


## Article

# Molecular Characterization of Burned Organic Matter at Different Soil Depths and Its Relationship with Soil Water Repellency: A Preliminary Result

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**Abstract:** Soil water repellency (hydrophobicity) prevents water from wetting or infiltrating soils, triggering changes in the ecosystems. This physical property is directly correlated to the erodibility grade of a soil. Wildfire events may develop, enhance, or destroy soil hydrophobicity, modifying the erodibility grade of a soil and increasing the loss of its most reactive layer (organic matter). To assess the main organic family of compounds (biomarkers) surrogates to fire-induced water repellency, a study was carried out on a fire-affected soil under eucalyptus canopy at two depths (0–2 and 2–5 cm) from Portugal. The potential soil water repellency was measured using the water drop penetration time (WDPT) test. The molecular characterization of hydrophobic biomarkers was carried out using analytical pyrolysis (Py-GC/MS) in combination with multivariate statistical analysis (PCA, MLR). The upper burned soil layer (0–2 cm) displayed a significant contribution of fresh biomass (lignin and polysaccharides), while the deepest (2–5 cm) one showed more humified organic matter (lipids). The soil hydrophobicity was directly correlated to non-polar organic compounds, such as lipids and polycyclic aromatic hydrocarbons (PAHs), and inversely to unspecific aromatic compounds. The combination of mass spectrometry techniques and chemometric analysis allowed obtaining a preliminary forecast model of hydrophobicity degree in fire-affected soil samples under eucalyptus canopy. This analytical approach opens the door to developing more sensitive mathematical models using molecular organic compounds to predict the alteration of hydrophobicity and other soil physical properties induced by fires.

**Keywords:** chemometrics; eucalyptus; hydrophobicity; Portugal; soil organic matter; wildfire



**Citation:** de Deus, M.; Miller, A.Z.; Jiménez-Morillo, N.T. Molecular Characterization of Burned Organic Matter at Different Soil Depths and Its Relationship with Soil Water Repellency: A Preliminary Result. *Agronomy* **2021**, *11*, 2560. <https://doi.org/10.3390/agronomy11122560>

Academic Editor: Geoff Anderson

Received: 12 November 2021

Accepted: 14 December 2021

Published: 16 December 2021

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## 1. Introduction

Wildfires are a global phenomenon, which annually affects 300–460 Mha in tropical, temperate, and boreal regions worldwide [1,2]. This generates significant biomass loss, as well as several changes in the physical, chemical, and biological properties of soil [3,4]. Therefore, wildfires are considered one of the major threats to soil health and ecosystem stability. In addition, forest fires increase the alteration of geomorphological and hydrological processes in the topsoil, which displays a significant direct impact on socio-economic aspects and climate change [5]. Wildfires also aggravate the erodibility degree of soil, as well as the quantitative and qualitative alteration of soil organic matter (SOM) [6,7].

Traditionally, forest fires are known to be one of the main drivers of soil loss (erosion) and land degradation [8]. The current climate change models displayed a noticeable increase in mega-fires (fire events with explosive character), which will lead to significant impacts on ecosystems and society in the near future [9]. Soil water repellency (SWR)

is a physical property playing a key role in hydrological and erosional processes or soil water dynamics [10]. The increase in SWR leads to an increase in surface flow processes, which directly affect the soil erosion risk (soil erodibility) [11]. Several research works showed a clear and direct correlation between SWR and erodibility rates of control and fire-affected soils [10,11]. Jordan et al. [12] reported that water repellency caused by forest fire was the major contributor to soil erosion after a long period of drought. Soil loss may have a significant impact on the climate change issue, owing to the increase in CO<sub>2</sub> emitted by eroded sediments. Therefore, the identification of the main compounds surrogated to SWR is mandatory to help the decision-makers in choosing the best practices to optimize soil post-fire stabilization. A considerable number of research works have demonstrated the relationship between SWR and a set of biotic and abiotic factors, such as soil texture (percentage of sand and clay fraction), temperature, water content, and organic matter [10]. However, the accumulation of hydrophobic organic compounds, mainly in the shallow soil layer, is the main factor influencing SWR [10–14]. These organic molecules may be gradually released into the soil from different sources, such as root exudates, soil microorganisms, and decomposed SOM [13]. The hydrophobic compounds can be located either as coatings on the surface of soil minerals or in the interstitial region of mineral particles [7]. Lipid-like compounds have been traditionally considered as the main hydrophobic organic family [7,14–16]. In addition, humic substances displayed a marked hydrophobic character [16].

