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Short-term effects of post-fire soil mulching with wheat straw and wood chips on the enzymatic activities in a Mediterranean pine forest

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ABSTRACT

Soils of Mediterranean forests can be severely degraded due to wildfire. However, post-fire management techniques, such as soil mulching with vegetal residues, can limit degradation and increase functionality of burned soils. The effects of post-fire mulching on soil functionality have been little studied in Mediterranean forests, and it is still unclear whether the application of straw or wood residues is beneficial. This study explores the changes in important soil chemical and biochemical properties in a pine forest of Central Eastern Spain after a wildfire and post-fire mulching with straw or wood chips. Only basal soil respiration (BSR), dehydrogenase activity

(DHA), pH and water field capacity (WFC) significantly changed after the fire and mulching. In contrast, the other enzymatic activities - urease (UA), alkaline phosphatase (Alk-PA) and β-glucosidase (BGA), - total organic carbon (TOC) and electrical conductivity (EC) were not influenced by these soil disturbances. Time from fire and soil conditions (due to burning and management) were significant variability factors for BSR, pH, BGA, UA, TOC, EC. Mulching increased BSR compared to burned areas, especially in soils with straw (+30%), thanks to addition of fresh organic residues, quickly incorporated in the soil. Soil pH showed a low variability among the four soil conditions, and TOC was higher in mulched soils (on average +20% compared to the burned soils), and this was correlated to the increased BSR. The role of mulching was essential with reference to WFC, as the post-fire management limited its reduction after the fire (on average from –30% to –20%). Finally, the Principal Component Analysis coupled to the Analytical Hierarchical Cluster Analysis confirmed the significant influence of the post-fire management on some enzymatic activities, although a sharp discrimination among the four soil conditions was only evident between unburned and burned sites, regardless of the management. Overall, it has been shown that mulching promotes conservation of fragile Mediterranean soils, indicating its effectiveness at preserving soil functionality in areas affected by forest fires.

KEYWORDS: enzymatic activities; chemical properties; post-fire management; wildfire; soil respiration.

1. INTRODUCTION

The Mediterranean forests are particularly exposed to the wildfire risk (Shakesby, 2011; Wagenbrenner et al., 2021). Wildfire leaves the forest soil bare due to vegetation removal (Bodí et al., 2012; Shakesby and Doerr, 2006), changes several properties of soils (e.g., the aggregate stability, the contents of organic matter and nutrients, and the microbial community composition (Certini, 2005; Zavala et al., 2014), and induces water repellency, with increased surface runoff and soil erosion (Zema et al., 2021a, 2021b). These fire effects mainly depend on the soil burn severity (Lucas - Borja et al., 2022), which is directly linked to forest fuel amount and type, and fire characteristics (frequency, duration, energy). The pre-fire soil conditions recovery after several years or even some decades, when the fire severity is extremely high (Certini, 2005; Glenn and Finley, 2010).

To support plant regeneration and limit the off-site effects of wildfires, forest managers adopt post-fire management actions on both hillslopes and channels draining the burned catchments (Robichaud et al., 2010). These actions are generally effective at reducing soil's exposure to hydrological risk and quality degradation (Girona-García et al., 2021; Lucas-Borja et al., 2020b). Mulching is one of the most common management actions on the hillslope scale after a wildfire (Fernández and Vega, 2016; Lucas-Borja, 2021). Burned soils are covered by a layer of vegetal residues, which limit the rainfall erosivity and enhance vegetation regrowth (Prats et al., 2012; Prosdocimi et al., 2016). The most common mulch material is the agricultural straw, but its use can bring some problems, such as the removal or accumulation of straw in different sites due to wind displacement (Carrà et al., 2021; Robichaud et al., 2020) or the plant disease and parasite invasion into mulched forests (Jordán et al., 2010; Prosdocimi et al., 2016). As alternative mulch materials, forest residues, such as wood chips or strands, can be applied to soils of burned forests, but their use has been less experimented compared to straw.

The scientific literature has mainly focused the hydrological effects of mulching (e.g., reduction in runoff and soil erosion), while its impacts on other components of the forest ecosystems have been less investigated. This is an important issues, since post-fire management may generate changes in biological, chemical and physical properties of soil in burned forests, which influence biota composition and activity (Badía et al., 2015; Inbar et al., 2014; Killham, 1994; Lucas-Borja et al., 2020c). Moreover, few studies have explored the impacts of mulching materials on those properties that are related to soil functionality. On this regard, enzymes and respiration of soil have been widely used to evaluate the functionality of soil (Fioretto et al., 2009; Utobo and Tewari, 2015). Soil enzymes and microbiome regulate organic matter decomposition and stabilization, post-fire nutrient dynamics and rhizosphere function (Nelson et al., 2022). Several enzyme activities, specifically related to the cycles of carbon, nitrogen and phosphorous (β-glucosidase, urease, alkaline and acid phosphatase, respectively (Lucas-Borja et al., 2020) and some general microbial indicators (e.g., dehydrogenase activity and soil respiration) have been proposed as specific indicators of soil functionality (Bastida et al., 2008). Moreover, as key microbiological soil properties, soil respiration and enzyme activities are considered as closely associated to organic matter decomposition and formation (Cerdà et al., 2016; Gutknecht et al., 2010).

In general, the impacts of the different vegetal materials adopted for soil mulching on soil properties can not be the same, since the application rates, soil covers and material sizes are different. For instance, straw and woody chips may play differentiated effects on the functionality of treated soils (Díaz et al., 2022; Prosdocimi et al., 2016) and, therefore, on enzymes and respiration of soil. Moreover, the biochemical properties of the soils mulched by these materials, such as organic matter content, microbial biomass carbon, respiration, enzymatic activities or nutrient content of soil (Bastida et al., 2008; Entry and Emmingham, 1998), may be affected by a large variability, as a response to the amounts and quality of nutrients and organic matter in soils generated by wood chips or straw and to the different decomposition rates. This variability requires a specific knowledge about the effects of these mulch materials on soil functionality in wildfire-affected forest soils, in order to plan possible countermeasures against soil degradation (Gómez-Sánchez et al., 2019; Lucas-Borja et al., 2022). However, to the authors' best knowledge, no studies are available about the changes in the enzymatic activities of burned soils after post-fire management. Since soil functionality may be severely altered by wildfires and mulching may reduce this disturbance in the short term, a quantitative assessment is essential to measure the effectiveness of this practice.

To fill this gap, this study explores the changes in important chemical and biochemical properties of soils in a forest of Castilla La Mancha (Central Eastern Spain) after a wildfire and post-fire mulching with straw or wood chips. The main objective of the research is the evaluation of effects of these two mulch materials on the enzymatic activities in severely-burned forests under Mediterranean conditions six months and one year after burning. We hypothesize that: (i) the changes in soil enzymatic activities depend on the soil condition (burned and untreated sites, and burned and mulched areas); (ii) the differences in these activities are less important with reference to the two mulch materials; and (iii) the differences in soil functionality among the soil conditions are pronounced.

2. MATERIALS AND METHODS

2.1. Study area

The study area is the Sierra de Los Donceles forest (municipality of Liétor, province of Albacete, region of Castilla-La Mancha, Spain, 38º30'41" N; 1º56'35"W) at an elevation between 520 and 770 m above the mean sea level (Figure 1) and north-west aspect. The climate is typically semi-arid Mediterranean (BSk type, according to the Köppen classification (Kottek et al., 2006)). Mean annual values of temperature and precipitation are equal to 16.6 °C and 321 mm, respectively, from the last 20 years of weather data collected at the meteorological station of Hellín, about 20 km far from Liétor (historical records of the Spanish Meteorogical Agency, AEMET). Soils are Calcic Aridisols (Nachtergaele, 2001), and their texture is sandy loamy. The geology is typical pre-Baetic Mountains, with limestone and dolomite outcrops alternating with marly intercalations that date back to the quaternary.

