

## Postprint of: Catena 145: 266- 273 (2016)

1 Fire effects in the molecular structure of soil organic matter fractions under *Quercus suber*  
2 cover.

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11

### 12 **Abstract**

13 Forest fires cause immediate and lasting environmental impacts in soil organic matter (SOM)  
14 by modifying existing chemical structures, forming new ones, or adding/removing materials  
15 such as fresh or charred biomass. In this paper, the information provided by analytical  
16 pyrolysis (Py-GC/MS) of whole soil samples and two particle-size fractions (coarse and fine) is  
17 analysed in a sandy soil from a typical Mediterranean oak forest close to Doñana National Park  
18 (Southwest Spain) that was affected by a severe wildfire. The pyrograms from unburnt whole  
19 soil samples show a prevalence of compounds derived from polysaccharides, proteins and  
20 lignin, whereas in pyrograms from burnt soils lignin markers prevail followed by proteins,  
21 alkylaromatic and polycyclic aromatic hydrocarbons. In fact, it seems clear that fire  
22 preferentially removes thermolabile biogenic materials with a concomitant selective  
23 preservation of lignin. Concerning particle size fractions, SOM in the unburnt coarse fraction  
24 had a major impact on vegetation, whereas the fine fraction consisted of comparatively  
25 humified SOM with lignin degradation and microbial-derived compounds. The pyrolysis  
26 products from the burnt soil coarse fraction revealed post-fire litter inputs from stressed  
27 vegetation, whereas the fine fraction shares chemical characteristics with those from the  
28 unburnt soil, with additional signs of fire alteration (less labile carbohydrate structures and  
29 higher relative abundance of PAHs). Alkane changes observed in the carbon preference index  
30 indicate that fire affected SOM mostly in the fine fraction, whereas the short/long chain ratio  
31 points to the cracking of long-chain alkanes. In addition, when plotting O/C and H/C values in a  
32 Van Krevelen diagram, additional information about the main chemical modifications  
33 produced by fire in SOM are described, including condensation (oxidation and hydrogenation  
34 paralleling) and dealkylation (associated with oxidation and dehydrogenation) processes. This  
35 is the first time that Py-GC/MS molecular information from soil samples is analysed using a Van

36 Krevelen graphical approach and together with other classic geochemical proxies it proves to  
37 be a valuable and intuitive tool in facilitating the interpretation of complex geochemical data.

38

## 39 **1. Introduction**

40

41 Forest fires represent a recurrent disturbance factor in the Mediterranean basin and they are  
42 the cause of immediate and lasting environmental impacts (Naveh, 1990). This is in part  
43 caused by changes in the soil organic matter (SOM), which is the most reactive and functional  
44 soil fraction. These changes include the transformation of biogenic chemical structures,  
45 accumulation of newly-formed structures, as well as a complex balance between the input and  
46 diminution of materials with differential resistance to thermal degradation, i.e. fresh or  
47 charred biomass (González-Pérez et al., 2004, 2008; Knicker, 2007; Atanassova and Doerr,  
48 2011; Faria et al., 2015). The pyrogenic signature in SOM is in the focus of research, not only in  
49 the Mediterranean area, but also in other parts of the world (Dyrness et al., 1989; Fernández  
50 et al., 1997; Neff et al., 2005; Dymov and Gabov, 2015). One out of the major alterations  
51 exerted by fire to SOM chemical composition is the formation of recalcitrant structural  
52 domains which are believed to include severely defunctionalized condensed aromatics in  
53 addition to fire-surviving alkyl structures probably including cross-linked lipid compounds. Such  
54 recalcitrant core, show characteristic 'bimodal, protokerogen-like' <sup>13</sup>CNMR spectra, and in  
55 general is resistant to drastic chemical hydrolysis (Almendros et al., 1988; González-Pérez et  
56 al., 2008; De la Rosa et al., 2012). Apart from the 'fire-induced structural stabilization', fire may  
57 also produce the removal of volatile structures and the transformation of labile compounds. A  
58 common example of this is the modification of polysaccharides to pyrogenic compounds, i.e.  
59 from levoglucosan and furans to benzenic compounds (Simoneit et al., 1999; Templier et al.,  
60 2005; Fabbri et al., 2009).

61 Nowadays, there is a wide array of analytical techniques available for the study of  
62 complex matrices, such as SOM. Among these, Fourier -Transform infrared (FT-IR) and nuclear  
63 magnetic resonance (NMR) spectroscopies, gas chromatography-mass spectrometry (GC/MS)  
64 or analytical pyrolysis (Py-GC/MS), have successfully been used to characterise the effects of  
65 fire on SOM (Almendros et al., 1988; Tinoco et al., 2006; González-Pérez et al., 2008; De la  
66 Rosa et al., 2011; Knicker, 2011; Atanassova et al., 2012; Aznar et al., 2013; Badía et al., 2014a;  
67 Wiedemeier et al., 2015).

68 Analytical pyrolysis, defined as the thermochemical decomposition of organic  
69 materials at elevated temperatures in the absence of oxygen (Irwin, 1982), is a fast and useful  
70 'fingerprinting' tool for the direct structural characterisation of SOM. The products of pyrolysis

71 (pyrolysate) are amenable to GC/MS separation and identification, yielding detailed molecular  
72 information about the complex mixtures of natural macromolecular substances (González-  
73 Pérez et al., 2013). Other well-known advantages of the technique include the requirement of  
74 small sample sizes and little to no sample preparation needs.

75 The products released by pyrolysis of SOM can be related to its biogenic origin;  
76 methoxyphenols from lignin, furans from polysaccharides and certain Nitrogen-containing  
77 molecules from proteins (Leinweber and Schulten, 1995; González-Vila et al., 2001; Arias et al.,  
78 2005 and references therein). Long-chain *n*-alkanes are derived from the epicuticular waxes of  
79 plants (Eglinton et al., 1962; Eglinton and Hamilton, 1967) and indexes such as *n*-alkane  
80 average chain length (ACL) and carbon preference index (CPI) are used as surrogates for source  
81 and evolution of organic matter (Cranwell, 1973). In fact, after deposition, *n*-alkanes are  
82 affected by factors such as decay from microbial activities or fire, which normally reduce ACL  
83 values in soils (Bull et al., 2000; González-Pérez et al., 2008, 2004).

