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The energetic and chemical signatures of persistent soil organic matter

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Abstract A large fraction of soil organic matter (OM) resists decomposition over decades to centuries as indicated by long radiocarbon residence times, but the mechanisms responsible for the long-term (multi-decadal) persistence are debated. The current lack of mechanistic understanding limits our ability to accurately predict soil OM stock evolution under climate and land-use changes. Using a unique set of historic soil samples from five long-term (27–79 years) bare fallow experiments, we demonstrate that despite wide pedo-climatic diversity, persistent OM shows specific

energetic signatures, but no uniform chemical composition. From an energetic point of view, thermal analyses revealed that combustion of persistent OM occurred at higher temperature and provided less energy than combustion of more labile OM. In terms of chemical composition, persistent OM was H-depleted compared to OM present at the start of bare fallow, but spectroscopic analyses of OM functional groups did not reflect a consistent chemical composition of OM across sites, nor substantial modifications with bare fallow duration. The low energy content of persistent OM may be attributed to a combination of reduced content of energetic C–H bonds or stronger interactions between OM and the mineral matrix. Soil microorganisms thus appear to preferentially mineralize high-energy OM, leaving behind material with

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low energy content. This study provides the first direct link between long-term persistence of OM in soil and the energetic barriers experienced by the decomposer community.

Keywords Carbon cycling · Long-term bare fallow · Rock–Eval 6 · NEXAFS · TG-DSC

Introduction

While the importance of soil organic matter (OM) as a global C pool is recognized, the mechanisms controlling OM turnover are still actively debated (Dungait et al. 2012; Lehmann and Kleber 2015; Schmidt et al. 2011). Soil OM encompasses compounds with residence times ranging from days to millennia (Trumbore 2000). For decades, the chemical composition of OM was considered to be the main controlling factor for soil C turnover because of the presumed intrinsic recalcitrance of certain molecules. Most current compartmental models simulating C turnover in the soil–plant system subscribe, at least partially, to such concepts of chemical recalcitrance (Dungait et al. 2012). However, these concepts are challenged by recent research (Bahri et al. 2006; Gleixner et al. 2002; Kleber 2010), and it is now recognized that the molecular structure of OM components alone cannot fully explain the long-term persistence of OM in soil (Lehmann and Kleber 2015). Current alternatives emphasize organo-mineral interactions as a dominant factor for long-term persistence in soil of otherwise labile organic compounds (Baldock and Skjemstad 2000; Lehmann and Kleber 2015; Schmidt et al. 2011). While the revised paradigm suggests that persistent OM consists of microbially-derived substances enriched in smaller molecules and intimately

associated with the mineral matrix (e.g., Kiem and Kögel-Knabner 2003; Lehmann and Kleber, 2015), the biogeochemical characteristics of persistent OM remain elusive.

Decomposition of OM in soil provides C, nutrients and energy for the maintenance and growth of microbial decomposers (Swift et al. 1979). Therefore, information on the energy status of OM is a highly relevant characteristic to explain its persistence in soils because OM decomposition must yield greater energy to the decomposers than they invest to acquire this energy. The energetic demands of, and barriers to, decomposers may thus relate to OM persistence. This has spawned interest in methods to measure the energy status of OM in soil (Currie 2003; De la Rosa et al. 2008; Plante et al. 2009). Recently, data from thermal analyses have been linked to the short-term stability of C in soil (Gregorich et al. 2015; Plante et al. 2011), and thermal indices of long-term OM biogeochemical stability have been proposed (Lopez-Capel et al. 2005; Saenger et al. 2013). However, the persistence of OM in soil has not yet been linked directly to its energy status.

One difficulty in linking the energy status or chemical composition of OM to different biogeochemical stability arises from the lack of methods that allow a complete isolation of persistent OM from faster cycling OM (*sensu* Smith et al. 2002). Particle-size fractionation generally separates fractions with contrasting mean residence times, but even fractions enriched in persistent OM still contain fast-cycling compounds (e.g. Puget et al. 2000). Similarly, chemical hydrolysis or oxidation procedures have been extensively used in attempts to isolate persistent soil OM, but with limited success (e.g. Lutfalla et al. 2014; Plante et al. 2004, 2006). Moreover, the potential formation of artefact OM components during chemical treatments limits the perspective of a detailed characterizing of chemically-extracted OM (Lehmann and Kleber 2015).