The dominant overstory vegetation consists of a tree layer of natural and reforested (about 60-70 years ago) Aleppo pine (*Pinus halepensis* Mill.) and a shrub layer of kermes oak (*Quercus cocciferae*) (Peinado et al., 2008). Before the wildfire, the stand density and tree height were in the range 500 - 650 trees/ha and 7 - 14 m, respectively. The understory vegetation consists of *Rosmarinus officinalis* L., *Brachypodium retusum* (Pers.) Beauv., *Cistus clusii* Dunal, *Lavandula latifolia* Medik., *Thymus vulgaris* L., *Helichrysum stoechas* L., *Stipa tenacissima* L., *Quercus coccifera* L. and *Plantago albicans* L.

In July 2021, a wildfire burned about 2500 ha in the studied forest. In order to limit the expected increases in surface runoff and erosion after the fire, the Forest Service of the Castilla La Mancha region immediately applied wheat straw and wood chips to the soils of the burned forest area as post-fire mulching action.

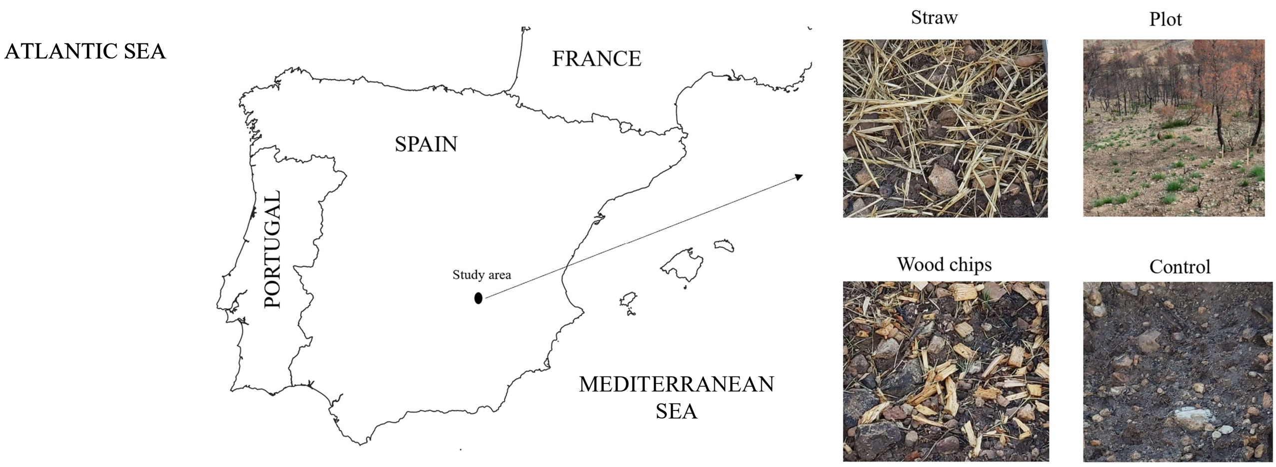


Figure 1 – Geographical location and aerial map of the study area (Liétor, Castilla La Mancha, Central Eastern Spain).

2.2. Experimental design

One week after the wildfire, a study area of 700 ha was selected, including both unburned and burned forest soils (the latter affected by crown fire with 100% tree mortality). In this burned area, a site with profile slope between 30.1 ± 3.9% and 48.1 ± 4.7% was identified. Soils with low slope (< 20%) were excluded, since these hillslopes are less prone to erosion, and the same was done for soils with high slope (> 60%), where pine forests commonly do not grow, at least in Central Eastern Spain. In each site, four blocks of eighteen plots, each one with an area of 2 square metres, were installed. One block of four plots was not burned (hereafter indicated as "unburned", U), and considered as control. A second block of eight plots ("burned", B) was burned but not treated. The third and four blocks were mulched with straw, M(WS) (four plots per block) or wood chips, M(WC) (four plots per block).

Mulching with both materials was carried in January 2022, six months after the wildfire. This choice, which is in contrast with previous experiences, where soils were mulched immediately after the wildfire, was adopted, in order to let rainfalls in the wet periods (late summer, autumn and winter) erode soil and leach ash. The main characteristics of the mulch materials were the following:

- wood cheap (mean values): dose of 0.3 kg/m^2; length: 3-10 cm; width: 2-4 cm; thickness: 1-2 cm; density: 500-550 kg/m^3;
- straw (mean values): dose or 2 kg/m^2; length: 5-25 cm; width: 0.25-1.0 cm; thickness: 0.1-0.7 cm; density: 80-100 kg/m^3.

These application rates are those suggested by the forest services of the Iberian Peninsula, and widely used in literature (e.g., Girona-García et al., 2021; Kim et al., 2008; Lucas-Borja et al., 2019).

Therefore, the experimental design consisted of four soil conditions (unburned soil, burned soil, soil mulched with straw, and soil mulched with wood chips) × two survey dates for soil analysis

(January and July 2022, see section 2.3) × eight (in burned soils) or four (in unburned sites) replicated plots.

2.3. Soil sampling

Soils in each of the 24 plots were sampled in January (six months after the wildfire) and again in July 2022 (six months after the post-fire treatments). The two sampling operations were carried out in very close points each other. Twenty-four samples of 600 g, one sample per plot, were collected from the top 10 cm of surface soil. Each soil sample was made up of six 100-g sub-samples from randomly selected points (at a reciprocal distance higher than 5 m), in order to capture the potential variability of soil conditions within each plot. The litter layer was removed from the soil surface before sampling. Each sample was brought to laboratory, passed through a 2 mm sieve and then stored at 4 °C prior of the subsequent analyses in the following day.

2.4. Analysis of soil functionality

One day after sampling, each soil sample was air dried, homogenised and sieved (< 2 mm) before analysis. The following soil chemical properties were determined on the collected samples: (i) pH and electrical conductivity (EC), determined in distilled water, at a soil:solution ratio of 1:2.5 and 1:5 respectively, using a a digital pHmeter (LAQUA PH1100, HORIBA, Tokio, Japan) and conductivity meter (Crison 522, Barcelona, Spain); (iii) Total Organic Carbon (TOC) was determined by the Walkey and Black (1934) method modified by Mingorance et al., (2007) and measured in a spectrophotometer (Spectronic Helios Gamma UV-Vis, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Water field capacity (WFC) were determined as pF -33 KPa by Richards membrane method (Richards, 1941).

Basal soil respiration (BSR, expressed as mg C-CO_2 kg^{-1} day^{-1} of dry soil), was measured with using an infrared CO_2 sensor (IRGA S151; Qubit Systems Inc., Canada). Soil dehydrogenase activity (DHA, expressed as µg INTF $hour^{-1}$ g^{-1} of dry soil) was determined by the reduction of p-iodonitrotetrazolium chloride (INT) to p-iodonitrotetrazolium formazan (INTF) following García et al. (1997). Urease activity (UA), expressed as µmol N-NH^{4+} $hour^{-1}$ g^{-1} of dry soil) was measured using urea as a substrate and a borate buffer at pH = 10 (Kandeler and Gerber, 1988). The activity of alkaline phosphatase (Alk-PA) and β-glucosidase (BGA), both expressed as µmol pNP $hour^{-1}$ g^{-1} of dry soil, were determined using the methods of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1988) , respectively.

