Changes in soil organic matter molecular structure after five-years mimicking climate change scenarios in a Mediterranean savannah

Layla M. San-Emeterio [a,b], Nicasio T. Jiménez-Morillo [c], Ignacio M. Pérez-Ramos [a], María T. Domínguez [b], José A. González-Pérez [a,*]

[a] Instituto de Recursos Naturales y Agrobiología de Sevilla, Consejo Superior de Investigaciones Científicas (IRNAS-CSIC), Av. Reina Mercedes 10, 41012 Sevilla, Spain
[b] Universidad de Sevilla, MED Soil Res. Group, Dpt. Cristalografía, Mineralogía y Química Agrícola, Facultad de Química, C/Prof Garcia Gonzalez 1, 41012 Sevilla, Spain
[c] University of Évora, Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento (MED), Núcleo da Mitra, Ap. 94, 7006-554 Évora, Portugal

HIGHLIGHTS

- Mediterranean savannahs "dehesas" are threatened by climate change.
- A five-years field soil warming and drought experiment was evaluated.
- Py-GC/MS was used for assessing SOM changes and biomarkers identification.
- Specific changes in SOM molecular structure and functioning were detected.
- Climate change is expected to affect SOM composition and dynamics in the short term.

GRAPHICAL ABSTRACT
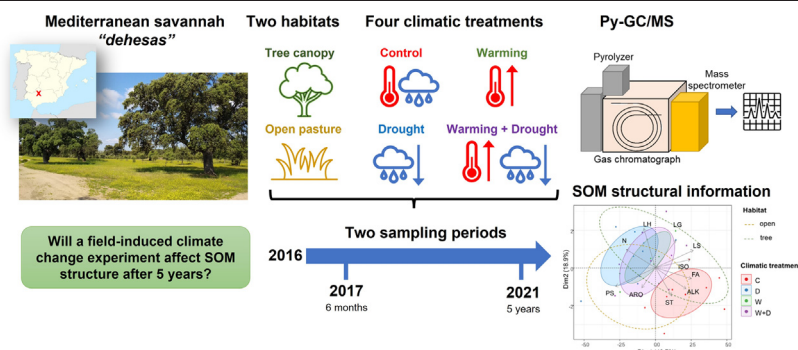
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ABSTRACT

Mediterranean savannahs (dehesas) are agro-sylvo-pastoral systems with a marked seasonality, with severe summer drought and favourable rainy spring and autumn. These conditions are forecasted to become more extreme due to the ongoing global climate change. Under such conditions, it is key to understand soil organic matter (SOM) dynamics at a molecular level. Here, analytical pyrolysis (Py-GC/MS) combined with chemometric statistical approaches was used for the molecular characterization of SOM in a five-years field manipulative experiment of single and combined rainfall exclusion (drought) and increased temperature (warming). The results indicate that SOM molecular composition in dehesas is mainly determined by the effect of the tree canopy. After only five years of the climatic experiment, the differences caused by the warming, drought and the combination of warming + drought forced climate scenarios became statistically significant with respect to the untreated controls, notably in the open pasture habitat. The climatic treatments mimicking foreseen climate changes affected mainly the lignocellulose dynamics, but also other SOM compounds (alkanes, fatty acids, isoprenoids and nitrogen compounds) pointing to accelerated humification processes and SOM degradation when soils are under warmer and dryer conditions. Therefore, it is expected that, in the short term, the foreseen climate change scenarios will exert changes in the Mediterranean savannah SOM molecular structure and in its dynamic.

* Corresponding author.
E-mail address: jag@irnase.csic.es (J.A. González-Pérez).

1. Introduction

Soil organic matter (SOM) plays an essential role in ecosystems dynamics: it influences a large set of soil chemical, physical and biological properties (Feng and Simpson, 2011), including soil structure, productivity, and erodibility among others (Cotrufo and Lavallee, 2022). These properties have an impact on vegetation, fauna, and ecosystem services such as carbon sequestration (Lal, 2007; Sanderman et al., 2017). Accounting for soils representing the second largest terrestrial carbon sink (Lal, 2004), carbon sequestration potential by soils is one of the cornerstones of future environmental policies in terms of climate change. Therefore, it is crucial to enhance our understanding about how climate change will modify carbon turnover rates in soils, especially in Mediterranean areas -an ecotone between temperate and tropical climate- where the sensitivity to these changes is expected to be high (Thuiller et al., 2006).

One of the most representatives Mediterranean ecosystems are Mediterranean-climate oak savannas, commonly known as "dehesas". *Dehesas* consist in typical agro-sylvo-pastoral systems, characterized by the scattered presence of oak trees (*Quercus ilex, Quercus suber*) without a regular pattern along with scattered pastures and crops. These ecosystems combine livestock, forest, and agricultural practices, with a continuous herbaceous cover (Moreno et al., 2016). This type of landscape covers about 3.5 million ha in Spain and are distinguished by their adaption to the unpredictability of the Mediterranean climate (Simón et al., 2013). The particularity of these ecosystems is expected to be reflected in SOM structure due to the induced effects of tree canopies, mitigating high radiation and temperature stress and favouring water availability (Gallardo, 2003). On the other hand, *Dehesas* are subjected to a marked seasonality that imposes severe summer droughts after favourable rainy autumns and springs, that is reflected in soil microbial dynamics (Aponte et al., 2010; Quilchano and Marañón, 2002). In addition, Mediterranean forests have large environmental heterogeneity (species composition, canopy structure, soil properties) that might affect microbial spatial patterns (Quilchano et al., 2008). The interplay of driving factors on the microbial dynamics - climate, vegetation, and soil - is key to understanding the biogeochemical cycles in Mediterranean forests. Changes in climatic and environmental conditions can affect both, plant biomass chemical and isotope composition that will eventually be reflected also in soil organic matter structure. Understanding SOM dynamic in this environment is important to forecast possible effects caused by climatic environmental changes and land use.

In 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 (González-Pérez et al., 2004). Under such conditions, the relative importance of abiotic constraints such as temperature warming, irreversible dehydration favoured by intense solar radiation and drastic drying cycles, are becoming important factors in the formation of stable organic matter in soil (Campo et al., 2011). Also, in areas with non-favourable SOM decomposition conditions, aromatics and short and mid-chain aliphatic compounds can accumulate in the soil (Vancampenhout et al., 2009).

