

Response of humic acid structure to soil tillage management as revealed by analytical pyrolysis

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10 11 **ABSTRACT**

12
13 The effects on the structural features of humic acids (HA) from dryland farming soils under
14 long term management practices have been approached by analytical pyrolysis (Curie-point
15 pyrolysis-gas chromatography/mass spectrometry, Py-GC/MS). The field experiments
16 (started in 1987) include conventional, minimum and no-tillage plots, as well as non
17 cultivated plots. The HA isolated from the various plots showed significant differences in
18 their pyrolytic behaviour, in particular regarding the total abundances of alkyl pyrolysis
19 compounds (fatty acids, alkenes and alkanes). The occurrence of very short-chain fatty acids
20 (C₅ to C₁₁) in uncultivated plots could be indicative of constitutional alkyl structures in the
21 relictual HA from undisturbed soil. The effect of soil tillage managements substantially
22 increased total abundances of fatty acids in plots under conservation practices (mainly no-
23 tillage).

24 The HAs from uncultivated soils showed the greatest percentages of alkanes and alkenes.
25 This was associated to the increased proportions of even C-numbered alkene homologues
26 from C₁₂ to C₁₈, possibly related to the incorporation of microbial compounds during the
27 humification process. High percentage of alkylbenzenes and catechols were also
28 characteristic of the uncultivated plots. The increased proportions of methoxyphenols, in
29 special of the syringyl (dimethoxyphenyl) type compounds, in HAs from plots subjected to
30 conventional tillage pointed out to humification processes based on progressive alteration of
31 plant lignins. From the viewpoint of soil quality, the results suggest comparatively advanced

32 transformation stages of the HA from uncultivated plots, which means that conservation
33 tillage practices seems to lead to increasing soil C levels, at expenses of the accumulation
34 of comparatively recent organic matter derived either from altered lignins and/or microbial
35 biomass.

36

37 *Keywords:* Humic acid; Soil organic matter; Dryland management; Minimum tillage; No-
38 tillage; Curie-point pyrolysis

39

40 **1. Introduction**

41

42 Current agroecological research is paying increasing attention to the assessment of soil
43 quality descriptors useful in forecasting sustainability of productive fields [1–4]. Among such
44 descriptors total soil C has been considered a key parameter to assess soil, since it is
45 generally accepted that soil organic matter (SOM) influences many soil properties related to
46 its productivity, including water holding capacity, bulk density, aggregate stability, cation
47 exchange and biological activity [5,6]. However, at this respect, a new trend deal with the
48 convenience of studying the quality of SOM instead of only considering the total amount of
49 soil organic C. In fact, even the quantitative composition of the major SOM fractions (i.e.,
50 free organic matter, humic acid (HA), fulvic acid (FA), humin) shows a limited value as
51 indicator of soil perturbation processes [7–9]. In addition, it has also been suggested that in
52 agricultural soils under contrasted tillage practices the different fractions may have similar
53 turnover rates [10].

54 One recognized way to approach the quality of SOM is the study of the different organic
55 fractions at a molecular level. At this respect, the potential of analytical pyrolysis in analysing
56 the molecular composition of soil organic matter, with similar success than alternative, time-
57 consuming, wet chemical methods has been largely recognized [9,11].

58 In fact, previous pyrolytic analyses has succeeded in revealing the impact of no-till
59 cropping systems on the composition of SOM, whereas complementary spectroscopic data
60 (CP/MAS ¹³C NMR measurements) failed in showing substantial effect due to the cropping
61 systems [12].

62 In the present study, a pyrolytic assessment of the impact of tillage practices on dryland
63 farming systems from Central Spain is carried out. The field experiment, involving
64 conventional, minimum and no-tillage as well as uncultivated plots, was designed in 1987. To
65 date, no previous molecular-level characterization of the differences in the organic matter
66 composition was done in this long-term experimental field.

67 Due to the massive incorporation of crop residues in agricultural fields subjected to
68 minimum tillage practices, and in order to preclude trivial pyrolytical results mainly reflecting
69 the quantitative contribution by non-decomposed crop residues (accumulated in the
70 fractions referred to as free organic matter and humins) the study was focused in the
71 comparatively stabilized HA fraction, which is considered to display a recalcitrant nature,
72 mainly after its interaction with the mineral fraction.

73

74 **2. Experimental**

75

76 *2.1. Field location and experiment design*

77

78 The CSIC experimental farm “La Higuera” (UTM coordinates: zone 30; 4434290 m N;
79 377738 m E) under semi-arid continental climate (average temperatures of 6°C and 23°C in
80 winter and summer, respectively; mean annual rainfall, *ca.* 400 mm, with a extended dry
81 season from June to September [13]) is located in Toledo, Central Spain. The soil samples
82 were collected from the 20 upper cm of a Calcic Haploxeralf [14] with loam-sandy texture
83 (total sand = $783 \pm 29 \text{ g kg}^{-1}$, clay = $135 \pm 22 \text{ g kg}^{-1}$).

84 The experiment to compare the effect of different tillage systems was a randomized
85 block design with three replications and two blocks. Plot size was 40 · 9 m. The tillage
86 treatments consisted of: i) conventional plow tillage (CT), i.e., tilling the soil with
87 mouldboard plow to a 20–22 cm depth, then using a rotovator; ii) chisel (minimal) tillage
88 (MT), i.e., chiselling the soil to a depth 14–16 cm and using the rotovator before sowing; iii)
89 no-tillage (NT), i.e., implementing direct drilling. In the NT plots, volunteer barley plants and
90 weeds were sprayed with 0.54 kg ha^{-1} glyphosate [*N*-(phosphonomethyl) glycine] before
91 seeding with a triple-disk seed drill.

92 Samples from uncultivated plots near the experimental plots, with typical Mediterranean
93 shrub consisting mainly of *Retama sphaerocarpa* (L.) Boiss and dense herbaceous layer, were
94 also taken as a reference in order to assess the impact of the above agricultural practices.

95

96 *2.2. Soil general analyses*

97

98 Air-dried soil samples were homogenised to 2 mm. The chemical analyses included
99 determination of pH (water suspension, 1:2.5 w:w), total N (micro-Kjeldahl digestion),
100 available P [15], and available Na, K, Ca and Mg (1 M NH₄OAc [pH = 7] extraction). Oxidizable
101 soil C was determined according to Walkley and Black method [16].

