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# Diversified crop rotations and organic amendments as strategies for increasing soil carbon storage and stabilisation in UK arable systems

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Adaptations in crop rotation with the inclusion of temporary grass-clover leys and organic amendments, have been promoted as effective ways to improve soil carbon (C) sequestration and mitigate climate change in agricultural systems. However, there are still a lot of uncertainties related to i) the combined effects of different crop rotations and different fertilisation sources, e.g., organic amendments, on soil C stocks; and ii) their potential effect on C stabilisation. The objective of this study was to evaluate the effect of different arable crop rotations with varying degrees of diversity in crop type and lengths of grass-clover ley periods and fertilisation sources on soil C stocks and C stabilisation down to 0.60 m soil depth. This was investigated in a long-term factorial field experiment-combining different crop rotation (cereal-intensive conventional vs. diversified legume-intensive organic) with different lengths of grass-clover ley periods (2 vs. 3 years), fertilisation sources (mineral vs. compost), and years (samples taken at the beginning and at the last year of one complete cycle of rotation; 8 years apart)-to explore their individual and interactive effect on soil C stock and C stabilisation at two soil depths (0–0.30 and 0.30–0.60 m). Soil C stabilisation was assessed using a unique combination of three different techniques: physical fractionation for separation of C associated to organic and mineral fractions, thermal analysis combined with differential scanning calorimetry and a quadrupole mass spectrometry (TG-DSC-QMS) for physical-chemical aspects, and pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC/MS) for molecular structural information. The findings showed higher soil C stocks under the diversified organic rotation with 3 years of grass-clover ley period at both soil depths, regardless of the fertilisation source or sampling year. However, the organic rotation seemed to deliver stable soil C stocks only in the subsoil layer. Compost fertilisation, in turn, increased topsoil C stocks between the two sample dates under both rotations, and it appears to be stable. These results suggested that combining a diversified organic rotation with 3 years grass-clover ley with compost fertilisation could be one way for agricultural systems to deliver stable soil C sequestration.

## KEYWORDS

carbon persistence, carbon stocks, crop rotation, fertilisation sources, interactions, management practices

## 1 Introduction

Accumulation of soil organic carbon (SOC) has been highlighted as a biological approach to draw down atmospheric CO<sub>2</sub> and reduce associated climate change effects (Paustian et al., 2016). Grasslands are frequently proposed as important ecosystems for soil carbon (C) cycling and consequently their storage (Bai and Cotrufo, 2022), while agricultural systems have been deemed C sources (Lal, 2004). In this sense, the inclusion of temporary grass-clover leys in a more diverse crop rotation, and other soil management practices, e.g., organic amendments, have been promoted as strategies for agricultural systems to increase soil organic matter (SOM) levels and improving soil quality aspects that foster SOC accumulation mechanisms (Zani et al., 2021; 2022; Guest et al., 2022). In addition to potential SOC benefits, diversified crop rotation, ley periods, and organic amendments are also related to other functions and ecosystem services that are essential for achieving sustainable management of agricultural soils, including but not limited to, high-quality crops, water resources, and nutrient cycling, among others (Tuomisto et al., 2012; Chen et al., 2022; Zhao et al., 2022; Zhu et al., 2022). While previous studies have confirmed that higher SOM inputs result in higher SOC stocks, further evidence is needed to understand the relative impacts of rotational crop-grass-clover leys and fertilisation strategies and especially how these practices might affect SOM quality, i.e., its composition and C stability.

Soil C stability can be simply defined as the resistance of SOC to decomposition. The decomposition of SOC is affected by the nature and composition (chemical and physical) of the input material, soil properties (e.g., physical), biological activities, and environmental conditions, as well as the quantity of the inputs to the given ecosystem (Dixon et al., 1994; Trumbore, 1997). The resistance of SOC to decomposition can be controlled by various biological, physicochemical and structural factors including its disconnection from microbes, soil aggregation and physical protection as well as chemical recalcitrance, where the SOC-mineral association is considered a significant factor controlling stability (Lützow et al., 2006; Schmidt et al., 2011; Basile-Doelsch et al., 2020). Overall, it has been suggested that if SOM consists of easily degradable material, e.g., due to its low C:N ratio, its decomposition rate is high because microorganisms decompose it relatively rapidly, while the opposite holds true for SOM with higher C:N ratio (excess of carbon) (Powlson et al., 2011; Li et al., 2018). This is, of course, also dependent on several other factors such as temperature, soil moisture, drying-wetting cycles, aeration, etc (Lal et al., 2015). Different crop rotations with different lengths of grass-clover leys and fertilisation sources are expected to show variation in these factors, as well as the quantity and quality of inputs, i.e., the amount of organic materials that will become SOM, and its composition. Such change can hence have a consequential effect on losses of SOM (through decomposition processes) and consequently SOC stocks and C stabilisation (Lal et al., 2015). Understanding how management practices affect SOM composition and proportions of SOC within pools with differing stability is crucial as this will help to design sustainable farming systems in the future which build stocks of soil C that are stable and permanent.

Several methods have been developed to assess the C content, composition and stability within SOM. Examples of analytical techniques used include i) physical fractionation of SOM into

organic and mineral-associated fractions (Christensen, 1992), ii) thermogravimetry-differential scanning calorimetry coupled with quadrupole mass spectrometry (TG-DSC-QMS) (Lopez-Capel et al., 2005a; Fernández et al., 2012), and iii) pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC-MS) (Mason et al., 2012; Abbott et al., 2013). The quantification of organic and mineral-associated fractions through physical fractionation of the SOM is used for understanding soil C dynamics, turnover and stability (Christensen, 1992). In this particular approach, SOM fractions can be associated with either a cellulosic material (composed of polysaccharides) or with a lignin-like material (composed of a mixture of aromatic, cross-linked phenolic C compounds) (Dell'Abate et al., 2002). Particulate organic matter fractions (POM) (i.e., the more labile available component for decomposition) represents the former, while the latter is composed of a more refractory (resistant to decomposition) material characterised by mineral-associated organic matter (Manning et al., 2005). Thermal analytical techniques (i.e., TG-DSC-QMS), in turn, involve programmed temperature change to monitor physical and/or chemical properties of a sample (Langier-Kuźniarowa, 2002). In this sense, TG-DSC-QMS can define SOM stability as a function of its bulk chemical composition and the degree of humification and mineral association of the SOM (Plante et al., 2009). As for the fractionation approach, this technique can provide insight into the proportions of active and more stable SOM components (Lopez-Capel et al., 2005b). Coupling TG-DSC to a QMS allows the chemical identification, characterisation and proportions of major evolved gas species from the target sample (Lopez-Capel et al., 2006). Variability in shape, area, and temperature of TG-DSC-QMS traces can reveal differences in thermal stability and chemical structure of the sample. As stated by Manning et al. (2005), this technique allows the determination of all the C present within a sample in a single heating analysis. Lastly, the use of Py-GC-MS can provide detailed molecular structural information, which is not provided by the other two techniques, in a simple and rapid manner. It aims to degrade macromolecules into small fragments (relative to the large macromolecules) and simultaneously identify structural information (Meier and Faix, 1992; Leinweber and Schulten, 1993).

In this context, in order to fully understand the potential for crop rotations with different lengths of grass-clover leys and fertilisation sources in delivering SOC accumulation, which is potentially stable and persistent, qualitative (size separation of SOM into fractions and chemical composition of SOM) and quantitative (SOC stocks) data must be investigated. Combining qualitative and quantitative data can provide novel insights about the composition of SOC stocks that might control stability and are still unknown. It could also help to elucidate the largely unknown processes in subsoil layers (i.e., > 0.30 m depth), which might represent more stable and long SOC turnovers as a result of reduced microbial activity, suboptimal environmental conditions, energy scarcity and less accessibility to the SOM (Rumpel and Kögel-Knabner, 2010). Until now, there have not been any investigations of SOM composition and C stability that compare crop rotation with different lengths of grass-clover leys and fertilisation sources that include both qualitative and quantitative data, specifically for long-term experiments (i.e., potential temporal changes) and subsoil layers.

The objectives of this study were to i) assess SOC stock changes in the top- (0–0.30 m) and subsoil layers (0.30–0.60 m) after one complete cycle of contrasting rotations (conventional *vs.* organic) that included either 2 or 3 years under grass-clover ley periods associated with different fertilisation sources (mineral *vs.* compost), and ii) characterise the SOM composition and make inferences about C stabilisation in these contrasting systems using SOM physical fractionation, TG-DSC-QMS, and Py-GC/MS-TMAH analyses. It was hypothesised that such management practices will significantly change SOC stocks on top- and subsoil layers, for which the unique combination of techniques will allow inferences about the processes of C stabilisation.

## 2 Materials and methods

### 2.1 Field site, experimental design, and treatments

The study used the Nafferton Factorial Systems Comparison (NFSC) trial based at Newcastle University's Nafferton Farm, located 12 miles west of Newcastle-upon-Tyne in North-East England (54°59'09"N; 1°43'56"W, 60 m a.s.l). According to the Köppen classification system, the site experiences a marine west coast climatic condition, with the average annual temperature and total annual precipitation 8.6°C and 638.6 mm respectively, with a maximum monthly temperature of 22°C and a minimum of 0°C (Supplementary Figure S1, average record between a weather station located ~5 miles away from the farm and by an on-site automated weather station). The soil in the experiment is classified predominantly as a uniform Dystric Stagnosol (WRB, 2015), slowly permeable, seasonally wet, acidic loamy and clayey soil that is naturally low in fertility (Farewell et al., 2011; Cranfield University, 2021); Cambic Stagnogley (Mückenhausen, 1981); Stagnic Cambisol (WRB, 2015). Analysis at the establishment of the experiment indicated an average SOC content of ~3%, pH of 6.3,  $p = 62.9 \text{ mg kg}^{-1}$ , C/N = 8.6, and particle-size distribution with an average of 60.5, 22.5, and 17% of sand, silt and clay, respectively (sandy clay loam) (Cooper J. et al., 2011; Bilsborrow et al., 2013). The soil mineralogical composition across the farm is predominantly composed of quartz, with 1:1 clay minerals (kaolinite) and minor illite, and alkali feldspar, particularly in subsoil layers (>0.30 m) (Supplementary Table S1). Coal fragments, derived from till via glacial erosion of Carboniferous rocks, occurs in the soil, but insufficiently to confound measurement of non-geological SOC (Wang et al., 2022).

The experiment was established in 2001 in an area previously in a conventional arable rotation. It compares organic *versus* conventional crop rotation (RT), fertility sources (FS) and crop protection (CP) in a factorial design following either current United Kingdom conventional farming best practices (Red Tractor Assurance, 2015) or certified organic production practices (Soil Association, 2019). Full details on the design and past management of the trial can be found in several previous publications (Cooper J. M. et al., 2011; Cooper et al., 2011 J.; Eyre et al., 2011; 2012; McKenzie et al., 2011; Bilsborrow et al., 2013). Briefly, there are four replicate blocks in the field, each containing four sub-experiments with rotations beginning in different years.

