FIRE EFFECTS ON C AND N ISOTOPIC COMPOSITION IN PLANT BIOMASS AND SOIL: BULK AND PARTICLE SIZE FRACTIONS.

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Abstract

This work studies carbon (C) and hydrogen (H) isotope composition of plant biomass and soil organic matter (SOM) in an attempt to assess both, changes exerted by fire and possible inputs of charred materials to the soil after a wildfire. Isotope composition of bulk soil, soil particle size fractions and biomass of the dominant standing vegetation in the area (*Quercus suber*) from Doñana National Park (SW-Spain) were studied by isotope ratio mass spectrometry (IRMS). SOM C isotope composition indicates the occurrence of two SOM pools with different degree of alteration. Coarse soil fractions (>0.5 mm) were found ^{13}C depleted with δ^{13}C values close to those in leaf biomass, pointing to a predominance of poorly transformed SOM. Conversely, fine fractions (<0.1 mm) were found enriched in ^{13}C as corresponds to a more humified SOM. The fire produced no changes in this trend, although a consistent ^{13}C enrichment (c. 1 ‰) was observed in all soil fractions with decreasing size. Concerning H isotopes, the coarse fractions (>0.5 mm) displayed significant lower δ^{2}H values than the intermediate and fine ones (<0.5 mm), again similar to those in leaf biomass (c. -80 ‰), whereas the fine fractions were found deuterium (^{2}H)-enriched with significant higher δ^{2}H values (c. 50 ‰), suggesting physical speciation of H depending on soil particle size. The fire produced a significant ^{2}H depletion (Δ^{2}H c. -10 ‰) in the finer fractions (<0.1mm). The study of stable isotope analysis added new information and complements the results obtained by other proxies to better understand the effect of fire on SOM.

Keywords: Charred biomass, δ^{13}C, δ^{2}H, Isotope fractionation, Mediterranean soil, Sieved soil fractions, Soil organic matter, Wildfire

1 Introduction

Fire is widely considered as one of the main disturbing factors in terrestrial ecosystems (Zavala et al., 2014). Mediterranean ecosystems, mainly dominated by dry and hot summers, are particularly prone to fire effects (Moreno and Oechel, 1995; De la Rosa et al., 2008). On average up to 50,000 forest fires per year are recorded in Mediterranean countries, affecting approximately 400,000 ha (San-Miguel-Ayanz et al., 2013). Nevertheless, due to global warming and climate change, in the next years, these numbers are expected to increase (Turco et al., 2014; Kalabokidis et al., 2015).

Fires produce changes in water dynamics, soil, flora, fauna and air (Neary et al., 2005). It is widely considered that fire exerts two main types of impacts on soils: i) direct impacts through the combustion of vegetation and chemical alteration of soil organic matter (SOM) and other impacts on physical and chemical properties; and ii) indirect effects, such as the additions and presence and of charred biomass and ash and the erosive processes and soil losses occurring in steep slope areas that are concomitant to changes in soil permeability, water repellence and water holding capacity (González-Pérez et al., 2004; Bodi et al., 2014; Zavala et al., 2014).

Usually, the effects of fire are only noticeable in the uppermost centimetres of the soil profile, with little or non-effect on deeper layers (Aznar et al., 2016). This fact is due to the remarkable heat insulator character of soils (DeBano et al., 1998; Badía et al., 2014). However, this thin shallow layer is where the largest amount of organic material is stored in most soils. Wildfires produce both, quantitative and qualitative changes to SOM (González-Pérez et al., 2004) and in related properties such as soil aggregation (Mataix-Solera et al., 2011; Jiménez-Pinilla et al., 2016). However, these transformations and their extent largely depend upon fire severity (Santín et al., 2016; Francos et al., 2018; Pereira et al., 2012) and in turn on complex soil and terrain

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physicochemical factors (the previous land uses, climatic conditions prior and during the fire, geomorphology and slope, temperature, amount and type of fuel, type of ecosystem and season) (Certini et al., 2005; De la Rosa et al., 2008; Pereira et al., 2012). Alterations of SOM quality are owed to lose and/or modification of labile organic compounds (e.g. removal of carbohydrates and defunctionalization of SOM fractions), as well as to additional inputs of partly burnt material (char and black carbon (BC)) from the vegetation and soil biomass (De la Rosa et al., 2018, 2019; Jiménez-Morillo et al., 2018).