2.5. Statistical analysis

A 2-way ANOVA was applied to the soil properties (dependent or response variables), in order to evaluate the statistical significance of the differences among the four soil conditions (unburned soils, burned and untreated soils, soils burned and mulched with straw or wood chips) and between the two survey dates (six and twelve months after fire, which means immediately after mulching and six months after) (independent variables or factors), and their interactions (soil condition x survey date). The differences in each soil property among factors were evaluated using the pairwise comparison by Tukey's tests (at $p < 0.05$). The equality of variance and normal distribution are assumptions of the statistical tests; these assumptions were evaluated by normality tests or were square root-transformed, when necessary. In this case, the Shapiro-Wilk test was again applied, to check the normal distribution of the samples.

Following this, a Principal Component Analysis (PCA) was applied to the soil samples collected in the last survey (July 2022), in order to identify the existence of meaningful derivative variables (Principal Components, PCs) (Lee Rodgers and Nicewander, 1988) and simplify the analysis of the large number of soil properties and conditions, losing as little information as possible. In this study, PCA was carried out by standardizing the original variables (expressed by different measuring units) and using Pearson's method to compute the correlation matrix. This matrix allowed the identification of relationships among the soil properties analysed. The first PCs that explain at least 70% of the original variance were retained.

Finally, the soil samples were grouped in clusters using Agglomerative Hierarchical Cluster Analysis (AHCA), a distribution-free ordination technique to group samples with similar characteristics by considering an original group of variables. As similarity-dissimilarity measure the Euclidean distance was used (Zema et al., 2015).

The statistical analysis was carried out using the XLSTAT software (release 2019, Addinsoft, Paris, France).

3. RESULTS

ANOVA revealed that, among the soil properties, only BSR was significantly different among both the four soil conditions and survey dates (but not with their interaction). The soil condition alone significantly influenced DHA, pH, and WFC, and the survey date alone influenced BGA, UA, TOC, end EC. The interaction of soil condition with the survey date was significant for pH. Finally, the Alk-PA was never significantly different (either among the soil conditions, survey dates and their interactions) (Table 1).

Table 1 – Results of two-way ANOVA applied to the properties of soil samples collected under four conditions (unburned, U, burned, B, mulched with wood chips, M(WC), and mulched with wheat straw, M(WS)) and at two dates (January and July 2022) in Liétor (Castilla La Mancha, Central Eastern Spain).

Factor	**Degrees of freedom**	**Sum of squares**	**Mean squares**	**F**	**Pr > F**
	BSR				
Soil condition	3	1802	601	4.23	**0.010**
Date	1	8925	8925	62.9	**< 0.0001**
Soil condition x date	3	78.7	26.2	0.18	0.906
	DHA				
Soil condition	3	11.4	3.81	5.19	**0.003**
Date	1	0.38	0.38	0.51	0.479
Soil condition x date	3	3.99	1.33	1.81	0.158
	BGA				
Soil condition	3	0.14	0.05	0.46	0.712
Date	1	11.7	11.7	118	**< 0.0001**
Soil condition x date	3	0.13	0.04	0.45	0.719
	Alk-PA				
Soil condition	3	12.7	4.25	0.78	0.510
Date	1	16.7	16.7	3.08	0.086
Soil condition x date	3	5.89	1.96	0.36	0.781
	UA				
Soil condition	3	4.66	1.55	2.52	0.069

Date	1	2.77	2.77	4.49	**0.039**
Soil condition x date	3	0.06	0.02	0.03	0.992
	TOC				
Soil condition	3	10.1	3.36	2.40	0.079
Date	1	16.1	16.1	11.5	**0.001**
Soil condition x date	3	2.85	0.95	0.68	0.570
	pH				
Soil condition	3	0.12	0.04	3.56	**0.021**
Date	1	0.01	0.01	0.51	0.477
Soil condition x date	3	0.15	0.05	4.24	**0.010**
	EC				
Soil condition	3	0.04	0.01	1.89	0.144
Date	1	0.03	0.03	4.65	**0.036**
Soil condition x date	3	0.02	0.01	0.86	0.471
	WFC				
Soil condition	3	433	144	3.95	**0.013**
Date	1	75.7	75.7	2.07	0.156
Soil condition x date	3	48.8	16.3	0.45	0.722

Notes: WFC = water field capacity; DHA = dehydrogenase activity; BGA = β-glucosidase activity; Alk-PA = alkaline phosphatase activity; UA = urease activity; TOC = total organic matter; EC = electrical conductivity; BSR = basal soil respiration.

In January 2022, BSR was the highest in the treated soils (79.7 ± 4.9 mg of C-CO_2 kg^{-1} day^{-1}, for WC plots, and 83.7 ± 6.56 mg of C-CO_2 kg^{-1} day^{-1}, for WS plots) and the lowest in the B plots (68.8 ± 5.45 mg of C-CO_2 kg^{-1} day^{-1}). The same BSR patterns were surveyed in July 2022, but at this date, the values of U (46.2 ± 0.12 mg of C-CO_2 kg^{-1} day^{-1}) and B (45.5 ± 4.93 mg of C-CO_2 kg^{-1} day^{-1}) plots were practically the same (Figure 2). After 12 months from the wildfire (July 2022), the BSR significantly decreased under all soil conditions (Figure 2).

About the enzymatic activities, the U plots showed the highest value of DHA at both the survey dates, with a maximum in July (6.54 ± 0.17 µmol of INTF g^{-1} of soil h^{-1}). Under the other soil conditions, this parameter was lower, particularly in July (although not significantly), with the minimum value (4.30 ± 0.41 µmol of INTF g^{-1} of soil h^{-1}) measured in the B soils (Figure 2).

