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

18

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31

32 **ABSTRACT**

33

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

41 (DHA), pH and water field capacity (WFC) significantly changed after the fire and mulching. In
42 contrast, the other enzymatic activities - urease (UA), alkaline phosphatase (Alk-PA) and β -
43 glucosidase (BGA), - total organic carbon (TOC) and electrical conductivity (EC) were not
44 influenced by these soil disturbances. Time from fire and soil conditions (due to burning and
45 management) were significant variability factors for BSR, pH, BGA, UA, TOC, EC. Mulching
46 increased BSR compared to burned areas, especially in soils with straw (+30%), thanks to addition
47 of fresh organic residues, quickly incorporated in the soil. Soil pH showed a low variability among
48 the four soil conditions, and TOC was higher in mulched soils (on average +20% compared to the
49 burned soils), and this was correlated to the increased BSR. The role of mulching was essential with
50 reference to WFC, as the post-fire management limited its reduction after the fire (on average from
51 -30% to -20%). Finally, the Principal Component Analysis coupled to the Analytical Hierarchical
52 Cluster Analysis confirmed the significant influence of the post-fire management on some
53 enzymatic activities, although a sharp discrimination among the four soil conditions was only
54 evident between unburned and burned sites, regardless of the management. Overall, it has been
55 shown that mulching promotes conservation of fragile Mediterranean soils, indicating its
56 effectiveness at preserving soil functionality in areas affected by forest fires.

57

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

60

61 **1. INTRODUCTION**

62

63 The Mediterranean forests are particularly exposed to the wildfire risk (Shakesby, 2011;
64 Wagenbrenner et al., 2021). Wildfire leaves the forest soil bare due to vegetation removal (Bodí et
65 al., 2012; Shakesby and Doerr, 2006), changes several properties of soils (e.g., the aggregate

66 stability, the contents of organic matter and nutrients, and the microbial community composition
67 (Certini, 2005; Zavala et al., 2014), and induces water repellency, with increased surface runoff and
68 soil erosion (Zema et al., 2021a, 2021b). These fire effects mainly depend on the soil burn severity
69 (Lucas - Borja et al., 2022), which is directly linked to forest fuel amount and type, and fire
70 characteristics (frequency, duration, energy). The pre-fire soil conditions recovery after several
71 years or even some decades, when the fire severity is extremely high (Certini, 2005; Glenn and
72 Finley, 2010).

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

86 The scientific literature has mainly focused the hydrological effects of mulching (e.g., reduction in
87 runoff and soil erosion), while its impacts on other components of the forest ecosystems have been
88 less investigated. This is an important issues, since post-fire management may generate changes in
89 biological, chemical and physical properties of soil in burned forests, which influence biota
90 composition and activity (Badía et al., 2015; Inbar et al., 2014; Killham, 1994; Lucas-Borja et al.,

91 2020c). Moreover, few studies have explored the impacts of mulching materials on those properties
92 that are related to soil functionality. On this regard, enzymes and respiration of soil have been
93 widely used to evaluate the functionality of soil (Fioretto et al., 2009; Utobo and Tewari, 2015). Soil
94 enzymes and microbiome regulate organic matter decomposition and stabilization, post-fire nutrient dynamics and
95 rhizosphere function (Nelson et al., 2022). Several enzyme activities, specifically related to the cycles
96 of carbon, nitrogen and phosphorous (β -glucosidase, urease, alkaline and acid phosphatase,
97 respectively (Lucas-Borja et al., 2020) and some general microbial indicators (e.g., dehydrogenase
98 activity and soil respiration) have been proposed as specific indicators of soil functionality (Bastida
99 et al., 2008). Moreover, as key microbiological soil properties, soil respiration and enzyme activities
100 are considered as closely associated to organic matter decomposition and formation (Cerdà et al.,
101 2016; Gutknecht et al., 2010).

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

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

125

126 **2. MATERIALS AND METHODS**

127

128 **2.1. Study area**

129

130 The study area is the Sierra de Los Donceles forest (municipality of Liétor, province of Albacete,
131 region of Castilla-La Mancha, Spain, 38°30'41" N; 1°56'35" W) at an elevation between 520 and
132 770 m above the mean sea level (Figure 1) and north-west aspect. The climate is typically semi-arid
133 Mediterranean (BSk type, according to the Köppen classification (Kottek et al., 2006)). Mean
134 annual values of temperature and precipitation are equal to 16.6 °C and 321 mm, respectively, from
135 the last 20 years of weather data collected at the meteorological station of Hellín, about 20 km far
136 from Liétor (historical records of the Spanish Meteorological Agency, AEMET). Soils are Calcic
137 Aridisols (Nachtergaele, 2001), and their texture is sandy loamy. The geology is typical pre-Baetic
138 Mountains, with limestone and dolomite outcrops alternating with marly intercalations that date
139 back to the quaternary.