The molecular alteration of SOM induced by wildfires may affect SWR due to the changes produced by fire on SOM composition at the molecular level [6,7,17]. Jiménez-Morillo et al. [7] and Mao et al. [14] demonstrated a significant correlation between altered lipid composition and different SWR persistence degrees. However, other studies reported strong water repellency in burned and unburned soils after lipid removal [7,15]. Therefore, other molecular families than lipids are affecting SWR.

The main goal of this work was to assess the biomarkers subrogated to water repellency in Portuguese fire-affected soil under eucalyptus cover at two different depths. For this purpose, analytical pyrolysis was conducted in combination with multivariate statistical tools to generate for the first time an SWR forecast model focused on the quality (molecular composition) of organic matter in coarse-textured soil. Currently, there is a knowledge gap in the post-fire soil erodibility models, as the quality of SOM has not been considered for the generation of soil erosion risk models due to its complexity. To avoid the influence of soil texture in the soil hydrophobicity rate, a soil with a relatively low percentage of clay fraction (<3%) was selected.

## 2. Materials and Methods

### 2.1. Study Area

The study area (Figure 1) is located in the Aljezur region (Faro, Southwest Portugal), dominated by a Mediterranean climate (Köppen climate classification: Csa) with a strong oceanic influence, and characterized by mild temperatures (annual average: 18 °C), high relative humidity and an annual average rainfall of 500 mm. Precipitation is distributed irregularly year-round, with the highest rainfall in December and the lowest in July. The soil sampled was classified as Podzol [18]. These soils are characterized by the presence of gray subsurface upper horizon (eluvial, albic horizon), discolored by the loss of organic matter and iron oxides, overlapping a dark horizon (illuvial, spodic horizon) of accumulation with brown, reddish, or black illuvial humus.

Soil samples were collected one week after the fire event in June 2020, which affected more than 22,000 ha of Mediterranean forest, dominated by *Quercus suber*, *Pinus maritimus*, *Pinus pinea*, and *Eucalyptus* sp. According to the remote sensing data obtained by Sentinel-2, different fire intensities were observed. For this work, a high-intensity burned area under *Eucalyptus* sp. cover (37°08'25.4" N 8°50'55.9" W) was selected. In an area of ca. 10 m<sup>2</sup> under fire-affected eucalyptus trees, 4 sub-samples from the shallowest layer (0–2 cm) and deeper layer (2–5 cm) were collected to obtain a significant representation of the fire

impact on this soil. No samples of deeper layers (>5 cm) were obtained since previous studies showed that SOM is not affected below 5 cm [19]. Each of the soil samples was transported to the laboratory in paper bags, thus avoiding contamination by phthalates (plastic bags). The samples were air-dried at 25 °C for 1 week. They were then sifted to separate the entire sample from the fine soil fraction (<2 mm), removing the coarse mineral fraction (>2 mm) and the visible, coarse plant remnants (e.g., little branches, leaves, roots). In this case study, it was not possible to compare the hydrophobicity degree and molecular composition of burned soils with unburned (control) samples, under *Eucalyptus* sp. cover, due to the absence of a control area close (<100 m) to the fire-affected area, with the same edaphologic and climatic characteristics.

## 2.2. Physicochemical Analysis

The organic C content ( $C_{\text{org}}$ ) and Total Nitrogen ( $N_{\text{tot}}$ ) in the burned soil samples at two depths were analyzed by a Flash 2000 HT (C, H, N, O, and S) elemental micro-analyzer equipped with a thermal conductivity detector (TCD) (Thermo Scientific, Bremen, Germany). Each soil sample (~1.5 mg) was measured in duplicate. The calibration curve was made for carbon and nitrogen using standard materials (e.g., acetanilide, nicotinamide, and aspartic acid). Prior to analysis, the samples were treated with a strong acid (HCl 1M) to remove carbonates.