The study of the natural abundance of light elements stable isotopes has taken great importance to increase the knowledge of ecosystems functioning (Michener and Lajtha, 2007). Particularly C isotope analysis has been widely used to investigate the sources, evolution and dynamics (including turn-over time) of organic materials in soils (Balesdent and Mariotti, 1987; Camino-Serrano et al., 2019). This is primarily based in that plants (autotrophic organisms) with distinct photosynthetic pathways (C3, C4 and CAM) discriminate differently against the heavier isotope when fixing atmospheric CO_2 to produce biomass enriched in ^{12}C. Subsequent plant necromass transformations by the heterotrophic activity, usually produce ^{13}C enriched organic products (Brüggemann et al., 2011). However, other factors may also alter the isotopic composition of soil organic C such are wildfires. In fire-affected systems, this has been studied mainly in laboratory burning experiments or under a fire ecology point of view. Bird et al. (2000) investigated the effects of fire on soil organic C isotopic composition and concluded that this responds rapidly to changes in fire regime and that this is related to changes in soil texture, they observed $\delta^{13}C$ increases with soil size fractions in the range 5-2000 µm. Isotopic fractionation produced by severe burning (smouldering combustion) on biomass have been also observed and related to the destruction of isotopically heavy ^{13}C

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enriched cellulose and selective accumulation of ^{13}C depleted lignin compounds (Czimczik et al., 2002). Another source of C isotope fractionation after a forest fire has been linked to post-fire vegetation succession and changes in C3 and C4 photosystem plant species and to the type of fuel (Resco et al., 2011; Ascough et al., 2008). In any case, there is controversy about the origin of the different isotopic fractionation trends observed in the burnt biomass, either under natural or laboratory conditions. The current knowledge about the effect of fire on the different soil C pools due to inputs of charred and/or fresh biomass from the vegetation is still poorly understood. This gap in knowledge may be partly due to the high chemical complexity of SOM together with the huge variability of fire behaviour.

Large variation occurs also shows in the proportion of the two H stable isotopes (protium ^{1}H and deuterium ^{2}H) present in biomass. During evaporation, water molecules bearing the light (^{1}H) isotope are first released to the vapour phase, resulting into the concomitant increase in the proportion of the heavy (^{2}H) isotope in the remaining water (Sachse et al., 2012). In fact, research on SOM H isotope composition and its relation with the water cycle has served to enlighten past and modern climate changes (Yang and Leng, 2009; Négrel and Petelet-Giraud, 2011). In addition, H stable isotopes studies can provide information about water dynamics in the ecosystems through the soil-plant-atmosphere continuum (Gat and Matsui, 1991; Yepez et al., 2003). However, to the best of our knowledge, data of H isotopic composition have not been used before to assess alterations produced by fire on SOM nor in burnt/charred biomass.

In this work, we made a comprehensive study of the effect of fire in the isotopic composition of the major light elements (C and H) in organic matter. Burnt and unburnt foliar and cork biomass, bulk soil and soil particle size fractions, were studied. Both

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elements provide distinct information; whereas C isotopes are informative about biomass contributions to SOM and its evolution status, H provides information about water pools and dynamics. This study will add new information and complement the results obtained by other proxies. Therefore, our working hypothesis is that with this approach, we will achieve a better understanding of SOM composition and of the effect of a wildfire in the different soil size fractions and a better evaluation of fire effects on biomass and SOM.

2   Materials and methods

2.1   Sampling area

Soil samples were collected in the Doñana National Park (SW Spain), where the fire is a recurrent perturbation and the cause of severe impacts in ecosystem functions and in the spatial distribution patterns of vegetation (Granados, 1985). The sampling site (Fig. 1), known as 'Matasgordas' place (37.11636 ^{o}N, -6.43177 ^{o}W), was under Mediterranean climate with oceanic influence; mild temperatures and relatively high humidity together with an amount of rainfall compared to inland (Siljeström and Clemente, 1990). The average annual rainfall is 830 mm, with 77% of the precipitation concentrated between October and March and mean air temperature of 16,9 ^{o}C. The main characteristics of the soil can be found in Tables 1 and S1. Soil pH in the area ranges between 6 and 7, without carbonated material (Jiménez-Morillo et al., 2016a). The sampling point was affected by a high severity fire as assessed *de visu* (Úbeda et al., 2009) just after the fire in August of 2012. The soil type is Arenosol (IUSS Working Group WRB, 2015) with more than 99% of aeolian sand (Holocene) covering gravels and other sandy sediments (Pliocene-Pleistocene). This relatively non-complex soil was considered an advantage for this study, as homogeneity of substrate limits the number

of variables that may affect the isotope composition of the SOM and helps to better understand the specific effects of forest fires in soil.

Composite soil samples were collected two years after the fire in October 2014 under the canopy of cork oaks (*Quercus suber*; QS) in a burnt (B) area and as a control in an adjacent unburnt (UB) one with no recent histories of forest fires, under the same vegetation cover, physiographic and climatologic characteristics. The sampling was done only once and the results correspond to a single-sampling. Each composite soil sample was obtained by combining five sub-samples (0–30 mm depth) collected after removing the uppermost litter layer within a circular area of approx. 20 m^2 under the cork oak trees. At the same time, five leaf and cork biomass samples were directly collected from well-developed trees in the B and UB sites and combined in two composite biomass samples. Plant and soil materials were transported in glass containers, to avoid contamination from plastic containers. Samples were air-dried under laboratory conditions (25 ^{o}C and *ca.* 50% relative humidity) during one week. Soil samples were sieved (< 2 mm) to discard gravel and litter fragments. Each bulk soil sample was further dry-sieved into six sub-fractions; two that can be considered as coarse (2–1, 1–0.5 mm), two intermediate (0.5–0.25, 0.25–0.1 mm) and two fine soil fractions (0.1–0.05, < 0.05 mm). For isotope analysis, each sample, including bulk soil, soil size fractions, leaf and cork, were finely ground and homogenized using a planetary mixer ball mill (Retsch GmbH Mod. MM 400, Haan, Germany).