An alternative way to isolate OM of different persistence would be to eliminate all new C inputs to soil, allow the more labile compounds to decompose, and sample the soil with the residual OM after different periods of decomposition. From this perspective, monitoring OM decomposition during laboratory incubations provides an effective biological means of fractionating OM. However, even long-term laboratory incubations (>2 years) are insufficient to

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assess decadal-scale C turnover. *In situ*, soil incubation studies spanning several decades would provide an ideal approach, allowing faster-cycling OM components to decompose while ensuring all other soil characteristics remain unaltered (Rühlmann 1999). This has been achieved in a number of field experiments with bare fallow conditions (*i.e.*, soil kept free of vegetation and other organic amendments) in which the OM initially present has been allowed to decompose under field conditions with no further inputs of OM. During the fallow period, soils have gradually become depleted in more labile OM components as these decompose, and enriched in persistent OM. The bare fallow treatments have been incorporated into several long-term field experiments in which soils have been sampled regularly over the years, generating precious archives of historic soil samples and a unique research platform for studies of persistent OM (Barré et al. 2010; Lefèvre et al. 2014; Menichetti et al. 2015). At each long-term bare fallow (LTBF) site, the soil archive provides a series of soil samples with OM of increasing persistence but originating from the same pedo-climatic conditions (*i.e.*, climate and mineral matrix). In the present study, we used soils from five LTBF experiments located in northwestern Europe and representing a wide variation in geological origins and climatic settings to characterize the energetic and chemical signatures of persistent soil OM.

Materials and methods

Study sites and soil samples

We used soils from five LTBF field experiments located across northwestern Europe. The soils were from the INRA “42 parcels” experiment located in the grounds of the Château de Versailles in Versailles, France; the long-term bare fallow adjacent to a Ley-arable experiment located at Rothamsted Research, Harpenden, United Kingdom; the long-term field experiment located at the Swedish University of Agricultural Sciences at Ultuna in Uppsala, Sweden; the “36-plots” experiment in the gardens of the Château de Grignon in Grignon, France; and the Lermarken site at Askov Experimental Station, Denmark. These experiments are described in more detail in Barré et al. (2010) and references cited therein. Soil

samples collected just before and during the bare fallow period were air-dried and archived in the dark at room temperatures. Selected site characteristics and the list of samples used in the present work are given in Table 1.

Analytical thermal analyses

Two analytical thermal analysis techniques were used to characterize the energetic composition of the soil samples. Neither of these methods generates specific and explicit information about chemical composition, but both are useful in quantifying and indexing the amount of energy contained in the OM and the amount of energy required for its thermal decomposition.

Coupled differential scanning calorimetry (DSC) and evolved gas analysis (EGA) during ramped combustion of soil samples was performed to measure the net energy released during OM combustion, knowing that some of the energy applied to the sample is consumed by the mineral phase and the breakdown of the organo-mineral associations. However, Fernández et al. (2011) previously showed that the exothermic energy released by OM combustion was substantially greater than the energetic reactions of the mineral phase. Ramped combustion was performed using a Netzsch STA 409PC Luxx thermal analyzer (Netzsch-Gertebau GmbH) coupled to a LI-840 infrared gas analyzer (Licor Biosciences) (Fernández et al. 2012). Samples were heated from ambient (~ 25 °C) to 105 °C at 10 °C min⁻¹, held for 15 min to drive off sample moisture, then heated to 700 °C at 10 °C min⁻¹ under flowing CO₂-free air. DSC thermograms were baseline corrected using the non-parametric baseline fitting function of Peakfit (Systat Software). The energy density of OM was determined by integrating the exothermic region of the DSC thermogram associated with OM combustion to generate energy content (J) and dividing by the total CO₂-C evolved (J mg⁻¹ C).

Samples were also characterized using Rock-Eval (RE) technology using a RE6 Turbo device (Vinci Technologies) (Disnar et al. 2003). RE analyses provides several measurements from the sequential ramped pyrolysis (200–650 °C) and oxidation (300–850 °C) of a sample. Details of the application of Rock-Eval to soils are provided elsewhere (*e.g.*, Disnar et al. 2003; Sebag et al. 2006). RE was used to determine the amount of energy required for the

