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The pyrolytical fingerprint of nitrogen compounds reflects the content and quality of soil organic carbon

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ABSTRACT

The increasing land degradation is a problem that affects many soils in countries with a Mediterranean climate. In this aspect the soil organic matter (SOM) plays an important role, due to its progressive biodegradation parallels to desertification and the concomitant emissions of CO_2 to the atmosphere. These facts make basic research on the structure and composition of SOM important for soil conservation. Organic *N*-compounds in soil are of particular interest due to their chemical structure and speciation status in the SOM which can play an important role in soil N bioavailability and in the whole biogeochemical activity of the soil. For this reason, studying the possible relationships between the different *N*-compounds and soil properties, such as SOM content and its chemical characteristics, can provide new information on the stabilization and storage of organic C in soil. For this research, 30 soils from Spanish ecosystems with a wide range of SOM content were selected. The molecular composition of SOM in whole soil samples including *N*-compounds, was analyzed by analytical pyrolysis (Py-GC/MS). A parallel characterization of SOM quality was carried out using solid state ^{13}C NMR and UV–vis spectroscopy. Based on their chemical structure, the *N*-compounds identified by Py-GC/MS were classified into seven main groups: indoles, pyridines, pyrazoles, benzonitriles, imidazoles, pyrroles and quinolines. Multivariate statistical analyses were used to explore the relationship between the distribution of the above compounds and the SOM content. A significant predictive model was obtained for the SOM using partial least squares (PLS) regression, which was used to predict SOM content using the pyrolytic *N*-compounds as descriptors. This would show that there is a relationship between the patterns of *N*-compounds and the biogeochemical mechanisms involved in the different C storage levels the soils. Also, multidimensional scaling (MDS) and principal components analysis (PCA) showed to what extent the individual *N*-compounds are informative of status and quality of the humic acid fraction of SOM. As a whole, the results obtained by Py-GC/MS suggest that indoles, alkylindoles, alkylbenzimidazoles and alkylpyridines could be indicators of SOM accumulation while unsubstituted benzonitrile and pyridine are related to SOM quality.

1. Introduction

There is a global concern to predict and control the effects of climatic variability on the soils. The organic matter present in soils is the largest carbon reservoir in Earth's surface and one of its most dynamic pools (Lal, 2004). This soil organic matter (SOM) plays an important role in the physical and chemical properties of the soil (pH, cation exchange capacity (CEC), hydrological properties, etc), with an outstanding role in soil fertility (Tiessen et al., 1994). Apart from this, the structure and composition of the SOM is highly responsive to environmental changes (Jiménez-González et al., 2020a; Parton et al., 1987). At present, there is great concern about soil degradation, especially in the Mediterranean area where the risk of desertification is more pronounced. Desertification is an important problem closely associated to the loss of SOM, which has generated great interest on the part of the research community to understand the dynamics of SOM, in particular the factors that influence

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its stability or resistance to biodegradation (Jiménez-González et al., 2020a, Solomon et al., 2007). The variable stability of SOM against biological degradation depends on different soils and is an important factor in defining SOM quality (Knicker, 2007; Savage et al., 2008). In this context, organo-mineral interactions have been invoked as a major factor in the stabilization of SOM (Grant et al., 2022; Schmidt et al., 2011), while other authors have highlighted the importance of the chemical structure of the SOM in its persistence in the soil (Jiménez-González et al., 2018; 2020b; Marschner et al., 2008).

Historically soil nitrogen levels have been considered an indicator of SOM quality (Rice et al., 1997; Schloter et al., 2003; Sikora and Stott, 1997). For this reason, many researchers focus their attention on practical studies on soil management techniques and optimizing the incorporation of nitrogen in stable forms in the SOM (Granada et al., 1991; Leinweber et al., 2009; Melero et al., 2011; Sharma et al. 2005; Van Eerd et al., 2014), but few of them focus their attention on studying the resulting molecular composition of the *N*-compounds in soil (Derenne and Quénéa, 2015; Jämtgård et al., 2010; Jiménez-González et al., 2020b; Leinweber et al., 2013). Despite the relatively small proportions of organic *N*-compounds in the soil system (Schulten et al., 1995; Schulten and Schnitzer, 1997), these structures play an essential role in soil biogeochemistry and fertility. Understanding the biogeochemical transformation of the SOM requires further studies on the role of the chemical structures of *N*-compounds. In particular, pyrolytic compounds can provide information on the origin of biomass constituents that are stabilized in the soil, corresponding to various compositional groups: protein derivatives, pyrogenic organic matter, amino sugars (Derenne and Quénéa, 2015; Song and Peng, 2009; Schulten and Schnitzer, 1997). Many studies focus their attention on the origin of these pyrolytic *N*-compounds (vegetation, microorganisms, black carbon…), but the possible relation or effect on the SOM quantity and quality remains little explored. Several techniques have been used to describe the molecular composition of the SOM, e.g., visible, infrared, nuclear magnetic resonance spectroscopy which can provide valid information about the *N*-domain in SOM structure. Analytical pyrolysis is particularly useful because it provides a characteristic *N*-fingerprint that allows differentiating the SOM according to its origin (Buurman et al., 2007; Jiménez-González et al., 2020a; Song and Peng, 2009).