2.2 Soil characterization

Soil texture analysis was performed after confirming the absence of $CaCO_3$ by adding a drop of HCl (1:1) and removing organic matter by oxidation (6% H_2O_2). The sand fraction was determined by sieving (0·05–2 mm) and clay (<0·02 mm) by the Bouyoucos method (Guitián and Carballas, 1976). Silt fraction (0·02–0·05 mm) was

determined by the difference between 100% and the proportion of clay and sand fractions. Soil pH was measured in the aqueous extract in de-ionised water (1:2·5 soil:water) at 25 °C. SOM content was analysed by dichromate oxidation of organic carbon (Walkley and Black, 1934) and ultraviolet-visible spectroscopy. Organic N was determined by Kjeldahl's method (Bremmer and Mulvaney, 1982).

2.3 Soil C and H stable isotope analysis (IRMS)

The C and H isotope composition (δ^{13}C and δ^{2}H respectively) of B and UB bulk soil and sieve fractions as well as of biomass were determined by elemental analysis/isotope ratio mass spectrometry (EA/IRMS). The EA/IRMS system consisted of a Flash 2000 HT elemental analyzer (Thermo Scientific, Bremen, Germany) with reactors for combustion (C, N and S determinations) and for pyrolysis (H and O determinations). The elemental analyzer is coupled by a ConFlo IV (Thermo Scientific) continuous flow open split interface to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific). Helium (He) was used as carrier gas at a flow rate of 80 mL min^{-1} for C and at 120 mL min^{-1} for H isotope analyses. The samples (0.5 to 5 mg) were weighed in metallic cups (IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany) made of tin for C and of silver for H analyses. The cups were closed, folded and pressed to a small size and loaded in a MAS 200R (Thermo Scientific) automatic sampling carrousel. Appropriate calibration standards were prepared in the same manner and placed within batches of samples.

For C isotope analysis the cups were flush-combusted and flush-reduced concurrently under a He carrier steam and oxygen pulse at 1020 °C in a quartz combustion reactor filled with chromium oxide (Cr_2O_3), silvered cobaltous/cobaltic oxide (Ag-Co_3O_4) and reduced copper (Cu). The combustion gases were dried through a 10 cm long glass column filled with anhydrous magnesium perchlorate ($Mg(ClO_4)_2$) and

then directed through a stainless steel gas chromatography column (3 m long, 4 mm in diameter) packed with Porapak stationary phase at 40 $^{\circ}$C for separation of CO_2, whose isotopic composition was analysed in the isotope ratio mass spectrometer. Pure CO_2 gas was inserted into the He carrier flow as pulses of the reference gas (250 mL min^{-1}).

For H isotopes the samples were analysed using a pyrolysis reactor. This consists of an outer ceramic (Al_2O_3) tube and an inner glassy carbon reactor tube filled with high-purity glassy carbon granulates and silver and quartz wool. The silver cups were dropped under steam of He into the pyrolysis reactor tube held at 1450 $^{\circ}$C. The pyrolysis evolved gases were passed through a 10 cm long glass column filled with a mixture of anhydrous magnesium perchlorate ($Mg(ClO_4)_2$), to dry the gas, and Carbo-sorb®, to trap any CO_2 generated during the pyrolysis reaction. The dry pyrolysis gases were directed through a stainless steel gas chromatography column (3 m in length and 4 mm in diameter) packed with Porapak stationary phase at 70 $^{\circ}$C for the separation of H_2, whose isotopic composition was analysed in the isotope ratio mass spectrometer. Pure H_2 gas was inserted into the He carrier flow as pulses of the reference gas (250 mL min^{-1}).