This was done to allow a diversity of crops to be present in the field in any given year. This study used samples from experiment No. 1 only. The 8-year conventional rotation (CONV-RT) is cereal-intensive beginning with 2 years of grass-clover ley while the organic rotation (ORG-RT) is more diverse consisting of 3 years of grass-clover ley followed by a mixture of cereals, spring beans, potatoes and vegetables (Table 1). The two levels of fertility source consist of mineral (MINE-FS) inorganic NPK fertiliser applied at rates recommended in the England Nutrient Management Guide (RB209, 2011) *vs.* compost (COMP-FS) fertilisation where only composted dairy manure is applied to the arable crops in the rotation at N rates equivalent to the MINE-FS treatment based on compost total N contents. While the NFSC includes both organic and conventional crop protection treatments, for the purposes of this study only plots under conventional crop protection were included (Supplementary Table S2). The main crop rotation plots are each 12 m × 96 m in size. These are subdivided into two crop protection sub-plots (12 m × 48 m) and further subdivided into two fertility source sub-sub-plots (12 m × 24 m). Figure 1 shows the layout of these plots in Block 1 of the experiment as an example. A full layout of the NFSC experiment is provided in Supplementary Figure S2. Further details of treatments used during the timeframe of this study including crops grown in the rotations every year and rates of fertilisation, are given in Table 1. Crop protection details are shown in Supplementary Table S2.

### 2.2 Soil sampling and sample preparation for soil analyses

Soil sampling was conducted in the same sub-experiment No. 1 at the beginning of the rotation (March 2011) and at the last year of the rotation (March 2018). In both years, soil sampling was conducted during the grass-clover ley periods (i.e., in 2011, it was carried out just before wheat planting and in 2018, just before the ley phase-out). In each one of the 16-target treatment plots, six intact soil cores (0–0.60 m depth) were taken in 2 × 3 grid spaced at 6 m apart to obtain a representative sample for the plot (Figure 1). The soil cores were collected using a hydraulic soil sampler (Atlas Copco Ltd., Hemel Hempstead, Hertfordshire, United Kingdom) and a metallic tube (1 m length, 0.30 m inner diameter). The collected cores were separated into two soil depth intervals (0–0.30 and 0.30–0.60 m) totalling 384 soil samples (192 collected in 2011 and 192 collected in 2018). Each one of the soil samples was gently mixed and passed through a 4 mm sieve; large stones were removed and weighed plant remains were discarded. The weight of the sieved fresh soil was then recorded. A subsample of the sieved soil (~5 g) was used for determination of gravimetric water content. The soil bulk density (BD) was calculated using the core method adjusting for the weight and volume of large stones (Blake and Hartge, 1986). Soil pH of samples was measured in H<sub>2</sub>O (1: 2.5 soil: solution), following analytical procedures described in Mc Lean (1982). After BD determination and pH measurement, the six soil samples taken from the same plot, sampling year and depth interval were pooled into a composite sample. This resulted in 64 composite soil samples, 32 for each sampled year, which were wet sieved through a 2 mm sieve and air-dried before further analyses.

**TABLE 1** Crop sequence, fertility inputs, and management details for conventional and organic rotations (conventional-CONV-RT vs. organic-ORG-RT) and fertility sources (mineral-MINE-FS vs. compost-COMP-FS) in the NFSC trial between 2008 and 2018. GC: perennial ryegrass/white clover ley; W = winter; S = spring; AN = ammonium nitrate; TSP = triple super phosphate; KCl = muriate of potash; 0-20-30 compound fertiliser containing 20% P<sub>2</sub>O<sub>5</sub> and 30% K<sub>2</sub>O.

	Year	2008 <sup>a</sup>	2009 2010	2011	2012	2013	2014	2015	2016	2017 2018
Fertility Source	CONV-RT	W Barley	GC <sup>b</sup>	S Wheat	W Wheat	W Barley	Potato	W Wheat	Spelt/Rye <sup>c</sup>	GC
MINE-FS	AN (kg N ha <sup>-1</sup> )	170	284	180	210	170	250	170	100	284
	0-20-30 (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	64			64	64		64		
	0-20-30 (kg K <sub>2</sub> O ha <sup>-1</sup> )	96			96	96		96		
	TSP (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		50				134			50
	KCl (kg K <sub>2</sub> O ha <sup>-1</sup> )		127				200			127
COMP-FS	C:N ratio	10.8		14.4	13 <sup>d</sup>	13	13			
	Compost (t DM ha <sup>-1</sup> )	4.4		5.7	6.5	5.3	5.6			
	Carbon (t C ha <sup>-1</sup> )	1.8		2.4	2.7	2.2	2.3			
	ORG-RT	GC	GC	S Wheat	Potato	S Beans	Cabbage	S Barley	GC	GC
MINE-FS	AN (kg N ha <sup>-1</sup> )	284	284	180	250		250	120	284	284
	0-20-30 (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )					60	100			
	0-20-30 (kg K <sub>2</sub> O ha <sup>-1</sup> )					90	150			
	TSP (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	50	50		134				50	50
	KCl (kg K <sub>2</sub> O ha <sup>-1</sup> )	127	127		200				127	127
COMP-FS	C:N ratio			14.4	13		13	13		
	Compost (t DM ha <sup>-1</sup> )			5.7	7.8		7.8	3.8		
	Carbon (t C ha <sup>-1</sup> )			2.4	3.2		3.2	1.6		

<sup>a</sup>For autumn sown crops year represents the harvest season.

<sup>b</sup>N fertilizer was split into three applications.

<sup>c</sup>W wheat was replaced with spelt or rye in the conventional rotation only in 2016.

<sup>d</sup>For 2012, 2013 and 2014 harvest seasons mean values for compost from the study period were used. Management for all crops: For all cereal crops straw was baled and removed from the plots following harvest. For grass and clover following the establishment year plots were harvested for silage three times. Potato residues were desiccated and left in the field. Soil preparation for all crops consisted of mouldboard ploughing (~25 cm depth) and discing prior to planting.

## 2.3 Total carbon and stocks calculation

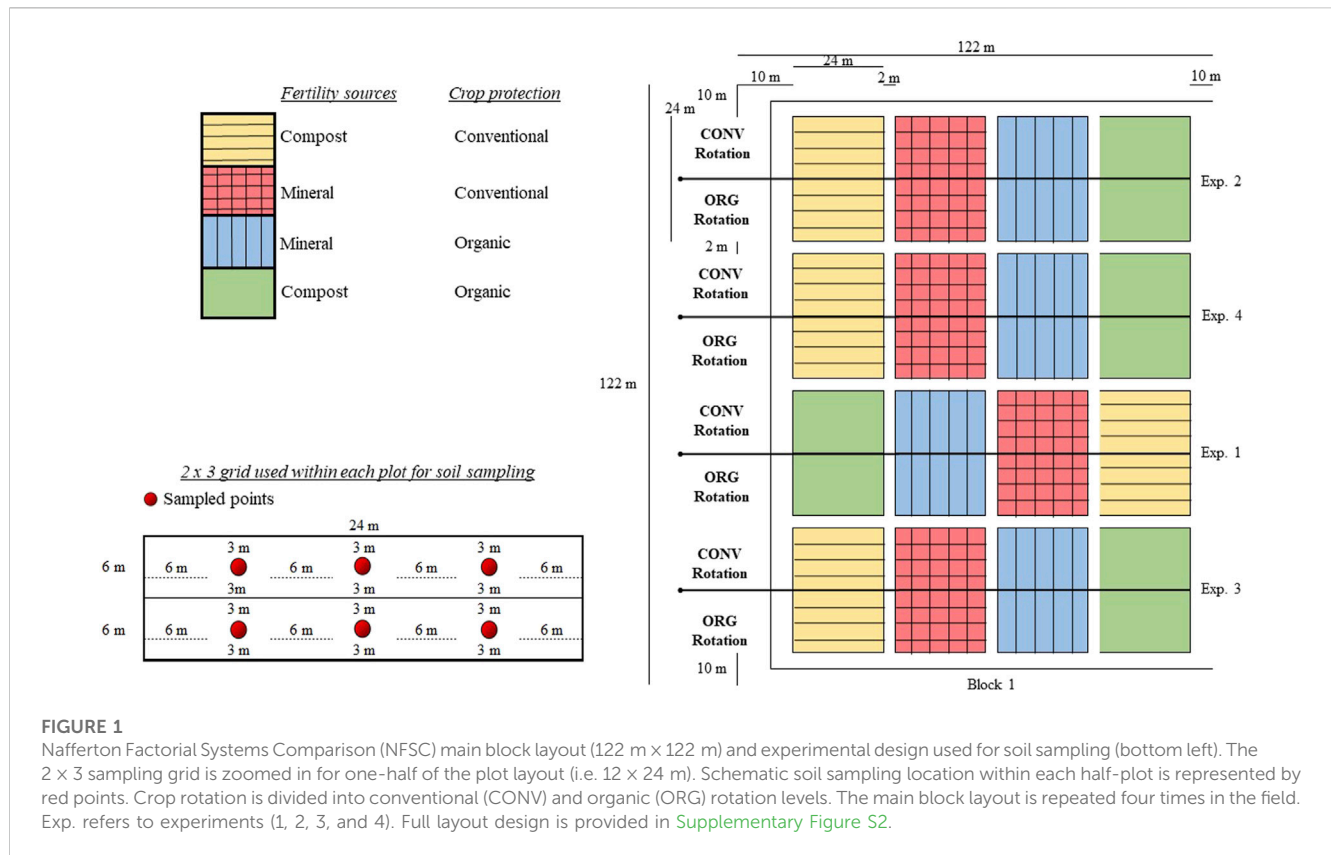
Soil carbon (C) concentration was determined by dry combustion (Nelson and Sommers, 1996), post-combustion and reduction tubes in an Elementar Vario Macro Cube analyser (furnace at 960°C in pure oxygen). SOC stocks per unit of area (Mg ha<sup>-1</sup>) were calculated for each depth interval (i.e. 0-0.30 and 0.30-0.60 m) on an equivalent soil mass basis (Wendt and Hauser, 2013) using the 2011 samples as a reference. The difference in SOC stocks between 2011 and 2018 samples were used to calculate accumulation or reduction rate during one rotation cycle (i.e., in 8-year period).

## 2.4 Physical fractionation of SOM

The method of SOM fractionation used in this study was primarily used by Christensen (1985), later described by Cambardella and Elliott (1992) and further adjusted by C. Feller and Beare (1997). The method is also further discussed and clarified in Christensen (1992). The method separates soil particles, often by

using high-density liquids (often between 1.6–2.0 g cm<sup>-3</sup>), into organic (particulate organic matter; POM >53 μm) and mineral-associated fractions (heavy fraction and silt and clay fraction; HF > 53 μm and SC < 53 μm, respectively) by dispersion, wet sieving, flotation and sedimentation, followed by a subsequent mass balance check (Christensen, 1992; 2001). In this study, however, it was chosen to not use any chemicals-based high density liquid but only Milli-Q water during the procedures, to minimise disruption of the chemical structure of the original SOM and reduce risk of chemical contamination (Lehmann and Kleber, 2015). This approach also avoids possible interferences in TG-DSC-QMS and Py-GC/MS-TMAH analysis. While the use of Milli-Q water, rather than high-density liquids, has raised some concerns, Poeplau et al. (2018) pointed out that the use of a liquid with density of 1 g cm<sup>-3</sup> performed in the best top 5 out of 20 methods. The only potential drawback is that soil particles might not be completely dispersed, which can result in inconsequential retention of mass and/or C in fractions to which they do not belong (von Lützow et al., 2007; Lavalley et al., 2019).

In summary, 20 g of each air-dried soil sample (i.e., for all 64 samples) was sonicated in 70 mL of Milli-Q water at 500 W for



**FIGURE 1**

Nafferton Factorial Systems Comparison (NFSC) main block layout (122 m × 122 m) and experimental design used for soil sampling (bottom left). The 2 × 3 sampling grid is zoomed in for one-half of the plot layout (i.e. 12 × 24 m). Schematic soil sampling location within each half-plot is represented by red points. Crop rotation is divided into conventional (CONV) and organic (ORG) rotation levels. The main block layout is repeated four times in the field. Exp. refers to experiments (1, 2, 3, and 4). Full layout design is provided in [Supplementary Figure S2](#).

