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Title: Soil microbiological properties and enzymatic activities of long-term post-fire recovery in dry and semiarid Aleppo pine (*Pinus halepensis* M.) forest stands.

Authors: Javier Hedo de Santiago; Manuel Esteban Lucas-Borja, Dr.; Consuelo Wic-Baena; Manuela András Abellán, Dr.; Jorge De las Heras, Prof.

Solid Earth

Dear reviewer 2,

We would like to thank you for the revision process of our work. We have addressed all the comments made by the reviewer with the aim to improve the quality of our manuscript. The last version of the document contains the comments of both reviewers.

We hope these and other modifications (see below) help to improve the quality of work.

Best regards,

Javier Hedo de Santiago

Interactive comment on “Soil microbiological properties and enzymatic activities of long-term post-fire recovery in dry and semiarid Aleppo pine (Pinus halepensis M.) forest stands” by J. Hedo et al.

Anonymous Referee #2

Received and published: 22 November 2014

The research paper entitled “Soil microbiological properties and enzymatic activities of long-term postfire recovery in dry and semiarid Aleppo pine (*Pinus halepensis* M.) forest stands” by J. Hedo, M. E. Lucas- Borja, C. Wic, M. Andrés Abellán, and J. de Las Heras has been revised for publication in Solid Earth.

The topic of the manuscript falls within the scope of the journal. The recovery of natural forest affected by wildfires is an issue of concern mostly in sensitive areas to the impacts of climate change (e.g. increasing temperatures and frequency of drought which may enhance the risk of wildfires). The MS was written in good English and itis well structured. However, there are several queries from this referee that must be addressed prior being considered for publication in a scientific journal.

(Authors) Thank you very much for all your comments and suggestions. We have addressed all of them in the new version of the manuscript and you can find a detailed response below.

Major comments:

There are contradictory statements in the abstract that may lead to misunderstand- ´ ing of the key message of this piece of work. It is said that “the long-term consequences and post-fire silvicultural management in the form of thinning have a significant effect on the site recovery after fire.”, however, at the same time authors are ending the abstract with, to my understanding, the main outcome of their study: “We conclude that total vegetation restoration normalises microbial parameters, and that wildfire and post-fire silvicultural treatments are not significant factors of soil properties after 17 years.”

(Authors) We erased the sentence “the long-term consequences and post-fire silvicultural management in the form of thinning have a significant effect on the site recovery after fire”.

Please, rewrite the abstract trying to be coherent with your conclusions.

The hypothesis must be reformulated. As it is stated now, it seems that authors were just referring to their own results after getting them.

(Authors) We changed the second hypothesis by: “microbiological soil properties and enzymatic activities recovered after the wildfire and the thinning at the mid-term”

My main concern in the design of the experiment is referred to stationarity of the study. Can the authors of this work justify the decision of carrying out the experiment in winter? Why did you choose this season? Logistics may be? Are there scientific reasons for this choice?

(Authors) Seasonality is an important factor to keep in mind, because it affects soil properties as enzymatic activities or microbial biomass, as many studies have showed. As Ferguson et al.

(2007) stated, late fall or early winter is a good time for the soil sampling. (Ferguson, R.B., G.W. Hergert, C.S. Shapiro, and C.S. Wortmann. 2007.Guidelines for Soil Sampling. NebGuide G1740. University of Nebraska–Lincoln). We sampled during the early winter season, when the variations of soil properties hit average values and also because it is the usual season used by different authors to carry out this type of research works in Mediterranean forest areas is early winter (Lucas-Borja et al., 2010, 2011 & 2012). Nevertheless, the effect of season should be further studied in the future. In fact, we are now involved in a new manuscript redaction related to seasonal effects.

Some statements in the Discussion section should be extended: ´

“Furthermore, Bastida et al. (2008) indicated that seasonality affects enzymatic activities or microbial biomass, and in this work only we sampled in early winter, so it would be suitable to conduct sampling in different seasons.” Please, indicate why you choose winter.

“Wic-Baena et al. (2013) have recently shown that soil enzymatic activities did not diminish 6 years after thinning.”

Please, extend your discussion at this point and try to support your findings with more sources of information available in the scientific literature.

(Authors) We extended our explanation about these statements. Please see discussion section.

“(. . .) Our results also indicate lower C/N values at Yeste, but no significant differences among treatments.” Could you explain why?

“Lower C/N rates have been associated with higher respiration rates and microbiological properties (Schmitz et al., 1998).”

Please, extend your discussion. Is there any limitation with the use of words that avoids you to do so?

(Authors) We extended our explanation about these statements. Please see discussion section.

The last sentence of the Conclusions section is again confusing about the main outcomes of your work:

“Forest management guidelines should consider the effect of thinning treatments and forest site in order to preserve soil quality under the adaptative forest management context.”

I may point out that forest site play a very important role in forest recovery after wildfire. Therefore, forest management policies should have aspect into account when designing (and budgeting) restoration plans.

(Authors) We changed the last sentence in the conclusion section.

Minor comments:

Abstract:

I am not sure if “normalises” is a good term to be used here.

(Authors) I think that is a good term, because the meaning of “normalize” is “resume a normal state”.

“(. . .) wildfire and post-fire silvicultural treatments are not significant factors of soil properties after 17 years”. Substitute “of” by “affecting”.

(Authors) It has been changed.

Introduction: ´

I do not understand the term “exposed” within this context.

(Authors) It has been changed by “…and runoff and surface erosion rates can greatly increase”.

Please, add a sentence about the importance of your work in the context of climate change and the vulnerability of natural forests against wildfires in sensitive (to the con sequences of Climate Change) Mediterranean areas.