The main objective of this study is to explore the possible relationships between the composition of *N*-compounds and the characteristics of the SOM accumulated in the corresponding ecosystems. For this purpose, the SOM of whole soil samples is studied using analytical pyrolysis (Py-GC/MS) to further analyse the information provided by the individual *N*-compounds and establish their value as surrogates of the different humification mechanisms that are reflected in the chemical structure of the HA fraction of SOM and the total content of the SOM.

2. Material and methods

2.1. Study area

Thirty soils from different ecosystems from Spain were selected for this study. The soils were classified according to the IUSS Working Group WRB (2014) system (Table 1). The soil samples used in this research were collected from the topsoil (0–10 cm) and composite samples were prepared by mixing soil material collected from three different sampling points. Finally, the soil samples were air-dried, homogenized and sieved to fine earth (< 2 mm) before analysis. The sampling sites, which show a wide range in soil organic carbon (SOC) content and quality, are described elsewhere (Jiménez-González et al., 2017, 2018, 2020).

2.2. Physical and chemical properties of soils

Soil texture was determined following the Bouyoucos (1927) method. Soil pH was measured in soil–water suspension (1:2.5), using a pH7 pH-meter (XS Instruments). The determination of the electrical conductivity (EC) was carried out in soil–water slurry (1:5) and a COND7 (XS instruments). The SOC content was determined by wet chemical oxidation (Walkley and Black, 1934) and the N by micro-Kjeldahl digestion (Prince, 1945). The CEC was measured with ammonium acetate according to Juo et al. (1976). A chemical fractionation of the SOM was carried out according to its solubility in alkaline and acid solutions. In a first step the free organic matter (FOM) was removed by flotation with *2 M* H_3PO_4 (d = 1.12 g·cm^{-3}). The extractable SOM was isolated using 0.1 *M* sodium pyrophosphate and 0.1 *M* sodium

Table 1
Classification and general characteristics of the soils.

Sample No.	Soil classification (World Reference Base for Soil Resources, 2014)	Soil texture	Vegetation
1	Dystric Cambisol (Humic)	Sandy loam	*Quercus pyrenaica*
2	Haplic Umbrisol (Hyperhumic)	Sandy loam	*Pinus sylvestris*
3	Calcaric Cambisol (Humic)	Silt loam	*Quercus ilex*
4	Gleyic Cambisol (Humic)	Sandy loam	*Fraxinus angustifolia*
5	Dystric Cambisol (Humic)	Sandy loam	*Paeonia coriacea,*
6	Dystric Cambisol (Ochric)	Sandy loam	*Quercus rotundifolia*
7	Leptic Kastanozems (Hyperhumic)	Clay loam	*Quercus rotundifolia*
8	Leptic Podzol (Arenic)	Sandy loam	*Fagus sylvatica*
9	Dystric Cambisol (Humic)	Sandy loam	*Pinus sylvestris*
10	Dystric Cambisol (Humic)	Loamy sand	*Pinus pinea*
11	Dystric Cambisol (Loamic)	Loamy sand	*Quercus rotundifolia*
12	Eutric Cambisol (Humic)	Sandy loam	*Juniperus oxycedrus*
13	Eutric Cambisol (Humic)	Sandy loam	*Juniperus oxycedrus*
14	Dystric Cambisol (Humic)	Sandy loam	*Pinus pinaster*
15	Dystric Cambisol (Humic)	Sandy clay loam	*Quercus pyrenaica*
16	Dystric Cambisol (Colluvic)	Sandy loam	*Pinus sylvestris*
17	Leptic Cambisol (Humic)	Loam	*Quercus ilex*
18	Leptic Umbrisol (Loamic)	Loam	Pastureland for grazing: *Brachypodium retusum, Lolium perenne, Trifolium repens*
19	Haplic Luvisol (Humic)	Silty clay loam	*Fagus sylvatica*
20	Eutric Cambisol (Humic)	Clay loam	Pastureland for grazing: *Brachypodium retusum, Cynosurus cristatus, Trifolium repens*
21	Haplic Umbrisol (Loamic)	Loam	*Pinus radiata*
22	Leptic Regosol (Humic)	Sandy loam	*Pinus pinaster*
23	Leptic Regosol (Humic)	Sandy loam	*Pinus pinaster*
24	Leptic Umbrisol (Hyperhumic)	Loam	*Pinus pinaster*
25	Dystric Regosol (Arenic)	Sand	*Pinus pinea*
26	Eutric Cambisol (Humic)	Sand	*Pinus pinea*
27	Eutric Cambisol (Arenic)	Sandy loam	*Quercus rotundifolia*
28	Eutric Cambisol (Humic)	Silt loam	*Quercus rotundifolia*
29	Eutric Cambisol (Humic)	Sandy loam	*Juniperus thurifera*
30	Dystric Leptosol (Humic)	Loam	*Juniperus thurifera*