Table 1 Selected site characteristics of samples included in the study

	Versailles (FR)	Rothamsted (UK)	Ultuna (SW)	Grignon (FR)	Askov (DK)
Location	48°48' N 2°08' E	51°82' N 0°35' E	59°49' N 17°38' E	48°51' N 1°55' E	55°28' N 9°07' E
Experiment initiation year	1929	1959	1956	1959	1956
Last sampling year	2008	2008	2009	2007	1983
Duration (years)	79	49	53	48	27
Mean annual temperature (°C)	10.7	9.5	5.5	10.7	7.8
Mean annual precipitation (mm)	628	712	533	649	862
FAO soil classification	Luvisol	Chromic Luvisol	Eutric Cambisol	Luvisol	Orthic Luvisol
Soil textural class (% sand/silt/clay)	Silty loam (26/57/17)	Flinty silty clay loam (7/68/25)	Clay loam (23/41/ 36)	Carbonated silty loam (30/54/16)	Coarse sandy loam (82/11/7)
Sampling depth (cm)	25	23	20	25	20
Sampling years included in study*	1929, 1934, 1939, 1950, 1960, 1968, 1980, 1991, 2002, 2008	1959, 1963, 1971, 1987, 2000, 2008	1956, 1967, 1974, 1985, 1995, 2009	1959, 1962, 1971, 1980, 2007	1956, 1962, 1968, 1976, 1983
Number of field replicate plots	2	2	2	2	3

* NEXAFS analyses were performed on a representative subset of 47 samples

thermal decomposition of OM. The temperature at which 50 % of the residual OM was oxidized to CO₂ during the oxidation phase (T₅₀ CO₂ oxidation, °C) was used as an index of the overall thermal stability of OM. The method also provides data on the elemental/chemical composition of the OM. The pyrolysis effluents (hydrocarbons; CH) are detected and quantified with flame ionization detection, while CO and CO₂ are quantified by infrared (IR) detection. This technique determines the total organic C concentration (TOC) of soil samples without the carbonate removal pre-treatment required for classical combustion techniques. It also provides information on the composition of OM, especially through the hydrogen and oxygen index values (HI and OI). The HI corresponds to the amount of hydrocarbons that formed during thermal pyrolysis of the sample (S2 peak in mg CH g⁻¹) divided by the TOC content of the sample, expressed as a percentage (%). The OI corresponds to the CO₂ yield during thermal pyrolysis of the sample (S3 peak in mg CO₂ g⁻¹) divided by the TOC of the sample, expressed as a percentage (%). These indices are well correlated with elemental H/C and O/C ratios (Espitalié et al. 1985). The analysis of biologically relevant standards showed, for example, that lipids have high HI values whereas polyphenols and carbohydrates have higher OI values (Carrie et al. 2012). An upper oxidation temperature of 611 °C was chosen for signal integration to obtain a CO₂ signal evolved from OM without interference from carbonates.

Chemical characterization techniques

NEXAFS analyses

The C speciation of soil samples was investigated by near-edge X-ray absorption fine structure (NEXAFS) spectroscopy at the carbon K-edge (270–330 eV) using the 11-ID-1 Spherical Grating Monochromator (SGM) beamline located at the Canadian Light Source (CLS) (Regier et al. 2007). For the present study, about 5 mg of air-dried and <200 μm ground LTBF soil samples were pressed onto gold foils (99.99 % purity, Sigma Aldrich) which were then mounted on a copper sample holder. Energy calibration was performed using a set of pure reference materials (calcium carbonate, benzoic acid and histidine, >99 % purity, Sigma Aldrich). Each spectrum reported in the present study corresponds to the average of 50 measurements. Of note, only the first

100 nm of the sample surface are probed using the SGM setup. Spectra were averaged, background subtracted and normalized using the Igor Pro software. Spectra were then normalized to TOC concentration by normalizing each spectrum to its own area calculated up to 291.5 eV (the ionization energy of C in OM). This treatment ensures that the amount of C is accounted for and that the spectra are chemically consistent, i.e., a spectrum showing a more prominent absorption than others at a given energy must have a less intense absorption at the energy of the other functional groups.

After normalization, spectra were fitted using multiple Gaussian functions placed at fixed positions corresponding to known functional groups (Bernard et al. 2012; Braun et al. 2006; Carravetta et al. 1998; Le Guillou et al. 2014). Full-width at half maximum (FWHM) of 0.3 eV below 295 eV and of 2 eV above 295 eV, to take into account the multiple scattering effects, were considered. The following positions were used: 284.4, quinones; 285, 285.4 eV, aromatic; 285.8 eV, amines; 286.2, 286.6, 287.1 eV, carbonyl; 287.7 eV, aliphatics; 288.2 eV, amides; 288.6 eV, carboxylic; 289.1 eV, aldehydes; 289.4 eV, hydroxyls; 289.9 eV, aliphatics; 290.3 eV, carbonates). Additional bands at 290.8, 291.2, 291.7, 292.2, 292.7, 293.4, 294, 295, 297.1, 297.5, 299.7, 300, 302.5, 305, 307.5, 310 and 312.5 eV were also introduced. Most of the latter Gaussian functions used for such deconvolutions do not account for the presence of specific functional groups but for broad spectral features corresponding to highly delocalized excited states, sometimes referred to as 1 s-σ* virtual state transitions, or for the overlapping contribution of Feshbach resonances (Stöhr 1992). Peak FWHM and position (energy) for all Gaussian curves were constrained, only the heights of the Gaussian curves were free to vary. The extracted peak heights were then used to compare the relative abundances of various functional groups. Only functional groups corresponding to visible Gaussian peaks were considered (aromatic, carbonyl, aliphatic and carboxylic).