102

103 *2.3. Soil organic matter fractionation and humic acid purification*

104

105 The isolation and quantitative analysis of the soil organic fractions included the previous
106 removal of the not-yet decomposed organic particles (*free organic matter*), which was
107 separated by flotation in 2 M H₃PO₄. The resulting soil residue was treated with 0.1 M
108 Na₄P₂O₇ and 0.1 M NaOH, and the extraction was repeated up to 5 times with each solution.
109 After centrifuging (2600 g) the successive suspensions obtained with the use of these
110 extractants, the supernatant fractions were aggregated and the final volume was measured.
111 Aliquots were taken from this total extract for quantitative determination of the C in the
112 acid-insoluble HA separated after dropwise precipitation with H₂SO₄ (1:1 by vol.) whereas
113 the acid-soluble FA was determined as the difference in C concentration with the whole
114 humic extract [17, 18].

115 For preparative isolation of the HAs, the remaining humic extract was precipitated (pH =
116 1) with 12 M HCl. The precipitate (HA) was redissolved in 0.25 M NaOH and centrifuged (43
117 500 g) to remove the particulate impurities. The resulting sodium humate solution was
118 reprecipitated with HCl overnight, purified with 1 M HCl-HF, dialysed in cellophane bags for
119 one week against distilled water, and desiccated at 36 °C.

120

121

122

123 2.4. Spectroscopic characteristics of the humic acids

124

125 The optical densities at 465 nm (E_4) and 665 (E_6) nm of HAs were determined from
126 solutions of 200 mg C L⁻¹ of HA in 0.02 M NaHCO₃ [19] with a Shimadzu UV-240
127 spectrophotometer. The E_4 is classically considered to be related with the aromaticity of HAs
128 [20,21], whereas the E_4/E_6 ratio gives information about the polydispersity of the HAs [22].

129

130 2.5. Curie-point pyrolysis-gas chromatography/mass spectrometry

131

132 The HAs were analysed by Py-GC/MS in a Horizon Instruments unit attached to a Varian
133 Saturn 2000 gas chromatography-mass spectrometry system (GC/MS). The samples on
134 ferromagnetic wires were heated at the Curie temperature of 510 °C for 5 s. The pyrolytic
135 interface was set to 250 °C, and the chromatographic temperature was programmed from
136 50 to 100 °C at 32 °C min⁻¹ and then up to 320 °C using a rate of 6 °C min⁻¹. In order to
137 enhance the chromatographic resolution, a liquid CO₂ cryogenic unit, fitted to the injection
138 port, was adjusted from -30 °C (1 min) to 300 °C at 20 °C min⁻¹. A 25-m · 0.32 mm · 0.4 (m
139 fused-silica capillary J&W CP-Sil 5 CB column (Agilent Technologies Spain S.L., Las Rozas,
140 Madrid) was used.

141 The pyrolysis compounds were identified on basis to their electron impact mass spectra
142 (70 eV) and by comparison with those in the Wiley (1986) spectral database. The ion traces
143 for the main series of homologous compounds were obtained (e.g., 85 for alkanes, 69 for
144 alkenes, 60 for fatty acids). In the case or aromatic compounds reconstructed ion
145 chromatograms displaying the joint intensity of the major diagnostic fragments, in general
146 $[M^+]$ and $[M^+ - CH_3]$, were used to identify the expected pyrolysis products in the whole
147 chromatogram (e.g., m/z 109+124 for guaiacol, m/z 139+154 for syringol, etc).

148

149 2.6. Data analyses

150

151 For comparison purposes, the amounts of the different compounds were considered to be
152 proportional to the peak areas of the total ion chromatographic trace. These data were
153 subjected to multivariate analyses in order to recognize some trends in the compound

154 assemblages released for the HAs from the different experimental plots. The variables
155 (descriptors) processed were the main groups of pyrolysis products from soil HAs, whereas
156 the observations (samples, individuals) were the different spatial replications of the HAs
157 isolated from soils managed with the different tillage systems. The program used
158 (correspondence analysis, two-way table, no supplementary observations [23]) converts the
159 original frequency table into a plot in which samples and variables are depicted as points in
160 the space defined by factorial axes.

161

162 **3. Results and discussion**

163

164 Several general analytical characteristics of the soils are illustrated in Table 1, showing
165 differences due to management. Thus, some significant ($P < 0.05$) improvements in soil
166 fertility with conservation tillage practices were noted. Available P and K in minimum and
167 no-tillage plots were in significantly higher concentrations as regards to conventional tillage
168 plots. The conservation practices also lead to significant increase in soil organic C as regards
169 conventional tillage, mainly with no-tillage, where the C content was found even higher than
170 in the uncultivated soil. In addition, a relative enrichment of the different humus fractions
171 (HA+FA) was found after conservation practices—very significant in the case of no-tillage
172 plots—with respect to conventional ones. Furthermore, the increased values of the HA/FA
173 ratio in minimum and no-tillage plots betrays a trend for accumulation of the HA fraction in
174 the soil managed with these tillage systems. On the other hand, the values for non-
175 extractable humin (highest values in the uncultivated plots) point to progressive
176 insolubilisation of the organic matter in soils under conservation practices (mainly in no-
177 tillage).

178 The lower E_4 values of the HAs from no-tillage plots as regards uncultivated plots could
179 indicate that surface straw mulch has led to soil enrichment in comparatively young and
180 aliphatic organic matter. In this sense, the changes observed in the E_4/E_6 ratio—an index
181 considered to decrease with the molecular size of humic macromolecules [22]—point out to
182 microbial fragmentation of the humic like substances in no-tillage plots when compared with
183 uncultivated plots.

184 Changes in HA characteristics after tillage were also observed, particularly the lower
185 HA/FA ratio and the higher E_4/E_6 ratio, than in the minimum tillage system, both suggesting
186 a lower molecular size of soil humic fractions with conventional tillage than with the
187 minimum tillage system.

188 Table 2 shows the major Curie-point pyrolysis products released from the HA samples
189 expressed as total abundances (relative percentage area of total ion chromatogram). All of
190 them yielded series of alkyl compounds (mainly series of fatty acids, alkenes and alkanes) as
191 well as aromatic compounds (mainly phenols and alkylbenzenes) as major compounds.