hydroxide. From this total humic extract, humic acid (HA) and fulvic acid were separated by precipitation with 6 *M* HCl (Duchaufour and Jacquin, 1975). The chemical characterization of HA fraction, corresponding to the humic fraction with high degree of transformation, was used to compare the quality of SOM of the soil samples. The determination of the optical density was carried out by preparing HA solutions in 0.01 *M* NaOH with a concentration of 0.2 mg·cm^{-3} to record the visible spectra where the absorbance at 465 nm (E4) is selected. The selection of the classical index E4 is due to its correlation with the amount of aromatic carbon in the HA (Tinoco et al., 2015). This index is used to indirectly assess the stability or maturity of SOM, being related to important characteristics of the whole soil, such as the degree of alteration of lignin and other plant macromolecules, and the general biogeochemical activity of the soil (Kögel-Knabner and Rumpel, 2018). In fact, in the course of humification, biogenic precursors are transformed into complex substances with a chaotic structure and undefined chemical composition (Hayes and Swift, 2020; Piccolo, 2001) as well as a high content of stable free radicals that absorb light (Chen et al., 1977). The main HA structural groups were analysed using solid-state ^{13}C NMR spectroscopy (Bruker Avance III HD 400 MHz instrument), operating at a frequency of 100.64 MHz and using ZrO_2 rotors of 4 mm OD with Kel-F caps. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane (0 ppm). The spectra were quantified by subdividing them into the following chemical shift regions (González-Vila et al., 1983; Knicker, 2011): alkyl C (0–45 ppm); *N*-alkyl + OCH_3 (45–60 ppm); *O*-alkyl C (60–110 ppm); aromatic C (110–160 ppm); carbonyl C (mainly carboxyl + amide, 160–220 ppm).

2.3. Analytical pyrolysis

The identification of *N*-compounds in the SOM was carried out by Py-GC/MS after grinding soil samples to fine powder (< 0.01 mm). A low quantity (1–2 mg) of soil was pyrolyzed into a preheated micro-furnace at 500 °C for 1 min. For the analysis, a multi-shot pyrolyzer (Frontier Laboratories ltd., Fukushima, Japan, Mod. PY-2020iD) coupled to a gas chromatograph (Agilent 6890 N GC) was used. The GC oven temperature was set at 50 °C for 1 min, then increased to 100 °C at 30 °C·min^{-1}; from 100 to 300 °C to a rate of 10 °C·min^{-1}, and finally isothermal at 300 °C for 10 min, with a total analysis time of 32 min. The carrier gas used was helium at a flow rate of 1 cm^3·min^{-1}. The mass spectra were acquired using a quadrupole mass spectrometer (Agilent 5973 N MSD) at 70 eV ionizing energy. The NIST and Wiley digital libraries were used for compound assignment. The peaks areas in the chromatogram were integrated and expressed as % chromatogram area. Only clearly identified *N*-compounds present in all samples and were selected for this work.

2.4. Data analysis

Several multivariate data treatments were applied to study the relationship between total abundances of 14 *N*-compounds (independent variables) and soil properties including the SOC content as predicted (dependent) variable using the software Statgraphics Centurion XV and partial least squares (PLS) regression. To check for overfitting of the final model, the PLS study was repeated using a completely random permutation of SOC values as dependent variables (models were discarded as overfitting if some significant ($P < 0.05$) model is also obtained with any random dependent variable).

Also, the relationships between the 14 *N*-compounds and other soil and HA properties were examined by principal component analysis (PCA) using total abundances of the 14 *N*-compounds as active variables. A series of soil and HA properties were processed as supplementary variables, i.e., not used to calculate the ordination axes, but represented in the space according to the components calculated from the principal variables (Legendre and Legendre, 1998).

Finally, multidimensional scaling (MDS) (Kruskal, 1964) using Statistica software ver. 7.1 was used for simultaneous ordination of different soil properties and *N*-compounds distribution, illustrating their mutual relationships, using the 1-Pearson *r* correlation index as a measure of dissimilarity for the cluster analysis.