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

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

151

152

153



154 Figure 1 – Geographical location and aerial map of the study area (Liétor, Castilla La Mancha,
155 Central Eastern Spain).
156

157

158 2.2. Experimental design

159

160 One week after the wildfire, a study area of 700 ha was selected, including both unburned and
161 burned forest soils (the latter affected by crown fire with 100% tree mortality). In this burned area, a
162 site with profile slope between $30.1 \pm 3.9\%$ and $48.1 \pm 4.7\%$ was identified. Soils with low slope (<
163 20%) were excluded, since these hillslopes are less prone to erosion, and the same was done for
164 soils with high slope (> 60%), where pine forests commonly do not grow, at least in Central Eastern
165 Spain. In each site, four blocks of eighteen plots, each one with an area of 2 square metres, were
166 installed. One block of four plots was not burned (hereafter indicated as “unburned”, U), and
167 considered as control. A second block of eight plots (“burned”, B) was burned but not treated. The
168 third and four blocks were mulched with straw, M(WS) (four plots per block) or wood chips,
169 M(WC) (four plots per block).

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

175 wood cheap (mean values): dose of 0.3 kg/m^2 ; length: 3-10 cm; width: 2-4 cm;
176 thickness: 1-2 cm; density: $500\text{-}550 \text{ kg/m}^3$;

177 straw (mean values): dose or 2 kg/m^2 ; length: 5-25 cm; width: 0.25-1.0 cm; thickness:
178 0.1-0.7 cm; density: $80\text{-}100 \text{ kg/m}^3$.

179 These application rates are those suggested by the forest services of the Iberian Peninsula, and
180 widely used in literature (e.g., Girona-García et al., 2021; Kim et al., 2008; Lucas-Borja et al.,
181 2019).

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

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

186

187 **2.3. Soil sampling**

188

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

197

198 **2.4. Analysis of soil functionality**

199

200 One day after sampling, each soil sample was air dried, homogenised and sieved (< 2 mm) before
201 analysis. The following soil chemical properties were determined on the collected samples: (i) pH
202 and electrical conductivity (EC), determined in distilled water, at a soil:solution ratio of 1:2.5 and
203 1:5 respectively, using a a digital pHmeter (LAQUA PH1100, HORIBA, Tokio, Japan) and
204 conductivity meter (Crison 522, Barcelona, Spain); (iii) Total Organic Carbon (TOC) was
205 determined by the [Walkey and Black \(1934\)](#) method modified by [Mingorance et al., \(2007\)](#) and
206 measured in a spectrophotometer (Spectronic Helios Gamma UV-Vis, Thermo Fisher Scientific,
207 Waltham, Massachusetts, USA). Water field capacity (WFC) were determined as pF -33 KPa by
208 Richards membrane method (Richards, 1941).

209 Basal soil respiration (BSR, expressed as $\text{mg C-CO}_2 \text{ kg}^{-1} \text{ day}^{-1}$ of dry soil), was measured with
210 using an infrared CO_2 sensor (IRGA S151; Qubit Systems Inc., Canada). Soil dehydrogenase
211 activity (DHA, expressed as $\mu\text{g INTF hour}^{-1} \text{ g}^{-1}$ of dry soil) was determined by the reduction of p-
212 iodinitrotetrazolium chloride (INT) to p-iodinitrotetrazolium formazan (INTF) following García et
213 al. (1997). Urease activity (UA), expressed as $\mu\text{mol N-NH}_4^+ \text{ hour}^{-1} \text{ g}^{-1}$ of dry soil) was measured
214 using urea as a substrate and a borate buffer at $\text{pH} = 10$ (Kandeler and Gerber, 1988). The activity
215 of alkaline phosphatase (Alk-PA) and β -glucosidase (BGA), both expressed as $\mu\text{mol pNP hour}^{-1} \text{ g}^{-1}$
216 of dry soil, were determined using the methods of Tabatabai and Bremner (1969) and Eivazi and
217 Tabatabai (1988) , respectively.

218

219 **2.5. Statistical analysis**

220

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

231 Following this, a Principal Component Analysis (PCA) was applied to the soil samples collected in
232 the last survey (July 2022), in order to identify the existence of meaningful derivative variables
233 (Principal Components, PCs) (Lee Rodgers and Nicewander, 1988) and simplify the analysis of the
234 large number of soil properties and conditions, losing as little information as possible. In this study,

235 PCA was carried out by standardizing the original variables (expressed by different measuring
236 units) and using Pearson's method to compute the correlation matrix. This matrix allowed the
237 identification of relationships among the soil properties analysed. The first PCs that explain at least
238 70% of the original variance were retained.