84 The complex molecular assemblages released from SOM by pyrolysis are often  
85 explored by multivariate data methods, but can also be analysed with the help of graphic tools,  
86 such as the classic Van Krevelen diagram (Van Krevelen, 1950). This was initially intended to  
87 study the degree of maturity of coal by comparing the relationships between H/C and O/C  
88 ratios of samples. Nowadays, an extended Van Krevelen approach is being used by researchers  
89 who are working with large chemical data matrices, such as those produced by ultrahigh  
90 resolution techniques, such as Fourier Transform ion cyclotron resonance mass spectrometry  
91 (FT-ICR-MS) (Kim et al., 2003; Kramer et al., 2004; Waggoner et al., 2015). In this case the  
92 atomic ratios (H/C and O/C) are calculated from the chemical molecular formulas that are  
93 inferred from the mass spectrometry data.

94 The majority of studies undertaken to evaluate the effect of fire on SOM have been  
95 undertaken in fine earth soil samples (<2 mm) or in the main textural soil fractions (sand, loam  
96 and clay). Although it is known that SOM differs among particle-size fractions (Nocentini et al.,  
97 2010; Jimenez-Morillo et al., 2014, 2015), the information available is still scarce. Therefore,  
98 the present study is focused on the information provided by the direct analytical pyrolysis of  
99 whole soil and two sieve size fractions (coarse and fine) with the aim of shedding light on the  
100 alterations that SOM undergoes after a fire episode. To accomplish this, the main goals of this  
101 study are: i) to assess the chemical composition of organic matter in whole soil and in size  
102 fractions before and after a wildfire; and ii) to assess the alterations and processes undergone  
103 in each of the fractions impacted by fire.

104

105        2. **Material and methods**

106

107        2.1 Study area and sampling

108                During a forest fire, apart from the heat itself, there are a number of interconnected  
109 variables of different nature (biological, environmental, physicochemical) acting at different  
110 levels (atmosphere, vegetation and fuels, soil) that eventually influence the way in which a fire  
111 affects SOM. Those variables may include factors such as pH, texture, conductivity, fuel  
112 availability, soil water/atmosphere, climatic conditions, etc. (Pyne et al., 1996). In order to  
113 simplify our natural experimental setting, the effect of a wildfire on SOM is studied in low  
114 complexity soils that are located in a well-known environment at Doñana National Park  
115 (Southwest Spain), where fire is a known recurrent factor and is the cause of changes in its  
116 geomorphology and vegetation (Granados, 1985).

117                A sandy soil showing relative spatial homogeneity and properties was selected at the  
118 site known as “Matasgorda”, having a typical Mediterranean climate with an oceanic influence,  
119 which produces mild temperatures, and relatively high humidity and rainfall compared to the  
120 inland climate (Siljeström and Clemente, 1990). The average annual rainfall is 830 mm with  
121 77% of precipitation (between October and March) and a mean air temperature of 16.9 °C. The  
122 soil pH is slightly acid to neutral (pH 6-7.0). The soil was classified as Arenosol (WRB 2006) with  
123 more than 99% sand from aeolian sediments (Holocene) covering gravels and other sandy  
124 sediments (Pliocene-Pleistocene). The study area was affected by a wildfire in the summer of  
125 2012 that affected more than 300 ha. The fire severity could be considered severe as assessed  
126 *de visu* just after the fire.

127                Composite soil samples were taken in October 2014 under Cork oak (*Quercus suber*)  
128 “QS” canopy. Burnt soil “B” was affected by the wildfire (Burnt: 37° 7’21.95”N; 6° 26’53.44”W)  
129 and a nearby control of unburnt soil “UB” (Unburnt: 37° 7’23.69”N; 6° 26’51.53”W), with the  
130 same vegetation cover, soil type, physiographic characteristics, and no recent histories of  
131 forest fires, were sampled. Each sample was made by combining five to six sub-samples taken  
132 within a circular area of ca. 20 m<sup>2</sup> under a well-developed cork oak stand. The samples were  
133 taken from the first 3 cm of soil after removing the litter layer. Earlier studies demonstrated  
134 that no effects are detected at deeper depths in the soil (Aznar et al., 2013; Badía et al.,  
135 2014a,b). The soil material was then transported in glass containers, air-dried under laboratory  
136 conditions (at 25 °C and approximately 50% relative humidity) during a 1-week period and  
137 sieved to fine earth (<2 mm) to remove gravel and litter fragments. Each combined fine earth  
138 sample (total sample) was further divided by dry sieving in six fractions (1–2 mm “coarse”, 0.5-  
139 1, 0.25–0.5, 0.1–0.25, 0.05-0.1 and <0.05 mm “fine”).

140

## 141 2.2 Elemental analysis

142 Total Carbon (C) and Total Nitrogen (N), in the total samples and in each one of its sieve size  
143 fractions, were analysed using a Flash 2000 HT (C, H, N, O and S) elemental micro-analyser  
144 coupled to a thermal conductivity detector (TCD) (Thermo Scientific, Bremen, Germany). Each  
145 sample (weight range of 1–1.5 mg) was measured in triplicate. The calibration curve was made  
146 for each element using recommended standard materials, acetanilide, nicotinamide and  
147 aspartic acid (Thermo Scientific, Bremen, Germany) in triplicate.

148

## 149 2.3 Analytical pyrolysis (Py-GC/MS)

150 Pyrolysis-gas GC/MS was performed using a double-shot pyrolyser (Frontier Laboratories,  
151 model 2020i) attached to an Agilent 6890N gas chromatograph. Soil samples (1–2 mg) were  
152 placed in small crucible capsules and introduced into a pre-heated furnace at 500 °C for 1 min.  
153 The evolved gases were directly injected into the GC/MS for analysis. The GC was equipped  
154 with a low-polarity fused silica capillary DB-5 column (J&W Scientific) of 30 m × 250 µm × 0.25  
155 µm film thickness. The oven temperature was held at 50 °C for 1 min and then increased to  
156 100 °C at 30 °C min<sup>-1</sup>, from 100 °C to 300 °C at 10 °C min<sup>-1</sup>, and stabilised at 300 °C for 10 min.  
157 The carrier gas was helium at a controlled flow of 1 mL min<sup>-1</sup>. The detector consisted of an  
158 Agilent 5973 MSD (mass selective detector), and mass spectra were acquired with a 70 eV  
159 ionising energy. Compound assignment was achieved via single ion monitoring for various  
160 homologous series, via low-resolution MS and via comparison with published and stored (NIST  
161 and Wiley libraries) data.