192 The most important difference in terms of tillage was observed in the distribution
193 patterns of alkyl compounds.

194 The homologous series of alkenes and alkanes peaks eluting as doublets are frequently
195 considered to be derived from non-hydrolyzable aliphatic polymers, such as cutan and
196 suberan. This would lead to hypothesize that most of these compounds could derive from
197 biopolyester domains in the HA structure more than being individual fatty acids trapped in
198 humic substances as free or esterified acids, as frequently reported in the literature [24].

199 The total abundances of C_{14} to C_{18} fatty acids (mainly palmitic) were significantly ($P <$
200 0.05) higher in cultivated plots compared to the uncultivated ones (Fig. 1). This fact could be
201 interpreted as an aliphatic enhancement of the HA structure at expenses of acids $> C_{14}$ of
202 recent origin from plants or microorganisms. In all cases, the most important effect of soil
203 tillage on the proportions of fatty acids was a relative increase in their total abundances in
204 the plots under conservation practices compared to plots with conventional tillage (Table 2).
205 This agree with the findings by Sleutel et al. [25], who described larger proportions of free
206 fatty acids, sterols, and N-containing compounds in reduced tillage soils than in the
207 conventional tillage soil.

208 In addition to the n -chain alkanes, the 60 m/z ion trace from the three chromatograms
209 (Fig. 1) clearly suggests peaks due to bacterial *iso*- and *anteiso*- C_{15} acids before the n - C_{15} (to
210 much lower extent, and only in some samples, similar doublets for branched fatty acids
211 seem to elute before the peaks for C_{17} and C_{13} n -acids). Assuming that these b -fatty acids
212 are not inherited from aliphatic biomacromolecules but derive from microbial metabolism,
213 the differences between their total abundances in terms of soil management could be

214 interpreted. Compared to the conventional tillage plots, the plots subjected to practices of
215 no tillage and minimum tillage showed proportions of these bacterial acids higher than for
216 the corresponding *n*-chain acids, which could be interpreted as an enhanced size of the
217 microbial biomass degrading the crop wastes left to decompose on these plots.

218 As a difference to fatty acids, the alkane homologue series (C₈ to C₂₂) showed no
219 differences attributable to agricultural practices. Apparently, alkane compounds behaved
220 relatively stable against microbial activity and consequently less responsive to perturbations
221 produced in cultivated plots. Due to the relatively short chain of the alkanes, this could be
222 justified by a biodegradation compensated for by a continuous microbial production,
223 although the comparatively complex *n*-alkane degradation pathways, generating fatty acid
224 intermediates ought not to be ruled out. As regards the alkene series, it was found a
225 substantial proportion of even C-numbered homologues from C₁₂ to C₁₈ in uncultivated
226 plots, possibly derived from from the above-indicated accumulation of alkanes from
227 microbial activity [26].

228 Total abundances of alkanes and alkenes were significantly ($P < 0.05$) higher in HAs from
229 uncultivated than in cultivated plots (Fig. 2) and qualitative differences were observed when
230 the data from alkyl compounds were examined by means of multivariate data analysis.

231 Figure 3 illustrates alkene/alkane distributions versus chain length after correspondence
232 analysis of a data matrix including HA samples, using as descriptors the total abundances of
233 pyrolytic alkyl compounds. The main changes in the fatty acid pattern observed in the plane
234 defined by the two first axes (up to 92% total variance explained) caused by the agricultural
235 practices (Fig. 3a) were a relative increase in proportions of fatty acids with comparatively
236 high C-number (from C₁₂ to C₁₈) in cultivated plots, and the concomitant increase of the
237 proportions of short-chain fatty acids (up to C₁₁) in the uncultivated soil.

238 The alkanes and alkenes (Fig. 3b, c) did not show clear trends in terms of tillage systems
239 but some tendency observed to relative accumulation of short-chain alkanes C₈ and C₉ in
240 uncultivated plots could be pointing out to an origin of these compounds from cutans,
241 suberans or epicuticular waxes which could be a major source of the aliphatic moiety of the
242 HA.

243 The total abundances of alkylbenzenes, commonly described as typical fragments of HAs

244 [27], and considered as indicator of stability of the soil organic matter in soils under different
245 management practices [28] were higher in uncultivated as regards to cultivated plots (Fig.
246 2). These compounds behaved also differently as regards the tillage system, showing low
247 relative proportions under minimum and no-tillage systems.

248 The patterns of phenolic compounds also showed substantial changes depending on soil
249 management practices. Most of the phenols (up to 8% of the total volatile products) showed
250 methoxyl substitutions typical of the lignin structural units (Table 2), which inform about the
251 preservation extent of this biomacromolecule in different soil conditions [29,30]. Therefore,
252 an increase in the yields of methoxyphenols in plots with conventional tillage (Fig. 2) point
253 out to prevailing processes of formation of HAs based on direct transformation of plant
254 lignin. In addition, the high syringyl/guaiacyl ratio (data not shown) calculated for these HAs
255 could betray to comparatively rapid incorporation of lignin to the HA fraction when the soil
256 is managed with conventional tillage in comparison with conservation tillage or uncultivated
257 plots. As a whole, the pyrolytic results suggest that the continuous incorporation of crop
258 waste on soil surface of reduced tillage plots would lead to somewhat higher inputs of
259 microbial metabolites as starting materials for HA formation. In fact, the guaiacyl-type lignins
260 have higher degree of internal cross-linking than those of syringyl-type, showing
261 comparatively higher resistance to biodegradation [31].

262 These results could be compared with those found in HAs from soils under different
263 tillage practices [32], showing an increase in the relative amounts of aromatic structures in
264 minimum tillage plots. In this study, analytical pyrolysis lead to a more accurate
265 differentiation between the origin of the aromatic moieties (Fig. 2) reflecting a dual origin
266 of aromatic structures in HAs, either consisting of methoxyl-containing non-decomposed
267 lignin structures or comparatively more condensed aromatic structures yielding
268 alkylphenols upon pyrolysis [33].

269 Total abundances of catechols upon pyrolysis was higher in uncultivated soil than in soil
270 under conservation tillage, as could correspond to the most intense oxidative transformation
271 of the lignin in the former. Conversely, the lowest relative amounts of catechols amounts
272 were found in the HAs from conventional tillage plots.

