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**Abstract**

Understanding the changes in physicochemical and microbiological soil properties induced by straw soil mulching is very important in the Mediterranean environment, where forest ecosystems are particularly prone to erosion and degradation risks. To fill this gap, this study has evaluated the seasonal changes (from spring to autumn) in important physicochemical soil properties, such as pH or soil organic matter, and enzymatic activities, such as urease or dehydrogenase activities, in burned (treated with mulching or not) plots, compared to non-burned soils, after a wildfire occurred in a Pinus halepensis M. forest. Monitoring activity has confirmed that the treatment of burned soils with straw mulching improves its functionality in the short term. More specifically, although soil pH was stable and electric conductivity noticeably reduced, the organic matter was higher in burned and mulched soils compared to non-burned soils. The increases of basal respiration as well as microbial carbon and glomalin contents after mulching indicated higher activity of soil microorganisms and increased carbon and nitrogen storage. Moreover, all microbiological and enzymatic activities improved, except for dehydrogenase activity (DHA). Overall, this study highlights that soil functionality of wildfire-affected areas significantly benefits with straw mulching treatment.

**Keywords** High-severity fire; Mediterranean forest; soil enzymes; soil respiration; soil organic matter.

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Dear editor of FORECO journal,

I have uploaded to the managing platform of FORECO a version of the manuscript entitled "**Short-term changes in soil functionality after wildfire and straw mulching in a *Pinus halepensis* M. forest"**. Understanding the changes in physicochemical and microbiological soil properties induced by straw soil mulching is very important in the Mediterranean environment, where forest ecosystems are particularly prone to erosion and degradation risks. To fill this gap, this study has evaluated the seasonal changes (from spring to autumn) in important physicochemical soil properties, such as pH or soil organic matter, and enzymatic activities, such as urease or dehydrogenase activities, in burned (treated with mulching or not) plots, compared to non-burned soils, after a wildfire occurred in a Pinus halepensis M. forest.

Thank you very much for the opportunity of Submitting our work to FORECO journal

Yours sincerely,

Dr. Manuel Esteban Lucas-Borja

Corresponding author

Highlights

- Burned soils with straw mulching improves its functionality in the short term.
- SOM was higher in burned and mulched soils compared to non-burned soils.
- All microbiological and enzymatic activities improved in mulched plots.
- Soil functionality significantly benefits with straw mulching treatment.

**Short-term changes in soil functionality after wildfire and straw mulching in a *Pinus halepensis* M. forest**

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**Abstract**

Understanding the changes in physicochemical and microbiological soil properties induced by straw soil mulching is very important in the Mediterranean environment, where forest ecosystems are particularly prone to erosion and degradation risks. To fill this gap, this study has evaluated the seasonal changes (from spring to autumn) in important physicochemical soil properties, such as pH or soil organic matter, and enzymatic activities, such as urease or dehydrogenase activities, in burned (treated with mulching or not) plots, compared to non-burned soils, after a wildfire occurred in a *Pinus halepensis* M. forest. Monitoring activity has confirmed that the treatment of burned soils with straw mulching improves its functionality in the short term. More specifically, although soil pH was stable and electric conductivity noticeably reduced, the organic matter was higher in burned and mulched soils compared to non-burned soils. The increases of basal respiration as well as microbial carbon and glomalin contents after mulching indicated higher activity of soil microorganisms and increased carbon and nitrogen storage. Moreover, all microbiological and enzymatic activities improved, except for dehydrogenase activity (DHA). Overall, this study highlights that soil functionality of wildfire-affected areas significantly benefits with straw mulching treatment.

**Keywords:** High-severity fire; Mediterranean forest; soil enzymes; soil respiration; soil organic matter.

## Introduction

Wildfires are a natural disturbance factor in Mediterranean forest ecosystems, where climate change and fire suppression have altered natural fire patterns (Kaufman et al. 2001). High-intensity fires modify the hydrologic response of soil and enhance its degradation, removing vegetation and altering chemical, physical and biological soil properties (DeBano 2000). For instance, as regards soil hydrology, decreased infiltration and increased overland flow exist after wildfires, leading to increasing erosion rates and soil degradation (e.g., Robichaud and Waldrup 1994). Therefore, mitigation of post-fire effects is needed to reduce soil's exposure to hydrological and quality degradation. Mitigating post-fire effects on soil has resulted in the increased use of post-fire treatments, which include soil stabilisation treatments that are crucial for diminishing soil degradation (Gómez et al. 2019).

Post-fire treatments may be divided into three categories: (i) emergency stabilisation; (ii) rehabilitation; and (iii) restoration (Lucas-Borja et al. 2019). Many experiments developed in the United States and Europe have shown that long-term rehabilitation and restoration actions are often focused on the biotic components of the ecosystem (Beschta et al. 2004; Fernandez and Vega 2016; Gómez et al. 2019; Hessburg and Agee 2003; Lucas-Borja et al. 2019; Robichaud 2005, 2010). For these activities, recovery of native plant communities and habitats, maintenance of plant biodiversity, reestablishment of timber or grazing species and control of invasive weeds are the most important targets. Regarding emergency stabilisation actions, mulching is considered one of the most efficient treatments to stabilise the soil of the burned area and to reduce additional damage to soil and vegetation immediately after a wildfire. This treatment consists of spreading organic material (e.g., wheat straw or woodchips) over soil immediately after a fire and just before the first autumn rainfall. The benefits of mulching have been largely demonstrated in the literature (e.g. Prosdocimi et al. 2016). Focusing strictly on the hydrological aspects, Smets et al. (2008) have shown that mulching provides a suitable soil cover that reduces raindrop impact, prevents soil sealing, promotes infiltration and slows runoff. Therefore, post-fire mulching is critical for reducing runoff and soil erosion, especially after clear-cutting in areas affected by crown fire, where the soil is exposed to rainfall action, and the amounts of logging debris on the soil surface may be low (Lucas-Borja et al. 2019).

Despite these benefits, using straw or woodchip mulching as post-fire emergency treatment has some problems, such as straw blowing under strong winds, quick decomposition and emergence of non-native plant species (Cerdà et al. 2016; Prosdocimi et al. 2016). Luna et al. (2018) found that these types of mulch (straw or woodchips) did not favour vertical water movement towards deeper horizons and so was not useful to increase soil water storage despite that woodchip mulch is appropriate for reducing erosion and runoff in restored soils. In general, mulching may alter soil moisture and temperature, since the mulch layer can obstruct emerging natural and seeded vegetation by sunlight interception or plant recovery (Lombao et al. 2014). Moreover, straw mulching can cause changes in soil properties, because straw can act as a new source of vegetal material to be incorporated into the soil. Straw applied to soil with mulching can influence organic matter content, microbial biomass carbon, respiration, enzymatic activities or nutrient content of soil whether directly or indirectly linked to vegetal input into the soil (Bastida et al. 2007; Doran and Parkin 1994; Entry and Emmingham 1998; Hedo et al. 2015; Larson and Pierce 1994).

