Javier Hedo de Santiago
Department of Plant Production and Agricultural Technology
School of Advanced Agricultural Engineering
Castilla La Mancha University, 02071. Albacete, Spain.

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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,

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.

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

Best regards,

Javier Hedo de Santiago

1. GENERAL COMMENTS

The aims of this research were to investigate microbiological and enzymatic activities in two Aleppo pine forest ecosystems (Pinus halepensis Mill.) affected by a wildfire event 17 years earlier. Pinus forests growing under two contrasting climates (dry and semi-arid ombroclimates). The authors intended to study the effect of climate on soil activity, and they also studied the effect of post-fire forest treatments (thinning) on soil. The woodlands studied at each site are the following: a 17-year-old forest stand treated 5 years after the fire (thinning), and a forest stand of the same age without thinning treatment. The authors hypothesized that i) microbiological properties and enzymatic activities are influenced by the climatic conditions recorded at each site; ii) there were no significant differences between burned and thinned plots, control (burned and no thinned plots) and mature (unburned and unthinned plots) because of the soil and vegetation recovery capacity.

Despite its ecological importance, information on soil functioning in semi-arid Mediterranean ecosystems is still very scarce. Studies that focus on post-fire silvicultural treatments, and which also attempt to improve the forest and soil structure, are very important. The availability of data of the fires that occurred almost 20 years ago can allow us to analyze the differences in soil functioning between mature and post-fire young regenerated stands in two contrasting climates. Thus, the aims of this study are very interesting. Nevertheless there are some questions I would like to discuss with the authors.

As to the climate effects on soil activity, previous studies have demonstrated the positive effects of rainfall on soil activity if soil temperature is adequate. The study of Criquet et al. (2004) indicated that rainfall is the most important factor to regulate production and the activity of numerous enzymes in sclerophyllous forest litter. Hence with the heaviest rainfall, increased enzymatic activity at the site was expected. Thus I generally agree with the results obtained in this research and with the conclusions drawn after analyzing this hypothesis.

(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.

However, the results of the soil parameters used within each site based on woodland maturity and silvicultural treatment should be better discussed by the authors. As the authors have well mentioned in the Introduction section, silvicultural treatments (thinning) modify microclimatic conditions at the ground level, as well as the amount and quality of litterfall andorganic matter. However, the results of this research appear to reflect the opposite since no difference in organic matter and soil functioning were found between treatments at each site. This conclusion should be discussed in more depth.

(Authors) Thank you very much for all your comments. We think that our results did not reflect the avobe mentioned trend because of the time period since the thinning was applied. As Wic-Baena et al. (2013) stated, the time period since the silvicultural treatment was applied seemed to significantly affect soil properties. Our soil samples were collected more than 10 years since the thinning was applied.

In this sense, perhaps the experimental design was not designed sufficiently to test the second hypothesis. The seasonality of both vegetation and soil functioning in Mediterranean ecosystems require study soil activity during different seasons before relevant conclusions can be drawn. So the method that the authors used to test this hypothesis raises doubts, especially when choosing winter as the sampling season, and also due to lack of clarity when identifying the sampling point.

Referring to the first question, testing significant differences in soil, it would have been better to have taken soil samples during seasons other than winter. Soil samples should be taken at least during the most important periods that influence growth, for example, in spring when vegetation is stressed by lack of water (summer), or in autumn. Furthermore in these seasons, litter fall occurs, which accelerates soil activity. Previous studies have demonstrated major seasonality in soil functioning under Mediterranean species (for example, see Sardans et al., 2005, Bastida et al., 2008, Aponte et al., 2010). The sampling done may have been insufficient to study the woodland maturity effect on soil functioning, and the results in winter could be biased.

Furthermore, both soil temperature and soil moisture are decisive factors for controlling soil activity, and high temperatures accelerate the decomposition and soil respiration processed (Waring and Running, 1998). Consequently, the authors should have justified the selection of winter as the sampling season. Due to this seasonality, a soil activity measure in winter may not be representative due to low temperatures. Soil temperature must also be included in the data analysis.