273 Figure 4 illustrates some significant ($P < 0.05$) trends in the methoxyphenol pattern
274 depending on tillage systems. It was observed the relative increase in the relative
275 proportions of syringyl-type (methyl-, ethyl-, vinyl- and propenyl-) methoxyphenols from HAs
276 in plots under conventional tillage and the concomitant increase of the guaiacyl-type
277 compounds in no-tillage and uncultivated plots. This would betray the intense lignin
278 demethoxylation expected in comparatively mature humus from undisturbed soil. Similar
279 patterns were found in no-tillage plots, where crop wastes are not mechanically
280 incorporated to the soil, and leave to decompose in the surface. On the opposite site, the
281 significant increase in the total abundances of methoxyphenols and the frequent occurrence
282 of syringyl-type phenols in HAs from conventional tillage plots with respect to reduced tillage
283 and uncultivated plots is probably pointing out to active incorporation to HAs of slightly-
284 transformed lignin from crop wastes. As expected, the HAs from plots subjected to minimum
285 tillage showed average methoxyphenol composition, their most diagnostic feature—apart
286 from the balanced syringyl/guaiacyl composition—being the dominance of non-alkylated
287 methoxyphenols, which depict a specific, intermediate fate of lignin alteration in these plots.

288 In addition, and considering that conventional tillage plots showed lower C/N ratio and
289 comparatively higher proportions of methoxyphenols than plots under the other soil
290 management practices, the above differences could be interpreted as a higher performance
291 of the lignin biodegradation processes in the N-limited systems, a circumstance widely
292 reported in the literature [34].

293 Figure 5 summarizes the effects of the different tillage managements based on the
294 whole pyrolytic data processed by multivariate data treatment. The correspondence analysis
295 suggests an increase after cultivation in the proportions of fatty acids (mainly in plots under
296 conservation tillage) and phenols (mainly in plots under conventional tillage). On the other
297 hand, the uncultivated plots show a HA pattern more similar to those from undisturbed
298 semiarid soils. This could be reflecting comparatively higher maturity degree, with
299 comparatively low proportions of lignin-derived methoxyphenols and the substantial
300 proportions of alkanes, alkenes, alkylbenzenes and catechols.

301
302

303 **4. Conclusions**

304

305 In general, maturity surrogates of the HAs (e.g., aromaticity, intense transformation of
306 lignins), defined by spectroscopic analyses and descriptors based in their pyrolytic
307 behaviour, were comparatively higher in uncultivated than in cultivated plots. In spite of
308 conservation practices lead to significantly increased levels of soil organic C, we found the
309 highest diagenetic transformation of the HAs in undisturbed or minimum tillage plots. This is
310 explained in terms of rapid incorporation of relatively unaltered organic fractions derived
311 either from microbial biomass or plant biomacromolecules to the soil HA fraction in
312 conventional tillage plots. On the other hand, comparatively intense biodegradation and
313 humification processes in the uppermost soil layer of undisturbed soil have led to some
314 common patterns with reduced tillage plots. This situation could be considered as a
315 consequence of the environmental constraints in semiarid Mediterranean scenarios, where
316 pyrolytical characterization of HAs could successfully betray accumulation of raw organic
317 materials in those treatments receiving continuous inputs of crop residues such as the
318 conservation tillage practices.

319

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321

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415 **Figure legends**

416

417 **Figure 1.** Mass fragmentograms showing the intensity of the m/z 60 ion, characteristic for
418 fatty acids, in pyrograms from humic acid isolated from uncultivated soil and soils managed
419 with conventional, minimum and no-tillage systems. Carbon number of the homologous
420 compounds is indicated on the peaks.

421

422 **Figure 2.** Cumulative values for the main groups (relative peak areas as regards total ion
423 chromatogram) of pyrolysis products from humic acid of uncultivated soil (U) and soils
424 managed with different tillage systems: conventional (CT), minimum (MT) and no-tillage
425 (NT). Error bars indicate the standard deviations between replicated spatial samples. Within
426 a subplot, bars labelled with the same letter are not significantly different at $P < 0.05$.

427

428 **Figure 3.** Correspondence analyses showing changes in the distribution patterns of: **a)** fatty
429 acids, **b)** alkanes and **c)** alkenes, in terms of the tillage systems (bold labels): CT =
430 conventional tillage; MT = minimum tillage; NT = no-tillage; U = uncultivated. Error bars
431 indicate the variability ranges defined by triplicate runs; average values (centroids) are
432 drawn with circles. The percentage of the total variance accounted for by the two first
433 components is shown in the corresponding axis. Compounds are indicated by their C atom
434 number.

435

436 **Figure 4.** Correspondence analysis showing changes in the distribution patterns of
437 methoxyphenols in terms of the tillage systems (bold labels): CT = conventional tillage; MT =
438 minimum tillage; NT = no-tillage; U = uncultivated. Error bars indicate the variability ranges
439 defined by triplicate runs; average values (centroids) are drawn with circles. The percentage
440 of the total variance accounted for by the two first components is shown in the
441 corresponding axes.

442

443 **Figure 5.** Correspondence analysis of cumulative data of all types of pyrolysis compounds,
444 showing the effects of tillage practices (bold labels: CT = conventional tillage; MT = minimum
445 tillage; NT = no-tillage; U = uncultivated) on the relative yields of the main groups of

446 pyrolysis compounds. Error bars indicate the variability ranges defined by triplicate runs;
447 average values (centroids) are drawn with circles. The percentage of the total variance
448 accounted for by the two first components is shown in the corresponding axes.
449

450

451

452 Table 1

453 General analytical characteristics in uncultivated soil (U) and soils managed with

454 conventional tillage (CT), minimum tillage (MT) and no-tillage (NT) systems

	CT	MT	NT	U	<i>LSD</i> ^a
Total organic C (g kg ⁻¹)	5.6	7.1	12.5	11.5	1.0
Total N (g kg ⁻¹)	0.7	0.7	1.3	1.2	0.2
C/N ratio	8.6	10.1	9.6	9.9	2.3
pH	5.1	5.7	5.0	7.2	0.7
Available K (mg 100 g ⁻¹)	22	30	39	44	8
Available Ca (mg 100 g ⁻¹)	171	170	116	251	24
Available Na (mg 100 g ⁻¹)	4	3	2	1	2
Available Mg (mg 100 g ⁻¹)	26	25	20	20	6
Free organic matter (g C kg ⁻¹)	0.6	0.4	0.9	1.0	0.2
Humic acid (HA) (g C kg ⁻¹)	0.5	1.1	2.6	1.2	0.3
Fulvic acid (FA) (g C kg ⁻¹)	0.9	1.2	3.3	3.1	0.4
Non-extractable humin (g C kg ⁻¹)	3.6	4.3	5.7	6.3	0.5
HA/FA ratio	0.57	0.92	0.79	0.39	0.33
E ₄ (optical density of HA at 465 nm)	0.95	1.03	0.76	1.13	0.35
E ₄ /E ₆ optical density ratio	4.93	4.50	5.06	4.55	0.85

455 ^a Least significant difference at *P* = 0.05.

