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Application of pyrolysis-gas chromatography/mass spectrometry to study changes in the organic matter of macro- and microaggregates of a Mediterranean soil upon heating

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ABSTRACT

The heating effect on the soil organic matter (SOM) of a Mediterranean soil was studied in two fractions (macro- and microaggregates) and in two environments (soil under canopy of *Quercus coccifera* and bare soil between plants). Samples were heated under laboratory conditions at different temperatures (220, 380 and 500 °C) to establish their effects on the SOM quality and quantity by comparison with unheated control samples (25° C). The SOM content in the soil under canopy was higher than in the bare one and in the microaggregate fractions than in the macroaggregate ones. Increasing temperatures caused, in general, the decrease of SOM content in both soils as well as in both aggregate classes. The quality of SOM was determined after extraction with 0.1 M NaOH and analysed by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Obtained pyrolysates were characterized by the presence of polyphenols and other aromatic pyrolysis products (lipids, polysaccharides, proteins and lignin derivatives). Some of the products in these control samples, and furthermore the presence of black carbon (BC) markers (e.g. benzene, pyridine and toluene), confirmed the occurrence of past wildfires in the study zone. The composition of the SOM extracted from the soils heated at 220 °C, was quite similar to that obtained from unheated soils. The products derived from polysaccharides and lignin, and some coming from polyphenols, were not detected in the pyrolysates of the soil heated at 380 and 500 °C.

1. Introduction

Forest fires can be considered as one of the main disturbances to soils and vegetation in the Mediterranean basin [1–3]. One of the effects of forest fires on soil properties is the alteration of SOM, affecting both its content and composition [4,5]. The effect of fire on the total SOM content is highly variable and depends on several factors (fire intensity, vegetation type, etc.). Numerous studies have attempted to quantify the fire changes on SOM content (i) under laboratory conditions [6], (ii) after controlled fires, and (iii) after wildfires [7]. In the laboratory, significant SOM losses are usually reported [8], meanwhile, after controlled and wildfires the effects may range from the destruction of the organic layer and SOM [9,10] to its increase in the surface layers due to external inputs, mainly from partially burnt materials or litter from decaying plants [11–13].

In contrast, the study of the changes in SOM composition is a complicated knowledge field that still represents a challenge to soil researchers. It has made great progress in recent years due to the use of techniques such as solid-state nuclear magnetic resonance (NMR), pyrolysis gas chromatography/mass spectrometry (Py-GC/MS), thermally assisted hydrolysis and methylation (THM), etc. [14–16]. Field and laboratory studies applying ^{13}C and ^{15}N NMR indicate that the thermal modifications of organic matter during charring include dehydration, aromatisation, loss of methoxylic and carboxylic functional groups, condensation of carbohydrates into furan-like structures and enrichment of heterocyclic aromatic N-compounds. In terms of spectroscopic properties, the effect of heating on SOM leads to the loss of the O-alkyl and di-O-alkyl structures that dominate wood and a large increase in aromatic C [4,13,17]. However, the number of studies is still low and the results obtained scarce.

Py-GC/MS is an approach that involves thermal degradation of organic molecules into small fragments that are analysed by GC/MS, providing specific information related to their structure, which can be used for studying thermal alterations of SOM. The advantage of using this technique is that it allows for a rapid screening of the chemical composition of a sample at the molecular level. Especially mixtures of complex compounds, such as SOM, can be analysed to provide an overview (or a chemical fingerprint) of a given sam-

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ple. Thus, pyrolysis products released from unburned soils include a wide variety of molecules derived from carbohydrates, lignin, lipids and proteins. On the contrary, in soils affected by wildfires, most of the pyrolysis products released from unburned soils are absent, and the dominance of charred "non-pyrolysable" refractory carbonaceous material is evident [4].

Similarly, there are many studies that have tried to establish differences in the SOM content according to aggregation and environment characteristics that have indicated that: (i) macroaggregates (⌀ > 0.25 mm) have greater concentrations of organic C than microaggregates (⌀ < 0.25 mm), because of the organic matter binding microaggregates into macroaggregates [18–20] and; (ii) soils under canopy showed, in general, higher SOM content than bare soils [21], as it has been found in Mediterranean environments [11,12,22–24].

As it can be appreciated in recent years, a growing number of researchers have worked on the quantification and/or analytical description of the organic matter in different soil types, affected or not by the impact of fires. Some of the studies that have been carried out in the Mediterranean area have proposed to differentiate the soil by environments or fractions for these determinations, but in this research a first attempt is made to give a joint description of the temperature effects on the quantity and quality of SOM as a function of the size of soil aggregates and the environment in which it is located. Thereby, this study aims (a) to assess the effect of the vegetation cover in the SOM content and composition of a Mediterranean forest soil; (b) to study the SOM quantity and quality of its macro and microaggregates fractions and; (c) to investigate the changes produced by different heating temperatures on the initial SOM characteristics of this soil.

2. Materials and methods

2.1. Study area and sampling

A Mediterranean forest soil located in the Sierra de La Calderona (Valencia province, Eastern Spain: 39°45′ N, 0°43′ W) was selected for this study. The area is forested and is situated on a lightly concave hillside with a SSE aspect, 22° of slope and an altitude around 575 m a.s.l. that last burned in 1978. The soil is a Rendzic Leptosol [25] with variable depth, smaller than 40 cm, developed on Jurassic limestone with a gravelly sandy loam to silty loam (45% gravel).

The Sierra de La Calderona is a large forest area covered by Mediterranean vegetation dominated by *Pinus halepensis, Quercus ilex* and shrubland in different wildfire related degradation stages such as *Quercus coccifera*, *Rosmarinus officinalis*, *Ulex parviflorus*, *Rhamnus lycioides*, *Stipa tenacissima*, *Globularia alypum* and *Thymus vulgaris.*

The climate of the area is Mediterranean (mean annual precipitation of 400 mm), with a maximum precipitation in autumn (51.7 mm in October) and a second, but less rainy period in spring (34.1 mm in April). The dry period, in which usually forest fires occur, ranges from April to September with a mean daily maximum temperature of 34.0 °C. The mean annual temperature is 17.2 °C.