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

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

245

246 **3. RESULTS**

247

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

254

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

259

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

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

263

264

265 In January 2022, BSR was the highest in the treated soils (79.7 ± 4.9 mg of C-CO₂ kg⁻¹ day⁻¹, for
266 WC plots, and 83.7 ± 6.56 mg of C-CO₂ kg⁻¹ day⁻¹, for WS plots) and the lowest in the B plots (68.8
267 ± 5.45 mg of C-CO₂ kg⁻¹ day⁻¹). The same BSR patterns were surveyed in July 2022, but at this

268 date, the values of U (46.2 ± 0.12 mg of C-CO₂ kg⁻¹ day⁻¹) and B (45.5 ± 4.93 mg of C-CO₂ kg⁻¹
269 day⁻¹) plots were practically the same (Figure 2). After 12 months from the wildfire (July 2022), the
270 BSR significantly decreased under all soil conditions (Figure 2).

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

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

279 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
280 7.14 ± 0.04 μmol of PNF h⁻¹ g⁻¹ of soil (U soils in January), but this enzymatic activity was very
281 similar among the soil conditions and the survey dates (Figure 2).

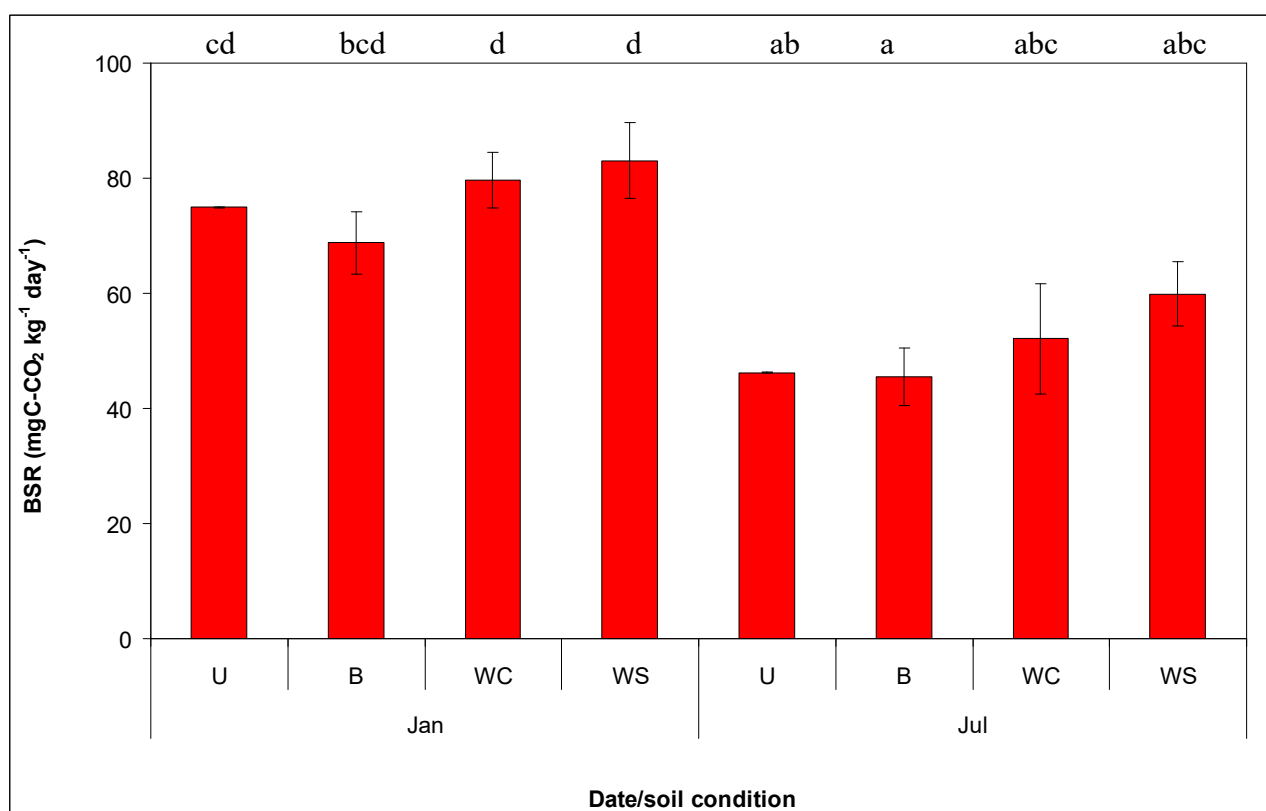
282 A gradient U > WC > B > WS plots was observed for the UA both in January and in July, with the
283 minimum and maximum values observed in the WS soils in July (1.55 ± 0.50 μmol of N-NH₄ h⁻¹g⁻¹
284 of soil) and in U plots in January (2.98 ± 0.01 μmol of N-NH₄ h⁻¹g⁻¹ of soil) (Figure 2). The
285 temporal difference in UA was significant, while that gradient not (Table 1).