162 From the relative abundances of the chromatographic areas for *n*-alkane peaks, a  
163 number of molecular markers were inferred:

164 S/L (Short/Large ratio) is an estimation of the relative abundance of short to long C  
165 length in a hydrocarbon chain and was calculated according to the following the formula:

$$S/L = \frac{\sum [C_n]_{10-23}}{\sum [C_n]_{24-31}}$$

166 Where *n* is the number of carbon atoms and [C<sub>*n*</sub>] is the concentration of each alkane  
167 or alkene.

168 CPI (24–31) (carbon preference index) is an estimation of the relative abundance of  
169 odd to even C chain numbers. In this research, CPI was calculated in the range from 24 to 31  
170 carbons. This index allows us to know the original source of SOM; CPI > 1 means that odd C  
171 chain numbers prevail over even C chain numbers and that the main input of organic matter to  
172 the soil is of plant origin. In this research, CPI was calculated as:

$$CPI (24-31) = \frac{\sum [C_n \text{ odd}]_{24-31}}{\sum [C_n \text{ even}]_{24-31}}$$

173 Where  $[C_n \text{ odd}]$  is the hydrocarbon abundance with odd-numbered C chain and  $[C_n$   
174 even] is the hydrocarbon abundance with even-numbered C chain (Bray and Evans, 1961).

175 Finally, the molecular information obtained by analytical pyrolysis was studied using  
176 Van Krevelen diagrams. The atomic ratios (H / C and O / C) were calculated from the chemical  
177 molecular formulas inferred from the mass spectra. Only compounds identified with a  
178 chromatographic area >0.2% of the total ion chromatographic area were included in the  
179 analyses.

### 181 3. Results and discussion

#### 182 3.1 Elemental Analysis

183 Elemental C and N contents and C/N ratios are shown in Table 1. The whole (total) B soil shows  
184 a 4.1% higher C content than the whole UB soil. We found that, in general, higher C contents  
185 are found in the burnt soil fractions, but the 1–0.5 mm fraction has a 4.4% lower C content  
186 than the corresponding fraction from the UB soil. This is in line with previous findings where  
187 the carbon content on burned topsoil usually decreases immediately after a wildfire (Badía et  
188 al., 2014b). Higher C contents found in the burnt size fractions have been previously observed  
189 and could be caused by an additional input of particulate biomass including partially charred  
190 materials, particularly adding to the coarse (>2–1 mm) fractions (Chandler et al., 1983; Bird et  
191 al., 2000; Nocentini et al., 2010) and to the smaller (<0.5 mm) size fractions where fine char  
192 particles accumulate (Skjemstad et al., 1996). The total N content in the whole (total) burnt  
193 soil (B = 1.3%) was higher than that in the unburnt soil (UB = 1.0%). As regards the different  
194 size fractions, no significant differences were found for total N content.

195 The C/N ratio shows the same tendency with that for total C content with higher  
196 values for B soil. In general, this C/N trend is not in agreement with the results obtained by  
197 other researchers (Almendros et al., 1984 a,b; Badía et al., 2014b; Viro, 1974; Vega, 1986), who  
198 observed that after a fire, there is usually a decrease of C and in turn a decrease of C/N ratio.  
199 The decreasing of C may be due to a loss of humified material by fire (González-Pérez et al.,  
200 2004) and this effect, while still present, can be also masked by black carbon additions to the  
201 B soil from charred biomass.

#### 202 3.2 Pyrolysis-GC/MS

203 Total ion current (TIC) chromatogram from the whole and sieve size fractions of B and UB soils  
204 with an indication of the origin of the major pyrolysis compounds is shown in Fig. 1. The  
205 pyrograms from whole soil samples showed conspicuous differences between B and UB sites.

208 The higher intensity peaks in the B soil pyrogram appear in the range of 4–10 min of retention  
209 time showing unimodal normal distribution, whereas for the total UB soil, the compound  
210 distribution is biased towards compound with low MW, and shows the highest total  
211 abundance in the range of 2–6 min retention time. When screening the pyrolysates produced  
212 by the different soil size sieve fractions, we were able to clearly differentiate two main  
213 contrasting patterns corresponding with the coarse (2–1 mm) and fine (< 0.05 mm) fractions,  
214 respectively, whereas the intermediate fractions showed pyrograms with intermediate  
215 characteristics between both fractions. Therefore, these two fractions together with the bulk  
216 fraction were chosen for further study in detail.

217 The pyrogram from the coarse fraction, in both scenarios, showed a normal  
218 distribution of the high intensity peaks, which are mainly lignin-derived methoxyphenols,  
219 unspecific aromatic compounds and carbohydrate-derived compounds. In contrast, in the fine  
220 fraction, major peaks distribution shifts towards the low MW compounds tailing to the high  
221 MW compounds, with alkyl compounds, peptide-derived and unspecific aromatic compounds  
222 being dominant in this fraction. In addition, there were differences between B and UB soil  
223 samples in each fraction with the main differences in the range of 23–30 min of retention time;  
224 whereas no chromatographic peaks are found in this region in the B samples, several peaks are  
225 clearly seen in this region in the UB samples. These peaks corresponded to high molecular  
226 weight alkane/alkene chains, the lack of which being consistent with a thermal cracking of high  
227 molecular weight hydrocarbon chains into smaller compounds, as usually observed in fire-  
228 affected SOM (Almendros et al., 1988; Tinoco et al., 2006; González-Pérez et al., 2008; De la  
229 Rosa et al., 2013; Aznar et al., 2013; Faria et al., 2015).