In spite of this close link between physicochemical and microbiological properties of soil and mulching, little is known about the effects of straw application on soil functionality of Mediterranean forest ecosystems, although its positive influence on soil hydrology is well documented. At this point, several enzyme activities, specifically related to the cycles of N, P, C and S (urease, alkaline and acid phosphatase, β-glucosidase and arylsulfatase, respectively) and some general microbial indicators, such as dehydrogenase activity (DHA) and soil respiration, have been proposed as specific indicators of soil functionality (Bastida et al. 2008; Hedo et al. 2015; Lucas-Borja et al. 2011). In addition, the C/N ratio (Hedo et al. 2015; Lucas-Borja et al. 2012), soil pH (Lucas-Borja et al. 2012), soil texture (Fterich et al. 2014), nutrient status (Burgess and Wetzel 2000; Santa-Regina and Tarazona 2001) or microbiological communities (Wu et al. 2013) have been used as meaningful indicators of soil functionality. Despite this knowledge, more research is needed to better understand whether, and to what extent, soil functionality is influenced by straw mulching, particularly as related to the Mediterranean forests where soils are especially prone to erosion and degradation, and the risk of fire is intense. Plant and soil cover may affect the equilibrium of these ecosystems, which, in consequence, could alter soil properties and functionality. In these delicate ecosystems, soil functionality plays an important role in soil fertility and stability by enhancing growth and proliferation of microorganisms, which help facilitate reactions to release soil nutrients for vegetation development (Hannam et al. 2006). Forest managers and policy makers should know more extensively about how straw mulching affects soil functionality in wildfire-affected areas to establish proper management guidelines (Gómez et al. 2019). This study aims to determine whether post-fire straw mulching alters specific indicators of soil functionality in the short term after a wildfire in a Mediterranean forest of *Pinus halepensis* M. More specifically, straw was applied as a mulching treatment immediately after a wildfire in different areas, and then soil microbiological properties were monitored throughout one year in spring and autumn. We hypothesised that straw mulching enhanced soil functionality in the short term because it increases soil's organic matter content, which plays an important role in controlling its metabolic processes.

## Materials and Methods

### *Study site*

The study was carried out in the Sierra de las Quebradas forest (Liétor, Castilla-La Mancha region, province of Albacete, Central Spain (W1º56'35.02'', N38º30'40.79''; Figure 1). Elevation ranges between 520 and 770 m, and the aspect is W-SW. The climate of the area, located on the meso-Mediterranean bioclimatic belt (Rivas-Martínez et al. 2002), is semiarid, or type BSk according to the Köppen classification (Kottek et al. 2006). The mean annual temperature and precipitation are 16.6°C and 321 mm, respectively. According to historical data (1990–2014) provided by the Spanish Meteorological Agency, the maximum precipitation is concentrated in October (44.5 mm) and the minimum in May (39.6 mm); from June to September a hot and dry period (air relative humidity below 50%) occurs. According to the Soil Taxonomy system, soils are *Calcid Aridisol*, with a sandy loam soil texture. Vegetation belongs to the *Querco cocciferae-Pino halepensis* S. series, with a tree cover of Aleppo pine and a shrub layer of kermes oak (Peinado et al., 2008). The current vegetation of the forest area mainly consists of *Pinus halepensis* M. stands. In the study site, the mean density and height of forest trees before the wildfire were about 500–650 trees/ha and 7–14 m, respectively. The main shrubs and herbaceous species were *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. The use of such species was an economic driver of the area from the seventeenth century until the middle of the twentieth century. Its progressive abandonment and the reforestation by the local public authorities have shaped a forest landscape composed of Aleppo pines of natural origin growing in shaded areas and along watercourses.

*Experimental design*

This study was carried out during 2017 inside a drainage basin of the approximately 700 ha affected by a wildfire that occurred in July 2016. Immediately after the wildfire, one site of about 1 $km^2$, totally covered by *Pinus halepensis* M. and affected by crown fire (tree mortality of 100%), was selected for study (Figure 1). In the burned area, nine rectangular experimental plots (each one being 20 x 10 m) were randomly selected with their longest dimension along the maximum slope. Plots were distributed by selecting certain site characteristics, slopes and aspects to ensure comparability among the nine plots used in this study. The distance between plots was always greater than 200 m. Soil burn severity, measured using the methodology proposed by Vega et al. (2013) and Fernandez et al. (2017), was high in each plot, allowing the comparison of the plots. A weather station (WatchDog 2000 Series model), purposely placed in the study area during the study period, measured precipitation depth and intensity and air temperature (Table 1).

Three of the nine experimental plots were located in an unburned area 1 km away from the burned site as controls. Three plots were located in the burned area but not treated. Mulching treatment was conducted in September 2016 in the remaining three plots, also located in the burned area. Mulching consisted of the manual application of straw (0.2 kg/m$^2$ of dry weight) on plot soils at an initial depth of 3 cm. This amount was proposed by different authors to achieve a cover over 80% in plots located in the north of Spain (e.g. Vega et al. 2014). Moreover, such an amount of straw is also successfully used (from the biophysical point of view) in agricultural land affected by intolerable erosion rates (Cerdà et al. 2017). To summarise, three replicated plots had non-burned soils (hereafter "NB,); three plots had burned and non-mulched soils (three replicates, hereafter "B+NM"); and three plots had burned and mulched soils (three replicates, hereafter "B+M"). Prior to soil sampling, the percentages of vegetation cover, rock fragments, dead matter, bare soil and ash on the plots were measured one day after the mulching application (in September 2016), in the middle of the study period (in March 2017) and at the end of the experiment (in July 2017). More extra details about soil cover measuring methods and results are reported in Lucas-Borja et al. (2019).