(Authors) We added the sentence: Moreover, global change is affecting fire regime, increasing fire frequency and area burned, its destructiveness to Mediterranean ecosystems (Pausas 2004).

“thinning in young”, Is there a “more scientific” term to refer to this practice.

(Authors) “Thinning” means selective removal of trees, primarily undertaken to improve the growth rate or health of the remaining trees. When is “thinning in young”, in a post-fire context, the selective removal of trees is carried out when the trees are still young.

“physical–chemical”. Use “Physicochemical” and be uniform within your MS. Please, add a more recent reference to “Nannipieri et al., 1990”. You said that “Some long-term studies appreciated that soil organic matter and microbial communities can recover to the pre-fire levels (Guénon et al., 2013).” Please, rephrase this statement indicating the differences with your study and their singularities. Otherwise, it seems that the work was already done.

(Authors) We homogenized the term “physicochemical” in the manuscript. We added another reference (Bastida et al. 2008). We specify that Guénon et al. (2013) worked with different species. Please see Introduction section.

“It is noteworthy 5 that we define recovery as a scenario which returns to the same soil functioning activity levels between the burnt or thinned and mature plots.” I see this sentence more as “Materials and Methods information”.

(Authors) We moved this sentence to Material and Methods, in Experimental design.

Section 2.3. Add a reference to “organic matter (OM) was inferred by multiplying the TOC content by 1.728.”

(Authors) The reference have been added.

Was a weather station placed in the experimental sites during the campaign?

(Authors) No, there was a official weather station closet o the study areas.

Section 3.1. ´

In “Soil texture (Table 1) and electrical conductivity (Table 2) were also similar for both study sites and for the different treatments.” Delete “also” to give coherence to the entire paragraph.

(Authors) It has been deleted.

“In relation to the experimental treatments, enzymatic activities presented similar values in the “BT”, “MAT” and “BNOT” plots (Fig. 1).” I guess this was already mentioned at the beginning of the sub-section.

(Authors) It has been deleted the second sentence.

Section 3.3. Delete “and also among the microbiological variables,”

(Authors) It has been deleted.

Discussion

“Gutknecht et al. (2010) recently showed” Delete “recently”. “(. . .) soil moisture and temperature showed no significant differences in the “BT”, “MAT” and “BNOT” plots,” Change “in” by “between”.

(Authors) We erased “recently”. We wrote “between” instead “in”.

Please, look for a different term to avoid “a large part” or rephrase the sentence.

(Authors) We finally wrote “may largely explain”.

There two times in the discussion in which you refer to “15 years”. This is confusing since you mentioned a period of 17 years before. Please, clarify this point.

(Authors) We apologize about this mistake. The correct period is 17 years.

“This long-term study demonstrated that soil parameters might recover to the pre-fire levels 15 years after the fire event and thinning operations.” Please, add “at least” before “15 years” and change “15” by “17”.

(Authors) We changed 15 by 17; and we added “at least” into the sentence.

Based on the stated above, I conclude that a major revision is needed prior considering the paper to be published in Solid Earth.

Yours sincerely,

Soil microbiological properties and enzymatic activities of long-term post-fire recovery in dry and semiarid Aleppo pine (*Pinus halepensis* M.) forest stands

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Abstract

Wildfires affecting forest ecosystems and post-fire silvicultural treatments may cause considerable changes in soil properties. The capacity of different microbial groups to recolonize soil after disturbances is crucial for proper soil functioning. The aim of this work was to investigate some microbial soil properties and enzyme activities in semiarid and dry Aleppo pine (*Pinus halepensis* M.) forest stands. Different plots affected by a wildfire event 17 years ago without or with post-fire silvicultural treatments five years after the fire event were selected. A mature Aleppo pine stand unaffected by wildfire and not thinned was used as a control. Physicochemical soil properties (soil texture, pH, carbonates, organic matter, electrical conductivity, total N and P), soil enzymes (urease, phosphatase, β-glucosidase and dehydrogenase activities), soil respiration and soil microbial biomass carbon were analysed in the selected forests areas and plots. The main finding was that long time after this fire event produces no differences in the microbiological soil properties and enzyme activities of soil after comparing burned and thinned, burned and not thinned, and mature plots. Moreover, significant site variation was generally seen in soil enzyme activities and microbiological parameters. We conclude that total vegetation recovery normalises post-fire soil microbial parameters, and that wildfire and post-fire silvicultural treatments are not significant factors affecting soil properties after 17 years.

Keywords: soil recovery; enzyme activity; forest fire; climate change; forest management; microbiological properties, semiarid climate, Aleppo pine.

1 Introduction.

Fire is one of the most important disturbances in the Mediterranean region as it shapes and structures many plant communities, forest ecosystems and landscapes (Boydak et al. 2006). After a fire event, forest functions, nutrient cycling, and the physical, chemical and biological properties of soils are significantly affected (Wic-Baena et al. 2013) and runoff and surface erosion rates can greatly increase (Alegre-Prats et al. 2013). Moreover, global change is affecting fire regime, increasing fire frequency and area burned, its destructiveness to Mediterranean ecosystems (Pausas 2004). On this context, post-fire forest management is useful to accelerate the recovery of soil forest functions, and to improve health, growth and reproductive processes (Moya et al. 2008). For fire-adapted pines, such as *Pinus pinaster* Ait. (Maritime pine) and *Pinus halepensis* Mill. (Aleppo pine), three main forest management guidelines have been proposed as proper post-fire silvicultural treatments. The guidelines are in accordance with the success of natural regeneration: (1) no treatments if natural regeneration is achieved after the fire event; (2) assisted natural regeneration or (3) active restoration (De las Heras et al. 2012). Moreover, several studies have shown that “thinning in young” reduces both intra-specific competition and fire recurrence events (Espelta et al. 2008).

