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1 **Microbial and plant-derived compounds both contribute to persistent soil organic**
2 **carbon in temperate soils**

3

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25

26 **Abstract**

27 Our study tests the emerging paradigm that biochemical recalcitrance does not affect
28 substantially long-term (50 years) SOC persistence. We analyzed the molecular composition of
29 SOC in archived soils originating from four European long-term bare fallow experiments
30 (Askov, Rothamsted, Versailles and Ultuna). The soils had been collected after various periods
31 (up to 53 years) under bare fallow. With increasing duration of bare fallow without new organic
32 inputs, the relative abundance of cutin- and suberin-derived compounds declined substantially,
33 and the abundance of lignin-derived compounds was close to zero. Conversely, the relative
34 abundance of plant-derived long-chain alkanes remained almost constant or increased during
35 the bare fallow period. The relative abundance of N-containing compounds, considered to be
36 abundant in SOC derived from microbial activity, increased consistently illustrating that
37 microbial turnover of SOC continues even when plant inputs are stopped. The persistence of
38 the different families of plant-derived compounds differed markedly over the scale of half a
39 century, which may be ascribed to their contrasting chemical characteristics and recalcitrance,
40 or to differences in their interactions with the soil mineral matrix, and likely some combination
41 since chemical composition drives the degree of mineral association. Using soil from this
42 unique set of long-term bare fallow experiments, we provide direct evidence that multi-decadal
43 scale persistent SOC is enriched in microbe-derived compounds but also includes a substantial
44 fraction of plant-derived compounds.

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47

48 **Keywords:** TMAH-Py-GC-MS, long-term bare fallow, plant-derived compounds, soil

49 organic carbon persistence

50

51 **Introduction**

52 In spite of their importance for soil quality and climate regulation, the mechanisms responsible
53 for long-term storage of soil organic carbon (SOC) remain insufficiently understood
54 (Stockmann et al. 2013). Emerging paradigms suggest that the persistence of SOC is less
55 defined by its chemical composition and recalcitrance than previously conceived (Schmidt et
56 al. 2011; Lehmann and Kleber, 2015). It is envisioned that the chemical composition of organic
57 inputs influence short-term C dynamics whereas the long-term persistence of SOC is defined
58 mainly by its interactions with the mineral matrix and environmental constraints. Evidence
59 supporting the reduced role of inherent biochemical recalcitrance as the primary mechanism for
60 SOC persistence is derived from studies showing shorter residence times of specific compounds
61 presumed to be recalcitrant compared to bulk SOC (*e.g.* Amelung et al. 2008; Schmidt et al.
62 2011), and the predominance of SOC of microbial rather than plant origin. Specifically, plant-
63 derived compounds such as lignin show much shorter residence times than previously
64 anticipated (*e.g.* Dignac et al. 2005; Amelung et al., 2008; Hofmann et al. 2009), and recent
65 molecular level characterization suggest that microbial metabolites and necromass are
66 important constituents of the most persistent fractions of SOC (Miltner et al. 2012; Kallenbach
67 et al. 2016). Although biochemical recalcitrance may not play a primary and direct role in SOC
68 stabilization, the molecular composition of SOC remains an essential feature that plays
69 important indirect roles in its long-term persistence in soil. For example, it has been
70 demonstrated that diverse soil minerals provide selective chemical affinities and induce
71 chemical fractionation of SOC through differential sorption processes (*e.g.* Sollins et al. 2009;
72 Mitchell et al. 2013; Coward et al. 2018).

73 Nonetheless, the link between SOC persistence and molecular composition remains
74 obscured partly because of lack of analytical procedures that allow persistent SOC to be
75 physically separated from labile SOC. One obvious way to assure that only persistent SOC is

76 present in a soil is to cease C inputs and allow the more decomposable compounds to
77 decompose. Long-term bare fallow experiments (LTBF) provide such conditions. LTBF
78 experiments consist of soil plots that have been kept free of organic inputs and vegetation for
79 decades by manual or chemical means. New C inputs to the soils have therefore been negligible
80 during the fallow period, and the SOC remaining has consequently persisted for at least the
81 duration of the fallow period. With regular sampling and archiving of soils, LTBF experiments
82 now provide a unique research platform for studies of the characteristics of SOC that has
83 persisted in soils for decades (Barré et al. 2010; 2016). It is noteworthy that the persistence over
84 decades of a C atom in the LTBF soils does not necessarily imply that the organic molecule
85 containing this C atom also has persisted for decades. Soil microbial activity in LTBF plots
86 may transfer a C atom, initially present in a plant-derived molecule, into a microbe-derived
87 molecule during the bare fallow period. Consequently, while our experimental set-up allows
88 for studies on the persistence of C entering the soil before the fallow period, it does not reflect
89 the persistence of pre-fallow organic matter.