456

457 Table 2

458 Relative amounts^a of the major pyrolysis products of humic acids isolated from uncultivated soil
 459 (U) and soils managed with conventional tillage (CT), minimum tillage (MT) and no-tillage (NT)
 460 systems

	CT	MT	NT	U	LSD ^b
Alkylbenzenes					
Methylbenzene (toluene)	0.77	0.76	0.65	1.46	0.45
Styrene	0.44	0.24	0.19	0.72	0.33
C ₃ -Alkylbenzene	0.34	0.20	0.13	0.91	0.22
C ₄ -Alkylbenzene	0.44	0.23	0.39	0.13	0.34
C ₅ -Alkylbenzene	0.00	0.02	0.06	0.00	0.08
Catechols					
Catechol	0.00	0.22	0.24	1.21	0.14
Methylcatechol	0.00	0.09	0.00	0.00	0.04
C ₂ -Catechol	0.00	0.02	0.00	0.00	0.02
Phenols					
Phenol	0.89	0.59	0.27	1.39	0.33
Methylphenol (cresol)	0.39	0.25	0.26	0.17	0.20
C ₂ -Alkylphenol	0.16	0.05	0.12	0.18	0.14
C ₃ -Alkylphenol	0.00	0.04	0.11	0.00	0.09
C ₄ -Alkylphenol	0.00	0.02	0.04	0.00	0.05
Methoxyphenols					
Guaiacol	1.25	0.81	0.50	0.69	0.76
Methylguaiacol	0.26	0.26	0.22	0.39	0.17
Ethylguaiacol	0.38	0.25	0.53	0.50	0.12
Vinylguaiacol	1.41	1.16	1.43	0.90	1.12
Syringol	0.65	0.48	0.33	0.19	0.57
Methylsyringol	0.14	0.12	0.10	0.13	0.08
Propenylguaiacol	0.12	0.12	0.10	0.14	0.12
Acetoguaiacone	0.59	0.33	0.23	0.23	0.28
Ethylsyringol	0.47	0.22	0.11	0.19	0.15
Vinylsyringol	0.43	0.24	0.26	0.18	0.16
Propenylsyringol	0.14	0.13	0.11	0.08	0.13
Acetosyringone	0.55	0.41	0.61	0.68	0.50
Polycyclic aromatic					
Naphthalene	0.00	0.00	0.02	0.00	0.02
Methylnaphthalene	0.00	0.00	0.02	0.00	0.03
C ₂ -Alkyl-naphthalene	0.00	0.00	0.03	0.00	0.04
Alkenes					
C ₈ -Alkene	0.03	0.03	0.00	0.16	0.09
C ₉ -Alkene	0.13	0.03	0.12	0.25	0.07
C ₁₀ -Alkene	0.16	0.09	0.15	0.32	0.10

C ₁₁ -Alkene	0.10	0.05	0.07	0.20	0.09
C ₁₂ -Alkene	0.96	0.18	0.06	1.03	0.15
C ₁₃ -Alkene	0.24	0.14	0.20	0.27	0.22
C ₁₄ -Alkene	0.68	0.41	0.17	1.33	0.86
C ₁₅ -Alkene	0.17	0.11	0.13	0.14	0.09
C ₁₆ -Alkene	0.79	2.29	1.91	4.20	1.72
C ₁₇ -Alkene	0.76	0.76	0.60	0.36	0.24
C ₁₈ -Alkene	2.29	1.75	1.58	6.12	1.11
C ₁₉ -Alkene	0.29	0.12	0.12	0.23	0.43
C ₂₀ -Alkene	0.04	0.55	0.59	0.31	0.71
C ₂₁ -Alkene	0.09	0.20	0.07	0.23	0.19
C ₂₂ -Alkene	0.00	0.55	0.00	0.74	0.34
Alkanes					
C ₈ -Alkane	0.23	0.46	0.52	1.10	0.39
C ₉ -Alkane	0.20	0.32	0.22	0.86	0.30
C ₁₀ -Alkane	0.04	0.04	0.05	0.04	0.06
C ₁₁ -Alkane	0.27	0.07	0.15	0.35	0.20
C ₁₂ -Alkane	0.17	0.07	0.07	0.19	0.08
C ₁₃ -Alkane	0.11	0.27	0.18	0.16	0.18
C ₁₄ -Alkane	0.16	0.11	0.37	0.50	0.15
C ₁₅ -Alkane	0.13	0.16	0.13	0.26	0.07
C ₁₆ -Alkane	0.31	0.20	0.32	0.25	0.20
C ₁₇ -Alkane	0.66	0.31	0.46	0.46	
C ₁₈ -Alkane	0.70	0.57	0.57	0.92	0.36
C ₁₉ -Alkane	0.59	0.35	0.54	0.66	0.51
C ₂₀ -Alkane	0.24	0.19	0.36	0.93	0.49
C ₂₁ -Alkane	0.09	0.34	0.02	0.39	0.18
C ₂₂ -Alkane	0.00	0.58	0.00	0.40	0.20
Fatty acids					
Heptanoic acid	0.23	0.07	0.14	0.18	0.21
Octanoic (caprylic) acid	0.79	0.28	0.66	0.81	0.13
Nonanoic (pelargonic) acid	1.02	0.44	0.44	1.05	0.57
Decanoic (capric) acid	0.38	0.31	0.33	0.23	0.14
Undecanoic acid	0.10	0.02	0.09	0.98	0.08
Dodecanoic (lauric) acid	0.96	0.59	0.77	0.26	0.38
Tridecanoic acid	0.22	0.22	0.11	0.13	0.16
Tetradecanoic (myristic) acid	3.87	3.70	4.06	0.25	1.27
<i>iso</i> -Pentadecanoic acid	1.56	3.35	1.47	0.41	2.09
<i>anteiso</i> -Pentadecanoic acid	1.83	2.87	2.56	0.90	0.37
Pentadecanoic acid	0.83	1.52	1.34	0.25	0.79
Hexadecanoic (palmitoleic) acid	0.66	1.74	2.13	0.00	1.25
Hexadecanoic (palmitic) acid	14.30	18.84	24.88	0.32	8.41
<i>iso</i> -Heptadecanoic acid	0.45	1.87	0.61	0.00	0.91
<i>anteiso</i> -Heptadecanoic acid	0.48	1.08	0.48	0.00	1.36
Heptadecanoic (margaric) acid	0.33	0.27	0.80	0.00	0.24