Regarding the second question, the authors could have ignored the effects of plant cover (arboreal or herbaceous cover) on soil functioning. It is well recognized that woodland maturity can significantly affect soil activity, and previous studies have shown that herbaceous vegetation can have a different influence on soil activity than trees (Lucas-Borja et al., 2010).

(Authors) We thank you for these comments. Seasonality is an important factor that generally affects soil properties as enzymatic activities or microbial biomass, as many studies have showed. However, 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 make 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.

Related to the sampling point, we randomly established the plots in the study area, because we wanted to study the effect of the variables in the whole ecosystem. The main objective of the study was not to compare points depending on their cover.

Other doubts arise with the findings on early recovery of soil functioning in post-fire regenerated forest. I agree with authors that long-term studies are necessary to reach reasonable conclusions on the impacts that fire events or post-fire silvicultural treatments have on soil properties in Mediterranean ecosystems. In this sense, a chronosequence of forests of distincts age might be more adequate. The time in which soil recovery is completed can really only be known when this study is performed.

(Authors) We agree with this comment. It is true that a chronosequence study would be adequate to achieve a more complete study about when is the exact moment where the soil can be considered recovered. We agree that this kind of works are necessary. We are trying to enlarge the knowledge about the mid- and long-term studies of burned soil properties with this study.

The study also contains soil parameter values that must be reviewed (and probably modified) since outliers or erroneous data may be present (carbonates, P, N, organic matter, C:N ratio). This could bias the conclusions drawn from the data analysis. The authors have not discussed the magnitude of the calculated enzymatic activity, and the magnitudes of the principal enzymes have not been compared with similar studies. I believe that this is very important to validate data measured in winter. Some conclusions have also been drawn that are not supported by the results in the Discussion section (see specific comments in this revision).

(Authors) We thank you for these comments. All the values presented in this study have been newly studied and we can say that there are not outliers or erroneous data. We rely on international research articles published in different journals. For example, respect to the **total carbonates**, studies of Lucas-Borja et al., (2010, 2011) related to the genus *Pinus* showed total carbonates values ranging between 2.80 to 5 g 100 g^{-1}. With regard to **phosphorus**, Bastida et al., (2008) and Lucas-Borja et al., (2011a, 2011b, 2012) showed in their studies P values ranging around 2 to 45 mg kg^{-1}. **Total nitrogen** values were between 1.93 and 5.70 % in the Lucas-Borja et al., (2012) study, and between 0.2 and 0.3 in another study of the same authors (Lucas-Borja et al., 2011a). As regards the **organic matter**, Bastida et al., (2006) and Lucas-Borja et al., (2012) found organic matter values between 1.3 and 4.13 g 100 g-1. Finally, and according to the comment about the **C/N ratio** values, Lucas-Borja et al., (2012) found values ranging between 16 and 55 in pine and mediterranean forest ecosystems.

Finally, I consider this is an interesting study, but it should be amply reviewed and the experimental design needs to be better justified. The Discussion should also be improved by focusing mainly on the principal results obtained, and by also considering the scarcity of the data analyzed and the sampling season. Only in this way can the authors draw objective conclusions from the available data. The literature review should be increased by focusing on the species or the genus studied (Pinus).

So, I consider this paper need a major revision. I also make specific comments, which are reflected below.

I hope that the suggestions I have indicated in this review allow the authors to write an excellent paper.

(Authors) Thank you very much for all your work and suggestions.

2. SPECIFIC COMMENTS

(Authors) We thank you for all the specific comments.

2.1 INTRODUCTION SECTION

More detailed previous information on soil functioning related to conifer forest in Mediterranean climate is required.

(Authors) We added some sentences related to this comment to the manuscript. Please see introduction section.