90 This study examines changes in the molecular composition of SOC after decades of *in-*
91 *situ* decomposition without new organic inputs. We hypothesize that the molecular composition
92 of SOC is dominated by plant residues in the early phase of LTBF (~10-30 years), whereas
93 plant-derived compounds contribute insignificantly to SOC that persists for half a century, and
94 that this persistent SOC is dominated by SOC of microbial origin. We tested the hypotheses by
95 analyzing the molecular composition of soils collected after different periods under bare fallow
96 from four experiments located at Askov (Denmark), Rothamsted (United Kingdom), Ultuna
97 (Sweden) and Versailles (France). The soils were analyzed using pyrolysis gas-chromatography
98 mass-spectrometry after tetramethylammonium hydroxide derivatization (TMAH-Py-GC-MS).
99 Molecular characterization of SOC using Py-GC-MS is a well-established method that allows
100 identification of plant-derived compounds such as lignin, cutin, suberin and long-chain aliphatic

101 compounds and compounds of microbial origin such as short-chain or ramified alkanes
102 (Derenne & Quenea 2015). Results obtained by Py-GC-MS have been used to compare soils
103 from different biomes (*e.g.* Carr et al. 2013), and soils under contrasting land-use (*e.g.*
104 Schellekens et al. 2013; Oliveira et al.. 2016) or land management practices (Buurman &
105 Roscoe 2011).

106

107 **Materials and Methods**

108 Study sites and soil samples

109 The LTBF experiments were initiated at different times at four sites across NW Europe: (1)
110 INRA “42 parcels” experiment initiated in 1928 and located in the grounds of the Château de
111 Versailles in Versailles, France; (2) the long-term bare fallow initiated in 1959 and located
112 adjacent to the Ley-Arable experiment at Rothamsted Research, Harpenden, United Kingdom;
113 (3) the Ultuna long-term field experiment initiated in 1956 and located at the Swedish
114 University of Agricultural Sciences, Uppsala, Sweden; and (4) the Lermarken long-term bare
115 fallow initiated in 1956 and located at Askov Experimental Station, Denmark (Table 1). More
116 details on the LTBF sites are available in Barré et al. (2010) and references cited therein.
117 Surface (up to 25 cm deep) soil samples collected just before and periodically during the bare
118 fallow period were ground and sieved at 2 mm, air-dried and archived in the dark at ambient
119 temperatures. In total, 26 samples were included in the study: eight from each of Rothamsted
120 and Versailles (4 dates × 2 plots), four from Ultuna (4 dates × 1 plot) and six from Askov (3
121 dates × 2 plots). Sampling dates of samples included in the study are mentioned in Table 1. For
122 the Versailles LTBF experiment, only soils sampled until 1980 (up to 51 years of bare fallow)
123 were included in this study because molecular analyses of soils sampled after 1980 yielded
124 compound abundances that were too low for quantification due to low SOC contents (6-8 mg
125 C g⁻¹ soil).

126

127

128 TMAH-Py-GC-MS TMAH analysis

129 All samples were subjected to Curie point pyrolysis in the presence of TMAH. Samples (5–10
130 mg, depending on OC content) were impregnated with a few drops of TMAH in 25% MeOH
131 (1/1, w/w) prior to loading into ferromagnetic tubes. Samples were pyrolysed at a Curie
132 temperature of 650 °C for 10 s using a pyrolysis unit (Pilodist), coupled to a gas chromatograph
133 (GC, Trace Ultra Thermo electron) and a mass spectrometer (MS, DSQ 1 Thermo). After 10
134 min at 50 °C, the GC oven temperature gradually increased from 50 °C to 310 °C at 2°C min⁻¹.
135 For the sake of clarity, the carbon number of the parent compound is reported for aliphatic
136 products since TMAH induces *in situ* methylation. Semi-quantitative information was derived
137 from TMAH-Py-GC-MS using the Agilent ChemStation software by manually subtracting the
138 background signal from the total ion current (TIC) of each peak of interest, and integrating each
139 peak area. The relative abundance of each compound was calculated by dividing its peak area
140 by the sum of all of the areas of identified pyrolysis products. Compounds corresponding to
141 each peak were identified by MS using the NIST compound library and published data. Only
142 results for elution times greater than 30 minutes were used because of strong co-elution and
143 artefacts due to TMAH elution before 30 minutes. Consequently, only part of SOC (generally
144 50-60%) is recovered as pyrolysis effluents and characterized by Py-GC-MS (Haddix et al.
145 2016). Identified pyrolysis products or groups of products, and their probable origins (*i.e.*,
146 plants, microbes or ubiquitous) are reported in Table 2 with key references.

147

148 Statistical analyses

149 A principal component analysis (PCA) was performed to assess similarities and differences in
150 molecular composition among the 26 soil samples (*i.e.*, all sites, years of LTBF duration, and

151 plots) based on the 18 selected mean-centered and variance-scaled Py-GC-MS TIC relative
152 abundances using the multivariate analysis R package Factoextra (<http://www.sthda.com>).
153 Trends in the relative abundance of each identified compound were investigated using least-
154 squares linear regression analysis with bare fallow duration as the independent variable using
155 the lm R statistical package.

156

157 **Results and Discussion**

158 Molecular characteristics of soils at the start of bare fallow

159 The thermo-chemolysates of samples collected at the start of the bare fallow periods (Figure 1)
160 are consistent with the products commonly identified in Py-GC-MS using similar acquisition
161 parameters (Vidal et al. 2016). At all sites, aromatic compounds are mainly represented by
162 lignin derived compounds and to a lesser extent by phenolic compounds. Aliphatic compounds
163 comprise *n*-alkanoic acids, ω -hydroxyalkanoic acids, *n*-alkanedioic acids, *n*-alkanes, *n*-
164 alkanols, 8,16 - 9,16 and 10, 16 or 18 dihydroxyhexadecanoic acids (DiOH) and 9,10,18
165 trihydroxyoctadecanoic acid (TriOH). Carbohydrates and N-containing compounds were also
166 observed.