Rising soil temperature may increase the decomposition of SOM, including recent plant-derived material or relatively stable pools (Ofiti et al., 2021). Also, warming may change topsoil microbial community composition and functioning. Thus, SOM including plant and microbial fractions in soils responses to warming, especially in Mediterranean soils, are key in future predictions of the carbon cycle (Bradford et al., 2016). The relevance of temperature is closely related to soil moisture and the point at which it may become a limiting factor, at least during a large part of the year. In semi-arid ecosystems, the limited water can suppress root and microbial activities (Ayangbenro and Babalola, 2021). Nevertheless, it has been difficult to estimate these changes in SOM properties due to its heterogeneity and pre-existing SOM stocks. Therefore, it is necessary to overcome these limitations with analytical approaches that allow us to follow semi-quantitative alterations in composition, sources and processes contributing to SOM dynamics.

Most studies have focused on the mechanisms and processes involved in the formation of SOM fractions, which should be closely related to the performance of C storage, which subsequently require time-consuming analytical procedures such as sample preparation, fractionation, extraction, etc. The SOM is quite heterogeneous, and its origin mainly relies on microbial transformation of plant biomacromolecules (biopolymers): i.e., polysaccharides (celluloses), polyphenols (lignin), polypeptides (proteins), polyesters (waxes). (Jiménez-González et al., 2017). In SOM characterization, analytical pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) is a powerful tool enabling a detailed separation, identification, and relative quantification of individual SOM constituents providing also detailed molecular structural information. In short, by using Py-GC/MS we are able to directly analyse the organic matter in soil or sediments by fractioning the biopolymers into smaller molecular fragments –representative to the large macromolecules– and made them amenable to chromatographic separation and identification (San-Emeterio et al., 2021; Soria et al., 2022). The technique has allowed the comparison of SOM produced under different environments and land uses (Buurman and Roscoe, 2011; da Silva Oliveira et al., 2016; Santana et al., 2015; Jiménez-González et al., 2020), elucidating the origin of SOM and in the identification of molecules that could be used as biomarkers compounds (Jiménez-Morillo et al., 2017; Kumar et al., 2019, 2020).

A known drawback of analytical pyrolysis when applied to soil and sediment analyses, is the possible influence of clay minerals that may act as catalysts of the pyrolytic rearrangement reactions (Faure et al., 2006; Bu et al., 2017). However, due to the low clay content in the analysed soils (<7 %), together with the experimental design and the way the molecular data is managed, minimizes the possible effects of this known pyrolysis artefact.

In this study, analytical pyrolysis (Py-GC/MS) is used to investigate changes in SOM composition under an evergreen oak Mediterranean savannah ecosystem (*dehesa*) with simulated warmer and drier treatments, mimicking possible future climatic scenarios. We hypothesized that future climate change scenarios, that include soil temperature increases and more severe drought conditions, would affect the SOM dynamics and the molecular composition of such agroforestry system.

2. Materials and methods

2.1. Site description

The study was carried out in an evergreen oak *dehesa* in Pozoblanco, Córdoba, southwestern Spain (38°20′47.8″N 4°48′57.0″W), located at an elevation of 675 m a.s.l. Climate in this area is dry Mediterranean-type with warm, dry summers and humid, cold winters. Mean annual temperature is 15.7 °C and mean annual rainfall is 439 mm yr^{-1}, most occurring from October to May (IFAPA meteorological station, Hinojosa del Duque; data from the 2017–2021 period).

The soil is classified as Eutric Cambisol (IUSS Working Group WRB, 2014), characterized by a sandy-loamy texture (74.2 % sand; 19.0 % lime; 6.9 % clay) and the absence of carbonates. Soil pH in the area ranged between 6.0 and 6.5, with no significant changes among the different experimental plots and habitats. Total C content was determined by dry combustion and varied from 1.6 to 3.5 % (Table S1). Significantly higher C content was always found under the tree canopy compared to the open pasture plots and no significant differences were observed during the course of the experiment among the climatic treatments but for treatment "drought" where C content was found significantly higher in 2021 under the tree canopy.

The vegetation is dominated by sclerophyllous evergreen oak (*Quercus ilex* L.) with a tree density of 14.5 ± 1.3 trees ha^{-1} and an herbaceous understory of annual native pasture dominated by *Hordeum murinum* (L.), *Senecio vulgaris* (L.), *Bromus madritensis* (L.) and *Sinapis alba* (L.) (Matías et al., 2021).

2.2. Experimental design and soil sampling

The experimental design was a full factorial field experiment started in 2016 during autumn, where the main factors were canopy cover and

climate scenarios. The effect of canopy, designated hereafter as 'habitat' was under *Quercus ilex* canopy (*tree, T*) and open grasslands between trees (*open, O*). Four permanent plots were selected at each habitat. In each habitat, four different climate scenarios were simulated: 1) *control* (C), without any manipulation; 2) *warming* (W), using methacrylate open top chambers to force an increase of 2 °C based on climate forecasting models according to the Intergovernmental Panel on Climate Change (IPCC, 2013); 3) *drought* (D), using rainfall-exclusion shelters covering 30 % of the surface based on the same IPCC scenario; and 4) a combined *warming and drought* (W + D). In total 32 experimental units (2 canopy covers x 4 climatic scenarios x 4 replicates) were sampled.

A first sampling was conducted in 2017 late spring and a second sampling in summer 2021. In both cases, the 10 uppermost cm of soil were extracted with an auger, five samples per experimental unit and combined in a composite sample. The resulting 64 samples were air-dried, sieved to fine earth (2 mm) and stored at room temperature. Prior to any analysis, all samples were ground to fine powder and homogenized using an agate mortar.

2.3. Analytical pyrolysis (Py-GC/MS)

Direct pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS) analysis was performed as described in San-Emeterio et al. (2021), using a double-shot pyrolizer (model 2020i; Frontier Laboratories Ltd., Fukushima, Japan) attached to a GC Agilent 6890 N (Agilent Technologies, Santa Clara, CA, USA) and an Agilent 5973 mass selective detector.