Mid infrared spectroscopy

Aliphatic C composition was investigated using diffuse reflectance Fourier transform mid infrared spectroscopy (DRIFT-MIRS). The LTBF site at Grignon was not considered for this technique due to the presence of soil carbonates, which generate a number of overlapping and interfering bands (Reeves

2012). Spectral regions other than the saturated hydrocarbon region were discarded because they are all subjected to strong interferences due to soil mineral matrix which impedes drawing clear conclusions from them (Reeves 2012). Air-dried and <200 μm ground LTBF soil samples were further dried overnight at 40 °C prior to MIRS analysis. Mid infrared spectra were obtained using a Thermo iS10 spectrometer (ThermoScientific, USA) by averaging 16 scans per sample at a 4 cm^{-1} spectral resolution over the spectral range 4000–400 cm^{-1} . Spectra were baseline corrected and the 3030–2780 cm^{-1} waveband region corresponding to aliphatic C groups (alkyl CH_2 and CH_3) in non-carbonate soils was selected. Spectra were normalized according to their OM content by setting the intensity of the peak at 2851 cm^{-1} to 1. Spectral pre-treatment (baseline correction and normalization) was performed with the R environment software v.3.1 (R Core Team 2013) using the hyperSpec (Beleites and Sergio 2014) and ptw (Bloemberg et al. 2010) R packages.

Statistical analyses

Changes in indices of energetics and chemical composition were examined over the durations of the various LTBF experiments. Trends in the Rock–Eval (temperature required to combust 50 % of initial sample C, HI and OI indexes), DSC (energy density), and NEXAFS (Gaussian peak heights for aromatic, carboxylic, aliphatic and carbonyl components) measurements with bare fallow duration were investigated using least-squares linear regression analysis with bare fallow duration as the independent variable. NEXAFS spectra were trimmed to include only the C K-edge (283–291.5 eV) and mean-centered prior to principal components analyses (PCA) using R software v.3.1 and the ade4 (Chessel et al. 2004) R package. Mean-centered MIRS-DRIFT data were similarly analyzed by PCA using the same package.

Results

Changes in soil OM concentrations in bare fallow soils

At all sites, concentrations of OM consistently decreased during the bare fallow period (Barré et al.

2010; Supplementary Fig. 1). At Rothamsted and Versailles, only one-third of the initial C concentration remained after 49 and 79 years of bare fallow, respectively. The decrease in C concentrations was smaller at Ultuna and Grignon (60 % of initial C left after about 50 years). The smallest decrease was observed at Askov, which had the shortest period under bare fallow (27 years; 75 % of initial C remaining).

Energy density

Combustion being exothermic, the energy density of persistent OM can be measured as the total energy liberated per gram of combusted organic C using DSC during ramped combustion. This energy density consistently decreased with bare fallow duration ($p < 0.05$ for all sites; Fig. 1a). While the trends were consistent

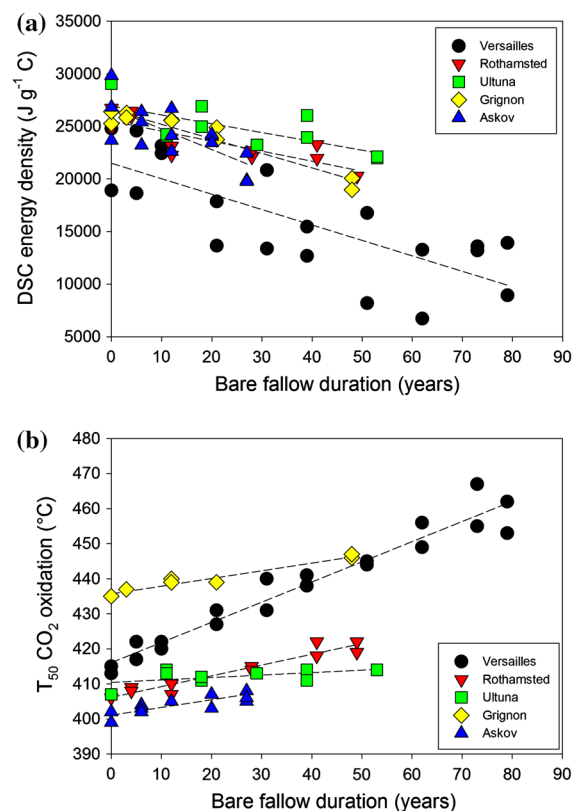


Fig. 1 **a** Temperature at which 50 % of SOC combusted during the Rock–Eval 6 oxidation step as a function of bare fallow duration. **b** Energy density measured by differential scanning calorimetry (DSC) during ramped combustion as a function of bare fallow duration