Soil plays an essential role in the forest ecosystem’s fertility and stability (Smith et al. 1993) and specifically soil microorganisms, which accomplish reactions to release soil nutrients for vegetation development (Hannam et al. 2006). As Rutigliano et al. (2004) reported, microbial biomass and activity increased from younger to later stages of ecological succession and the introduction of pine into Mediterranean areas retards soil development. However, soil properties and plant cover relationship can be in various ways at various rates and since different studies were not replicated across a range of site types, conclusions cannot be generalized (Muscolo et al. 2007). Forest fires and post-fire silvicultural treatments may significantly change forest and soil properties (Grady and Hart, 2006; Wic-Baena et al. 2013). After forest fires, changes in vegetation dynamics and soil properties are expected to occur due to the plant-soil feedback (Van der Putten et al. 2013; Brandt et al. 2013). Soil erosion is a key process as redistribute the soil particles, the seeds and the nutrients (Cerdà and Lasanta, 2005; Lasanta and Cerdà, 2005). Fire may alter physico-chemical soil properties (i.e., soil organic matter content and structure, hydrophobicity, pH and nutrient cycles), and microbiological or biochemical soil properties (i.e., microbial biomass, microbial activity, soil enzymes activities) (Mataix-Solera et al. 2009). These changes mostly occur below 5 cm of the surface, where the soil temperature rarely overtakes 100ºC (Úbeda and Outeiro, 2009; Aznar et al. 2013). Post-fire silvicultural treatments may also modify the soil microbiological and biochemical variables, such as belowground biological activity and soil nutrients availability (Grady and Hart, 2006) or enzyme activities

(Wic-Baena et al. 2013). Tree felling or shrub clearing modifies microclimatic conditions at the ground level, as well as the amount and quality of potential organic inputs to soil (Grady and Hart, 2006). The magnitude of the changes occurring after wildfire events or post-fire silvicultural treatments depends on forest characteristics, such as the recovery capacity of vegetation (Irvine et al. 2007), climatic factors (Almagro et al. 2009) and post-fire soil rehabilitation management (Fernández et al. 2012; Alegre-Prats et al. 2013).

Given the fundamental importance of soil microbial communities in soil ecosystem sustainability, information on how microbial functionality is affected by fire or post-fire silvicultural treatments under semiarid climatic conditions is required. Estimation of microbiota and soil status are necessary to determine optimal management strategies (Mabuhay et al. 2003, Mataix-Solera et al. 2009). In this context, the use of one parameter is not consistent because soil quality depends on a wide range of chemical, physical, biochemical and microbiological variables (Nannipieri et al. 1990; Bastida et al. 2008b). Thus, many authors have proposed using a combination of several variables as indicators of soil status (Dick et al. 1996). Specific indicators of microbial activity, such as variables relating to nutrient cycles (nitrogen, carbon and phosphorus) and enzymatic activities (urease, β-glucosidase and phosphatase), have been proposed to evaluate soil status (Trasar-Cepeda et al. 1998). Moreover, general indicators of microbial activity have been extensively used in forest and agricultural soil status characterization (Armas et al. 2007; García-Orenes et al. 2010; Fterich et al. 2014; Câmara-Ferreira et al. 2014).

Long-term studies into soil quality or those that evaluate soil recovery capacity are scarce. However, long term studies are necessary to reach reasonable conclusions on the impacts that fire events and post-fire silvicultural treatments have on soil properties, particularly in Mediterranean ecosystems (Wic-Baena et al. 2013). Some long-term studies appreciated that soil organic matter and microbial communities can recover to the pre-fire levels in the Mediterranean region, taking into account study areas dominated by *Quercus ilex* L., *Quercus suber* L. and *Pinus pinaster* Aiton subsp. *pinaster* (Guénon et al. 2013). The aim of this study is to investigate soil microbiological and soil enzymatic activities in different semiarid and dry Aleppo pine forest ecosystems affected by: (i) a wildfire event 17 years earlier; (ii) a wildfire event 17 years ago and treated with early thinning 12 years earlier; (iii) an Aleppo pine mature stand not affected by wildfire with no silvicultural treatments. We hypothesised that: 1) microbiological soil properties and enzymatic activities are influenced by the climatic conditions recorded at each semiarid and dry location; 2) microbiological soil properties and enzymatic activities recover after the wildfire and the thinning at the mid-term.

2 Material and Methods

2.1 Study area

The study was conducted at two sites burnt in the summer of 1994, Yeste and Calasparra (in the provinces of Albacete and Murcia, respectively) in SE Spain. The total burnt area covered about 44,000 ha in both provinces. The forest tree composition in the study area was dominated by mature even-aged Aleppo pine stands, with shrubs and herbaceous vegetation in the understory (Table 1). Natural post-fire regeneration took place at both sites (45,000 saplings/ha in Calasparra and 7,000 saplings/ha in Yeste) (Table 1). The climate of both experimental areas is classified as Mediterranean (Allué, 1990), with Yeste and Calasparra classed as a dry site and a semi-arid ombroclimate site, respectively (Rivas-Martínez, 1987). Average annual rainfall and temperature for the last 30 years were respectively 503 mm and 13.5ºC in Yeste as compared to 282 mm and 16.3ºC in Calasparra. According to the Spanish Soil Map (Guerra Delgado, 1968), Yeste and Calasparra soils are classified as Inceptisols and Aridisols, respectively (Table 1). Soil texture at both sites is classified as loam/clay-loam (Table 1).