167 The molecular composition of SOC varies substantially among experimental sites
168 (Figure 2a) as evidenced by the lack of clustering of the year-zero samples in PC space, and
169 conversely the clustering of samples by site and separation of sites in PC space. Differences in
170 the molecular composition among sites are illustrated by separation along PC 1 in the scores
171 plot (Figure 2a), where soil from Versailles and Ultuna are relatively similar but differ from
172 those from Rothamsted and Askov. These differences between sites are maintained over the
173 duration of the LTBF experiments, suggesting that variability in molecular composition among
174 sites may be greater than changes over time. The relative abundance of ω -hydroxyalkanoic
175 acids was greater at Rothamsted and Askov compared to Versailles and Ultuna (Figure 2,

176 Supplementary Table 1). Long chain ω -hydroxyalkanoic acids (C₂₂ and C₂₄) are recognized as
177 suberin-derived compounds, *i.e.* a typical biopolymer mainly present in plant roots
178 (Kolattukudy 1980; Nierop 2001; Kögel-Knabner et al. 2002). Compared to other sites, Askov
179 also shows greater relative abundances of DiOH, TriOH, and long chain *n*-alkanols. These
180 compounds are typical plant-derived compounds, with TriOH and DiOH being commonly
181 identified as cutin monomer-derived compounds (Riederer et al. 1993; Del Rio and Hatcher
182 1998; Otto and Simpson 2006). As such, Askov and to some extent also Rothamsted exhibit
183 greater plant contributions compared to Versailles and Ultuna which in turn have greater
184 abundances of N-containing compounds (especially Versailles) than Askov and Rothamsted.
185 The greater proportion of N-containing compounds suggests a higher contribution of microbial
186 products to SOM (Grandy & Neff 2007; Vancampenhout et al. 2009). The observed N-
187 containing compounds are dominated by pyrrole and pyridine which are usually associated with
188 microbial material whereas N-containing compounds from plant origin (for instance
189 diketodipyrrole or diketopiperazines) were not observed. The different contribution of N-
190 containing compounds may also be related to the different abundance of legumes in the
191 vegetation before bare fallow implementation. However, information on plant community
192 compositions before bare fallow implementation is not available. The addition of N fertilizers
193 does not seem to play an important role for the relative content of N-containing compounds as
194 a higher contribution of N-containing compounds was not observed at Askov where fertilizers
195 were added throughout the bare fallow.

196 A number of edaphic factors may explain the observed differences among sites in
197 molecular composition, including climate (temperature, precipitation), management history,
198 pre-fallow vegetation and productivity, and soil properties such as texture, mineralogy and pH.
199 The SOC associated with fine mineral fractions has been ascribed to microbial origin (Nierop
200 et al. 2005; Grandy & Neff 2008), partly because of the affinity of reactive mineral surfaces for

201 N-containing functional groups. The long agricultural history and sandy texture at Askov
202 compared to the three other sites (Table 1), may explain why compounds usually associated
203 with fine fractions (*i.e.*, microbe-derived N-containing compounds) are recovered in smaller
204 relative proportions at Askov. Conversely, high proportions of fine particles in Versailles and
205 Ultuna soils may explain the greater proportions of microbial products. Despite the high
206 proportion of fine particles in soils at Rothamsted, results indicate a large plant-derived
207 contribution. Before being converted to fallow, the Rothamsted site had a long history of
208 permanent grassland and probably a greater accumulation of plant inputs than the other sites,
209 as reflected by the larger initial soil C contents (Table 1). The larger pre-fallow plant inputs at
210 Rothamsted might explain the greater relative proportion of plant-derived compounds in the
211 fallowed soil.

212

213 Evolution of SOC molecular composition during the bare fallow period

214 The degree to which samples from the same site cluster in PCA scores reflects the degree to
215 which SOC molecular composition changed relative to other sites. Tight clustering of samples
216 from the same site but different bare fallow durations would indicate less change in chemical
217 composition compared to more scattered samples (Figure 2a). As such, changes in SOC
218 composition appears to be greatest at Rothamsted, followed by Versailles, Ultuna, and Askov.
219 PCA loadings (Figure 2b) indicate that most plant-derived compounds (ω -hydroxyalkanoic
220 acids, long chain *n*-alkanedioic acids, dihydroxyhexadecanoic acids, trihydroxyoctadecanoic
221 acid and long chain *n*-alkanoic acids) contribute negatively to PC 1 whereas microbial-derived
222 compounds (FAMicro) have a positive contribution to PC 1. N-containing compounds are
223 considered to be mainly of microbial origin and have a positive contribution to PC 1, whereas
224 ubiquitous compounds considered to be mostly derived from plants such as short chain diacids
225 (C₈, C₉) contribute negatively to PC 1. The changes in SOC molecular composition with time

226 under bare fallow for samples from Versailles and Ultuna are illustrated by PCA trends toward
227 more positive PC 1 and more negative PC 2 scores (*i.e.*, towards the bottom-right quadrant;
228 Figure 2a). For samples from Rothamsted, only the trend towards more negative PC2 scores is
229 observed. Lack of divergence in PC scores for samples within sites during the first 25 years of
230 bare fallow suggests that changes in the chemical composition of SOM were limited for all sites
231 except Rothamsted. This site showed the greatest loss of SOC during the first 25 years (from
232 30 mg C g⁻¹ soil to 15 mg C g⁻¹ soil). Conversely, no temporal evolution is observed in PCA
233 scores for Askov samples.