Soil samples were taken in two environments (Table 1): under the canopy (UC) and between plants (bare soil: BS). The "under canopy soil" was sampled under *Quercus coccifera* (considered to be the most dominant species in the area). Soil material was sampled from the first 5 cm of the topsoil in summer 2008 by taking three independent sub-samples within an area of approximately 20 m^2 (trees were separated at least 80 cm), and both the litter layer (±3 cm) as well as rock fragments >1 cm were excluded and removed. The sub-samples from each environment were mixed together, homogenized, transported in hermetically sealed plastic containers, and next dried at room temperature and passed through a sieve with 2 mm width (fine earth fraction).

Table 1
Physico-chemical characteristics of the studied soils ($n = 4$). Values with different superscripts (a, b) indicate significant differences between environments detected by Student's t-test ($p < 0.05$).

	Under canopy soil	Bare soil
Structural stability (%)	31.49[a]	24.12[b]
Organic matter content (%)	12.11[a]	8.50[b]
$CaCO_3$ content (%)	45.42[a]	50.15[b]
Mean weight diameter (mm)	0.87[a]	0.71[b]
pH	7.52[a]	7.65[b]
Electrical conductivity (dS/m)	1.10[a]	0.70[b]

2.2. Laboratory tests and analyses

The UC and BS samples were heated in a rapid heating chamber furnace (Carbolite®) that allowed for heating steps of 3 °C min^{-1}, to prevent sudden combustion, and 2 min of heating at the selected temperature [26]. A sample amount of less than 1 cm thickness was used to avoid a temperature gradient in the sample. Four heating steps were applied according to known thresholds in which significant changes can occur in both mineral and organic soil components: (a) 25 °C: control (room temperature), (b) 220 °C: increased hydrophobicity and dehydration of the gel forms [27,28], (c) 380 °C: SOM combustion and particles re-aggregation [7,29] and (d) 500 °C: loss of –OH groups from the clays [8]. Once heated, samples were separated by gentle sieving in macroaggregates (⌀ > 0.25 mm) and microaggregates (⌀ < 0.25 mm).

Standard laboratory analyses were performed in duplicate to differentiate UC and BS samples (Table 1). Soil pH was measured in water and 1 M KCl, and electric conductivity was determined in the saturation extract of soil [30]. To assess soil aggregate stability Wet-sieving (0.25 mm mesh) was used to assess soil aggregate stability [31]. Total carbonates were measured using the Bernard calcimeter method [32].

SOM content was determined in duplicate, using the wet oxidation by potassium dichromate method, following Walkley–Black procedure [33] for all samples (under canopy and bare soil), temperature treatments (25, 220, 380 and 500 °C) and both aggregate fractions (macro and micro). Therefore, a total of 16 soil samples were considered in this study. The factor used to convert C into organic matter was 1.724 [32]. SOM contents were related to the dried soil weight.

Analyses of Student's t-tests at $\alpha = 0.05$ were performed to detect differences between soil characteristics (under canopy and bare soils) before thermal treatments. A General Linear Model (GLM) uni-variate procedure (95% significance level) was performed (SPSS 15®) to study possible differences in organic matter content with regard to environment, both aggregate fractions and heat treatment. Additionally, a principal component analysis (PCA) was also conducted to determine whether it could be possible to use a small number of compounds to explain the likely influence of the environment, aggregates fraction and temperature in the measured intensity of each compound found in the chromatograms.

2.3. Isolation of organic matter

The 16 samples were prepared based on a modified method of Nierop et al. [34] to concentrate the soil organic matter. Soil samples were shaken for 5 min in 0.1 M NaOH (10 g soil: 50 mL aqueous NaOH) (twice), after which they were centrifuged at ~2000 × g for 10 min and then decanted. The residual sand was washed with water, at least ten times, until the water was colourless. All extracts of each sample were combined and acidified to reach pH 1.5–2.5

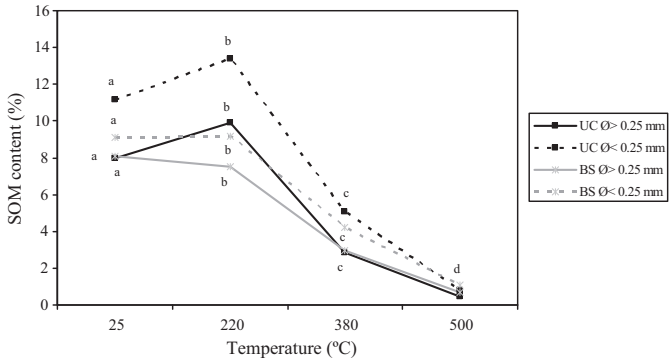

Fig. 1. Mean soil organic matter related to dried soil weight for macroaggregates (⌀ > 0.25 mm) and microaggregates (⌀ < 0.25 mm) in under *Quercus coccifera* soil and bare soil. Different letters (a–d) indicate significant differences in each soil sample.

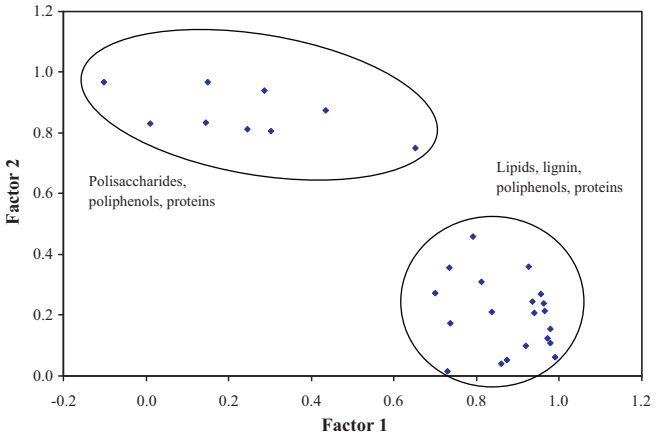

Fig. 2. Factor plot of the PCA which explains about 85% of the variability observed in the 35 identified compounds characterizing the extracted SOM.

by dropwise addition of 10:4 M HF:HCl solution. The suspensions containing the extracted organic matter were gently manipulated through a 63 μm mesh-size sieve to remove any material that may still exist in the extracts (63 μm to 2 mm). The suspensions were desalinized by dialysis against distilled water and freeze-dried in an alcohol bath at −60 °C to produce a powder of extracted SOM.