3. Results

Table 2 shows the general properties of the soils, which illustrate the large variability in SOC content (from 18 to 157 g · kg^{-1}) and the C/N ratio ranging between 8.9 and 31. The proportions corresponding to different functional groups were determined from the solid-state ^{13}C NMR spectra. The aromatic-C content in HAs ranged between 16.6 and 27.8 %. Observing the *O*-alkyl C type, the sample with the lowest content had a value of 18.6 % while the highest one reached 27.4 %. In the case of *N*-alkyl + OCH_3 groups, the proportion showed low variation (8.4–11.8 %). Finally, the alkyl C and the carbonyl region presented values ranging from 28.4 to 41.5 % and 9.4 to 14.6 %, respectively. A total of 14 *N*-compounds were clearly identified in all the samples: indole, 7-methyl-1*H*-indole, pyrazole, methylbenzonitrile, C2-alkylbenzimidazole, pyridine, methylpyridine I and II, dimethylpyridine, pyrrole, 1-methyl-1*H*-pyrrole, 3-methyl-1*H*-pyrrole, benzonitrile and quinoline. These *N*-compounds belong to seven principal chemical structures: indoles, pyridines, pyrazoles, benzonitriles, imidazoles, pyrroles and quinolines.

The PLS model obtained using total abundances of *N*-compounds as descriptors and including the first five PLS components led to predict the SOC content with a high significance level ($P < 0.01$). In Fig. 1a, it is possible to observe the cross validation plot where the observed values are represented *vs* the predicted values. The model was validated by comparison with possible alternative models computed from the fully randomized SOC values (different SOC values that do not correspond to the experimental value were assigned to each soil sample). In Fig. 1b it is also shown that this model with the randomized values had a poor correlation ($P > 0.05$). The standardized coefficients obtained by PLS for the *N*-compounds are shown in Fig. 1c. These coefficients obtained in the model generated for the SOC content showed positive values in the case of indole, 7-methyl-1*H*-indole, methylpyridine I and II, pyrazole, methylbenzonitrile and C2-alkylbenzimidazole. Other compounds showed negative standardized coefficients in the prediction of SOC: pyridine, dimethylpyridine, pyrrole, 1-methyl-1*H*-pyrrole, 3-methyl-1*H*-pyrrole, benzonitrile and quinoline.

The results of the PCA are shown in Fig. 2. The two first components calculated from the abundances of the *N*-compounds explains 63.8 % of the total inertia, 45.5 % (first component) and 18.3 % (second component). The properties of SOM are represented as supplementary variables (red vectors). The PCA shows two well-differentiated groups. The first group is formed by properties related with the quality or degree of transformation of the SOM, such as E4 or aromatic- and carbonyl-C content, which are projected close to benzonitrile, 1-methyl-1*H*-pyrrole, and pyridine. Observing the localization of SOC content, it is possible to detect a second group located in the opposite side of the group described above around the value of E4. In this second group, it is possible to observe how properties as the SOC content, *O*-alkyl C and (*N*-alkyl + OCH_3) content are located close other *N*-compounds: methylpyridine I, methylpyridine II, quinoline, dimethylpyridine, C2-alkylbenzimidazole, methylbenzonitrile, indole, 7-methyl-1*H*-indole, and pyrrole.

Fig. 3 shows the scatter diagram obtained after MDS in which the variables (represented by green circles in the case of soil properties and by orange squares for the *N*-compounds) are arranged according to the correlations between them, i.e., the variables located closely are more correlated. The variables that showed a highly significant correlation with the SOC content (or the E4) are encircled with dotted lines ($P < 0.01$) and dashed lines ($P < 0.05$). The "stress" level in the diagram was 0.1587, indicating excellent reliability of the scatterplot (Kruskal, 1964).

Table 2
Soil physical and chemical properties.