286 Regarding the main chemical properties, TOC significantly increased throughout the survey dates.
287 Although the differences in this parameter were not significant among the four soil conditions, the
288 lowest TOC was measured in U plots in January ($2.53 \pm 0.19\%$), while the highest value was
289 detected in WC soils in July ($4.78 \pm 0.59\%$) (Figure 2). The pH was similar at the two survey dates,
290 but not among the analysed soil conditions. The U (9.22 ± 0.04) and WC (8.92 ± 0.04) plots showed
291 the highest and lowest values, respectively, in January, while the minimum and maximum pH in
292 July was measured in the WS (9 ± 0.06) and B (9.03 ± 0.07) soils, respectively (Figure 2). The U

293 soils showed the lowest EC at both survey dates (0.16 ± 0.01 mS/cm in January, and 0.15 ± 0.001
294 mS/cm in July). However, while in January the highest EC was measured in the WC and WS plots
295 (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
296 mS/cm) (Figure 2).

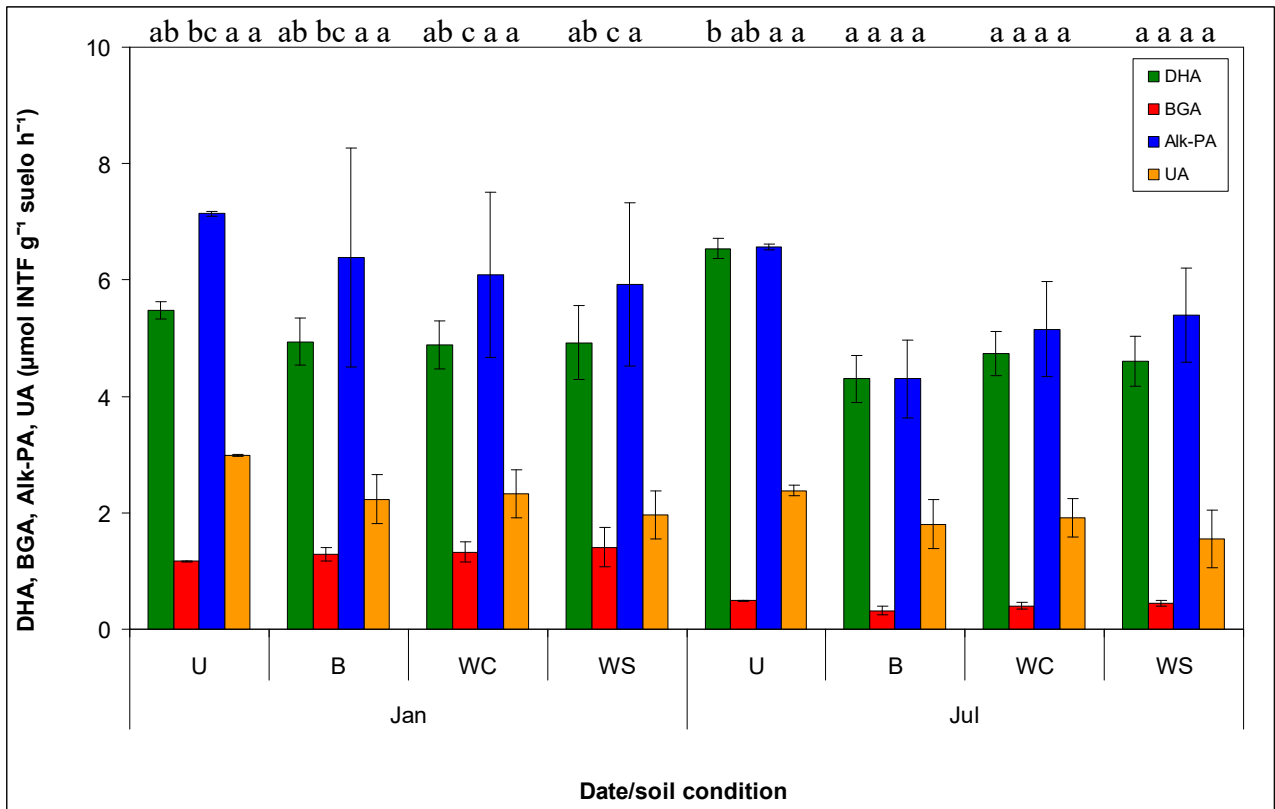
297 The WFC, which was not significantly variable over time, was always higher in the U soils ($38.1 \pm$
298 0.36% in January, and $31.2 \pm 0.08\%$ in July), and lower in the B plots ($26.7 \pm 3.54\%$) compared to
299 the treated soils. The latter showed a WFC closer to the B soils than to the U plots ($28.7 \pm 2.15\%$ in
300 January, and $27 \pm 3.38\%$ in July for WS soils, $28.5 \pm 2.97\%$ in January, and $27.3 \pm 3.07\%$ in July
301 for WC soils) (Figure 2).