*Soil sampling and analyses*

For soil sampling, three soil samples (each of 600 g) were collected in each plot in two seasons (May 2017 and November 2017) in one year after the wildfire for a total of 18 soil samples, 3 treatments (NB, B+M, B+NM), two seasons (autumn and spring) and three replicates. Soil samples consisted of the composition of further six sub-samples, randomly distributed over each plot, to take into account the spatial variability of plot soils. Each soil sample was collected from the upper soil layer (depth of 5 cm) after litter removal, then sieved (at 2 mm) and kept at 4º C. Soil analyses were carried out one day after sampling. Sampled soil was analysed for its main physical, chemical and microbiological properties. In order to evaluate its physical and chemical properties, texture (soil contents of sand, silt and clay) was analysed according to the method of Guitián and Carballás (1976). Soil pH and electrical conductivity (EC, µS/cm) were determined in a 1:5 (w/v) aqueous solution by portable analyser with dedicated probes. Organic matter content (OM, %) was measured by the potassium dichromate oxidation method (Nelson and Sommers 1996). Organic carbon (OC, %) was calculated by dividing OM by 1.72 (Lucas-Borja et al., 2018). The C/N ratio (-) was calculated according to methods in Lucas-Borja et al. (2012). Total nitrogen (TN, %) was determined using the Kjeldahl method (Bremner and Mulvaney 1982). With respect to the microbiological properties of soils, microbial carbon (MC, expressed as mg C $kg^{-1}$ dry soil) was measured by the fumigation-extraction methods (Vance et al. 1987). Basal soil respiration (BSR, expressed as the $CO_2$ rate µg $hour^{-1}$ $g^{-1}$ of dry soil), was determined in a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA). Soil dehydrogenase activity (expressed as µg INTF $hour^{-1}$ $g^{-1}$ of dry soil) was determined as the reduction of p-iodonitrotetrazolium chloride (INT) to piodonitrotetrazolium formazan using the modified method of Von Mersi and Schinner (1991). Urease activity (UA, expressed as µmol N-NH4+ $hour^{-1}$ $g^{-1}$ of dry soil) was measured according to the method of Tabatabai (1994) using urea as a substrate and borate buffer (at pH = 10; Kandeler and Gerber 1988). Acid phosphatase (acid-PA) and β-glucosidase (BGA) activities, both expressed as µmol pNP $hour^{-1}$ $g^{-1}$ of dry soil, were determined according to the methods of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977), respectively. Glomalin-related soil protein (GPRS, expressed as $g^{-1}$ dry soil) content was evaluated according to the techniques of Lozano et al. (2016).

*Statistical analyses*

Statistical differences on physical, chemical and microbiological soil variables of non-burned spring (NB-spring), non-burned autumn (NB-autumn), burned and non-mulched spring (B+NM-spring), burned and non-mulched autumn (B+NM-autumn), burned and mulched spring (B+M-spring) and burned and mulched autumn (B+M-autumn) samples were evaluated with univariate and multivariate permutational analysis of variance (PERANOVA and PERMANOVA, Anderson 2001) using a three-factor design: (i) fire occurrence, (ii) mulch addition, (iii) season of the year. We used Pearson's correlation analysis to study the relationships between these soil properties and a Canonical Analysis of Principal Coordinates (CAP) after normalizing the data to assess the similarities among the soils samples of each treatment. CAP is a constrained nonparametric ordination procedure, widely used as ecology ordering method because it allows the use of any distance or dissimilarity measure, and, at the same time, takes into account correlation structure among response variables (Anderson and Willis 2003). This analysis consists of the following steps: (i) principal coordinate analysis (PCA) on the data matrix Y, using a similarity measure (Euclidean distance in this study), which yields orthogonal Q; (ii) selection, based on in minimum misclassification error or minimum residual sum of squares, of an appropriate number of axes $m$ as a subset of Q, thus defining a matrix $Q_m$; (iii) application of a traditional canonical analysis (e.g., Canonical Correlation Analysis, since it contains X quantitative variables) on the first *m* axes of Q. The software used for the statistical analyses was PRIMER V 7® with PERMANOVA add-on (Anderson et al. 2008) and Statgraphics Centurion XVI ® (StatPoint Technologies, Inc.).

## Results

*Effects of wildfire and mulching on physicochemical and microbiological soil properties*

PERMANOVA analysis showed significant differences ($p < 0.001$) among soils sampled in NB (spring and autumn), B+NM (spring and autumn) and B+M (spring and autumn) plots (Table 2). The results of CAP evidenced that the soil samples were put into the following six categories: (i) NB soils sampled in spring field campaign; (ii) B+NM soils sampled in spring; (iii) B+M soils sampled in spring; (iv) NB soils sampled in autumn; (v) B+NM soils sampled in autumn; (vi) B+M soils sampled in autumn. Selecting the first 10 axes of the PCA (that is, choosing $m = 10$), 99.97% of the variance of the samples was explained, and 100% of correct assignations (12 on 12) of the soil samples in each cluster of treatments was achieved (Table 3). The results of the cross validation correctly allocated all the observations to original groups for the choice of $m = 10$ (Table 4). All clusters were significantly different from each other with the exception of the soils sampled in B+NM in autumn, which did not differ from previous samples in spring and from those sampled in B+M in autumn. Moreover, the microbiological parameters (BSR, MC and the enzymatic activities) as well as GPRS and the contents of silt, OM, TN and C/N are mainly oriented to the clusters grouping the soil samples treated with straw mulching after fire (Figure 2). On the contrary, DHA, content of clay and pH were oriented to the clusters consisting of non-burned samples. Finally, it is noteworthy that OM and TN content of soils have higher loadings on axis 1 (CAP1, Figure 2), while important microbiological parameters (BGA and UA) have higher weights on axis 2 (CAP2, Figure 2).

*Differences among treatments and temporal changes in physicochemical soil properties*

The texture of NB plots was loam-clay-sandy, while both the burned soils (B+M and B+NM) were sandy-loam both in spring and autumn 2017 (Table 5). While the textural properties of NB soils remained practically constant in time, some significant changes in textural content were monitored from autumn to spring in the other experimental plots. Moreover, the clay content significantly decreased (by 48%) compared to the first field campaign in spring, and the percentage of silt simultaneously increased (by 27%) in B+NM plots. In B+M soils, the percentage of silt significantly increased (by 26%), and the sand content decreased (by 12%) from spring to autumn (Table 5). In general, most of the physicochemical properties (contents in OM, OC, TN and EC) were significantly different among the three analysed treatments in both field campaigns. Regarding their time evolution, OM and OC contents were almost stable in NB soils, while they significantly increased (by 30% both) in B+NM plots and decreased (by 16% and not significantly) in B+M soils from spring to autumn. In this period, TN increased in NB (by 28%) and B+NM plots (by 14%) and decreased (by 18%) in B+M soils, although not significantly (Table 5). Both in autumn and spring, the highest EC, OC and OM contents were detected in B+M plots, while the lowest values of these properties were found in NB soils, except for EC, which showed the lowest value (81.35±19.85 μS/cm) in B+NM soils sampled in autumn. High reductions in EC values from spring to autumn were found in NB soils (-18%) and mainly in B+M (-57%) and B+NM plots (in the latter the value was practically halved from autumn to spring). EC of NB soils was significantly different from the values measured in burned (B+NM and B+M) plots in spring, while in autumn the value recorded in B+M soils become significantly different from the other treatments. On the other hand, there were no significant differences in soil pH in every season and treatment. All soils showed a slightly alkaline pH (on the average in the range 8.4-8.7) during each season, and low variability was found for soil pH between the monitored seasons (Table 5). The lowest C/N ratio was found in B+NM in spring; this value was significantly different from the other five samples (B+M and B+NM in spring as well as NB, B+M and B+NM in autumn, all showing insignificant differences). More specifically, the NB soils showed the lowest C/N ratio (13.9±0.73) in autumn and the highest value (22.1±2.23) in spring. In autumn, the maximum value of C/N ratio (16.3±1.38) was measured in B+M soils, while in spring the minimum C/N ratio (12.5±2.33) was found in B+NM plots (Table 5). From these changes, a large reduction in the C/N ratio (by 37%) was estimated in NB plots, and an increase was calculated in both burned soils, more noticeably in B+NM plots (+20% against a 4% in B+M soil; Table 5).

