

## TEMPERATURE-INDUCED CHANGES IN HUMUS QUALITY AND $\delta^{13}\text{C}$ SIGNATURES AS A PROXY INDICATOR OF SOIL BURN INTENSITIES AFTER FOREST WILDFIRES

*Irene Fernandez, Ana Cabaneiro*

Departamento de Bioquímica del Suelo, Instituto de Investigaciones Agrobiológicas de Galicia, Consejo Superior de Investigaciones Científicas (IIAG-CSIC) - Santiago de Compostela

### Abstract

Due to the increasing number and virulence of forest wildfires recently observed around the world, the establishment of a simple, accurate and reliable index that would correctly evaluate the fire effects on soil quality as a support for a suitable forest recovery management is becoming progressively more necessary. This objective is addressed here by using both  $\delta^{13}\text{C}$  isotope ratio mass spectrometry and traditional solvent fractionation methods (widely used to assess soil biogenic components or humus fractions) to quantify the temperature-induced changes in soil chemical and isotopic composition. Soil samples from the upper 5 cm layer of two Cambisols developed over granite under pine forest in the NW of Spain were heated in an oven under controlled conditions to attain moderate or intense soil burn severity levels by using two different temperatures (220°C or 350°C). Biochemical changes induced by the heating process appreciably differed according to the intensity of the temperature applied. Multilinear regression modelling not only showed a significant relationship between soil C isotopic signature shifts ( $\Delta \text{soil}\delta^{13}\text{C}$ ) with temperature increases but also revealed other key outcomes: i.e. >96 or >81% of its total variance can be predicted by changes in lignin or non-humified organic matter, respectively. Indeed,  $\Delta \text{soil}\delta^{13}\text{C}$  explained by itself  $\approx 60\%$  of thermal variance, pointing to the aptness of using  $^{13}\text{C}$  shifts as a valid index for soil burn severity estimation in wildfires.

**Key words:** controlled heating, edaphic thermal alteration, soil burn severity, fire effects, soil organic matter, stable C isotopes

### INTRODUCTION

The enormous social and environmental consequences of forest wildfires that are directly derived from the economic and ecological losses caused by the burning, or the posterior related effects, clearly justify the increase of interest observed during the last decades around any research field that encompasses the study of the impacts of the fire on diverse soil properties (Mataix-Solera et al., 2011; Stavi et al., 2017), especially those that deal with the effects on the soil organic matter (SOM) quality, due to its particular thermal vulnerability and its importance on the soil structure and fertility (Fernandez et al., 1997; Fernandez et al., 2005; Certini et al., 2011; Almendros et al., 2012; Merino et al., 2014) as well as its influence on the subsequent post-fire soil recovery rate (Fernandez et al., 1999; Jimenez-González et al., 2016). Countless difficulties for measuring the exact values of the temperature reached by the soil during wildfires, closely related to

their virulence or unpredictability in addition to other possible obstacles generated by safety and logistics aspects (Santín et al., 2016), are exceedingly common and are usually preventing the successful collection of thermal data. That is why many authors have tried to estimate *a posteriori* the fire intensity or the soil burn severity level by examining the fire-induced changes on several characteristics of the ecosystem (Amato et al., 2013; Zheng et al., 2016). Accordingly, some of the early attempts to indirectly determine the intensity of forest wildfires, in order to relate this intensity with the corresponding effects on the soil, were based on diverse physical characteristics such as the surface ash colour or the charred layer thickness. Afterwards, even with the apparent inaccuracy of these visual inspection methods, this approach proved to have a factual correspondence with the pyrogenic transformations caused by the fire (derived from the lower or higher fuel combustion completeness) and to be sustained on scientific evidences as currently endorsed by investigations on this subject (Bodí et al., 2014). Even so, some scientists convincingly highlight the fact that these types of methods are essentially subjective, having little precision too and hence little effectiveness in practice when trying to translate observations into numerical ratings of the fire intensity (Bodí et al., 2014).

Although, there is still confusion and ambiguity when predicting the ways in which environmental conditions influences fires effects and post-fire soil response according to the last bibliographical reviews on fire or burn severity, defined as the immediate and longer-term ecological effects of the fire (Keeley 2009; Morgan et al., 2014), many scientists are nowadays taking into account some burn severity classification for their research works on fire events (Jain et al., 2012; Fernández, Vega 2016; Whittier, Gray 2016; Quintano et al., 2017; Fernandez-García et al., 2018) some of which pay specific attention to the study of diverse soil characteristics in wildfires or experimental burning (Weber et al., 2014; Merino et al., 2018) or to the subsequent post-fire recovery process of the soil (Owen et al., 2017; Meng et al., 2018).

Analytical techniques such as different SOM solvent fractionation methods were traditionally and are still used to characterize the SOM structure and composition in burned soils (Fernandez et al., 1997; Mastrodonardo et al., 2015). More recently stable C isotope mass spectrometry has also been successfully utilized to monitor organic matter transformations in different compartments of the ecosystems (Fernández et al., 2004; Fernandez et al., 2006a, b; Mastrodonardo et al., 2015; Jiménez-Morillo, 2017) since isotopic techniques applied to diverse research fields can provide an integrated and quantitative view of chemical, biological and ecological interactions in nature (Griffiths et al., 1999; Fernandez et al., 2012a). Given their accuracy and efficiency, a large number of studies are now using several isotopic techniques, including the determination of the  $^{13}\text{C}/^{12}\text{C}$  ratio at natural abundance levels, to monitor the C cycle in a variety of biochemical processes, such as the photosynthetic fixation of atmospheric  $\text{CO}_2$  (Fernandez et al., 2005; Cabaneiro, Fernandez, 2015) or the decomposition of complex plant debris (Fernandez et al., 2003; Carrasco et al., 2017). Moreover, the proven fact that SOM biodegradative processes affect its  $^{13}\text{C}$  isotopic composition (Fernandez et al., 2003; Fernandez, Cadisch, 2003) is generating great expectations as regards to the application of C isotope mass

spectrometry in many studies aimed at assessing the changes in soils as a consequence of fire-induced alterations, opening opportunities to establish new indexes of soil burn intensity after forest wildfires that are able to better predict empirically observed post-fire SOM behaviours and hence to more precisely adjust to what will actually happens as a result of the particular characteristics of each forest wildfire (Merino et al., 2018).