Sample No.	SOC[a] (g·kg^{-1})	C/N	pH	WHC[b] (g·kg^{-1})	EC[c] (dS·m^{-1})	CEC[d] (cmol$_c$·kg^{-1})	FOM[e] (g C · 100 C g^{-1} soil)	FA[f] (g C · 100 C g^{-1} soil)	HA[g] (g C · 100 C g^{-1} soil)	Humin (g C · 100 C g^{-1} soil)	E4[h] (AU)	^{13}C NMR HA arom. C (%)	^{13}C NMR HA *O*-alkyl C (%)	^{13}C NMR HA *N*-alkyl + OCH_3 C (%)	^{13}C NMR HA alkyl C (%)	^{13}C NMR HA carbonyl C (%)
1	41	11.3	5.2	619	0.488	16.4	3.5	9.9	46.0	35.4	0.78	18.9	27.4	10.8	28.4	14.6
2	67	14.8	3.9	652	0.385	24.4	6.6	10.8	47.1	28.3	1.14	27.8	18.7	8.4	32.7	12.5
3	96	15.3	7.2	814	1.143	30.8	3.4	11.0	31.5	49.7	0.66	21.9	24.2	11.3	31.4	11.3
4	87	13.3	6.2	894	0.747	22.0	6.9	15.6	35.4	38.2	0.38	16.8	24.8	11.7	35.4	11.3
5	48	13.1	5.3	657	0.418	14.4	8.1	9.3	38.3	40.5	0.51	19.1	24.8	11.8	31.6	12.7
6	18	16.0	5.2	428	0.290	4.5	3.9	12.3	28.0	49.8	0.68	18.1	21.0	9.7	41.1	10.1
7	87	13.3	6.8	833	1.326	21.5	5.4	5.5	37.0	46.4	0.86	22.1	22.2	9.9	32.9	12.9
8	32	16.4	5.7	511	0.490	11.6	5.2	16.0	28.2	42.9	0.78	18.1	21.4	10.4	37.2	12.9
9	140	18.1	5.1	1122	0.549	14.2	13.8	8.0	26.5	49.1	0.71	24.1	23.1	9.6	32.4	10.9
10	117	26.7	4.9	842	0.499	13.9	23.7	5.0	13.0	56.7	0.59	22.4	25.2	11.0	31.0	10.5
11	93	8.9	6.4	851	0.693	20.1	5.1	12.6	35.7	44.1	0.53	16.6	26.6	10.7	33.2	12.8
12	134	12.1	7.0	1273	1.356	19.8	14.0	10.0	39.3	31.8	0.51	18.1	24.6	9.9	36.0	11.5
13	104	18.5	6.5	1007	0.786	22.2	16.3	8.1	36.3	36.6	0.61	21.0	26.3	9.4	31.0	12.2
14	81	16.9	6.0	910	0.552	17.1	14.6	13.5	38.9	30.0	0.48	19.2	25.2	10.2	34.8	10.6
15	55	18.0	5.7	676	0.309	15.9	10.8	11.9	30.3	44.0	0.55	17.4	25.7	10.2	36.1	10.7
16	39	13.0	5.6	480	0.186	13.8	8.8	14.0	47.0	24.8	0.80	18.9	25.3	10.5	33.9	11.5
17	105	17.0	7.2	1206	0.767	41.9	8.5	9.1	36.6	42.8	0.51	18.6	24.7	11.8	32.3	12.7
18	41	15.8	4.6	624	0.381	15.8	4.7	10.7	46.8	29.9	0.95	22.6	20.1	8.9	37.0	11.4
19	44	13.4	5.1	704	0.491	11.3	9.1	15.1	34.0	34.2	0.61	18.2	25.7	11.2	33.8	11.1
20	57	13.9	6.9	943	0.645	32.8	7.0	17.5	48.7	21.1	0.66	19.1	26.5	10.3	31.3	12.8
21	27	17.0	4.2	613	0.324	13.3	6.0	12.4	38.0	32.1	0.95	20.6	18.6	8.8	41.5	10.5
22	133	31.0	3.5	1124	0.485	32.4	15.0	12.0	43.3	25.3	1.18	26.4	20.2	8.8	32.0	12.7
23	90	20.0	3.7	783	0.361	22.6	7.5	11.3	48.8	26.9	0.76	21.7	22.3	8.9	35.0	12.0
24	132	18.0	4.2	1420	0.527	31.7	8.9	11.6	39.1	31.9	0.76	21.9	26.0	10.3	29.7	12.1
25	35	20.0	5.7	467	0.327	4.9	17.4	12.6	21.3	45.5	0.66	18.4	21.4	10.5	39.5	10.3
26	99	25.9	5.7	991	0.561	9.3	23.4	7.1	10.1	56.6	0.58	20.8	22.4	10.2	36.8	9.8
27	46	16.8	5.4	567	0.376	12.8	5.1	9.3	44.7	37.2	1.13	25.0	21.3	8.7	31.5	13.6
28	89	23.7	6.2	971	0.759	17.2	23.5	11.8	18.0	41.6	0.64	20.3	24.1	10.3	35.4	9.8
29	157	21.6	6.6	1197	1.087	18.0	24.9	6.3	12.1	54.4	0.46	18.8	23.7	10.9	37.3	9.4
30	92	13.9	7.4	743	0.820	25.9	3.4	13.9	37.5	40.3	0.62	17.8	25.0	11.0	34.8	11.5

[a]SOC: soil organic carbon, [b]WHC: water holding capacity, [c]EC: electrical conductivity, [d]CEC: cation exchange capacity, [e]FOM: free organic matter, [f]FA: fulvic acid, [g]HA: humic acid, [h]E4: optical density of HA at 465 nm.