*Differences among treatments and temporal changes in microbiological properties of soils*

Both in spring and autumn, the B+M soils generally showed the highest values in all the microbiological properties in comparison to other treatments, except for DHA and BSR (in autumn); the highest DHA and BSR in autumn were detected in NB and B+NM soils as well as in B+NM, respectively. Moreover, the differences in the enzymatic activity between B+M soils and the other treatments were significant for BGA and UA in both seasons and for MC in autumn. Most of the surveyed microbiological properties attained the lowest values in the NB plots (e.g., BGA and BSR in spring and autumn, acid-PA and GPRS in autumn as well as DHA and MC in spring; Table 6). The soils sampled in B+NM plots showed the lowest UA (in both seasons), BGA, acid-PA and GPRS (in spring) and MC (in autumn), whereas DHA and BSR were the highest among the treatments in spring and in autumn, respectively. Compared to the control soils, the differences are significant only for acid-PA in both seasons, UA in spring as well as GPRS and MC in autumn (Table 6).

*Correlations among physical, chemical and microbiological soil properties*

The Pearson's correlation analysis among the physical, chemical and microbiological soil properties surveyed in the experimental site showed interesting correlations (Table 7). In regards to soil texture, the clay content was negatively correlated with silt ($r = -0.93$) and sand ($r = -0.63$) content. The soil pH was significantly linked with glomalin content ($r = -0.48$), while EC showed a higher correlation with chemical content (OM, OC and TN; $r > 0.77$) than with microbiological properties (BGA and UA). The highest correlations ($r > 0.98$) were found among OC, OM and TN content of the soils. As expected, a noticeable and significant correlation coefficient ($r > 0.55$) was found between the C/N ratio and the OC and TN contents. Microbiological soil properties, with the exception of DHA, showed high significant correlations with several physical and chemical parameters (Table 7). The enzymatic activity BGA showed the greatest number of positive correlations ($r > 0.49$) with physical and chemical soil properties, but it was negatively correlated with the clay content ($r = -0.68$). Moreover, BGA, UA and acid-PA were positively correlated with each other with a minimum r of 0.61 between BGA and UA and a maximum r of 0.77 between BGA and acid-PA. GPRS was positively correlated with OM and OC contents (r = 0.47 for both) and with BGA, and negatively correlated with DHA (r = -0.53). A correlation coefficient of 0.54 was found between the BSR and TN, OC and OM content, while the MC was only positively correlated with GPRS (r = 0.50; Table 7).

## Discussion

Studying the incidence of post-fire management actions on soil properties and the related changes is very important to identify the magnitude of these effects and plan possible countermeasures against soil degradation, but, due to the number and complexity of these effects, very little guidance is currently available. Therefore, it is necessary to select a set of soil parameters about physical, chemical and biological soils properties for use in evaluation of soil status and functions (Muñoz-Rojas et al. 2016). The extent of post-fire changes in some soil properties, directly attributed to heating, is usually related to burn severity (Mataix-Solera et al. 2009). Many authors have found changes in the soil quality and organic matter content in these soils (González-Pérez et al. 2004), increases in soil pH (Mataix-Solera et al. 2002; Ulery et al. 1993), decay of soil structure and thus the stability of aggregates, formation of hydrophobic films on soil aggregates (DeBano 2000), changes in the nutrient availability and water retention (Certini 2005) and modifications of the enzymatic activities (Mataix-Solera et al. 2009). Because enzymatic activities have an important role in catalysing biological reactions, there is a particular need for information about reaction rates related to production of essential elements in biogeochemical cycles (Mataix-Solera et al. 2009).

This study has explored the effects of straw mulching application immediately after wildfire on some meaningful physical, chemical and microbiological soil properties in comparison to unthreatened and control plots with particular regard on microbial activity, which has not yet been adequately investigated in Mediterranean environments (D'Ascoli et al. 2005; Pourreza et al. 2014; Rincón and Pueyo 2010) and especially in forests. More specifically, we have investigated whether the soil treatments with straw mulching could be beneficial for soil quality in terms of changes in physicochemical and microbiological soil properties in the short term after fire occurrence to mitigate fire-induced soil degradation.

In the same plots we selected for outr study, Lucas-Borja et al. (2019) observed noticeable variations in vegetation cover, dead matter and bare soils for one year after fire in each experimental condition (NB, B+NM and B+M soils). According to Lucas-Borja et al. (2019), in spring 2017, soil became bare for about 30% of the time, and a vegetation cover of 10% was detected in the B+NM experimental plots. The vegetation and dead matter (coming from straw application) cover were about 25% and 55%, respectively, in the B+M plots. By the following autumn, the amount of bare soil increased to 55% and the vegetation cover increased to 25% in B+NM soils, whereas the vegetation cover was 53% and dead matter cover was 22% in B+M soils. In addition, straw mulching was found to promote a higher water content and a lower temperature of soil, thus determining sunlight interception (Lucas-Borja et al. 2019). From these findings, we suspect that these changes in soil vegetation cover and soil microclimatic conditions may have significantly altered the physicochemical and microbiological soil properties during the two sampled periods. Moreover, because all the experimental plots were set up on sites characterised by the same burn severity, changes in soil properties cannot be attributed to burn severity.

It is well known that of all the physical and chemical soil properties, OM content is one of the most important quality indicators, given its influence on plant growth-related functions (e.g., humidity retention and reservoir and nutrient exchange; Muñoz-Rojas et al. 2016) and also on the maintenance of productivity, biodiversity and other ecosystem services (Lucas-Borja et al. 2016). In this study in both autumn and spring, OM showed the highest value in B+M soils; this parameter was significantly lower in B+NM and NB plots. Also, variations in TN content were detected after wildfire and mulching, inducing significant increases in B+NM and B+M plots. It is expected that soil treatment (in our case, fire and mulching) modify the C/N ratios compared to the values recorded before the fire. The simultaneous changes in OM and TN significantly reduced the C/N ratio only in B+NM soils immediately after the wildfire because the C/N ratio is related with OM decomposition and N mineralisation (Lucas-Borja et al. 2016). After one year, none of the experimental soils showed significant differences in C/N ratio, although a slight increase was recorded in B+M plots. This is in accordance with previous studies in burned pine forests, indicating that, after the initial C/N drop caused by fire and owing to new forms of recalcitrant N accumulation and to the volatilisation of C compounds immediately after fire (Carballas et al. 2009; Rodríguez et al. 2017), the C/N ratio recovers its pre-fire values (Jiménez-González et al. 2016). A higher C/N ratio for the hillslope stabilisation-treated plots (as in our B+M soils) indicates low activity and disintegration speed for OM as well as a lower degree of N mineralisation, which may be due to a more recalcitrant chemical composition of litter and low litter quality (high C/N ratio; Martín-Peinado et al. 2016).