2.2 Experimental design

Two experimental sites of 3 ha were selected in both Yeste (2º20'W 38º21'S) and Calasparra (1º38'W 38º16'S). Three plots were set up inside each site, one of which (1 ha) was naturally burnt in summer 1994 and was then occupied by high Aleppo pine post-fire natural. The second plot of 1 ha was naturally burnt in summer 1994 and then thinned in 1999. The post-fire silvicultural treatment and thinning operations left 1,600 saplings/ha at both the Calasparra and Yeste sites. The third plot was a mature stand of 1 ha used as a control. The mature Aleppo pine stand was located adjacent to the fire perimeter at both the Calasparra and Yeste sites and has not been affected by either forest-fire or silvicultural treatments in the last 20 years. It is noteworthy that we define recovery as a scenario which returns to the same soil functioning activity levels between the burnt or thinned and mature plots. All the plots were selected in areas with a low slope (< 5%). Sampling was carried out in early winter as Ferguson et al. (2007) recommended in their guidelines for soil sampling.

In December 2011, six soil samples (1,000 g) were randomly taken from each plot: (i) the plot affected by a wildfire event and post-fire silvicultural treatments 17 and 12 years earlier, respectively (burned and thinned, hereafter named "BT"); (ii) the plot affected by a wildfire event 17 years earlier with no post-fire silvicultural treatments (burned and not thinner, hereafter named "BNOT"); (iii) the plot occupied by a mature Aleppo pine stand (hereafter named "MAT"). Each soil sample was composed of six subsamples collected in a 5 x 5 m subplot area, which were thoroughly mixed to obtain a composite sample (Andrés et al. 2011). Finally 36 samples were obtained: 2 experimental sites x 3 treatments x 6 replicates. The results shown are the average of the samples taken at each subplot. Soil samples were taken from the uppermost mineral layer (0-15 cm) after removing litter. Samples were passed through a 2-mm sieve and were kept at 4ºC during one month to avoid any influence on the parameters analysed in the laboratory (Andrés et al. 2011).

2.3 Physical and chemical variables

Five hundred grams of the collected soil samples were used to analyse some physical and chemical soil properties. pH and electrical conductivity (EC) were measured in a 1/5 (w/v) aqueous solution using a pH-meter (Navi Horiba model). Total organic carbon (TOC) was determined by wet oxidation with K_2CrO_7 and titration of dichromate excess with Mohr´s salt (Yeomans and Bremner, 1989), while organic matter (OM) was inferred by multiplying the TOC content by 1.728 (Lucas-Borja et al. 2010b). Total carbonates (CO_3^{2-}) were measured in a Bernard calcimeter according to the method of Guitián & Carballas (1976). Bioavailable phosphorus (P) was determined using the method described by Olsen and Sommers (1982). Total nitrogen (total N) was measured following Kjeldhal's method modified by Bremner (1965). The texture analysis was performed using the method of Guitián and Carballas (1976). Soil moisture and temperatures were recorded during the sampling season (winter 2011) using a soil moisture sensor (ECHO EC-10 model), a soil temperature sensor (TMC6-HD model) and a data-logger (Hobo U12-006 model). Soil temperature and humidity sensors were installed at a depth of 10 cm in each plot.

2.4 Biochemical and microbiological variables

Soil dehydrogenase activity (DHA) was determined by using 1 g of soil, and the reduction of p-iodonitrotetrazolium chloride (INT) to p-iodonitrotetrazolium formazan was measured by a modified version of the method reported by García et al. (1993). Soil dehydrogenase activity was expressed as μmol INTF g^{-1} soil h^{-1}. Urease activity (UA) was determined as the NH_4^+ released in the hydrolysis reaction (Kandeler et al. 1999). Alkaline phosphatase (PA) and β-glucosidase (BA) activities were measured following the methods reported by Tabatabai and Bremner (1969) and Tabatabai (1982), respectively. Basal soil respiration (RESP) was analysed by placing 50 g of soil moistened to 40-50% of its water-holding capacity (water potential: 0.055 MPa) in hermetically sealed flasks and by incubating for 20 days at 28°C. Released CO_2 was periodically measured (daily for the first 4 days and then weekly) using an infrared gas analyzer (Toray PG-100, Toray Engineering Co. Ltd., Japan). The data were summed to give a cumulative amount of released CO_2 after a 20-day incubation. Basal soil respiration was expressed as mg CO_2-C kg^{-1} soil per day. Microbial biomass carbon (CB) was determined by Vance et al. (1987) following the method adapted by García et al. (2003).

2.5 Statistical analysis

Data were analysed by a two-way ANOVA at which site level (Yeste and Calasparra) and the silvicultural management level ("BT", "MAT" and "BNOT") were selected as the factors. All the subplots were assumed to be spatially independent. The *post hoc* test applied was Fisher's least significant difference. A $P<0.05$ level of significance was adopted throughout, unless otherwise stated.

Moreover, a multivariate statistical method using a principal component analysis (PCA) was carried out to study the structure of the dependence and correlation between the physicochemical and microbiological soil properties at the different sites and for the various treatments. Another multivariate statistical method (correlation analysis) was carried out. To satisfy the assumptions of the statistical test (equality of variance and normal distribution), variables were square root-transformed whenever necessary. The statistical analyses were done with the Statgraphics Centurion software.