Soil samples were weighted (15 mg) in small ultra-alloy deactivated pyrolysis capsules and pyrolized into a preheated micro-furnace at 400 °C for 1 min. The evolved gases were directly transferred into the GC/MS for analysis. The gas chromatograph was equipped with a low polar-fused silica (5 % phenyl-methylpolysiloxane) capillary column Agilent J&W HP-5 ms Ultra Inert, of 30 m × 250 μm × 0.25 μm film thickness. The oven temperature was held at 50 °C for 1 min and then increased to 100 °C at 20 °C min^{-1}, from 100 °C to 300 °C at 10 °C min^{-1} and stabilised at 300 °C for the last 10 min. The carrier gas used was He at a controlled flow of 1 mL min^{-1} and the total chromatographic time was 32,50 min. The mass spectra were acquired in the scan mode with positive IE at 70 eV. To ensure a clean system, to evaluate contamination and that no carryover occurred from successive samples, method blanks using dichloromethane and hexane were included every three samples.

The identification and relative quantification of the pyrolysis products was carried out by the following features: 1) selecting traces in ion chromatograms for major compound series; 2) comparing with published spectra reported in the literature for lignin and plant material (Ralph and Hatfield, 1991) and 3) with comparison with stored commercial mass spectra NIST and Wiley libraries.

For comparison among samples, a semi-quantification analysis based on the peak area of each product released by pyrolysis was done using the total ion chromatogram (TIC). Minor compounds (area < 0.2 % of total chromatographic area) were excluded. For each soil sample, the peak area of each identified compound was calculated as a percentage of the total integrated area. The different compounds identified were then clustered in nine main biogenic groups: N-containing compounds (N); polysaccharide derived (PS); aromatics of unknown origin (ARO); lignin derived methoxyphenols (LIG: LH, LG, LS,); fatty acids (FA); aliphatic hydrocarbons, mainly alkane/alkene pairs (ALK); isoprenoids (ISO), sterols (ST) and unidentified compounds (UNK).

2.4. Biogeochemical proxies calculation

Several molecular proxies were applied to assess the source and degradation of organic matter in soil from the relative abundance of the aliphatic compounds (ALK) released directly after pyrolysis.

Hydrocarbon average chain length (ACL), was calculated for the long *n*-alkane range detected (C_{26-31}) as Eq. (2):

$$ACL = \sum (C_n * n)_{26-31} / \sum (C_n)_{26-31} \qquad (2)$$

where *n* is the number of carbons and C_n is the relative abundance of the respective alkane with *n* carbons. Microbial-derived or reworked organic matter is characterized by shorter ACL than fresh plant-derived organic matter. Therefore, ACL can be used as molecular proxy for the source and degradation of SOM (Gonzalez-Vila et al., 2003).

Chain length ratio (short/long) (S/L) is the relative abundance of short to long hydrocarbon chains and was calculated according to the following formula Eq. (3):

$$S/L = \sum (C_n)_{10-23} / (C_n)_{24-32} \qquad (3)$$

where *n* is the number of carbons and C_n is the relative abundance of the respective alkane with *n* carbons.

Fresh plant-derived organic matter is characterized by odd-over-ever dominance for *n*-alkanes C number. The carbon preference index (CPI) thus indicates input of plant-derived organic matter (high CPI > 10) or to which degree it has been degraded (values close to 1). Here, CPI index

Fig. 1. Example of a chromatogram obtained from the direct pyrolysis of a "dehesa" soil (15 mg) under the tree canopy in a control (C) plot. Labels on the peaks refer to compounds listed in Table S2. A detail of the selected ion chromatograms for alkane/alkene doublets (*m/z* 85), FA (*m/z* 73) and FAME (*m/z* 74) is also depicted.

was calculated considering the *n*-alkanes with 24 to 31 carbons as described in Jiménez-Morillo et al. (2016) as Eq. (4):

$$CPI = \left[\left(\sum C_{25-31}\, odd / \sum C_{24-32}\, even \right) + \left(\sum C_{25-31}\, odd / \sum C_{26-32}\, even \right) \right] / 2 \quad (4)$$

where [Cn odd] is the hydrocarbon abundance with odd-numbered C and [Cn even] is the hydrocarbon abundance with even numbered C chain.

2.5. Data analysis

Normality and homoscedasticity were checked prior to any statistical analysis using the Kolmogorov-Smirnov test and the Levene test, respectively. Data did not meet the normality or homogeneity requirements even after a log transformation was applied. and sn analysis of variance (ANOVA) was found not appropriate for the available data. Therefore, non-parametric tests were used for treatment comparisons i.e., the Kruskal-Wallis test along with a Dunn post-hoc test for multiple non-parametric comparisons, and the Mann-Whitney U test. These statistical analyses were made with a 95 % confidence level using the statistical package SPSS 20.0 (SPSS Inc., Chicago, USA).

Factor Analysis of Mixed Data (FAMD) was applied as a function of principal component analysis (PCA), where both quantitative and qualitative data are analysed (Lê et al., 2008). In this case, FAMD was used to assess the effect of categorical variables ("habitat" and "climatic treatments") on the average relative abundance of each family compound identified by pyrolysis. RStudio (version 2022.02.3) and "FactoMineR" and "ggplot2" packages were used for graphical representation of multivariate data.

3. Results and discussion

3.1. Analytical pyrolysis (Py-GC/MS)

The direct pyrolysis of soil samples at 400 °C resulted in well-resolved, chromatograms for all treatments and habitats (Fig. 1). A total of 117 pyrolysis products were identified in the total ion current chromatograms (TIC) (Table S1), the total abundances are summarized according to their biogenic origin in Table 1. As depicted in Fig. 1, the first 13 min of the chromatograms were dominated by carbohydrate-derived pyrolysis products (furan derivatives and levoglucosan), some aromatic compounds with unknown origin (phenol, benzopyranone, benzofuran, etc.), N-containing compounds (pyrroles and indoles), lignin-derived methoxyphenols (hydroxyphenyl —H—, guaiacyl -G- and syringyl —S— units). The second part of the chromatogram is composed mainly by series of alkyl doublets, fatty acids, and fatty acid alkyl esters (mainly methyl but also butyl esters). It is also noticeable a bimodal distribution in the alkyl-pair series as shown when m/z 85 is extracted (Fig. 1). Generally, carbohydrates (31 %), N-containing products (25 %), *n*-alkanes (9 %) and guayacil units from lignin (12 %) were dominant in all samples, accounting together for c. 75 % of the chromatographic area. This figure peaks c. 97 % when adding fatty acids and aromatic compounds of unknown origin.

