25.8 (0.1) AB	19.6	29.83 (0.67) b	−25.1 (0.6) B
L1-3	**4669**	**33.5**	**53.87 (2.40) a**	**−26.3 (0.4) A**	**32.1**	**53.42 (0.22) a**	**−26.3 (0.3) A**	**31.8**	**47.48 (1.14) b**	**−25.8 (0.1) A**	**33.0**	**49.36 (1.39) b**	**−25.0 (0.7) B**
L1-4	5553	39.4	58.98 (1.54) a	−26.2 (0.3) A	38.1	58.37 (0.64) a	−26.2 (0.3) A	37.7	52.71 (1.04) b	−25.8 (0.1) A	38.8	54.07 (1.43) b	−25.0 (0.7) B
L1-5	8690	59.4	71.60 (2.29) a	−26.1 (0.3) A	58.1	71.59 (0.13) a	−26.1 (0.3) A	57.8	66.72 (2.17) b	−25.8 (0.1) A	58.8	67.60 (2.04) b	−25.1 (0.6) B

Values in brackets are SDs. Letters indicate significant differences ($P < 0.05$) between rotations (lower case: SOC; upper case: $\delta^{13}C_w$). Bold values correspond to the old ploughed layer.

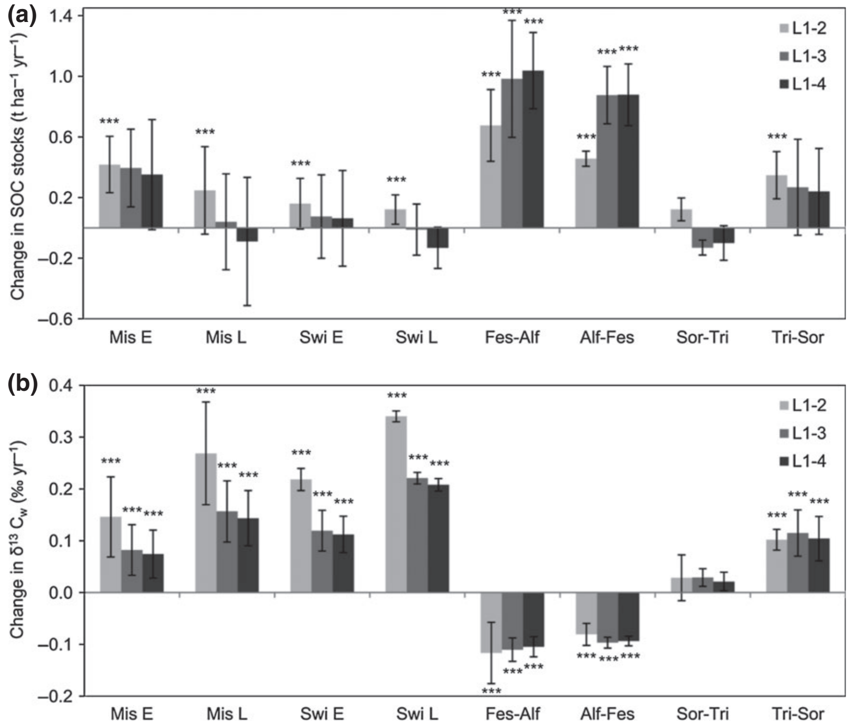

Fig. 5 Change in (a) soil organic carbon (SOC) stocks (t C ha^{-1} yr^{-1}) and (b) mean weighted $\delta^{13}C$ (‰ yr^{-1}) on equivalent soil mass (ESM) basis in layers L1-2 (2667 t ha^{-1}), L1-3 (4669 t ha^{-1}), and L1-4 (5553 t ha^{-1}) between 2006 and 2011 for perennial crops or 2012 for semi-perennial and annual crops. Bars represent the SDs. Asterisks indicate the probability of a significant change during the 5 or 6 year period: ***$P < 0.001$.

Table 6 Change in new (C_{New}) and old (C_{Old}) soil organic carbon (SOC) stocks (t C ha^{-1} yr^{-1}) calculated from the $\delta^{13}C$ analyses [Eqns (11–13)] on equivalent soil mass (ESM) basis between 2006 and 2011 for perennial crops or 2012 for semi-perennial crops (mean of N− and N+)

Soil layer	Soil mass (t ha^{-1})		Mis E	Mis L	Swi E	Swi L	Fes-Alf	Alf-Fes
L1	667	C_{New}	0.24 (0.11) c	0.47 (0.15) ab	0.38 (0.03) abc	0.55 (0.11) a	0.25 (0.07) bc	0.18 (0.08) c
		C_{Old}	0.12 (0.23) a	−0.09 (0.08) ab	−0.08 (0.07) ab	−0.22 (0.00) b	−0.16 (0.09) b	−0.11 (0.06) ab
L1-2	2667	C_{New}	0.36 (0.17) b	0.67 (0.30) ab	0.53 (0.03) ab	0.83 (0.07) ab	1.08 (0.50) a	0.68 (0.24) ab
		C_{Old}	0.06 (0.36) a	−0.42 (0.27) ab	−0.37 (0.15) ab	−0.71 (0.04) b	−0.40 (0.57) ab	−0.22 (0.19) ab
L1-3	**4669**	**C_{New}**	**0.33 (0.18) d**	**0.63 (0.28) cd**	**0.48 (0.16) cd**	**0.89 (0.03) bc**	**1.65 (0.28) a**	**1.36 (0.23) ab**
		C_{Old}	**0.06 (0.43) a**	**−0.59 (0.38) ab**	**−0.40 (0.24) ab**	**−0.91 (0.18) b**	**−0.67 (0.61) ab**	**−0.49 (0.42) ab**
L1-4	5553	C_{New}	0.32 (0.19) d	0.63 (0.27) cd	0.49 (0.15) cd	0.91 (0.04) bc	1.71 (0.31) a	1.42 (0.21) ab
		C_{Old}	0.03 (0.54) a	−0.72 (0.45) ab	−0.43 (0.26) ab	−1.04 (0.17) b	−0.68 (0.47) ab	−0.55 (0.40) ab

Values in brackets are SDs. Letters indicate significant differences ($P < 0.05$) between rotations. Bold values correspond to the old ploughed layer.

als) and outputs (C mineralized), respectively, and their balance determines the net change in SOC content.