230 The differences between samples can be best seen when studying the abundances of  
231 the main pyrolysis compound relative to the total chromatographic area (Fig. 2). In the UB  
232 total soil sample the majority of structures detected in the SOM pyrolysates correspond to  
233 carbohydrate (polysaccharide)-derived compounds (22.7%), aromatic compounds of unspecific  
234 origin (17.2%, mainly alkyl phenols and alkyl benzenes), peptide-derived N-compounds (20.8%)  
235 and lignin methoxyphenols (17.7%, mainly guaiacol and alkyl derivatives). Also, lipids (alkane  
236 alkene pairs and fatty acids) and N-compounds of unknown origin were detected but at low  
237 relative abundance (<10% total chromatographic area). In the B soil samples the pyrograms  
238 are dominated by lignin-derived compounds (40.1%), followed by N compounds from peptides  
239 (15.9%) and aromatics of unspecific origin (12.9%). Further, a number of polycyclic aromatic  
240 hydrocarbons (PAHs 1.2%, mainly derived from naphthalene) were detected. The latter  
241 distribution, with a general depletion of carbohydrates and proteins and enrichment in lignin-  
242 derived compounds, indicates that fire effectively affected SOM, and preferentially removed

243 thermolabile biogenic materials alongside a selective preservation of the more fire-resistant  
244 lignin moieties. In fact, carbohydrate moieties are known to be thermolabile components in  
245 soils (Almendros et al., 1990, Pastorova et al., 1994, Knicker et al., 1996; Baldock and Smernik,  
246 2002; De la Rosa et al., 2008). Soil lignin has been reported to be particularly resistant to fire  
247 (De la Rosa et al., 2008), to the point that additional char particles containing methoxyphenol  
248 from partially burnt lignin could add to the observed increase (Skjemstad et al., 1996).  
249 However, a decrease in the relative abundance of lignin markers and in other typical  
250 vegetation markers (terpenes, resinic acids) with fire has also been observed (Badía et al.,  
251 2014a). It is suggested that the chemical changes observed in burned SOM may play important  
252 roles in the colloidal properties and the water-repellency observed in the post-fire soil area  
253 (Almendros et al., 1992; Atanassova et al., 2012).

254         The chemical compositions of the two (coarse and fine) soil size fractions studied were  
255 different and, as with the whole soil samples, fire was able to induce chemical changes in the  
256 SOM of the different fractions (Fig. 2). The coarse fraction (2–1 mm) of UB soil showed a clear  
257 chemical signature for fresh plant biomass; high abundance of lignin-derived compounds  
258 (30.7%), carbohydrate (19.7%) and peptides (12.3%) and a proportion of fatty acids (5.5%) and  
259 alkyl compounds (8.6%), probably derived from epicuticular waxes (Eglinton et al., 1962;  
260 Eglinton and Hamilton, 1997). This indicates that the SOM in this fraction retains a major  
261 influence from the vegetation litter. In contrast, SOM in the UB fine (<0.05 mm) soil fraction  
262 shows the lowest relative amount of lignin-derived compounds (12%), being mainly composed  
263 of protein (24.7%) and carbohydrates (22.1%) with a substantial proportion of unspecific  
264 aromatic compounds (22.9%), pointing to the presence of microbial-derived compounds, i.e.  
265 glycoproteins such as glomalin, that are known to be abundant in mineral soils with low  
266 organic content (Lozano et al., 2015 and references therein), and to high microbial activity.  
267 This activity is prone to produce humic-type SOM and lignin degradation. In fact, the  
268 abundance of unspecific aromatic compounds could also have an origin in the fungal alteration  
269 of plant lignins (Kellner et al., 2014).

270         The B coarse fractions showed the highest relative proportion of lignin-derived  
271 compounds (51.9%) in comparison with the rest of the studied samples. This points to inputs  
272 of new litter, probably from the standing vegetation that is affected by fire (De la Rosa et al.,  
273 2008). This is further supported by the observed high relative proportion of carbohydrate-  
274 derived structures (17.3%). This fraction also showed the lowest amount of unspecific aromatic  
275 compounds and alkyl compounds (alkane/alkene pairs and fatty acids). The low proportions of  
276 alkyl compounds may be a direct effect of fire that is known to produce thermo-evaporation of  
277 free lipid, in addition to thermal cracking producing shorter chain length compounds



278 (González-Pérez et al., 2008). The B fine fraction shares chemical characteristics with the UB  
279 fine fraction, viz., low lignin (16.6%) and high protein (25.1%)-derived compounds, mainly  
280 pyridine and indole derivatives (see supplementary material) and unspecific aromatic  
281 structures (19.9%), suggesting that SOM in this fraction may be more evolved and subjected to  
282 alteration by microorganisms. However, the pyrolytic signature also indicates that this SOM  
283 has been affected by fire in different ways. First, it is noteworthy that this fraction shows the  
284 highest relative amount of PAHs (1.7%) that, besides a direct condensation effect of fire on this  
285 SOM fraction (González-Pérez et al., 2014), could be also explained in part as inherited SOM  
286 from soot or charred inputs from the coarse SOM fractions or by past wildfire episodes  
287 (Jiménez-Morillo et al., 2014). Note that a small proportion of PAHs were detected in all burnt  
288 soil samples but also, with a very small contribution, in the U fractions that were unaffected by  
289 the studied fire episode (coarse 0.43%; fine 0.58%). These PAHs are robust surrogate measures  
290 of fire intensity in fire episodes (De la Rosa et al., 2012, González-Pérez et al., 2014) and their  
291 presence in the control soil may indicate past fires or aeolian transport of black carbon  
292 aerosols from the nearby burned site. The existence of fine char fine particles was also  
293 confirmed by optical microscopy (data not shown). Second, there is a substantial reduction of  
294 labile carbohydrate-derived compounds (12.7%) that are known to be responsive to fire (De la  
295 Rosa et al., 2008). However, polysaccharide-derived compounds, i.e. levoglucosan, glycosides  
296 and mono-saccharides, have been previously found to be abundant in the soluble SOM  
297 fractions of heated soils (Atanassova and Doerr, 2011), that could have resulted from their *de*  
298 *nov*o formation, volatilisation from particulate organic matter, or from the breakdown of  
299 polysaccharides. The fact that we find a reduction of these compounds may also respond to a  
300 loss by lixiviation and/or to the fast turn-over after the fire of the easily available C source,  
301 represented by the soluble saccharides from the activity of heterotrophic organisms.