234 Temporal trends in the relative abundances of pyrolysis products were compound
235 specific (Figures 2a and 3; supplementary Table 1). Lignin-derived compounds decreased with
236 bare fallow duration at all sites, though the trend is not statistically significant for Askov (Figure
237 3; supplementary Table 1). Lignin-derived compounds are not observed after 50 years of bare
238 fallow at Versailles, Ultuna and Rothamsted, suggesting that the downward trend might have
239 been more pronounced at Askov after a longer period of bare fallow. These results are consistent
240 with previous results showing a low persistence of lignin compounds in soils (Dignac et al.
241 2005; Kelleher et al. 2006; Hofmann et al. 2009). Cutin- and suberin-derived compounds
242 (ω OH, diacids long chain, TriOH and DiOH) contributed similarly to the PCA axis (Figure 2b).
243 As for lignin-derived compounds, the sum of cutin- and suberin-derived compounds during the
244 bare fallow period showed that their relative contributions decreased with duration of bare
245 fallow, except at Askov with the shortest bare-fallow period (Figure 3; Supplementary Table
246 1). The decrease in cutin+suberin derived compounds is statistically significant for Rothamsted
247 and Versailles, but not for Ultuna (Supplementary Table 2), likely because of the smaller
248 number of samples retrieved at this site. A rapid decay of cutin- and suberin-derived compounds
249 (water repellent wax-like substances from the cuticle) has previously been reported (Armas-
250 Herrera et al. 2016). Conversely, the relative abundance of long chain alkanes, also of plant

251 origin, showed no trends with bare fallow duration, with the exception of Versailles where a
252 statistically significant increase was observed. However, the significance of this trend relies on
253 the last sampling date (Figure 3; Supplementary Table 2). The different slopes of the regressions
254 models for the various groups of plant-derived compounds (Supplementary Table 2) suggest
255 that alkanes have a greater persistence than cutin and suberin, which in turn are more persistent
256 than lignin. Thus, the chemistry of these plant-derived compounds affects their persistence in
257 soil for at least 50 years.

258 The relative abundance of N-containing compounds tended to increase with bare fallow
259 duration at all sites except Askov (Figure 3), the increases being statistically significant for
260 Rothamsted and Versailles (Supplementary Table 2). This is consistent with Carr et al. (2013),
261 who observed a relative decrease in lignin-derived compounds and a relative increase in N-
262 containing compounds in soils with degraded SOM retrieved from arid and semi-arid regions
263 in South Africa. For all other compounds, the trends with duration of bare fallow were minor,
264 not statistically significant or not consistent across sites (supplementary Table 2).

265

266 Plant and microbial contributions to persistent SOC

267 Differences in the relative abundance of plant-derived, microbial-derived, and N-containing
268 compounds between the start of the bare fallow period and the last sampling varied by
269 compound group and site (Figure 4). Except at Askov, the relative abundances of plant-derived
270 compounds decreased and microbial products and N-containing compounds increased after
271 decades of bare fallow. This illustrates that the microbial turnover of SOC continues even when
272 plant inputs are stopped and may promote an ongoing relative increase of microbial products in
273 the bare fallow soils compared to plant-derived compounds. However, compounds of plant
274 origin are still recovered in substantial quantities after 50 years without new organic inputs. At
275 Rothamsted plant-derived compounds represent about 50% of relative peak areas while they

276 accounted for 25% at Versailles and Ultuna at the last sampling. The presence of plant-derived
277 compounds after 50 years of bare fallow substantiates the influence of initial plant chemistry
278 on the resulting SOC composition. Contrary to our hypothesis, this demonstrates that persistent
279 SOC is not composed exclusively of compounds derived from microbial activity but that plant-
280 derived compounds contribute to SOC even after several decades of degradation. Our findings
281 contribute to the current debate on the composition of persistent SOC and the importance of
282 substrate quality for the chemical composition of SOC that is formed during consecutive cycles
283 of microbial turnover (Kallenbach et al. 2016; Liang et al. 2017). Thus, biochemical
284 characteristics remain a mechanism to consider for long-term (>50 years) persistence of SOC.
285 Chemically complex plant components undergo substantial alteration during microbial
286 decomposition, and decomposition products, as well as compounds originating from the
287 microbial population, are retained in organo-mineral associations (Christensen 2001). Indeed,
288 previous studies demonstrated negligible amounts of non-mineral associated particulate OM
289 (Balabane & Plante 2004).