Thus, the **aim** of the present study is to take advantages of the isotopic techniques sensitivity by carrying out  $^{13}\text{C}/^{12}\text{C}$  ratio determinations at natural abundance levels in order to evaluate the potential of using the fire-induced shifts of soil  $\delta^{13}\text{C}$  signatures as a proxy, objective and reliable soil burn intensity index. The expected outcomes would therefore contribute to a better evaluation of the SOM disturbances caused by wildfires by providing a rapid and easy tool, which could be useful for the optimization of silvicultural management practices oriented to post-fire recovery of damaged forest soils.

## MATERIAL AND METHODS

### Forest site description

The ecosystems selected for this study were two pine forests located in Galicia (NW Spain) with soils, developed over acidic bedrock (granite), that can be classified as Cambisols (IUSS Working Group WRR, 2014). Soil 1 (S1) was collected from a *Pinus sylvestris* L. forest, located at an altitude of 1740 m a.s.l. in the province of Ourense, with an annual rainfall of approximately 2000 mm and annual mean temperature 6°C (monthly  $\bar{T}_{\min} = -4^\circ\text{C}$  and  $\bar{T}_{\max} = 22^\circ\text{C}$ ). Soil 2 (S2) was collected from a forest with *Pinus pinaster* Aiton as the dominant tree species, located at an altitude of 190 m in the province of Pontevedra where the annual rainfall was approximately 1800 mm and the annual mean temperature 14°C (monthly  $\bar{T}_{\min} = 5^\circ\text{C}$  and  $\bar{T}_{\max} = 26^\circ\text{C}$ ). According to these different climatic conditions, the S1 undergrowth vegetation was mainly composed of *Chamaespartium tridentatum* (L.) P. Gibbs, along with *Vaccinium myrtillus* L., *Erica* spp and species of Poaceae; whereas in S2 it was mostly composed of *Ulex europaeus* L. and *Pteridium aquilinum* (L.) Kuhn, accompanied by diverse herbaceous plants.

### Controlled soil heating

After the removal of the whole litter and duff layers, the soil sampling was performed by assembling up to 50 random subsamples (totalizing >10 kg of each soil), samples being collected from the A horizon by taking only the upper 0-5 cm soil layer, because below this depth soils are rarely affected by natural fires (Raison et al., 1986; Badía et al., 2017). Soil samples were air dried, sieved (<4 mm), homogenised and stored in air-tight plastic bags before laboratory analyses.

The heating procedure was performed in the laboratory by using an oven with a timer and a heating rate programme. As a previous essay aimed at choosing the laboratory heating intensities at which SOM losses were comparable to those already reported for forest wildfires (Fernandez et al., 1999), a wide temperature interval was tested covering from 150°C to 490°C, in order to select those corresponding to fires of moderate or high

intensity levels. In all cases, the heating conditions applied were: a temperature increase of  $3^{\circ}\text{C min}^{-1}$  (to prevent the sudden combustion produced when reaching the soil ignition temperature) and 30 min of heating after reaching the final intended temperature inside the bulk volume of the soil. To minimize possible vertical thermal gradients, the thickness of the soil sample placed in the oven tray was less than 0.5 cm and soil temperature was controlled by using a thermocouple located inside each soil sample. In order to discern the heat-induced effects, unheated soil subsamples were always used as controls.

### **Soil characterization**

Methods described by Fernandez et al. (2006a) were used to determine soil pH, total C and alteration compounds, whereas total N was assessed by acid Kjeldahl digestion (Fernandez et al. 2012a, b). All results were expressed as means from at least three replicates on a dry soil basis ( $105^{\circ}\text{C}$ ).

### **SOM composition**

Since, conventionally, SOM can be systematized by using different chemical approaches according to the biogenic essence or acid/alkali solubility of the diverse complex fractions (i.e. soil organic substances of individual nature or humic compounds, Kononova 1982), two solvent fractionation methodologies were used to quantify the heat-induced changes in SOM quality.

Firstly, to estimate the biochemical components or soil organic substances of individual nature, SOM fractionation was performed using the Kononova's (1961) method, except for the hydrosoluble fraction that was extracted by refluxing with boiling water for 2 h, as recommended by Stevenson (1965). Thus, lipids were extracted with ethanol-benzene for 24 h in a Soxhlet; subsequently, holocellulose (cellulose+hemicellulose) was solubilized with  $\text{H}_2\text{SO}_4$  (72%), leaving lignin and mineral material in the solid remainder. The lignin content was calculated by difference from the final residue weight, after ignition in an oven at  $500^{\circ}\text{C}$  for 6 h.