2.4 Calculation of stable isotope abundances

The stable isotope abundances are reported in the delta (δ) notation (i.e., $\delta^{13}C$ and δ^2H) in variations relative to the international standards Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW) (Coplen, 2011):

$$\delta^i E_{sample} = \frac{R\left(\frac{^i_a E}{^j_a E}\right)_{sample}}{R\left(\frac{^i_a E}{^j_a E}\right)_{standard}} - 1 \qquad \text{1)}$$

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Where R is the molar ratio of the heavy (^iE) to light (^jE) most abundant isotope of chemical element E (e.g. $^{13}C/^{12}C$, $^2H/^1H$). The δ values are reported in part per thousand (‰). The stable isotope standards values used in this work were those with certified δ recognized by the International Atomic Energy Agency (IAEA) (Valkiers et al., 2007). For C, we used cellulose (IAEA-CH-3, $\delta^{13}C$ = -24.7 ‰), sucrose (IAEA-CH-6, $\delta^{13}C$ = -10.449 ‰) and caffeine (IAEA-600, $\delta^{13}C$ = -27.771 ‰). For H, we used polyethylene (IAEA-CH-7, δ^2H = -100.3 ‰) and oil (IAEA-NBS-22, δ^2H = -120 ‰). The analytical standard deviation of $\delta^{13}C$ and δ^2H were typically lower than $\pm$ 0.1 and 1.0 ‰ respectively. Duplicate samples were analysed to obtain the sample average and standard deviations.

The isotopic fractionation (Δ) defined as the partitioning of stable isotopes between two substances A and B (Hoefs, 1997) was also calculated as in the next equation:

$$\Delta_{A-B} = \delta_A - \delta_B)\qquad\qquad\qquad 2)$$

2.5 Statistical analysis

One-way analysis of variance (ANOVA) with Tukey's *post hoc* testing was used to evaluate the differences in the C and H stable isotope composition between i) biomass between and among unburnt and burnt scenarios; and ii) soil samples (bulk) and soil particle size fractions between and among unburnt and burnt scenarios. The differences were considered statistically significant when the calculated P-value was less than 0.05. Statgraphics Centurion XVI statistical package was used for data analysis.

3   Results

The main characteristics of the burnt and unburnt bulk soil (texture, C and N elemental composition and C/N ratio) and of the corresponding soil particle size fractions are shown in Table 1 and S1.

3.1   Biomass C isotope composition (δ^{13}C)

The stable C isotope composition and isotope fractionation values after fire obtained for the main possible litter sources (cork bark and leaves) are shown in Figure 2. Both B and UB materials showed δ^{13}C values between -25 ‰ and -30 ‰, that is in the range for C_3 vegetation. Cork was significantly enriched in ^{13}C, as compared with leaves. Burnt leaves showed the lowest δ^{13}C value ($\delta^{13}C_{\text{B leaf}}$= -29.2 $\pm$ 0.3 ‰), in contrast to the burnt cork ($\delta^{13}C_{\text{B cork}}$= -25.3 $\pm$ 0.1 ‰) (Fig. 2 and Table S2). The fire produced a significant ^{13}C enrichment in the suberized cork tissue ($\Delta^{13}C_{\text{B cork-UB cork}}$ = 1.9 ‰) and a conspicuous, although not significant, ^{13}C depletion in leaf ($\Delta^{13}C_{\text{B leaf-UB leaf}}$ = -0.7 ‰) (Fig. 2).

3.2   Bulk soil and soil fractions C isotope composition (δ^{13}C)

The C isotope composition of SOM in bulk soil samples was not found significantly different between burnt and control soil samples (Fig. 3 & Table S3). Nonetheless, the UB bulk soil shows a lower ^{13}C composition than B bulk soil ($\Delta^{13}C_{\text{B soil-UB soil}}$ = 1.3‰) and this trend is consistent for all soil sieve fractions, with B samples ^{13}C enriched with respect to UB counterparts ($\Delta^{13}C_{\text{B-UB soil fractions}}$ > 0‰).

Significant differences were also observed among sieved soil fractions. In UB soil, the coarse sieve fractions (2-1 and 1-0.5 mm) showed the lowest δ^{13}C (-28.8 $\pm$ 0.1 and -28.7 $\pm$ 0.3 ‰, respectively), while, the fine fractions (0.1–0.05, < 0.05 mm) showed the highest (-27.7 $\pm$ 0.0 and -27.3 $\pm$ 0.0 ‰) (Fig. 3). This trend was similar for

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both B and UB soil samples ($\Delta^{13}C_{< 0.05\ mm–2–1\ mm} \approx 1.2$ ‰). On the other hand, the average values of $\delta^{13}C$ for B and UB soil sieve fractions, are coincident with that of bulk B and UB soil ($\delta^{13}C_{B\ soil}$ = -26.9 ± 0.5‰ and $\delta^{13}C_{B\ soil\ fractions}$ = -27.1 ± 0.7‰; $\delta^{13}C_{UB\ soil}$ = -28.2 ± 0.1‰ and $\delta^{13}C_{UB\ soil\ fractions}$ = -28.1 ± 0.6‰).

3.3 Biomass H isotope composition (δ^2H)

There were relevant differences between the stable H isotope composition and isotope fractionation values of the main burnt litter sources (cork and leaf) (Fig. 4 and Table S4). Unburnt leaves showed ^2H enrichment (δ^2H = -76.2 ± 0.5‰), with a marked difference with the unburnt cork tissue ($\Delta^2H_{UB\ cork-UB\ leaves}$ = -76.3‰). Burnt leaves and cork showed similar behaviour to that observed in the unburnt samples with small but no significant ^2H-enrichment ($\Delta^2H_{B\ cork-UB\ cork}$ = 3.5‰ and $\Delta^2H_{B\ leaf-UB\ leaf}$ = 4.9‰) (Fig. 4).