3 Results

3.1 Physical and chemical variables

Soil temperatures and soil moisture differed significantly ($P<0.05$) between both experimental sites (Yeste and Calasparra), but not between different treatments ("BT", "MAT" and "BNOT") (Table 1). Soil texture (Table 1) and electrical conductivity (Table 2) were similar for both study sites and for the different treatments. The percentage of carbonates, organic matter, phosphorus and total nitrogen differed between sites, with higher values recorded for Yeste. Significant differences were also observed ($P<0.05$) in the pH values and C/N ratio between sites, with Yeste obtaining lower values. Under the experimental conditions ("BT", "MAT" and "BNOT"), the physical and chemical variables showed a different behaviour depending on the site (Yeste and Calasparra; Table 2). There were not significant differences in any of the studied parameters taking into account the interaction.

3.2 Biochemical and microbiological variables

The experimental treatments considered in this study and the interaction between sites and experimental treatments did not significantly (*P<0.05*) influence the microbiological properties and enzyme activities (Table 3, Figure 1). The experimental site was the only influential factor (*P<0.05*) found for microbial biomass carbon, soil respiration and enzymatic activities (Table 3). Urease activity showed higher values in Calasparra than in Yeste, whereas β-glucosidase and dehydrogenase activities displayed higher values in Yeste than in Calasparra (Figure 1). No significant differences for phosphatase activity were found (Figure 1).

3.3 Correlation analysis

Positive and significant correlation coefficients were found between organic matter and some microbiological and biochemical variables (dehydrogenase, β-glucosidase and soil respiration). Negative and significant correlation coefficients were observed between organic matter and the physical-chemical variables, such as pH and C/N ratio and with the urease activity (Table 4). pH also showed a positive correlation and a significant coefficient with urease activity. pH negatively and significantly correlated with soil respiration, dehydrogenase and β-glucosidase activity. Urease activity presented different correlation coefficients, and positively and significantly correlated with phosphatase activity, pH and C/N ratio, while a negative and significant correlation was observed with dehydrogenase and β-glucosidase activities and total carbonates, phosphorus and total nitrogen. Conversely, a positive and significant correlation was seen between dehydrogenase and β–glucosidase activity. pH and C/N ratio correlated significantly and negatively with dehydrogenase and β-glucosidase activities (Table 4).

3.4 PCA analysis

The multivariate PCA analysis showed differences between the two study sites by separating into homogeneous groups (Figure 3). Conversely, the PCA did not separate among different treatments. The PCA analysis clustered the plots located in Yeste on the negative axis of PC 2 (Figure 3), which explained about 13.81% of variability. PC 1 explained around 42.22% of variability. The plots located in Calasparra were clustered on the positive axis of PC 2. Urease activity, C/N ratio and pH had a positive weight on PC 1, whereas dehydrogenase, β-glucosidase and organic matter had a negative weight (Table 5). Moreover, respiration, phosphatase and electrical conductivity had a positive weight on PC 2, while phosphorus and biomass carbon had a negative weight. The other loading factors of the different variables appear in Table 5.

4 Discussion

Vegetation and soil type are key factors that can modify soil characteristics and are responsible for maintaining a stable microbial community (Bastida et al. 2008a). Since Aleppo pine forest dominates both experimental sites, variations in soil properties can be related mainly to site-specific differences, such as soil temperature and moisture and soil type (soil organic matter, C/N ratio, pH and P, soil texture). Micro-climatic factors influence microbial enzymes, and also change the quality and quantity of the substrate upon which they act (Kumar et al. 1992). Different authors have demonstrated that scarce soil moisture generate lower soil respiration rates, microbial biomass carbon values and dehydrogenase, phosphatase and β-glucosidase enzymatic activities (Criquet et al. 2004; Sardans and Peñuelas, 2005; Baldrian et al. 2010; Lucas-Borja et al. 2012). Our results coincide with these trends since Calasparra (higher temperatures at lower soil moisture values) obtained lower values of microbiological parameters, β-glucosidase and dehydrogenase activities, but higher values for urease and phosphatase enzymes. The latter may be explained by quantity of total N and P present at each site. Given the lower total N and P values found in Calasparra, greater urease and phosphatase activity may be required to produce inorganic N and P ready for plant development. Gutknecht et al. (2010) showed that decreased N and P results in greater urease and phosphatase activity and higher enzyme production through soil microorganisms. On the other hand, Bastida et al. (2008a) indicated that seasonality affects enzymatic activities or microbial biomass, and in this work only we sampled in early winter, so it would be suitable to conduct sampling in different seasons.

In relation to fire and post-fire silvicultural treatments, soil moisture and temperature showed no significant differences between the “BT”, “MAT” and “BNOT” plots, thus may largely explain similar microbiological parameters values and enzymatic activities. Moreover, Aleppo pine is a pyrophyte species that exhibits good post-fire natural regeneration, being observed good post-fire seedling recruitment during the first growth season after the wildfire event (Leone et al. 2000). Thus, initial vegetation recovery is promptly ensured after a wildfire event (De las Heras et al. 2012). In this context, temporary plant cover loss and subsequent plant recruitment after a fire event may enhance the microbiological soil properties recovery. According to our results, the microbiological soil properties and enzymatic activities capacity recovery should be achieve 17 years after the wildfire event and the post-fire silvicultural treatment. This long-term study demonstrated that soil parameters might recover, at least, to the pre-fire levels 17 years after the fire event and thinning operations. Wic-Baena et al., (2013) have recently shown that soil enzymatic activities recovered 6 years after thinning. The same authors stated that the time period since the silvicultural treatment was applied seemed to significantly affect soil properties. It may be explained because long-term effects on soil processes are likely driven by changes in the quality of the organic matter inputs (Hart et al., 2005), and the relationship between post-fire recovery of Aleppo pine (dominant species in both experimental sites) and soil properties.

