



Hydrophobicity of soils affected by fires: An assessment using molecular markers from ultra-high resolution mass spectrometry

Nicasio T. Jiménez-Morillo^{a,*,} Gonzalo Almendros^{b,} Ana Z. Miller^{c, d,} Patrick G. Hatcher^{e,} José A. González-Pérez^d

^a MED, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

^b MNCN-CSIC, c/Serrano 115-B, 28006 Madrid, Spain.

^c HERCULES-Lab, Universidade de Évora, Palácio do Vimioso, 7000-089 Évora, Portugal.

^d IRNAS-CSIC, Av. Reina Mercedes, 10, 41012 Seville, Spain.

^e Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529, USA.

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ABSTRACT

Soil water repellency (SWR) is a physical property due to a complex interaction of factors (e.g., fire, soil organic matter, soil texture) that reduces the soil water infiltration capacity. Traditionally, SWR is attributed to the accumulation and redistribution of hydrophobic compounds within soil profile. To obtain further insight into chemical compounds, which could be associated with SWR, a study was done on coarse (1–2 mm) and fine (< 0.05 mm) granulometric fractions of burned and unburned sandy soils under two Mediterranean vegetation biomes from Doñana National Park (Spain). The water drop penetration time (WDPT) test was used to assess the SWR. The molecular composition of extracted humic substances from the soil organic matter (SOM) was determined by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS). Partial least squares (PLS) regressions showed that the SWR can be predicted ($P = 0.006$) solely based on the abundances of approximately 1200 common compounds determined by FT-ICR/MS. This model confirmed the significant correlation between a specific SOM molecular composition and the SWR. The comparative analysis revealed that the SWR in the burned samples was significantly ($P < 0.05$) related to the abundance of aromatic and condensed compounds, while in the unburned samples there was a significant influence of aromatic hydrocarbons and lignin compounds. In the fine fraction, lipid compounds were significantly associated with the SWR. Contrastingly, the coarse fraction did not show any correlation. Alternatively, soils with a high SWR were significantly related to the presence of lipids and lignin. This analysis showed that combining FT-ICR/MS molecular characterizations with statistical treatments is a powerful approach for exploratory analysis suggesting that the structural features associated with SWR in the studied soils are different depending on the types of vegetation or the soil physical fractions with different particle size.

1. Introduction

Soil water repellency (SWR) is a reduction in the rate of wetting and retention of water in soil caused by the presence of hydrophobic coatings on soil particles (Hallett, 2007). This property is associated with some important hydrological and geomorphological consequences (Zavala et al., 2014), such as the increase of water flow on shallow soil layers, the increase of erosion risks (erodibility), and the loss of soil nutrients (Imenson et al., 1992). However, SWR, when not excessively high, can also have a positive effect, such as increasing the stability of soil aggregates that favours soil C sequestration processes (Almendros,

2008; Arcenegui et al., 2018; Mataix-Solera and Doerr, 2004; Jordán et al., 2013).

Forest fires may induce, enhance, and even remove SWR depending on temperature and duration of the event, as well as soil type, which may generate temporary changes in the hydrological properties of soils (Granged et al., 2011; Jiménez-Pinilla et al., 2016).

The SWR is traditionally assumed to be associated with hydrophobic organic substances (Doerr et al., 2000; Jiménez-Morillo et al., 2016a, 2017; Wu et al., 2020), as well as with soil texture (Bisdom et al., 1993; DeBano 1981). Currently, there is controversy regarding which soil textural fraction has the greatest influence on SWR, and several observa-

* Corresponding author.

E-mail address: ntjm@uevora.pt (N.T. Jiménez-Morillo).

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tions have suggested that coarse fractions are more repellent than fine ones. Some previous studies have indicated that water repellency depends significantly on soil particle size (DeBano 1981). In fact, García-García et al. (2018) and Jiménez-Morillo et al. (2017) observed that fine-size fractions showed a higher degree of water repellency than the coarse ones. However, this “natural” behaviour can be significantly altered by wildfires. The ensuing combustion of organic matter produces volatile organic molecules that preferably condense in the deeper layers of the soil. This condensation is due to the existence of a negative temperature gradient (cooler sites), which leads to the coating of soil particles with hydrophobic organic compounds (Jordán et al., 2010).

One of the main hydrophobic organic compounds are lipids, which are progressively released from plants to the soil (epicuticular waxes) and/or from microorganisms (membrane lipids). The lipids are mainly made up of *n*-alkanoic acids (González-Peñaloza et al., 2013; Jiménez-Morillo et al., 2016a, 2017); *n*-alcohols (Mao et al., 2016); *n*-alkanes and α -alkenes (de Blas et al., 2013); terpenes and sterols (Franco et al., 2000); and complex polar lipids (Atanassova and Doerr, 2010). Traditionally, research studies have used different extraction methods and analytical techniques focused on the assessment of the lipid fraction of SOM (Mao et al., 2015, 2016). However, up to date, the biomarkers that act as surrogates of the SWR as well as the impact of fire on these organic molecules, are still unknown.