15 min (providing approximately 13 J per sample or 144 J mL<sup>-1</sup>) using an ultrasonic processor (Model VC-505; Sonics Vibra Cell). After sonication, the sample was wet sieved through a 53 μm sieve using Milli-Q water. The HF and POM fractions were retained in the sieve and were separated by flotation and sedimentation using Milli-Q water (1 g cm<sup>-3</sup>). This procedure generated 192 fraction samples (64 samples × 3 fractions). Each fraction was oven-dried at 40°C and their weights recorded. SOC concentration of each fraction was determined following the preparation and dry combustion methods described above. For quality assurance, the final recovery of the soil mass was checked against the original 20 g and the recovery of the elemental analysis for the fractions was checked against SOC concentrations from the <2 mm samples ([Supplementary Table S3](#) and [Supplementary Figures S3, S4](#)). SOC concentration and the masses of each fraction were used for the calculation of SOC in each fraction and the results were reported on a per kilogram bulk soil basis (g C kg<sup>-1</sup>). SOC concentrations of the individual fractions and their recovery soil masses are given in [Supplementary Table S4](#).

## 2.5 Thermogravimetry-differential scanning calorimetry-quadrupole mass spectrometry (TG-DSC-QMS)

Thermal analysis was used to examine the relative proportions of different “fractions” of C in the soil samples, termed the labile, recalcitrant and refractory fractions following the methods described by [Lopez-Capel et al. \(2005a\)](#); [Fernández et al. \(2012\)](#). The samples were analysed using thermogravimetry (TG) and

differential scanning calorimetry (DSC), combined with quadrupole mass spectrometry (QMS) analysis of the gas evolved during thermal decomposition. While TG and DSC data quantifies the weight change and the gain/loss in energy of the sample during heating, QMS analysis provides data on the chemical composition of the gaseous combustion products, which can be used to characterise the sample in terms of its organic and inorganic components.

Sixteen soil samples were selected for TG-DSC-QMS analysis, one composite soil sample per treatment per depth per year (i.e., considering the combination of treatment factors): i) conventional crop rotation with mineral fertilisation source (CONV-M); ii) conventional crop rotation with compost fertilisation source (CONV-C); iii) organic crop rotation with mineral fertilisation source (ORG-M); and iv) organic crop rotation with compost fertilisation source (ORG-C). The samples were selected with reference to the mean total C content obtained by the dry combustion method such that the sample selected for analysis had a total C content closest to the mean ([Supplementary Table S5](#)).

An aliquot of each sample (ca. 50 mg) was weighed accurately into an alumina crucible and analysed using a Netzsch Jupiter STA 449C thermogravimetry-differential scanning calorimetry (TG-DSC) analyser. Samples were heated from 25°C to 1,000°C at a rate of 10°C min<sup>-1</sup> in an (oxidizing) atmosphere of 20% oxygen and 80% helium (purge gas, flow rate 30 mL min<sup>-1</sup>). The protective gas was helium (flow rate 20 mL min<sup>-1</sup>). TG and DSC data were acquired and processed using Netzsch *Proteus* 61 software. For mass spectrometric analysis, the evolved gas stream was sampled continuously through a fused silica capillary transfer line

connected to a Netzsch Aeolos 403C quadrupole mass spectrometer (QMS). Adapter heads and the transfer line (between the Jupiter and Aeolos) were at 150°C. The QMS was operated in full scan mode over the range  $m/z$  10–160 and the dwell time was 0.2 s, giving a sampling rate of ca. 1 scan per 5°C increase in temperature. Mass spectrometric data were acquired and processed using Aeolos software.

In short, TG-DSC was used to determine the relative proportions of labile, recalcitrant and refractory C fractions by comparing the total weight loss over the temperature range 200°C–750°C (Exotot) with its relative proportions from the defined intervals: i) 200°C–350°C (Exo 1), ii) 350°C–500°C (Exo 2) and iii) 500°C–750°C (Exo 3). These temperatures were established based on the first derivatives of the DSC traces (i.e., distinct exothermic reactions), in accordance with the methods described by Dell'Abate et al. (2000); Dell'Abate et al. (2002). Additionally, we have used  $m/z$  18 (water) to distinguish the different organic matter pools (Supplementary Figure S5). Briefly, thermal decomposition between 200°C to approximately 350°C will release relatively volatile and labile forms of C, whilst decomposition between 350°C–650°C will release more recalcitrant and refractory C forms, such as lignin and related biopolymers (Plante et al., 2009). Soil carbonate minerals, if any, decompose at 750°C–800°C. The curves of the gas evolution (i.e., the QMS data) were interpreted in order to assess the contribution of individual peaks into the overall trace (Arenillas et al., 1999). The main ion of interest in the QMS analysis was  $m/z$  44 (carbon dioxide). For each sample, the QMS data for the selected ion ( $m/z$  44) were normalised to the total ion intensity to allow comparison of different samples (Arenillas et al., 1999). The corresponding variation in abundance of the  $m/z$  44 with the variation in TG and DSC curves was used to verify the organic origin of the three fractions and differentiate these from the decomposition of carbonate minerals (inorganic carbon). The same intervals considered in the TG-DSC approach (200°C–350°C, 350°C–500°C, 500°C–750°C) were used to seek CO<sub>2</sub> peaks and to calculate the area under the peaks, representing the proportion of C released.

## 2.6 Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

The same sixteen soil samples used for TG-DSC-QMS were subjected to Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) analysis (Supplementary Table S5). Whilst Py-GC-MS has allowed comparison of SOM produced under different environments and land uses (Buurman and Roscoe, 2011; Oliveira et al., 2016), the highly polar pyrolysis products from biopolymers can be either difficult or impossible to detect by Py-GC-MS analysis (Challinor, 1989; Kaal and Janssen, 2008). To address this potential deficiency, on-line thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) has been used together with Py-GC-MS. Accordingly, phenolic compounds formed from the TMAH-induced cleavage of ether and ester bonds, which are present in soils from plant-derived

macromolecular organic C, can be also characterised (Mason et al., 2012). In this sense, all Py-GC-MS analyses in this study were conducted by THM in the presence of TMAH following adapted analytical procedures described by Abbott et al. (2013).

Analytical procedures involved the use of a Frontier Laboratories Single-shot Pyrolyser Model PY-3030S. The pyrolyser was connected to an HP 6890 gas chromatograph (GC) and interfaced to an HP 5973 MSD. The pyrolysis temperature and time were 610°C and 1 min, respectively. The GC inlet was heated at 320°C and the sample was injected in split mode with a split ratio of 30:1. Gas chromatographic separation of compounds was performed using a Phenomenex ZB-5MS (Torrance, CA, United States of America) fused silica capillary column (60 m × 0.25 mm i.D. × 0.25 μm film thickness). The GC oven temperature program was 50°C (initial hold time 1 min) then 4°C min<sup>-1</sup> to 320°C (final hold time 10 min). Helium was used as carrier gas at a constant flow rate of 1 mL min<sup>-1</sup>. The GC-MS was operated in full scan mode, scanning the range  $m/z$  50–650. Operating conditions were; electron voltage 70 eV, emission current 35 μA, source temperature 230°C, quadrupole temperature 150°C, multiplier voltage 2200 V and interface temperature 320°C. All the analytical procedures were conducted in triplicate so that analytical reproducibility could be checked.

Data acquisition and processing were performed using Agilent Chemstation software and pyrolysis products were identified using the Chemstation NIST05 library of mass spectra. All prominent, identifiable, products of each sample were quantified relative to the internal standard and reported as a proportion of the total peak area of the identified characteristic ions (i.e.,  $m/z$  values). The identified products were grouped into *n*-alkanes, *n*-alkenes, aromatics, benzofurans, carbohydrates, fatty acids, lignin phenols, N containing compounds, phenols and polycyclic aromatic hydrocarbons (polyaromatics). These groups were defined based on the origin and chemical similarity of the identifiable products.

## 2.7 Statistical analyses

Linear mixed-effects models were fitted to test the main effects of crop rotation (RT); i) conventional (CONV-RT) vs. organic (ORG-RT); fertility sources (FS); mineral (MINE-FS) vs. compost (COMP-FS), year of sampling (YR) (2011 and 2018) and their interactions (RT\*FS\*YR) on soil organic C stocks (SOC stock) and C in the SOM fractions (POM >53 μm, HF > 53 μm and SC < 53 μm). Results from the Thermogravimetry-Differential Scanning Calorimetry-Quadrupole Mass Spectrometry (TG-DSC-QMS) and the Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) analyses were only used to elucidate processes, and therefore were not statistically assessed.

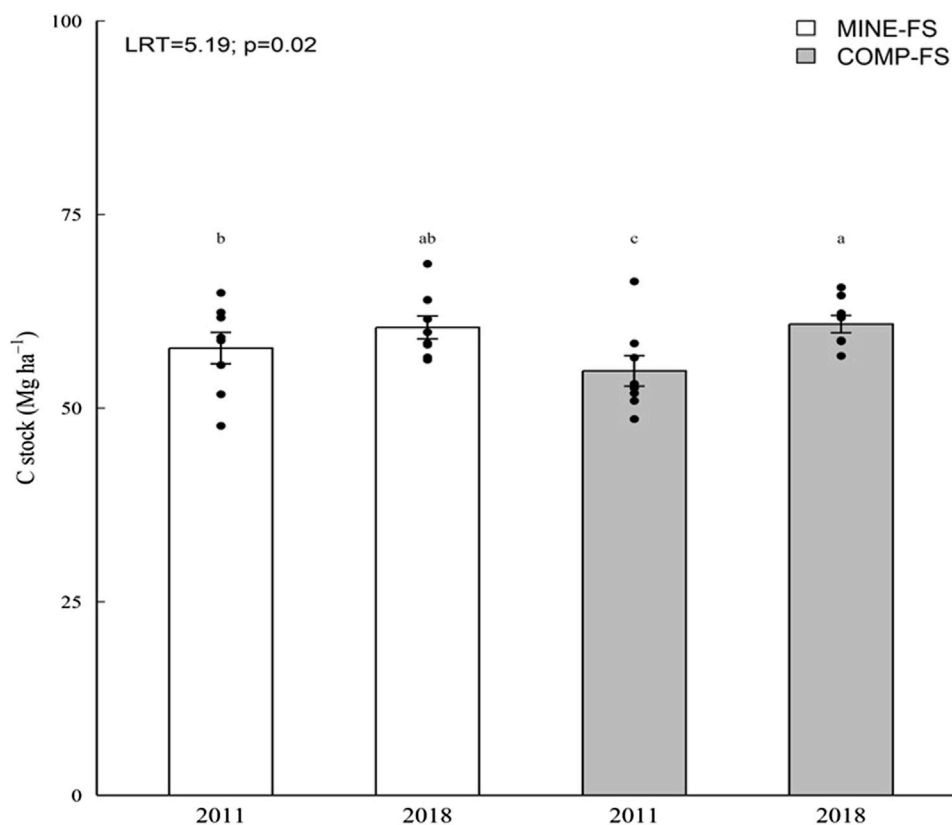
For all models, fixed effects were crop rotation, fertility sources, year of sampling and their three-way interaction. The random effect was defined as block, with plot nested to it to account for repeated measures (i.e., years of sampling). The analyses were conducted separately for each depth interval (i.e. 0–0.30 and 0.30–0.60 m). Assumptions were checked for normality and equal variances by examining the QQ plots of

**TABLE 2** Main effect means for crop rotation (RT) (conventional-CONV-RT vs. organic-ORG-RT), fertility sources (FS) (mineral-MINE-FS vs. compost-COMP-FS), and years of sampling (YR) (2011 and 2018). Significant of the main effects and their interactions on soil organic C stocks (SOC stock) at 0–0.30 and 0.30–0.60 m soil depth intervals.

