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

Lucas-Borja, M.E.[a,*], Plaza-Álvarez, P.A[a], Ortega, R.[b], Miralles, I.[b], González-Romero, J[a]., Sagra, J.[a], Moya, D.[a], Zema, D.A.[c], de las Heras, J.[a]

[a] Higher Technical School of Agricultural and Forestry Engineering, Castilla-La Mancha University, Campus Universitario s/n, 02071 Albacete, Spain.

[b] Department of Agronomy & Center for Intensive Mediterranean Agrosystems and Agri-food Biotechnology (CIAIMBITAL), University of Almeria, E-04120, Almería, Spain.

[c] Mediterranean University of Reggio Calabria, Department AGRARIA, Località Feo di Vito, I-89122 Reggio Calabria, Italy.

*Email: ManuelEsteban.Lucas@uclm.es

Abstract

Understanding the changes in physico-chemical and microbiological soil properties induced by wildfire and post-fire soil restoration technique (e.g., soil mulching with straw) is very important in the Mediterranean environment, where the forest ecosystems are particularly prone to erosion and degradation risks. Nevertheless, the studies about the effects of straw application on functionality of burned soils in Mediterranean forest ecosystems are scarce. To fill this gap, this study has evaluated the seasonal changes (from spring to autumn) in important physical, and chemical soil properties and enzymatic activities in burned (treated with mulching or not) plots, compared to non-burned soils, after a wildfire occurred in a *Pinus halepensis* M. forest. The monitoring activity has confirmed that the treatment of burned soils with straw mulching improves its functionality in the short-term, assumed as working hypothesis. More specifically, compared to non-burned soils, although soil pH was stable and the electric conductivity noticeably reduced the organic matter content increased and the soil C/N ratio recoveries in one year in burned and mulched soils. The increases of basal respiration as well as microbial carbon and glomaline contents after mulching indicated higher activity of soil microorganisms and increased carbon and nitrogen storage. Moreover, all the microbiological and enzymatic activities improved, except for dehydrogenase activity. Finally, the Canonical Analysis of Principal Coordinates confirmed the differentiated functionality of non-burned, burned and non-treated, and burned and straw-mulched soils. Overall, this study highlights that soil functionality of wildfire-affected areas significantly benefits with straw mulching treatment, which could be adopted as countermeasure against soil quality decay in the Mediterranean forest ecosystems.

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

1. 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, it is well documented that decreased infiltration and increased overland flow after wildfires, leading to increasing erosion rates and soil degradation (e.g., Robichaud and Waldrup, 1994). Therefore, mitigation of post-fire effects is compulsory in order to reduce the soil exposure to hydrological and quality degradation. Mitigating the post-fire effects on soil has resulted in the increased use of post-fire treatments, in which soil stabilization treatments are crucial for diminishing soil degradation (Gómez et al., 2019).

Post-fire treatments may be divided into three categories: (i) emergency stabilization; (ii) rehabilitation; and (iii) restoration (Lucas-Borja et al., 2019). Many experiments developed in USA and Europe have shown that long-term rehabilitation and restoration actions are often focused on the biotic components of the ecosystem (Hessburg and Agee 2003; Beschta et al. 2004; Robichaud, 2005; 2010; Fernandez and Vega, 2016; Gómez et al., 2019; Lucas-Borja et al, 2019). For these activities, recovery of native plant communities and habitats, maintenance of plant biodiversity, re-establishment of timber or grazing species and control of invasive weeds are the most important targets. As regards the emergency stabilization actions, mulching is considered as one of the most efficient treatment to stabilize the soil of the burned area and to reduce additional damage to soil and vegetation immediately after wildfire. This treatment consists in 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 literature (e.g. Prosdocimi et al., 2016). Strictly speaking about 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 the rainfall action and the amounts of logging debris on the soil surface may be low (Lucas-Borja et al., 2019).

Beside these benefits, some problems using straw or woodships mulching as post-fire emergency treatment, such as, for example, straw blowing under strong winds, quick decomposition or emergence of non-native plant species (Cerdà et al., 2016; Prosdocimi et al., 2016). Luna et al. (2018) found that, despite woodships mulch was appropriate for reducing erosion and runoff in restored soils, this type of mulch did not favour vertical water movement towards deeper horizons and then was not useful in order to increase soil water storage. 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 generate changes in soil properties, since straw can act as a new source of vegetal material to be incorporated into the soil. On this regard, organic matter, microbial biomass carbon, respiration, enzymatic activities or nutrient content of soil, directly or indirectly linked to vegetal input into the soil (Doran and Parkin 1994; Larson and Pierce 1994; Entry and Emmingham, 1998; Bastida et al., 2007; Hedo et al., 2015), can be influenced by straw doses applied to soil with mulching.