302

### 303 3.3. Alkane CPI and S/L ratios

304 Informative biogeochemical proxies for the effect of fire in SOM are provided by the  
305 alkyl series that are easily identified by analytical pyrolysis. It is known that after a forest fire  
306 the aliphatic compounds undergo a thermal cracking, producing hydrocarbon chains that are  
307 shorter in length (González-Pérez et al., 2008; Badía et al., 2014a; De la Rosa et al., 2012;  
308 Eckmeier and Wiesenberg, 2009; Tinoco et al., 2006). Also, in addition to the shortening in the  
309 average length of alkyl chains, conspicuous changes in the C preference index (ratio of alkyl  
310 molecules with odd-to-even number of C atoms) also represent a valid surrogate of the  
311 damage levels of soils affected by fires (Almendros and González-Vila, 2012 and references  
312 therein). In fact, these effects have also been observed under laboratory conditions

313 (Almendros et al., 1988; Almendros et al., 1992; González-Vila et al., 2001) and a  
314 predominance of short-chain and even carbon numbered *n*-alkanes, as a result of thermal  
315 degradation of biomass, and has been found to be a valid molecular marker in archaeological  
316 or palaeoenvironmental research (Eckmeier and Wiesenberg, 2009). In the current study, we  
317 use this proxy to assess the impact of fire on SOM, i.e. the indexes tentatively used were: i)  
318 chain carbon preference index (CPI (C<sub>24</sub>-C<sub>31</sub>)) and ii) short to long chain ratio (S/L).

319 For CPI (C<sub>24</sub>-C<sub>31</sub>) (Fig. 3A), the values obtained were always higher than one, indicating  
320 SOM that are mainly of plant origin. Higher plants waxes contain hydrocarbon chains,  
321 preferentially with odd-over-even carbon preference number (Eglinton et al., 1962). Shifts in  
322 this biogenic trend indicate alteration to SOM. Our results point to the fact that fire-affected  
323 SOM were mostly in the fine fraction where CPI was almost lost and the value shifted from 1.7  
324 to 1.1. There were no clear differences in CPI for the total or for the coarse fractions. Post-fire  
325 CPI values are affected by inputs of biomass enriched in long-chain and odd-numbered alkane  
326 chains, hiding the effect of fire. This may be the reason why the differences in the total soil and  
327 coarse fractions were not high.

328 For all burned samples the S/L (Fig. 3B) ratios were higher than for the unburned  
329 samples, indicating cracking of long-chain alkanes. This effect was very clear for the coarse  
330 fraction (UB = 2.04 and B = 3.24). As previously described, it seems that there was a post-fire  
331 input of litter adding to the B coarse SOM fraction and was probably including, not only fresh  
332 biomass with a high CPI, but also char or partially-charred particles with a high proportion of  
333 short-chain alkanes, that may explain in part the finding that no substantial difference in S/L  
334 values between B and UB for the total and fine fractions were found.

335

### 336 3.4 Van Krevelen diagram

337 Here we used a Van Krevelen graphical approach to gain information about main  
338 chemical modifications produced by fire in SOM. The plot interpretation in relation to possible  
339 SOM transformation processes used here is based on that proposed by Kim et al. (2003).  
340 Figure 4 represents the Van Krevelen diagram with the average (centroid) O/C and H/C values  
341 of the pyrolysis compounds released from the total sample and the coarse and fine size  
342 fractions selected for this study. The values were inferred from the structural (molecular)  
343 information obtained by Py-GC/MS (Table S1 and S2).

344 The samples show only small differences with values plotted close together in a  
345 narrow area. However, when zooming in on this region, a similar behaviour is observed for the  
346 total soil and fine fractions that show an increase in O/C and H/C ratios, indicating the  
347 occurrence of a condensation process in the change from UB to B samples. When

348 decomposing the imaginary UB-B linking line into X- and Y-axes the condensation process  
349 occurring when burning could be further separated into two sub-processes: i) oxidation and ii)  
350 hydrogenation. This result is in agreement with a higher relative abundance of lignin-derived  
351 methoxyphenols in the B (total and coarse) soils compared with the UB soils and to a lesser  
352 extent to fatty acids (increasing of oxygen atoms) and the comparatively lower proportions of  
353 unspecific aromatic compounds, and PAHs (increasing of hydrogen atoms) to a lesser extent as  
354 seen in Fig. 2.

355 The transition from the coarse UB to the B sample shows an increase in O/C values and  
356 a decrease in H/C values which indicate demethylation/dealkylation processes. Decomposing  
357 the imaginary UB-B linking line, as with the total samples, the two principal processes induced  
358 by fire would be: i) oxidation, as for the total and fine B fractions, and ii) dehydrogenation. This  
359 shift that is observed in the coarse fractions could be best explained by an input of new litter  
360 and partially charred particles after a fire, which is also in agreement with the high relative  
361 contribution of methoxyphenols in the coarse B sample that may be in part responsible for the  
362 observed increase of O/C ratio. On the other hand, the observed H/C ratio decrease could be  
363 explained by a low relative contribution of alkane/alkene pairs in the B coarse fraction and to a  
364 lesser extent to demethylation of lignin-derived methoxyphenols and a higher relative  
365 contribution of PAHs with lower number of hydrogen atoms due to their condensed structure.

366 Although the total sample and the fine fraction show a similar trend when burned, the  
367 global value for O/C ratio was minor for the fine fraction, reflecting a lower relative proportion  
368 of plant derived methoxyphenols.