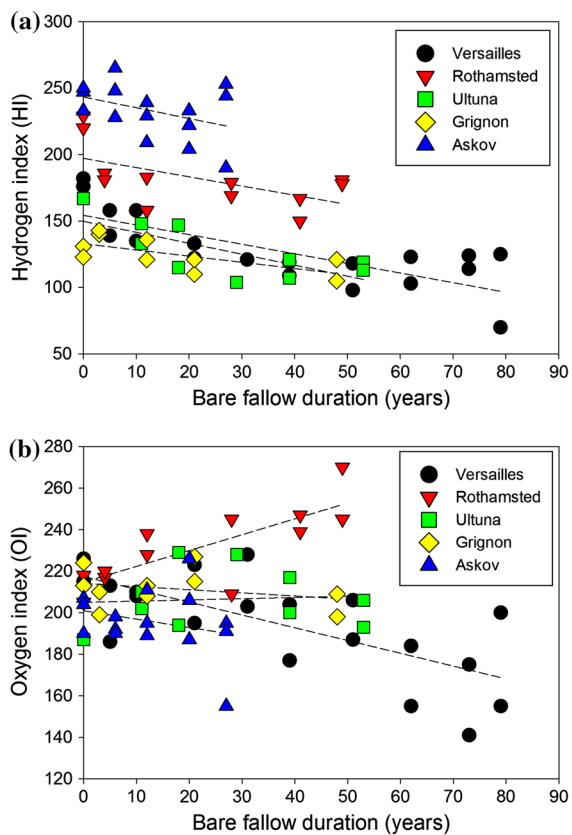


Fig. 2 **a** Hydrogen index (HI) and **b** oxygen index (OI) measured by Rock–Eval 6 as a function of bare fallow duration

across sites, initial values and rates of decrease varied among them (Supplementary Table 1). As mineralogy plays an important role in determining the combustion profile of a soil (Plante et al. 2009), the differences among sites likely reflect the specific mineral compositions of the soils at each site.

Rock–Eval 6

Thermal analysis by Rock–Eval 6 (RE6) showed a systematic increase in thermal stability with increasing bare fallow duration ($p < 0.01$ for all sites, except Ultuna $p = 0.07$; Supplementary Table 1), as measured by the combustion temperature required to reach 50 % of the total CO_2 evolution during the oxidation step (Fig. 1b). The initial T_{50} values were similar for all sites except Grignon. Greater T_{50} at Grignon was generated by a specific peak around 525 °C (not shown). The hydrogen index measured using RE6 (HI; which is related to the H/C atomic ratio) of OM

consistently decreased during the bare fallow period ($p < 0.05$) except at Askov ($p = 0.14$; Supplementary Table 1), which had the shortest bare fallow duration (Fig. 2a). The oxygen index (OI; which is related to the O/C atomic ratio) increased with bare fallow duration at Rothamsted ($p < 0.05$) and decreased at Versailles ($p < 0.05$), with no observable trends observed at the three other sites (Fig. 2b; Supplementary Table 1).

NEXAFS

The atomic environment of C in OM in the carbonate-free LTBF sites (*i.e.*, excluding Grignon) observed by NEXAFS spectroscopy at the C K-edge was dominated aliphatic and carboxylic functional groups, with additional aromatic groups (Supplementary Fig. 2a). NEXAFS spectra showed little difference among sites and with bare fallow duration as illustrated by the wide scatter and overlap in the PCA scores (Supplementary Fig. 2b), although samples from Askov did plot apart in a separate quadrant. The general features of these spectra are consistent with a previous study that examined soils under different land-uses and climatic conditions, and contrasting C contents (Purton et al. 2015).