The organic matter greatly differed, obtaining higher values for Yeste than for Calasparra. Higher values for the general soil microbial activity indicators (i.e., soil respiration and dehydrogenase activity) and for β-glucosidase and phosphatase activity have been reported by Lucas-Borja et al. (2010a, 2011) in forest soil at a higher organic matter concentration. Some organic matter fractions contain readily metabolisable compounds, which can act as energy sources for microorganisms. In relation to fire and post-fire silvicultural treatments, the organic matter content was similar when comparing "BT", "MAT" and "BNOT" plots, which may be explained by the Aleppo pine post-fire initial recruitment. The organic matter derived from new trees may be the responsible of the similarities comparing "BT", "MAT" and "BNOT" plots. We found significant positive correlations between microbiological measurements (soil respiration) and enzymatic activities (dehydrogenase and β–glucosidase activities) and organic matter content. Our results also indicate lower C/N values at Yeste, but no significant differences among treatments and we found significant negative correlations between microbiological measurements and enzymatic activities (except urease enzyme) with the C/N ratio. As Merilä et al. (2002) have shown, substrate quality, as determined by C/N, generally influences microbial biomass and respiration, so the main substrate of the litterfall were pine needles, which have high content of lignin. Berg (1986) stated that higher C/N ratios may be an indicator of the more recalcitrant nature of the soil organic matter. In Yeste and Calasparra the Aleppo pine was the dominant species, so the main explanation of this different behaviour in each site may be the contrasting climatic conditions, which let the litterfall degrade faster. On the other hand, the experimental treatments showed the same climatic conditions and the same dominant tree species at each site, which can explain the absence of differences on C/N values depending on the post-fire treatment . Moreover, lower C/N rates have been associated with higher respiration rates and microbiological properties (Schmitz et al. 1998).

Regarding pH, some authors have denoted its influence on soil microbial biomass properties (Bååth and Anderson, 2003). According to Sinsabaugh (2008), soil pH has direct biochemical effects on the activity of the extracellular enzymes immobilised in the soil matrix. The same author has also argued that soil pH reflects climatic controls in soil and plant community composition, which may affect the large-scale distribution of extracellular enzymatic activities through changes in nutrient availability, soil organic composition and microbial community composition. Our results agree with this trend and indicate that pH correlates negatively with soil enzymes activities (except urease activity), soil respiration and organic matter.

Finally, the PCA results reveal that the sites were significantly discriminated. The higher soil temperatures and lower soil moisture values recorded at Calasparra provide unfavourable conditions for balanced soil functional diversity, as reflected by poorer enzyme activities, soil respiration and biomass carbon if compared with Yeste. On the contrary, treatments were not significantly discriminated, which reflects that vegetation recovery after a wild-fire event and the time elapsed since the post-silvicultural treatments applied were enough to achieve the initial soil property values found in mature and unaffected plots.

5 Conclusions

Biochemical, microbiological and physicochemical variables are affected by site, but not by post-fire silvicultural treatment, under dry and semiarid conditions. Seventeen years after the wildfire event and the post-fire silvicultural treatment, microbiological soil properties may recover the initial status and values shown for mature and undisturbed Aleppo pine forest stands. The micro-climatic conditions, higher soil temperature and lower soil moisture values obtained at Calasparra indicate unfavourable conditions for microbiological properties and enzyme activities if compared with Yeste. Our results provide data on the long-term recovery pattern of microbiological and enzymatic activities, and clearly distinguish between sites with different microclimatic conditions (temperature and moisture), but not among burnt/unburnt or post-fire thinned/unthinned Aleppo pine forests stands for more than 17 years after the wildfire and silvicultural treatment. Forest management guidelines should consider that forest site plays an important role in forest recovery after wildfire, and therefore in soil quality. Thus, forest management policies should have these aspects into account when designing (and budgeting) restoration plans.

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Table 1. Soil, climatic and stand characteristics of each experimental site*.

Site	**Exp. condition**	**Altitude (m a.s.l.)**	**Vegetation cover***	**Aleppo pine density (trees/ha)**	**Tsoil (ºC)**	**Soil moisture (%)**	**Mean age (years)**	**Shrub and herbaceous vegetation**	**Soil order (suborder) / texture****
Calasparra	MAT	330	90 % Ph 10% Shrub and herbaceous	400	12.0±1.1	5.9±2.0	70-80	*Macrochloa tenacissima (L.) Kunth; Rosmarinus officinalis*	Aridisol (Orthid) Loam
	BNOT	430	80 % Ph 20% Shrub and herbaceous	45000	9.2±1.8	7.5±1.1	17	*Macrochloa tenacissima (L.) Kunth; Rosmarinus. officinalis,; Brachypodium. retusum; Thymus vulgaris L.*	Aridisol (Orthid) Loam
	BT	330	70 % Ph 30% Shrub and herbaceous	1600	9.5±1.4	5.2±0.9	17	*Macrochloa tenacissima (L.) Kunth; Rosmarinus officinalis Brachypodium. retusum; Thymus vulgaris L.*	Aridisol (Orthid) Loam
Yeste	MAT	1010	90 % Ph 10% Shrub and herbaceous	500	8,0±1.2	10.6±1.8	70-80	*Rosmarinus officinalis L., Brachypodium retusum*	Inceptisol (Ochrept) Loam
	BNOT	860	80 % Ph 20% Shrub and herbaceous	7000	7.1±0.9	14.6±3.1	17	*Rosmarinus officinalis L., Brachypodium retusum*	Inceptisol (Ochrept) Loam
	BT	1010	70 % Ph 30% Shrub and herbaceous	1600	7.5±1.3	12.4±2.6	17	*Rosmarinus officinalis L., Brachypodium retusum.*	Inceptisol (Ochrept) Clay loam

*Ph: Aleppo pine; T: soil temperature (mean ± standard error) during the sampling period; H: soil moisture (mean ± standard error) during the season of sampling.