Soil acidity (pH) was measured in aqueous soil extract in deionized water (1:5 soil:water) at 25 °C using a pH 1100 L equipment. The sand fraction of soil samples was assessed by sieving (>0.05 mm). The clay (<0.02 mm) fraction was measured by a Bouyoucos hydrometer. The silt (0.02–0.05 mm) fraction was determined by the difference between 100% and the sum of sand and clay fractions. Before texture analysis, the organic matter was removed by oxidation (6%  $H_2O_2$ ).

## 2.3. Persistence of Soil Water Repellency

The study of persistence in soil water repellency (SWR) was carried out using the Water Drop Penetration Time test (WDPT). Five water distilled drops were placed on the soil surface (<2 mm), and the time for complete infiltration was registered [20,21]. The average WDPT value of each sample was classified according to Bisdom et al. [22]: wettable (WDPT < 5 s), slight WR (5 ≤ WDPT < 60 s), strong WR (60 ≤ WDPT < 600 s), severe WR (600 ≤ WDPT < 3600 s) and extreme WR (WDPT ≥ 3600 s). Water drops were applied with a Pasteur pipette onto the soil sample surface from a height of approximately 5 mm to avoid excess kinetic energy affecting soil-droplet interactions [6,7].

## 2.4. Analytical Pyrolysis

Pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) is a fast and reproducible technique widely used for structural characterization of SOM. The technique involves a thermolytic degradation of macromolecules into small fragments that can be separated and identified. Py-GC/MS was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i), working at 500 °C, attached to a Shimadzu GC2010 gas chromatographer with a Shimadzu GCMS-QP2010 Plus mass spectrometer working at 70 eV. A capillary column Zebron-ZB-5HT (30 m length, 0.25 mm internal diameter, 0.10 µm film thickness) was used for separation, with helium as a carrier gas, adjusted to a flow rate of 1.2 mL min<sup>-1</sup>. The splitless injector operated at a temperature of 250 °C. The chromatographic ramp of temperature was previously described by Jiménez-Morillo et al. [23]: 50 °C for 1 min and then increased to 100 °C at 30 °C min<sup>-1</sup>, from 100 to 300 °C at 10 °C min<sup>-1</sup>, and stabilized at 300 °C for 10 min. The mass spectrometer was programmed to acquire data between 40 and 850 m/z. The compound assignment was achieved by monitoring diagnostic ions for the main homologous series, via low-resolution MS and via comparison with published and stored (NIST and Wiley libraries) data. To facilitate comparisons between samples, the compounds released by Py-GC/MS were categorized into 8 families with standardized biogenic origin: unspecific aromatic compounds, polysaccharides,

peptides, lignin, lipids, nitrogen compounds (N-comp), polycyclic aromatic hydrocarbons (PAH), and steranes.

### 2.5. Statistical Analysis

The WDPT values were normalized using  $\text{Log}_{10}$  to be compared with the relative proportion (%) of the main organic families. The univariate statistical (one-way ANOVA) and multivariate analyses (principal component analysis “PCA” and multiple linear regression “MLR”) were performed with Statgraphics Centurion XV software (Statgraphics Technologies, Inc., The Plains, VA, USA). The PCA was used for the simultaneous ordering of different families of organic compounds and the persistence of SWR, illustrating their mutual relationships. The MLR was applied to generate a hydrophobicity prediction model, using the main families of compounds released by analytical pyrolysis. The validation of the model was confirmed by the production of spurious models calculated from the totally random dependent variable.