3.1.1. Polysaccharides

Plant carbohydrates are primarily comprised of glucose from cellulose and hemicellulose being the primary source of energy in soil (Paul, 2016). It is noticeable the high abundance of polysaccharide derived compounds found in the soils, accounting for an approximate 30 % of the total compounds identified (Table 1). It is also noteworthy the presence of furfural, anhydrosugars (levoglucosan and glucopyranose), cyclopentenones and other furan derivatives that are all typical lignocellulose pyrolysis compounds (Cavallo et al., 2018). The relative abundance of these compounds was found significantly higher at the beginning of the experiment (29.9 ± 0.9 in 2017, 21.4 ± 1.1 in 2021; $p = 0.001$), where there were no statistical differences driven by habitat factor (Table 1). In 2021 it was still not observed any differences by habitat. Regarding the climatic treatments, there was no effect detected in the abundance of PS. Carbohydrate compounds in

Table 1
Total abundances of the pyrolytic products classified by compound families according to all environmental factors. Values are means ± SE (n = 4). Uppercase different letters indicate a significant difference of the indexes among years; lowercase letters indicate differences among habitats; "*" indicates differences among climate treatments (*p* < 0.05).

Year	Habitat	Climatic treatment	N	PS	ARO	LH	LG	LS	FA	ALK	ISO	ST	UNK
2017	TREE	C	22.6 ± 1.0	28.6 ± 2.7^B	4.3 ± 0.2^A	5.7 ± 0.4	12.8 ± 1.1^Ab	6.2 ± 1.0^Ab	7.7 ± 0.7^Ab	8.8 ± 0.9	1.2 ± 0.3^b	0.03 ± 0.01^A	0.86 ± 0.07
		W	23.3 ± 0.3	31.9 ± 1.6^B	4.7 ± 0.1^A	5.5 ± 0.2	12.8 ± 1.2^Ab	4.4 ± 0.6^Ab	6.3 ± 0.5^Ab	8.9 ± 1.0	0.75 ± 0.02^b	0.08 ± 0.05^A	0.59 ± 0.09
		D	24.0 ± 1.2	28.3 ± 1.4^B	4.3 ± 0.2^A	5.9 ± 0.7	12.0 ± 0.9^Ab	6.9 ± 0.6^Ab*	6.7 ± 1.0^Ab	9.0 ± 0.8	1.1 ± 0.1^b	0.13 ± 0.06^A	0.67 ± 0.07
		W + D	23.9 ± 2.2	27.4 ± 0.7^B	3.7 ± 0.5^A	5.3 ± 0.3	13.0 ± 0.8^Ab	5.8 ± 0.6^Ab	7.1 ± 1.0^Ab	10.6 ± 1.9	1.1 ± 0.2^b	0.04 ± 0.01^A	0.91 ± 0.18
	OPEN	C	23.7 ± 1.2	33.5 ± 2.5^B	4.6 ± 1.2^A	6.0 ± 0.5	9.6 ± 0.8^Aa	3.5 ± 0.1^Aa	4.6 ± 1.0^Aa	11.5 ± 2.6	0.65 ± 0.19^a	0.20 ± 0.02^A	0.61 ± 0.23
		W	24.0 ± 1.9	31.7 ± 5.0^B	5.3 ± 0.7^A	4.4 ± 0.8	9.4 ± 1.2^Aa	3.6 ± 0.6^Aa	6.1 ± 2.0^Aa	12.4 ± 2.7	0.66 ± 0.24^a	0.00 ± 0.00^a*	0.71 ± 0.28
		D	26.2 ± 1.3	26.9 ± 2.0^B	5.0 ± 1.6^A	4.9 ± 0.7	13.2 ± 1.6^Aa*	5.2 ± 0.4^Aa*	4.9 ± 1.3^Aa	10.7 ± 1.1	1.1 ± 0.3^a	0.00 ± 0.00^A	0.57 ± 0.17
		W + D	25.0 ± 1.8	30.9 ± 2.8^B	4.5 ± 0.5^A	6.6 ± 0.6	12.3 ± 1.1^Aa	4.4 ± 0.4^Aa	5.3 ± 0.8^Aa	8.6 ± 2.2	1.0 ± 0.3^a	0.11 ± 0.06^A	0.54 ± 0.13
2021	TREE	C	19.6 ± 0.4	16.3 ± 0.6^A	7.0 ± 1.1^B	5.9 ± 0.7	17.7 ± 1.8^B	9.5 ± 0.3^Bb	11.7 ± 1.0^B*	11.7 ± 1.8	1.7 ± 0.3^b	0.20 ± 0.11^B	1.1 ± 0.1
		W	22.3 ± 0.4	19.8 ± 1.9^A	5.1 ± 0.1^B	5.9 ± 0.0	16.5 ± 0.5^Bb	7.6 ± 0.6^Bb	10.6 ± 1.0^B	9.2 ± 0.4	0.87 ± 0.11^b	0.10 ± 0.06^B	0.78 ± 0.04
		D	24.7 ± 0.6	20.8 ± 2.2^A	5.8 ± 0.4^B	6.6 ± 0.6	16.0 ± 1.0^Ba	7.1 ± 0.7^Bb	7.9 ± 1.3^B	8.4 ± 1.3	0.87 ± 0.14^b	0.09 ± 0.03^B	0.85 ± 0.16
		W + D	22.4 ± 0.9	19.4 ± 1.2^A	5.4 ± 0.2^B	6.1 ± 1.5	17.3 ± 0.6^Bb	8.6 ± 0.8^Bb	8.5 ± 1.0^B	9.1 ± 0.9	1.1 ± 0.2^b	0.11 ± 0.04^B	0.84 ± 0.12
	OPEN	C	22.5 ± 0.2	21.7 ± 0.9^A	6.2 ± 0.8^B	4.1 ± 0.5	15.4 ± 0.6^Ba	6.8 ± 0.4^Ba	10.6 ± 0.9^B*	9.8 ± 0.3	0.72 ± 0.13^a	0.33 ± 0.15^B	0.77 ± 0.11
		W	24.8 ± 1.6	22.2 ± 1.7^A	7.0 ± 1.1^B	3.6 ± 0.4	15.9 ± 1.2^Ba	6.9 ± 0.9^Ba	8.8 ± 0.5^B	8.5 ± 0.3	0.60 ± 0.17^a	0.00 ± 0.00^B*	0.46 ± 0.11
		D	22.3 ± 0.9	26.8 ± 3.0^A	6.1 ± 0.7^B	4.7 ± 0.9	15.2 ± 1.0^Ba	5.9 ± 1.0^Ba	7.8 ± 1.0^B	8.7 ± 0.9	0.58 ± 0.14^a	0.04 ± 0.01^B	0.52 ± 0.18
		W + D	22.1 ± 0.7	24.2 ± 1.6^A	7.7 ± 0.5^B	4.4 ± 0.7	15.3 ± 0.8^Ba	6.3 ± 0.8^Ba	7.9 ± 1.1^B	9.6 ± 0.8	0.57 ± 0.13^a	0.09 ± 0.05^B	0.57 ± 0.19