Therefore, the use of state-of-the-art analytical techniques such as ultrahigh resolution mass spectrometry, e.g., Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR/MS), would be highly beneficial. The amount of information it provides (identification of thousands of molecular formulas, some of which can be used as molecular markers) is considerably high compared to traditional techniques such as GC/MS. FT-ICR/MS has been employed to study the impact and the chemical reactions induced by fire on SOM (Ikeya et al., 2015; Jiménez-Morillo et al., 2018; Kramer et al., 2004). The main advantage of FT-ICR/MS is the huge number of different chemical formulas that are produced, without significant fractionation, however, the major problem is associated with the large number of compounds, i.e., their interpretation. The use of graphical-statistical tools is mandatory to facilitate the comparison among analysed samples. One of the most widely employed tools is the classical van Krevelen diagram (Kim et al., 2003; van Krevelen, 1950). This graphical-statistical approach plots the H/C vs O/C atomic ratios of each singular compound in a x,y scatter diagram. The exact location of each molecular formula allows its assignment to a specific family of biogeochemical compounds, as proposed by Kim et al. (2003), and has been used to evaluate the degree of soil recovery after forest fires (Almendros et al., 2018a, 2018b; Jiménez-Morillo et al., 2020a).

This study proposes to simplify the interpretation of the complex molecular patterns obtained by FTICRMS of soil samples, by means of the representation of the quantitative differences between the proportion of the different formulas in the space defined by their atomic ratios H/C and O/C, in order to visualize the significant changes in the molecular composition as a function of the effect of fire, the particle size and its reflection in the resulting hydrophobicity.

For the above purpose, the FTICRMS data were analysed in order to: i) identify surrogate molecular markers for SWR in soil fractions of different particle size affected or not by fire; ii) examine by means of PLS forecasting models if the SWR depends on the different molecular composition of the SOM, recognized by FT-ICR/MS; iii) assess quantitative differences in the main families of soil organic compounds related to SWR; and iv) investigate whether the SWR correlated compounds are the same in fire-affected samples as in fire un-affected samples.

2. Materials and methods

2.1. Sampling sites

The sites chosen for this work are located at Doñana National Park (south-western, Spain), one of the most important biosphere reserves in Europe. Doñana displays a mild, typically Mediterranean climate (Csa. Köppen classification, Köppen, 1918). It is characterized by hot, dry summers and comparatively humid winters with significant average rainfall (> 800 mm) compared to inland regions and a mean air temperature of ca. 17 °C (Jiménez-Morillo et al., 2016a). Soil samples were collected in October 2014 from a Typic Xeropsamment/Haplic Arenosols (IUSS, 2015) under two types of vegetation covers: cork oak (*Quercus suber* L.) “QS”, and the association *Erico scopariae-Ulicetum australis*, known locally as “Monte negro”, shrubland (i.e., wet shrubland) “MN”. The MN is dominated by taxa such as heather *Erica scoparia* L., *Erica umbellata* Loefl. ex L., *Erica ciliaris* Loefl. ex L., *Calluna vulgaris* (L.) Hull, gorse or Moorish gorse (*Ulex australis* Clemente), myrtle (*Myrtus communis* L.), labyrinth (*Phillyrea angustifolia* L.), brambles (*Rubus ulmifolius* Schott.) and flax-leaved daphne (*Daphne gnidium* L.). This nature reserve has been repeatedly affected by wildfires. The previous studies have suggested the existence of a recurrence of fires between 1552 and 4099 days (approx. 4 and 11 years) in the study area (García-Novo et al., 2018). Recurrent fires have been considered the key cause of changes in vegetation patterns and geomorphology during the last several centuries (Granados, 1985). The studied area dominated by QS (37°7'21.95"N; 6°26'53.44"W) was affected by a severe wildfire (> 300 ha) during the summer of 2012, while the MN area (37°7'41.19"N; 6°38'0.29"O) was affected in the summer of 2013, by burning of over 60 ha, according to the information (severity of the fire and number of hectares of forest burned) provided by the forest managers of the Doñana National Park. The wildfire severity was evaluated by remote sensing approaches conducted by the governmental “Agencia de Medio Ambiente y Agua de Andalucía (AMAYA)”. The sampling campaign was conducted in November of 2014, one year after MN fire (2013) and two years after QS fire (2012). This work follows the classic study model where the burned area (B) was comparatively studied with an unburned one (UB, control). The UB and B soil samples, under QS and MN canopies, consisted of composite samples obtained by combining five sub-samples collected within a circular area of ca. 20 m² under canopies unaffected and affected by fire. We selected these areas because there was no water erosion process, due to the absence of slope. There were wind erosion processes, but it has not been considered relevant for this work. In addition, there was a firebreak between the areas, which helped stopping the wildfire. The samples were collected from the shallow layer of soil (uppermost 3 cm) after removing the litter layer. We randomly sampled within a circular area of ca. 20 m². In total, we collected approximately 3 kg of combined soil samples for each area. We tried to take approximately the same weight (0.5 kg) of soil for each subsample. The sampling site did not present any slope, so we avoided the impact of erosion. Although the SWR is usually very heterogeneous, in this case, due to the low complexity of the soil (99.9%) of the burned areas under QS and MN, we assume that the 20 m² area is representative. In addition, each area was dominated by QS and MN, respectively, hydrophobicity tests (water drop penetration time, WDPT) were carried out in-situ in the selected area. The WDPT values in whole sandy soil showed no differences in each area. Therefore, it can be assumed that SWR values are representative of each area. Each composite sample was transported in clean glass flasks, air-dried under laboratory conditions (at 25 °C and approximately 50% relative humidity) for 1 week and sieved to fine earth (< 2 mm) to remove large fragments of litter and rocks. The QS and MN fine earth samples (total sample) were further divided by dry sieving into two particle-size fractions: coarse (1–2 mm, CF) and fine (< 0.05 mm, FF). The intermediate fractions (0.06–0.09 mm) were discarded because previous studies indicate that

SOM in these fractions presents intermediate trivial chemical characteristics between the coarse and fine fractions (Jiménez-Morillo et al., 2020a, 2020b). Therefore, a total of 8 composite samples collected around a target were analysed to evaluate the molecular composition: 1) unburned QS coarse fraction (UBQSCF); 2) unburned QS fine fraction (UBQSFF); 3) burned QS coarse fraction (BQSCF); 4) burned QS fine fraction (BQSFF), 5) unburned MN coarse fraction (UBMNCF), 6) unburned MN fine fraction (UBMNFF), 7) burned MN coarse fraction (BMNCF), and 8) burned MN fine fraction (BMNFF).