290 Wickings et al. (2012) proposed three hypotheses to describe changes in the chemical
291 composition of litter during decomposition: (1) the chemical convergence hypothesis (“all
292 litters will converge eventually towards a common chemistry”); (2) the initial litter quality
293 hypothesis (“the initial litter quality has persistent effects on litter chemical composition”); (3)
294 the decomposer control hypothesis (“changes of litter chemistry during decomposition do not
295 simply reflect the effects of time or initial litter quality, but are also strongly influenced by the
296 functional attributes of distinct decomposer communities”). Our dataset allows for testing these
297 hypotheses of organic matter decomposition in soil. Previous results from spectroscopic
298 analyses (MIRS and NEXAFS) of LTBF soils failed to provide clear evidence of chemical
299 trends and convergence in SOC composition over time (Barré et al. 2016). Conversely, the Py-
300 GC-MS data reported here demonstrate that, despite contrasting initial molecular

301 characteristics, SOC in soils subject to more than 25 years of bare fallow had similar chemical
302 trajectories during degradation. The trajectory was characterized by a decrease in lignin-, cutin-
303 and suberin-derived compounds and a relative increase in N-containing compounds and alkanes
304 (Figure 3). However, initial differences in SOC composition among the sites were reflected
305 even after 50 years of decomposition, and these differences among sites appear to be greater