Where N: nitrogen-containing compounds; PS: polysaccharides; ARO: aromatics from unknown origin; LH: hydroxyphenyl-derived lignin units; LG: guayacil-derived lignin units; LS: syringyl-derived lignin units; FA: fatty acids; ALK: *n*-alkane/alkene pairs; ISO: isoprenoids; ST: sterols; UNK: unknown compounds.

soils have been associated with microbial material (Barreto et al., 2021) and to microbial activity as fungal walls are composed of carbohydrates. However, the PS derived compounds released by a direct soil pyrolysis can be better assigned to the decomposition of plant lignocellulose. The diminished proportion of polysaccharides observed between 2017 and 2021 could be explained by a fast turnover of this PS labile carbon fraction resulting in a more biologically transformed material (Krull et al., 2003; Jiménez-Morillo, 2017).

3.1.2. N-containing compounds

The second most abundant group of compounds (c. 25 %) corresponded to N-containing compounds. These compounds are identified as pyrolysis products of organic matter, mainly released from peptides and proteins. Pyridine, methyl hydantoin, acetamidofuran, indoles and mainly diketopyperazines may have a protein/polypeptide origin (Fabbri et al., 2012). Also, previous studies point out that the presence of N heterocycles in soil e.j. pyrrols, have a probable pyrogenic origin during forest fires (Knicker et al., 2005). Several authors state that N-compounds in SOM are generally linked with a high microbial activity and a high degree of decomposition (Wild et al., 2014, 2016; Lozano-Guardiola, 2017). Here, the abundance of N compounds remained unchanged during the experiment, without significant differences exerted by habitat, or climatic treatments (Table 1).

3.1.3. Lignin methoxyphenols

Typical pyrolysis products from lignin moieties were abundant, including both guayacil (LG) and syringyl (LS) units and their methyl-, ethyl-, vinyl-, propenyl- and aceto- derivatives, in addition to hydroxyphenyl (LH)-lignin units (vinylphenol and phenol, x-methyl) derived mainly from grass lignins (Bahri et al., 2006; Vancampenhout et al., 2009).

The major peaks of lignin-derived compounds found were the LG units, the most abundant in proportion and higher abundance was found in 2021. Similarly, the effect of habitat led to a higher proportion under tree canopy than in the open pasture at both sampling times. Whereas at the beginning of the experiment there were no differences under tree canopy, in open pasture a higher relative abundance of LG units in "D" plots compared to control plots was found (Table 1). On the other hand, after five years of experiment we were unable to detect any effect driven by the climatic treatments for LG in any of the habitats. Consequently, regarding LG relative abundance in soil, it seems that the effect of the induced climate changes diminishes with time.

For the LS units, also higher abundance is observed in 2021 (Table 1). Again, the effect of habitat remained constant during the experiment, with higher relative abundance under the tree canopy. In 2017, under the tree canopy and in the open pasture, there was a higher proportion in "D" treatment compared to the "C" and "W". However, in 2021 there were no significant effect caused by the induced climate treatments, though an increase of LS units is detected in the control plots. Again, the effect of the induced climate factors seems to be diminished with time. This may indicate an adaptation of ligninolytic microbial populations to the new soil environmental conditions.

Lastly, LH units did not show any remarkable shift regarding any of the factors, though an increasing trend is also observed in "D" treatments.

To explain the ongoing processes of SOM transformation, specifically regarding lignocellulose dynamics, the lignin/carbohydrate (LG/PS) and guayacil/syringil ratios (LG/LS) were calculated (Fig. 2). Generally, the LG/PS ratio increased during the experiment period, along with more pronounced differences over time between habitats, which may indicate a preferential microbial degradation of carbohydrates (Rodriguez et al., 1997; Arias et al., 2005). In 2017, a significant higher LG/PS ratio was observed in "D" treatment in open habitat but no for 2021.Therefore, it can be drawn that preferential degradation is mainly driven by the time course of the experiment and partially influenced by the effect of canopy. This is also supported by the tendency observed for the LG/LS ratio a significant decrease of LG units is observed at expenses of the LS during the experiment. Higher LG/LS ratio was found in the open pasture than under the tree canopy. Moreover, a significant increase in this ratio was found in "W" plots, only under the tree canopy. Nevertheless, after five years of experiment, the effect of "W" treatment disappeared, although the effect of habitat remained constant and within the same trend. Such LG/LS increase under induced warming under the tree canopy may indicate preservation of lignin under such conditions.

3.1.4. Alkyl compounds

Plants are known to preferentially produce odd-numbered carbon long chain alkanes (Eglinton et al., 1962) and this odd-over-even C predominance was also observed in *dehesa* SOM. Generally, the *n*-alkane length distribution can be divided into three main distributions: C_{14} to C_{16} range (short length, indicating microbial sources); C_{17} to C_{24} range (medium chain length, indicating degradation products); C_{25} to C_{32} range (long chain length, epicuticular waxes) (Gonzalez-Vila et al., 2003; Wang et al., 2010). Accounting for this trimodal distribution, the *n*-alkane series peaks at C_{15}, C_{24} and C_{22} (in 2017 and 2021, respectively), and C_{29}.

We were unable to find significant differences in the ALK relative abundance along time although there were slightly higher in 2021 (Table 1). Therefore, several ALK proxies were analysed.