Depth m		C Stock
		— Mg ha <sup>-1</sup> —
0–0.30	<i>Crop rotation</i>	
	CONV-RT	57.20 (1.08)
	ORG-RT	59.71 (1.44)
	<i>Fertility source</i>	
	MINE-FS	59.08 (1.26)
	COMP-FS	57.83 (1.35)
	<i>Year</i>	
	2011	56.27 (1.42)
	2018	60.64 (0.90)
	<i>Main effects and interactions</i>	<i>Likelihood ratio tests and p-value</i>
	RT	LRT = 4.45; <b>p = 0.03</b>
	FS	LRT = 5.02; <b>p = 0.02</b>
	YR	LRT = 16.95; <b>p &lt; 0.01</b>
	RT*FS	LRT = 2.24; <i>p</i> = 0.13
RT*YR	LRT = 0.14; <i>p</i> = 0.71	
FS*YR	LRT = 5.19; <b>p = 0.02</b>	
RT*FS*YR	LRT = 0.05; <i>p</i> = 0.83	
0.30–0.60	<i>Crop rotation</i>	
	CONV-RT	33.84 (1.46)
	ORG-RT	36.70 (1.28)
	<i>Fertility source</i>	
	MINE-FS	35.47 (1.17)
	COMP-FS	35.06 (1.64)
	<i>Year</i>	
	2011	34.23 (1.31)
	2018	36.30 (1.48)
	<i>Main effects and interactions</i>	<i>Likelihood ratio tests and p-value</i>
	RT	LRT = 6.41; <b>p = 0.01</b>
	FS	LRT = 0.14; <i>p</i> = 0.71
	YR	LRT = 3.47; <i>p</i> = 0.06
	RT*FS	LRT = 0.42; <i>p</i> = 0.52
RT*YR	LRT = 0.04; <i>p</i> = 0.85	
FS*YR	LRT = 0.62; <i>p</i> = 0.43	
RT*FS*YR	LRT = 0.57; <i>p</i> = 0.45	

Data are measured mean values of main effects ( $n = 32$  for crop rotation, fertility sources and years of sampling within individual soil depth intervals). The standard error of the mean is in parentheses. Significance tests, using likelihood ratio tests (LRT), are comparing models with or without the parameter of interest. Significant effects ( $p < 0.05$ ) are shown in bold.





**FIGURE 2**

Interactive effects between fertility sources (mineral-MINE-FS and compost-COMP-FS) and years of sampling (2011 and 2018) on soil organic C stocks (C stock Mg ha<sup>-1</sup>) in the 0–0.30 m. Data are measured mean values  $\pm$ SE ( $n = 8$  for crop rotation schemes, fertility sources and years of sampling). Significance tests, using likelihood ratio test (LRT), are comparing models with or without the parameter of interest.

residuals (for both fixed and random effects compartments of the model) and scatterplots of standardised against fitted values. The data were Tukey's Ladder of Powers transformed when visual breakdowns in the model assumptions were revealed by residual plots. To test the significance of the fixed effects on the dependent variables, models were compared with and without the factor of interest using the likelihood ratio tests (LRT) approach. When the interaction term in the model was significant, Tukey's HSD *post hoc* test was carried out and a significant effect was determined at  $p < 0.05$ . All statistical analysis was carried out in the R programming language 3.4.3 (Team, 2019) using the additional packages, ape (Paradis et al., 2004), nlme (Pinheiro et al., 2018), plyr (Wickham, 2011), ggplot2 (Wickham, 2009), and multcomp (Hothorn et al., 2008) and following statistical approaches described by Zuur et al. (2009).

## 3 Results

### 3.1 SOC stocks

For the 0–0.30 m depth, there was an interactive effect between FS and YR affecting SOC stocks (LRT = 5.19;  $p = 0.02$ , respectively; Table 2). This has shown that COMP-FS significantly increased SOC

stocks over time (i.e., from 2011 to 2018) from  $54.81 \pm 1.98$  to  $60.86 \pm 1.11$  Mg ha<sup>-1</sup>, suggesting an accumulation mean of 11% at a rate of  $0.76$  Mg ha yr<sup>-1</sup>. MINE-FS, on the other hand, increased SOC stocks over the years from  $57.74 \pm 2.03$  to  $60.42 \pm 1.48$  Mg ha<sup>-1</sup>, i.e., SOC stock accumulation mean of approximately 5% and C accumulation rate of  $0.33$  Mg ha yr<sup>-1</sup>, which were not statistically verified (Figure 2). SOC stocks were also higher in the ORG-RT compared with CONV-RT (approximately 5%), regardless of the FS (MINE or COMP) or YR (2011 or 2018) (LRT = 4.45;  $p = 0.03$ ) (Table 2). In an 8-year rotation, this translates into SOC accumulation rate of  $0.31$  Mg ha yr<sup>-1</sup>.

For deeper soil layers (0.30–0.60 m), SOC stocks were only affected by RT (LRT = 6.41;  $p = 0.01$ ), where ORG-RT showed higher SOC stocks than CONV-RT regardless of the FS or YR (Table 2).

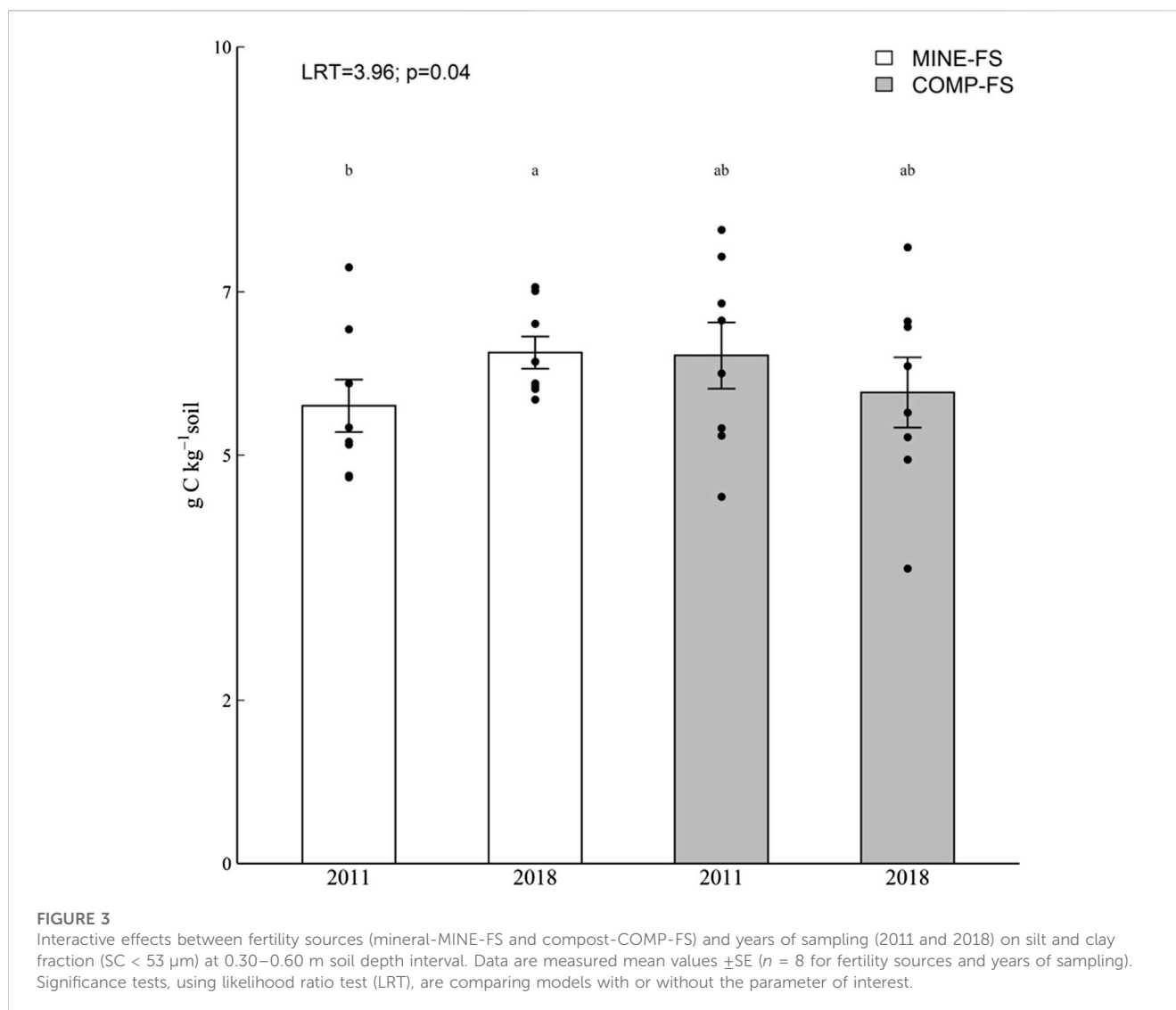
### 3.2 SOC distribution in SOM fractions

The average mass balance recovery of physical fractionation ranged between 97% and 98% (Supplementary Table S3), which indicates technique reliability, particularly in terms of mass recovery, for assessing SOM fractions. Whilst most of the soil mass was found in the HF fraction, higher SOC concentration

**TABLE 3** Main effect means for crop rotation (RT) (conventional-CONV-RT vs. organic-ORG-RT), fertility sources (FS) (mineral-MINE-FS vs. compost-COMP-FS), years of sampling (YR) (2011 and 2018). Significance of the main effects and their interactions on soil organic C concentrations (g per kg<sup>-1</sup> soil) in the organic fraction (particulate organic matter-POM >53 μm), heavy fraction (HF > 53 μm) and mineral-associated fraction (silt and clay fraction-SC < 53 μm) at 0–0.30 and 0.30–0.60 m soil depth intervals.