**Soil Taxonomy (USDA)

Table 2. Soil physicochemical parameters for each site and experimental condition.

Site	Exp. condition	pH	Electrical conductivity ($\mu S\ cm^{-1}$)	Organic matter (%)	Total carbonates (%)	P ($mg\ kg^{-1}$)	Total N (%)	C/N
Calasparra	**BT**	8.66 (0.07) aA	21.15 (0.78) aA	6.73 (0.66) aB	2.72 (0.07) aB	11.32 (1.35) aB	0.18 (0.00) aB	53.5 (4.26) bA
	BNOT	8.75 (0.06) aA	20.28 (1.03) aA	5.87 (0.38) bB	2.13 (0.02) bB	12.74 (3.84) aB	0.11 (0.00) bB	83 (5.95) aA
	MAT	8.39 (0.02) bA	23.48 (2.69) aA	5.35 (0.68) bB	1.92 (0.20) bB	16.95 (1.12) aB	0.20 (0.03) aB	44 (3.73) bA
Yeste	**BT**	8.30 (0.17) aB	20.85 (0.02) aA	8.24 (0.60) aA	2.94 (0.01) aA	27.99 (0.57) aA	0.98 (0.22) aA	16.5 (4.22) aB
	BNOT	7.83 (0.17) aB	21.15 (0.73) aA	9.17 (0.19) aA	2.94 (0.01) aA	14.24 (2.38) cA	1.09 (0.26) aA	15 (3.71) aB
	MAT	8.37 (0.22) aB	21.89 (2.28) aA	6.42 (0.22) bA	2.88 (0.02) bA	20.63 (2.67) bA	0.76 (0.27) aA	33 (12.07) aB

For each parameter values represent mean (standard error). Data followed by the same small letter are not significantly different according to the LSD test ($P<0.05$) for each experimental condition. For each experimental site, data followed by the same capital letter are not significantly different according to the LSD test ($P<0.05$).

Table 3. Result of the two-factor ANOVA (site and experimental condition) for the microbiological properties and enzymatic activities analysis.

	Dehydrogenase activity		Urease activity		Phosphatase activity		β-Glucosidase activity		Basal respiration		Microbial biomass carbon	
	F-ratio	*p-value*	F-ratio	*p-value*	F-ratio	*p-value*	F-ratio	*p-value*	F-ratio	*p-value*	F-ratio	*p-value*
Factors												
S	170.21	0.0001	45.15	0.0001	0.37	0.5486	65.14	0.0001	14.88	0.0006	4.61	0.0399
T	0.34	0.7137	0.01	0.9932	0.29	0.754	1.70	0.1993	1.35	0.2741	0.20	0.8202
S x T	2.16	0.1334	0.02	0.9819	0.05	0.9519	0.72	0.4948	0.01	0.9885	0.07	0.9363

S: Site; T: Experimental treatment; S x T: interaction between S and T.

Tabla 4. Correlation matrix between the different variables determined.

	UA	PA	DHA	BA	BC	RESP	H	OM	P	pH	EC	Total N	CO_3^{2-}
PA	**0.38***												
DHA	**-0.66*****	**-0.14ns**											
BA	**-0.58*****	**-0.06ns**	**0.67*****										
CB	**-0.26ns**	**-0.11ns**	**0.36***	**0.28ns**									
RESP	**-0.12ns**	**0.18ns**	**0.61*****	**0.42***	**0.10ns**								
H	**-0.62*****	**0.01ns**	**0.77*****	**0.76*****	**0.28ns**	**0.41***							
OM	**-0.38***	**0.18ns**	**0.50****	**0.56*****	**0.02ns**	**0.54*****	**0.47****						
P	**-0.41***	**-0.13ns**	**0.26ns**	**0.52****	**0.19ns**	**0.10ns**	**0.43****	**0.11ns**					
pH	**0.39***	**-0.15ns**	**-0.39***	**-0.57*****	**-0.19ns**	**-0.40***	**-0.50***	**-0.63*****	**-0.11ns**				
EC	**0.18ns**	**0.10ns**	**0.10ns**	**-0.13ns**	**-0.03ns**	**0.43****	**-0.09ns**	**0.07ns**	**-0.16ns**	**0.16ns**			
Total N	**-0.51****	**-0.07ns**	**0.73*****	**0.54*****	**0.35***	**0.64***	**0.62*****	**0.27ns**	**0.06ns**	**-0.21ns**	**0.33***		
CO_3^{2-}	**-0.58*****	**-0.06ns**	**0.61*****	**0.58*****	**0.27ns**	**0.45****	**0.46****	**0.38***	**0.30ns**	**-0.63*****	**-0.26ns**	**0.20ns**	
C/N	**0.50****	**0.12ns**	**-0.67*****	**-0.55*****	**-0.27ns**	**-0.41***	**-0.57*****	**-0.33***	**-0.27ns**	**0.25ns**	**-0.40***	**-0.82*****	**-0.26ns**

UA: Urease activity, **PA**: Phosphatase activity, **DHA**: Dehydrogenase activity, **BA**: β-Glucosidase activity, **CB**: Biomass carbon, **RESP**: Soil respiration, **H**: Soil moisture, **OM**: Organic matter, **P**: Phosphorus, **pH**, **CE**: Electrical conductivity, **Total N**: Total nitrogen, **CO_3^{2-}:** Total carbonates, **C/N**: Carbon nitrogen ratio. Significant correlations; ns: non-significant; *P≤0.05; **P≤0.01; ***P≤0.001