3.1.5. n-Alkane molecular proxies

These proxies were calculated to help estimating the effect of the habitat and induced climate factors in SOM. A summary of the calculated ALK proxies can be found in Fig. 3. In 2017, all indexes showed statistical differences: whereas S/L values were higher in open than under the tree, ACL and CPI indexes followed the opposite trend with higher values under the tree than in the open pasture.

ACL_{26-32} values ranged between 27.7 and 28.8. For 2017, ACL values did not show any significant differences with habitat or climatic treatments (Fig. 3a). However, lower values and more pronounced were observed for "W" and "D" treatments in the open pasture. This possibly suggests a varied source of *n*-alkanes, indicating a higher proportion of microbial-derived carbon in open grassland (Gonzalez-Vila et al., 2003). After 5 years of experiment (2021), a shift in the ACL trend is observed, now with lower values under the tree for the climatic treatments pointing to a possible
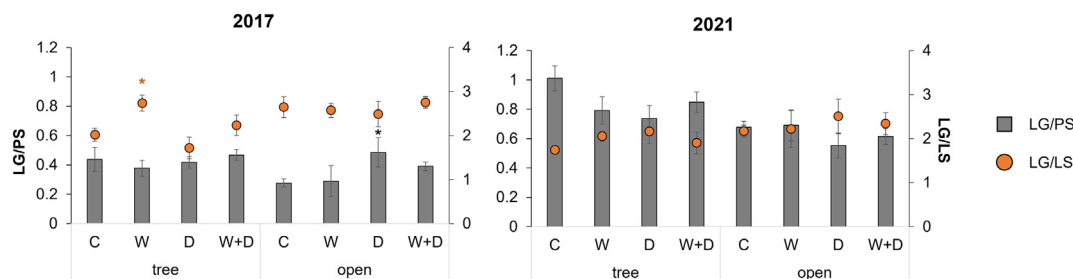

Fig. 2. Semiquantitative analysis of polysaccharides and lignin compounds (LG/PS and LG/LS ratios) released after Py- GC/MS. Values are means ± SE (n = 4). "*" indicates differences among climate treatments ($p < 0.05$) for that variable.
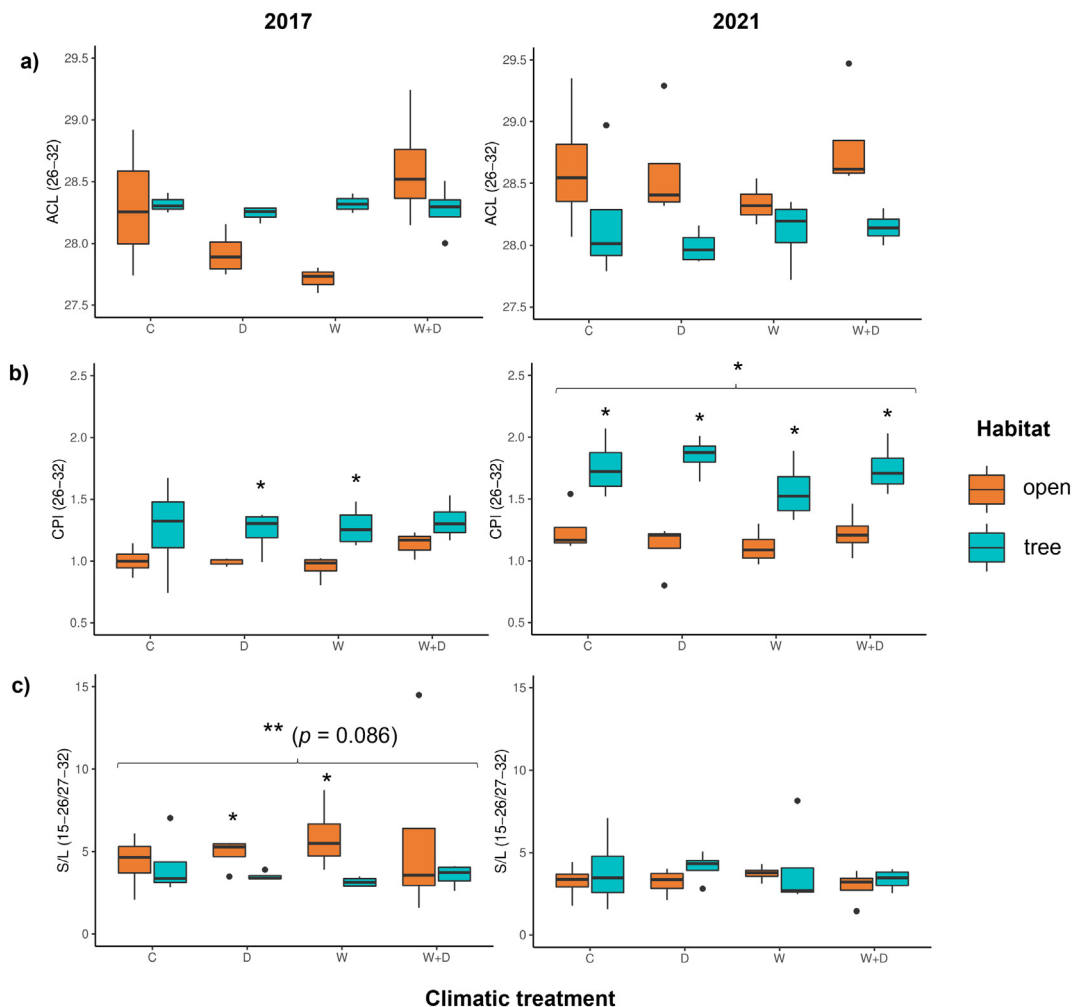
Fig. 3. Average chain length (ACL) of *n*-alkanes (a), carbon preference index (CPI) (b) and short-to-long chain index (S/L (15–26/27–32)) (c) in soil from all climatic plots and habitats (mean ± SE, n = 4). Significant effects are indicated with *; those in braces represent differences between years.

enhancement of the microbial activity exerted by the climatic treatments under the tree canopy.