Depth		POM (>53 μm)	HF (>53 μm)	SC (<53 μm)
m		g kg <sup>-1</sup>		
0–0.30	<i>Crop rotation</i>			
	CONV-RT	1.24 (0.05)	6.68 (1.03)	9.59 (0.55)
	ORG-RT	1.34 (0.09)	6.78 (0.95)	9.14 (0.37)
	<i>Fertility source</i>			
	MINE-FS	1.28 (0.06)	7.86 (1.17)	9.22 (0.42)
	COMP-FS	1.30 (0.08)	5.60 (0.64)	9.51 (0.52)
	<i>Year</i>			
	2011	1.34 (0.09)	6.27 (1.06)	8.86 (0.53)
	2018	1.23 (0.04)	7.19 (0.90)	9.87 (0.37)
	<i>Main effects and interactions</i>	<i>Likelihood ratio and p-value</i>		
	RT	LRT = 0.44; <i>p</i> = 0.50	LRT = 0.12; <i>p</i> = 0.73	LRT = 1.05; <i>p</i> = 0.30
	FS	LRT = 0.01; <i>p</i> = 0.95	LRT = 3.71; <b><i>p</i> = 0.05</b>	LRT = 0.39; <i>p</i> = 0.53
	YR	LRT = 0.37; <i>p</i> = 0.54	LRT = 1.05; <i>p</i> = 0.30	LRT = 4.63; <b><i>p</i> = 0.03</b>
	RT*FS	LRT = 0.27; <i>p</i> = 0.60	LRT = 1.27; <i>p</i> = 0.26	LRT = 3.00; <i>p</i> = 0.08
	RT*YR	LRT = 0.01; <i>p</i> = 0.98	LRT = 0.01; <i>p</i> = 0.93	LRT = 0.74; <i>p</i> = 0.39
	FS*YR	LRT = 0.01; <i>p</i> = 0.95	LRT = 0.73; <i>p</i> = 0.39	LRT = 3.04; <i>p</i> = 0.08
	RT*FS*YR	LRT = 0.20; <i>p</i> = 0.66	LRT = 2.40; <i>p</i> = 0.12	LRT = 1.81; <i>p</i> = 0.18
0.30–0.60	<i>Crop rotation</i>			
	CONV-RT	0.61 (0.11)	5.41 (1.00)	6.10 (0.30)
	ORG-RT	0.44 (0.03)	4.24 (0.47)	5.83 (0.19)
	<i>Fertility source</i>			
	MINE-FS	0.45 (0.03)	5.69 (0.98)	5.93 (0.20)
	COMP-FS	0.61 (0.11)	3.96 (0.45)	5.99 (0.29)
	<i>Year</i>			
	2011	0.47 (0.04)	5.66 (0.98)	5.91 (0.26)
	2018	0.58 (0.10)	3.99 (0.45)	6.01 (0.24)
	<i>Main effects and interactions</i>	<i>Likelihood ratio and p-value</i>		
	RT	LRT = 2.19; <i>p</i> = 0.14	LRT = 0.10; <i>p</i> = 0.75	LRT = 1.42; <i>p</i> = 0.23
	FS	LRT = 1.36; <i>p</i> = 0.24	LRT = 2.51; <i>p</i> = 0.11	LRT = 0.17; <i>p</i> = 0.68
	YR	LRT = 0.60; <i>p</i> = 0.44	LRT = 4.20; <b><i>p</i> = 0.04</b>	LRT = 1.36; <i>p</i> = 0.24
	RT*FS	LRT = 0.02; <i>p</i> = 0.88	LRT = 2.68; <i>p</i> = 0.10	LRT = 0.03; <i>p</i> = 0.86
	RT*YR	LRT = 1.08; <i>p</i> = 0.30	LRT = 1.34; <i>p</i> = 0.25	LRT = 1.90; <i>p</i> = 0.17
	FS*YR	LRT = 2.35; <i>p</i> = 0.12	LRT = 2.05; <i>p</i> = 0.15	LRT = 3.96; <b><i>p</i> = 0.04</b>
	RT*FS*YR	LRT = 0.07; <i>p</i> = 0.79	LRT = 3.80; <i>p</i> = 0.06	LRT = 2.03; <i>p</i> = 0.15

Data are measured mean values of main effects (*n* = 32 for crop rotation, fertility sources and years of sampling within individual fractions and soil depth intervals). Standard error of the mean is in parentheses. Significance tests, using likelihood ratio tests (LRT), are comparing models with or without the parameter of interest. Significant effects (*p* < 0.05) are shown in bold.



was found in the SC (<53 μm) than either of the other >53 μm fractions (POM or HF), regardless of soil depth interval (0–0.30 or 0.30–0.60 m), RT, FS or YR (Table 3; Supplementary Figure S4).

For the 0–0.30 m depth, although SOC concentration in the POM was numerically higher in 2011 than in 2018 samples, the difference was not statistically significant (LRT = 0.37, *p* = 0.54). The MINE-FS had higher SOC concentration in the HF (>53 μm) fraction compared to the COMP-FS (LRT = 3.71; *p* = 0.05). In 2018, SOC concentration was higher in the SC fraction compared to 2011 (LRT = 4.63; *p* = 0.03) (Table 3).

For the 0.30–0.60 m depth, SOC concentration in the POM was not affected by RT, FS or YR (*p* > 0.05). SOC concentration in the HF (>53 μm) fraction was affected by YR, showing a significant increase from 2011 to 2018 (LRT = 4.20; *p* = 0.04) irrespective of the RT and FS (Table 3). In the same depth interval (i.e. 0.30–0.60 m), FS and YR interacted resulting in an increased SOC concentration in the SC over time (i.e., from 2011 to 2018) from  $5.60 \pm 0.48$  to  $6.26 \pm 0.27$  g C kg<sup>-1</sup> under MINE-FS, whilst under COMP-FS decreased SOC concentration in the SC fraction from  $6.22 \pm 0.53$  to  $5.77 \pm 0.65$  g C kg<sup>-1</sup> (LRT = 3.96; *p* = 0.04) (Figure 3).

### 3.3 Thermogravimetry-differential scanning calorimetry-quadrupole mass spectrometry (TG-DSC-QMS)

Total weight loss and relative weight loss values from different temperature intervals (Exo 1 – 200°C–350°C; Exo 2 – 350°C–500°C; and Exo 3 – 500°C–750°C), which represent material loss during heating (e.g., labile, recalcitrant and refractory), are given in Table 4.

For the 0–0.30 m depth, soil samples showed discrete weight loss variability between the treatments, with labile (Exo 1) and recalcitrant plus refractory (i.e., the sum of Exo 2 + Exo 3) fractions being evenly distributed within the samples (approximately 50/50). In general, ORG-RT, MINE-FS and samples collected in 2018 showed a slightly more weight loss in the labile fraction compared to their counterparts CONV-RT, COMP-FS and samples collected in 2011 (Exo 1). Likewise, ORG-RT and samples collected in 2018 showed a slightly more weight loss in the refractory fraction compared to their counterparts CONV-RT and samples collected in 2011 (i.e., Exo 2 + Exo 3), while

**TABLE 4** Changes in total weight loss (50°C–800°C), weight loss for the temperature interval 200°C–750°C (Exotot) and relative weight losses of temperature intervals 200°C–350°C (Exo 1), 350°C–500°C (Exo 2) and 500°C–750°C (Exo 3) as a result of different crop rotation (conventional-CONV-RT or organic-ORG-RT), fertility sources (mineral-MINE-FS or compost-COMP-FS) and years of sampling (YR) (2011 and 2018).

Depth		Total weight loss	Exotot	Exo 1	Exo 2	Exo 3
m		(50°C–800°C)	(200°C–750°C)	(200°C–350°C)	(350°C–500°C)	(500°C–750°C)
		%				
0–0.30	<i>Crop rotation</i>					
	CONV-RT	5.58 (0.26)	4.69 (0.19)	46.79 (0.51)	36.85 (0.38)	15.63 (0.28)
	ORG-RT	5.82 (0.12)	4.87 (0.09)	47.52 (0.34)	37.15 (0.27)	16.06 (0.71)
	<i>Fertility source</i>					
	MINE-FS	5.85 (0.18)	4.89 (0.12)	47.79 (0.33)	36.97 (0.42)	15.24 (0.43)
	COMP-FS	5.55 (0.21)	4.67 (0.16)	46.52 (0.28)	37.03 (0.24)	16.45 (0.44)
	<i>Year</i>					
	2011	5.44 (0.21)	4.59 (0.15)	46.95 (0.50)	36.63 (0.36)	16.41 (0.44)
	2018	5.96 (0.07)	4.97 (0.05)	47.35 (0.43)	37.37 (0.10)	15.28 (0.46)
	0.30–0.60	<i>Crop rotation</i>				
CONV-RT		4.29 (0.33)	3.48 (0.25)	34.80 (2.70)	38.76 (1.01)	26.43 (1.90)
ORG-RT		3.98 (0.26)	3.25 (0.18)	27.26 (1.74)	41.04 (0.72)	31.69 (1.05)
<i>Fertility source</i>						
MINE-FS		4.04 (0.29)	3.28 (0.20)	31.97 (3.37)	39.88 (0.93)	28.15 (2.51)
COMP-FS		4.23 (0.32)	3.44 (0.24)	30.09 (2.79)	39.93 (1.24)	29.97 (1.57)
<i>Year</i>						
2011		3.74 (0.28)	3.07 (0.20)	33.62 (3.48)	38.97 (1.18)	27.40 (2.43)
2018		4.54 (0.09)	3.65 (0.09)	28.44 (1.78)	40.83 (0.65)	30.72 (1.25)

Data are measured mean values of main effects ( $n = 8$  for crop rotation, fertility sources and years of sampling within soil depth intervals). Standard error of the mean is in parentheses.

COMP-FS outperformed MINE-FS at the same temperature intervals (i.e., Exo 2 + Exo 3) (Table 4).

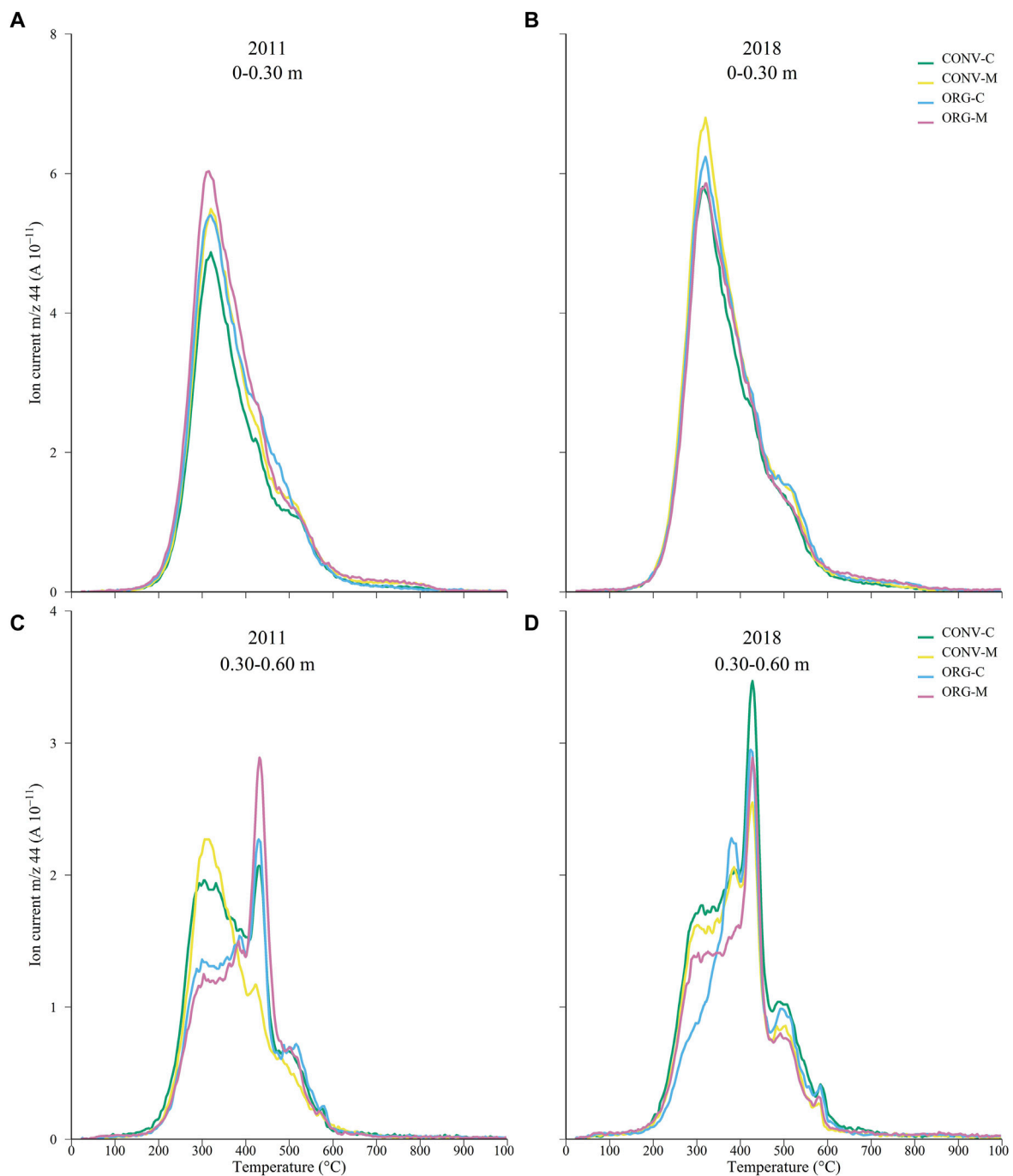
For deeper soil layers (0.30–0.60 m), more disparity in weight loss was observed between the treatments, with recalcitrant plus refractory fractions (Exo 2 + Exo 3) dominating over the labile fractions (Exo 1) (Table 4). CONV-RT, MINE-FS and samples collected in 2011 showed more weight loss in the labile fraction compared to their counterparts ORG-RT, COMP-FS and samples collected in 2018 (Exo 1). The opposite was observed for the refractory organic matter (Exo 2 + Exo 3), i.e., ORG-RT, COMP-FS and samples collected in 2018 showing more weight loss in the refractory fraction than CONV-RT, MINE-FS and samples collected in 2011 (Table 4).