For the second approach based on acid/alkali solubility of SOM, humic substances were extracted using a modified method of Duchaufour, Jacquin (1966), which was proven suitable for the study of organic matter in forest soils from the same geographic area (Fernandez et al., 2006a). After a densitometric removal of the unhumified SOM using a mixture of bromoform-ethanol with a density of  $1.8\text{ g cm}^{-3}$ , the fulvic acids (FA) and the humic acids (HA) fractions were successively extracted with 1%  $\text{Na}_4\text{P}_2\text{O}_7$  (pH 9.8) and 0.1N NaOH (pH 13) by shaking the corresponding soil-reagent mixture (1:100) for 1 h, followed by centrifugation (repeating the cycle until total extraction). The final residues, which constitute the humin fraction (H), were washed and dried (at  $40^{\circ}\text{C}$ ) for C content determination. The HA fraction was separated from the FA by precipitation with  $\text{H}_2\text{SO}_4$  (at pH 1) and the C content in these fractions was determined by using the modified Sauerlandt's method (Fernandez et al., 1997). The amounts of Fe and Al linked to FA in  $\text{Na}_4\text{P}_2\text{O}_7$  and NaOH extracts were assessed by atomic spectrophotometry. The SOM humification degree ( $\text{C}_{\text{FA+HA+H}}/\text{total C}$ ) and polymerisation index (FA/HA ratio) were also calculated.

### Stable isotope $^{13}\text{C}/^{12}\text{C}$ ratio mass spectrometry

Soil isotopic composition was determined using a FlashEA1112 elemental analyzer (ThermoFinnigan, Italy) coupled to a DeltaPlus isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) through a ConFlo II interface. As part of each analytical batch run, sets of international reference materials (NBS 22, IAEA-CH-6, USGS 24) were analyzed. The isotopic composition, based on the  $^{13}\text{C}/^{12}\text{C}$  ratio deviation from VPDB (Vienna Pee Dee Belemnite) reference standard, was expressed as  $\delta^{13}\text{C}$  signature according to the following equation (Eq. 1):

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3 \quad (1)$$

where  $R = ^{13}\text{C}/^{12}\text{C}$ . Replicate measurements carried out in order to establish the instrument precision for the isotopic determinations showed that the standard deviation (SD) for the  $\delta^{13}\text{C}$  analysis of the laboratory standard (acetanilide) was lower than  $\pm 0.15\text{‰}$  ( $n=10$ ).

### Statistical analyses

The results were statistically analysed by one-way ANOVA and Tukey test was performed to detect significant differences between means ( $P \leq 0.05$ ). Pearson correlation coefficients were determined in order to obtain possible relationships between variables. Multiple linear regression models were developed to predict the relative importance of the variables included in the models that were effective to describe soil burn severity levels. All statistical analyses were carried out using the 2016 version (v.24.0) of the IBM® SPSS® Statistics software package for Windows (IBM Corp., New York).

## RESULTS AND DISCUSSION

### Soil characteristics

The two forest ecosystems selected for this study are developed over sandy, unsaturated and acidic soils, with soil  $\text{pH}_{\text{H}_2\text{O}}$  lower than 4.5 and with strong potential acidities (Table 1a). Before heating, the main characteristics of both soils were similar to the results previously reported for pine forest soils from the same region (Fernandez et al. 2006a, b) with organic matter contents above  $100 \text{ gC kg}^{-1}_{\text{d.s.}}$  and  $5 \text{ gN kg}^{-1}_{\text{d.s.}}$ . The soil C-to-N ratio ranged from 14 to 19, indicating that their humus could be considered from a *moder* to an intermediate type between *moder* and *mull* in accordance with the temperate forest humus classification proposed by Duchaufour (1977). S1 exhibited a lower presence of alteration compounds than S2, especially for the content of extractable Al oxides (S1:  $5.7 \pm 0.1 \text{ gFe}_2\text{O}_3/\text{kg}_{\text{d.s.}}$  and  $6.6 \pm 0.1 \text{ gAl}_2\text{O}_3/\text{kg}_{\text{d.s.}}$ ; S2:  $12.6 \pm 0.1 \text{ gFe}_2\text{O}_3/\text{kg}_{\text{d.s.}}$  and  $18.2 \pm 0.1 \text{ gAl}_2\text{O}_3/\text{kg}_{\text{d.s.}}$ ).

### SOM composition

Limited differences between both unheated soils were found in the percentage of biochemical components according to their biogenic nature. Thus, lignin was the more

**Table 1.** Main soil characteristics (a) and temperature-induced changes (b) in soil properties and SOM composition (expressed as differences between heated and unheated samples) for the upper 0-5 cm layer of each one of the two forest soils (Soil 1 and Soil 2) that were treated at 220 °C or 350 °C

<b>a)</b>	<b>Soil 1</b>	<b>Soil 2</b>
<b>Soil textural class</b>	<b>Sandy loam</b>	<b>Sandy loam</b>
pH <sub>H<sub>2</sub>O</sub>	4.35 ± 0.03	4.20 ± 0.02
pH <sub>KCl</sub>	3.30 ± 0.01	3.60 ± 0.02
Total soil C, g/kg <sub>g<sub>d.s.</sub></sub>	102.9 ± 1.1	196.8 ± 2.4
Total soil N, g/kg <sub>g<sub>d.s.</sub></sub>	5.5 ± 0.1	14.3 ± 0.2
C-to-N ratio	19	14
Soil alteration comp., g/kg <sub>g<sub>d.s.</sub></sub> (Fe <sub>2</sub> O <sub>3</sub> +Al <sub>2</sub> O <sub>3</sub> )	12.3 ± 0.2	30.8 ± 0.2