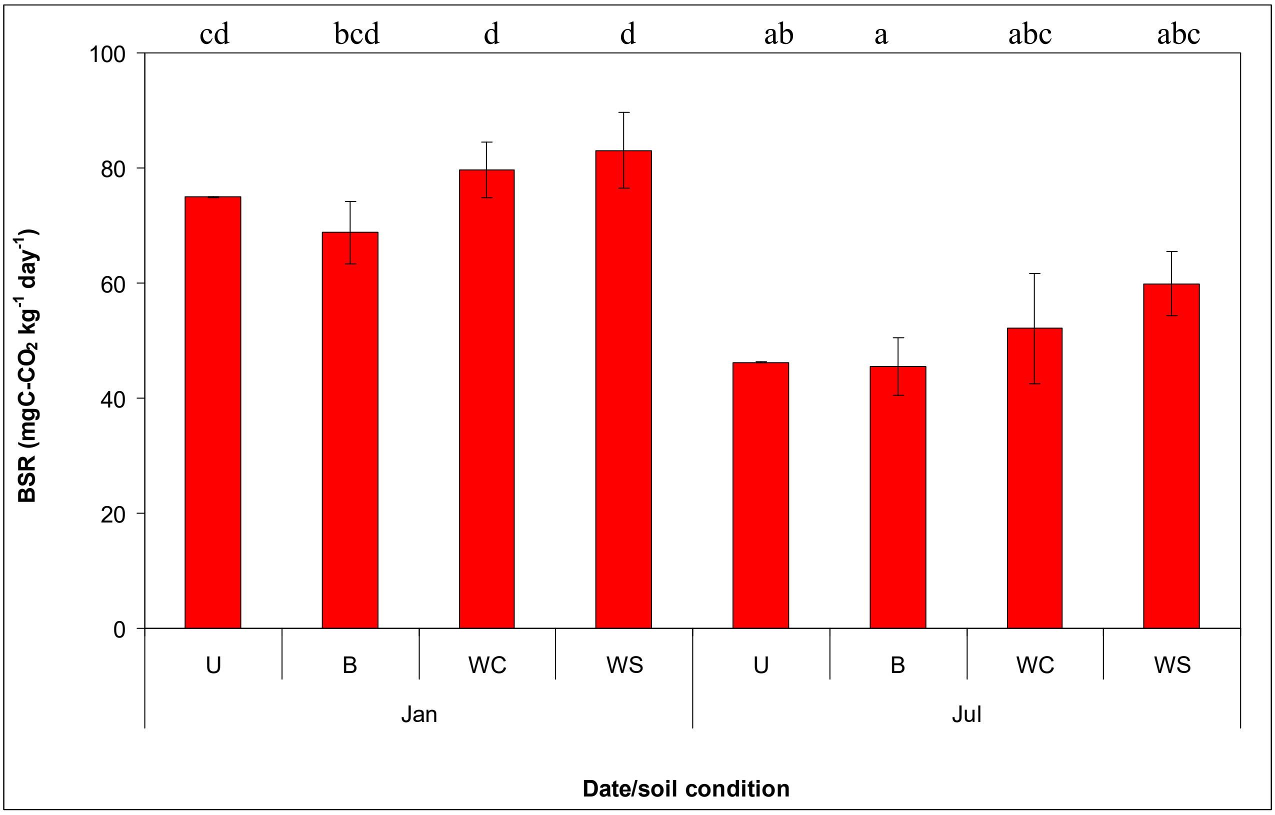
The BGA was significantly higher in January compared to the survey in July for all soil conditions. The absolute lowest value was measured in the B plots in July (0.32 ± 0.07 µmol of PNF h^{-1} g^{-1} of soil), while the absolute highest BGA was detected in the WS soils in January (1.41 ± 0.01 µmol of PNF h^{-1} g^{-1} of soil, although without significant differences compared to the other plots) (Figure 2).

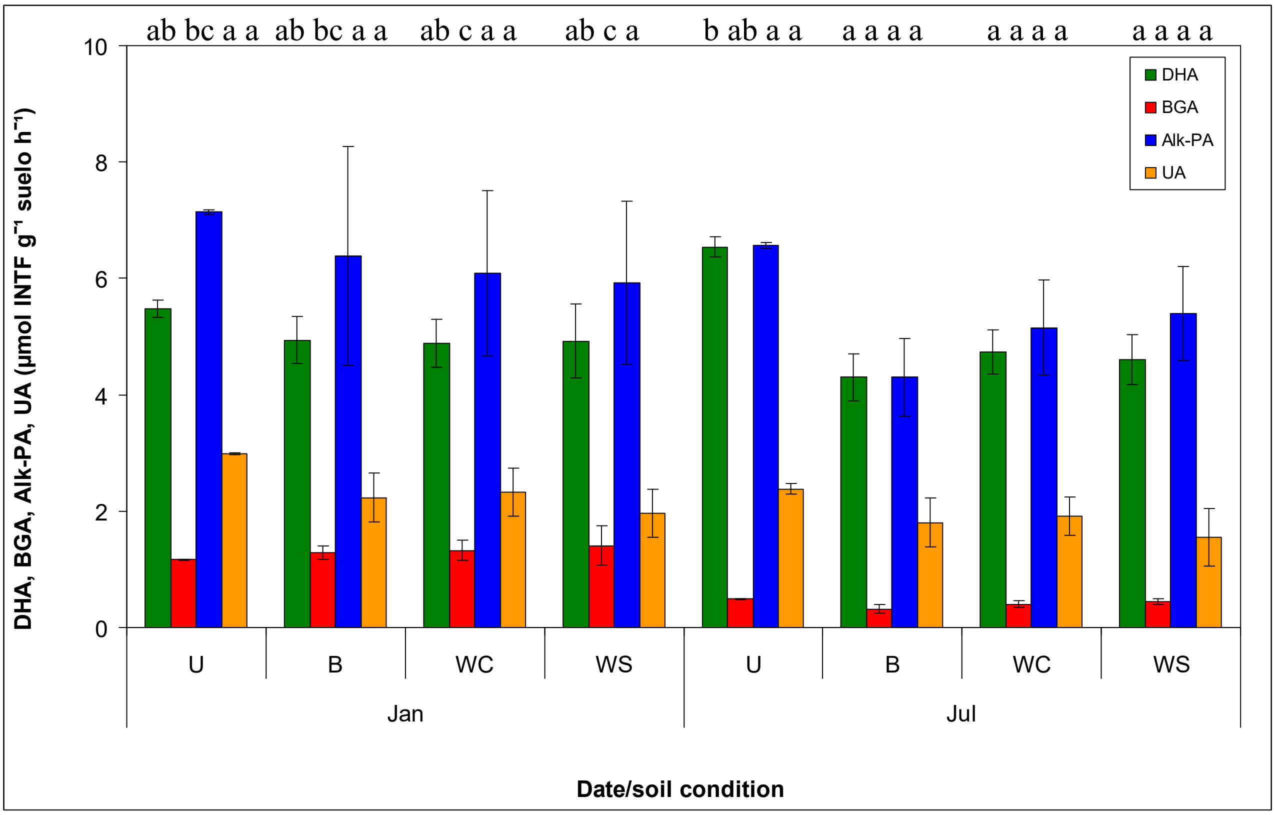
The values of Alk-PA were in the range 4.3 ± 0.67 µmol of PNF h^{-1} g^{-1} of soil (B plots in July) to 7.14 ± 0.04 µmol of PNF h^{-1} g^{-1} of soil (U soils in January), but this enzymatic activity was very similar among the soil conditions and the survey dates (Figure 2).

A gradient U > WC > B > WS plots was observed for the UA both in January and in July, with the minimum and maximum values observed in the WS soils in July (1.55 ± 0.50 µmol of N-NH_4 $h^{-1}g^{-1}$ of soil) and in U plots in January (2.98 ± 0.01 µmol of N-NH_4 $h^{-1}g^{-1}$ of soil) (Figure 2). The temporal difference in UA was significant, while that gradient not (Table 1).

Regarding the main chemical properties, TOC significantly increased throughout the survey dates. Although the differences in this parameter were not significant among the four soil conditions, the lowest TOC was measured in U plots in January (2.53 ± 0.19%), while the highest value was detected in WC soils in July (4.78 ± 0.59%) (Figure 2). The pH was similar at the two survey dates, but not among the analysed soil conditions. The U (9.22 ± 0.04) and WC (8.92 ± 0.04) plots showed the highest and lowest values, respectively, in January, while the minimum and maximum pH in July was measured in the WS (9 ± 0.06) and B (9.03 ± 0.07) soils, respectively (Figure 2). The U soils showed the lowest EC at both survey dates (0.16 ± 0.01 mS/cm in January, and 0.15 ± 0.001 mS/cm in July). However, while in January the highest EC was measured in the WC and WS plots (0.27 ± 0.04 and 0.27 ± 0.06 mS/cm), in July the EC was maximum in the B soils (0.21 ± 0.07 mS/cm) (Figure 2).