## 3. Results and Discussion

### 3.1. Physicochemical Characterization of Fire-Affected Soil Samples under Eucalyptus Cover

Table 1 shows the  $C_{\text{org}}$ ,  $N_{\text{tot}}$ , ratio C vs. N (C/N), pH, as well as the percentage of sand, silt, and clay of burned soil samples (<2 mm) at two depths (0–2 cm and 2–5 cm). The highest content of  $C_{\text{org}}$  and  $N_{\text{tot}}$  were observed in the upper soil layer (9.80% and 0.39%, respectively). This may be due to a deposition of necromass, partially burned, from the stressed trees [24]. The C/N ratio showed no significant differences between depths. Soil acidity (pH value) varied significantly between 6.7 (0–2 cm) and 7.5 (2–5 cm). The highest acidity observed for the upper layer may be due to the denaturalization of organic acids after fire events [25]. Soil texture was dominated by sand (>75%), which is in line with the stabilized value of Podzols by WRB [18]. However, the percentage of sand in the deepest layer is significantly higher than in the upper one. The fire may produce the degradation of large particle size fractions (sand) [25], increasing the silt fraction in the first centimeters of soil.

**Table 1.** Physicochemical characterization of fire-affected fine earth (<2 mm) soil samples at two depths (0–2 cm and 2–5 cm) under eucalyptus cover.

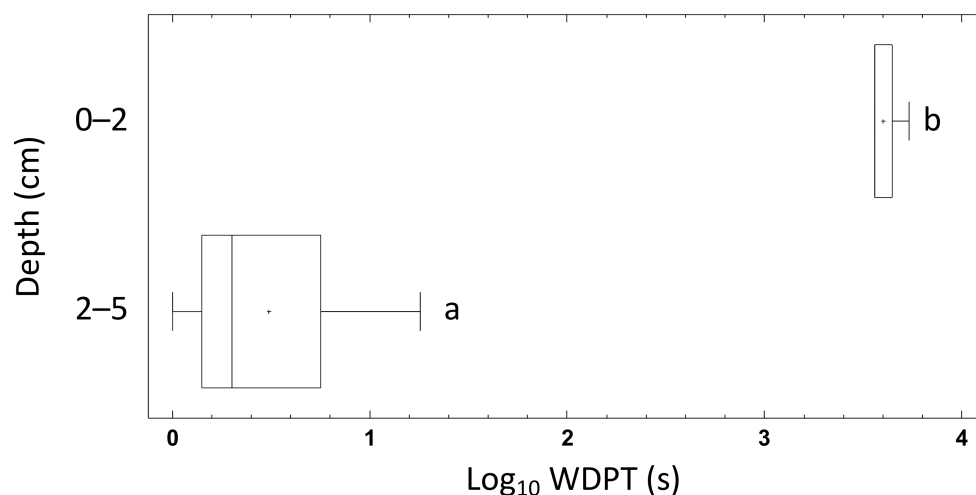
Sample	$N_{\text{tot}}$ (%)	$C_{\text{org}}$ (%)	C/N	pH	Sand (%)	Silt (%)	Clay (%)
0–2 R <sub>1</sub>	0.47 ± 0.05	11.92 ± 0.40	25.68 ± 2.15	6.1 ± 0.1	76.46	21.68	1.86
0–2 R <sub>2</sub>	0.34 ± 0.01	9.44 ± 0.01	28.12 ± 1.20	6.2 ± 0.1	77.28	19.97	2.75
0–2 R <sub>3</sub>	0.50 ± 0.04	11.11 ± 0.80	22.12 ± 0.36	7.2 ± 0.1	75.87	22.56	1.57
0–2 R <sub>4</sub>	0.24 ± 0.07	6.72 ± 1.81	27.80 ± 0.62	7.2 ± 0.0	75.05	23.66	1.29
2–5 R <sub>1</sub>	0.07 ± 0.00	1.62 ± 0.29	23.69 ± 2.83	7.4 ± 0.1	79.32	18.26	2.42
2–5 R <sub>2</sub>	0.07 ± 0.02	1.60 ± 0.21	23.54 ± 2.17	7.3 ± 0.1	85.57	12.22	2.21
2–5 R <sub>3</sub>	0.06 ± 0.00	1.98 ± 0.30	30.76 ± 2.56	7.7 ± 0.1	82.35	15.98	1.67
2–5 R <sub>4</sub>	0.05 ± 0.01	1.44 ± 0.36	30.84 ± 3.81	7.7 ± 0.1	83.47	14.34	2.19
0–2	0.39 ± 0.12b	9.80 ± 2.26b	25.93 ± 2.74a	6.7 ± 0.6a	76.15 ± 0.94a	21.97 ± 1.56b	1.87 ± 0.63
2–5	0.06 ± 0.01a	1.66 ± 0.31a	27.21 ± 4.42a	7.5 ± 0.2b	82.68 ± 1.87b	15.20 ± 2.45a	2.12 ± 2.12