2.4. Pyrolysis GC/MS

The Py-GC/MS analyses were conducted according to the analytical procedure of Nierop and Verstraten [35]. The freeze-dried organic matter was pyrolysed using a Horizon Instruments Curie-Point pyrolyser attached to a Thermo-Quest Trace GC 2000 system (Py-GC). Each sample was deposited on a ferromagnetic wire and heated at 600 °C for 5 s. The pyrolysis products were separated on a fused silica column (J&W, 30 m × 0.32 mm i.d.) coated with DB-1 (film thickness 0.50 μm) and used Helium as the carrier gas. The oven was initially kept at 40 °C for 1 min, next it was heated at a rate of 7 °C min^{-1} to 320 °C and maintained at that temperature for 15 min. The column was coupled to a Finnigan Trace MS mass spectrometer (mass range *m*/*z* 45–600, ionization energy 70 eV, cycle time 1 s).

Individual compounds were identified by interpretation of their mass spectra and relative retention times using NIST98 and Wiley07 libraries or by comparison with published data.

3. Results and discussion

3.1. SOM quantification

The results of the statistical analyses show in Table 2 that there are differences in SOM content as a function of the environment, aggregate fraction, and temperature treatment, and further double interactions (fraction × environment, environment × temperature, fraction × temperature) and triple interaction (environment × fraction × temperature). These differences are well reflected in Fig. 1 that shows the SOM content of both aggregate fractions in UC and BS, heated at the different temperatures.

According to the mentioned analyses, the SOM content in the UC was higher than in the BS. This result is in agreement with those of other researchers [11,12,23,24]. The impact of different temperatures changed the SOM content in both fractions and environments. In the BS, and in both aggregate sizes, the SOM content decreased with increasing temperatures, which has also been reported in several laboratory studies [8,26].

However, in all fractions of the UC samples, SOM firstly increased from to 25 °C to 220 °C and then decreased in the highest temperature ranges (Fig. 1). This increase in SOM is difficult to explain but it is possible that the increased temperature has liberated SOM compounds that were prior to heating, occluded in the microaggregates [36] and not accessible for oxidation under the wet oxidation procedure.

The SOM content was higher in the microaggregates than in the macroaggregates, even when they were analysed separately according to the environment (UC and BS). This fact suggest that the role of SOM in keeping microaggregates together in macroaggregates was of limited importance and contradicts the results reported by other authors [18–20], according to which macroaggregates showed higher SOM content than microaggregates.

3.2. SOM composition

The use of Py-GC/MS to characterize the SOM allows the identification of 34 pyrolysis products that accounted for most of the peaks found in the total ion current (TIC). Based on previous research and published data, these 34 compounds were grouped according to their probable origin into the following component classes: polysaccharides, polyphenols, proteins/amino acids, lignin, black carbon (BC) and lipids. Some contaminants were also observed as 2,4-bis (1,1-dimethylethyl-phenol), phthalate or broxyquinoline. Identified peaks are listed in Table 3.

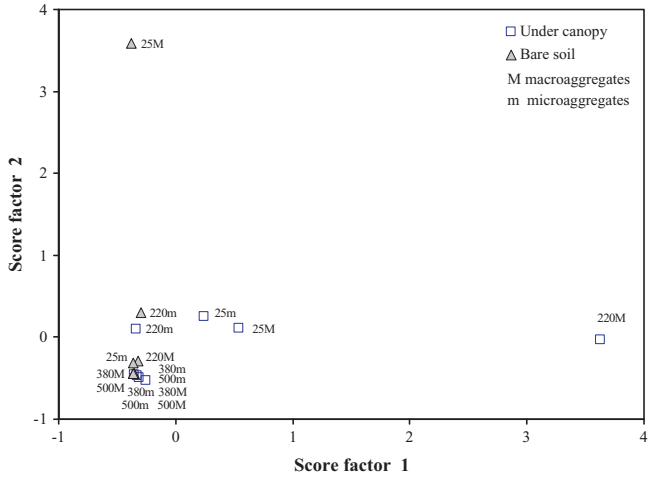

Fig. 3. Bi-plot diagram from the PCA of peak intensities of the identified compounds versus the environment, aggregate fraction and temperature.

Table 2
Differences in the organic matter content of the studied soil in function of its environment (under canopy or bare), aggregate size distribution (macro and microaggregates) and heat treatment (different temperatures reached in the laboratory) according to univariate General Linear Model ($n = 16$). Differences were reported at 95% significance level.

Source	Type III sum of squares	Degrees of freedom	Square mean	F[a]	Significance
Corrected model	518.318[b]	15	34.555	275.857	0.000
Intercept	1114.392	1	1114.392	8896.454	0.000
Size	22.646	1	22.646	180.792	0.000
Environment	9.680	1	9.680	77.278	0.000
Temperature	461.661	3	153.887	1228.516	0.000
Size × environment	2.928	1	2.928	23.377	0.000
Size × temperature	5.383	3	1.794	14.325	0.000
Environment × temperature	14.495	3	4.832	38.572	0.000
Size × environment × temperature	1.524	3	0.508	4.057	0.025
Error	2.004	16	0.125		
Total	1634.714	32			
Corrected total	520.322	31			

[a] F: ratio of two quadratic means.
[b] R square = .996 (Adjusted R square = .993).

According to the PCA results, it can be said that the 34 initial compounds, which characterize the SOM in both environments and fractions heated at different temperatures, can be reduced to two components that explain about 85% of the variability observed in the original compounds. As can be seen in Fig. 2, the factor 1, which includes most of the compounds, would explain the variability experienced by lipids, lignin and some polyphenols and proteins, while the factor 2 would describe it with the polysaccharides and the other polyphenols and proteins.

Based on the PCA the scores of the studied treatments (environment, fraction, temperature) were plotted in Fig. 3, in agreement with the above defined factors. According to this figure there are no clear differences in the variability of the studied compounds intensity depending on the environment or the aggregate fraction that are analysed. However, it seems that major changes in these intensities were due to the application of the different temperatures. In fact, it could be said that the changes produced by them can be classified into two groups: on the one hand would be the observable changes at low temperatures (25 and 220 °C), and on the other hand those that occurred at high temperatures (380 and 500 °C). It can be even noted that the dispersion of scores in the group of low temperatures indicate that, in this temperature range,

Table 3
List of compounds identified by GC/MS in the studied soils, containing compound code, compound name, characteristic fragment ions (m/z) and average retention time (RT).