In spite of this close linkage between physico-chemical 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. As far as now, 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 and soil respiration, have been proposed as specific indicators of soil functionality (Bastida et al., 2008; Lucas-Borja et al., 2011; Hedo et al., 2015). In addition, the C/N ratio (Lucas-Borja et al., 2012; Hedo et al., 2015), soil pH (Lucas-Borja et al., 2012), soil texture (Fterich et al., 2014), nutrients 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. In spite of this knowledge, more research is needed to better understand whether and to what extent soil functionality is influenced by straw mulching, with particular reference to the Mediterranean forests, where soils are particularly prone to erosion and degradation and the fire risk is very 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 accomplish reactions to release soil nutrients for vegetation development (Hannam et al., 2006). Forest managers and policy makers should know more deeply how straw mulching may affect 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 alter specific indicators of soil functionality in the short-terms after a wildfire in a Mediterranean forest of *Pinus halepensis* M. More specifically, straw was applied as mulching treatment immediately after the wildfire in different areas and then soil microbiological properties were monitored throughout one year in spring and autumn. We hypothesized that the straw mulching may enhance soil functionality in the short-term, because it increases the soil organic matter content, which plays an important role in controlling its metabolic processes.

2. Methods

2.1. 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 semi-arid, "BSk" according to the Koppen classification (Kottek et al., 2006). The mean annual temperature and precipitation are 16.6°C and 321 mm, respectively. According to the historical data (1990-2014) provided by the Spanish Meteorological Agency (AEMET), 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 17th century until the middle of the 20th 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 watercourses.

2.2. Experimental design

This study was carried out during 2017 inside a drainage basin of the approximately 700 ha affected by a wildfire in July 2016. Immediately after the wildfire, one site of about one 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 of 20 x 10 m) were randomly installed with their longest dimension along the maximum slope. Plots were distributed selecting certain sites characteristics, slopes and aspects to ensure comparability among the nine plots used in this study. Distance between plots was always higher 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, thus allowing to compare our experimental 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 placed in an unburned area, one km away from the burned site, and assumed as control. Three other plots were located in the burned area, but not treated.

Mulching treatment was assigned in September 2016 to the remaining three plots located in the burned area. Mulching consisted of manual application of straw (0.2 kg/m^2 of dry weight) on plot soils at an initial depth of three centimetres. This dose was proposed by different authors to achieve a cover over 80% in plots located in the north of Spain (Vega et al., 2014). Moreover, such amount of straw is also successfully (the biophysical point of view) used in agricultural land affected by intolerable erosion rates (Cerdà et al., 2017). To summarize, three replicated plots were non-burned soils (and hereinafter indicated as "NB"), three plots were burned and non-mulched soils (three replicates, hereinafter "B+NM"), and three plots were burned and then mulched soils (three replicates, "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 mid of the study period (in March 2017) and at the end of the experiment (in July 2017). More details about soil cover measuring methods and results are reported in Lucas-Borja et al. (2019).

2.3. Soil sampling and analyses

As regards soil sampling, three soil samples (each of 600 g) were collected in each plot in two seasons (May 2017 and November 2017) throughout one year after the wildfire for a total of 18 soil samples, 3 treatments (NB, B+M, B+NM) x two seasons (autumn and spring) x three replicates. Soil samples consisted of the composition of further six sub-samples, randomly distributed over each plot, in order 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 4 ºC. Soil analyses were carried out 1 day after sampling. Sampled soil was analysed for the main physical, chemical and microbiological properties. Concerning the physical and chemical properties, texture (soil contents of sand, silt and clay) was analysed according to the methods by 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 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

Lucas-Borja et al. (2012). Total nitrogen (TN, %) was determined using the Kjeldahl (Bremner and Mulvaney, 1982). As regards 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 (DHA, 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 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 Lozano et al. (2016).

2.4. 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. To study the relationships between these soil properties was used a Pearson's correlation analysis and to assess the similarities among the soils samples of each treatment was used a Canonical Analysis of Principal Coordinates (CAP) after normalizing the data. The CAP analysis is a constrained nonparametric ordination procedure, widely used as ecology ordering method, since 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 (in this study using Euclidean distance), 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 $\mathbf{Q}_m$; (iii) application of a traditional canonical analysis (e.g., a Canonical Correlation Analysis, since it **X** contains 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.).

3. Results

3.1. Effects of widlfire and mulching on physico-chemical and microbiological soil properties

The 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 constricted in the six treatments analyzed: (i) NB soils sampled in spring field campaign; (ii) B+NM soils sampled in spring; (iii)

Commento [YYY1]: No es claro; por supuesto, los mostreos tienen que estar en diferientes clusters.

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 axis 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 the 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 equal to 10 (Table 4). All clusters were significantly different each other with the exception of the soils sampled in B+NM both in autumn and spring and 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 axe 1 (CAP1), while important microbiological parameters (BGA and UA) have higher weights on axe 2 (CAP2).