369

#### 370 **4. Conclusions**

371

372 Forest fires cause qualitative impacts in SOM either by transforming existing chemical  
373 structures or by forming new chemical structures, but forest fires also quantitatively modify  
374 the balance between the addition and removal of materials in the course of, or after, the fire  
375 event. In this research, the information provided by the direct analytical pyrolysis (Py-GC/MS)  
376 of whole soil and sieve size samples under a *Quercus suber* cover showed that fire caused an  
377 increase in the C and C/N ratio, both in total soil and in coarse and fine fractions, which is  
378 interpreted as an input of new biomass after the fire, i.e. ashes and charred material from the  
379 scorched canopy.

380 When studying total soil, major chemical differences were found between the unburnt  
381 (UB) soil and the burnt (B) soil. In the UB soil the organic compounds are found to derive  
382 mainly from polysaccharides, proteins and lignin, whereas for the B soil the organic

383 compounds are found mainly derived from lignin and the presence of pyrogenic polycyclic  
384 aromatic hydrocarbons. This allows us to make an assessment, in the different particle size-  
385 fractions, of the extent to which fire-induced depletion of thermolabile biogenic materials  
386 resulted in the concomitant selective preservation of more heat-resilient lignin moieties. In  
387 fact, the UB coarse (2–1 mm) fraction SOM was found to have a major influence on the  
388 vegetation, whereas the chemical structure of the fine (<0.05 mm) fraction points to SOM with  
389 higher proportions of humic substances, evidenced by a high degree of lignin demethoxylation  
390 and accumulation of microbial compounds. The organic signature for the B coarse fraction  
391 indicates new litter inputs after the fire, probably from stressed vegetation, and the  
392 corresponding fine fraction, share chemical characteristics with the UB fine fraction, but with  
393 additional signs of fire alteration: a reduction of labile carbohydrate structures, and a higher  
394 relative abundance of PAHs.

395 In this study, and for the first time, the high amount of molecular information that is  
396 usually obtained with direct analytical pyrolysis of environmental samples is analysed using a  
397 classical Van Krevelen graphical-statistical approach. This is an intuitive approach that is  
398 routinely being used to simplify the interpretation of a very large number of compounds that  
399 are detected by modern ultrahigh resolution mass spectrometry, i.e. Fourier Transform ion  
400 cyclotron resonance mass spectrometry (FT-ICR-MS) (Kim et al., 2003; Sleighter and Hatcher,  
401 2007; DiDonato et al., 2016 and references therein). Here, we used a Van Krevelen diagram  
402 representing atomic H/C and O/C ratios of the pyrolysis compounds in the basal plane (x,y  
403 axes), together with the use of classic geochemical proxies like the alkane carbon preference  
404 index (CPI) and a short/long chain ratio (S/L) proved to be a valuable tool in enlightening upon  
405 relevant chemical processes and a transformation that occurs in SOM due to heat or fire.

406 Under the natural conditions studied here, we found that fire affected most SOM in  
407 the fine soil fraction and, in general, exerted significant cracking of long-chain alkyl SOM  
408 components in addition to other SOM modifications, fire-exerted oxidation in all samples  
409 studied and condensation and demethylation processes in the coarse fraction.

410

#### 411 **Acknowledgements**

412

413 This study is a part of the results of the GEOFIRE Project (CGL2012-38655-C04-01)  
414 funded by the Spanish Ministry for Economy and Competitiveness. N.T Jiménez-Morillo is  
415 funded by a FPI research grant (BES-2013-062573). Dr. J.M. de la Rosa thanks the Marie  
416 Skłodowska-Curie actions (REA grant agreement n° PCIG12-GA-2012- 333784—Biocharisma

417 project). Alba Carmona Navarro and Desire Monis Carrere are thanked for their invaluable  
418 technical assistance in Py/GC-MS analysis.

419

420 **Appendix 1. Supplementary data**

421

422 List of compounds released by Py-GC/MS.

423

424

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624

625 **Tables**

626

627 Table 1. Percentage of total carbon (C) and total nitrogen (N) with standard deviation (SD) for  
628 the whole soil (Total) and sieve fractions from unburned (UB) and burned (B) soils under  
629 *Quercus suber* cover.

630

<b>Scenario</b>	<b>Sieve fraction (mm)</b>	<b>% C</b>	<b>SD</b>	<b>% N</b>	<b>SD</b>	<b>C/N</b>
UB	Total	6.7	1.3	1.0	0.1	6.8
	2–1	15.7	2.7	1.5	0.2	10.3
	1–0.5	14.0	2.0	1.4	0.1	10.3
	0.5–0.25	6.5	0.3	1.0	0.0	6.8
	0.25–0.1	5.8	0.1	0.9	0.0	6.3
	0.1–0.05	6.3	0.4	1.0	0.0	6.5
	<0.05	7.3	0.0	1.1	0.0	6.9
B	Total	10.8	1.1	1.3	0.1	8.3
	2–1	27.9	0.9	1.3	0.0	21.4
	1–0.5	9.6	0.1	1.2	0.0	8.2
	0.5–0.25	7.7	1.0	1.1	0.1	7.2
	0.25–0.1	7.4	0.1	1.1	0.0	6.9
	0.1–0.05	7.5	1.1	1.1	0.1	6.9
	<0.05	9.6	0.3	1.3	0.0	7.4

631

632

633 **Figure captions**

634

635 Fig. 1. Py-GC/MS Total ion current chromatogram from the total and sieve size fractions of  
636 unburned (UB) and burned (B) soils. Labels on the peaks indicate the origin of major pyrolysis  
637 compounds: A: Aromatic; S: sugar; P: polypeptides; Lg: lignin; Isop: Isoprenoid; number on  
638 peaks indicates C number alkene/alkane chains.

639

640 Fig. 2. Relative percentage of the main chemical families identified by Py/GC/MS from  
641 unburned (UB) and burned (B) total soil (Total) and two main sieve size fractions; coarse (1-0.5  
642 mm) and fine (<0.05 mm).

643

644 Fig. 3. Geochemical markers for unburned (UB) and burned (B) soils in the total sample (Total)  
645 and two main soil sieve size fractions; coarse (2–1 mm) and fine (<0.05 mm): A) CPI (24–31):  
646 Carbon Preference Index for chains with 24 to 31 carbons. B) S/L: Alkane short to long chain  
647 length ratio.