Number	Compound	m/z	RT	Source
1	Acetone	43 + 58	1.75	Ps
2	Furan	68	1.78	Ps
3	2-/3-Methylfuran	82	2.25	Ps
4	Acetic acid	45 + 60	3.00	Ps
5	2-Furfural	95 + 96	6.95	Ps
6	5-Methyl-2-furfural	109 + 110	8.85	Ps
7	Levoglucosenone	96 + 97 + 98	10.88	Ps
8	Anhydroglucosan (Levoglucosan)	60 + 73	20.52	Ps
9	*N*-methylpyrrole	80 + 81	3.50	Pr
10	Pyridine	52 + 79	3.90	Pr/BC?
11	Vinylbenzene (Styrene)	78 + 104	6.50	Pr/BC
12	2-Methylbenzenamine	106 + 107	8.48	Pr
13	Benzene	78	2.70	Pp/BC?
14	Toluene	91 + 92	4.10	Pp/Pr/BC?
15	Ethylbenzene	91 + 106	6.00	Pp/BC
16	Phenol	66 + 94	9.52	Pp/Lg/Pr
17	3-/4-Methylphenol	107 + 108	11.45	Pp/Lg/Pr
18	2,4-Dimethylphenol	107 + 122	13.44	Pp
19	2-Methoxyphenol (Guaiacol)	109 + 124	10.87	Lg
20	4-Methylguaiacol	123 + 138	13.60	Lg
21	4-Vinylguaiacol	135 + 150	15.63	Lg
22	*trans*-Isoeugenol	149 + 164	18.35	Lg
23	4-Acetylguaiacol	151 + 166	19.20	Lg
24	Butane	29 + 43	1.70	Lp
26	1-Tridecene	41 + 43 + 55	15.42	Lp
27	C_{14} alkanol	55 + 69 + 83	23.13	Lp
28	C_{14} alkanoic acid	60 + 73	23.70	Lp
29	C_{16} alkanoic acid	60 + 73	26.60	Lp
30	Oleic acid ($C_{18:1}$ FA)	41 + 55 + 69	28.95	Lp
31	C_{18} alkanoic acid	60 + 73	29.30	Lp
32	Hexadecane	57 + 59	34.50	Lp
32	Heptadecane	57 + 71	35.10	Lp
33	Squalane	57 + 71 + 85	36.20	Lp
34	Squalene	69 + 81	36.60	Lp
*	2,4-bis (1,1-dimethylethyl)-phenol (contaminant)	191	23.80	
*	Broxyquinoline (contaminant)	303	28.03	
*	Phtalate (contaminant)	149 + 167	35.70	

Ps: polysaccharide; Pr: proteins/amino acids; Pp: polyphenols; BC: black carbon; Lg: lignin; Lp: lipids.

changes would have occurred differently in different compounds. In contrast, the clustering of scores in the second group would indicate that at high temperatures the changes would have affected similarly to the same compounds.

As the PCA results have suggested, at the control temperature of 25 °C the SOM extracted from macro- and microaggregates of the UC and BS samples showed similar compositions. In all the pyrolysates (Figs. 4–7; Table 3) products derived from polysaccharides [1, furans and reduced furans (2, 3, 5 and 6), acids (4) and anhydrosugars (7, 8)]; proteins (9–12), polyphenols and other aromatic compounds [i.e. benzenes (13–15), phenols (16–18)]; products derived from lignin (19–23) and lipids [alkanoic acids (27–30), alkanol (26), *n*-alkane and *n*-alkene (24, 25, 31–34)] were observed.

In soils heated at 220 °C some compounds were not detected but, in general, all pyrolysates showed the same compounds obtained from soils maintained at 25 °C (Figs. 4–7; Table 3) confirming the initial results obtained from the PCA. In the macroaggregates some compounds were not detected such as furan (2) in the UC and *N*-methylpyrrole (9) and C_{14} alkanoic acid (27) in the BS, indicating the partial degradation of proteins and lipids. In the microaggregates of the BS samples, 4-methylguaiacol (20) and butane (25) were not detected. This degradation could be related to the structural configuration of the various biomolecules in plants, litter or SOM, since lipids are at the outer part of a plant leaf, and thus will receive the heat impact first.

It should be also observed that macroaggregates of the BS heated at 200 °C also presented significant reductions in the intensities of their compounds. Most of these were higher than 90% except for butane, *n*-alkanes and *n*-alkenes, which intensities decreased 40–70%. The compounds of the UC soil microaggregates also showed lower intensities at 220 °C than at 25 °C. In this fraction, lignin product intensities were the least reduced (10–40%), while polyphenol and protein product intensities decreased between 40 and 90%, and those of the polysaccharides between 10 and 95%.

On the other hand, most of the compounds in the macroaggregates of the UC soil showed augmented intensities when heated at 220 °C. Such increases ranged from 4% for some lipids (32, 33) to ~350% in some lignin products (19, 21 or 22). Although these higher intensities could be related to the increased SOM content measured in this soil fraction, at this temperature, the fact that much of the compounds identified in the microaggregates of the BS soil have also increased their intensities from 25 to 220 °C, while the SOM content of this soil decreased, does not support this hypothesis. Most of the changes measured in the compound intensities of the BS soil also varied widely ranging from 10% in some lipids (27, 29) to ~650% in some polysaccharides or polyphenols (5 or 14).

Significant changes in the extracted SOM composition were observed in the soils heated at 380 °C (Figs. 4–7; Table 3) as the PCA results have already suggested. Pyrolysates of the different soil samples generally revealed a decrease in the number of compounds, and a greatly reduced intensity of most signals compared to those obtained from control samples. This could suggest that labile organic matter (lipids, proteins and polysaccharides mainly) was destroyed under the higher temperature regime. Thus, in the UC macroaggregate fraction, several products that should be derived from polysaccharides (1, 3 and 4) were not present in the pyrolysates, as well as to some of those derived from proteins (9 and 10), polyphenols (14 and 16), lignin (19, 20 and 22), and lipids (27). In the microaggregate fraction the pyrolysis products of polysaccharides (1–6) and proteins (9–11) were not detected, just as those compounds derived from polyphenols (16) and lignin (19). The few products that were still observed in the pyrolysates of both fractions showed intensities that were reduced by over 80% in relation to their initial values. Only in some lipid derivatives this decrease was less marked (compound 31 in both fractions and compound 33 in the macroaggregates).