302

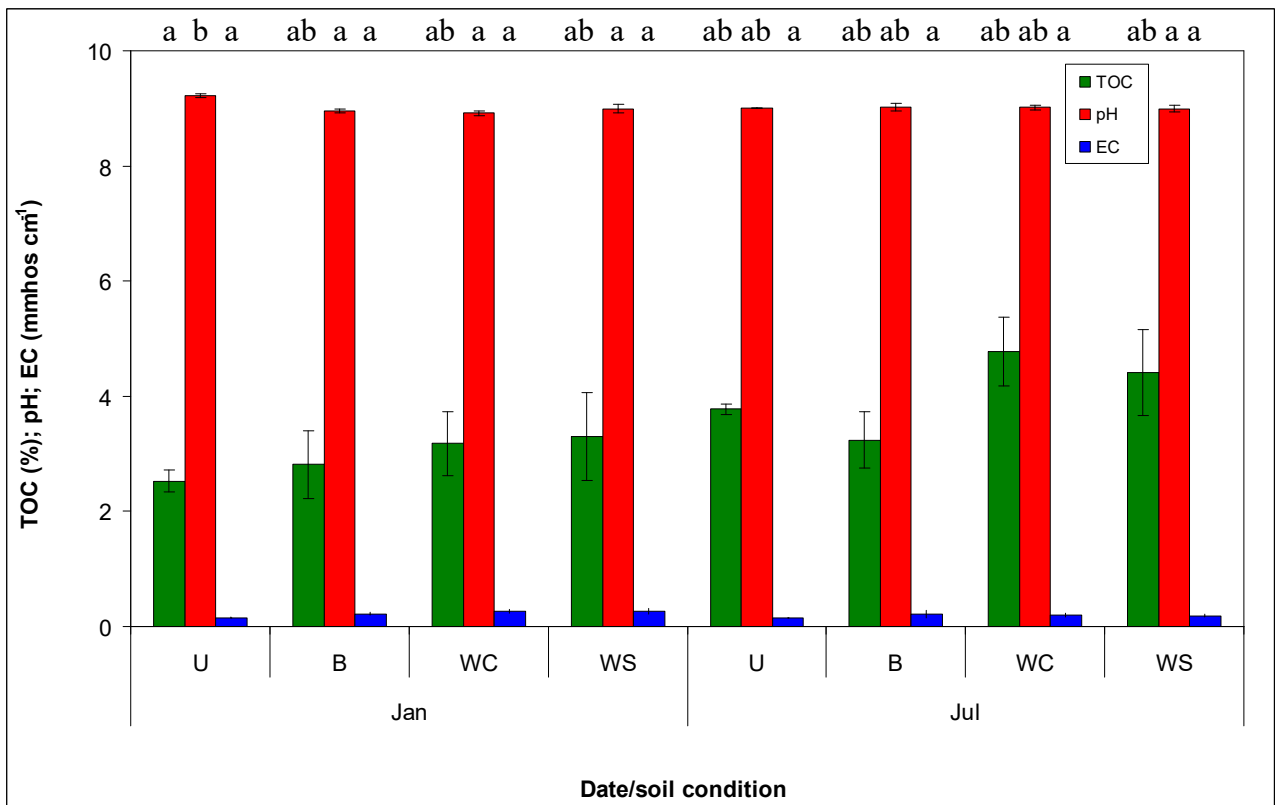


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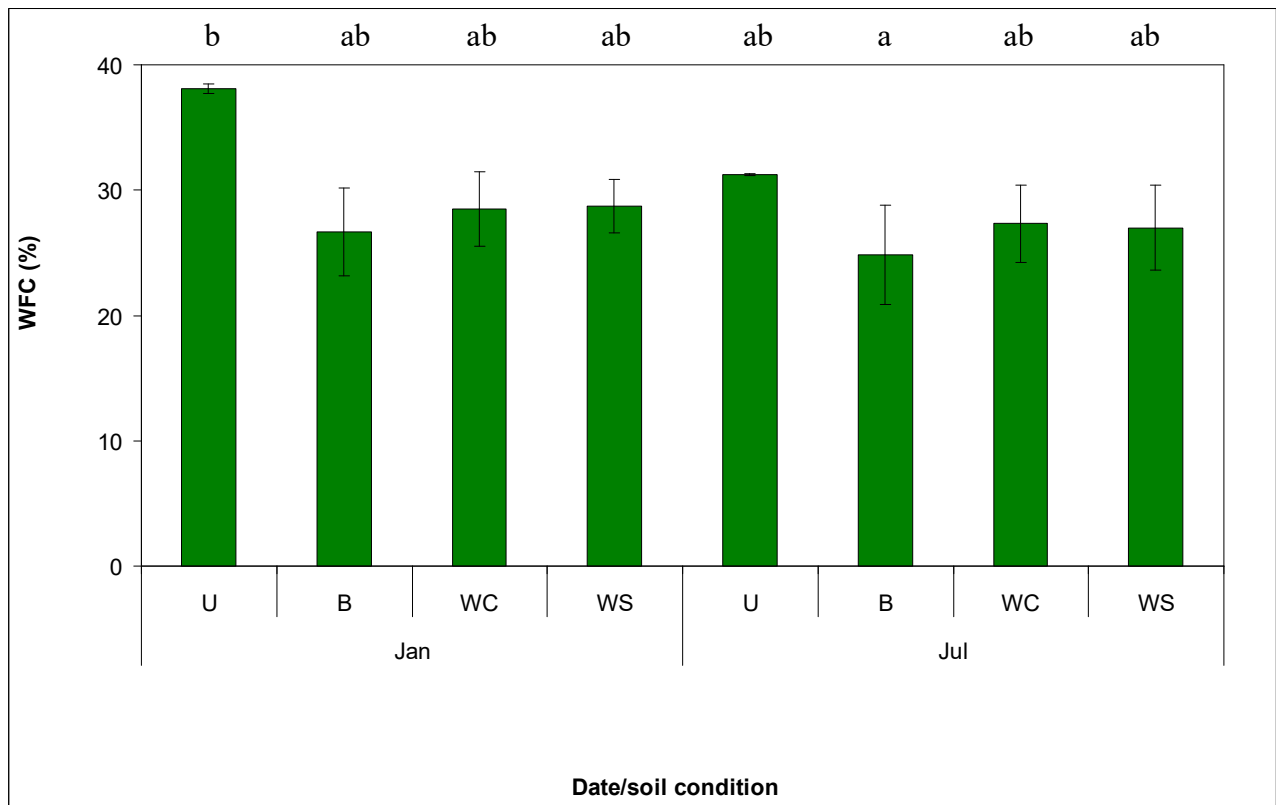
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307

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

315

316

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

321

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

325

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

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

327

328

329 The PCA identified three main Principal Components (PCs), which explain together 75.7% of the
 330 total variance of the original variables. PC1 and PC2 explain 30.1% and 28.1% of this variance. In