648

649 Fig. 4. Van Krevelen diagram for unburned (UB) and burned (B) soils in the total sample (Total)  
650 and two main soil sieve size fractions; coarse (2–1 mm) and fine (<0.05 mm).

651

- SOM molecular alterations after a wildfire are studied using Py-GC/MS
- Van Krevelen plots & Alkane CPI, S/L indicated about fire mediated transformations
- Fire exerted oxidation, chain cracking, polysaccharide removal and lignin preservation

Figure 1  
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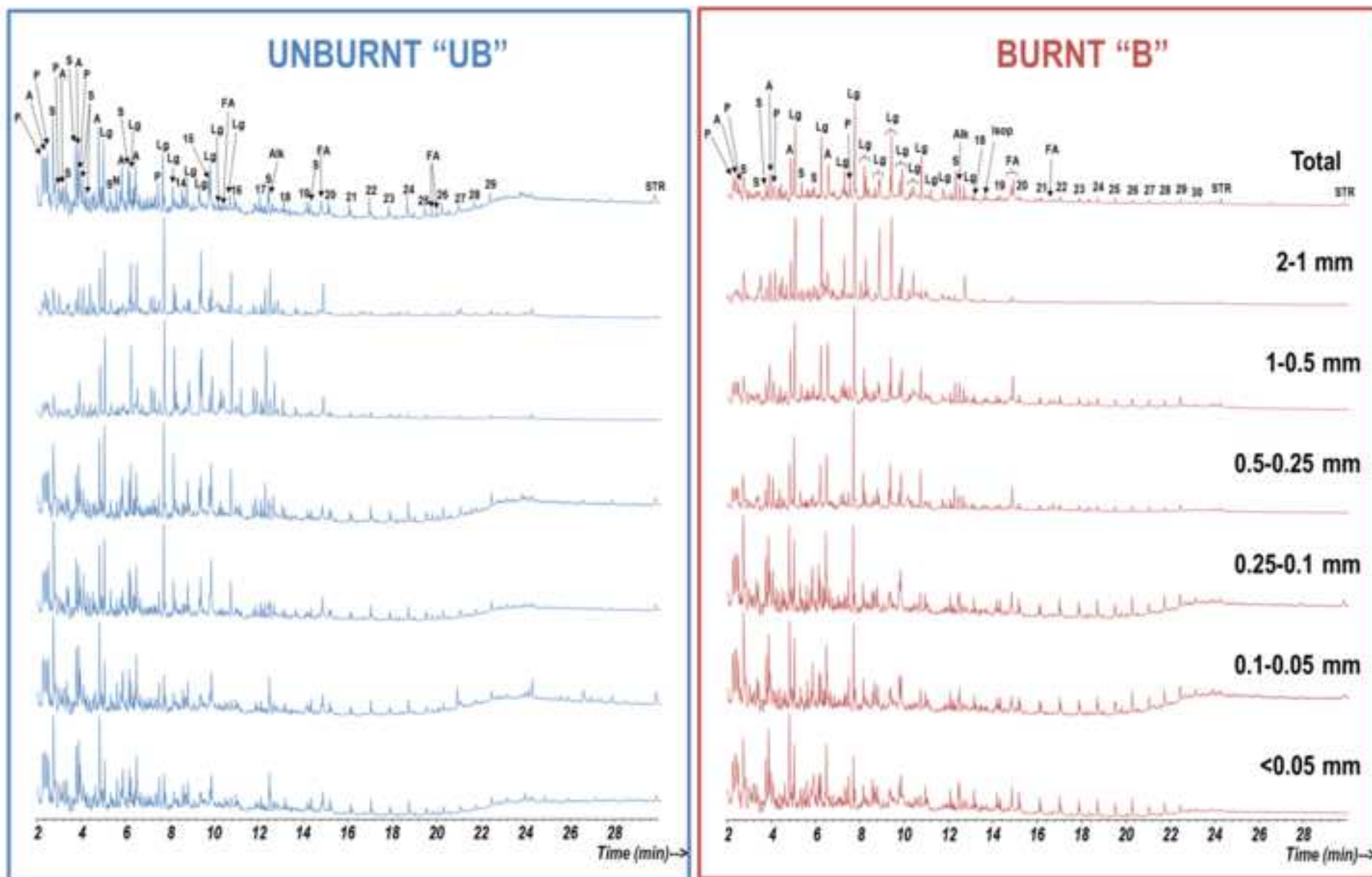