These differences are highlighted by the differential scanning calorimetry analysis (DSC traces), which showed three exothermic peaks between 200°C and 600°C in the topsoil (0–0.30 m), characterised by a distinct peak at 300°C–350°C and two other broad peaks, one at 400°C–450°C and another at 500°C–550°C (Supplementary Figure S6A, B). Subsoil (0.30–0.60 m) samples also showed three exothermic peaks characterised by a distinct peak at 400°C–450°C and two other broad peaks, one at 300°C–350°C and another at 500°C–550°C (Supplementary Figure

S6C, D). Regardless of the RT, FS or YR, all samples showed an endothermic peak at approximately 570°C–580°C for both depth intervals assessed (Supplementary Figure S6).

Changes in the relative ion intensity for CO<sub>2</sub> ( $m/z$  44) clearly show the difference in the SOM character when comparing different depths (Figure 4). The shallow samples show a simple major peak around 300°C–350°C, whereas the deep samples show a more complex set of peaks with the strongest around 450°C.

For the 0–0.30 m depth, regardless of the YR, all samples showed a similar pattern with  $m/z$  44 reaching a maximum at around 300°C–350°C and with two minor shoulders at 400°C–450°C and 500°C–550°C (Figures 4A,B). Except for the ORG-M treatment (i.e., organic rotation with mineral fertilisation), all the other treatments showed a slight increase in the C released, particularly in the first temperature interval (Exo 1 – 200°C–350°C), in 2018 compared to 2011. The other two temperature intervals (Exo 2 + Exo 3 – 350°C–500°C and 500°C–750°C), which represent recalcitrant and refractory fractions, showed a similar release of C with the ORG-RT, COMP-FS and samples collected in 2018 being slightly predominant than their counterparts (CONV-RT, MINE-FS and 2011 samples) (Figures 4A,B). These results are especially



**FIGURE 4**

Ion current intensity for  $\text{CO}_2$  ( $m/z$  44) from the soil samples of combined treatment factors: conventional rotation with mineral fertilisation (CONV-M), conventional rotation with compost fertilisation (CONV-C), organic rotation with mineral fertilisation (ORG-M) and organic rotation with compost fertilisation (ORG-C) at 0–0.30 (A, B) and 0.30–0.60 m (C, D) soil depth intervals and different years of sampling 2011 (A, C) and 2018 (B, D).

highlighted when the amount of C released within each temperature interval was calculated using the  $m/z$  44 peak areas (Table 5). In general, there was a little variability between the treatments in the topsoil (0–0.30 m), with labile (Exo 1 – 200°C–350°C) and recalcitrant and refractory (Exo 2 + Exo 3 – 350°C–500°C and 500°C–750°C, respectively) fractions

showing similar C amounts (approximately 50/50). The only major difference observed was regarding the YR, where 2018 samples had higher soil C amounts than 2011 samples (Table 5).

For deeper soil layers (0.30–0.60 m), in both years (2011 and 2018), the  $m/z$  44 reached a maximum at around 400°C–450°C, with

**TABLE 5** Changes in carbon (C) released calculated from the  $m/z$  44 ( $\text{CO}_2$ ) peak areas in the temperature intervals 200°C–350°C (Exo 1), 350°C–500°C (Exo 2) and 500°C–750°C (Exo 3) as a result of different crop rotation (conventional-CONV-RT or organic-ORG-RT), fertility sources (mineral-MINE-FS or compost-COMP-FS) and years of sampling (YR) (2011 and 2018).

Depth m		Exo 1 (200°C–350°C)	Exo 2 (350°C–500°C)	Exo 3 (500°C–750°C)
		g C kg		
0–0.30	<i>Crop rotation</i>			
	CONV-RT	9.03 (0.37)	7.44 (0.25)	1.93 (0.05)
	ORG-RT	9.01 (0.10)	7.75 (0.18)	1.99 (0.12)
	<i>Fertility source</i>			
	MINE-FS	9.13 (0.30)	7.57 (0.26)	1.91 (0.11)
	COMP-FS	8.90 (0.22)	7.62 (0.21)	2.00 (0.06)
	<i>Year</i>			
	2011	8.66 (0.13)	7.30 (0.21)	1.86 (0.06)
	2018	9.37 (0.21)	7.89 (0.09)	2.06 (0.08)
0.30–0.60	<i>Crop rotation</i>			
	CONV-RT	3.60 (0.32)	4.34 (0.53)	0.96 (0.17)
	ORG-RT	2.50 (0.17)	4.53 (0.28)	0.97 (0.07)
	<i>Fertility source</i>			
	MINE-FS	3.21 (0.43)	4.32 (0.29)	0.87 (0.04)
	COMP-FS	2.89 (0.36)	4.55 (0.52)	1.06 (0.16)
	<i>Year</i>			
	2011	3.29 (0.44)	3.98 (0.34)	0.80 (0.04)
	2018	2.81 (0.32)	4.89 (0.34)	1.12 (0.12)

Data are measured mean values of main effects ( $n = 8$  for crop rotation, fertility sources and years of sampling within soil depth intervals). Standard error of the mean is in parentheses.

two other shoulders observed at 300°C–350°C and 500°C–550°C (Figures 4C,D). Under CONV-RT, there was a shift from 2011 to 2018 in C released to higher temperatures, particularly with the combination of CONV-RT and COMP-FS (CONV-C treatment), which resulted in the highest peak observed under the subsoil layer (Figures 4C,D). Under the ORG-RT, similar peaks were observed between the 2 years of sampling (2011 and 2018). However, it appears that the combination of ORG-RT and COMP-FS (ORG-C treatment) slightly shifted the release of C to higher temperatures resulting in a higher peak at 400°C–450°C whereas the peaks remained unchanged in the combination of ORG-RT and MINE-FS (Figures 4C,D). These results were confirmed by the amount of C released within each temperature interval using the  $m/z$  44 peak areas (Table 5). The CONV-RT, MINE-FS and samples collected in 2011 showed a higher release of C at the first interval (Exo 1 – 200°C–350°C) compared to ORG-RT, COMP-FS and samples collected in 2018. For the recalcitrant and refractory fractions (Exo 2 + Exo 3), 2018 samples showed higher soil C than 2011 samples (Table 5).

For both top- (0–0.30 m) and subsoil layers (0.30–0.60 m), there were no peaks between the 750°C–900°C temperature range, indicating that there were no detectable soil carbonate minerals present in the samples, therefore, total soil C concentration can be assumed to be equal to total SOC (Figure 4).

### 3.4 Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) in the presence of tetramethylammonium hydroxide (TMAH)

More than 300 pyrolysis product compounds were released of which 184 dominant product compounds were selected and quantified (Supplementary Table S6). All the quantified product compounds are listed in Supplementary Table S6, with their position in the chromatogram indicated by retention time (ReT). Supplementary Table S6 also provides information about the chemical group of the quantified product compounds and in which soil depth interval (0–0.30 and 0.30–0.60 m) they were found. Table 6 provides the relative abundance of the quantified pyrolysis product compounds by chemical groups.

For the 0–0.30 m soil depth, 161 quantified compounds were observed of the total 184 detected (Supplementary Table S6). More specifically, ORG-RT showed a higher relative abundance of benzofurans, carbohydrates, lignin phenols, phenols, and polyaromatics compared to the CONV-RT. The CONV-RT, on the other hand, showed a higher relative abundance of *n*-alkanes, *n*-alkenes, aromatics, fatty acids, and N compounds compared to ORG-RT (Table 6). In terms of fertilisation, COMP-FS showed a higher relative abundance of *n*-alkanes, *n*-alkenes, aromatics and polyaromatics compared to MINE-FS. The MINE-FS, on the other

**TABLE 6** Relative abundance of groups of pyrolysed product compounds released after Py-GC-MS-TMAH analytical procedures as a result of different crop rotation (conventional-CONV-RT or organic-ORG-RT), fertility sources (mineral-MINE-FS or compost-COMP-FS) and years of sampling (YR) (2011 and 2018).

Depth		<i>n</i> -alkanes	<i>n</i> -alkenes	Aromatics	Benzofurans	Carbohydrates	Fatty acids	Lignin phenols	N compounds	Phenols	Polyaromatics
m		%									
	<i>Crop rotation</i>										
0–0.30	CONV-RT	1.31 (0.02)	2.34 (0.04)	8.80 (0.06)	1.97 (0.05)	3.40 (0.04)	23.30 (0.02)	40.17 (0.04)	9.61 (0.04)	7.30 (0.08)	2.12 (0.04)
	ORG-RT	0.46 (0.01)	1.49 (0.04)	6.50 (0.12)	2.59 (0.04)	4.49 (0.07)	17.64 (0.02)	46.73 (0.05)	9.28 (0.03)	7.36 (0.08)	3.13 (0.03)
	<i>Fertility source</i>										
	MINE-FS	0.48 (0.01)	1.14 (0.05)	7.53 (0.08)	2.61 (0.05)	4.08 (0.04)	22.48 (0.02)	44.17 (0.05)	9.71 (0.03)	9.01 (0.10)	2.31 (0.03)
	COMP-FS	1.33 (0.02)	2.65 (0.04)	8.04 (0.08)	1.93 (0.04)	3.61 (0.06)	18.89 (0.02)	42.02 (0.04)	9.19 (0.03)	5.97 (0.07)	2.86 (0.04)
	<i>Year</i>										
	2011	0.56 (0.04)	2.50 (0.05)	6.47 (0.09)	3.35 (0.04)	3.52 (0.05)	20.60 (0.02)	42.77 (0.04)	9.18 (0.03)	8.08 (0.08)	2.97 (0.04)
	2018	1.39 (0.03)	1.39 (0.03)	9.32 (0.08)	0.98 (0.04)	4.25 (0.05)	21.19 (0.02)	43.22 (0.04)	9.69 (0.03)	6.48 (0.06)	2.09 (0.03)
	<i>Crop rotation</i>										
0.30–0.60	CONV-RT	0.00 (0.00)	0.81 (0.01)	19.92 (0.39)	7.25 (0.04)	7.70 (0.10)	2.72 (0.06)	19.29 (0.11)	19.82 (0.28)	9.08 (0.11)	13.41 (0.08)
	ORG-RT	0.00 (0.00)	1.05 (0.01)	39.11 (0.51)	27.82 (0.99)	5.25 (0.03)	0.00 (0.00)	5.91 (0.06)	4.16 (0.19)	1.08 (0.08)	15.62 (0.10)
	<i>Fertility source</i>										
	MINE-FS	0.00 (0.00)	0.00 (0.00)	30.30 (0.48)	2.24 (0.11)	7.86 (0.16)	3.00 (0.09)	20.19 (0.17)	20.72 (0.54)	4.98 (0.26)	17.59 (0.08)
	COMP-FS	0.00 (0.00)	1.73 (0.05)	25.34 (0.39)	27.79 (0.41)	5.66 (0.08)	0.36 (0.01)	8.08 (0.07)	6.84 (0.06)	6.61 (0.61)	10.71 (0.09)
	<i>Year</i>										
	2011	0.00 (0.00)	0.59 (0.01)	23.71 (0.30)	18.09 (0.37)	6.48 (0.09)	2.24 (0.05)	16.26 (0.08)	15.72 (0.26)	5.29 (0.12)	11.61 (0.05)
	2018	0.00 (0.00)	1.73 (0.01)	38.08 (0.74)	9.12 (0.10)	7.29 (0.13)	0.00 (0.00)	7.61 (0.24)	7.60 (0.19)	7.25 (0.43)	21.32 (0.20)