306 than the changes undergone during long-term decomposition. The degradation of SOC may
307 therefore be explained by a combination of the chemical convergence and the initial chemical
308 quality hypotheses proposed by Wickings et al. (2012). Our focus on the evolution of the
309 chemical and molecular composition of SOC during decomposition under long-term bare
310 fallow does not address the structure of the decomposer communities. Consequently, the
311 decomposer control hypothesis cannot be ruled out.

312

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322

323

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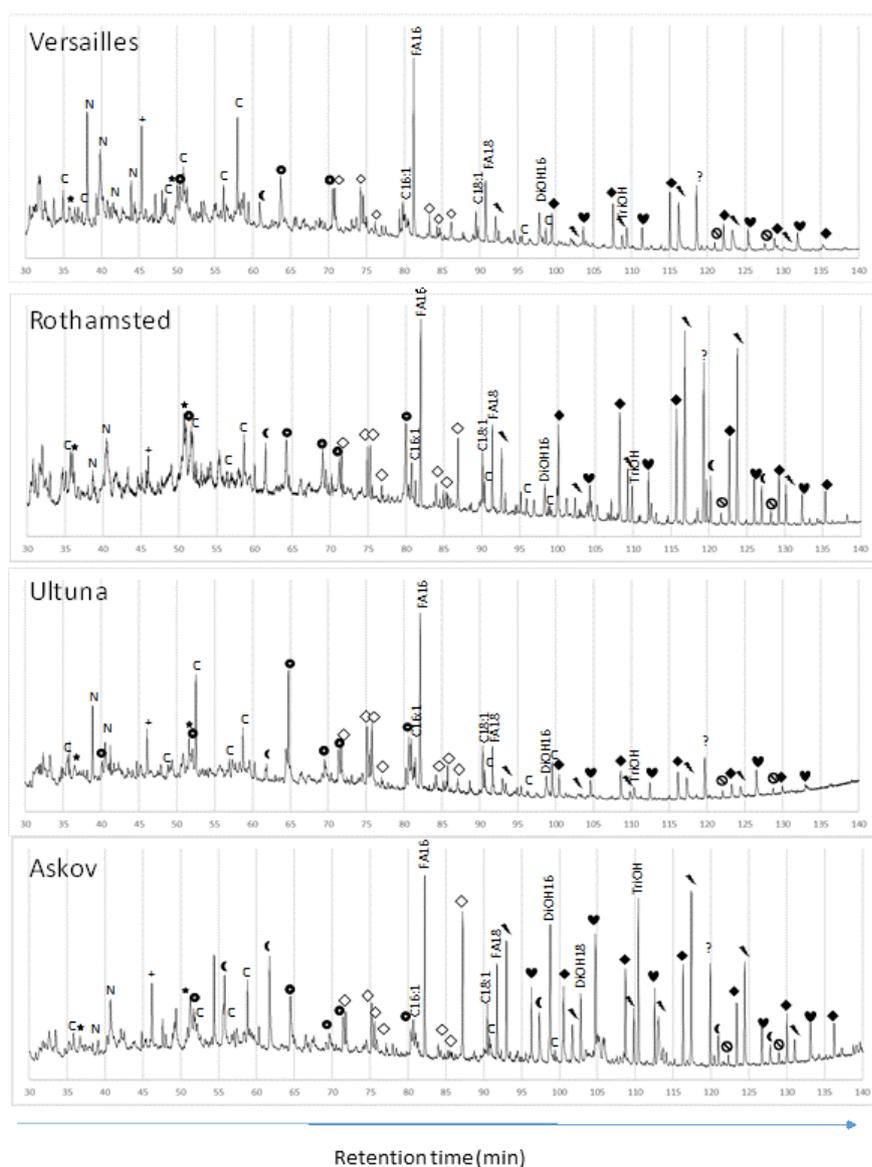
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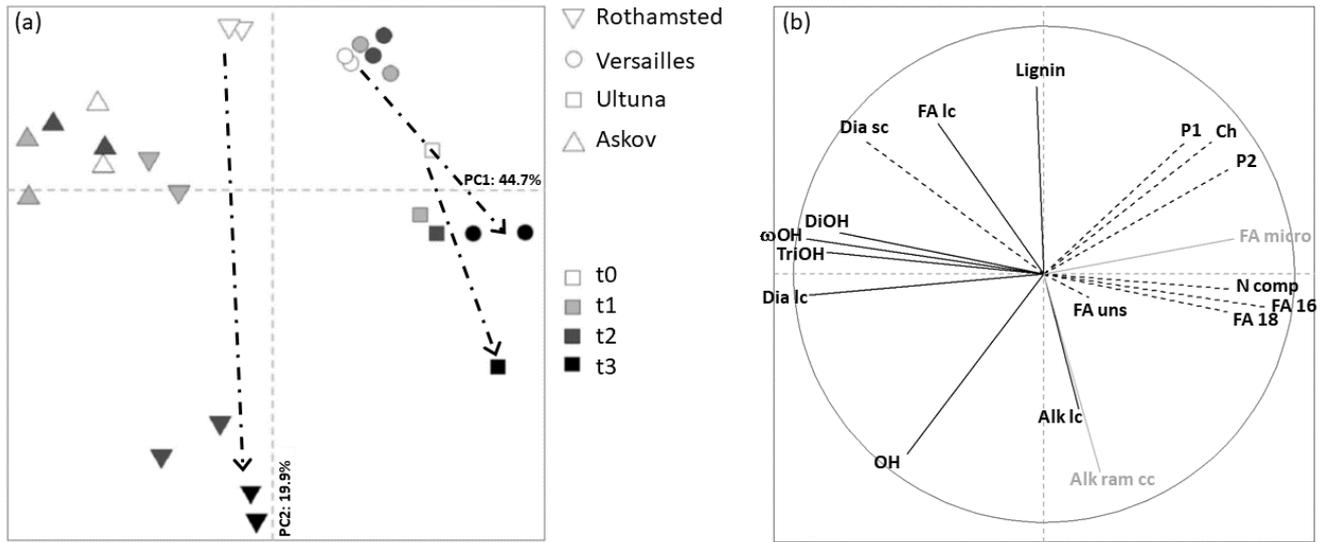
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 458 **Fig. 1** Total ion concentration (TIC) traces of 650°C Curie point pyrolysate of initial samples
 459 of each site. Compound families are identified using various symbols. **C** refers to
 460 “carbohydrates”, **N** to “N-containing compounds”, **●** to “lignin-derived” compounds and **★** to
 461 other phenolic compounds. Aliphatic compounds were identified as follows: methyl esters of
 462 microbe-derived fatty acids (◇), long-chain methyl esters of n-alkanoic acids (◆), ω-
 463 hydroxyalkanoic acids (♣), n-alkanedioic acids (●), n-alkanes (⊙), n-alkanols (♥), 8,16 - 9,16
 464 and 10, 16 or 18 dihydroxyhexadecanoic acids (DiOH16), 9,10,18 trihydroxyoctadecanoic acid
 465 (TriOH). + corresponds to a contaminant and ? to an unidentified compound.