On the other hand, in the BS macroaggregate fraction some compounds were not found at 380 °C as some derived from polysaccharides (1–4), proteins (10) and polyphenols (14–16). These compounds were also not detected in the pyrolysate of the BS microaggregate fraction, just as those derived from polysaccharides (5–8), proteins (9 and 11) and lignin (19, 23–25). The reduction in the intensities of the compounds still present in the pyrolysates of these fractions was also higher than 80% compared to those obtained from control samples. Lipid products showed again smaller intensity decreases of around 65% in the macroaggregates (31, 32 and 34) and between 20 and 40% in the microaggregates.

The pyrolysates of the extracted SOM heated at 500 °C were significantly different from those obtained under lower temperature treatments (Figs. 4–7; Table 3). At 500 °C, most of the previously observed compounds were not detected. The decreasing proportion of pyrolysed material could be partly due to the selective enrichment of heat-resistant aromatic components. This environmentally recalcitrant organic material is also known to become non-pyrolysable under the standard laboratory pyrolysis conditions [37]. As a consequence, in the UC macroaggregate fraction pyrolysate, besides the compounds not detected at lower temperatures, some products of proteins (12), polyphenols (15, 17 and 18) and lignin (21) were not observed. These compounds were also not present in the UC microaggregate fraction pyrolysates.

Regarding the organic matter extracted from the BS macroaggregate and microaggregate fractions heated at 500 °C, in addition to the compounds that were already not observed at lower temperatures, some products obtained from polyphenols (18) and lignin (21) were also not detected in their pyrolysates. The intensities of the few compounds still present in the pyrolysates of both fractions in the UC and BS soils at this temperature were reduced more than 95% in relation to the control samples. Only some lipid products (31, 33 and 34) presented smaller decreases, about 70% in both aggregate fractions of the UC soil and of about 40% in the BS soil microaggregates.

3.2.1. Polysaccharides

Small amounts of polysaccharides with a low abundance of intact form markers (i.e. mannose, glucose, galactose, pentose or rhamnose) suggest that a large part of such polysaccharides could not be formed by micro organisms and soil fauna *in situ*. On the other hand, little contributions of levoglucosan (8), a pyrolysis product of intact polysaccharide/cellulose [40], were detected and used as indicator of the wildfire that took place at the study site in 1978.

The reduction in the presence of typical pyrolysis products such as polysaccharides in the pyrolysates of the samples heated to 380 and 500 °C could indicate that the cellulose occurring in the soil was partially cracked by the heat treatment [41].

3.2.2. Proteins and other polypeptides

The presence of pyridine (10) in the pyrolysates of SOM extracted from the control samples would suggest an accumulation of heterocyclic N and the possible incidence of a fire, as is also reported in other Mediterranean fire-affected soils [13]. This N-containing compound is also considered to be the pyrolysis product of amino sugars and alanine [42].

In addition to these N-containing moieties, *N*-methylpyrrole (9), styrene (11), toluene (14), phenol (16), 3-methylphenol and 4-methylphenol (17) may also have a proteineous origin. The source of these proteins and polypeptides is ambiguous because all living species contain proteins, and related polypeptides are likely to be derived from them through transformation processes.