<b>b)</b>	<b>220°C</b>		<b>350°C</b>	
	<b>(heated values - unheated values)</b>		<b>(heated values - unheated values)</b>	
	<b>Δ Soil 1</b> <sub>220°C</sub>	<b>Δ Soil 2</b> <sub>220°C</sub>	<b>Δ Soil 1</b> <sub>350°C</sub>	<b>Δ Soil 2</b> <sub>350°C</sub>
<b>Basic soil characterization analyses</b>				
pH <sub>H<sub>2</sub>O</sub>	- 0.30	- 0.45	+ 1.35	+ 0.65
pH <sub>KCl</sub>	- 0.15	- 0.10	+ 1.45	+ 0.90
Total soil C, g/kg <sub>g<sub>d.s.</sub></sub>	- 38.1	- 90.1	- 92.2	- 181.6
Total soil N, g/kg <sub>g<sub>d.s.</sub></sub>	- 0.09	- 1.89	- 4.09	- 9.72
C-to-N ratio	- 7	- 5	- 11	- 10
Fe <sub>2</sub> O <sub>3</sub> , g/kg <sub>g<sub>d.s.</sub></sub>	- 0.7	- 4.9	- 0.3	+ 5.2
Al <sub>2</sub> O <sub>3</sub> , g/kg <sub>g<sub>d.s.</sub></sub>	- 0.4	- 7.4	+ 0.5	+ 9.9
Soil alteration comp., g/kg <sub>g<sub>d.s.</sub></sub> (Fe <sub>2</sub> O <sub>3</sub> +Al <sub>2</sub> O <sub>3</sub> )	- 1.1	- 12.3	+ 0.2	+ 15.1
Total mass (weight), %	- 10	- 25	- 17	- 35
<b>Soil biochemical fractionation</b>				
Hydrosoluble fraction, g/100g <sub>g<sub>d.s.</sub></sub>	- 0.7	- 1.6	- 1.1	- 1.8
Lipid fraction, g/100g <sub>g<sub>d.s.</sub></sub>	- 0.1	- 1.1	- 0.3	- 1.1
Holocellulose fraction, g/100g <sub>g<sub>d.s.</sub></sub>	- 4.6	- 8.1	- 5.3	-10.3
Lignin fraction, g/100g <sub>g<sub>d.s.</sub></sub>	- 1.1	- 4.7	- 9.2	-18.1
<b>Soil humus fractionation</b>				
uSOM <sup>a</sup> , gC/kg <sub>g<sub>d.s.</sub></sub>	- 2	- 12	- 8	- 23
Fulvic acids (FA), gC/kg <sub>g<sub>d.s.</sub></sub>	- 2	- 25	- 22	- 30
Fe in fulvic acids (Fe <sub>inFA</sub> )	+ 0.08	- 1.92	- 3.00	- 7.21

Al in fulvic acids ( $Al_{inFA}$ )	+ 0.90	+ 3.34	+ 3.86	+ 18.08
Humic acids (HA), gC/kg <sub>d.s.</sub>	- 9	- 4	- 49	- 29
Humins (H), gC/kg <sub>d.s.</sub>	+11	+ 30	+ 71	+ 60
SOM <sup>b</sup> humification degree, %	+13	- 1	+ 16	+ 14
FA/HA ratio	+ 0.06	- 0.53	+ 0.14	- 0.28
<b>Stable isotope-ratio spectrometry (<sup>13</sup>C/<sup>12</sup>C)</b>				
Soil δ <sup>13</sup> C (‰)	+ 0.45	+ 0.50	+ 1.05	+ 2.40

<sup>a</sup> uSOM: unhumified soil organic matter

<sup>b</sup> SOM: soil organic matter

abundant fraction with nearly 60% of SOM, whereas the holocellulose, hydrosoluble and lipidic fractions accounted for 30-31%, 6-7% and 4-5%, respectively.

Although the total quantity of SOM differed between S1 and S2, the total proportion of unhumified SOM (uSOM) was instead quite similar in both soils (around 8% of the total soil dry mass). However the amount of humic substances obtained by the fractionation method based on acid/alkali solubility and their percentual distribution differed for each soil. Soil S1 contained a lower percentage of humic substances and hence a lower SOM humification degree (54%) than S2 (76%). Also, the humified SOM of S1 had a lower proportion of FA, more HA and a similar percentage of H (26%, 56% and 18%, respectively) as compared with S2 (39%, 43% and 17%, respectively), with a lower FA/HA ratio in the former (0.46 *vs* 0.91). In relation to the organometallic solubility, the extracted Fe and Al linked to the FA was higher in S1 than in S2, suggesting a higher complexation quotient of the fulvic fraction with metals in the former, despite the relative minor abundance of alteration compounds in this soil.