Octadecenoic (oleic) acid	1.68	0.69	1.25	0.73	2.02
Octadecanoic (stearic) acid	0.81	0.66	1.66	0.00	1.47
Nonadecanoic acid	0.00	0.07	0.00	0.11	0.12
2-Propenoic acid, 3-(4-methoxyphenyl)-	0.85	0.00	1.01	3.39	1.83
Eicosanoic (arachidic) acid	0.00	0.16	0.00	0.00	0.17

461

462 ^a Percentage of the total peak area in the total ion chromatogram (compounds representing less
463 than 00.02% are not shown).

464 ^b Least significant difference at $P = 0.05$

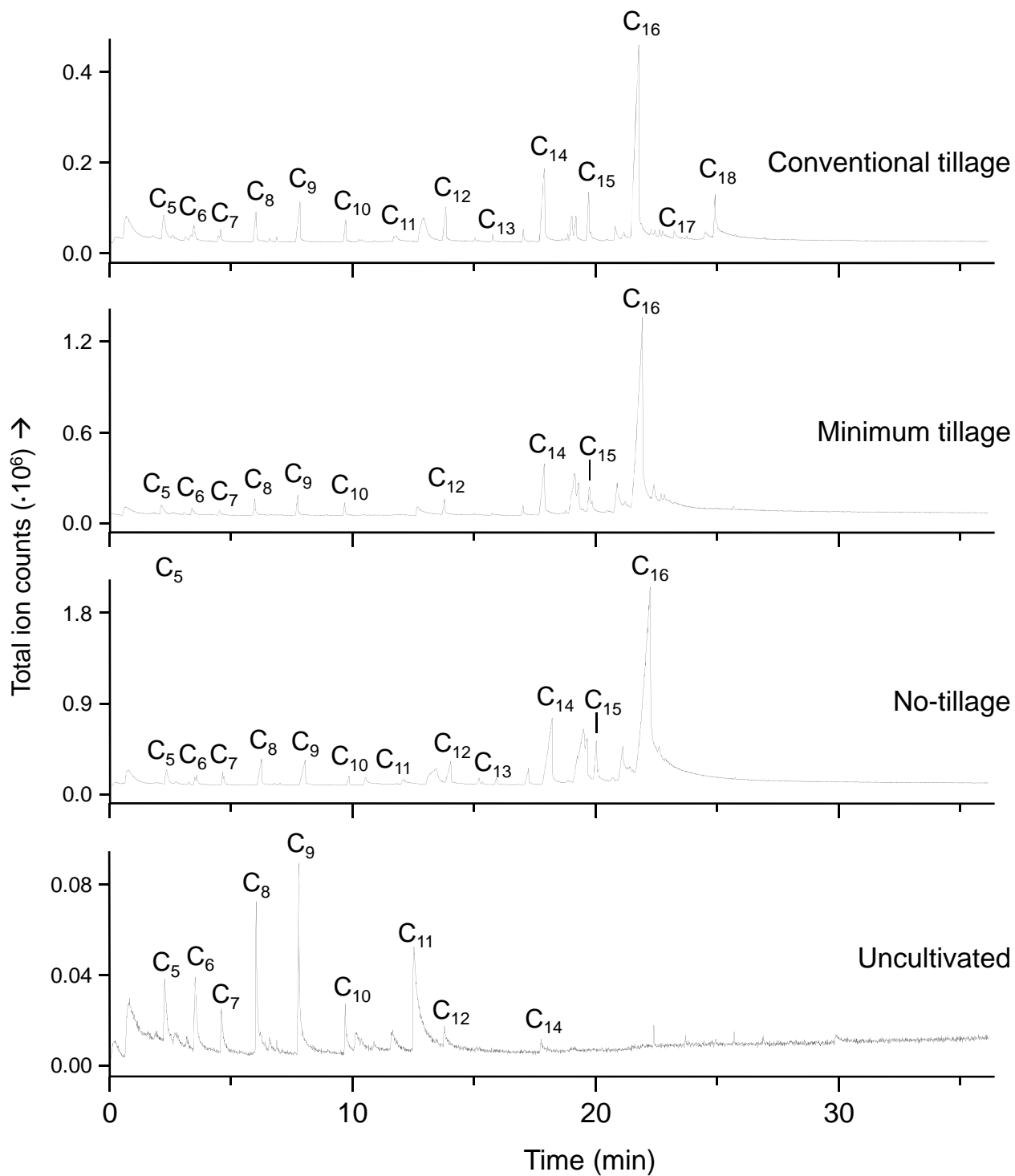


Figure 1. Mass fragmentograms showing the intensity of the m/z 60 ion, characteristic for fatty acids, in pyrograms from humic acid isolated from uncultivated soil and soils managed with conventional, minimum and no-tillage systems. Carbon number of the homologous compounds is indicated on the peaks.