One-way ANOVA. The different letters (a = lower value, and b = higher value) indicate significant ( $p < 0.05$ ) differences between samples according to the Tukey test.

### 3.2. Persistence of Soil Water Repellency

Figure 1 shows the one-way variance analysis (ANOVA) of  $\text{Log}_{10}$  (WDPT) values of burned soil samples under eucalyptus at two depths (0–2 and 2–5 cm). A significant difference ( $p < 0.01$ ) of hydrophobicity is observed between samples. The most superficial samples showed higher persistence in water repellency than the samples collected at greater depth (3.5 vs. 0.5, respectively). The same trend has been observed by other researchers [6,7,11,13]. They considered that the highest hydrophobicity degree in the

upper soil layer may be due to not only the huge amount of organic C but also the chemical transformation induced by fire of organic molecules. This may yield pyrogenic compounds with a remarkable hydrophobic character. Therefore, it is important to determine the molecular composition of organic matter for both depths.

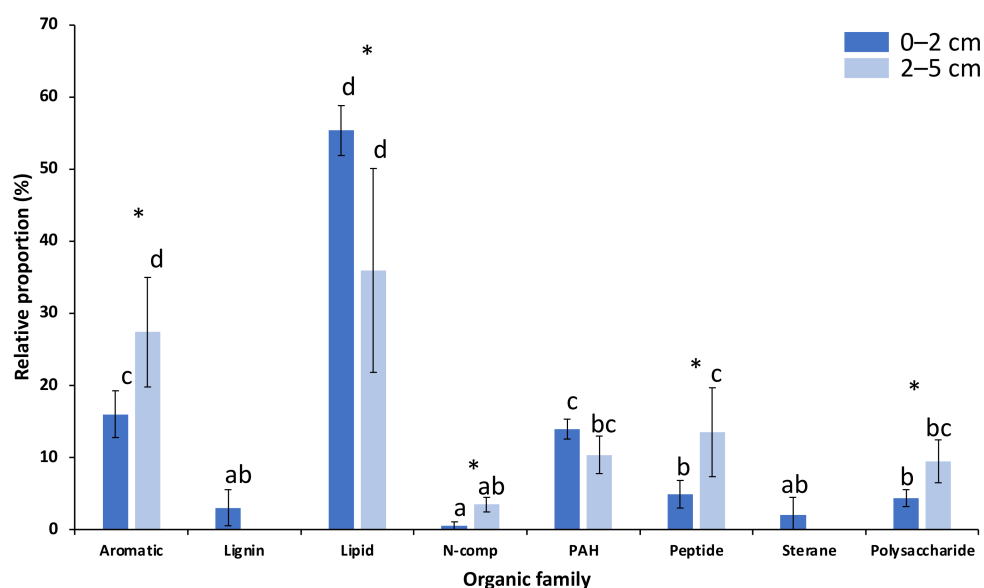


**Figure 1.** Boxplot Log<sub>10</sub> (WDPT) of soil as a function of depth. The plot shows the values of the largest and smallest quartiles, as well as the value of the median. The different letters observed in each sample indicate the existence of a significant difference between them (“one-way ANOVA”; means compared to the Tukey test,  $p = 0.05$ ).