Changes in soil texture of burned soils (decrease of clay and increase of silt in B+NM plots and increase of silt and decrease of sand in B+M) are to be expected after wildfire, as also detected in the same environment by Lucas-Borja et al. (2019). These changes must be monitored with caution, because a decrease of the soil finer fraction may lead us to suspect that burned but not treated soils may be more prone to erosion compared to non-burned and burned but mulched soils. Literature shows that soil pH and EC tend to rise after fire, although these properties gradually return to the original pre-fire values due to the washout effect (Mataix-Solera et al. 2009; Muñoz-Rojas et al. 2016). In the study area, pH did not respond to the described pattern, since its changes among treatments is stable, probably due to the higher buffering capacity of carbonated soils (Certini 2005; Mataix-Solera et al. 2009). Conversely, EC of burned soils evolved as predicted by literature, since this parameter strongly decreased with a more noticeable effect in B+NM plots after sudden increases immediately after fire compared to NB soils. This may be due to the effects of burning, which accumulates ash containing C and other nutrients from burned forest fuel (Caon et al. 2014). Mulching should have decreased EC, thanks to the progressive release of these compounds.

With regards to the monitoring of the soil's microbiological properties, we noticed that both the quantity and activity of microorganisms grew, as indicated by biomass carbon and BSR (the latter not being statistically different) parameters, respectively, increased in the burned soils compared to NB plots immediately after fire. Conversely, one year after the fire, the microbial carbon decreased in B+NM soils. These microbiological effects detected in B+M soils compared to untreated plots may be due to the accumulation of biodegradable plant material and the increase in exchangeable cations (Rodríguez et al. 2017), which continued until these mineralised materials had been consumed (Muñoz-Rojas et al. 2016) with a lower effect recorded in B+NM soils. The mulching treatment had a remarkable effect on all microbiological and enzymatic activities, except for DHA. This was due to the accumulation of OM and nutrients and their following decomposition in soil throughout one year. This result was further confirmed by the positive correlations among the BSR, OM and TN content shown by Pearson's correlation analysis.

Our results showed different trends of enzyme content of soil, depending on their function and nature. In more detail, the recovery, and even the increase, in acid-phosphatase activity in B+M soils could be explained by its close relationship (stronger compared to the other enzymes) with the progressive restoration of plant cover, with roots being the main resource (López-Poma and Bautista 2014). The lack of variation in DHA observed in the studied soils, beside the lack of response to the post-fire treatment with straw mulching and their absence of relationships with the most of physical and chemical soil parameters, complies with other studies conducted in Mediterranean areas, showing the lack of sensitivity of DHA to seasonality and site effects rather than management practices. 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, β-glucosidase, urease and acid-phosphatase (Blonska et al. 2017). Urease and ß-glucosidase activity was greater in B+M soils both in spring and autumn compared to both NB and B+NM plots. The soil response of urease may be related to the greater accumulation of nitrogen due to the application of straw, as indicated by the fairly positive correlation with TN content shown by Pearson's correlation analysis. The evolution of ß-glucosidasae is related to

OM decomposition velocity and to energy released by soil microorganisms as indicated by the positive high correlation with the OC. The progressive temporal changes among the analysed soil conditions suggest that mulching soils with straw could promote bacterial development, but the DHA could behave quite differently from other enzymes.

Moreover, the glomalin content resulting from arbuscular mycorrhizal fungi is an indicator of C and N storage, which play a key role in aggregate stability and water repellence of soils (Lozano et al. 2016). Although very few studies have explained glomalin's temporal evolution and response to post-fire mulching, some authors have demonstrated the its sensitivity to fire, even at low temperatures (e.g. Lozano et al. 2016). For instance, Rivas et al. (2016) showed the GPRS level recovery four years after fire due to species' rapid root colonisation that symbiosis occurs in the presence of arbuscular mycorrhizal fungi. Sansano (2016) confirmed the negative influence of cutting timber in the short term after fire. Our study has demonstrated that glomalin content quickly recovers after fire, especially in B+M soils (presumably thanks to the higher OM content and C/N ratio), in accordance with Sansano (2016). Finally, the Canonical Analysis of Principal Coordinates has clearly discriminated NB, B+NM and B+M soil samples in terms of their physical, chemical and microbiological soils properties. This suggests that one year after wildfire, burned soils not subject to any treatment present different physicochemical and microbiological soil properties compared to soils treated with straw mulching and to control plots.

**Conclusions**

To better understand the effects of an important post-fire soil restoration technique such as mulching with straw on soil functionality of Mediterranean forests, which are particularly prone to intense erosion and degradation, this study has evaluated the seasonal changes in the physical, chemical and microbiological properties of burned (treated with straw mulching or not) soils compared to non-burned plots throughout one year after a wildfire in a *Pinus halepensis* M. forest. Differentiated physical, chemical and microbiological properties between non-burned, burned and non-treated, and burned and straw-mulched soils were confirmed by CAP. The results of enzyme activity monitoring in the soils have confirmed the working hypothesis that straw mulching enhances soil functionality in the short term. In fact, organic matter content increased in burned and straw-mulched soils compared to non-burned soils, both in autumn and spring. After a decrease immediately after fire in burned and non-mulched plots, the C/N ratio did not show significant differences among the other treatments. pH values were stable, but a marked reduction in electric conductivity was noticed after one year, especially in burned soils both straw-mulched and not mulched. The quantity and activity of soil microorganisms grew (as shown by the increase of biomass carbon and BSR) in the burned and treated soils. Moreover, after the mulching treatment all microbiological and enzymatic activities improved, except for DHA. The glomalin content quickly recovered after fire, indicating a higher carbon and nitrogen storage of mulched soils. One year after fire, burned soils not subject to any treatment were in an intermediate stage between the characteristics they had immediately after fire and those of soils treated with straw mulching. Overall, the results of this study indicate the positive effects of straw application in areas burned by high-severity fire on soil functionality and support forest managers and policy makers in selecting the proper management actions against soil erosion and degradation in the delicate environmental ecosystems of Mediterranean pine forests.

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**FIGURES**

**Figure 1**. Location of the study area (A), aerial image of the wildfire affected area in red (B) and pictures from each experimental condition (C).