2.2. Persistence of soil water repellency measurement

The water drop penetration time (WDPT) test was used to measure the persistence of SWR (van't Woudt, 1959). The average soil water repellency was classified according to Bisdorn et al. (1993): 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). However, due to the remarkable differences in WDPT values, it was used a logarithmic transformation (Log₁₀) to obtain a better fit to the normal distribution, as well as to be compared among them, and with the relative proportion of the main organic families. This test was performed in the QS and MN particle size fractions in a laminar flow hood under constant temperature and relative humidity conditions (25 °C and 50%, respectively) in order to avoid external evaporation of the drop. The WDPT values determined under laboratory conditions on soil samples (fine soil, < 2 mm, or soil particle size fractions air-dried at room temperature) are the mean of 5 individual measurements (*n* = 5).

2.3. Extraction of humic substances from SOM

The extraction protocol used was that described in Jiménez-Morillo et al. (2018). Briefly, 100 mg of sample were mixed with 1 mL 1 M NaOH by gentle shaking (180 rpm) at room temperature. The extracts were filtered through a 0.2 μm poly(tetrafluoroethane) filter. Ion-exchange resin Dowex® 50WX8 (100–200 mesh H⁺-form) was employed to remove cations (Na⁺). The extracts were repeatedly re-purified with MilliQ® water until pH = 7 and stored in a freezer (-4 °C) until analysis. A blank sample was obtained following the same procedure but without sample.

2.4. Ultra-high resolution mass spectrometry analysis

The methodology used for the molecular composition assessment for each sample was described in detail by Jiménez-Morillo et al. (2018). In short, an Apollo II electrospray ionization (ESI) source, working in negative ion mode, direct injection at a flow rate of 120 μL h⁻¹, with a nebulizer gas pressure of 20 psi and a drying gas pressure of 15 psi, on a Bruker Daltonics 12 T Apex Qe FT-ICR/MS instrument (Bruker Daltonics, Bremen, Germany) was used. Aliquots of each extract (blank included) were mixed 1:1 (v:v) with chromatography grade methanol (MeOH) to improve the ionization efficiency. All mass spectra were calibrated externally and internally as described by Sleighter and Hatcher (2007). The external calibration was performed using a polyethylene glycol standard, while a fatty acid series common to natural organic matter, were used for the internal calibration. The peaks identified in the “blank sample” were subtracted from the sample peak list prior to formula assignment. Empirical molecular formulas were assigned in the mass range from 300 to 700 amu, using an in-house Matlab code (The MathWorks, Inc., Natick, MA) according to the following criteria: ¹²C₂₋₅₀, ¹H₅₋₁₀₀, ¹⁴N₀₋₆, ¹⁶O₁₋₃₀, ³²S₀₋₂, and ³¹P₀₋₂ within a maximum error of 1 ppm, and using the rules defined by Stubbins et al. (2010). This corresponds to the standard range of atomic composition for natural organic matter: i) O/C ≤ 1.2; ii) 0.35 ≤ H/C ≤ 2.25; iii) N/C ≤ 0.5; iv) S/C ≤ 0.2; v) Double bond equivalent ≥ 0 and an integer value.

In order to use appropriate data matrices for chemometric approaches, only molecular formulas identified in all samples corresponding to the different particle size fractions (common compounds) of the QS and MN soils were used to develop the water repellency forecasting model. For the selection of the common CHO compounds, after the correct assignment of the formulas, the exact mass of the different molecules was used, which allows differentiation.

The assigned molecular formulas of common CHO-containing compounds were examined using a van Krevelen diagram (van Krevelen, 1950). The van Krevelen diagram was generated from the atomic ratios (H/C and O/C) of each common molecular formula. The molecular formulas plotted within the diagram were categorized into 7 main regions as suggested by Kim et al. (2003). From the elemental composition of the different molecules identified by FT-ICR/MS it was possible to construct van Krevelen surfaces—or contour diagrams—as described by Almendros et al. (2018a, 2018b), using the authors' own ad-hoc computer program. Briefly, the atomic ratios (H/C and O/C) of specific organic compounds calculated from the empirical formulas inferred from the ultra-high resolution mass spectrometry information were represented by a 3D graph in the H/C vs O/C plane (*y*, *x* axes) and representing (*z* axis) the percentages of total intensity (normalized as total abundances) of the common formulas normalized as total abundances. As a result, density surfaces are obtained for the entire molecular assemblages in the *x*, *y* plane defined by atomic ratios H/C and O/C ratios. The surface density plots are calculated for mean values of different samples.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was carried out, comparing means using Tukey test, *P* = 0.05 using Statgraphics Centurion XV software, to assess significant differences among SWR values. Partial least squares (PLS) regression, by the ParLeS software (Viscarra Rossel, 2008), was employed to generate a statistical model to predict the persistence of SWR and the fire-induced changes, i.e., to explore the utility of the molecular descriptors (1221 common CHO compounds) used as independent variables to predict the dependent one (WDPT values). The root mean squared error (RMSE) and the Akaike's information criterion (Akaike, 1974) were employed to choose the minimum number of latent variables (LVs) of the PLS model. Spurious models due to overfitting were detected and discarded after comparing PLS models calculated with the fully randomized dependent variable (WDPT). The different validation methods would confirm that the common CHO compounds are valid predictors of the WDPT values in the case of obtaining a significant (*P* < 0.05) forecast model.