Commento [YYY2]: Porqué 12 y no 18 (3 replicaciones x 3 treatments x 2 estaciones)?

3.2. Differences among treatments and temporal changes in physical and chemical 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 contents were monitored from autumn to spring in the other experimental plots. Moreover, compared to the first field campaign in spring, the clay content significantly decreased (by 48%) 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 physico-chemical properties (contents in OM, OC, TN and EC) were significantly different among the three analyzed treatments in both field campaigns. As regards their time evolution, the 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 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 contrary, there were no significant differences in soil pH in every season and treatment. All soils showed always a slightly alkaline pH (on the average in the range 8.4-8.7) 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 and this value was significant 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 of which showing not significant differences). More specifically, the NB soils showed the lowest C/N ratio (13.9±0.73) in autumn and the highest values (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 C/N ratio (by 37%) was estimated in NB plots and an increase was calculated in both burned soils, more noticeable in B+NM plots (+20% against a 4% in B+M soil) (Table 5).

3.3. 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 (CONTROLAR Acid-PA y GPRS) and 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 season), 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 (CONTROLAR GPRS) in both seasons, UA in spring as well as GPRS and MC in autumn (Table 6).

3.4. 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). As regards soil texture, the clay content was negatively correlated with silt (r = -0.93) and sand (r = -0.63) contents. The soil pH was significantly linked with glomalin (r = -0.48), while EC showed a higher correlation with chemical (OM, OC and TN contents, r > 0.77) than with microbiological properties (BGA and UA). The highest correlations (r > 0.98) were found among OC, OM and TN contents of the soils. As expected, a noticeable and significant r (> 0.55) was found between the C/N ratio and the OC and TN contents. Microbiological soil properties, except DHA, showed high significant correlations with several physical and chemical parameters (Table 7). BGA was the enzymatic activity that 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 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 contents, while the MC was only positively correlated with GPRS (r = 0.50) (Table 7).

4. Discussions

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 background is available. Therefore, it is necessary to select a set of soil parameters about physical, chemical and biological soils properties, which suitably reflect its status and the functions that need evaluating (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 (Ulery et al., 1993; Mataix-Solera et al., 2002), 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). As regards the latter, since enzymatic activities have an important role in catalyzing biological reactions, there is a particular need of 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 meaningful of some physical, chemical and microbiological soil properties in comparison to unthreatened and control plots, with particular regard on microbial activity, previously not enough investigated in Mediterranean environments (D'Ascoli et al., 2005; Pourreza et al., 2014; Rincón and Pueyo, 2010) and especially in forests. More specifically, it has been investigated whether the soil treatments with straw mulching could be beneficial for soil quality in terms of changes in physico-chemical and microbiological soil properties at short-term after fire occurrence, in order to mitigate the well-known soil degradation induced by fire.

In the same environment and experimental plots than in this study, Lucas-Borja et al. (2019) observed noticeable variations in vegetation cover, dead matter and bare soils extent one year after fire for each experimental condition (non-burned, burned and non-treated as well as burned and mulched soils). According to Lucas-Borja et al. (2019) and in spring 2017, soil became bare for about 30% and a vegetation cover of 10% was detected in the B+NM experimental plots. The covers of vegetation and dead matter (coming from straw application) were about 25% and 55%, respectively, in B+M plots. In the following autumn, the extent of bare soils increased to 55% and vegetation cover increased to 25% in B+NM soils, whereas the latter parameter 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, determining sunlight interception (Lucas-Borja et al., 2019). From these findings, we suspected that these changes in soil vegetation cover and soil microclimatic conditions, significantly may have altered the physico-chemical and microbiological soil properties during the two sampled periods. Moreover, since all the experimental plots were set up on sites characterizes with the same burn severity, changes in soil properties may be not attributed to burn severity.

It is well known that, of all the physical and chemical soils properties, the OM content is one of the most important quality indicators, given its influence on plant growth-related functions (e.g., humidity being retained, 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 and both in autumn and spring, OM showed the highest values in B+M soils; this parameter was significantly lower in B+NM and NB plots. Also, variations in TN contents were detected after wildfire and mulching, inducing significant increases in B+NM and mainly in

B+M plots. It is expected that soil treatments (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, since the C/N ratio is related with OM decomposition and N mineralisation (Lucas-Borja et al., 2016). After one year of time span, all the experimental soils did not show 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 owning 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 mineralization, 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 on 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 quite expected after wildfire, as also detected in the same environment by Lucas-Borja et al. (2019). These changes must be monitored with caution, since a decrease of the finer fraction let us suspect that burned but not treated soils may be more prone to erosion compared to non-burned and burned but mulched soils.