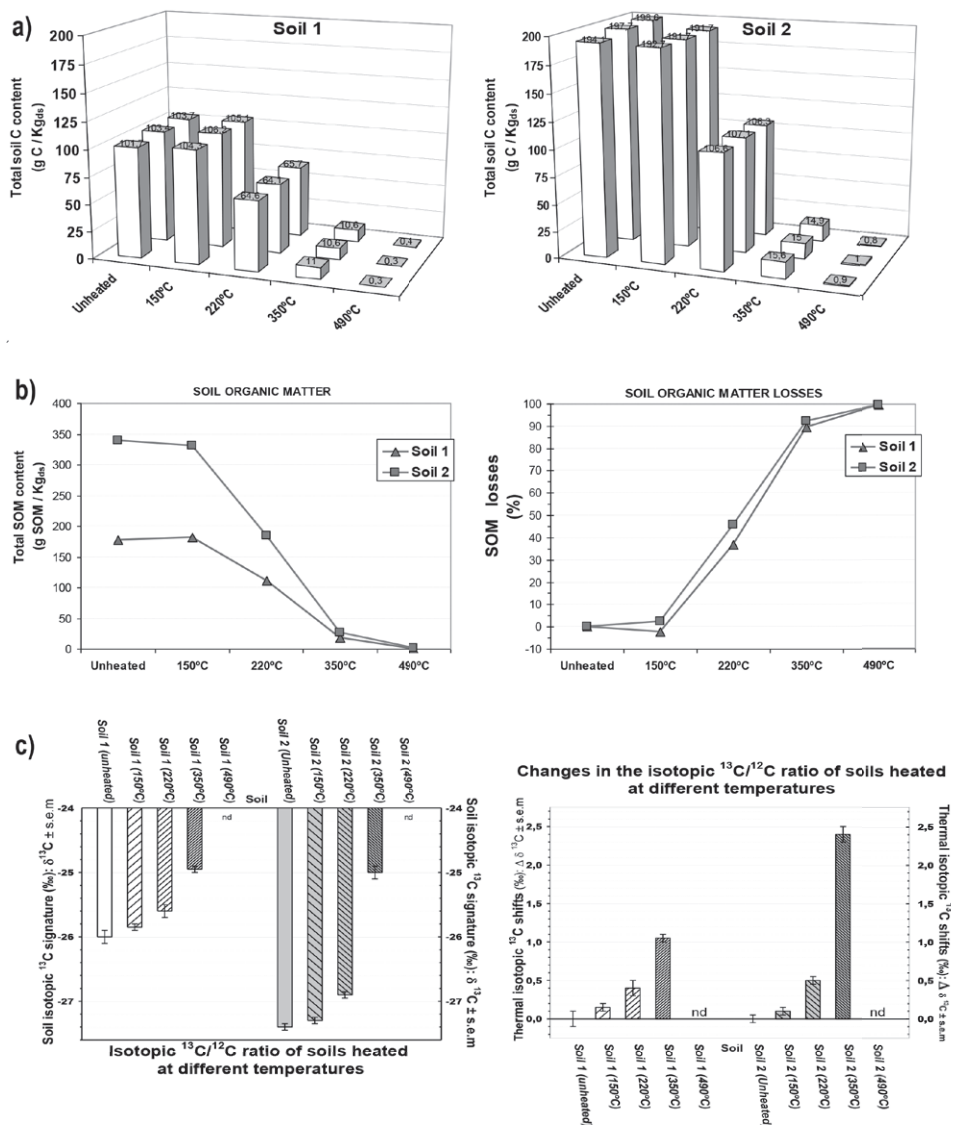
### Isotopic <sup>13</sup>C signature of the SOM (<sup>13</sup>C/<sup>12</sup>C ratio)

As expected according to the different pine species growing in each one of the two ecosystems selected, the stable C isotopic composition substantially differed in the two studied soils. S1, with *P. sylvestris* as the dominant tree species and located at more than 1700 m of altitude, showed a higher pre-heating δ<sup>13</sup>C value as compared with S2, with *P. pinaster* trees growing at less than 200 m (S1: δ<sup>13</sup>C=-26‰ and S2: δ<sup>13</sup>C=-27.5‰). In both cases these δ<sup>13</sup>C values were within the range of isotopic <sup>13</sup>C signatures reported for soil profiles developed under a C<sub>3</sub>-type vegetation cover (Arrouays et al., 1995; Spaccini et al., 2000; Fernandez et al., 2005; Fernandez et al., 2006b), as befits a mostly C<sub>3</sub>-derived SOM.

### Soil heating effects

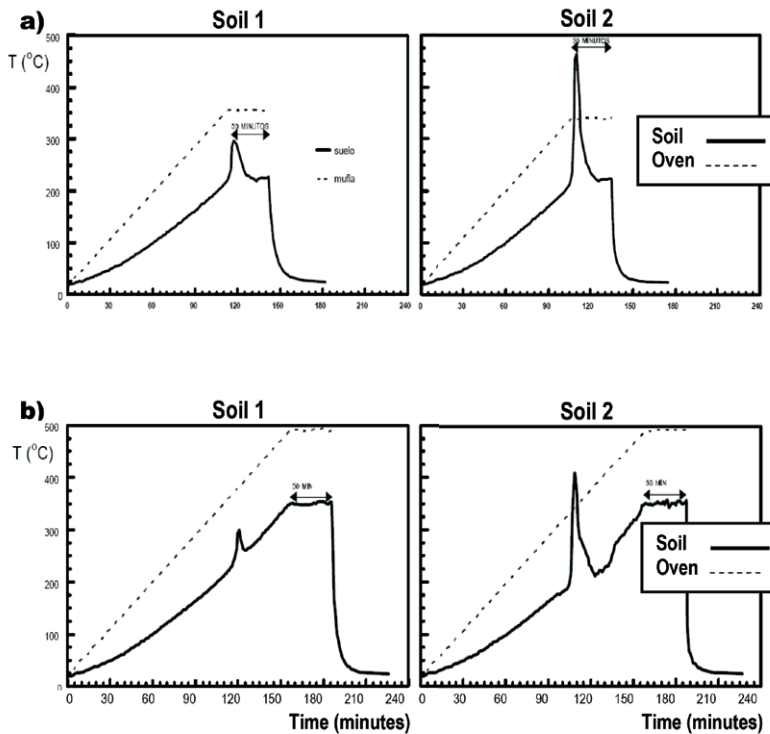
For the temperature spectrum analysed (from 150°C to 490°C), the control of the exact temperature reached inside soil samples during the heating procedure under controlled laboratory conditions as well as the strict stipulation of the total heating time allowed to circumvent the influence of other external factors (such as wind, fuel or soil

moisture) and to determine the actual heating effects produced on the soil at each specific temperature (by isolating the heating effects from other secondary or indirect disturbances that undoubtedly would occur in any forest wildfires), in order to clarify the interpretation of certain phenomena that may admit several explanations and to reject some erroneous or