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467 **Fig. 2** Scores (a) and loadings (b) of principal component analysis carried out with the relative
 468 abundance of the 18 compounds (see Table 2 for description) of soil samples from different
 469 durations of long-term bare fallow at four sites. Arrows on Figure 2a were added from visual
 470 interpretation. Variables in black and grey with solid lines correspond to plant-derived and
 471 microbe-derived compounds respectively. Variables in black with dashed lines correspond to
 472 ubiquitous compounds.

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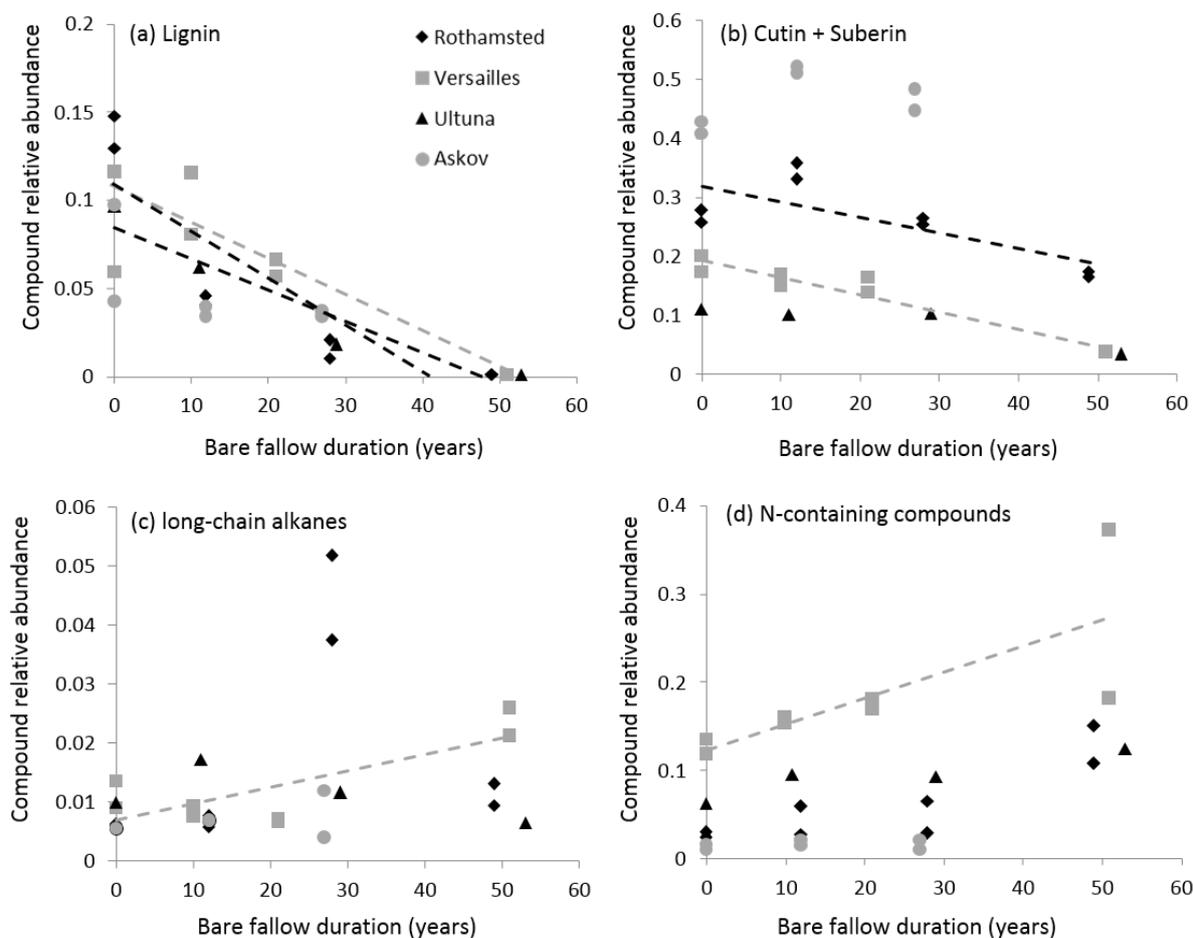
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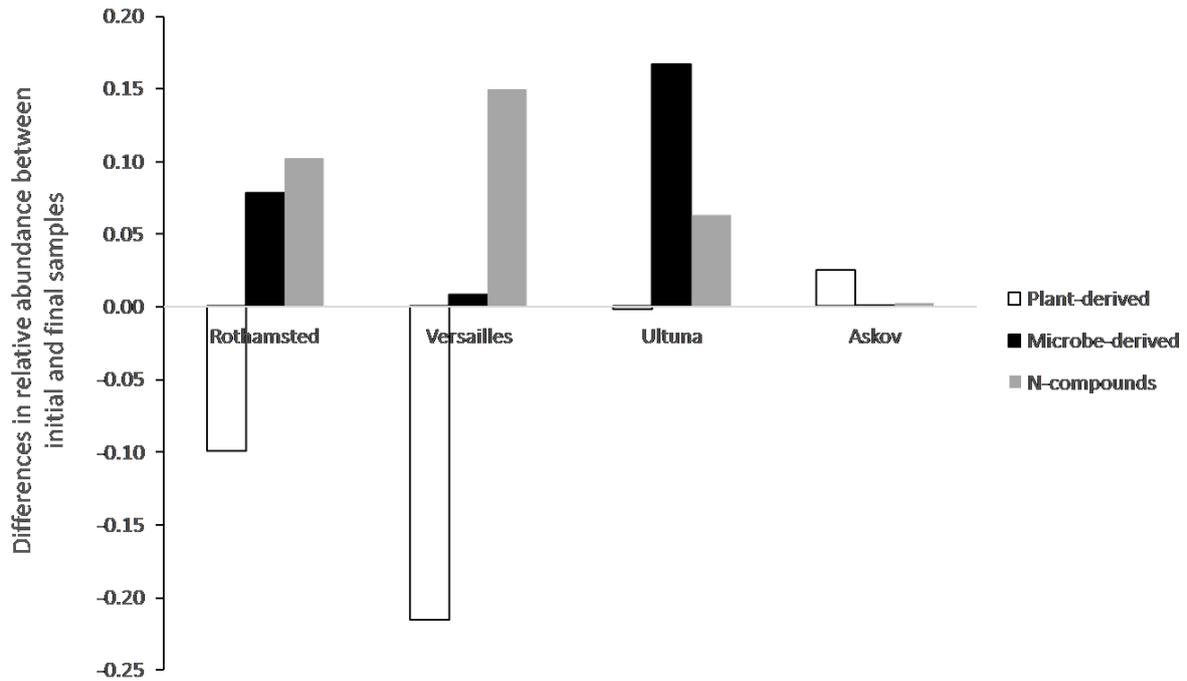
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484 **Fig. 3** Evolution with bare fallow duration of the relative abundances of lignin, cutin+suberin
 485 (sum of ω OH, diacids long chain, TriOH and DiOH), long-chain alkanes and N-containing
 486 compounds at four long-term bare fallow sites. As cutin- and suberin-derived products (ω -
 487 hydroxyalkanoic acids, *n*-alkanedioic acids, dihydroxyhexadecanoic acids,
 488 trihydroxyoctadecanoic acid) had a similar contribution to the PCA axis (Figure 2b), their
 489 relative abundances were combined in this figure. Dotted lines refer to statistically significant
 490 ($p < 0.05$) relationships.