3.4 Bulk soil and soil fractions H isotope composition (δ^2H)

The UB bulk soil was found ^2H enriched when compared with B bulk soil ($\Delta^2H_{B\ soil-UB\ soil}$ = -20.1 ‰) (Fig. 4 and Table S5). In contrast to C isotope composition (section 3.2), sieve fractions did not show the same trend; the UB coarse fractions (2–1 and 1–0.5 mm) show relatively depleted δ^2H values (-93.3 ± 1.8 ‰ and -85.1 ± 2.2 ‰, respectively), whereas the intermediate (0.5–0.25, 0.25–0.1 mm) and fine soil fractions (0.1–0.05, < 0.05 mm) were relatively ^2H enriched with δ^2H values ranging between -51.4 ± 11.0 ‰ (0.25–0.1 mm) and -45.0 ± 1.5 ‰ (0.1–0.05 mm). The δ^2H values in intermediate and fine sieve fractions were not significantly different to that in UB bulk soil samples (-42.7 ± 0.6 ‰).

The B sieve fractions showed two different tendencies: i) ^2H enrichment in the coarse (2–1, 1–0.5 mm) fractions (-80.1 ± 8.6 ‰); and, ii) ^2H depletion in one

intermediate (0.5–0.25 mm) and fine soil fractions (0.1–0.05, < 0.05 mm) (-56.4 ± 3.1‰). For the intermediate (0.25–0.1 mm) fraction, the UB and B samples showed similar δ^2H values (-51.4 ± 11.0 ‰ and -52.6 ± 9.1 ‰ respectively). This, together with the relatively high dispersion observed in both samples, may indicate that this fraction represents an inflexion point where a shift in δ^2H composition occurs between B and UB sieve fractions (Fig. 5).

4   Discussion

4.1   Effect of burning in δ^{13}C of biomass and SOM

In this study, cork oak leaves showed significant lower δ^{13}C value than cork (Fig. 2). This is explained by the fact that− although photosynthesis in leaves is responsible for strong discrimination against the heavy ^{13}CO$_2$ molecules in the stomata− there is an additional known ^{13}C-enrichment (typically 1-2‰) that occurs during transformation and translocation of photosynthates from leaves to other plant tissues (O´Leary et al., 1981; O´Leary 1988; Marshall et al., 2007).

Burnt biomass (cork and leaves) showed different tendencies (see Fig. 2) with cork samples significantly ^{13}C enriched after the passage of fire. Fire may have a selective effect on cork by breaking the three-dimensional structure of the aliphatic domain of suberin. This thermal cracking of lipid moieties is a well-known fire effect on biomass (Jiménez-Morillo et al., 2016b) and may be producing BC particles with the concomitant quantitative enhancement of comparatively thermostable SOM (e.g., lignin) with higher δ^{13}C values than unburnt cork. In this respect, De la Rosa et al. (2008) showed that BC particles produced from the burning of biomass still retain a noticeable amount of lignin-like compounds.

Burnt leaf samples showed a significant ^{13}C depletion compared with UB samples. This could be caused by a combination of selective removal of thermolabile components and a direct chemical transformation increasing the proportion of unspecific aromatic compounds in the burnt residue. High temperatures may primarily affect the most thermolabile molecules like polysaccharides, which are known to be ^{13}C-enriched (Gonález-Pérez et al., 2016; Schleser, 1990). Further, combustion may induce defunctionalization of lignin-like SOM producing aromatic moieties (González-Vila et al., 2001; Jiménez-Morillo et al., 2018) and poly-aromatization processes, with the formation of double covalent bonds (C=C) and the production of ^{13}C depleted aromatics (Cachier et al., 1985; Krull et al., 2003).

The isotopic composition of the SOM seems also to be affected by the fire. The alteration status of SOM is also known to be reflected in its C isotope composition; fresh organic material is usually ^{13}C depleted compared with transformed/humified (more altered) one (Benner et al., 1987). Bird and Pousai (1997) suggested that at least part of the ^{13}C enrichment in the topsoil may be caused by stabilization of a proportion of microbial processed organic material. In previous works, we studied the chemical composition of the same soil samples using mass spectrometry techniques (Jiménez-Morillo et al., 2016b; 2018). We found that fresh material prevailed in the coarser SOM fractions (> 1 mm), whereas the fine fractions are comparatively richer in humic substances, with the presence of degraded lignin and microbial-derived SOM. Consequently, the different chemical composition and characteristics of SOM stored in soil fractions of different sizes may explain the different δ ^{13}C isotope composition between coarse and fine fractions.