The CPI$_{26-32}$ values ranged between 0.95 and 1.85 (Fig. 3b). The CPI index significantly differed between years, with lower values observed in 2017 (p = 0.001). Both in 2017 and 2021, CPI was higher under the tree than in the open pasture (p = 0.001). Under tree canopy and in open grassland, there were no effects detected due to the climatic treatments. However, there was an increase in 2021 where the differences given by the habitat increased, again higher in tree than in open habitat. There were no effects of the climate treatments for any of the habitats. Values above 1 of long-chain CPI index may indicate the presence of epicuticular waxes of terrestrial vascular plants and fresh plant biomass. Therefore, lower CPI values found in open habitat may indicate main inputs from microorganisms, leading to higher degraded, recycled organic matter and degradation of SOM (Jansen et al., 2019; Ofiti et al., 2021), that in our experiment is pronounced over time.

The S/L ratio ranged between 2.94 and 5.90 with slightly higher values in 2017 (4.4 ± 0.8 in 2017, 3.6 ± 0.3 in 2021; p = 0.086**) (Fig. 3c). Significant differences were observed with habitat, being higher in open (5.2 ± 0.4) than under the tree (3.6 ± 0.2; p = 0.010). Under the tree canopy, there were no effects of the climatic treatments for any of the two sampling periods. However, in 2021 the differences given by the habitat diminish for the ratio S/L (p = 0.669). Short chain *n*-alkanes in soils are usually considered from microbial origin (Jansen et al., 2008), hence a decrease in S/L index indicate a predominant influence of the vegetation, other way an increase points to the microbial mediated conversion from the vegetation's

primary *n*-alkane assemblages into shorter chain *n*-alkanes (Kuhn et al., 2010). Therefore, less pronounced differences between habitats after five years of experiment may be pointing to a selective preservation of *n*-alkanes over time.

3.1.6. Other compounds

A series of non-specific aromatic compounds were also found in the soil pyrolizates: phenol and alkylbenzenes (benzeneacetaldehyde; 1,2-benzenediol, 3-methoxy-). There was a significant higher proportion of aromatics in 2021 (4.5 ± 0.2 in 2017, 6.3 ± 0.3 in 2021; p = 0.001) (Table 1), but not appreciable changes were found linked with any of the environmental factors from the experimental trial. This may suggest transformation of part of the SOM over time as consequence of an accelerated humification process (Simonetti et al., 2012; Jiménez-González et al., 2019; San-Emeterio et al., 2021).

In terms of fatty acids, this group of compounds is of interest given its association with various microbial groups and their preservation may indicate differences in its biomass composition (Assis et al., 2011), being also an indicator of suberin from tree barks (Spielvogel et al., 2014). At the beginning of the experiment, the differences observed in the FA abundance seems to be driven by the habitat with a higher presence under the tree canopy. There is an increase in the abundance of fatty acids over time (6.1 ± 0.4 in 2017, 9.2 ± 0.5 in 2021; p = 0.000) and in 2021 the attributed difference to habitat disappeared, although the trend is the same as in 2017 being abundant under the tree canopy. In contrast, after 5 years of the experiment, a remarkable difference is observed in the climatic treatments,

with higher FA abundance in "C" plots compared to "D" and "W + D". This, together with the reduction of labile components like PS (LG/PS ratio increases), may be attributed to a depleted microbial activity due to the presence of comparatively more reworked SOM in the plots where soils were subjected to water scarcity and the combination of this and warming climatic factors. Therefore, FA occurrence, mainly attributed to the microbial activity, is somehow driven by the SOM quality present in the plots.

The presence of isoprenoids (cis-2-Methyl-7-octadecene; hexahydro-farnesyl acetone) remained constant although a slight decrease was observed with time (Table 1). These compounds are probably rest of pigments side chains, mainly chlorophyll, frequently found in humic substances (de la Rosa et al., 2022). A significantly higher ISO abundance was detected under the tree canopy (1.13 ± 0.19 under the tree canopy; 0.61 ± 0.03 in open pasture, $p = 0.000$). Finally, there were no effects detected due to the climatic treatments, though in both habitats there is a slight decrease in the "W" plots. Therefore, since the beginning of the experiment, ISO were exclusively affected by the habitat without any noteworthy impact caused by the climate except for a minor decrease observed under soils subjected to warming treatment.

Lastly, the sterol *stigmastan-3,5-dien* was found in the soil pyrolizates. This type of compound is one of the main phytosterols commonly present in plant oils and lignocellulosic material and a known dehydratation product of β-sitosterol (Piironen et al., 2000; Benveniste, 2004). Therefore, the presence of this ST in the pyrolysate is probably the result of a thermal degradation during the pyrolysis process. The abundance of this ST increased over time (0.07 ± 0.02 in 2017; 0.12 ± 0.04 in 2021, $p = 0.001$) though, regardless time, it is remarkable the absence of this compound in the "W" plots in the open habitat.

3.2. Multivariate analysis

The application of factor analysis of mixed data (FAMD) allowed to assess the effect of habitat and climatic treatments as qualitative variables, on the main biogenic compounds (UNK excluded). The resulting pattern was defined by a series of clusters which are shown encircled in Fig. 4. Detailed data on the contribution of each variable to the two dimensions retrieved from the FAMD analysis are listed in Table S2.