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493 **Fig. 4** Differences in the relative abundances of plant-derived, microbe-derived and N-
 494 compounds between initial and final soil samples from four long-term bare fallow sites. All
 495 compounds within the plant and microbial origins groups were summed. For Rothamsted,
 496 Versailles and Askov, where two replicates were analyzed, the two values were averaged.
 497 Differences were obtained by subtracting the averaged relative abundances in the final samples
 498 from the averaged values in initial samples.

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	Versailles (FR)	Rothamsted (UK)	Ultuna (SE)	Askov (DK)
Location	48°48' N 2°08' E	51°82' N 0°35' E	59°49' N 17°38' E	55°28' N 9°07' E
Mean annual temperature (°C)	10.7	9.5	5.5	7.8
Mean annual precipitation (mm)	628	712	533	862
FAO soil classification	Luvisol	Chromic Luvisol	Eutric Cambisol	Orthic Luvisol
Previous land-use	Unmanaged grassland	Managed grassland	Arable	Arable
Soil textural class (% sand/ silt/clay)	Silty loam (26/57/17)	Flinty silty clay loam (13/62/25)	Clay loam (23/41/36)	Coarse sandy loam (79/11/10)
Sampling depth (cm)	25	23	20	20
Experiment initiation year	1928	1959	1956	1956
Number of field replicate plots	2	2	1	2
Sampling year (OC concentration mgC g ⁻¹ soil)	1929 (18.7 ; 16.2)	1959 (30.5 ; 30.1)	1956 (15.0)	1956 (17.5 ; 16.9)
	1939 (13.2 ; 11.9)	1971 (18.2 ; 18.1)	1967 (13.3)	1968 (11.8 ; 14.1)
	1950 (11.9 ; 11.3)	1987 (12.5 ; 13.9)	1985 (10.6)	1983 (11.4 ; 11.2)
	1980 (7.5 ; 7.4)	2008 (9.7 ; 10.3)	2009 (9.1)	

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510 **Table 1** Selected site characteristics of four long-term bare fallow field experiments, and the
511 identification of archived soil samples used for characterization of the molecular composition
512 of soil organic matter.

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Code (Figure 2)	Symbol (Figure 1)	Compound	Origin	Key references
Lignin	●	Lignin derived aromatic compounds	Higher plants	Clifford et al. (1995), Chefetz et al. (2000), Nierop (2001) and Otto et al. (2005)
<u>Other phenolic compounds</u>				
P1	★	1,4-Dimethoxy benzene	Higher plants	Chefetz et al. (2000)
P2	★	1,2,4-Trimethoxy benzene (4-methoxy catechol)	Higher plants	Fabbri & Helleur (1999)
<u>Aliphatic compounds</u>				
FAmicro	◇	C14, C14i, C15i,a and C17i,a n-alkanoic acids	Microorganisms	Wannigama et al. (1981), Quénéa et al. (2012)
FA_lc	◆	C20-32 even chain n-alkanoic acid	Higher plants	Chefetz et al. (2000), Wannigama et al. (1981)
FA16	FA16	C16 n-alkanoic acid	Ubiquitous	Nierop (2001)
FA18	FA18	C18 n-alkanoic acid	Ubiquitous	Wiesenberg et al. (2010)
FAuns	C16:1 C18:1	C16:1 and C18:1 n-alkanoic acid	Ubiquitous	Wiesenberg et al. (2010)
Alk_lc	⊙	C25–33 n-alkanes	Higher plants	Rieley et al. (1991), Wiesenberg et al. (2010)
Alk_ram_sc	⊙	C13–15 n-alkanes + ramified short chain alkanes	Microorganisms	Han and Calvin (1969)
OH	♥	C18–30 n-alkanols	Higher plants	Kolattukudy (1980), Quénéa et al. (2006)
ωOH	↘	C16–26 even chain x-hydroxyalkanoic acids	Higher plants (Cutin and Suberin)	Kolattukudy (1980) and Nierop (2001)
Dia_sc	☾	C8, C9 n-alkanedioic acids	Ubiquitous	Kolattukudy (1980) and Nierop (2001), Grasset & Ambès (1998)
Dia_lc	☾	C18, C22 and C24 n-alkanedioic acids	Higher plants (Cutin and Suberin)	Kolattukudy (1980) and Nierop (2001)
DiOH	DiOH16	8,16 - 9,16 and 10, 16 or 18 dihydroxyhexadecanoic acids	Higher plants (Cutin)	Riederer et al. (1993) and Del Río & Hatcher (1998)
TriOH	TriOH	9,10,18 trihydroxyoctadecanoic acid	Higher plants (Cutin)	Otto & Simpson (2006)
<u>Other compounds</u>				
Ch	C	Carbohydrates	Ubiquitous	
N-compounds	N	N-containing compounds	Ubiquitous	

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518 **Table 2** Identified pyrolysis products, the probable origins of each product or group of
519 products, and associated key references