2.6. Assessment of SWR biomarkers

To analyze the chemotaxonomic value of the molecular markers relevant to SWR persistence levels, new van Krevelen graphs were drawn comparing the average molecular composition of a discrete number of samples grouped according to the different criteria: [1] molecular assemblages from burned and unburned scenarios, [2] comparisons between different particle size fractions, and [3] differences in terms of hydrophobicity degree. The resulting van Krevelen plots after subtracting the normalized values of the sample sets (with positive and negative values) are useful to compare the molecular composition of the different samples. In addition, the Student's-*t*-test value was performed on the relative concentration of organic compounds, to evaluate the significance of the differences in the percentages of each compound between the groups of samples established according to the 3 criteria. The *t*-Student was calculated using the function = T.TEST (array1, array2, tail: 2, type: 3) of Microsoft Excel 365. The chemical compounds significantly different (*P* < 0.15, *P* < 0.10, and *P* < 0.05) are represented as a van Krevelen plot in the form of superimposed contour diagram on

the density map where the coloured levels indicate the different concentration of the groups of compounds (Martins et al., 2020).

3. Results and discussion

3.1. Soil water repellency

The one-way ANOVA of the WDPT values indicated that there were 4 different classes of SWR persistence (Fig. 1). The majority of burned (B) and unburned (UB) particle size fractions under MN canopy displayed the lowest WDPT level, while the soils under QS canopy showed the highest level. In fact, Jiménez-Morillo et al. (2017), comparing particle size fractions in soils affected and non-affected by wildfire under QS canopy, found that the SWR was directly correlated both with the amount of SOM, as well as the amount of extractable lipids, and the molecular composition of the extractable lipids. In this work, Jiménez-Morillo et al. (2017) observed a significant correlation between the proportion of long chain fatty acids and the SWR persistence. However, in this work, there were two unexpected behaviours: i) both B fractions of QS present lower values than their UB counterparts; and ii) the WDPT value of BMNFF is similar to the rest of soil samples under QS canopy. In the former case, in a recent research article, the authors showed as the WDPT value decreased in the BQSCF sample due to the elimination of an organic matrix, which consisted mainly of degraded organic matter, plant issues, and microbial structures, including extracellular polymeric substances (Jiménez-Morillo et al., 2017). These facts are consistent with the classical literature (DeBano, 2000) that indicates a decrease in SWR when soils experience temperatures higher than 280 °C, which should be an expected effect of condensations or fire-induced changes in the molecular composition of hydrophobic surfaces that remain stable at lower temperatures.

3.2. Molecular composition of SOM

The use of ultra-high resolution mass spectrometry has generated more than 7000 different chemical formulas for each analysed SOM sample. Nevertheless, in this work, only the compounds (1221) that are common to the 8 analysed samples, with a molecular weight ranging between 289 and 617 Da and composed by C, H and O, were considered. In this case, the H/C and O/C values of all common compounds identified ranged 0.4–2.0 and 0.1–1.2, respectively (Fig. 2). Therefore, there are compounds in the different families proposed by Kim et al. (2003), which include lignin, tannins, carbohydrates, proteins, lipids, aromatics, and condensed aromatics.

Fig. 2 shows the van Krevelen plots (surface density plots of the abundance of the compound families with different atomic ratios) represented as contour diagrams formed by the 1221 common CHO compounds. Despite the overall similarity of the contour plots corresponding to the different particle size fractions (i.e., the main groups of compounds in the different areas of the Krevelen graph are essentially the same for all samples), quantitative differences are observed in the magnitude of compounds in well-defined regions of the plot. A remarkable proportion of lignin-like compounds (H/C: 0.6–1.5; O/C: 0.1–0.6) characterized the coarse fraction of the soils unaffected by fire under QS canopy (Fig. 2A), while the corresponding fine fraction is composed mainly of lipid (i.e., H/C: 1.7–2.3; O/C: 0.0–0.2) and protein-like molecules (H/C: 1.5–2.3; O/C: 0.1–0.6).

The fire-affected samples (BQSCF and BQSFF) showed different compositional families. In the coarse and fine fractions there was a relatively high proportion of aromatic (H/C: <0.6–1.0)/condensed aromatic-like (H/C: <0.5–0.8) compounds.

Several researchers (e.g., Bird et al., 2000; Nocentini et al., 2010; Skjemstad et al., 1996) have reported a remarkable proportion of pyrogenic organic compounds in fire-affected particle size fractions of soil. Charred litter (black carbon, “BC”) particles are concentrated either in the coarse or fine fractions depending on the BC particle size. Alternatively, the presence of aromatic/condensed-like molecules in fine fractions may also be due to the chemical alteration and fragmentation of SOM in coarse fraction, which are transferred to the fine one (Jiménez-Morillo et al., 2018, 2020a) or to the fact that condensed aromatic compounds in soil can originate from humic substances or DOM via non-pyrolytic means (Chen et al., 2014; Waggoner et al., 2015). In the case of MN canopy (Fig. 2B), the surface density van Krevelen diagrams showed conspicuous differences compared to other samples. All diagrams displayed a relative high presence of aromatic/lignin-like compounds. However, there are several minor specific changes. The existence of a high proportion of hydrophobic compounds, such as lipids and condensed aromatic compounds, in the BMNFF could explain why this sample showed a WDPT value similar to the particle size fraction under QS canopy. Several researchers have observed the same trend in the fire-affected fine fractions (Jiménez-Morillo et al., 2016b, 2018). In any case, the sole FT-ICR/MS data do not allow us to fully explain the variability of SWR in the studied samples. That is the case with QS samples being less repellent in UB condition, compared to the MN samples, where an increase in SWR was instead observed from UB to B. In particular, the speciation of lipids in the soil organomineral matrix is an aspect that should be the subject of further studies, since several investigations (De Blas et al., 2010) showed that the extraction of free lipids