Focusing on 2017 data, a first component (Dim 1) is defined by major influences from LS, FA, and ISO compounds for the positive loading. The
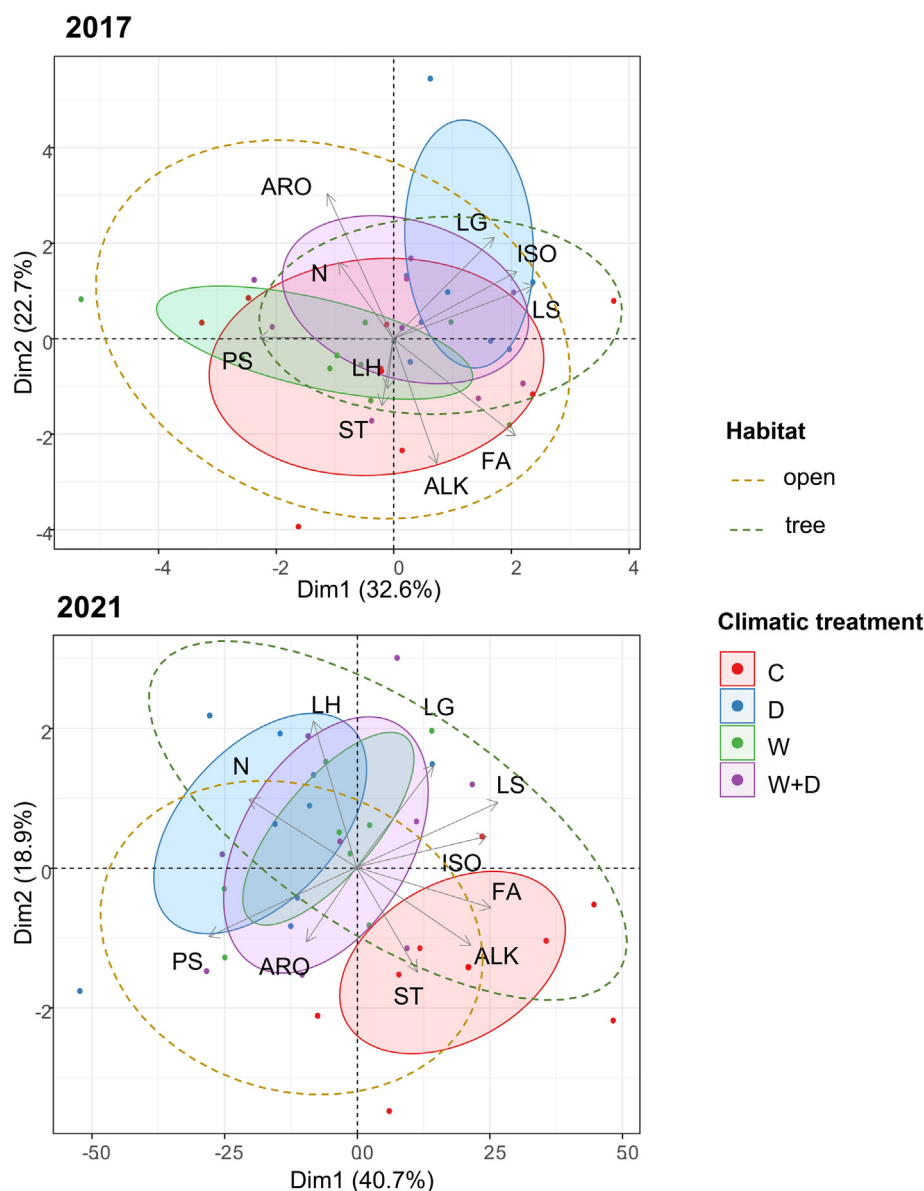

Fig. 4. FAMD corresponding to the Factor Analysis of Mixed Data analysis using mean values of families of pyrolytic compounds as quantitative factors, and habitat (dotted ellipses) and climatic treatments (solid ellipses) as categorical factors. Ellipses indicate 95 % confidence level (CI).

presence of ARO, PS and the effect of habitat were the variables driving the negative loading. It can be observed that the distribution of the diverse pyrolysis products is quite broad for all climatic treatments, including the control. Main differences are driven by the habitat as confirmed by previous analysis, with a greater relationship is spotted for ARO, N-containing and PS compounds for open habitat, and LG, LS and ISO compounds for tree habitat. These trends are in accordance with previous findings, as the compounds with discriminant value for the open pasture habitat (N, ARO, PS) are mainly indicative of microbial-derived processes whereas those for the tree habitat (LG, LS, ISO) indicate inputs from plant biomass.

Five years later, in 2021 again the Dim 1 is negatively driven by the abundance of PS, accounting for the N-containing compounds with an emergent influence; on the opposite, the positive loading is led by the presence of aliphatic compounds such as FA, ALK, and ISO, as well as LS compounds, all assigned to fresh biomass. Lastly, the Dim 2 is positively influenced by the effect of habitat and the abundance of LH and LG, and negatively influenced by the presence of ST and the effect of climatic treatments. Therefore, it seems that the abundance of aliphatic compounds, comprising FA, ALK, ISO and ST are clearly those with higher discriminant value for the control plots. On the contrary, N and LH compounds are the variables with higher discriminant value for the climatic treatments, especially the drought-induced one, probably related with the presence of a more recycled organic matter. Therefore, after only five years of the experiment mimicking climate change scenarios in a Mediterranean savannah, a neat change in the SOM molecular features is noticeable in *dehesas*.

4. Conclusions

During this research, the distinct contributions and distribution of the diverse biogenic compounds identified in the SOM pyrolysates allowed us to distinguish different SOM qualities; from fresh, less developed to more reworked and recalcitrant. This was particularly noticeable between the open pasture and tree canopy habitats, with more reworked and humified SOM characteristics in the former and less evolved, resembling fresh biomass in the later. Soil organic matter dynamics and hence its molecular features in Mediterranean soils are primarily caused by climatic conditions. In this sense, here we found that the effect of the main habitat (tree canopy and open pasture) is determinant in *dehesas'* SOM composition.

At the beginning of the experiment, no significant differences were found for the effect of the different climatic treatments. Five years later, the differences caused by the warming, drought and the combination W + D forced climate scenarios became statistically significant with respect to the untreated control, notably in the open pasture habitat. The climatic treatments mimicking the foreseen climate changes seem to be affecting mainly to the lignocellulose dynamics and the study of ALK biomarker proxies also points to an accelerated SOM degradation when soils are under warmer and dryer conditions. Therefore, it is expected that, in the short term, the foreseen climate change scenarios will exert changes in the Mediterranean savannah SOM molecular structure and dynamic.

In this study, the analytical pyrolysis combined with appropriate chemometric statistical approaches was found as a valuable direct approach for characterizing and assessing SOM molecular structural features. This method helped in shading some light in our understanding of how Mediterranean savannah may adapt to the foreseen climate change conditions. Further and deepest investigations on the effect of climate change in soil lignin dynamics related to other soil functions such as carbon sequestration are underway.

CRediT authorship contribution statement

Layla M. San-Emeterio: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Nicasio T. Jiménez-Morillo:** Methodology, Funding acquisition,

Writing – review & editing. **Ignacio M. Pérez-Ramos:** Methodology, Funding acquisition, Writing – review & editing. **María T. Domínguez:** Methodology, Funding acquisition, Writing – review & editing. **J.A. González Pérez:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that there is no conflict of interest for this research: no known competing financial interests or personal relationships had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159288.

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