 331 more detail, all the enzymatic activities showed high and positive loadings (> 0.544) on the PC1,
 332 while the chemical properties (TOC, pH and EC) significantly weigh on the PC2 (loadings over
 333 0.572), TOC and EC having positive loadings (> 0.572) and pH a negative loading (-0.839). BSR
 334 (loading of 0.881) and WFC (loading of -0.618) significantly influence the PC3 (Table 3 and Figure
 335 3a).

336

337

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

342

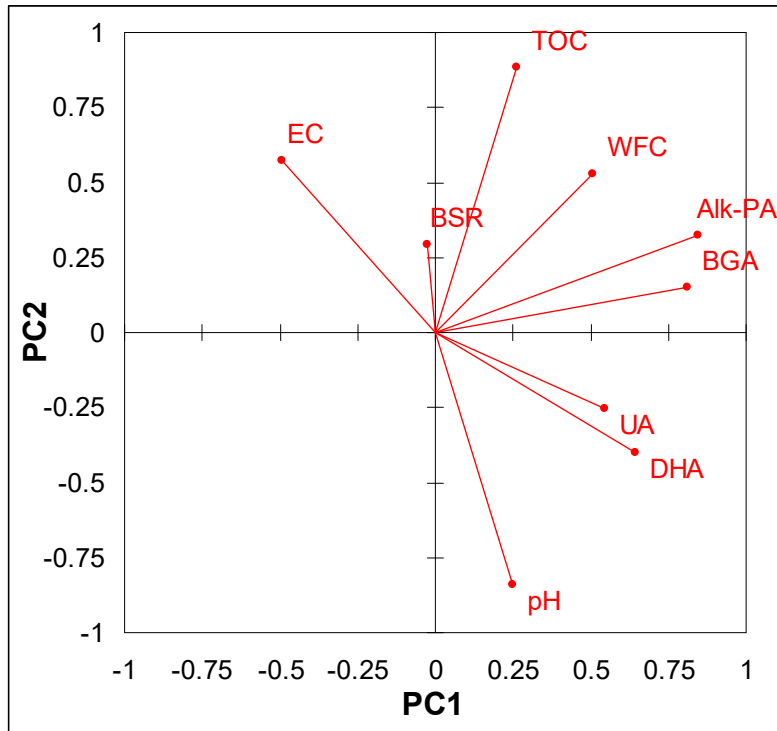
Soil property	Principal component		
	PC1	PC2	PC3
BSR	-0.027	0.291	0.881
DHA	0.643	-0.399	0.292
BGA	0.812	0.152	0.452
Alk-PA	0.846	0.323	0.034
UA	0.544	-0.253	-0.278
TOC	0.261	0.883	-0.041
pH	0.250	-0.839	0.048
EC	-0.494	0.572	0.198
WFC	0.504	0.530	-0.618