NEXAFS analyses revealed no consistent trends with bare fallow duration across all sites for the four specific C functional groups investigated (Fig. 3; Supplementary Table 2). Double bonded C (aromatic and olefinic groups) as well as carbonyl groups (C=O) decreased at all sites except Versailles, where double-bonded C significantly increased with bare fallow duration (Fig. 3a). Carboxylic C increased significantly with bare fallow duration at Rothamsted, but showed no significant trends at other sites (Fig. 3b). While carbonyl C appeared to also increase at Versailles (Fig. 3d), the trend was not statistically significant (Supplementary Table 2). NEXAFS data for aliphatic C showed a statistically significant decrease at Ultuna, downward trends ($P = 0.06$) at Versailles and Askov, and no trend at Rothamsted (Fig. 3c, Supplementary Table 2).

Mid-IR spectroscopy

Principal component analysis (PCA) on DRIFT spectra in the mineral free CH_3/CH_2 stretching absorption bands region ($2800\text{--}3000\text{ cm}^{-1}$) is reported in Fig. 4. The first two axes of the PCA of DRIFT spectra in the

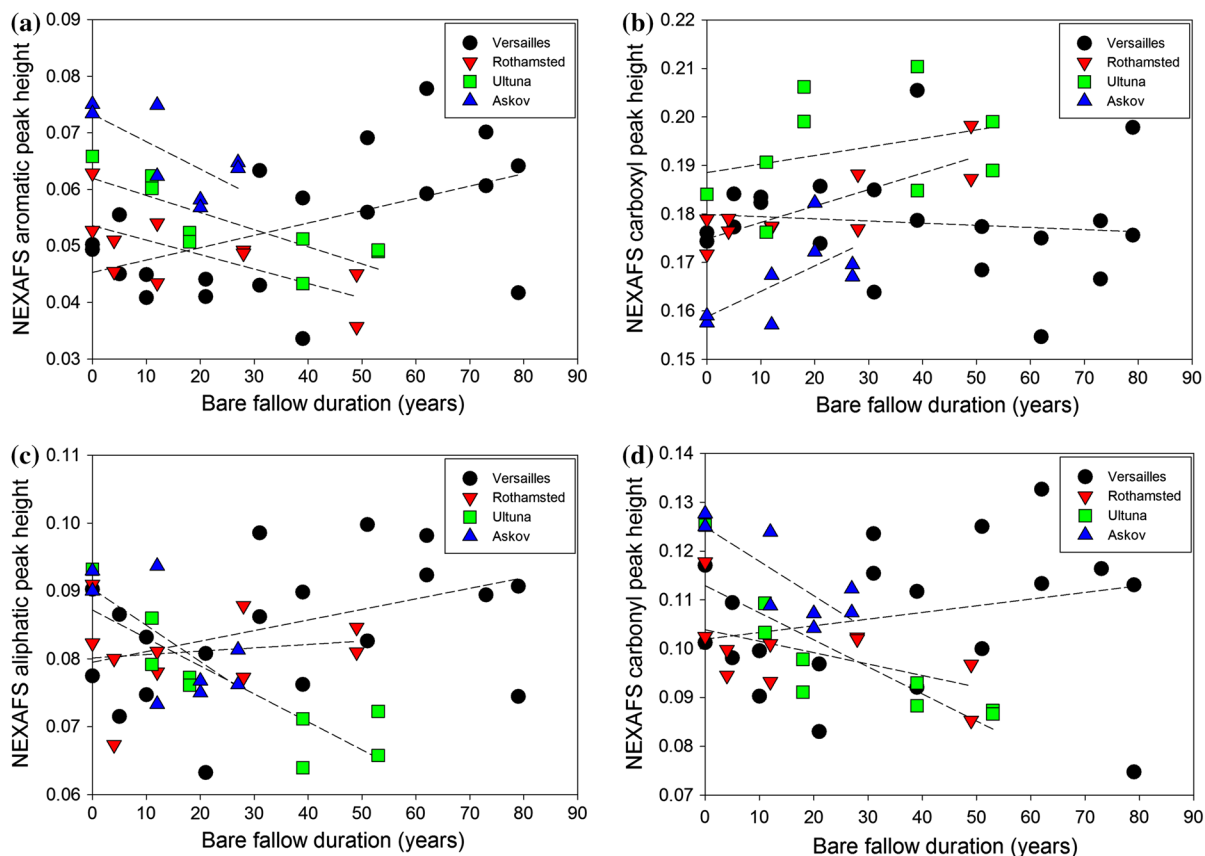
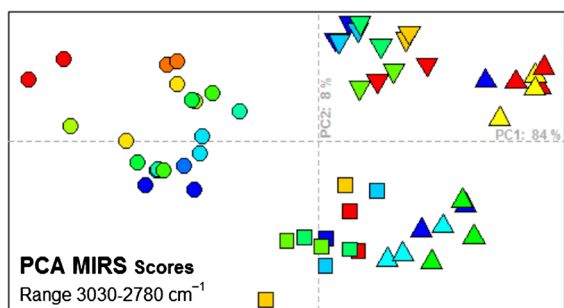