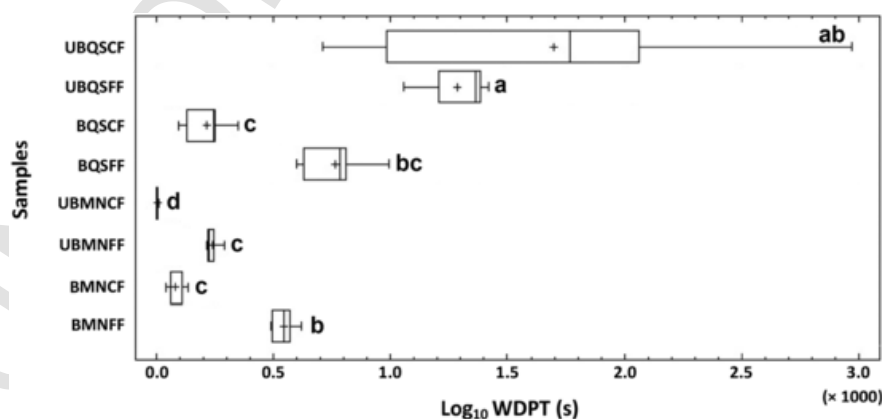


Fig. 1. Boxplots of the WDPT values ($N = 4$) of particle size fraction of burned and control soils under cork oak (QS) and Monte negro (MN) canopies. Boxplots display the ranges, lower and upper quartiles (Q1, Q4, and the median). The crosses inside the boxes represent the mean of data. Boxplots with different letters indicate significant ($p < 0.05$) difference. Unburned QS coarse fraction (UBQSCF); unburned QS fine fraction (UBQSFF); burned QS coarse fraction (BQSCF); burned QS fine fraction (BQSFF); unburned MN coarse fraction (UBMNCF); unburned MN fine fraction (UBMNFF); burned MN coarse fraction (BMNCF); and burned MN fine fraction (BMNFF).