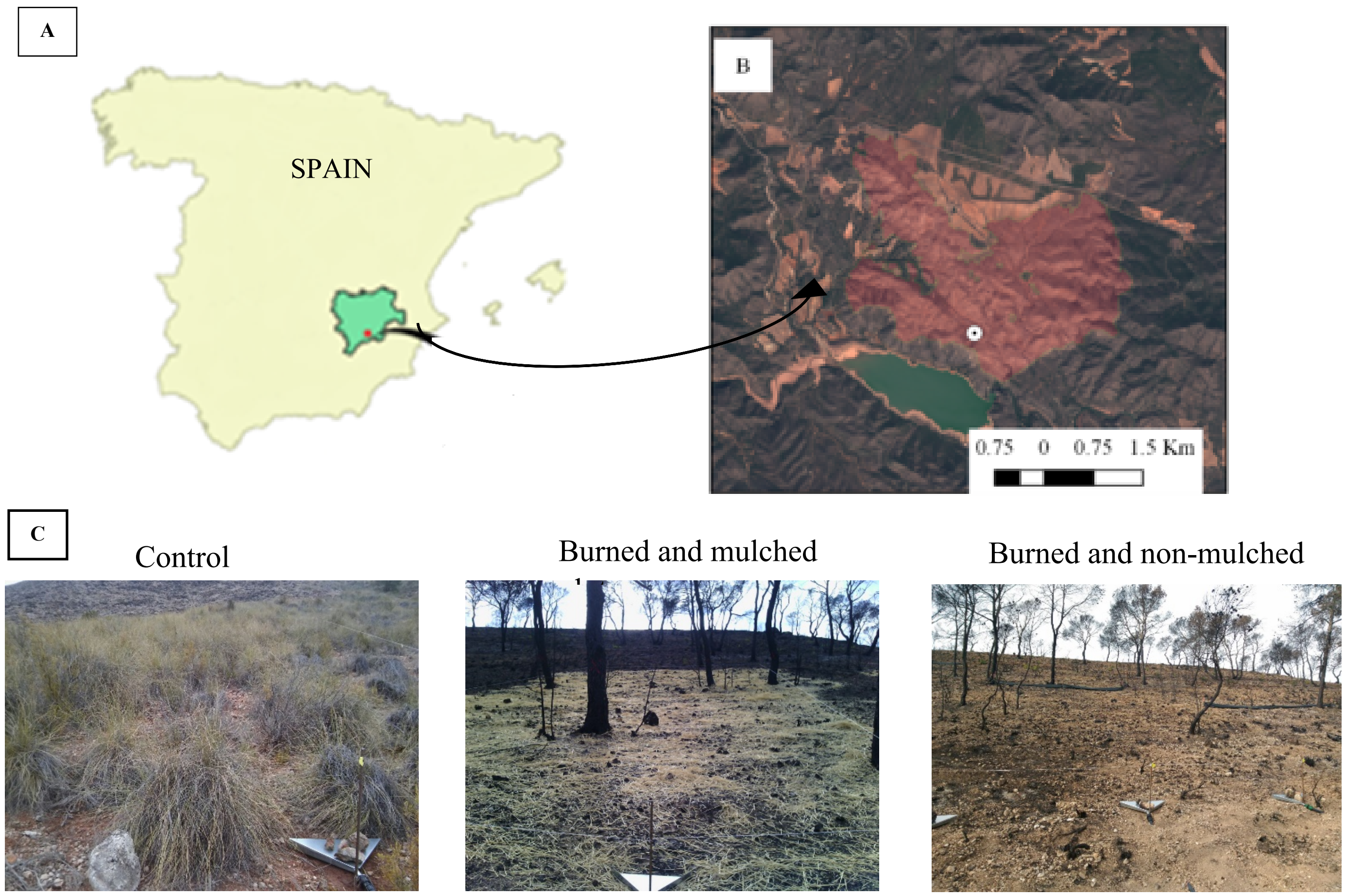
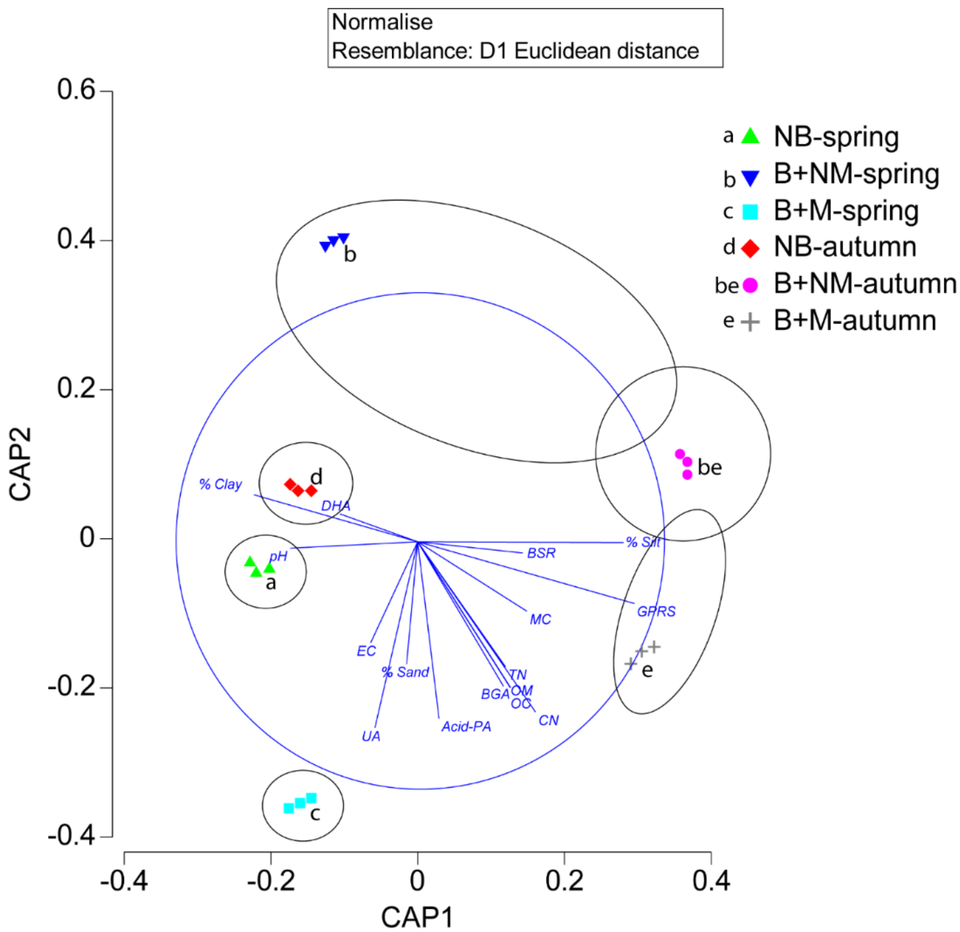


**Figure 2**. Canonical analysis of principal coordinates in non-burned spring (NB-spring), non-burned autumn (NB-autumn), burned and non-mulched spring (B+NM-spring), burned and non-mulched autumn (B+NM-autumn) plots, burned and mulched spring (B+M-spring) and burned and mulched autumn (B+M-autumn) (n=18). Different lower case letters and black ellipses indicating statistical significant differences among treatments. Blue circle. Blue vectors show the weights of variables with each of the CAP axis with the blue circle representing a value of 1.




Note: OC = Organic Carbon; OM = Organic Matter; EC = Electrical Conductivity; TN = Total Nitrogen; CN = ratio C/N; MC = Microbial Carbon; BSR = Basal Soil Respiration; DHA = DeHydrogenase Activity; UA = Urease Activity; Acid-PA = Acid Phosphatase Activity; BGA = β-Glucosidase Activities; GPRS = Glomalin-Related Soil Protein.