Fig. 3 Heights of the Gaussian curves derived from C K-edge NEXAFS analyses corresponding to **a** aromatic, **b** carboxylic, **c** aliphatic, and **d** carbonyl carbon functional groups as a function of bare fallow duration



Initial ■ ■ ■ ■ ■ ■ ■ Final

○ Versailles (FR) □ Ultuna (SW)

▽ Rothamsted (GB) △ Askov (D)

Fig. 4 Principal Component Analysis (PCA) on DRIFT spectra in the mineral free CH_3/CH_2 stretching absorption bands region ($2800\text{--}3000\text{ cm}^{-1}$)

alkyl-C region accounted for 91 % of total variance. High PC1 scores are characterized by an increase in CH_2 relative to CH_3 (Supplementary Fig. 3). High PC2 scores are characterized by an increase in CH_3 relative to CH_2 . Clear and opposite trends were observed for Versailles and Rothamsted samples: CH_2/CH_3 ratio increased with bare fallow duration at Rothamsted and decreased at Versailles. No trends were observed for Askov and Ultuna.

Discussion

Persistent OM is H-depleted, thermally stable, low-energy OM

As the more labile OM decomposed and the proportion of persistent OM increased in the LTBF soils over

the decades, increasing energy inputs (*i.e.*, higher temperatures, Fig. 1b) were required for OM combustion, while less energy was evolved during the combustion (Fig. 1a). Our results therefore show that in spite of differing characteristics among the five LTBF sites (e.g., texture, climate, past land-use), persistent OM had a clear energetic signature. Rovira et al. (2008) proposed that litter with low quality is decomposed more slowly because it requires greater input of energy (high T_{50}) for a low energy benefit (low energy density). OM in organo-mineral complexes requires substantial activation energy to be combusted and may release less energy upon mineralization because of endothermic soil OM desorption. These phenomena might be indicative of an energetic barrier to decomposition. Although the majority of OM that has persisted for decades in soil under bare fallow is intimately associated with soil minerals, the trends in energetics with bare fallow duration are consistent with the conceptual framework derived for litter. These results are also consistent with previous results obtained through laboratory incubation experiments (Plante et al. 2011), that generated similar energetic trends when comparing OM before and after a 588-day incubation at 35 °C, which resulted in a significant soil organic C depletion.

MIRS and NEXAFS results did not reveal any uniform chemical composition for persistent OM across sites, or changes in composition with bare fallow duration. All sites except Versailles showed a significant decrease in aromatic and olefinic functional groups (Fig. 3a). Gregorich et al. (2015) also observed a decrease in aromatic groups for an 11-year bare fallow treatment compared to an adjacent cropped soil. The increase in C = C bonds at Versailles is likely due to greater concentrations of pyrogenic OM, clearly observable in the sand sized fraction at this site. It suggests that the decrease in aromatic moieties with increasing persistence may be a general trend for soils with a low content in pyrogenic OM. Increases in carboxyl and OI were observed at Rothamsted, suggesting that persistent OM is more oxidized at this site. However, non-significant trends with bare fallow duration of OI and carboxyls at the other LTBF sites, and decreases in carbonyls at Askov and Ultuna, suggest that the greater oxidation of persistent OM cannot be viewed as a general trend. The significant decrease in OI with bare fallow duration at Versailles may also be explained by pyrogenic OM at this site.

Indeed, pyrogenic OM has very low OI (Saenger et al. 2013), and an increase in the relative proportion of OM present as pyrogenic OM in Versailles soil would decrease bulk soil OI values. Although persistent OM did not show a unique chemical composition, it was clearly associated with a specific chemical signature: persistent OM was strongly H-depleted, as seen from Rock-Eval 6 measurements. This trend was also suggested in a previous study (Gregorich et al. 2015). HI and peak heights of aliphatic group were correlated at Askov, Ultuna and Rothamsted (Supplementary Fig. 4), suggesting that a decrease in HI can be related to a decline in aliphatic groups. The decrease in HI at Versailles cannot be related to a modification in aliphatic groups (Supplementary Fig. 4), but may be related to an increased proportion of pyrogenic OM, which has low HI values (Saenger et al. 2013).