Commento [YYY3]: Citacion de nuestro articulo de JEMA

Literature shows that soil pH and EC tend to rise after fire, although, in any case, 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, after sudden increases immediately after fire compared to NB soils, this parameter strongly decreased with a more noticeable effect in B+NM plots. This may be due to the effects of burning, which accumulated ash that contains C and other nutrients from burned forest fuel (Caon et al., 2014). Mulching should have smoothed the decreasing trend of EC, thanks to the progressive release of these compounds.

With regards to the monitoring of the soil microbiological properties, we noticed that both the quantity and activity of microorganisms grew, as respectively indicated by biomass carbon and basal soil respiration (the later being not statistical different) parameters increased in the burned soils compared to NB plots immediately after fire. Conversely, one year after fire, the microbial carbon decreased in B+NM soils. These microbiological effects detected in B+M soils compared to non-treated 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 mineralized 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 dehydrogenase. 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 basal soil respiration, 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 restore of plant cover, with roots being the main resource (López-Poma and

Bautista, 2014). The lack of variation of dehydrogenase activity 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 dehydrogenase activity to seasonality and site effects than management practices. This could be related with the fact that dehydrogenases are not active as extracellular enzymes in soil, thus presenting a different pattern compared to the extracellular soil enzymes, that is, β-glucosidase, urease and acid-phosphatase (Blonska et al., 2017). The urease and ß-glucosidase activities were 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 with the greater accumulation of nitrogen due to the straw application, as indicated by the fair positive correlation with TN content shown by the Pearson's correlation analysis. The evolution of ß-glucosidasae is related with OM decomposition velocity and with energy released by soil microorganisms, as indicated by the positive high correlation with the OC. The progressive temporal changes among the analyzed soil conditions suggests that mulching soils with straw could promote bacterial development, but the dehydrogenase activity could behave quite differently from the other enzymes.

Moreover, the glomalin content as a result of arbuscular micorrizal fungi is an indicator of C and N storage, which plays a key role in aggregate stability and water repellence of soils (Lozano et al., 2016). Although very few studies have explained its temporal evolution and response to post-fire mulching, some authors have demonstrated the glomalin sensitivity to fire, even at low temperatures (Lozano et al., 2016). For instance, Rivas et al. (2016) showed the GPRS level recovery four years after fire due to species' rapid root colonization that symbiosis undertakes with arbuscular micorrizal fungi. Sansano (2016) confirmed the negative influence of cutting timber in the short term after fire. This 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 physico-chemical and microbiological soil properties compared to soils treated with straw mulching and to control plots.

5. Conclusions

In order to better understand the effects of an important post-fire soil restoration technique, such as mulching with straw, on soil functionality of the Mediterranean forests, 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 the Canonical Analysis of Principal Coordinates. The results of enzyme activity monitoring in the soils have confirmed the working hypothesis that the straw mulching enhances soil functionality on the short-term. As a matter of fact, compared to non-burned soils, both in autumn and spring, in burned and straw-mulched soils the organic matter content increased. The C/N ratio, after a decrease immediately after fire in burned and non-mulched plots, did not show significant differences among the other treatments. The values of pH were stable, but a marked reduction in the electric conductivity was noticed after one year, especially in burned soils both treated with straw-mulching and without mulching. The quantity and activity of soil microorganisms grew (as shown by the increase of biomass carbon and basal soil respiration) in the burned and treated soils. Moreover, after the mulching treatment all microbiological and enzymatic activities improved, except for dehydrogenase activity. 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, indicating the positive effects of straw application in areas burned by high-severity fire on soil functionality, 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.

Acknowledgements

We wish to thank the Regional Government of Castilla-La Mancha (Junta de Comunidades de Castilla-La Mancha) for field work support. This study was supported by funds provided by the University Castilla-La Mancha to the Forest Ecology Research Group and the Spanish Institute for Agricultural and Food Research and Technology (INIA) for the funding awarded through National Research Projects GEPRIF (RTA2014-00011-C06). This study was also funded by the Spanish Ministry of Economy, Industry and Competitiveness Research Project BIORESOC (CGL2017-88734-R) and the FEDER-Junta de Andalucía Research Project RESTAGRO

(UAL18-RNM-A021-B). Isabel Miralles is grateful for funding received from the Ramón y Cajal Research Grant (RYC-2016-21191) from the Spanish Ministry of Economy, Industry and Competitiveness (MINECO).

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46.

Anderson, M.J. and Willis, T.J. (2003). Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology 84(2)*, 511-525.

Blonska et al., 2017. The relationship between soil properties, enzyme activity and land use. Forest research papers, 78 (1):39-44.

Seber, G. A. F. (1984) *Multivariate Observations*, Wiley, New York.

Luna, L., Vignozzi, N., Miralles, I., Solé-Benet, A. 2018. Organic amendments and mulches modify soil porosity and infiltration in semiarid mine soils. Land degradation and development 29 (4), 1019-1030.