Soil properties such as water holding capacity (WHC) and FOM are located near the SOC content. Some compounds showed significant correlation with the SOC content: indole, 7-methyl-1*H*-indole, methylpyridine I, methylpyridine II, dimethylpyridine, methylbenzonitrile and C2-alkylbenzimidazole. On the other hand, two compounds are located close E4, benzonitrile and pyridine. Some chemical properties of the HA (aromatic C and carbonyl C content) composition showed a significant correlation with the E4.

The results of these statistical treatments suggest that six *N*-compounds are closely related to the SOC content (indole, 7-methyl-1*H*-indole, methylpyridine I, methylpyridine II, methylbenzonitrile, C2-alkylbenzimidazole), whereas mainly-two *N*-compounds have shown a relationship with the degree of transformation of SOM (expressed by the value of E4).

4. Discussion

The possibility of obtaining a good prediction model for the SOC content exclusively using the information corresponding to the abundances of *N*-compounds showed that there is a connection between the pyrolytic signature of *N*-compounds in soil and SOC accumulation.

The results of the PCA showed two marked groups defined by properties like the E4 and the SOC content. Properties as the aromatic C and carbonyl C region of the ^{13}C NMR spectra of HA lie in the factorial plane near E4, showing that the levels of these properties parallel HA fraction quality, defined here in terms of the classic E4 value. This E4 has been considered an integrating variable the values of which increase with the progress of humification, i.e., the formation of dark-coloured SOM (Kononova, 1982), which has been previously associated with the aromaticity in HA (Tinoco et al., 2015) and resistance to biodegradation. This result agrees with the location in the factorial plante of aromatic and carbonyl groups of ^{13}C NMR analysis of the HA fraction. Both variables appear close to E4 and this fact confirms the important relationship between them (Tinoco et al., 2015). This location in the factorial plane of aromaticity and high oxidation degree (carbonyl groups) corroborate the high degree of transformation and resistance to degradation attributed to high values of E4. Three compounds are related with the E4: benzonitrile, 1-methyl-1*H*-pyrrole, and pyridine, behaving as molecular markers of aromaticity even though they do not necessarily originate from aromatic N moieties. In previous studies, compounds such as pyridine and benzonitrile were identified as black carbon markers or representative compounds in soils affected by wildfires (Campo et al., 2011; De la Rosa et al., 2008; Derenne and Quénéa, 2015; Kaal et al., 2008; Song and Peng, 2009). The Iberian Peninsula is an area highly affected by frequent wildfires (Vázquez de la Cueva, 2012), this fact can explain the presence of these compounds due to the estimate reservoirs of black carbon (Jiménez-González et al., 2021). In the case of SOC, the variables corresponding to NMR regions of *O*-alkyl C and *N*-alkyl + OCH_3 are close in the factorial plane to SOC due to these functional groups are predominant in fresh organic matter structures (low transformation degree). This fact would explain the presence of several *N*-compounds close to SOC: methylpyridine I and II, quinoline, dimethylpyridine, C2-alkylbenzimidazole, methylbenzonitrile, indole, 1*H*-indole, 7-methyl- and pyrrole, which are pyrolytic products of plant and microorganisms (Schulten et al., 1995; Schulten and Schnitzer, 1997) that are factors related with SOC levels.

The MDS analysis showed relationships between variables that follow trends similar to those found in the PCA analysis. We again find