**Fig. 1.** Total C content of each one of the three replicate analyses of soil 1 (S1) and soil 2 (S2), for soil samples either unheated or heated at different temperatures in laboratory controlled conditions (a). Total soil organic matter (SOM) content of both soils at different heating temperatures and the corresponding percentage of SOM losses caused by the heating (b). Isotopic <sup>13</sup>C signatures exhibited by both unheated and heated soils at different temperatures and the respective thermal isotopic shifts of the <sup>13</sup>C/<sup>12</sup>C ratio with respect to the corresponding unheated soil (c)





**Fig. 2.** Temporal evolution of the air temperature in the muffle as well as of the temperature reached inside the bulk volume of the soil samples during the heating processes performed under controlled laboratory conditions, that were applied to both soil 1 (S1) and soil 2 (S2) in order to carry out wildfire simulations of two different intensities: 220°C (a) and 350°C (b)

misattributed hypotheses. The heating period length and the final values of temperature selected to quantify heat-induced chemical and isotopic changes were established according to the results obtained from previous essays, in which the soils under study were heated at 150°C, 220°C, 350°C or 490°C. According to these laboratory essays and in view of the observed soil C losses, temperatures of 220°C and 350°C can be properly assimilated to moderate and intense wildfire severity levels (Fernandez et al., 1997; Fernandez et al., 2001). Thus, for the subsequent SOM fractionations, the other two values tested were finally discarded (150°C and 490°C), since at 150°C there were no significant changes in the main soil characteristics and the effects on the total soil C content were negligible, whereas at 490°C almost all organic C has disappeared (Fig. 1a). So, for more detailed explorations of the heating effects on the SOM composition of both soils, only the following two heating temperatures were selected: 220°C, which is approximately the SOM ignition temperature (Salgado et al., 1995), and 350°C, which produces severe C losses. Furthermore, another important fact is that for both soils the percentage of heat-induced SOM losses was quite similar (Fig. 1b) either at 220°C ( $\approx 40\%$ ) or at 350°C ( $\approx 90\%$ ), even though before the heating process the total SOM content of S1 was practically half the amount of C stored in S2.

Fig. 2 that shows the temperature achieved by the muffle and by S1 or S2 soil samples during the heating at 220°C (Fig. 2a) or 350°C (Fig. 2b), clearly illustrates the differences between the air temperature inside the muffle and the temperature registered by the thermocouple introduced into the soil sample, with a thermal mismatch that sometimes exceeded 100°C. At ignition SOM temperature, the sudden energetic release from combustion produced a sharp, albeit brief, soil temperature rise, which for S2 was even momentary higher than the muffle air temperature. The big size of the exothermic peak found when heating S2 at the two selected temperatures (220°C and 350°C) may probably be related to its high total soil C content, which seems to have also some influence on the ignition temperature and timing.

The heating effects on the soil pH were different at each one of the two temperatures considered. Although the pH (in H<sub>2</sub>O and in KCl) slightly decreased at 220°C in both soils, this parameter showed a significant increase of nearly or even more than 1 unit at 350°C (Table 1b), as previously found not only for heated soils (Fernandez et al., 2004) but also for wildfire-affected soils (Fernandez et al., 1997). The C-to-N ratio decreased by more than 5 units after heating the soils at 220°C whereas a much higher decrease was observed at 350°C, suggesting a differentiate C and N volatilization or a partial conversion of the organic N into inorganic forms. Similarly to the pH behaviour, the effect of the controlled heating on the soil alteration complex differed according to the temperature and the soil considered, with both Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> diminishing at 220°C but generally augmenting at 350°C; even so, the increases seem to be only a relative enrichment derived from total weight loss as a direct consequence of SOM combustion, being hence specially acute in S2 due to its great initial organic richness (Table 1).

### **Heating effects on SOM composition**

All changes produced by the heating on the SOM quantity and quality were also strongly dependent on the values of the applied temperatures. Thus, although the losses of SOM were already high in both soils at 220°C (37% and 46% in S1 and S2, respectively), at 350°C SOM losses in both soils were close to 90% (Fig. 1b).

While the original differences in the SOM composition (either taken as biochemical components of individual nature, or as humic substances) between both unheated soils were not especially significant, the heating effect was slightly different on each one of the soil considered, despite which, some general deductions can be made. At 220°C the behaviour of the different organic fractions allowed a direct and straightforward identification of the less thermal resistant fractions that already disappeared at this temperature, whereas at 350°C the fractions with the highest thermal resistance were revealed. Thus, holocellulose and FA presented a more thermolabile character whereas humin was the more resistant fraction, being the only SOM fraction that exhibited a relative increase at 220°C that is even higher at 350°C (Table 1b). It can also be highlighted that the quantity of Al linked to AF increased significantly when the soil samples were heated, suggesting a higher protection of these compounds against high temperatures when bond to this metal, since it was not only observed at 220°C, but predominantly at 350°C; this fact was even more evident for S2 which initially exhibited a higher presence of alteration compounds (Fe<sub>2</sub>O<sub>3</sub>+Al<sub>2</sub>O<sub>3</sub>, and

especially  $\text{Al}_2\text{O}_3$ ). However, the degree of affectation of other organic components, such as the lipid fraction, was quite similar in magnitude for both heating intensities, being rather dependent on the soil characteristics and on its initial SOM quality (Table 1), since its intrinsic composition seems to be related to certain diversity on their thermal resistance, possibly associated to different proportions of relatively labile lipids (that disappeared at 220°C) and resistant lipids (that even remained at 350°C).