The chemical characterization techniques selected for this study operate at the functional group scale rather than the molecular scale. This was, in part, due to the need for methods that require very small amounts of sample (due to limitations from the LTBF experimental sample archives; a non-renewable resource), with no required pre-treatments. As a result, information on the nature of the chemical compounds at the molecular scale or the chemical links between organic molecules was not achieved. Analytical techniques that evaluate the spatial distribution of persistent OM within the mineral matrix would represent a useful supplement to our approach and an avenue for further research.

Mechanisms for the energetic signatures of persistent OM

(LaRowe and Van Cappellen 2011) previously reported various bond energies and the thermodynamics of OM decomposition, and noted the high energy content of C–H bonds compared to other molecular bonds. The evolution of OM from high energy C–H bonds to less energetic molecular bonds with increasing bare fallow duration, and thus increasing enrichment in persistent OM, may explain, at least partially, why combustion of persistent OM released less energy. Our results thus suggest that the chemical characteristics of OM remain a potential driver of long-term OM persistence in soil, and that this may result from specific bond energies at the molecular level.

The interaction between the mineral matrix and OM most likely contributes to the energetic signature of persistent OM. Previous studies have shown that the OM interacting with minerals is thermally more stable (see Feng et al. 2014 for TG-DSC; Tambach et al. 2009 for Rock–Eval). As the fraction of OM bound to minerals increases with bare fallow duration (no particulate organic matter can be found after 60 years of bare fallow at Versailles (Balabane and Plante 2004), persistent soil OM becomes more thermally resistant. Further research, including studies on model systems (pure minerals and organic molecules) is needed to disentangle the importance of mineral surface interactions and the intrinsic chemical nature of OM on the energetic signatures of organo-mineral complexes.

Implications of low-energy persistent OM for soil C dynamics

Observations of thermally stable, low-energy persistent OM have profound implications for our understanding of C dynamics in soil. If OM with long residence time is less energy-rich than faster-cycling OM, it implies from a thermodynamic perspective (*i.e.*, van't Hoff's law) that the relative temperature sensitivity of OM decomposition increases with OM residence time. This is consistent with recent results from laboratory incubations of LTBF soils (Lefèvre et al. 2014) and long-duration (but still relatively short-term) laboratory soil incubation studies (Conant et al. 2008; Craine et al. 2010). Thermally stable, low-energy OM also suggests that OM persistence may be a function of the high energetic cost-to-benefit ratio experienced by microbial decomposers. That is, while heterotrophic organisms gain energy through the catabolism of organic substrates, they must invest energy for anabolic processes for the production of exocellular enzymes needed for the depolymerization and degradation of substrates before the energy gains can be realized (Rovira et al. 2008). As a corollary, co-metabolism with more energy-rich OM compounds may be essential for microbes to decompose low energy OM, thereby providing mechanistic support for priming effects (Fontaine et al. 2007). Persistent OM from the Versailles bare fallow soil has indeed been shown to be sensitive to priming (Guenet et al. 2012). Our results therefore suggest that the forecasted increases in temperature and net primary production

(and thereby increased inputs of fresh energy-rich OM compounds to the soil) may increase the mineralization rate of persistent OM, inducing an additional forcing on the global C cycle. This positive feedback on climate change is currently poorly accounted for in global climate models.

Our quantification of the energetic signature of persistent OM is a static representation of the state of this pool. A more complete representation of the energetic dynamics of OM involves additional aspects. For instance, microbial enzymes modify the activation energy of reactions, and the decomposition of OM involves a suite of exo- and intra-cellular enzymes. This suggests that the activation energies measured by thermal analysis techniques are likely to be modified by soil microbial activity, and may not be directly transferable to the energetic of enzymatic decomposition (Leifeld and von Lützow 2014). At the present, it is not clear whether the energy density of OM can be considered to be a measure of the fuel for microbial metabolism, how soil microbial communities exploit this energy, and how OM energy density might be related to microbial C use efficiency (e.g., Geyer et al. 2016). Coupling our current results from ramped combustion and pyrolysis to isothermal microcalorimetry (Herrmann and Bolscher 2015; Herrmann et al. 2014) analysis of LTBF soils may yield compelling insights.

An energetics approach to interpreting and quantifying OM in soil turnover (Currie 2003) may prove valuable in making simulation models of C dynamics more mechanistic. The next generation of soil C dynamics simulation models should perhaps then include not only OM solubility, soil architecture (Lehmann and Kleber 2015) and geochemical factors (Doetterl et al. 2015), but also the microbial energy gains provided by OM (Herrmann et al. 2014) and the energetic barriers to decomposition. These model improvements would allow them to be more robust and capable of better predicting soil OM responses to disturbances such as climate change.

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