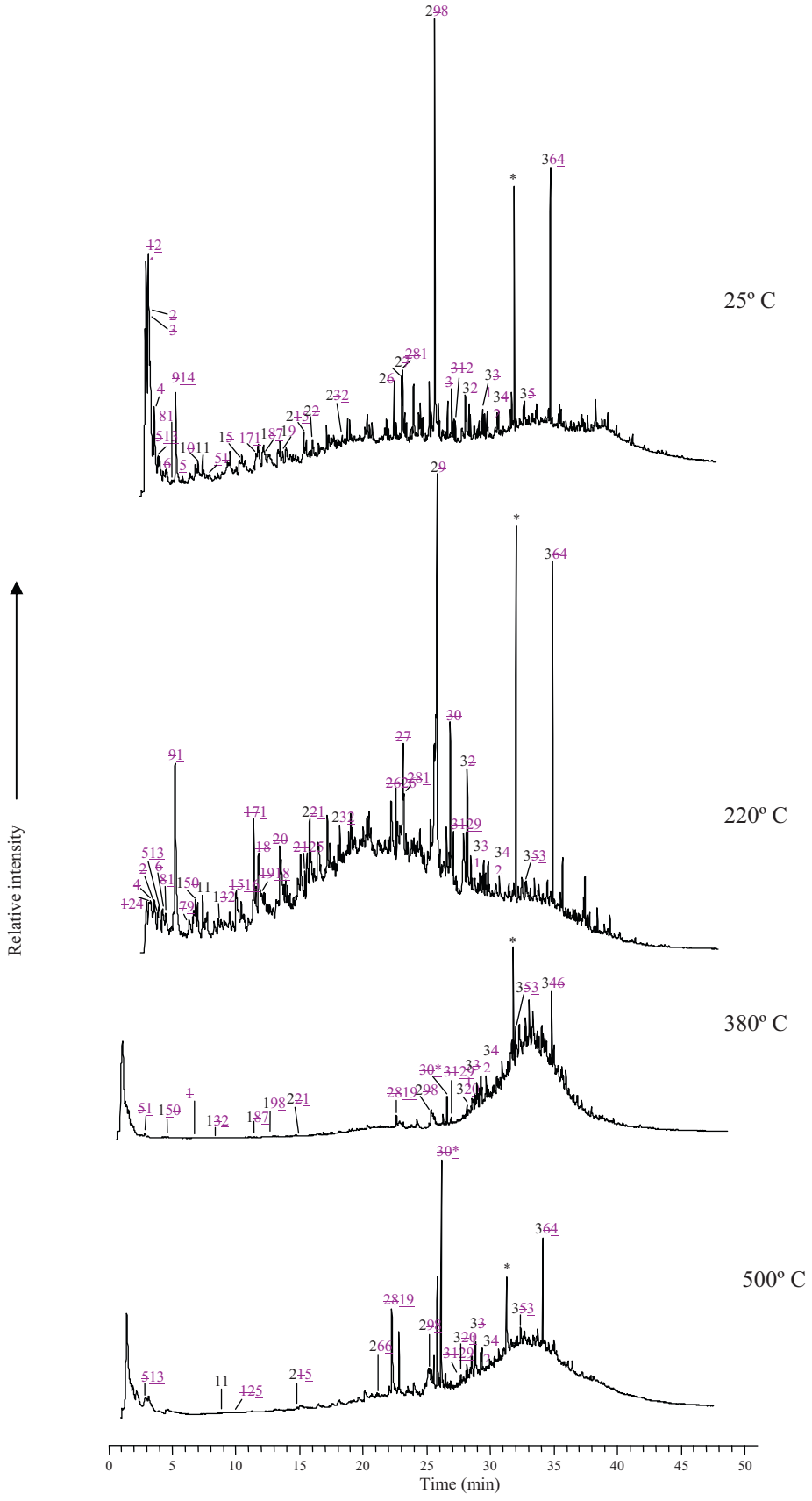

Fig. 4. Pyrolysis-GC trace of the organic matter extracted from macroaggregates in under *Quercus coccifera* soil at different temperatures.

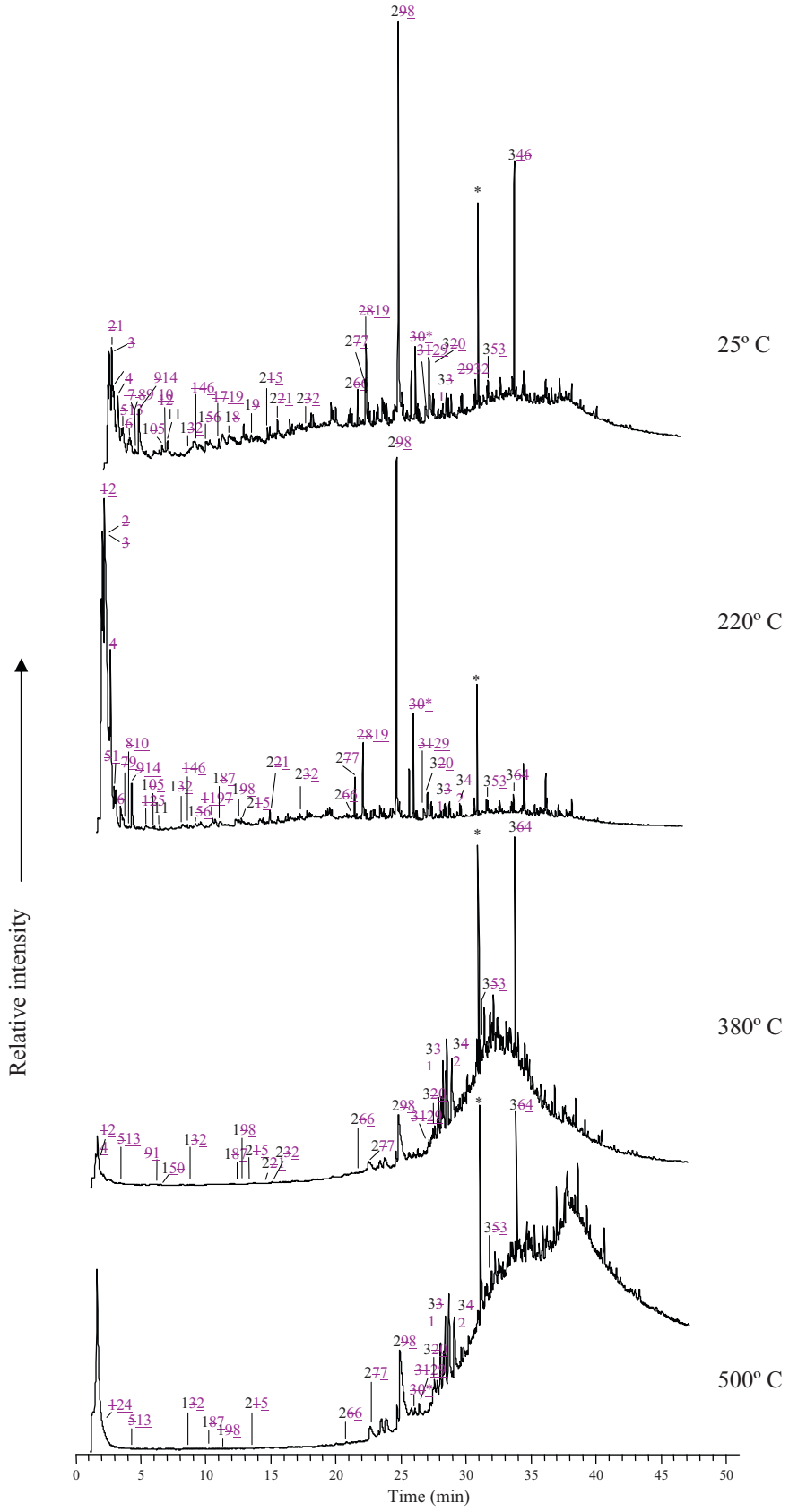

Fig. 5. Pyrolysis-GC trace of the organic matter extracted from microaggregates in under *Quercus coccifera* soil at different temperatures.