### Heating effects on the soil $^{13}\text{C}$ signatures

Despite of the previously mentioned initial differences on the  $^{13}\text{C}$  signature of both unheated soils (S1:  $\delta^{13}\text{C}=-26\text{‰}$  and S2:  $\delta^{13}\text{C}=-27.5\text{‰}$ ), the isotopic analyses of the  $^{13}\text{C}/^{12}\text{C}$  ratio at natural abundance levels of the soil samples heated at the two different temperatures applied showed an analogous behaviour of their stable C isotopes concentration ( $^{13}\text{C}/^{12}\text{C}$  ratio), with a general trend of  $^{13}\text{C}$  enrichment with temperature increase. Heat-induced isotopic enrichments were also found by other authors either for soil samples (from different types of herbaceous and forest ecosystems) heated in muffle furnaces (Dorodnikov et al., 2008; Araya et al., 2017) or for forest soils affected by wildfires (Jimenez Morillo, 2017).

As a consequence of the controlled heating carried out in the laboratory, the  $^{13}\text{C}$  content of S1 and S2 soils (with initial  $^{13}\text{C}$  isotopic signatures that differed by 1.5‰ in their natural abundance levels) showed in the two cases significant changes on their  $^{13}\text{C}/^{12}\text{C}$  ratio, which value was also strongly dependent on the intensity of the applied temperature (Fig. 1c). Although in both soils the  $\delta^{13}\text{C}$  increase was minor at 150°C, statistically significant increases between the  $^{13}\text{C}$  isotopic compositions of unheated soils and soils heated at 220°C were observed (ANOVA,  $P\leq 0.000$ ), these incremental shifts being much more extreme at 350°C (ANOVA,  $P\leq 0.000$ ). At 220°C the extent of these observed isotopic increases were quite comparable for both soils, with appreciable  $\delta^{13}\text{C}$  variations ( $\Delta_{\text{soil}}\delta^{13}\text{C}\approx 0.5\text{‰}$ ). At 350°C the observed isotopic  $^{13}\text{C}$  shifts were more evident, these shifts always surpassing  $\Delta_{\text{soil}}\delta^{13}\text{C}>1\text{‰}$  but being more acute in S2 as compared with S1 (Fig. 1c). Therefore, for the full temperature spectrum analysed, S1 and S2 samples exhibited an analogous thermal behaviour of their soil  $\delta^{13}\text{C}$  signatures, the difference in the magnitude of some of the temperature-induced changes being probably related to the previous differences in their initial soil  $^{13}\text{C}$  signatures, that seems to be notably conditioned by the vegetation cover and/or the altitude (S1: *P. sylvestris* at 1740 m; S2: *P. pinaster* at 190 m) of the forest ecosystems considered (Fernandez et al., 2005; Fernandez et al., 2006b). These soil  $\delta^{13}\text{C}$  values showed a strong negative correlation with total soil C ( $P\leq 0.000$ ) and N contents ( $P\leq 0.000$ ) as well as with the more abundant biogenic or humic fraction, i.e. with lignin ( $P\leq 0.000$ ) and with HA ( $P\leq 0.006$ ), which are among the fractions that underwent the greatest losses at high heating intensity levels (Table 1b).

### Modelling the temperature-induced edaphic changes

In order to establish the main driving factors responsible of soil isotopic changes caused by different burn intensities, several multiple linear regression models were tested. Overall, the best models to elucidate the soil burn intensity, which use the increase in

the soil temperature ( $\Delta T_{\text{soil}}$ ) as the dependent variable, were obtained when the soil C isotopic shift ( $\Delta \text{soil} \delta^{13}\text{C}$ ) was included among the independent variables, since alone this parameter was able to properly predict the temperature reached by the soil during the heating process (soil burn intensity) with nearly the 60% of the total variance explained. Moreover, the regression model based on only one variable appreciably improved when the quantity of soil alteration compounds was also added as a predictor variable, since this combined multilinear regression model that included  $\Delta \text{soil} \delta^{13}\text{C}$  and  $\Delta \text{soil}$  alteration compounds (variables with an acceptable tolerance) explained more than 80% of the total variance of the heating intensity (Table 2).

On the other hand, the best models to describe the response of the soil C isotopic signature to thermal events, which use the temperature-induced soil  $^{13}\text{C}$  changes ( $\Delta \text{soil} \delta^{13}\text{C}$ ) caused by the heating as the dependent variable, were obtained when some changes related to the SOM quantity or quality were included among the independent variables. Consequently, in accordance to the significance of the previously mentioned correlations exhibited by  $\Delta \text{soil} \delta^{13}\text{C}$ , changes in soil C and N contents as well as in the lignin fraction provide the best models to predict the temperature-induced soil  $^{13}\text{C}$  changes (Table 3). Finally, besides the importance of SOM characteristics, the relationship of SOM with the mineral soil composition also seems to exert certain influences. Thus, this thermal influence of the mineral soil composition on the temperature-induced soil  $^{13}\text{C}$  changes ( $\Delta \text{soil} \delta^{13}\text{C}$ ) is also endorsed by models using data of metal-FA complexation as a predictor variable, that explained a high percentage of the variance of the  $^{13}\text{C}$  changes produced by the heating (Table 3). This points towards the fulvic acids (FA) as organic molecules that could play a predominant role as ligands, mostly with aluminium ( $\text{Al}_{\text{inFA}}$ ) at temperatures around  $350^\circ\text{C}$ , suggesting the superior capacity of this metal (as compared to iron,  $\text{Fe}_{\text{inFA}}$ ) to form covalent bonds with C atoms of organyl groups for building organometallic complexes and hence to better act as an organic protector against thermal damage, especially at extremely high burn severity levels.