The WFC, which was not significantly variable over time, was always higher in the U soils (38.1 ± 0.36% in January, and 31.2 ± 0.08% in July), and lower in the B plots (26.7 ± 3.54%) compared to the treated soils. The latter showed a WFC closer to the B soils than to the U plots (28.7 ± 2.15% in January, and 27 ± 3.38% in July for WS soils, 28.5 ± 2.97% in January, and 27.3 ± 3.07% in July for WC soils) (Figure 2).

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DHA
BGA
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U
B
WC
WS
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Jan
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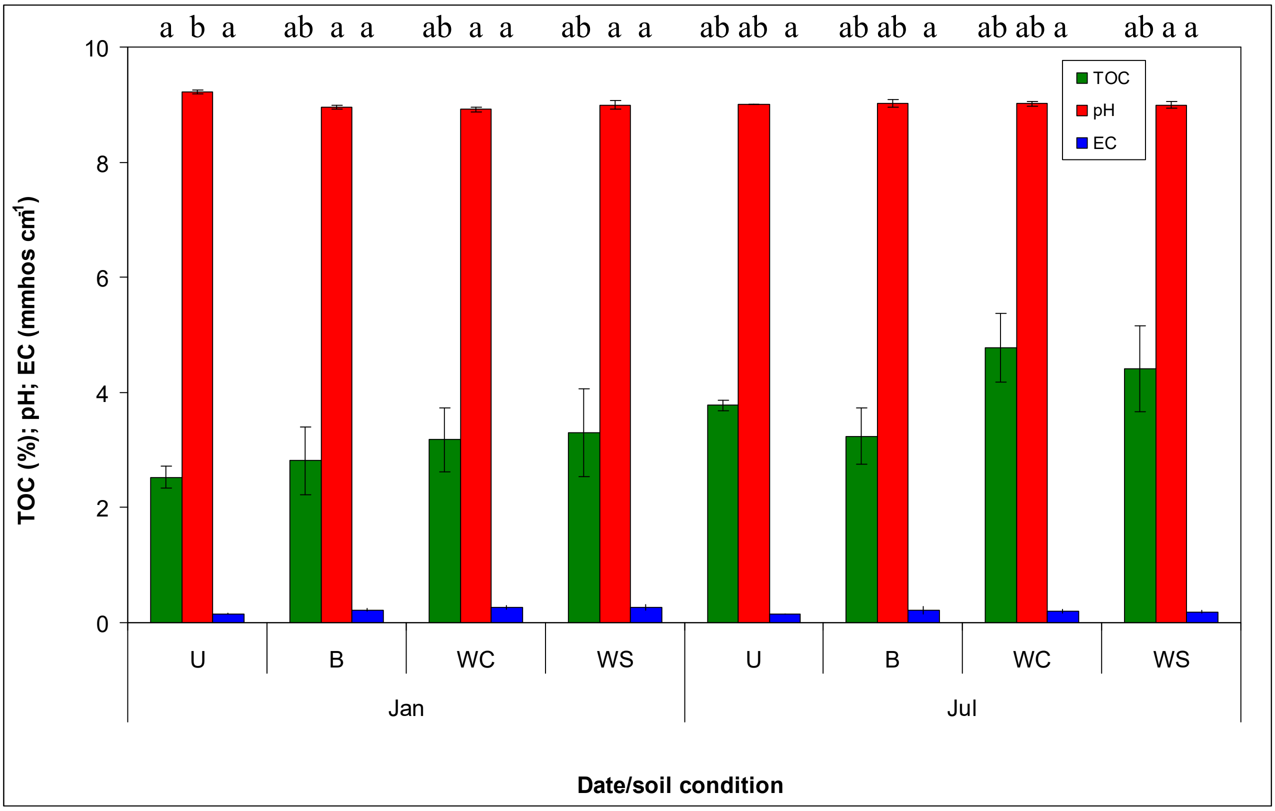
305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306

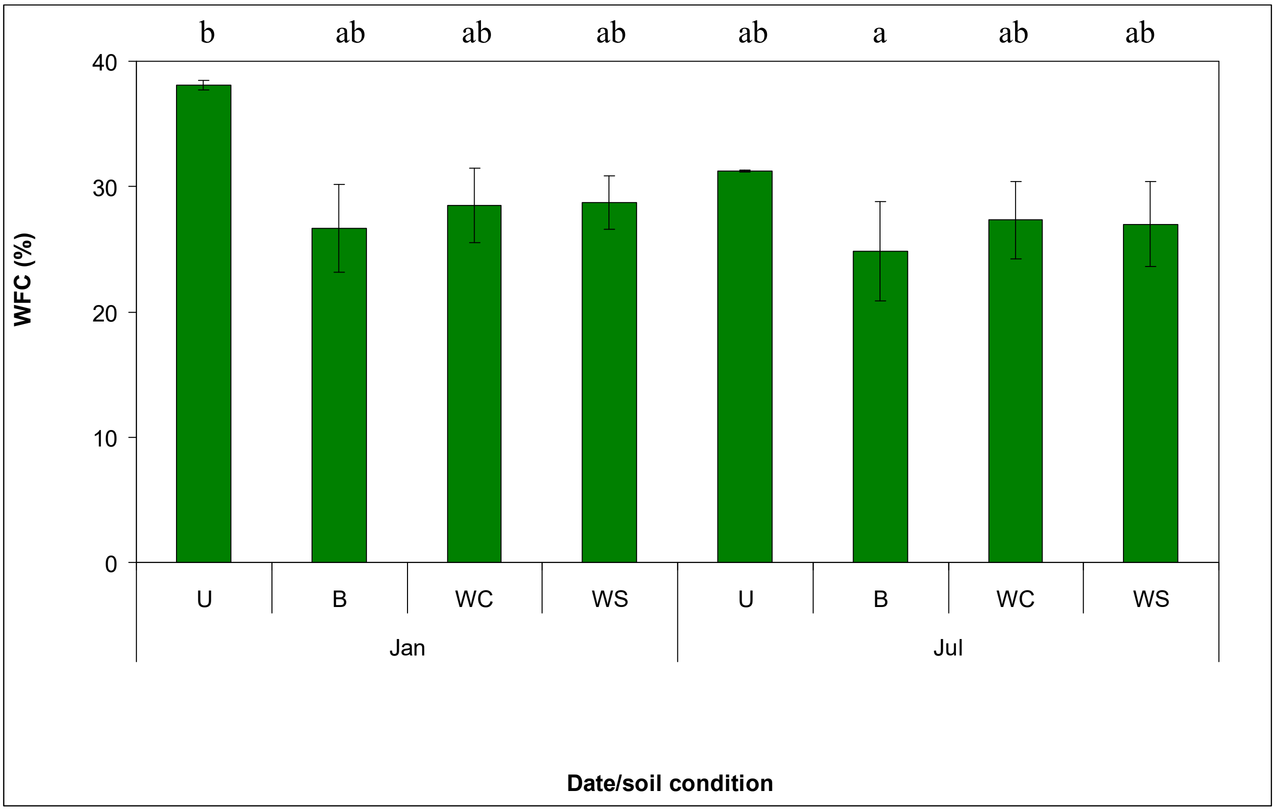


Figure 2 – Main properties of samples of soils collected under four conditions (unburned, U, burned, B, mulched with wood chips, M(WC), and mulched with wheat straw, M(WS)) and at two dates (January and July 2022) in Liétor (Castilla La Mancha, Central Eastern Spain). *Legend: WFC = water field capacity; DHA = dehydrogenase activity; BGA = β-glucosidase activity; Alk-PA = alkaline phosphatase activity; UA = urease activity; TOC = total organic matter; EC = electrical conductivity; BSR = basal soil respiration. Different letters indicate significant differences in the interaction soil condition x survey date at* $p < 0.05$ *of Tukey's test.*

Significant correlations were found among almost all the properties of soils measured under the four conditions. Some of these correlations were also high ($r > 0.50$), such as, for instance, the relationships between BGA and DHA ($r = 0.550$) or Alk-PA ($r = 0.726$), TOC and pH ($r = -0.564$) or WFC ($r = 0.656$), and pH and EC ($r = -0.732$) (Table 2).