Data are measured mean values of main effects ( $n = 8$  for crop rotation, fertility sources and years of sampling within soil depth intervals). Standard error of the mean is in parentheses.

hand, showed a higher relative abundance of benzofurans, carbohydrates, fatty acids, lignin phenols, N compounds, and phenols compared to the COMP-FS (Table 6). In relation of years of sampling, samples collected in 2018 showed a higher relative abundance of almost all groups expected for the benzofurans, phenols and polyaromatics than the samples collected in 2011 (Table 6).

For deeper soil layers (0.30–0.60 m), 72 quantified compounds were observed of the total 184 detected (Supplementary Table S6). Comparison between the treatments indicated that ORG-RT had a higher relative abundance of *n*-alkenes, aromatics, benzofurans, and polyaromatics compared to the CONV-RT. Consequently, the CONV-RT showed a higher relative abundance of carbohydrates, fatty acids, lignin phenols, N compounds and phenols. Concerning fertilisation, the COMP-FS showed a higher relative abundance of *n*-alkenes, benzofurans and phenols while the MINE-FS showed a higher relative abundance of aromatics, carbohydrates, fatty acids, lignin phenols, N compounds, and polyaromatics (Table 6). In relation of years of sampling, samples collected in 2018 showed a higher relative abundance of *n*-alkenes, aromatics, carbohydrates, phenols and polyaromatics while samples collected in 2011 showed a higher relative abundance of benzofurans, fatty acids, lignin phenols and N compound (Table 6).

Comparisons between the two depth intervals indicated an increased contribution from aromatics, benzofurans, carbohydrates, and polyaromatics at deeper soil layers (0.30–0.60 m), whereas *n*-alkenes, fatty acids, and lignin phenols decreased at topsoil layer (0–0.30 m), regardless of the RS, FS or YR (Table 6). It was also observed an increased contribution from N compounds at deeper soil layers (0.30–0.60 m) under CONV-RT, MINE-FS and in the samples collected in 2011 compared to the topsoil layer (0–0.30 m).

## 4 Discussion

### 4.1 Changes in soil C stocks due to differences in crop rotation, length of ley periods, and fertilisation sources

Organic rotation with the use of 3 years of temporary grass-clover leys (rather than 2 years, represented by the conventional rotation), and compost fertilisation led to SOC accumulation confirming our hypothesis. This also reflects findings from previous studies, which particularly compared conventional vs. organic systems as a whole (Gattinger et al., 2012; Triberti et al., 2016; Jian et al., 2020). However, while organic rotation showed higher SOC stocks than the conventional rotation under both sampled years (i.e. 2011 and 2018) and soil depth intervals (i.e. 0–0.30 m and 0.30–0.60 m) irrespective of the fertility sources (i.e., mineral or compost), compost fertilisation led to topsoil SOC accumulation (0–0.30 m) over the years (i.e., from 2011 to 2018) under both crop rotations. These results suggested that these two management practices are playing a strategic role in SOC accumulation.

The positive effect on SOC stocks by organic rotation may be partially ascribed to both the incorporation of legumes and the longer length of ley periods (3 years vs. 2 years under organic and

conventional rotation, respectively). Previous research has indicated that the mixture of grasses and legumes (e.g., grass-clover) on ley periods can provide additional yield benefits and thus increase SOC stocks via higher crop residue deposition to the soil surface (Persson et al., 2008; O'Dea et al., 2013). This was also the case in our experiment (data not shown, but published in Bilsborrow et al. (2013)). Greater above-ground biomass can also lead to greater below-ground biomass along with more rhizo-deposition, and soil microbial activities (Araujo et al., 2012; Balakrishna et al., 2017), all of which can further benefit SOC accumulation even at deeper soil layers. According to a recent meta-analysis conducted by Jian et al. (2020), a greater mass and activity of root biomass, rhizo-deposits, and soil microbes could enhance the availability of essential nutrients to plant growth (e.g., N, phosphorus, and potassium), which can be a mechanism explaining the positive effect in SOC stocks at both soil depth intervals. The positive effect of both the incorporation of legumes and the longer length of ley periods on SOC stocks is also in line with previous research that suggested a minimum period of three-years ley after five-years arable rotation to promote a significant increase in SOC concentration in topsoil layers (Johnston et al., 2017).

In turn, the topsoil SOC accumulation (0–0.30 m) in both crop rotations over the years (2011–2018) under compost fertilisation can be attributed to the highest and direct supply of SOM to the soil (Aguilera et al., 2013). Previous research also reported significant SOC stock increase under fields receiving organic amendments such as composted dairy manure due to the direct supply of organic C (Christensen, 1988; Gerzabek et al., 2001; Gattinger et al., 2012). Another important factor that may have favoured SOC accumulation under compost fertilisation is its potential to enhance soil aggregate stability (Haynes and Naidu, 1998; Whalen and Chang, 2002). Organic amendments were previous shown to have positive effects on soil biological activity (Maeder et al., 2002; Lori et al., 2017), which can foster the physical protection of C against decomposition through chemical-physical bindings processes (Six et al., 2002).

Whilst the mixture of grasses and legumes and the use of organic amendments often result in an increased SOC stock (Sainju et al., 2006; Jian et al., 2020), mixed results have been reported due to the use of grass or legume during the ley period phases as well as due to the application of different organic amendments source (Mazzoncini et al., 2011; Aguilera et al., 2013; O'Dea et al., 2013). This might be due to differences in biomass production, C:N ratios and lignin content of the crops in the rotation as well as persistence of the organic amendments source to degradability in soils (Tokarski et al., 2019; Zhou et al., 2019). In the organic rotation, along with the grass-clover ley periods, other legumes (e.g., peas and beans) and vegetables (e.g., cabbage, lettuces, onions, and carrots) were cultivated in an 8-year period (2011–2018), which might have provided the finest balance between biomass production and optimal C:N ratio inputs for SOC accumulation benefits. While legumes (usually low C:N ratios) provide soil N to plants by fixing atmospheric N, the grass provides high biomass with high C:N ratios (Jian et al., 2020). In turn, organic amendments such as farmyard manure can increase SOC stocks as it is a C source that offers strong resistance to microbial decomposition (Nardi et al., 2004; Li et al., 2018). In this sense, the combination of grass-clover ley periods, other legumes and vegetables, and compost fertilisation is presumed



to be the optimum for long-lasting SOC stock benefits. However, it is important to highlight that the amount of biomass and the characteristics of residues (i.e., C:N ratios, lignin content, as well as other molecular compounds) play a key role in SOM mineralisation (Tian et al., 1992; Triberti et al., 2016). Accordingly, crop choice in the rotation and organic amendment sources can either increase or decrease SOC stocks through effects not only related to residue deposition but also due to potential changes in soil properties, including chemical (nutrient availability), physical (soil structure), and biological (microbial biomass) properties (Campbell et al., 1991; Bandick and Dick, 1999; Sainju et al., 2006). Since this study evaluated SOC stocks in a relatively short period (only 8 years apart between sampling years), the results should be considered with caution as changes in SOC are expected to occur slowly and variations over the years are common regardless of the management practices deployed (Smith et al., 2020).

## 4.2 Effects of differences in crop rotation, length of ley periods, and fertilisation sources on SOM composition and soil C stabilisation

A unique combination of physical SOM fractionation, TG-DSC-QMS and Py-GC/MS-TMAH analyses allowed us to better understand potential changes in SOM composition and soil C stability brought about by differences in crop rotation, length of ley periods, and fertilisation sources strategies. The results of this study particularly suggested that the increased topsoil (0–0.30 m) SOC stocks under organic rotation with 3 years grass-clover ley period might be susceptible to losses since it occurs through a high contribution from fresh organic materials in the soil surface. On the other hand, the increased subsoil (0.30–0.60 m) SOC stocks under the same rotation have occurred through a higher contribution of more stable compounds, probably related to the set of crops grown, and thus different rooting patterns, implying a potential SOC stabilisation. Likewise, the increased topsoil SOC stocks over years under compost fertilisation showed a larger contribution from more stable compounds.

In this study, crop straw and debris have been always removed from the field under both crop rotations while organic amendments were applied mainly using composted dairy manure. In terms of organic rotation, this indicates that the increased SOC stock in both top- and deeper soil layers was a function of a more diverse rotation system, which is accompanied by a longer period under grass-clover leys (3 years vs 2 years in the conventional rotation). Previous studies have suggested that a more diverse rotation could supply higher C inputs from root biomass and crop stubble; materials acknowledged for their relevant amount of stable SOM (Triberti et al., 2016). In addition to the high C:N ratios of grasses (Jian et al., 2020), studies from Martens, (2000) and Lorenz et al. (2005) indicated that cereal roots and stubbles are slowly decomposed materials as they have high C:N ratios, lignin, and phenols contents. This could be a second mechanism to explain the enhanced SOC accumulation under the organic rotation, as legumes, grasses and cereal were all inserted over the rotation. On the other hand, as compost fertilisation such as farmyard manure *per se* offers a resistance option to biodegradability in

soils (Nardi et al., 2004; Li et al., 2018), it might benefit SOC accumulation, irrespective of the crop rotation, due to the presence of more stabilised C forms. This was confirmed by a meta-analysis study conducted by Aguilera et al. (2013), where the authors found that raw organic amendment materials have a lower capability to increase soil C sequestration than organic composted materials. We also speculate that both organic rotation and compost fertilisation resulted in enhanced faunal activity, particularly worms, which promotes stability of organomineral aggregates and consequently SOC stabilisation (Coq et al., 2007).