Figure 2  
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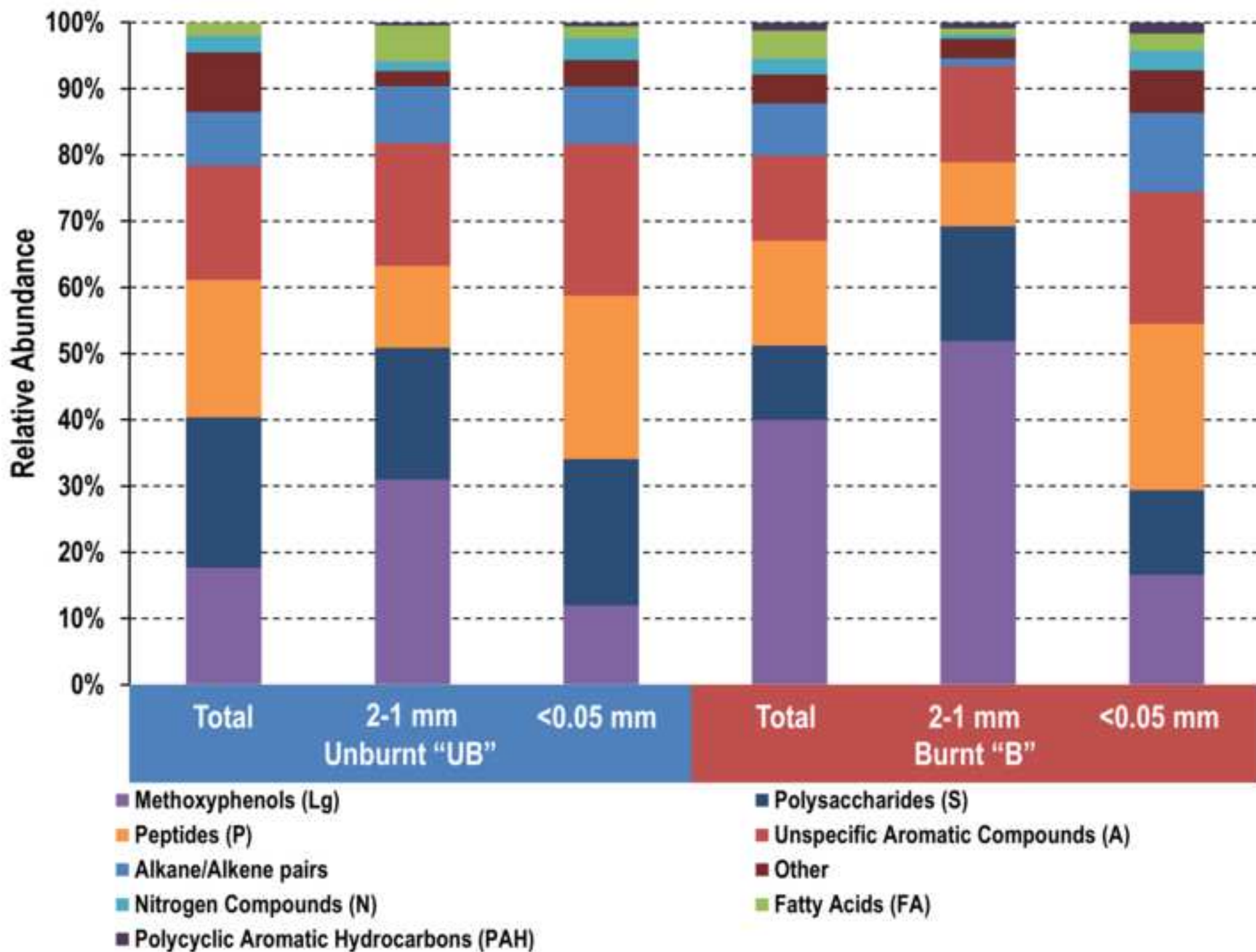




Figure 3  
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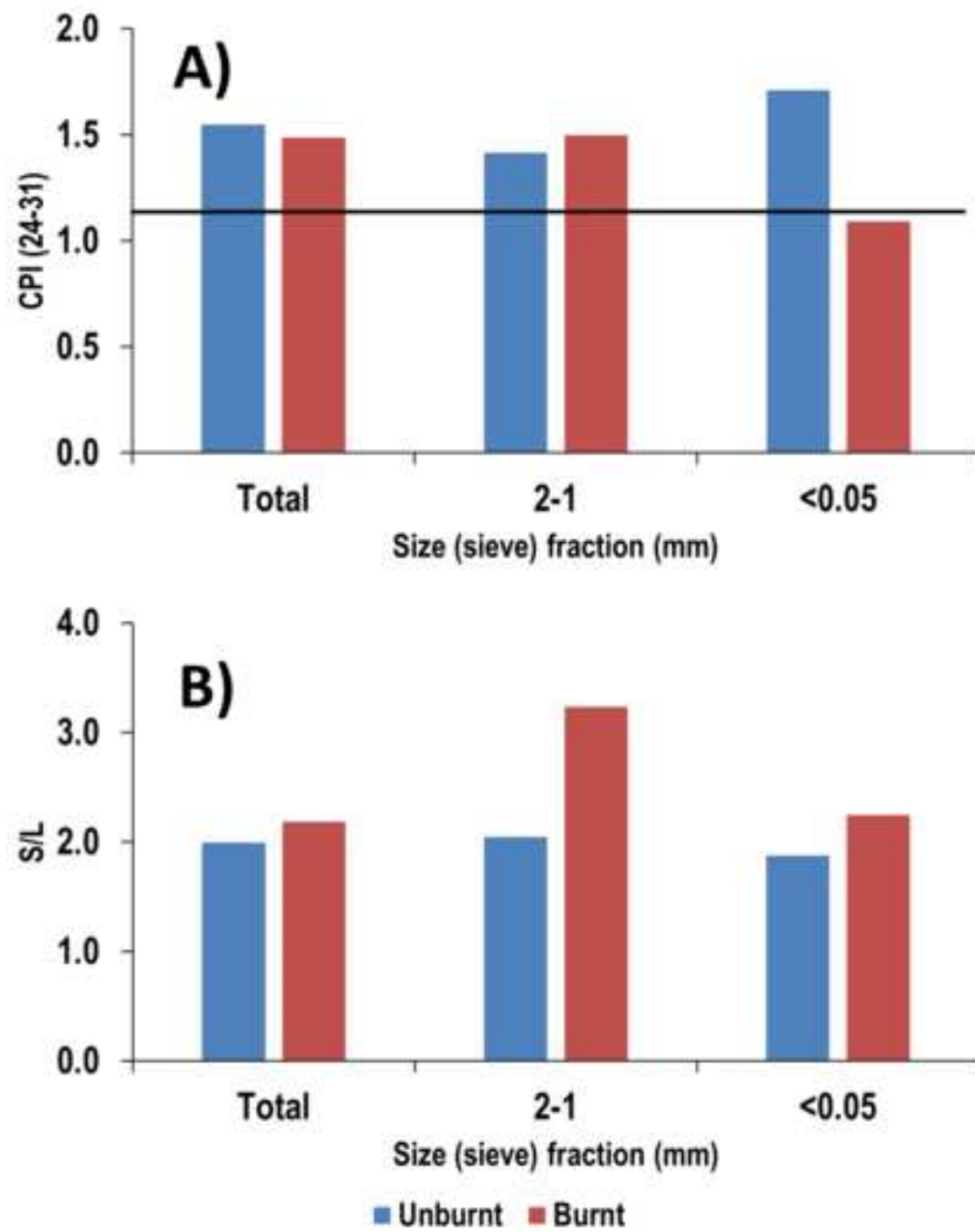


Figure 4  
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## Van Krevelen's diagram