This general pattern of δ^{13}C enrichment with decreasing soil particle size is also preserved in the burnt soil fractions (Fig. 3). These fractions showed a consistent ^{13}C

enrichment with consistently positive $\Delta^{13}C$ fractionation values ranging between 0.6 and 1.5 ‰. A possible reason may be the constant input of soot and BC particles from burnt aboveground vegetation with high $\delta^{13}C$ values (González-Pérez et al., 2004; Knicker et al., 2008).

In contrast with UB cork, the B cork studied in this work did not show a defined structure. The later included fine charred particles, black-coloured and spherical (soot). The B leaf tissue showed a defined three-dimensional structure, showing larger-sized particles. Therefore, after a fire and due to its smaller size, charred cork particles may have been preferentially associated to the fine soil fractions (< 0.25 mm) as previously suggested. On the other hand, the partly charred leaf larger particles may preferentially appear in the coarse fractions (> 0.5 mm). This is consistent with previous findings using mass spectrometry techniques (Jiménez-Morillo et al., 2016a, 2017, 2018).

Both B and UB bulk soil samples showed similar $\delta^{13}C$ values than the average of the different sieved soil fractions. Therefore, SOM in sieve fractions effectively reflects $\delta^{13}C$ composition of the bulk soil and small shifts in the composition of SOM in soil particle size fractions may affect the bulk soil stable C composition.

4.2 Effect of burning in δ^2H on biomass and soil

The values of δ^2H in plant tissues and soil are connected with the water sources (Ellsworth and Williams, 2007). The main sources of isotopic variation in continental water come from the isotopic variation in precipitation inputs and enrichments of the ^2H content in water from soil surface evaporation (Barnes and Allison, 1983) and leaves during evapotranspiration (Gonfiantini et al., 1965). In contrast, it is considered that there is no H isotope fractionation during absorption of soil water by plants through the roots (Dawson and Ehleringer, 1993; Ellsworth and Williams 2007). Organically bound

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H isotopes in plant tissues –particularly in alkyl chains- retain the isotopic composition of the water taken during its growth from the soil, which is also consistent with the δ^2H value of rainfall water (Dansgaard, 1964). Nevertheless, it is also well known that moisture in leaves is considerably ^{2}H enriched compared with precipitation water due to evapotranspiration (Gonfiantini et al., 1965). This may help to explain our findings in cork oak biomass with conspicuous δ^2H higher values for leaf biomass than for cork (Fig. 4).

Concerning the light δ^2H value found for cork tissue, this could be explained by its particular structure. Cork tissue to large extent consists of suberin, i.e., a biopolyester including long-chain aliphatic and lignin-like compounds (40% and 22% respectively, Pereira, 1988). Previous works have found that plant lipids and phenolic compounds display more negative δ^2H values than polysaccharides (the third more abundant compound in cork, 18%), and even than the uptake water by plant roots (Chikaraishi et al., 2004a, b). Although the fire did not affect largely the δ^2H signature of biomass samples, a small positive fractionation between B and UB samples was observed (Fig. 4). This fact suggests that other factors may be affecting the ^{2}H composition, such as the removal of a remaining fraction of light –deuterium depleted-, non-structural or unconfined water during burning (Chen et al., 2016).

With respect to bulk and soil fractions, it is known that fire is a disturbance factor on the soil hydrologic cycle, that affects a wide number of processes, such as rainfall interception, infiltration, water repellency, evaporation and soil water storage (Jordán et al., 2013; Zavala et al., 2014). Despite short-time impacts, reduction in plant cover and changes in soil surface structure may contribute to low evapotranspiration rates and increased soil water content (DeBano et al., 1998). Therefore, heavy H isotope content in burnt soils should theoretically be lower than in control, unaffected soils. This

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hypothesis could explain our findings concerning the negative fractionation values (-20.1 ‰) for H in bulk soil samples (Fig. 5). In relation to the observed trend in the sieved soil fractions, only one intermediate (0.5–0.25 mm) and the fine soil fractions (0.1–0.05, < 0.05 mm) exhibited the same trend than the bulk samples. In contrast, positive isotope H fractionation was observed in the coarser fractions (2–1 and 1–0.5 mm).

In general, sieved soil fractions displayed two ranges of δ^2H values, a low one for the coarser fractions (> 0.5 mm) and a high one for intermediate and fine fractions (< 0.5 mm). The former, with δ^2H values similar to that in leaves, may be associated with a predominance of fresh biomass as seen in Section 4.1. On the other hand, the enrichment in ^{2}H in intermediate/fine fractions may reflect the signature of soil water, not of biomass. After drying soil samples under laboratory conditions, only the gravitational (if any) and available water were likely to be removed. In contrast, hygroscopic water films with the same H isotope composition than meteoric water remains held by strong adhesion forces (Brook et al., 2010). In addition, water in the first millimetres of the soil profile is prone to further ^{2}H enrichment due to the intense evapotranspiration produced by grasses (Brook et al., 2003) which may explain in part the observed higher ^{2}H content in intermediate and fine soil fractions. Changes in this ^{2}H enriched water after the fire may also explain the significant decrease in δ^2H observed in the fine soil fractions (0.1–0.05, < 0.05 mm).