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

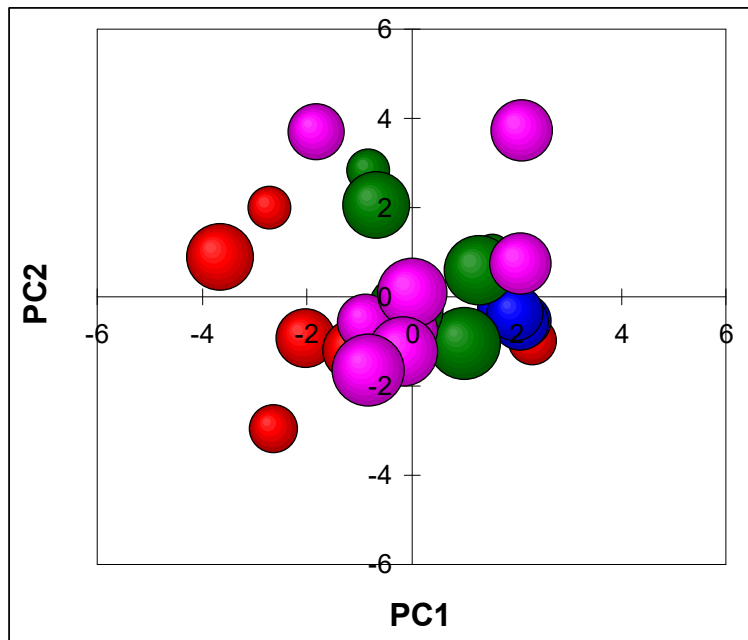
346

347

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



(a)



(b)