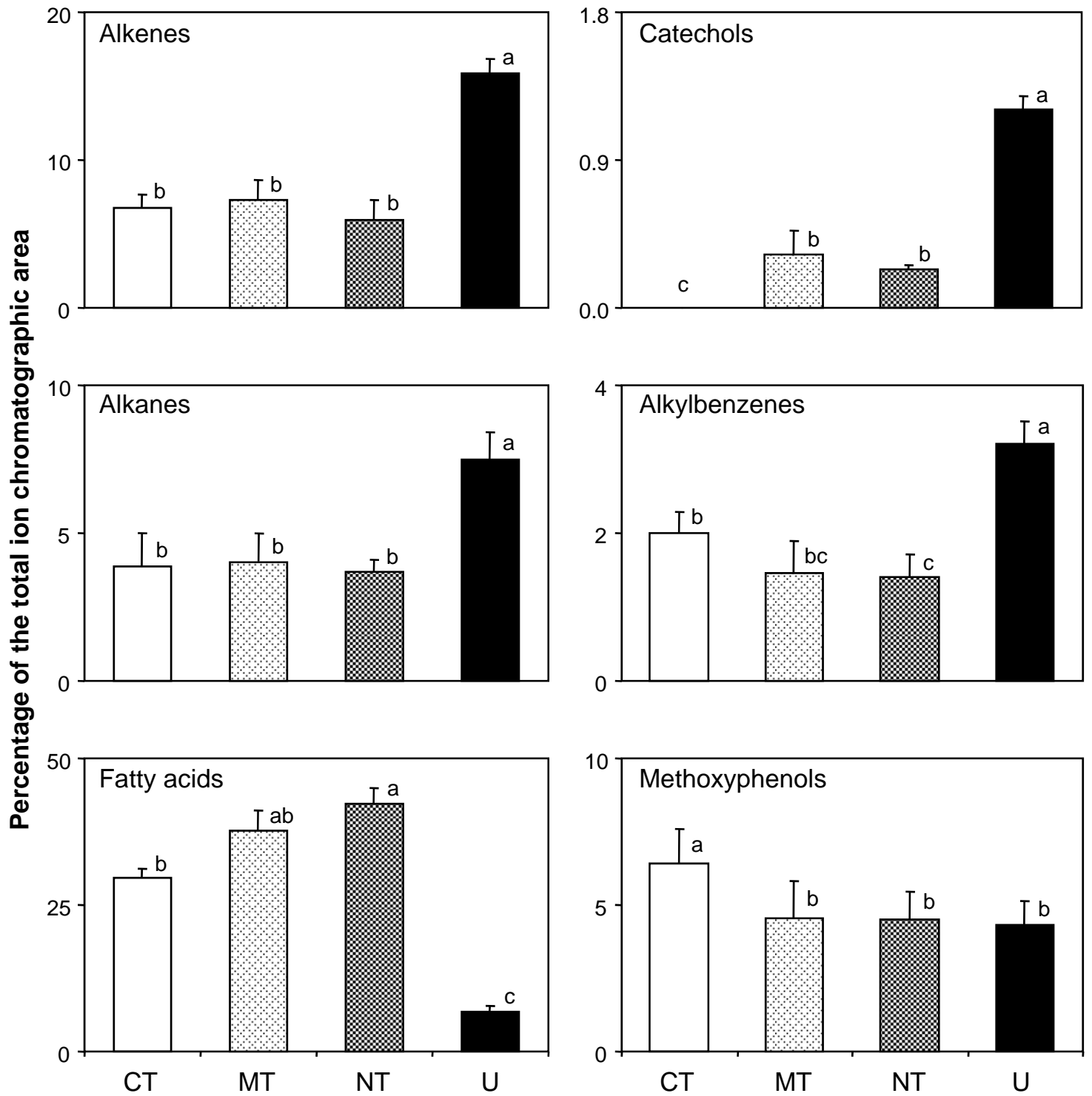


Figure 2. Cumulative values for the main groups (relative peak areas as regards total ion chromatogram) of pyrolysis products from humic acid of uncultivated soil (U) and soils managed with different tillage systems: conventional (CT), minimum (MT) and no-tillage (NT). Error bars indicate the standard deviations between replicated spatial samples. Within a subplot, bars labelled with the same letter are not significantly different at $P < 0.05$.