There is again a small but significant fractionation between B and UB soil samples (Fig. 5) with fire affecting differently the coarse and fine and fractions. The ^{2}H-enrichment registered for coarser fractions (> 0.5 mm) may be attributed, as previously, to the additional input of partially charred biomass, mainly from leaf tissue (with a higher particle size and heavier H composition). On the other hand, the divergent trend

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observed in medium-fine size fractions (< 0.5 mm) may be due to i) rupture of the absorption/evapotranspiration flow due to post-fire decreased vegetation cover and root depth and number; or ii) charred cork particles with low δ^2H values incorporated preferentially to the fine soil fractions during the post-fire period.

To the best of our knowledge, this is the first detailed study reporting the effect of a wildfire on the H and C isotope composition of SOM in different soil size fractions.

5   Conclusions

Soil C isotope composition largely depends on SOM maturity with a higher transformation degree observed in fine soil particle fractions (< 0.5 mm) whereas less altered organic matter is concentrated preferably in the coarser fractions (> 0.5 mm). In general, fire lead to a ^{13}C enrichment of soil SOM and soil particle sieve fractions. On the other hand, different water pools in the soil may be related to the differences observed in δ^2H that is reflected in the significant lighter composition of coarser fractions (> 0.5 mm) compared to the intermediate and fine (< 0.5 mm). Fire effectively lead to significant ^{2}H depletion, but only in the fine soil fractions (< 0.1 mm). This may be caused by shifts in SOM circulating water pool due to fire temperature and heating time, rather than to shifts in SOM composition.

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Tables

Table 1: Characterisation of unburnt and burnt bulk soil samples (<2 mm) under *Quercus suber* canopy.

	Sand (%)	Silt (%)	Clay (%)	pH	C (%)*	N (%)*	C/N
Unburnt	99.2	0.0	0.8	6.4	6.70±1.27	0.98±0.10	6.83
Burnt	99.2	0.1	0.7	6.3	10.80±1.05	1.30±0.06	8.33

*Mean ± standard deviation

Figure captions

Figure 1: Localization of study area in Doñana National Park (Spain) and sampling scheme. Image from Google Earth.

Figure 2: Cork oak (*Quercus suber*) biomass (cork and leaf) C isotope composition (δ^{13}C). Error bars indicate STD. Numbers below the plot indicate C isotope fractionation (Δ^{13}C) between burnt and unburnt samples. Different letters indicate significant differences between samples in each specific scenario. Significant differences between burnt and unburnt soil sample within the same biomass type is indicated with "*".

Figure 3: Bulk soil and corresponding soil particle size fractions C isotope composition (δ^{13}C). Error bars indicate STD. Numbers below the plot indicate C isotope fractionation (Δ^{13}C) between burnt and unburnt samples. Different letters indicate significant differences between samples in each specific scenario. Significant differences between burnt and unburnt soil sample within the same soil fraction is indicated with "*".

Figure 4: Cork oak (*Quercus suber*) biomass (cork and leaf) H isotope composition (δ^{2}H). Error bars indicate STD. Numbers below the plot indicate H isotope fractionation (Δ^{2}H) between burnt and unburnt samples. Different letters indicate significant differences between samples in each specific scenario. Significant differences between burnt and unburnt soil sample within the same biomass type is indicated with "*".

Figure 5: Bulk soil and corresponding soil particle size fractions H isotope composition (δ^2H). Error bars indicate STD. Numbers below the plot indicate H isotope fractionation (Δ^2H) between burnt and unburnt samples. Different letters indicate significant differences between samples in each specific scenario. Significant differences between burnt and unburnt soil sample within the same soil fraction is indicated with "*".

CONFLICT OF INTEREST STATMENT

FIRE EFFECTS ON C AND N ISOTOPIC COMPOSITION IN PLANT BIOMASS AND SOIL: BULK AND PARTICLE SIZE FRACTIONS.

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TO WHOM IT MAY CONCERN

Hereby, in the name of all authors of the above mentioned manuscript,

I DECLARE:

That there is no conflict of interests whatsoever.