**TABLES**

**Table 1.** Main precipitation data in the study period recorded at the meteorological station (Liétor, Castilla La Mancha, Spain).

| Month and year | Total monthly precipitation (mm) | Mean daily precipitation (mm) | Maximum daily precipitation (mm) | One-hour precipitation intensity (mm/h) |
|---|---|---|---|---|
| Oct 2016 | 38.6 | 1.2 | 17 | 7.1 |
| Nov 2016 | 64.7 | 2.1 | 27.2 | 11.3 |
| Dec 2016 | 132.5 | 4.2 | 36.8 | 15.3 |
| Jan 2017 | 26.5 | 0.9 | 19 | 7.9 |
| Feb 2017 | 16.4 | 0.5 | 9.7 | 4.0 |
| Mar 2017 | 51.7 | 1.6 | 41.9 | 17.5 |
| Apr 2017 | 27.9 | 0.9 | 17.1 | 7.1 |
| May 2017 | 0.3 | 0.01 | 0.1 | 0.0 |

**Table 2**. One-way Permutational Multivariate Analysis of Variance (PERMANOVA) applied to all variables of soil samples collected in non-burned, spring (NB-spring), non-burned, autumn (NB-autumn), burned and non-mulched, spring (B+NM-spring), burned and non-mulched, autumn (B+NM-autumn), burned and mulched, spring (B+M-spring) and burned and mulched, autumn (B+M-autumn) plots (n = 18) (Liétor, Castilla La Mancha, Spain).

| ~~Global~~ | Source | df | SS | MS | Pseudo-F | P(perm) | Unique perms |
|---|---|---|---|---|---|---|---|
| | | 5 | 217.61 | 43.52 | 9.6025 | 0.0001 | 9922 |
| | Residues | 12 | 54.38 | 4.532 | | | |
| | Total | 17 | 272 | | | | |

Notes: df = degree of freedom; SS = Sum of squares; MS = Mean squares; Unique perms = Number of unique values of the test statistic obtained under permutation.

**Table 3.** Canonical principal coordinates diagnosis among factors (season, burning/non-burning, mulching/non-mulching) applied to all variables of soil samples collected in non-burned, spring (NB-spring), non-burned, autumn (NB-autumn), burned and non-mulched, spring (B+NM-spring), burned and non-mulched, autumn (B+NM-autumn), burned and mulched, spring (B+M-spring) and burned and mulched, autumn (B+M-autumn) plots (n = 18) (Liétor, Castilla La Mancha, Spain).

| m | prop.G | ssres | d $1^2$ | d $2^2$ | d $3^2$ | d $4^2$ | d $5^2$ | %correct |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.5193 | 4.3268 | 0.9931 | 0 | 0 | 0 | 0 | 83.333 |
| 2 | 0.6763 | 3.5637 | 0.9976 | 0.8707 | 0 | 0 | 0 | 88.889 |
| 3 | 0.7644 | 3.1533 | 0.9982 | 0.876 | 0.6114 | 0 | 0 | 83.333 |
| 4 | 0.8323 | 3.5769 | 0.9986 | 0.9164 | 0.6139 | 0.0107 | 0 | 61.111 |
| 5 | 0.8904 | 2.814 | 0.9994 | 0.9758 | 0.9091 | 0.6003 | 0.0043 | 72.222 |
| 6 | 0.9374 | 2.2249 | 0.9995 | 0.9942 | 0.9673 | 0.9078 | 0.0745 | 88.889 |
| 7 | 0.971 | 3.3523 | 0.9997 | 0.9988 | 0.9941 | 0.9301 | 0.075 | 94.444 |
| 8 | 0.9909 | 3.0349 | 1 | 0.9997 | 0.9988 | 0.9941 | 0.3458 | 88.889 |
| 9 | 0.9971 | 1.2403 | 1 | 1 | 0.9997 | 0.9969 | 0.927 | 94.444 |
| 10 | 0.9997 | 0.50458 | 1 | 1 | 1 | 0.9996 | 0.9817 | 100 |
| 11 | 0.9999 | 0.37797 | 1 | 1 | 1 | 1 | 0.9995 | 100 |
| 12 | 1 | 0.37875 | 1 | 1 | 1 | 1 | 1 | 100 |

Notes: ‘prop.G’ = the proportion of variation in the data cloud described by the resemblance matrix explained by the first *m* PCO axes; ‘ssres’ = the leave-one-out residual sum of squares; ‘$d\_1^n$’ = the size of the n squared canonical correlation. ‘%correct’ = the percentage of the left-out samples that were correctly allocated to their own group using the first *m* PCO axes for the model.

**Table 4.** Results of the cross validation (leave-one-out allocation of observations to groups after choosing m = 10) applied to all variables of soil samples collected in non-burned, spring (NB-spring), non-burned, autumn (NB-autumn), burned and non-mulched, spring (B+NM-spring), burned and non-mulched, autumn (B+NM-autumn), burned and mulched, spring (B+M-spring) and burned and mulched, autumn (B+M-autumn) plots (n=18) (Liétor, Castilla La Mancha, Spain).

| Treatment-season | NB-spring | NB-autumn | B+NM-spring | B+NM-autumn | B+M-spring | B+M-autumn | Total | %correct |
|---|---|---|---|---|---|---|---|---|
| **NB-spring** | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 100 |
| **NB-autumn** | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 100 |
| **B+NM-spring** | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 100 |
| **B+NM-autumn** | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 100 |
| **B+M-spring** | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 100 |
| **B+M-autumn** | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 100 |

**Table 5.** Main physical and chemical properties of soils sampled in non-burned, spring (NB-spring), non-burned, autumn (NB-autumn), burned and non-mulched, spring (B+NM-spring), burned and non-mulched, autumn (B+NM-autumn) plots, burned and mulched, spring (B+M-spring) and burned and mulched, autumn (B+M-autumn) plots (n = 18) (Liétor, Castilla La Mancha, Spain).

| Treatment-season | Clay (%) | Silt (%) | Sand (%) | OC (%) | OM (%) | pH (-) | EC (µS/cm) | TN (%) | C/N |
|---|---|---|---|---|---|---|---|---|---|
| **NB-Spring** | 32.68±1.76a | 19.87±1.35b | 47.44±2.23b | 1.54±0.09a | 2.65±0.23d | 8.73±0.13a | 124.34±16.23b | 0.07±0.02c | 22.12±2.23a |
| **B+NM-Spring** | 14.88±1.65b | 32.97±4.74a | 52.14±2.40ab | 2.75±0.08b | 4.75±0.15c | 8.47±2.03a | 190.00±23.02a | 0.21±0.01b | 12.5±2.33b |
| **B+M-Spring** | 8.57±0.45c | 31.7±2.69a | 59.68±2.69a | 6.29±1.22d | 10.80±2.11a | 8.52±2.47a | 275.00±65.00a | 0.39±0.03a | 15.6±1.51a |
| **NB-Autumn** | 32.18±4.76a | 19.14±1.00b | 48.17±1.00b | 1.27±0.29a | 2.19±0.16d | 8.64±0.21a | 102.55±17.75b | 0.09±0.01c | 13.9±0.73a |
| **B+NM-Autumn** | 7.71±1.03c | 41.89±1.96c | 50.39±3.00ab | 3.60±0.42c | 6.20±0.73b | 8.45±0.90a | 81.35±19.85b | 0.24±0.03b | 15.0±0.41a |
| **B+M-Autumn** | 7.64±1.23c | 40.00±5.02c | 52.35±1.036ab | 5.26±0.21d | 9.05±0.37a | 8.41±0.04a | 189.60±50.40a | 0.32±0.01a | 16.3±1.38a |

Notes: OC = Organic carbon; OM = organic matter; EC = electrical conductivity; TN = total nitrogen. Lowercase letters indicate statistically significant differences among treatments and seasons.