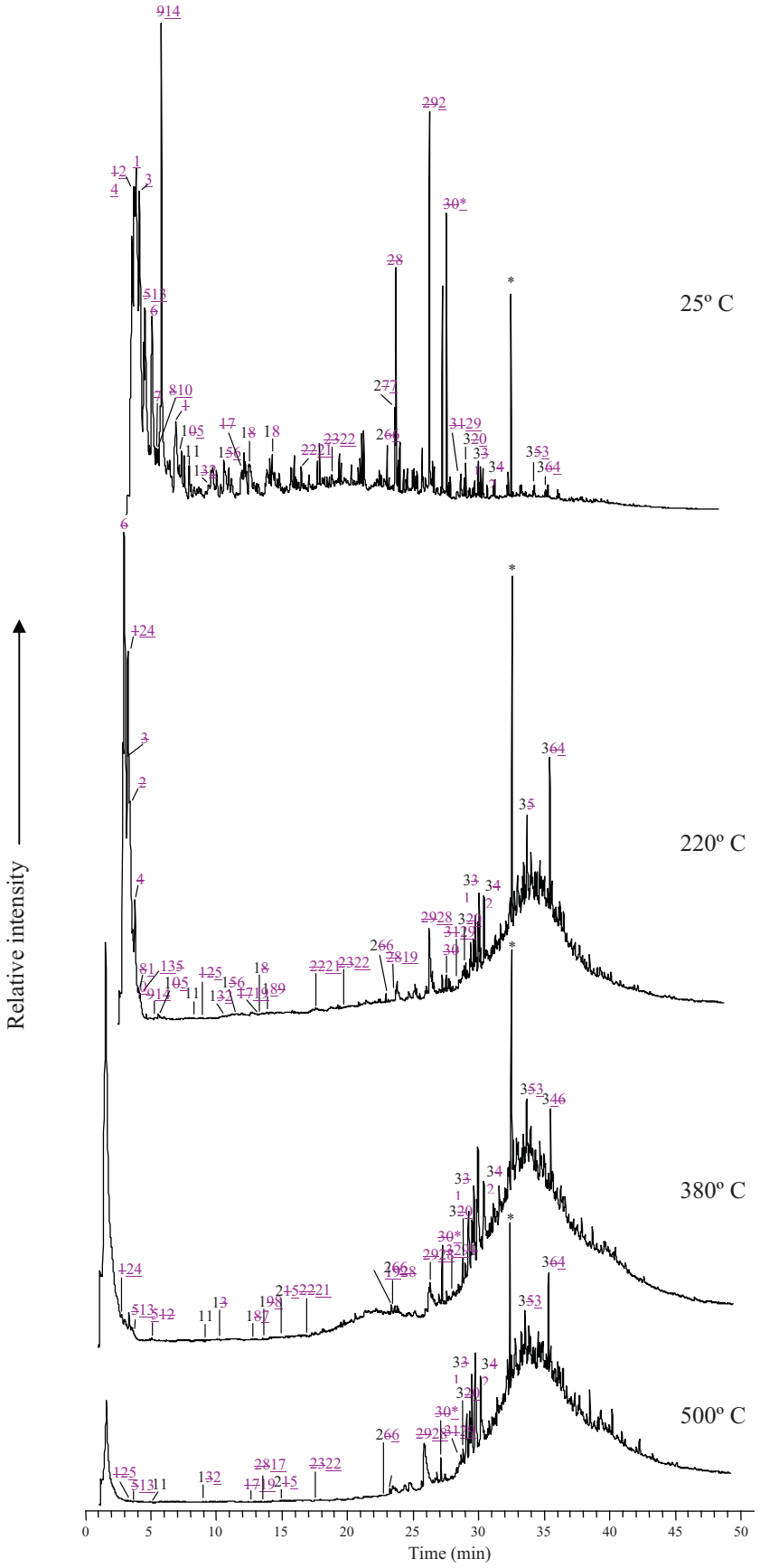

Fig. 6. Pyrolysis-GC trace of the organic matter extracted from macroaggregates in bare soil at different temperatures.

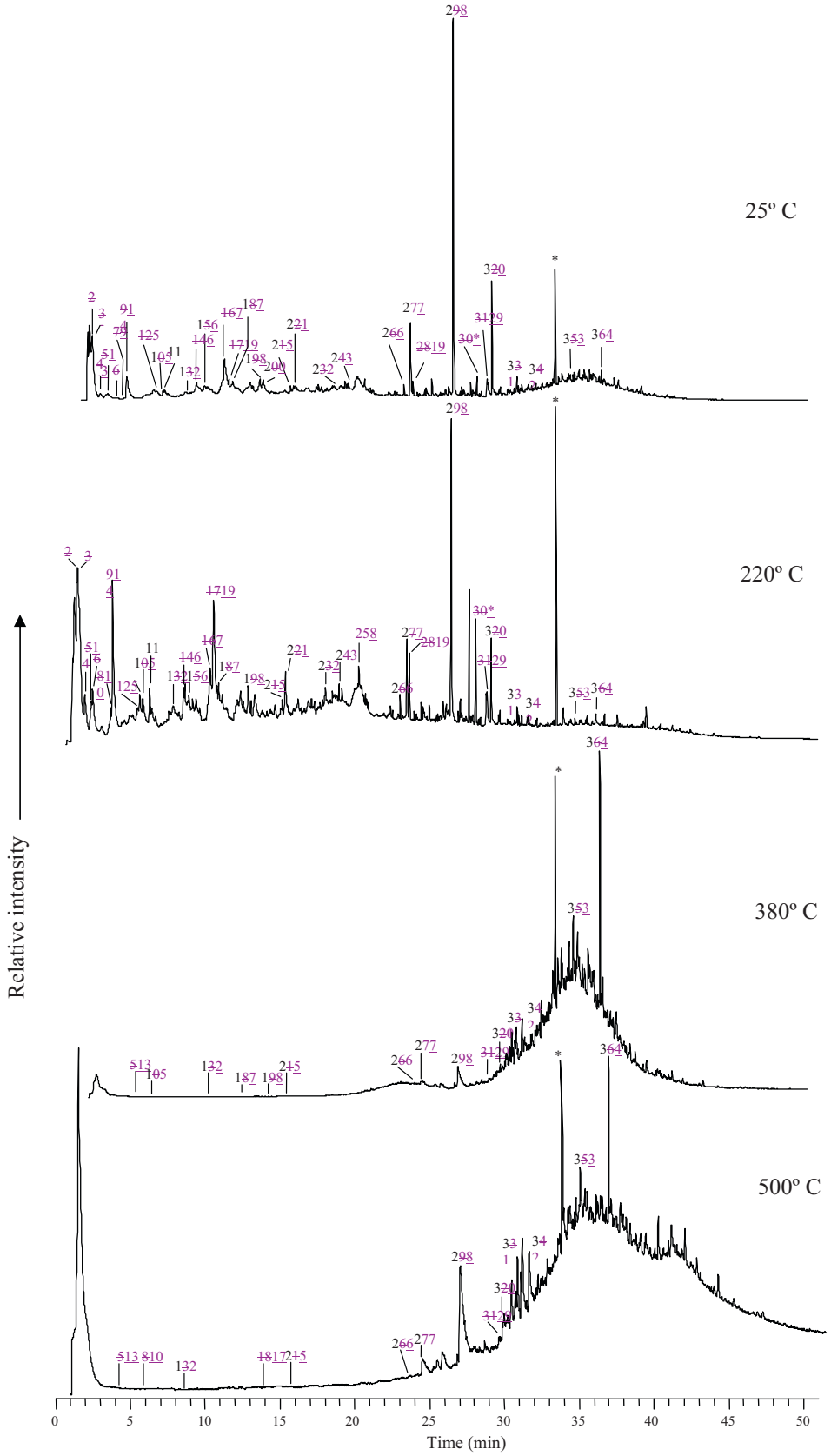

Fig. 7. Pyrolysis-GC trace of the organic matter extracted from microaggregates in bare soil at different temperatures.