### 3.3. Molecular Characterization of SOM

Due to the high number of organic compounds released by Py-GC/MS from each soil sample (Supplementary Materials, Tables S1 and S2), the different organic families were categorized into eight families to clearly distinguish significant differences in the molecular composition between both depths (Figure 2). The organic matter in the upper soil layer was mainly composed of lipids (>50% of total compounds), as well as lignin and polysaccharide-like compounds. The deposition of partially burned leaf biomass could be the main source of these organic compounds. González-Pérez et al. [24] and Jiménez-Morillo et al. [26] observed that, after a fire, there is a deposition of slightly burned material from the leaf necromass due to plant stress [6,7,23,26]. This biomass is rich in lipid-derived compounds, such as epicuticular waxes [27,28], as well as compounds derived from lignin and polysaccharides. In addition, there is a significant contribution of pyrogenic compounds (unspecific aromatic compounds and PAHs). These molecules are generated by the partial combustion of plant biomass and/or SOM [29–31]. In the case of the deepest (2–5 cm) soil samples, the organic matter displayed a well-humified character since it is dominated by lipid and unspecific aromatic compounds. In addition, there was a significant contribution of compounds derived from microbial activity, such as peptides and furfural (polysaccharide) compounds [32]. In addition, these samples displayed a relatively important contribution of recalcitrant compounds such as PAHs. Their origin may be due to: (i) the incorporation of small carbon particles (“soot” or “black carbon”) after the fire [33–36], or (ii) the inherited pyrogenic carbon pool in this layer from previous wildfire events. It is worth mentioning that this region experiments with recurrent wildfire events [37].





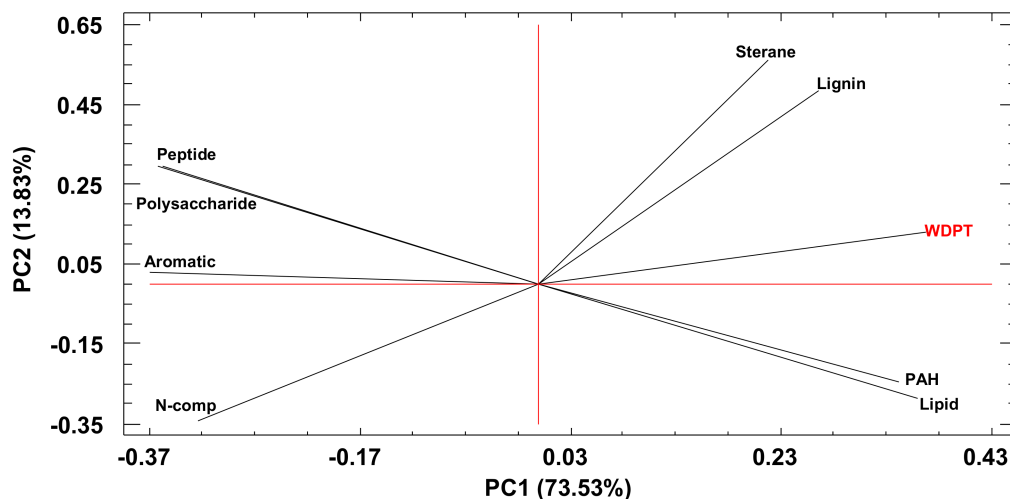
**Figure 2.** Relative proportion of the main organic families in which organic compounds released during analytical pyrolysis are ordered. The different letters in each sample indicate the existence of significant differences among organic families (“one-way ANOVA”; means compared to the Tukey test,  $p = 0.05$ ). Specific families with \* are significantly different between depths (“one-way ANOVA”; it means compared to the Tukey test,  $p = 0.05$ ).

One-way ANOVA analysis of the relative proportion of each family was carried out to establish differences between both soil depths (Figure 2). There were significant differences in almost all organic families. The 0–2 cm soil samples showed a significantly higher abundance of lipid, lignin, and sterane compounds. This result suggests the incorporation of slightly combusted leaf material, which may contain a relatively high proportion of epicuticular waxes (lipids and steranes) and methoxyphenol compounds (lignin monomers) [23]. In contrast, unspecific aromatic compounds, N-comp, peptides, and polysaccharides, make a greater contribution to the deepest soil layer. The main sources of the unspecific aromatic molecules may include: (i) the degradation of fresh material by microorganisms [38], (ii) the defunctionalization of lignin-derived compounds by fire [36], (iii) the cyclization of lipid compounds (Diels–Alder reaction) [36], or (iv) the incorporation of partially burned organic particles. The significant depletion of lipid compounds and the elimination of lignin-like and sterane molecules may support these hypotheses. On the other hand, the higher proportion of peptide and N-comp in the deepest layer may suggest the presence of either highly altered organic material or high microbial activity [31] since these organic families have been correlated to microbial alteration. It is well established that the highest concentration of microorganisms is found in the upper layer, which is related to the highest concentration of organic matter. However, fire can produce a sterilizing effect on the first centimeters of soil [39]. This trend may not have occurred in the deepest layer due to the heat protection provided by soils, which would allow the development of microbial communities. This same hypothesis could be applied to polysaccharide-derived compounds, which despite being thermally labile, can be preserved in deeper soil layers.