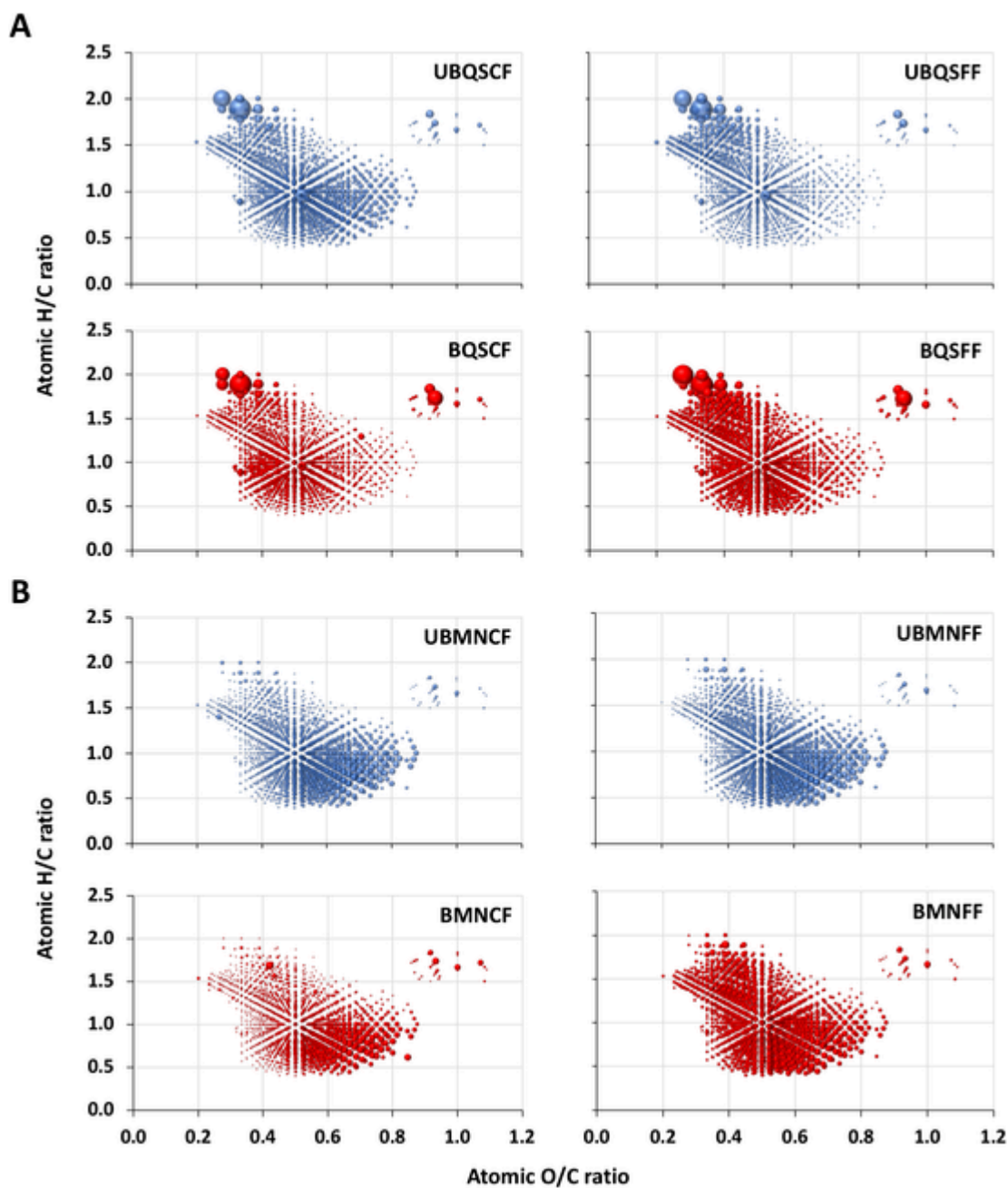


Fig. 2. van Krevelen diagram of common CHO compounds present in each soil samples collected under cork oak (*Quercus suber*, QS) forest and Monte negro shrubland (MN). Blue bubbles correspond to unburned (UB) samples, while red ones correspond to burned (B) samples. CF and FF refer to coarse and fine fractions, respectively. Sample labels refer to Section 2.1 “Sampling sites”. The bubbles diameter is related to the relative proportions of each compound. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from the soil with solvent organic compounds and even the removal of “fixed lipids” by successive acid and alkaline treatments only eliminates a part of the hydrophobicity of the soil, which seems to be largely due to changes in the surface properties of the macromolecular SOM.

3.3. Forecasting WDPT model

Fig. 3 shows the comparison of cross-validation plots generated in the PLS models from both the experimental and randomized WDPT values. The value of RMSE and AIC suggest that the best model, using the common compounds as independent variables, contain up to 6 LVs (Fig. 3 a,c). To confirm that the PLS model was not overfitted (i.e., that a greater number of LVs had been included than adequate), the Akaike criteria and the RSME values were considered (Viscarra Rossel, 2008)

and this was further confirmed by verifying that the model was no longer significant ($P = 0.932$; $R^2 = 0.133$) when it was repeated with randomized values of the dependent variable (Fig. 3f). In contrast, the observed vs predicted validation plot from experimental WDPT values did show a highly significant correlation ($P = 0.006$) with a value of $R^2 = 0.742$. This suggests the possibility of predicting the persistence of SWR from the molecular composition of the SOM defined by the 1221 common compounds.

Despite of the limited number of samples used in this work, the data analysis proposed to interpret the molecular composition of the SOM, defined by the 1221 common organic compounds, is presented as an exploratory study to examine their relationship with of SWR of soils affected by fire under two well-differentiated vegetation cover. The main interest of this pilot work, therefore, is to examine the potential of the