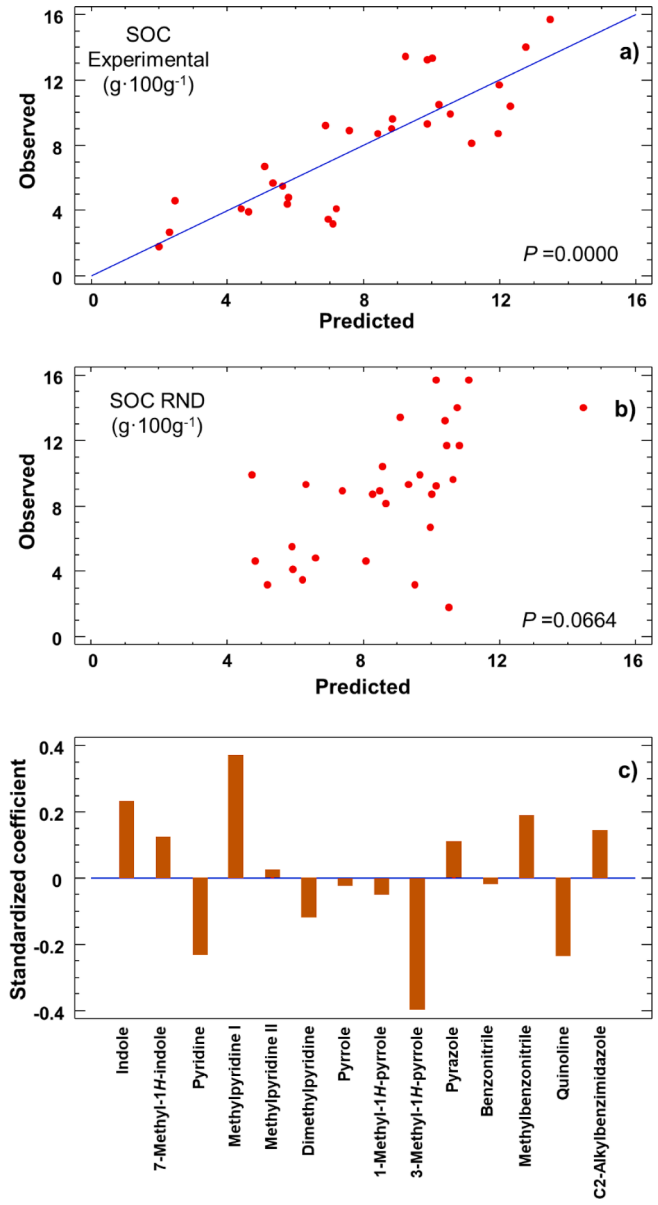

Fig. 1. Observed versus predicted values of soil organic C (SOC) content obtained by partial least squares regression (PLS) using pyrolytic *N*-compounds as descriptors to predict: a) experimental SOC values, b) randomized SOC values. Standardized coefficients of the *N*-compounds corresponding to the PLS forecasting model for the experimental SOC are represented in c).

variables as the regions of aromatic carbon and carbonyl groups of NMR correlated with the values of E4. This statistical treatment supports the idea of the degree of transformation and its correlation with E4, as was also observed in the PCA analysis. In this case, only pyridine and benzonitrile are located in the MDS plane close to E4, compounds that were also identified near E4 in the PCA analysis. This confirms the idea that these compounds can be molecular markers of transformation degree, as they have also been previously considered surrogates of pyrogenic aromatic material such as black carbon (Campo et al., 2011; Song and Peng, 2009). In the case of the second group (close to SOC) the FOM and the WHC are properties correlated with the SOC content. This fact suggests that SOM content strong depends on fresh organic matter supply and this amount of SOM plays an important role on the porosity and therefore on water retention of the soil. Methylpyridine I and II, dimethylpyridine, C2-alkylbenzimidazole, methylbenzonitrile, indole, 7-methyl-1*H*-indole, and pyrrole are the compounds which are located

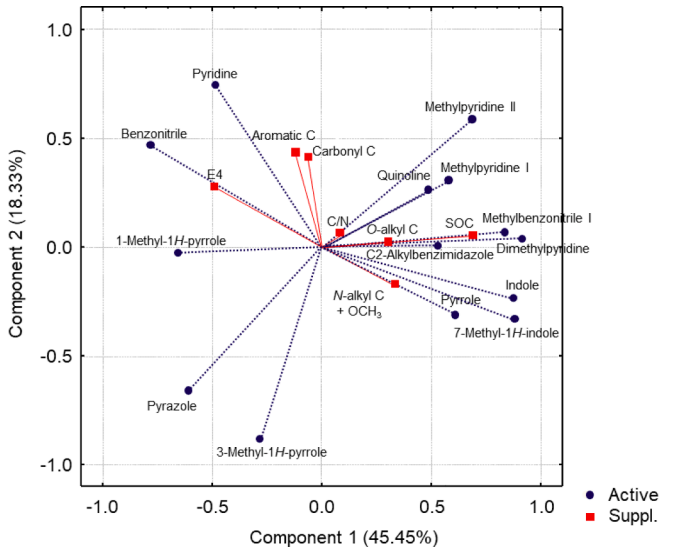

Fig. 2. Principal component analysis (PCA) plot with eigenvectors pointing to the direction of increasing values of the independent variables (*N*-compounds abundances, represented with blue dotted lines). Chemical properties of soil organic matter (SOM) (considered as dependent variables and processed as supplementary variables) are displayed with solid red lines. Variable labels refer to Table 2: soil organic C (SOC), optical density of HA at 465 nm (E4), different regions of ^{13}C NMR spectra (aromatic C, alkyl C, carbonyl C, *O*-alkyl C and *N*-alkyl + OCH_3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the MDS plane close to SOC content. These *N*-compounds also coincide with the compounds obtained in the PCA, and all of them are identified as pyrolytic products of plants and microorganisms (Schulten et al., 1995; Schulten and Schnitzer, 1997). For this reason, these *N*-compounds studied by analytical pyrolysis of bulk soil samples, can be a valuable source of information on the efficiency of C storage mechanisms in soil which remain mostly unknown. They may be indicators of transformation processes that are reflected in the final structure of the SOM, probably by the influence of plant biomass and microbial communities that determine its transformation in the soil.