Tabla 5. Weights of principal components analysis

	PC 1	PC 2
Dehydrogenase	-0.351	-0.072
β-glucosidase	-0.341	-0.092
Moisture	-0.334	-0.103
Organic matter	-0.283	0.359
Total nitrogen	-0.273	-0.079
Soil respiration	-0.247	0.338
Phosphorus	-0.161	-0.298
Carbon biomass	-0.143	-0.271
Electrical conductivity	-0.021	0.317
Phosphatase	0.017	0.444
pH	0.264	-0.168
Total carbonates	-0.271	-0.093
C/N	0.284	0.058
Urease	0.288	0.312

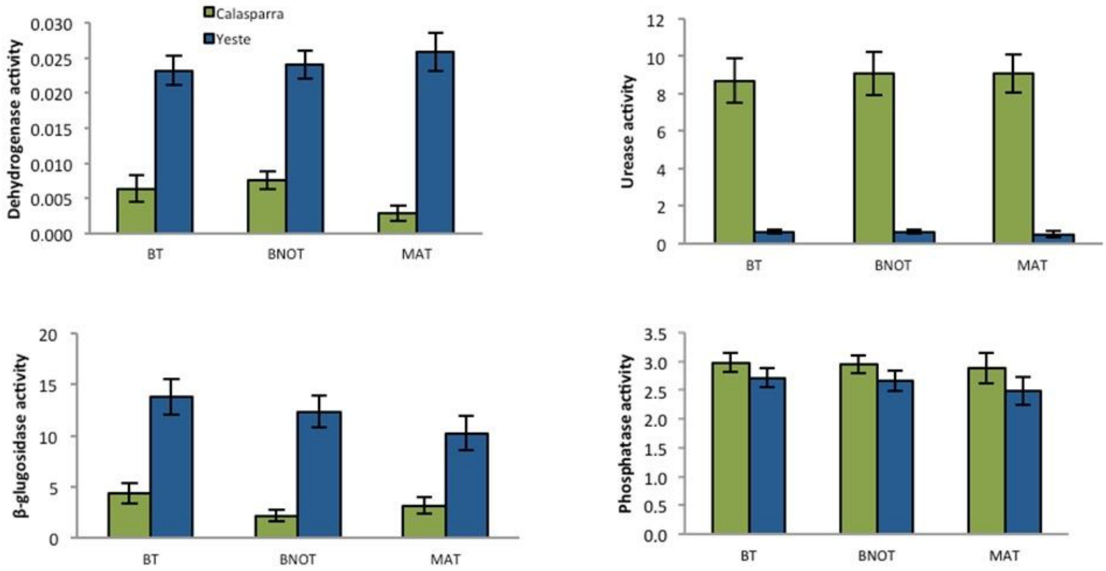

Figure 1. Dehydrogenase activity (μg (INTF) g^{-1} soil $hour^{-1}$), β-glucosidase activity (μmol PNP g^{-1} dry soil $hour^{-1}$), phosphatase activity (μmol PNP g^{-1} dry soil $hour^{-1}$) and urease activity (μmol $N\text{-}NH_4^+$ g^{-1} dry soil $hour^{-1}$) in relation to the experimental site. Error bars are the LSD intervals at *P <0.05*.

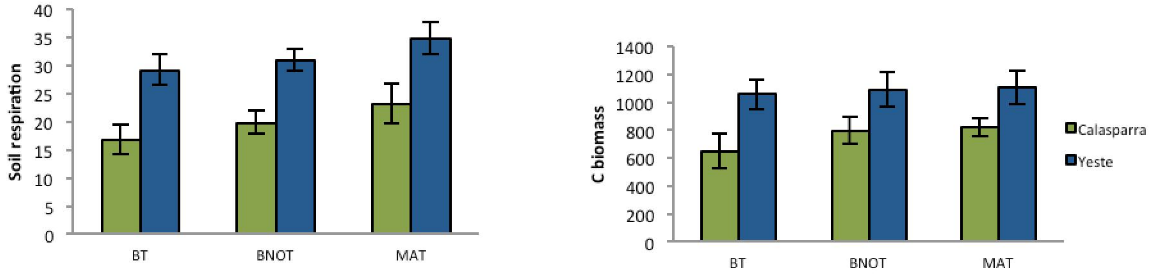

Figure 2. Basal soil respiration (mg CO_2 kg^{-1} soil) and microbial biomass carbon (mg kg^{-1}) in relation to the experimental site. Error bars are the LSD intervals at $P < 0.05$.

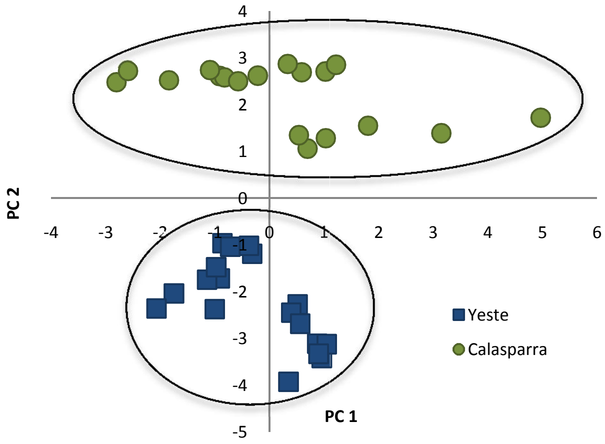

Figure 3. Principal components analysis of the experimental sites Yeste and Calasparra.