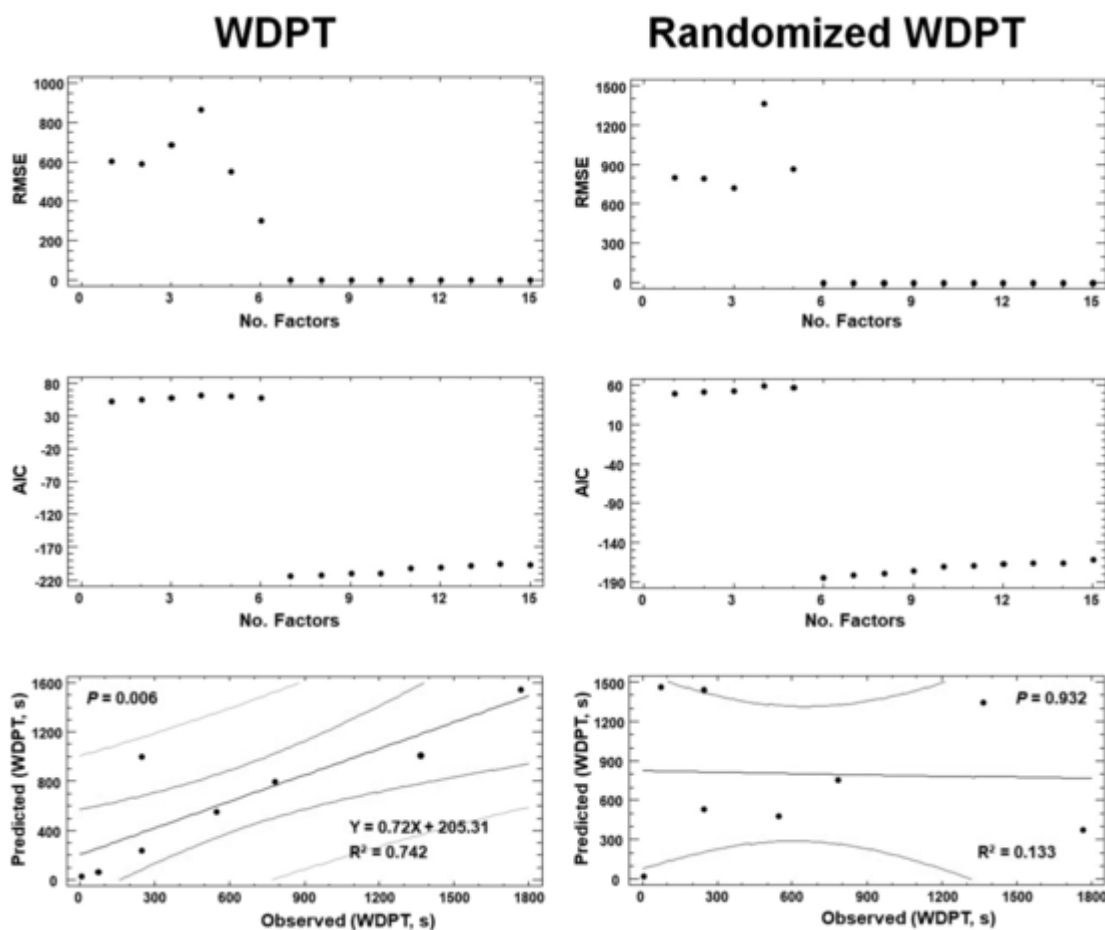


Fig. 3. Cross-validation plots (experimental vs predicted values) corresponding to Partial Least Squares (PLS) models to predict the SWR as measured by WDPT (s) values. Comparison of results of experimental values of WDPT (a, c, e) and randomized WDPT values (b, d, f). Root Mean Squared Error (RMSE) of WDPT (a) and randomized WDPT (b) respectively. Akaike Information Criterion (AIC) (c, d). Observed vs predicted WDPT values obtained by the PLS prediction model for WDPT (e) ($R^2 = 0.6799$) and randomized values of WDPT (f) ($R^2 = 0.0084$) using 5 latent variables or factors suggested by the RMSE and AIC values.

information provided by the FTCIRMS in relation to the SWR, based on a novel methodology that can be applied to extensive projects using a greater number of samples in other scenarios.

3.4. SWR biomarkers

In order to identify a series of SWR persistence biomarkers based on the 3 above-mentioned criteria, the presence or absence of the main families of compounds was compared using new subtracted surface density maps showing the comparative increase or decrease of specific organic families (Fig. 4). This methodological approach has been useful to identify molecular biomarkers in soils and other organic matrices (Almendros et al., 2018a; Jiménez-González et al., 2020). In addition, the *t-Student* test was used to determine the regions significantly different to 85%, 90% and 95% levels ($P < 0.15$, $P < 0.1$, and $P < 0.05$, respectively), and the results were plotted on the van Krevelen plots as contour maps (Fig. 4). In these combined plots, the coincidence of areas of the contour plot (showing significance levels) with those in the subtraction map areas indicates the family of compounds that may be used as SWR molecular markers according to each of the three criteria.

3.4.1. First comparison criterion: Differences between the molecular composition of soils affected or not affected by fires

Fig. 4A shows the most diagnostic compounds related to the hydrophobicity of burned and unburned soil samples. The contour van Krevelen maps corresponding to this criterion displays the existence of significant (95%) contributions of aromatic/condensed compounds

with a relative depletion in O ($O/C < 0.54$) in soil samples affected by fire ($0.5 > H/C < 1.2$, and $0.16 > O/C < 0.50$). On the other hand, and, at a lower level of significance, the molecular assemblages in soil samples from unburned areas were dominated by tannin-like and oxygen-containing aromatic compounds ($O/C > 0.54$). The above fire-induced changes in SOM were also reported by Knicker (2011), evidencing defunctionalization of oxygen-containing (carboxyl and hydroxyl) groups of lignin/tannin-like compounds with heat, while no variation in their aromaticity index was observed (no changes in H/C axis). This observation may be associated with a decrease in the colloidal properties of the SOM with a concomitant increase of hydrophobicity for fire-affected samples (Almendros et al., 2018a). Therefore, in fire-affected soil samples, aromatic/condensed compounds may be used as potential biomarkers of SWR persistence. In contrast, soil samples from areas unaffected by fire displayed a significant proportion of aromatic/lignin-derived compounds, which may indicate their potential as possible SWR biomarkers in the soil samples unaffected by fire. At this point, SWR should be addressed as an emergent feature ruled not only by SOM molecular composition, but also by its structure, speciation and surface properties that, equally, may impact the formation of hydrophobic coatings (de Blas et al., 2010)."

3.4.2. Second comparison criterion: differences in molecular composition as a function of soil particle size

The van Krevelen surface density plot obtained by subtracting the values of the proportions of the different compounds in samples of different particle size (Fig. 4B) showed statistically significant differences

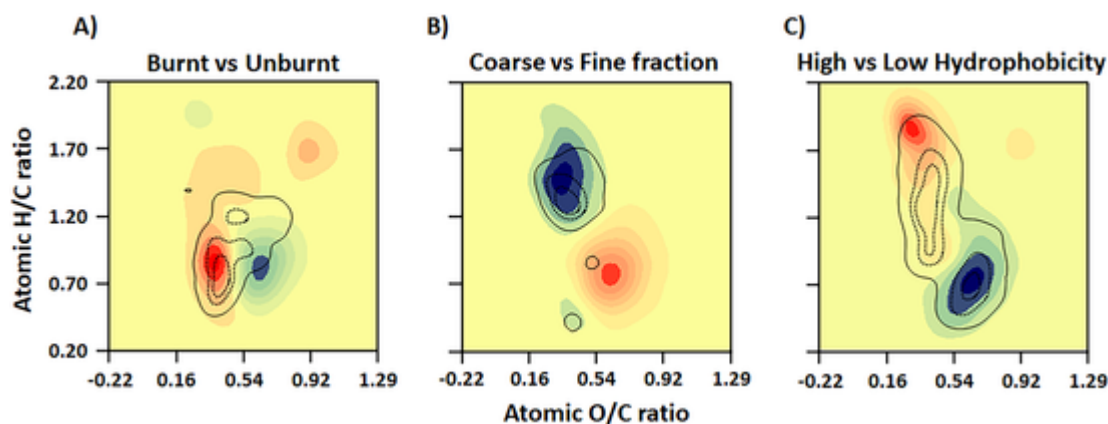


Fig. 4. van Krevelen subtraction density plots illustrating the difference between the molecular composition (and significance levels) of the soil organic matter in scenarios influenced by different factors: A) Burnt (red) vs Unburnt (blue) samples, B) Coarse (green) vs Fine (purple) fractions, and C) High (red) vs Low (blue) hydrophobicity. The common compounds were represented in the space defined by their atomic H/C and O/C ratios. Compounds significantly correlated with the SWR were represented by a contour plot superimposed on the subtraction plot (the most external contour indicates $p > 90\%$, and the internal contour indicates the $p > 95\%$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