5. Conclusions

The results show that a relation exists between the SOM molecular structure defined by the pyrolytic *N*-compounds and the amounts of C stored in the studied Spanish soils. This can be interpreted as a causal relationship between the SOM chemical composition and its resistance to biodegradation, or simply that information provided by the pyrolytic molecular assemblages reflects the processes and precursors that have influenced SOM accumulation, allowing an assessment of soil C sequestration. In any case, both hypotheses are not mutually exclusive. This coincides with the importance traditionally attributed to SOM maturity indexes involving N, such as the C/N ratio or the percentage of hydrolysable N, and illustrates the importance of the organic *N*-domain as a source of molecular descriptors of SOM resilience.

The SOC content tends to be related to indoles, alkylindoles, alkylbenzimidazoles, alkylpyridines, which are compatible with an origin from chitins and proteins in microbial or fungal biomass and plant biomass. On the other hand, soils with the HA fraction with higher aromaticity and maturity tends to be associated to an *N*-domain that includes unsubstituted aromatic and heterocyclic *N*-compounds, mainly benzonitrile and pyridine, compatible with proteins and photosynthetic plant pigments and even a pyrogenic origin, as may correspond to the effect of the frequent fires in the Iberian area.

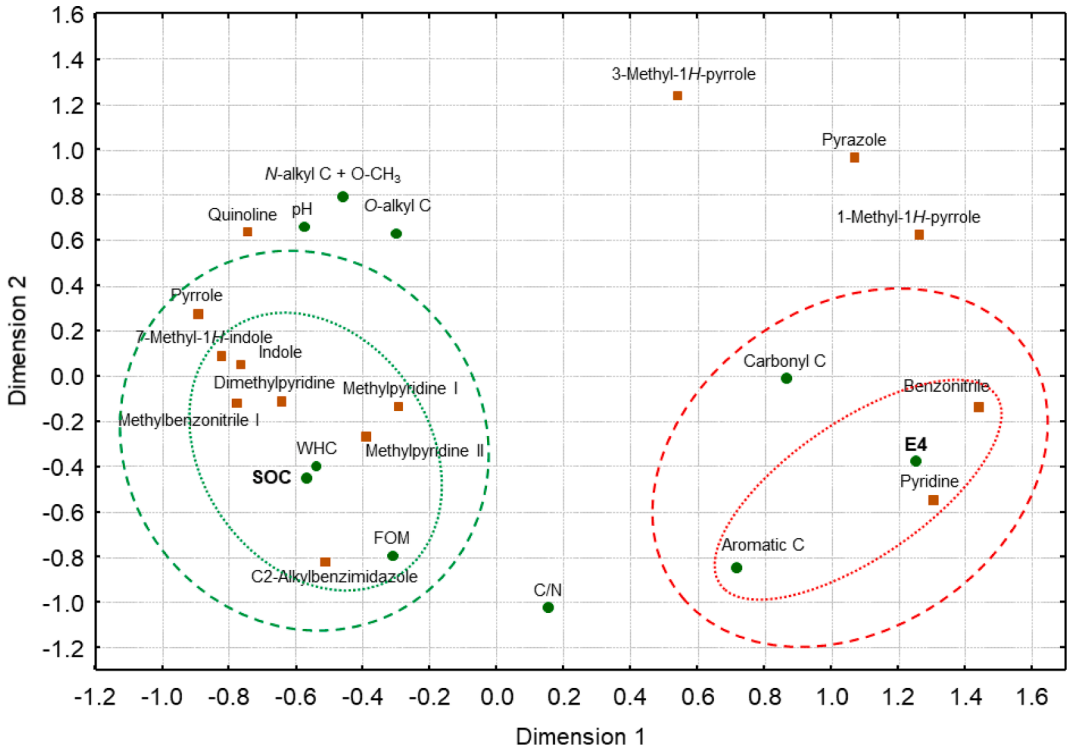

Fig. 3. Multidimensional scaling (MDS) analysis applied to show the relationships between different soil properties, quantity and quality descriptors of SOM. Concentric lines indicate the variables positively correlated with the SOC content at $P < 0.01$ (green dotted line) and at $P < 0.05$ (green dashed line), then variables positively correlated with E4 at $P < 0.01$ (red dotted line) and at $P < 0.05$ (red dashed line). Variable labels refer to Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marco Antonio Jiménez-González reports financial support was provided by Spanish Ministry of Economy and Competitiveness. Gonzalo Almendros reports financial support was provided by Spanish Ministry of Economy and Competitiveness.

Data availability

All data are described in the article and supplementary material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.116187.

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