**Table 2.** Multilinear regression models based on soil isotopic shifts ( $\Delta \text{soil} \delta^{13}\text{C}$ ) that significantly explained the soil burn intensity (i.e. using the increase in the temperature of the soil or  $\Delta T_{\text{soil}}$  as the dependent variable) showed by pine forest ecosystems from the northwestern corner of Spain after heating soil samples (upper 0-5 cm layer) under laboratory controlled conditions

Independent variables	R <sup>2</sup>	Adjusted R <sup>2</sup>	Standardized b	Significance	Tolerance
$\left\{ \begin{array}{l} \Delta \text{soil} \delta^{13}\text{C} \\ * \text{Regression model significance: 0.044} \end{array} \right.$	0.678	0.598	+0.823	0.044	1.000
$\left\{ \begin{array}{l} \Delta \text{soil} \delta^{13}\text{C} \\ \Delta \text{soil alteration compounds (Fe}_2\text{O}_3\text{+Al}_2\text{O}_3) \\ * \text{Regression model significance: 0.039} \end{array} \right.$	0.884	0.807	+1.222 -0.604	0.018 0.104	0.565 0.565

\* *Significance* of the regression model at the 0.05 probability level

**Table 3.** Multilinear regression models that significantly explained the changes or thermal shift observed in the soil  $\delta^{13}\text{C}$  signature ( $\Delta$  soil  $\delta^{13}\text{C}$ ) after heating soil samples from the upper 0-5 cm layer under laboratory controlled conditions at different temperatures

Independent variables	R <sup>2</sup>	Adjusted R <sup>2</sup>	Standardized b	Significance	Tolerance
<b>Basic soil characterization</b>					
{ $\Delta$ soil C	0.913	0.891	-0.956	0.003	1.000
{ ** Regression model significance: 0.003					
{ $\Delta$ soil N	0.969	0.961	-0.984	0.000	1.000
{ *** Regression model significance: 0.000					
{ $\Delta$ soil C	0.997	0.995	-0.745	0.000	0.760
{ $\Delta$ soil alteration compounds ( $\text{Fe}_2\text{O}_3+\text{Al}_2\text{O}_3$ )			+0.658	0.003	0.760
{ *** Regression model significance: 0.001					
<b>Soil biochemical composition</b>					
{ $\Delta$ soil holocellulose	0.685	0.606	-0.828	0.042	1.000
{ * Regression model significance: 0.042					
{ $\Delta$ soil holocellulose	0.978	0.963	-0.697	0.004	0.945
{ $\Delta$ soil alteration compounds ( $\text{Fe}_2\text{O}_3+\text{Al}_2\text{O}_3$ )			+0.557	0.008	0.945
{ ** Regression model significance: 0.003					
{ $\Delta$ soil lignin	0.970	0.962	-0.985	0.000	1.000
{ *** Regression model significance: 0.000					
<b>Soil humus composition</b>					
{ $\Delta$ soil uSOM <sup>a</sup>	0.852	0.816	-0.923	0.009	1.000
{ ** Regression model significance: 0.009					
{ $\Delta$ soil Fe in FA <sup>b</sup>	0.863	0.828	-0.929	0.007	1.000
{ ** Regression model significance: 0.007					
{ $\Delta$ soil Al in FA <sup>b</sup>	0.921	0.902	-0.960	0.002	1.000
{ *** Regression model significance: 0.000					
{ $\Delta$ soil Al in FA <sup>b</sup>	0.992	0.986	+0.744	0.002	0.603
{ $\Delta$ soil C-to-N ratio			-0.342	0.015	0.603
{ *** Regression model significance: 0.001					

<sup>a</sup> Unhumified soil organic matter (uSOM)

<sup>b</sup> Fulvic acids (FA)

\* *Significance* of the regression model at the 0.05 probability level

\*\* *Significance* of the regression model at the 0.01 probability level

\*\*\* *Significance* of the regression model at the 0.001 probability level

## CONCLUSIONS

The present research confirms the efficacy of isotopic  $^{13}\text{C}$  techniques at natural abundance levels for quantifying degradation levels in disturbed ecosystems, particularly to monitor SOM alterations. Our findings demonstrate that the changes in SOM isotopic  $^{13}\text{C}$  composition are clearly associated with the temperature reached during the heating process

and therefore with soil burn intensity. The modellization of the soil  $^{13}\text{C}$  changes caused by the heating showed parallel thermal behaviours for both soils (despite their initially different isotopic composition) and, although more extensive investigations are still needed, the outcome of this research points to the effectiveness of this parameter ( $\Delta_{\text{soil}}\delta^{13}\text{C}$ ) to evaluate the degree of the disturbance of different types of forest soils and can be considered a first step towards a possible generalization of using the soil  $\delta^{13}\text{C}$  shifts as a proxy indicator of soil burn intensities after forest wildfires that can be helpful as a support tool for *a posteriori* estimations of soil alteration in order to contribute not only to an adequate forest recovery management but also to improve post-fire soil restoration.

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**E-mail:** i.f@csic.es