**Table 6.** Main microbiological properties of soils sampled in non-burned, spring (NB-spring), non-burned, autumn (NB-autumn), burned and non-mulched, spring (B+NM-spring), burned and non-mulched, autumn (B+NM-autumn) plots, burned and mulched, spring (B+M-spring) and burned and mulched, autumn (B+M-autumn) plots (n = 18) (Liétor, Castilla La Mancha, Spain).

| Treatment-season | BGA (µmol p-NP hour$^{-1}$ g$^{-1}$) | UA (µmol N-NH4+ hour$^{-1}$ g$^{-1}$) | Acid-PA (µmol p-NP hour$^{-1}$ g$^{-1}$) | DHA (µg INTF hour$^{-1}$ g$^{-1}$) | BSR (µg$CO_2$ hour$^{-1}$ g$^{-1}$) | GPRS (µ g$^{-1}$ dry soil) | MC (mg C kg$^{-1}$ dry soil) |
|---|---|---|---|---|---|---|---|
| **NB-Spring** | 0.86±0.10b | 0.73±0.03b | 1.16±0.04ab | 0.10±0.01a | 1.94±0.05a | 1700±89.49c | 56.25±0.50d |
| **B+NM-Spring** | 0.86±0.21b | 0.53±0.09c | 0.77±0.04c | 0.12±0.01a | 3.73±0.73a | 1394±83.18cd | 191.04±9.63c |
| **B+M-Spring** | 1.33±0.02a | 1.46±0.51a | 1.38±0.09a | 0.12±0.01a | 4.04±0.05a | 1579±106.42c | 252.33±56.99c |
| **NB-Autumn** | 0.59±0.06b | 0.50±0.11c | 0.47±0.04d | 0.11±0.02a | 1.94±0.11a | 1030±118.20d | 369.36±20.43b |
| **B+NM-Autumn** | 0.96±0.14b | 0.44±0.05c | 0.93±0.03c | 0.11±0.06a | 4.47±2.57a | 2845±289.38b | 203.93±3.91c |
| **B+M-Autumn** | 1.38±0.35a | 0.96±0.10a | 1.16±0.06ab | 0.08±0.01a | 3.98±2.60a | 3534±216.8a | 559.05±84.47a |

Notes: MC = Microbial Carbon; BSR = Basal Soil Respiration; DHA = DeHydrogenase Activity; UA = Urease Activity; Acid-PA = Acid Phosphatase Activity; BGA = β-Glucosidase Activities; GPRS = Glomalin-Related Soil Protein. Lowercase letters indicate statistically significant differences among treatments and seasons.

**Table 7**. Correlation matrix among physical, chemical and microbiological soil properties in non-burned, spring (NB-spring), non-burned, autumn (NB-autumn), burned and non-mulched, spring (B+NM-spring), burned and non-mulched, autumn (B+NM-autumn) plots, burned and mulched, spring (B+M-spring) and burned and mulched, autumn (B+M-autumn) plots (n = 18) (Liétor, Castilla La Mancha, Spain).

| Soil property | Clay % | Silt % | Sand % | pH | EC | OC % | OM % | TN % | C/N | BGA | UA | Acid-PA | DHA | GPRS | BSR | MC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Clay %** | | **-0.93** | **-0.63** | **0.63** | -0.47 | **-0.84** | **-0.84** | **-0.88** | **-0.47** | **-0.68** | -0.34 | -0.46 | 0.07 | **-0.62** | **-0.60** | -0.34 |
| **Silt %** | | | 0.31 | **-0.68** | 0.26 | **0.69** | **0.69** | **0.71** | 0.46 | **0.55** | 0.04 | 0.28 | -0.20 | **0.76** | **0.63** | 0.34 |
| **Sand %** | | | | -0.18 | **0.69** | **0.72** | **0.72** | **0.77** | 0.25 | **0.62** | **0.81** | **0.62** | 0.23 | 0.02 | 0.21 | 0.16 |
| **pH** | | | | | -0.10 | -0.42 | -0.42 | -0.44 | -0.40 | -0.37 | -0.14 | -0.18 | -0.01 | **-0.48** | -0.36 | -0.28 |
| **EC** | | | | | | **0.77** | **0.77** | **0.77** | 0.36 | **0.49** | **0.53** | 0.46 | 0.09 | -0.05 | 0.42 | 0.15 |
| **OC %** | | | | | | | **0.99** | **0.98** | **0.68** | **0.78** | **0.56** | **0.63** | -0.09 | **0.47** | **0.54** | 0.38 |
| **OM %** | | | | | | | | **0.98** | **0.68** | **0.78** | **0.57** | **0.63** | -0.09 | **0.47** | **0.54** | 0.38 |
| **TN %** | | | | | | | | | **0.55** | **0.8** | **0.59** | **0.63** | -0.11 | 0.44 | **0.54** | 0.36 |
| **C/N** | | | | | | | | | | 0.46 | 0.30 | 0.44 | 0.01 | **0.54** | 0.38 | 0.38 |
| **BGA** | | | | | | | | | | | **0.61** | **0.77** | -0.13 | **0.58** | 0.06 | 0.41 |
| **UA** | | | | | | | | | | | | **0.76** | -0.01 | 0.08 | 0.10 | 0.22 |
| **Acid-PA** | | | | | | | | | | | | | -0.09 | 0.40 | 0.19 | -0.06 |
| **DHA** | | | | | | | | | | | | | | **-0.53** | -0.33 | -0.38 |
| **GPRS** | | | | | | | | | | | | | | | 0.38 | **0.50** |
| **BSR** | | | | | | | | | | | | | | | | 0.05 |
| **MC** | | | | | | | | | | | | | | | | |

Notes: values in bold are statistically significant at $p < 0.05$; C = Organic Carbon; OM = Organic Matter; EC = Electrical Conductivity; TN = Total Nitrogen; MC = Microbial Carbon; BSR = Basal Soil Respiration; DHA = DeHydrogenase Activity; UA = Urease Activity; Acid-PA = Acid Phosphatase Activity; BGA = β-Glucosidase Activities; GPRS = Glomalin-Related Soil Protein.