The intensity of the signals of most of the N-containing compounds in the pyrolysates of macro- and microaggregates, from samples of both environments, were greatly reduced at 220 and 500 °C compared to those obtained from control samples. Their presence at these high temperatures could be associated to the resistance of aromatic components to heat or to the fact that this organic material is recalcitrant and has become non-pyrolysable during analytical pyrolysis under standard laboratory conditions [37].

3.2.3. Black carbon (BC)

Pyrolysis-GC/MS may appear inadequate for the study of BC because an unknown fraction does not produce GC-amenable products upon pyrolysis [4,13], and because two thermal modifications that may be difficult to distinguish are involved (natural fire or laboratory heating initially and pyrolysis during analysis) [43]. Possibly for this reason, no polycyclic aromatic hydrocarbons (PAH's), that could be used as indicators of the wildfire occurred at the research area in 1978, were found in the studied samples.

The contribution to the pyrolysates of pyridine (10), benzene (13) and toluene (14) could be a first indication of the presence of BC in SOM [44]. However, these compounds are not true markers of BC (benzene and toluene can also be produced upon pyrolysis of lignin, tannin and proteins) but they are used in this study to suggest the likely presence of BC in both environments and aggregate classes, product of past fires in the zone.

3.2.4. Lignin

In the pyrolysates of non-heated samples (25 °C), the presence of some guaiacols with intact C_3 chains (compound 22) in both environments and fractions suggests that lignin did not undergo complete degradation in the form of side-chain oxidation prior to thermal treatment.

Products derived from lignin in the pyrolysates of the control samples in both environments revealed guaiacols and phenolic compounds in their composition. Since lignin from angiosperms, such as *Quercus coccifera*, contains syringyl lignin next to guaiacyl–lignin, and in its pyrolysates no syringols were detected, it may be due to contributions from other plants. The similar lignin signature in both soils may either signify syringyl–lignin complete degradation (e.g. demethoxylation producing phenols from guaiacols) yielding a similar residue or some kind of redistribution of the organic inputs related with runoff processes or even, lignin remnants of an earlier canopy (like grasses) over bare soil. It could also be attributed to root (decayed) systems of other plant species. Despite UC samples contained more SOM than BS samples; their lignin composition seemed to be very similar.

Degradation of lignin products can explain the lack of pyrolysates in the macro- and microaggregate fractions in both environments, heated over 220 °C, since beyond this temperature the lignin macromolecule can be altered to structures with decreasing number of methoxyl groups [37,39].

3.2.5. Lipids

In control soils, only the C_{16} alkanoic acid seemed to present a significant amount in the pyrolysates of macro and microaggregates in both environments. Other lipids were only present in relatively low amounts, probably because only extracts were analysed and lipids are generally poorly extractable with a NaOH solution [45].

Apart from this, the fact that *n*-alkanes and *n*-alkenes appeared to be limited in the studied soils prior to the thermal treatment suggests that residual accumulation of such compounds through preferential degradation of substances, such as polysaccharides, could have not occurred. It is hypothesized that high temperature and low soil moisture characteristics of Mediterranean areas, in some cases, could inhibit the microbial activity responsible for degrading polysaccharides. In fact, in areas with non favourable SOM decomposition conditions, aromatics and short and mid-chain aliphatics can accumulate in the soil [16].

Additionally, in some world's areas where extreme environmental conditions alternate through the year, like in Mediterranean regions, biological activity is not especially favourable to humification processes but more to intense mineralization [4]. Under such conditions, the relative importance of a-biotic constraints such as high temperatures produced by fires, irreversible dehydration further favoured by intense solar radiation and drastic drying cycles, are becoming important factors in the formation of stable organic matter in soil.

Most of the lipid derived compounds such as alkanols (26), alkanoic acids (28 and 30), *n*-alkanes and *n*-alkenes (25, 31–34) were detectable in all the pyrolysates irrespective of environments, aggregate fractions and heat treatments, implying a high resistance of these compounds to the thermal treatment [46].

4. Conclusions

The SOM extracted from both aggregate fractions in the control soils (no heat treatment), under canopy and bare, exhibits a rather similar quality but different quantity. The organic matter content was higher in the UC than in the BS, as well as in the microaggregates than in the macroaggregates. Natural homogenisation and mixing of the organic matter inputs in both environments or the possibility that parts of the SOM were not extracted by the applied method (the extracted fraction may not provide information about the SOM that is strongly bound to the mineral fraction) may explain the scarce differences on SOM quality.

In both aggregate fractions of control soils, the dominant constituents of the SOM comprised an array of polyphenols and polysaccharides with some amounts of compounds derived from a mixture of different plant groups, such as oxidised lignin and alkanols. Indications of wildfire occurrence in the study zone were found in the pyrolysates of the control soils (i.e. benzene, toluene, other methylbenzenes and pyridine). However, no polycyclic aromatic hydrocarbons, which could confirm this, were detected in this research.

The capacity of this Mediterranean soil to store considerable amounts of SOM may be related to a relative low decomposition rate of plant-derived material, generally the main source of SOM, rather than a SOM production *in situ*. Moreover, the low degree of oxidation observed for lignin and *n*-alkanes (*n*-2-alkanones were absent) suggest that these plant constituents, likely to be present in the UC and BS samples, are well preserved implying reduced SOM degradation in both soil environments.

With increasing temperatures SOM content decreased in both environments, as well as in both aggregate classes, suggesting that labile OM was likely destroyed by the higher temperatures applied. The decreasing proportion of pyrolysed material detected at 500 °C could be partly due to selective enrichment of heat-resistant aromatic components or to the presence of charred "non-pyrolysable" refractory carbonaceous material.

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