José A. González-Pérez   Seville, 30th July 2020

CREDIT AUTHOR STATMENT

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Graphical abstract

Highlights:

- Particle size fractions $\delta^{13}C$ reflects SOM maturity status
- Particle size fractions δ^2H reflects different water pools in the soil
- Fire lead to a general $\delta^{13}C$ enrichment and δ^2H depletion in fine soil fractions
- $\delta^{13}C$ enrichment is related to changes in SOM composition after the fire
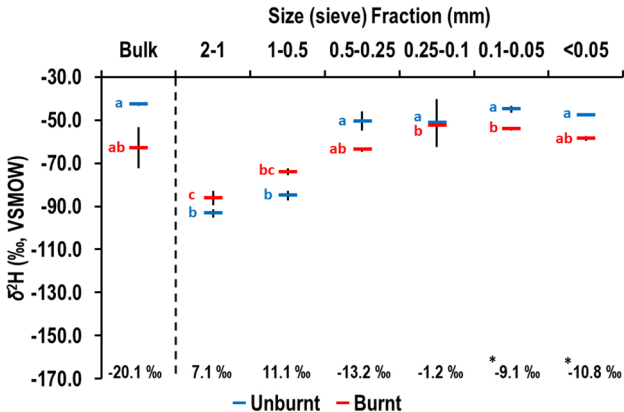- δ^2H depletion is caused by shifts in circulating water rather than to SOM changes
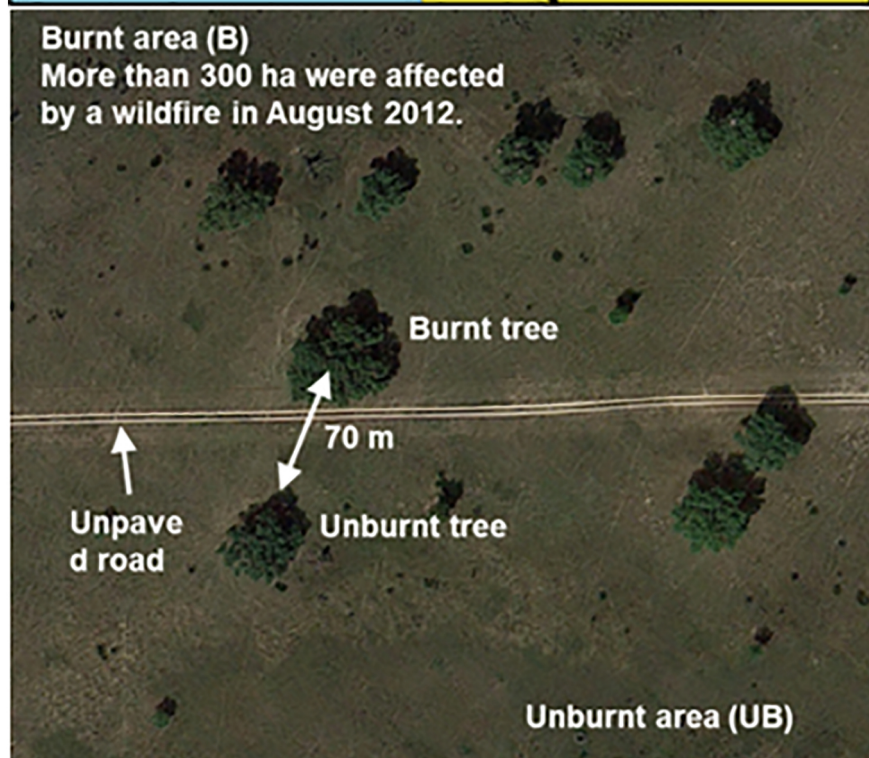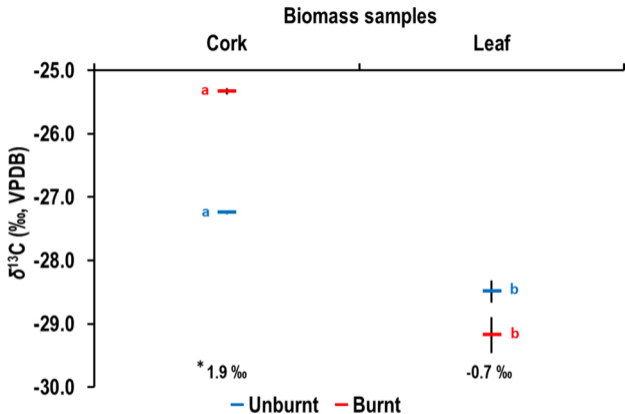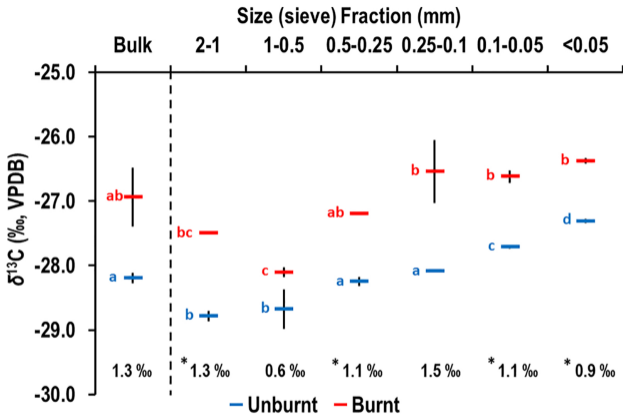
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5