Table 2 - Correlation matrix among the properties of soil samples collected under four conditions (unburned, U, burned, B, mulched with wood chips, M(WC), and mulched with wheat straw, M(WS)) in July 2022 in Liétor (Castilla La Mancha, Central Eastern Spain).

Soil properties	BSR	DHA	BGA	Alk-PA	UA	TOC	pH	EC	WFC
BSR	**1**	**0.032**	**0.347**	**0.076**	**-0.330**	**0.298**	**-0.132**	**0.198**	**-0.358**
DHA		**1**	**0.550**	**0.345**	**0.350**	**-0.261**	**0.327**	**-0.355**	**-0.032**
BGA			**1**	**0.726**	**0.335**	**0.289**	**0.070**	**-0.147**	**0.167**
Alk-PA				**1**	**0.271**	**0.437**	0.000	**-0.200**	**0.556**
UA					**1**	**-0.129**	**0.178**	**-0.203**	**0.169**
TOC						**1**	**-0.564**	**0.207**	**0.656**
pH							**1**	**-0.732**	**-0.304**
EC								**1**	**-0.166**
WFC									**1**

Notes: values in bold are different from zero with a p-level < 0.05.

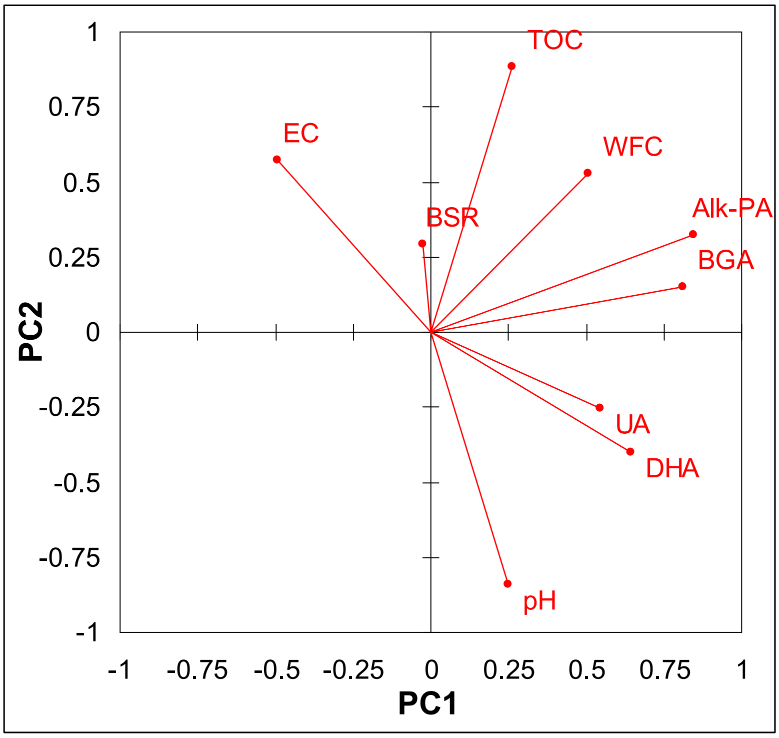
The PCA identified three main Principal Components (PCs), which explain together 75.7% of the total variance of the original variables. PC1 and PC2 explain 30.1% and 28.1% of this variance. In more detail, all the enzymatic activities showed high and positive loadings (> 0.544) on the PC1, while the chemical properties (TOC, pH and EC) significantly weigh on the PC2 (loadings over 0.572), TOC and EC having positive loadings (> 0.572) and pH a negative loading (-0.839). BSR (loading of 0.881) and WFC (loading of -0.618) significantly influence the PC3 (Table 3 and Figure 3a).

Table 3 - Factor loadings of the soil properties on the first two Principal Components provided by the Principal Component Analysis applied to soil samples collected under four conditions (unburned, U, burned, B, mulched with wood chips, M(WC), and mulched with wheat straw, M(WS)) in July 2022 in Liétor (Castilla La Mancha, Central Eastern Spain).

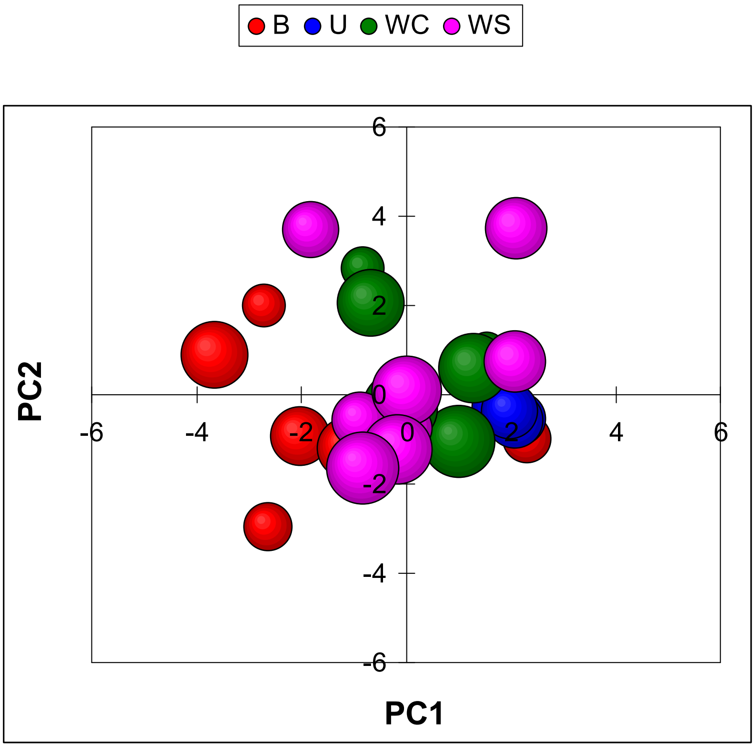
Soil property	**Principal component**		
	PC1	**PC2**	**PC3**
BSR	-0.027	0.291	**0.881**
DHA	**0.643**	-0.399	0.292
BGA	**0.812**	0.152	0.452
Alk-PA	**0.846**	0.323	0.034
UA	**0.544**	-0.253	-0.278
TOC	0.261	**0.883**	-0.041
pH	0.250	**-0.839**	0.048
EC	-0.494	**0.572**	0.198
WFC	0.504	0.530	**-0.618**

Notes: WFC = water field capacity; DHA = dehydrogenase activity; BGA = β-glucosidase activity; Alk-PA = alkaline phosphatase activity; UA = urease activity; TOC = total organic matter; EC = electrical conductivity; BSR = basal soil respiration; the values in bold are significant at $p < 0.05$.

The AHCA clustered the soil samples in three groups. A first cluster includes all samples collected in the U plots and three other samples of B, WC and WS plots. A second group consists of most samples collected in B, WC and WS plots (five samples for each of these soil conditions). Finally, the third cluster groups the remaining six samples with two pairs for each of B, WC and WS plots (Figures 3b and 4).