Such assumptions were partially validated by our physical fractionation of the SOM, TG-DSC-QMS and Py-GC-MS analyses. Regarding crop rotation, thermal analysis (i.e., TG-DSC-QMS) showed that organic rotation has a slightly higher relative weight loss and ion intensity for CO<sub>2</sub> (*m/z* 44) in the temperature intervals between 350°C–500°C and 500°C–750°C (Exo 2 and Exo 3) for both soil layers. Likewise, compost fertilisation also resulted in a slightly higher relative weight loss and ion intensity for CO<sub>2</sub> (*m/z* 44) in the same temperature intervals at both soil layers. The results of the present study are also in agreement with a recent study conducted by Tokarski et al. (2019), who observed that farmyard manure results in thermal mass losses mainly around 450°C. Previous studies using thermogravimetry (TG) and differential scanning calorimetry (DSC) analysis indicate that exothermic peaks up to 350°C are related to decomposition of organic matter rich in labile aliphatic and carboxyl groups, whereas identified peaks up to approximately 500°C represent dominance of stable aromatic component classes. However, although these findings may indicate high amounts of recalcitrant and refractory C fractions and therefore a potential SOC stabilisation under both organic rotation and compost fertilisation (Lopez-Capel et al., 2005b; 2006; Manning et al., 2005; Plante et al., 2009), some considerations should be carefully taken into account.

Under organic rotation and for both soil depth intervals, there was a trend (non-significant) towards a decreased SOC in the mineral-associated fractions (silt and clay fraction from physical fractionation technique; SC < 53 μm), i.e., less accessible to decomposers and thus more stable and long-lived SOM (von Lützwow et al., 2007). Although not statistically proven, this potential disparity between the physical fractionation of the SOM and the thermal analysis (TG-DSC-QMS) results might be due to either the similarity between the two rotations in terms of SOC associated with this fraction (also observed in the TG-DSC-QMS analysis) as well as potential discrepancies between the temperature intervals and soil fractions (Schiedung et al., 2017). At a molecular level, the organic rotation has shown a slightly higher relative abundance of benzofurans, carbohydrates, lignin-phenols, phenols, and polyaromatics in the top 0–0.30 m depth, in comparison to the conventional rotation. Conversely, in deeper soil layers (0.30–0.60 m), organic rotation showed a higher relative abundance of *n*-alkenes, aromatics, benzofurans, and polyaromatics as well as a much lower relative abundance of carbohydrates, fatty acids, lignin-phenols, N compounds, and phenols. Benzofurans, carbohydrates, lignin-phenols and phenols are products from relatively fresh plant materials while aromatics and polyaromatic compounds are products that originate from different sources, including lignin, carbohydrates proteins and

charred plant material (González-Pérez et al., 2004; Kaal et al., 2008; Mazzetto et al., 2019). Pyrolysis products from cutan and suberin result in *n*-alkanes and *n*-alkenes compounds, which are more resistant against degradation than lignin (Tegelaar et al., 1995; Klotzbücher et al., 2011). These results indicate that the use of one of these techniques alone can lead to a misleading conclusion about soil C stability. In this study, in particular, the unique combination of techniques allows us to confidently say that while the organic rotation has increased SOC stocks in the topsoil layers, it might be susceptible to losses, as there is a high contribution from fresh organic materials. This is most likely related to the potential higher yields under such crop rotation and hence a higher amount of fresh crop residue deposition to the soil surface (Persson et al., 2008; O'Dea et al., 2013). On the other hand, increased SOC stocks in deeper soil layers under organic rotation may be attributed to other factors rather than crop residue deposition. In particular, it can be attributed to the fact that organic rotation is more diversified with a completely different set of crops and rooting patterns, including deep-rooting crops, compared to the conventional rotation (Blanco-Canqui et al., 2017). Kutsch et al. (2010), highlighted the importance of root biomass, rhizo-deposits, and microbes as sources of below-ground C. The high relative abundance of *n*-alkenes, aromatics, and polyaromatics in deeper soil layers (i.e., > 0.30 m) under organic rotation is an important finding as it implies that SOC stabilisation may be occurring (Mazzetto et al., 2019).

Concerning the fertility sources, a significant higher SOC in the heavy fraction (HF > 53  $\mu\text{m}$ ), i.e., a more labile fraction than the mineral-associated fraction due to its weaker association with clay mineral matrix (Hassink, 1997), was observed in the topsoil layers under the mineral fertilisation in comparison to compost fertilisation treatment. Also, at the same soil depth interval, a trend (also non-significant) towards increased SOC in the mineral-associated fractions (silt and clay fraction; SC < 53  $\mu\text{m}$ ) was observed under the compost fertilisation treatment in comparison to mineral fertilisation. In subsoil layers (0.30–0.60 m), mineral fertilisation significantly increased SOC in the mineral-associated fractions (silt and clay fraction; SC < 53  $\mu\text{m}$ ) over time, while compost fertilisation decreased it. These results suggest that the observed increased topsoil SOC stocks (0–0.30 m) over 8 years (2011–2018) under compost fertilisation can potentially lead to a SOC stabilisation, but this effect might be limited to depths less than 0.30 m. In contrast, mineral fertilisation might have a positive effect on SOC stabilisation in subsoil layers. The mechanisms for this could be the same as those discussed under organic rotation, i.e., higher yields followed by a higher amount of fresh crop residue deposits, which are potentially incorporated to the soil through tillage events (Bilsborrow et al., 2013; Schellekens et al., 2013) and greater below-ground biomass followed by greater rhizo-deposition, and soil microbial activities (Araujo et al., 2012; Balakrishna et al., 2017). The Py-GC-MS results reflected such assumptions. For the depth range 0–0.30 m, mineral fertilisation showed a higher relative abundance of products originated from fresh plant materials, including lignin-phenols and phenols (i.e., relatively easy to decompose), while compost fertilisation showed a higher relative abundance of compounds that are relatively difficult to decompose including aliphatic compounds (*n*-alkanes and *n*-alkenes), aromatics, and polyaromatics. For the 0.30–0.60 m depth interval, although the mineral fertilisation continued to show higher relative abundance of products originated from fresh plant materials, it also showed higher relative abundance of recalcitrant products in

comparison to the compost fertilisation treatment (e.g., aromatics and polyaromatics).

Lastly, it is also worth noting a few further points: 1) there was a significant increase in the mineral-associated SOC fractions (SC < 53  $\mu\text{m}$ ) after a full rotation cycle, at topsoil layer (0–0.30 m) and irrespective of the crop rotation or fertility source. This is an important outcome as it indicates a potential stabilisation by the interaction of clay minerals and SOC. Previous studies have observed that the thermal behaviour of SOC stocks was affected by clay minerals interactions (Leinweber and Schulten, 1992; Plante et al., 2005). In particular, high clay content soils have a greater potential to stabilise SOC compared to sandy soils (Lützw et al., 2006; Schrumpf et al., 2013; Brandani et al., 2016). It is very unlikely, however, that clay content and soil mineralogy have changed over an 8-year crop rotation period, which ultimately suggests that both crop rotations and fertility sources are somehow stabilising SOC over-time at the 0–0.30 m depth; 2) although some disparities have been observed between physical fractionation of SOM and TG-DSC-QMS analysis, the results of both agreed with each other in relation to years of sampling (e.g., higher mineral-associated C fractions and higher mass losses and soil C released in 2018 in the Exo 2); and 3) at a molecular level, it was observed a substantial decrease in subsoil layers (0.30–0.60 m) was observed for *n*-alkenes, *n*-alkanes, fatty-acids, and lignin-phenols, whilst aromatics, benzofurans, carbohydrates, and polyaromatics increased for all treatments in topsoil layers (0–0.30 m). In addition, an increase contribution from N compounds was observed under conventional rotation, mineral fertilisation and samples collected in 2011. The decreases in *n*-alkenes, *n*-alkanes, fatty-acids, and lignin-phenols at depth as well as the high contribution from polyaromatics are acceptable findings as they are pyrolysis products from plant biopolymers/biological origin and black carbon, respectively (Ralph and Hatfield, 1991; Nierop et al., 2001; González-Pérez et al., 2014). However, the higher relative abundance of carbohydrates at this depth interval regardless of the treatment as well as the high contribution from N containing compounds under the conventional system practices (i.e., conventional rotation and mineral fertilisation) deserves particular attention. Upon pyrolysis, these are the main products of microbial activities (Derenne and Quéné, 2015) and thus it may suggest an enhanced SOM decomposition (Rumpel and Kögel-Knabner, 2010). Further research is still required to fully understand the impacts of management practices on SOM decomposition in subsoil layers (i.e., > 0.30 m depth).

## 5 Conclusion

This study has shown that SOC stocks, as well as soil organic matter (SOM) composition, differ between crop rotations that include 2- or 3-years grass-clover ley periods (i.e., conventional and organic crop rotations) and mineral and compost fertilisation sources with potential implications to C stabilisation. More specifically, a more diversified and legume-rich crop rotation system with 3 years grass-

clover ley period (organic rotation) has shown higher SOC stocks than a more simplified rotation characterised by cereal intensive cropping and 2 years grass-clover ley period (conventional rotation). This result was observed in both the topsoil and subsoil (i.e. 0–0.30 m and 0.30–0.60 m) regardless of the sampled year (i.e. 2011 and 2018) and applied fertility sources (mineral or compost). In turn, compost fertilisation increased topsoil SOC stocks over years (i.e., from 2011 to 2018) under both crop rotations. The innovative combination of SOM physical fractionation, TG-DSC-QMS, and Py-GC/MS-TMAH analyses helped to better understand the potential shifts in the composition of SOM and consequently, draw more confident conclusions about its C stability. In particular, the findings of this study suggested that the increased topsoil SOC stocks under organic rotation and thus longer grass-clover ley period might be susceptible to losses since it occurs through a high contribution from fresh organic materials in the soil surface, easily lost in thermal analysis and not attached to more stabilised soil fractions (SC < 53  $\mu\text{m}$ ). On the other hand, the increased subsoil SOC stocks under the same rotation have occurred through a higher contribution of more stable compounds, only lost at high temperatures in thermal analysis and associated with more stable soil fractions (SC < 53  $\mu\text{m}$ ). Likewise, the increased topsoil SOC stocks over years under compost fertilisation showed a larger contribution from more stable compounds (aliphatics, aromatics and polyaromatics), only lost at high temperatures in thermal analysis and soil C associated with more stable soil fractions (SC < 53  $\mu\text{m}$ ). These findings ultimately suggest that combining these two management practices (i.e., organic rotation with longer ley periods and compost) could be one of the ways to assist agricultural systems in delivering potential stable soil C sequestration. Along with the positive effect to soil C accumulation, it is important to underscore that the use of the legumes and longer period of grass-clover leys in the rotation are widely acknowledged for their benefits on weed control, disease break crop as well as production. However, despite its potential critical role in agroecosystem functioning, crop rotations have been broadly simplified in modern agricultural systems, which may jeopardise the provision of ecosystem services. It is, therefore, encouraged to use of such practices regardless of the potential benefits to soil C. Further data collection from this, as well other, long-term trials will help to confirm these effects of crop rotation, ley periods, and fertility sources on SOC stocks and stabilisation and further elucidate their relationship with other factors for instance changes in environmental variables. The results, therefore, should be considered carefully under different climate, specific managements, soil texture and type than those tested here, as all these factors can either assist or hinder towards physical protection of SOM and thus affect decomposition and stabilisation of SOC stocks.

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## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), raw data can also be provided on request from the corresponding author.

## Author contributions

The data collection in this study, including soil analyses, consists of original work undertaken by the first author, CFZ. The 2018 soil sampling was carried out by the first author, CFZ while the 2011 soil sampling was carried out by farmer technicians as a part of a previous project. DACM and EL-C led the thermal analysis, and GDA led the pyrolysis-GC-MS analysis. Statistical analyses, interpretation of the data, and writing of the manuscript were led by CFZ, with full contributions from all co-authors.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2023.1113026/full#supplementary-material>

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