### 3.4. Chemometric Analysis

Figure 3 displays the principal component analysis (PCA) scatterplot, which was used to identify the main biomarkers significantly (positively and negatively) correlated to water repellency in burned soil under eucalyptus cover. In this case study, over 85% of the total variance can be explained using the two first components (PC1: 73.53% and PC2: 13.83%). The persistence of SWR (WDPT value) was directly correlated to lipid, PAHs, sterane, and

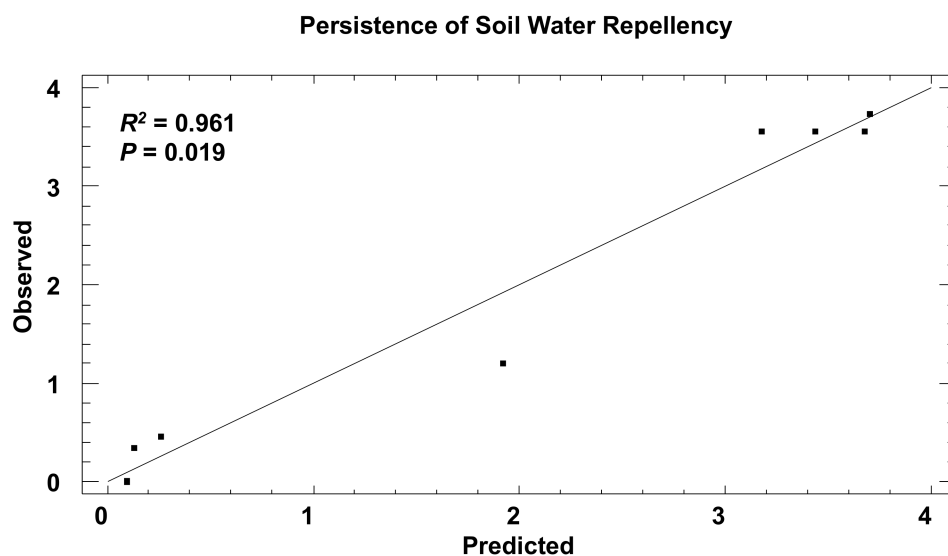
lignin-like compounds. In contrast, unspecific aromatic molecules, polysaccharides, and nitrogen-derived compounds (peptides and N-comp) are inversely correlated to this soil property. Several authors have observed that organic molecules derived from lipid-like compounds (with a noticeable hydrophobic character) were positively linked to higher soil hydrophobicity degrees [15,16,19]. Jiménez-Morillo et al. [7] found a direct correlation between SWR and the concentration of long-chain fatty acids in burned soils under *Quercus suber* from Doñana National Park (Spain). On the other hand, the significant positive correlation of SWR with PAH-derived compounds may be explained by its chemical structure. PAHs are made up of the combination of a relatively high number of aromatic rings without oxygenated (polar) functional groups, which gives a strong hydrophobic character. Almendros et al. [40] observed, in a laboratory-based combustion experiment, that the higher amount of pyrogenic organic matter was correlated to the SWR degree, which is due to the alteration of the colloidal property of organic matter. However, the significant negative correlation of SWR with unspecific aromatic compounds could be due to the contribution of aromatic molecules generated through lignin defunctionalization. These compounds, such as phenols, may display residual polar functional groups, which can perform a poor hydrophilic character.