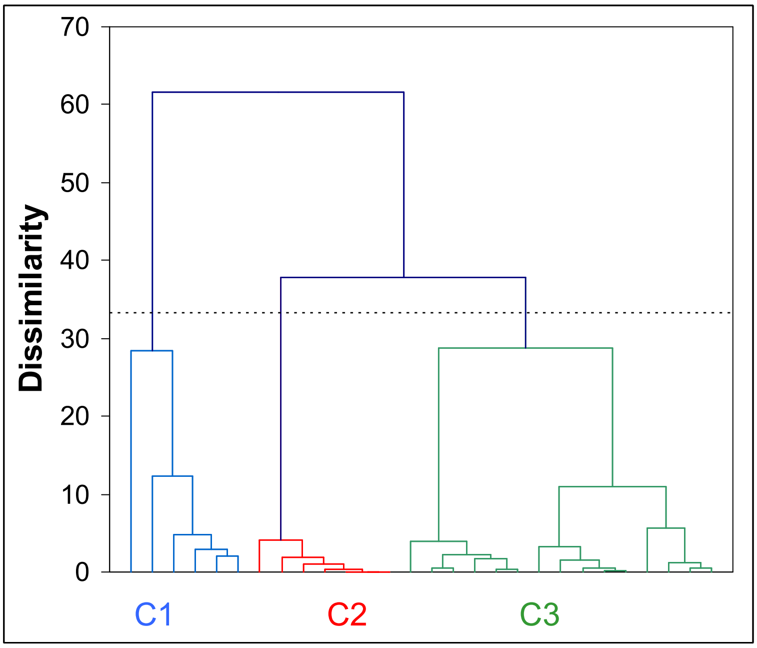
(a)



(b)

Figure 3 - Loadings of the original variables (a, soil properties), and scores (b) on the first three Principal Components (PC1, PC2 and PC3) provided by the Principal Component Analysis applied

to samples of soils collected under four conditions (unburned, U, burned, B, mulched with wood chips, M(WC), and mulched with wheat straw, M(WS)) in July 2022 in Liétor (Castilla La Mancha, Central Eastern Spain). *Legend: WFC = water field capacity; DHA = dehydrogenase activity; BGA = β-glucosidase activity; Alk-PA = alkaline phosphatase activity; UA = urease activity; TOC = total organic matter; EC = electrical conductivity; BSR = basal soil respiration; bubble diameter is proportional to the PC3. The diameter of bubbles in Figure 3b is proportional to the value of PC3.*



Cluster composition		
C1	**C2**	**C3**
B	B	B
WC	B	WC
WS	WC	WS
U	WC	WS
U	WS	B
U	WS	WC
U	B	
	B	
	B	
	WC	
	WC	
	WC	
	WS	
	WS	
	WS	

Figure 4 - Dendrogram and cluster composition provided by the Agglomerative Hierarchical Cluster Analysis (AHCA) applied to samples of soils collected under four conditions (unburned, U, burned, B, mulched with wood chips, M(WC), and mulched with wheat straw, M(WS)) in July 2022 in Liétor (Castilla La Mancha, Central Eastern Spain).

4. DISCUSSIONS

In degraded ecosystems, such as the severely-burned forests of the Mediterranean environment, soil functionality is a key aspect of ecosystem health, and its maintenance is challenging for land managers. Biochemical and microbiological indicators of soil activity are of paramount importance for maintaining its functionality, since these indicators are strongly asssociated to nutrient and organic matter dynamics (Gómez-Sánchez et al., 2019; Gutknecht et al., 2010). This study has shown that, among the biochemical and chemical parameters of soils under different conditions (unburned, burned, and burned and mulched with straw or wood chips), only the changes in BSR, DHA, pH and WFC due to the wildfire and treatments were significant compared to the control soils. Moreover, for two of these parameters (BSR and pH) and the other analysed soil properties (BGA, UA, TOC, and EC), the time elapsed from the treatment was also a significant factor of variability, while the Alk-PA was not affected by the fire or treatment disturbance and time from fire.

In general, all the enzymatic activities are well correlated with each other, and this agrees with findings of Lucas-Borja et al. (2022), also working in a burned pine forest of Central Eastern Spain, which was treated with different post-fire management techniques (namely log erosion barriers and contour felled log debris). In more detail, the mulching treatments increased soil respiration compared to the burned areas both in the soils mulched with wood chips or treated with straw).

However, the BSR values decreased in time (mainly due to the seasonality of chemical processes), and were higher (at both survey dates) in the mulched sites compared to both the burned plots and even to the unburned soils, especially in the case of treatment with straw. According to Bastian et al. (2009), straw mulching is a fresh organic residue that is incorporated in soil, and plays a noticeable and positive effect on all microbiological and enzymatic activities, such as the accumulation of organic matter and nutrients and their subsequent decomposition in soil (Lucas-Borja et al., 2020a). Increases in soil respiration and activity of microorganisms were found also after post-fire straw mulching by Lucas-Borja et al. (2022), Lucas-Borja et al. (2020b) and Lucas-Borja et al. (2020). These authors stated that the accumulation of organic matter coming from the burned plant material (Rodríguez et al., 2017) continue until these mineralised materials have been consumed (Muñoz-Rojas et al., 2016) and their decomposition (Lucas-Borja et al., 2020c).

The enzymatic activities in the unburned soils measured six months after mulching were generally greater compared to the burned sites, and this is expected (Lucas-Borja et al., 2021). The latter authors stated that the lower enzymatic activity in burned soils is a clear effect of wildfire, which, due to the high soil temperature, destroys a large amount of the enzymes (Barreiro et al., 2010). Moreover, again Lucas-Borja et al. (2021) attributed these differences to the nutrient cycling, climate regulation, waste decomposition, wood production, and water regulation functions, which were lower in the soils subject to wildfire. In the forest soils, the application of two post-fire management techniques, such as mulching with straw or wood chips, reduced the depletion of enzyme content due to wildfire, although soil enzymes generally do not recover to the pre-fire (unburned) conditions. A limited effect was noticed for soil DHA, which was comparable in the burned plots (treated or not) and significantly lower compared to the unburned soils, in particularly after six months from the treatments. Mulching soils with straw could promote bacterial development, but the DHA could behave quite differently from other enzymes (Lucas-Borja et al., 2020b). The latter authors found no changes reductions in soil DHA after a wildfire and straw mulching in pine forests of Spain, and this effect was ascribed to the lack of sensitivity of DHA to site effects rather than management practices (Gómez-Sánchez et al., 2019; Lucas-Borja et al., 2022; Quilchano and Marañón, 2002). This could be related to the fact that dehydrogenases are not active as extracellular enzymes in soil, thus presenting a different pattern compared to extracellular soil enzymes, that is, BGA, UA and acid-phosphatase (Błońska et al., 2017; Lucas-Borja et al., 2022, 2019).