C inputs under perennial crops were mainly located in the layer L1-2 (ca. 0–18 cm). This was consistent with other studies using ^{13}C abundance, which found that new SOC was concentrated above 30 cm (Schneckenberger & Kuzyakov, 2007; Collins *et al.*, 2010; Felten & Emmerling, 2012; Cattaneo *et al.*, 2014; Poeplau & Don, 2014). In our experiment, the rate of new SOC accumulation in the old ploughed layer of 0.63 t ha^{-1} yr^{-1} under *Miscanthus* L fell within the range of values compiled by Poeplau & Don (2014), that is 0.85 ± 0.68 t ha^{-1} yr^{-1} (0–30 cm). Results for switchgrass were also consistent with Collins *et al.* (2010) and Follett *et al.* (2012), who found new SOC accumulation of 1.0 and 0.5 t ha^{-1} yr^{-1}, respectively at 0–30 cm. The increase in new SOC stocks was higher for semi-perennial than for perennial crops, indicating higher C inputs under these rotations. In contrast to perennial crops, semi-perennials were destroyed twice during the experimental period. This led to the return of the whole crop biomass (roots and aboveground below cutting height) to the soil, which could be significant. For example, Justes *et al.* (2001) estimated the root biomass of 2-year-old alfalfa to be 2.8 t C ha^{-1}.

Reported changes in old SOC stocks under perennial crops are highly variable. Poeplau & Don (2014) calculated from experimental data a mean positive rate of change for old SOC of 0.83 ± 3.24 t ha^{-1} yr^{-1} under *Miscanthus* L. In the absence of other carbon sources (sewage sludge, C3 weeds, etc.), this change should be negative. Poeplau & Don (2014) used RothC to simulate a mean change of -0.60 ± 0.43 t ha^{-1} yr^{-1} (0–30 cm), which matches our results for *Miscanthus* L (-0.59 ± 0.38 t ha^{-1} yr^{-1}). The change in old SOC was not significantly different between perennial and semi-perennial crops. This result suggested that soil tillage associated with the periodic destruction of fescue and alfalfa did not increase SOC mineralization. The increase in SOC stocks observed under semi-perennial crops was therefore due to a higher C input rather than to a change in mineralization rate.

Effect of management practices

There have been few studies into the effects of bioenergy crop management practices on SOC stocks. In our study, N fertilization had a significant effect only on crop yields, but not on crop residues, belowground biomass, and SOC stocks measured in 2011–2012. This was in accordance with Cattaneo *et al.* (2014) for *Miscanthus*, but in contrast with Follett *et al.* (2012) and Lee *et al.* (2007), who found that an increase in mineral N fertilization enhances crop production and SOC stocks under switchgrass. In our experiment, N fertilization effects on *Miscanthus* E and switchgrass yields increased with time (Cadoux *et al.*, 2014); therefore, future effects on crop residues and SOC stocks could be expected. The effect of the harvest date of perennial crops has been investigated for switchgrass but not for *Miscanthus*. Follett *et al.* (2012) did not find any significant effect on SOC stocks under switchgrass, which is consistent with our findings. In our study, SOC stocks did not differ significantly between *Miscanthus* E and L. This result was surprising because *Miscanthus* L had higher C inputs to the soil due to leaf fall during winter. However, a large part of these leaves was found undecomposed at soil surface. New SOC accumulation was two times greater in the upper soil layer for *Miscanthus* L than for *Miscanthus* E, whereas old SOC change did not differ significantly between the two treatments. As for N fertilization, significant effects of the harvest date of *Miscanthus* on SOC stocks can be expected in the longer term and should be further studied. Finally, it is likely that crop management and particularly N fertilization also have a strong effect on GHG balance as a result of their impact on N_2O emissions. A complete GHG budget including SOC stocks changes and measured N_2O emissions will need to be established.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map representing the experimental design of the B&E long-term experiment.
Figure S2. Diagram showing the three steps defining the sampling strategy for SOC measurements in *Miscanthus* plots.
Figure S3. Map representing the spatial variability of SOC concentration in 2006.
Figure S4. Map representing the spatial variability of $\delta^{13}C$ in 2006.
Table S1. Physical and chemical soil characteristics measured in 2006.
Table S2. Carbon content in harvested biomass from 2006 to 2011.
Table S3. Soil bulk densities measured in 2006, 2011 and 2012 from 0 to 40 cm depth.
Table S4. Minimum and maximum values of belowground biomass (BG) of *Miscanthus* and switchgrass found in individual soil cores and the regression equation between BG and three variables: C (SOC concentration), $\delta^{13}C$ (SOC composition) and C_{New} (SOC concentration derived from the new crop).
Table S5. Statistical analysis of SOC concentrations and $\delta^{13}C$ on each date of measurement.
Table S6. Statistical analysis of cumulative SOC stocks and mean weighted $\delta^{13}C$ in 2006.
Table S7. Statistical analysis of cumulative SOC stocks and mean weighted $\delta^{13}C$ in 2011 and 2012.
Table S8. Statistical analysis of cumulative SOC stocks and mean weighted $\delta^{13}C$ testing the effects of rotation, nitrogen rate and year.
Table S9. Proportion of the SOC stock derived from the new crops in 2011 and 2012.