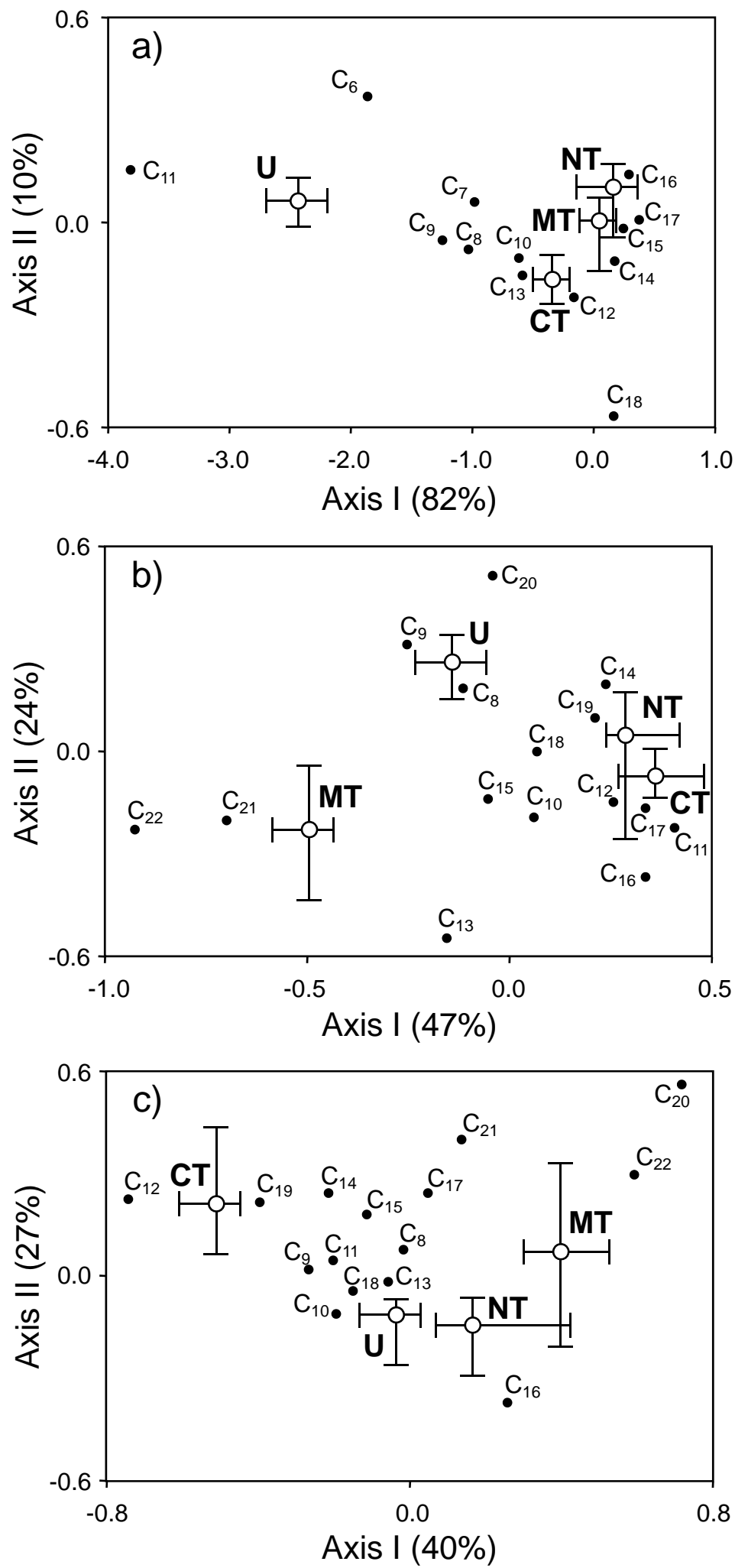


Figure 3. Correspondence analyses showing changes in the distribution patterns of: **a)** fatty acids, **b)** alkanes and **c)** alkenes, in terms of the tillage systems (bold labels): CT= conventional tillage; MT= minimum tillage; NT= no-tillage; U= uncultivated. Error bars indicate the variability ranges defined by triplicate runs; average values (centroids) are drawn with circles. The percentage of the total variance

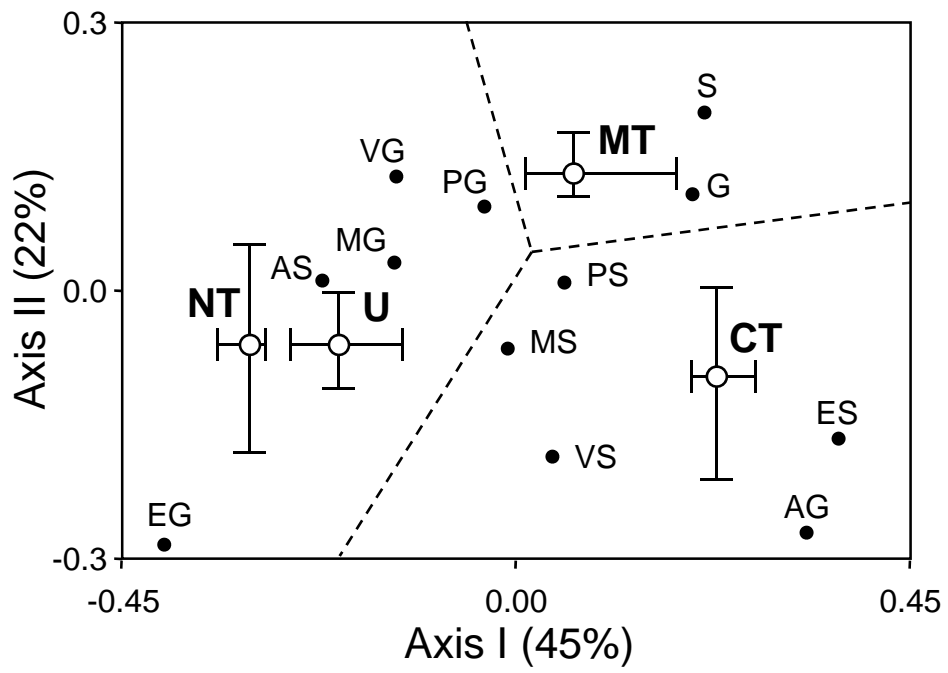


Figure 4. Correspondence analysis showing changes in the distribution patterns of methoxyphenols in terms of the tillage systems (bold labels): CT= conventional tillage; MT= minimum tillage; NT= no-tillage; U= uncultivated. Error bars indicate the variability ranges defined by triplicate runs; average values (centroids) are drawn with circles. The percentage of the total variance accounted for by the two first components is shown in the corresponding axes.

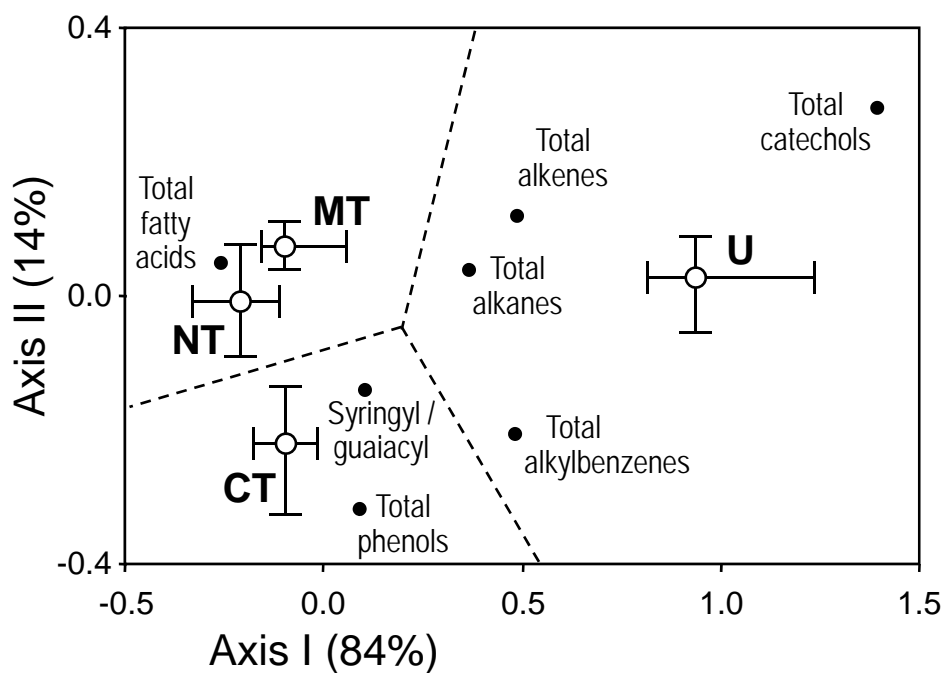


Figure 5. Correspondence analysis of cumulative data of all types of pyrolysis compounds, showing the effects of tillage practices (bold labels: CT = conventional tillage; MT = minimum tillage; NT = no-tillage; U = uncultivated) on the relative yields of the main groups of pyrolysis compounds. Error bars indicate the variability ranges defined by triplicate runs; average values (centroids) are drawn with circles. The percentage of the total variance accounted for by the two first components is shown in the corresponding axes.