In contrast to what observed for BSR, DHA, pH and WFC, the other enzymatic activities (BGA, UA and Alk-PA) as well as the other chemical properties (TOC and EC) of the studied soils were not affected either by the wildfire or soil mulching. More specifically, a clear and expected reduction in the BGA was noticed from January to July, and this decrease was common to all soil conditions. BGA, one of the enzymes that break down labile cellulose and other carbohydrate polymers, plays a fundamental action in order to liberate the nutrients and organic compounds through its role in the first phases of degradation of organic compounds. This reduces the molecular size and produces smaller organic structure, thus facilitating soil enzyme activities (Sardans et al., 2008). For UA and Alk-PA, the increases in burned and mulched soils were always negligible. The lack of soil response of UA and BGA to wildfire and mulching in our study is surprising, since the application of straw generally leads to the accumulation of nitrogen and the evolution of BGA is related to decomposition velocity of straw (Gómez-Sánchez et al., 2019), as shown by Lucas-Borja et al. (2020b), who reported that UA and BGA activity was greater in burned and mulched soils compared to both unburned, and burned and untreated sites. As Criquet et al. (2004) and Sardans and Peñuelas (2005) demonstrated, the progressive temporal and weather changes among the analysed soil conditions suggest that some enzymatic activities (e.g., UA, Acid and Alk-PA and BGA) substantially decrease in the dry season. It is also necessary to highlight that, when burned, either treated or not, differences for soil enzyme activities were hard to find and seasonality is not as an important factor (Lucas-Borja et al., 2020).

Regarding the main chemical properties of the studied soils, a low variability among the four soil conditions was noticed for pH, although this variability made this parameter significantly different. The fire and the mulching significantly reduced the pH of the soil immediately after the treatment, but this influence lost importance six months after the treatments. According to the literature, soil pH is increased by the heating as a result of denaturation of organic acids (Certini, 2005), and the increase of sodium and potassium oxides, carbonates and hydroxides from ash (Pereira et al., 2018; Ulery et al., 1993). Some authors have observed decreased pH also in soils exposed to high temperatures in the laboratory (e.g., Wondafrash et al. 2005), although experiments under laboratory conditions usually do not consider the effect of ash (Zavala et al., 2014).

The TOC significantly increased over time, while the reverse pattern was observed for EC. Although the differences among the four soil conditions were not significant, the mulched soils showed at both survey dates higher TOC contents. It should also be noticed that the temporal variability in this parameter was high also in the unburned soils, and this may be due to the seasonal differences in the organic matter dynamics. Increases in organic matter after straw mulching in burned soils compared to both unburned, and burned and non-mulched sites were also observed by (Lucas-Borja et al., 2020b). In general, significant changes in organic matter content in soils affected by wildfire compared to unburned soils are common (e.g., García-Orenes et al., 2017; González-Pérez et al., 2004), which indicates an improved soil fertility. The increases in organic matter may be due to accumulation of ash, which contains carbon and other nutrients from burned forest fuel (Bodí et al., 2012; Caon et al., 2014), as well as to the decomposition of organic compounds of the mulch material. Among the chemical properties, the organic matter amount is one of the most important quality indicators, since the organic compounds influence plant growth-related functions (e.g., retained humidity, nutrient reservoir and exchange) (Muñoz-Rojas et al., 2016), the maintenance of productivity, biodiversity and other ecosystem services (Gómez-Sánchez et al., 2019; Lucas-Borja et al., 2022), and an enhanced biological activity of soils (Robichaud, 2000). However, the soil functionality may not depend only on the quantity of the organic matter applied to the soil, but also on the quality of the organic compounds supplied with the restoration techniques (Lucas-Borja et al., 2022). Moreover, in line with findings of Lucas-Borja et al. (2020c), it is worth to notice that the increases in organic matter recorded in the burned soils did not generate a parallel increase in the DHA and BSR (see the coefficients of correlation between TOC on one side and DHA and BSR on the other side), showing an uncoupling of the soil microbial biomass and its activity. The EC, which was stable in the unburned and burned soils, significantly decreased over time in the mulched plots. However, in contrast with our findings, which showed the lack of significance of the increases in EC among unburned, burned and burned and mulched sites, the literature shows that wildfire significantly increases the EC of burned soils compared to the unburned soils (Mataix-Solera et al., 2002; Muñoz-Rojas et al., 2016).

About the hydrological properties of soils, the wildfire noticeably reduced the WFC of burned soils. This reduction was detected both immediately after mulching and six months after but mulching was effective at limiting this decrease. However, these differences smoothed over time, and this was mainly due to the increase in WFC in the unburned soils from January to July. WFC was basically stable in the burned and untreated, and in the mulched soils, although always lower compared to the control. The improvement of water availability due to mulching supports the enzymatic activity and microorganism growth, thanks to the reduced evaporation and the incorporation to soil of available nutrients and organic carbon compounds with straw (Siczek and Frac, 2012). This is a very important effect of mulching especially in the Mediterranean forests growing under semi-arid conditions, since, as Merilä et al. (2002) showed, low soil moisture is a major factor in controlling the activity of microbes and the seasonal changes in soil moisture are frequently reported to affect enzymatic activities in forest soils (Baldrian et al., 2010).

The multivariate statistical analysis using PCA and AHCA demonstrated the presence of three meaningful derivative variables (the first three PCs), which were separately influenced by the different types of soil parameters. More specifically, while the four enzymatic activities (DHA, BGA, UA and Alk-PA) together significantly weigh on the first PC, the chemical parameters (pH, TOC and EC) are strongly linked to the second PC, and the third PC is associated to the BSR and WFC. This means that a clear gradient on the first PC (linked to the enzymatic activities with special reference to BSR and DHA, which are significantly different among the soil conditions) is established between burned soils (low content of soil enzymes) on one side, and the unburned soils (where, in contrast, the enzymatic activities are high), the mulched soils being in an intermediate level of these biochemical properties). The other gradient, established along the second PC, which is mainly influenced by the soil chemical properties, has a much lower significance, since the TOC and EC are not significantly different among the four soil conditions. However, a noticeable overlapping among the three clusters evidenced by the AHCA, with the exception of the cluster grouping the samples of the burned soils, which appears clearly distinct from the other soil groups.

5. CONCLUSION

The study has shown that, in soils of pine forests in Central Eastern Spain, only BSR, DHA, pH and WFC significantly changed after the wildfire and mulching with straw and wood chips. In contrast, the other enzymatic activities (BGA, UA, and Alk-PA) and chemical properties (TOC and EC) were not influenced by burning and post-fire management. The time elapsed from the fire and mulching was a significant factor of variability for many studied parameters (BSR, pH, BGA, UA, TOC, and EC), mainly due to the seasonality of the chemical and biochemical processes. This finding replies to the first working hypothesis, showing which soil enzymatic activities is significantly altered by fire and post-fire mulching in Mediterranean forests.

Mulching increased soil respiration compared to the burned areas, especially in soils mulched with straw, thanks to the addition of fresh organic residues, quickly incorporated in the soil. Moreover, the treatments reduced the depletion of enzyme content noticed in the burned soils, except for DHA. While soil pH showed a low variability among the four soil conditions, TOC was higher in the mulched soils, and this was correlated to the increase in BSR. A key role by mulching was played with reference by WFC, since the treatments helped to limit its reduction after the wildfire. This improvement of water availability due to mulching supports the enzymatic activity and microorganism growth, and this is a very important effect of mulching especially in the Mediterranean forests growing under semi-arid conditions. In general, the differences in the analysed soil properties were less pronounced with reference to the much materials applied compared to the effects of the wildfire, and this confirms our second working hypothesis. Finally, the multivariate statistical analysis using PCA and AHCA confirmed the significant influence of the treatments on some enzymatic activities. However, a sharp discrimination among the four soil conditions was only evident between the unburned and burned (mulched or not) sites, thus partially rejecting the third working hypothesis that the differences in soil functionality among the soil conditions are pronounced.

Overall, the study helps forest managers to preserve soil functionality of fire-affected areas in the Mediterranean forests. This task is essential towards a quick vegetation recovery and soil conservation in these delicate ecosystems.

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