($P > 95\%$) between coarse and fine fractions. In fact, the overlap of the contour map showing Student- t values with the map showing subtraction values between compound concentration in sets of samples with different particle sizes showed a significant contribution from lipids and highly-condensed compounds in fine soil fractions (red clusters). This may be relevant with respect to water repellency of soils, since several works have suggested the remarkable contribution of lipid-like compounds in SWR (Atanassova et al., 2017; De Blas et al., 2013; Ma'shum et al., 1988), including macromolecular aliphatic constituents of vascular plants (Mao et al., 2014). Fig. 4 suggests how the factors associated with repellency in the soils under study, especially under QS vegetation, are different depending on the particle size: i.e., molecular constituents of biomass in the case of coarse fractions, or condensed aromatic constituents in the case of fine fractions. In fact, Almendros and González-Vila (2012) indicated the possibility that in the soil fractions strongly affected by fire, in which most of the thermolabile lipid products have been destroyed, water repellency may be associated with strongly condensed aromatic compounds in the form of black carbon, where the strong SWR is due to the loss of oxygen-containing hydrophilic groups.

3.4.3. Third comparison criterion: differences in molecular composition depending on the levels of soil hydrophobicity

Fig. 4C shows the main family of biogeochemical compounds (with markers of the persistence of hydrophobicity) whose abundance is significantly different in soils with extreme levels of SWR. The molecular composition of highly hydrophobic samples (red) shows significantly higher proportions of lipid-like compounds ($1.7 < H/C < 2.7$; $0.0 < O/C < 0.2$) as well as of lignin-derived compounds ($0.6 < H/C < 1.5$; $0.1 < O/C < 0.6$). Several authors have reported the typical correlation between SWR persistence and the quantity and quality of lipid-like compounds (González-Peñaloza et al., 2013; Lozano et al., 2013; Mao et al., 2015), highlighting the fact that the degree of maturity of the SOM may have a greater influence on the SWR than its total content (Teramura, 1980). In this sense, it seems well established that the composition of the organic matter contributed by the different types of vegetation is important in explaining the variability of the SWR (Arcenegui et al., 2008). On the other hand, Fig. 4C shows that, in all the samples, low hydrophobicity (blue) is also accompanied by high values of aromatic-type compounds ($H/C > 0.7$) with significant proportion of oxygen-containing groups ($O/C > 0.5$). Comparing the set of the three graphs in Fig. 4C illustrated that SWR can be significantly associated with different structural aspects, depending on the fraction or type of

soil under consideration, in some cases consisting of hydrophobic coatings, or of defunctionalized condensed aromatic matrices, or transformed forms of lignin not incorporated into the soil matrix.

4. Conclusions

Comparison of the abundances of the 1221 compounds common to all the samples studied, between samples with different hydrophobicity, has allowed us to recognize significant differences that could help to explain the different water repellency of the corresponding soils. In particular, significant differences were found between the molecular composition of the SOM of the studied soils, which made it possible to establish diagnostic compounds for different groups of soils classified according to 3 criteria: i) burned vs unburned samples, ii) coarse vs fine fractions, and iii) high vs low hydrophobicity. Under the first criterion, the molecular composition of samples from burned areas is significantly enriched in aromatic and condensed compounds with a low content of oxygen-containing groups. However, aromatic formulas corresponding to low number of rings and tannin-like compounds, suggesting weakly transformed lignin in SOM, were the dominant organic families in samples from the fire-unaffected areas. Based on the second criterion, the presence of lipid compounds was the most characteristic of fine soil fractions. Finally, lipid structures were also found the most diagnostic compounds for samples with greater repellency (criterion 3).

The combination of the FT-ICR/MS with graphical-statistical treatments is a powerful approach with great potential for exploratory analysis of the complex factors that result in changes in the water repellency of soils after a wildfire event. The combination of the FT-ICR/MS with graphical-statistical treatments is a powerful approach with great potential for exploratory analysis of the complex factors that result in changes in the SWR of soils after a wildfire event. In fact, the results illustrated that the present research presents a fundamentally methodological interest in future research with large number of samples, facilitating data reduction and the perceptual interpretation of complex molecular assemblages.

This exploratory model work may be helpful to manage the SOM quality of fire-affected soil samples under two type of vegetation cover. In addition, this approach gives valuable information about the molecular alteration of natural and anthropogenic factors on the SOM and their impact on the ecosystem.

Uncited references

Almendros, 2008

Martins et al., 2020
Wu et al., 2020

CRediT authorship contribution statement

Nicasio T. Jiménez-Morillo: Validation, Formal analysis, Visualization, Investigation, Resources, Funding acquisition, Writing – review & editing. **Gonzalo Almendros:** Conceptualization, Methodology, Resources, Supervision, Validation, Formal analysis, Visualization, Writing – review & editing. **Ana Z. Miller:** Validation, Formal analysis, Visualization, Investigation, Funding acquisition, Writing – review & editing. **Patrick G. Hatcher:** Validation, Formal analysis, Visualization, Investigation, Supervision, Writing – review & editing. **José A. González-Pérez:** Conceptualization, Methodology, Resources, Funding acquisition, Supervision, Validation, Formal analysis, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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