**Figure 3.** Plot of component weights for the soil water repellency variable (WDPT) and the main organic families identified by analytical pyrolysis: aromatic, polysaccharides, peptides, lignin, lipids, nitrogen compounds (N-comp), polycyclic aromatic hydrocarbons (PAH), and steranes.

The existence of a correlation between the persistence of SWR and the molecular composition of fire-affected organic matter at two depths was validated by a multilinear regression (MLR) approach. This statistical tool allowed generating a specific forecasting model ( $p = 0.019$ ) of the persistence of SWR (dependent variable), under burned eucalyptus cover, using some of the main organic families analyzed by analytical pyrolysis as independent variables (Figure 4). In addition, MLR analysis generated the equation (Equation (1)) of the prediction model, using automatic backward variable selection. The model validation was established by a strict comparison (MLR cross-validation) with the alternative model produced with fully randomized  $\text{Log}_{10}$  (WDPT) values of soil samples (data not shown). The lack of a meaningful model with randomly ordered data supports the viability and credibility of the forecast SWR model in this specific study case.

$$\text{Log}_{10}(\text{WDPT}) = 0.66 + 0.018 \times \text{Aromatic} + 0.07 \times \text{Lipid} - 0.69 \times \text{N-comp} - 0.05 \times \text{PAH} \quad (1)$$



**Figure 4.** Multiple linear regression (MLR) for persistence to SWR ( $\text{Log}_{10}$  WDPT), using the molecular composition of burned SOM at different depths as predictive variables.

#### 4. Conclusions

The SWR degree and the molecular composition of fire-altered SOM depend on the soil depth. The higher SWR degree found in the upper soil layer was related to the existence of a significantly high concentration of lipid and polycondensated (PAH) compounds. In contrast, polysaccharide-like, lipid-like, and aromatic compounds were the main organic families in the deepest soil layer, which displayed the lowest SWR value. In addition, this layer recorded the highest amount of peptide molecules, which may be generated by a relatively high microbial activity or preserved by the heat protection provided by soils.

The soil hydrophobicity degree was directly correlated to non-polar organic compounds, such as lipids and polycyclic aromatic hydrocarbons (PAHs), and inversely to unspecific aromatic compounds. This preliminary work demonstrated that SWR cannot be explained by a unique organic family (i.e., lipid-like compounds). Thus, further investigation is needed to fully understand this soil property.

The use of state-of-the-art analytical techniques, such as analytical pyrolysis, in combination with multivariate statistical tools (PCA and MLR), allowed generating for the first time a preliminary forecast model of the SWR persistence degree in fire-affected soil samples, at different depths, under eucalyptus canopy. Although this is a preliminary and specific case study, the model obtained will allow forecasting the trend of soil hydrophobicity in burned eucalyptus, which may be helpful to manage these specific fire-altered soils. This preliminary work prompts further investigations for developing soil erosion risk models based on the molecular composition of SOM to predict the alteration of soil physical properties after wildfire events, as well as its impact on the ecosystems.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11122560/s1>, Table S1: Organic compounds released by analytical pyrolysis for 0–2 cm burned soil sample, Table S2: Organic compounds released by analytical pyrolysis for 2–5 cm burned soil sample.

**Author Contributions:** M.d.D.—investigation, data treatment and writing original draft and review; A.Z.M.—data treatment, writing—original draft preparation, and supervision; N.T.J.-M.—conceptualization, formal analysis, data treatment, writing—original draft preparation, supervision, funding and project administration. All authors have read and agreed to the published version of the manuscript.



**Funding:** This research was funded by National Funds through the Portuguese Foundation for Science and Technology (FCT) through project “EROFIRE—Post-fire erosion risk assessment using molecular markers—PCIF-RPG-0079-2018”.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The Portuguese Foundation for Science and Technology (FCT) and the Spanish Ministry of Science and Innovation are acknowledged for funding the CEECIND/01147/2017 and “Ramón y Cajal” (RYC2019-026885-I) post-doctoral contracts, respectively).

**Conflicts of Interest:** The authors declare no conflict of interest.

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