353

354

355

356

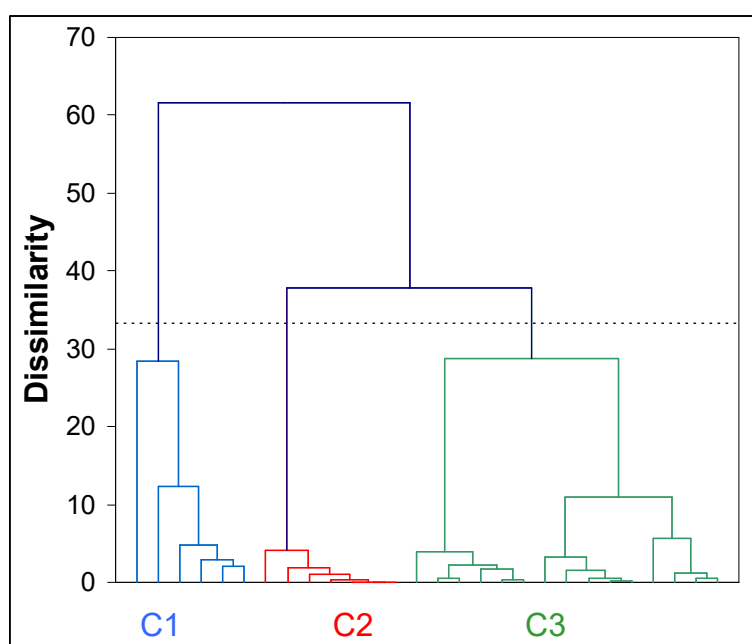
357

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

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

366

367



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	

368

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

373

374 **4. DISCUSSIONS**

375

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

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

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

404 The enzymatic activities in the unburned soils measured six months after mulching were generally
405 greater compared to the burned sites, and this is expected (Lucas-Borja et al., 2021). The latter
406 authors stated that the lower enzymatic activity in burned soils is a clear effect of wildfire, which,
407 due to the high soil temperature, destroys a large amount of the enzymes (Barreiro et al., 2010).
408 Moreover, again Lucas-Borja et al. (2021) attributed these differences to the nutrient cycling,
409 climate regulation, waste decomposition, wood production, and water regulation functions, which
410 were lower in the soils subject to wildfire. In the forest soils, the application of two post-fire
411 management techniques, such as mulching with straw or wood chips, reduced the depletion of
412 enzyme content due to wildfire, although soil enzymes generally do not recover to the pre-fire
413 (unburned) conditions. A limited effect was noticed for soil DHA, which was comparable in the
414 burned plots (treated or not) and significantly lower compared to the unburned soils, in particularly
415 after six months from the treatments. Mulching soils with straw could promote bacterial
416 development, but the DHA could behave quite differently from other enzymes (Lucas-Borja et al.,
417 2020b). The latter authors found no changes reductions in soil DHA after a wildfire and straw

418 mulching in pine forests of Spain, and this effect was ascribed to the lack of sensitivity of DHA to
419 site effects rather than management practices (Gómez-Sánchez et al., 2019; Lucas-Borja et al.,
420 2022; Quilchano and Marañón, 2002). This could be related to the fact that dehydrogenases are not
421 active as extracellular enzymes in soil, thus presenting a different pattern compared to extracellular
422 soil enzymes, that is, BGA, UA and acid-phosphatase (Błońska et al., 2017; Lucas-Borja et al.,
423 2022, 2019).

424 In contrast to what observed for BSR, DHA, pH and WFC, the other enzymatic activities (BGA,
425 UA and Alk-PA) as well as the other chemical properties (TOC and EC) of the studied soils were
426 not affected either by the wildfire or soil mulching. More specifically, a clear and expected
427 reduction in the BGA was noticed from January to July, and this decrease was common to all soil
428 conditions. BGA, one of the enzymes that break down labile cellulose and other carbohydrate
429 polymers, plays a fundamental action in order to liberate the nutrients and organic compounds
430 through its role in the first phases of degradation of organic compounds. This reduces the molecular
431 size and produces smaller organic structure, thus facilitating soil enzyme activities (Sardans et al.,
432 2008). For UA and Alk-PA, the increases in burned and mulched soils were always negligible. The
433 lack of soil response of UA and BGA to wildfire and mulching in our study is surprising, since the
434 application of straw generally leads to the accumulation of nitrogen and the evolution of BGA is
435 related to decomposition velocity of straw (Gómez-Sánchez et al., 2019), as shown by Lucas-Borja
436 et al. (2020b), who reported that UA and BGA activity was greater in burned and mulched soils
437 compared to both unburned, and burned and untreated sites. As Criquet et al. (2004) and Sardans
438 and Peñuelas (2005) demonstrated, the progressive temporal and weather changes among the
439 analysed soil conditions suggest that some enzymatic activities (e.g., UA, Acid and Alk-PA and
440 BGA) substantially decrease in the dry season. It is also necessary to highlight that, when burned,
441 either treated or not, differences for soil enzyme activities were hard to find and seasonality is not as
442 an important factor (Lucas-Borja et al., 2020).

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

452 The TOC significantly increased over time, while the reverse pattern was observed for EC.
453 Although the differences among the four soil conditions were not significant, the mulched soils
454 showed at both survey dates higher TOC contents. It should also be noticed that the temporal
455 variability in this parameter was high also in the unburned soils, and this may be due to the seasonal
456 differences in the organic matter dynamics. Increases in organic matter after straw mulching in
457 burned soils compared to both unburned, and burned and non-mulched sites were also observed by
458 (Lucas-Borja et al., 2020b). In general, significant changes in organic matter content in soils
459 affected by wildfire compared to unburned soils are common (e.g., García-Orenes et al., 2017;
460 González-Pérez et al., 2004), which indicates an improved soil fertility. The increases in organic
461 matter may be due to accumulation of ash, which contains carbon and other nutrients from burned
462 forest fuel (Bodí et al., 2012; Caon et al., 2014), as well as to the decomposition of organic
463 compounds of the mulch material. Among the chemical properties, the organic matter amount is one
464 of the most important quality indicators, since the organic compounds influence plant growth-
465 related functions (e.g., retained humidity, nutrient reservoir and exchange) (Muñoz-Rojas et al.,
466 2016), the maintenance of productivity, biodiversity and other ecosystem services (Gómez-Sánchez
467 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

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

505

506 **5. CONCLUSION**

507

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

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

530 rejecting the third working hypothesis that the differences in soil functionality among the soil
531 conditions are pronounced.

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

535

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537

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545

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