2.2 STUDY AREA

Pag 3029, Line 19: "According to the Spanish Soil Map, Yeste and Calasparra soils are classified as Inceptisols and Aridisols, respectively".

- Reference of the "Spanish Soil Map" and the scale of this map are necessary.

(Authors) The reference has been added to the References section:
Guerra Delgado, A. C.S.I.C. (1968). Leyenda del Mapa de Suelos de España (1:1.000.000). Inst. Nac. Edafol. CSIC, Madrid

- Reference of the Soil Classification System used is required.

(Authors) The reference is Soil Taxonomy (USDA).

- Inceptisols and Aridisols are soil orders; soil order not well describes the soil type. A more detailed classification level is required (Suborder level).

(Authors) Yeste soils are Inceptisol order and Ochrept suborder. Calasparra soils are Aridisol order and Orthid suborder. It has been added to the Table 1.

2.3 EXPERIMENTAL DESIGN

- Replication is a fundamental aspect in the experimental design. One hectare can be a small area to analyze the forest soil and the lack of significant differences may be partly due to this.

(Authors) We thank for the comment. These areas were selected due to their homogeneity and representativity. We thought that the area size of 1 ha was enough to achieve the main objectives of this work. Moreover, there are more studies with the same size plot, for example Wu et al., (2013).

- The method used testing the two hypotheses provides questions (or doubts) especially when choosing winter as the sampling season, and the lack of clarity identifying the sampling point. Authors should clarify whether soil sample under arboreal vegetation or under shrubs as conducted. Sampling without controlling the plant cover could generate a confounded effect and no significant conclusions can be drawn.

(Authors) We thank you for these comments. Seasonality is an important factor that generally affects soil properties as enzymatic activities or microbial biomass, as many studies have showed. However, 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 make 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.

Related to the sampling point, we randomly established the plots in the study area, because we wanted to study the effect of the variables in the whole ecosystem. According to Hedo et al., (2014). The main objective of the study was not to compare points depending on their cover. Nevertheless, the effect of the cover should be further studied in the future. In fact, we are now involved in a new manuscript redaction related to tree cover effects.

- As a recommendation, to determine the effects of woodland maturity on both enzymatic and microbiological activities metabolic and microbial quotients should be recommendable. Quotients derived from measurements of basal respiration, microbial biomass C have also been employed as indicators of microbial C use and soil activity

(Authors) We thank for the comment. We have been working in this quotients and we have recently published an article related to that (Hedo et al., 2014).

- It is necessary to indicate the repetitions for temperature and soil moisture sampling. Does soil temperature was measured only during few hours in one day? These measurements may not be representatives.

(Authors) We thank for the comment. We recorded soil moisture and soil temperature with data-loggers during the sampling season, and we calculated measures using all the records. The data-logger stored data every hour.

- The data collection was carried out in 2011 (1994 + 17 = 2011)

(Authors) Yes, the data collection (soil sampling) was carried out in 2011 (17 years after the fire event, and 12 years after thinning treatment).

2.4 STATISTICAL ANALYSIS

- Why the authors have not considered random effects?

(Authors) We thank for the comment. The considered factors were fix factors. Factors such as season or sampling point were not used so, there is no way to use ramdom factors.

- Interactions of factors that have been considered should be indicated.

(Authors) We carried out a two-way ANOVA, which included the interactions between both variables site and thinning.

- The study of correlation between variables should be included in this section.

(Authors) Thank for the comment. It has been revised.

2.5 RESULTS

2.5.1 Physical and chemical variables

- The paragraph "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")" is not well reflected in Table 1.

(Authors) Thank for the comment. We had added to the Table 1 means and standard errors.

- Study interactions for all the analyzed variables.

(Authors) Thank for the comment. It has been revised.

- Soil texture was not similar in Yeste for the Bt treatment (clay loam). Clay content is an important parameter which enhances soil microbial activity.

(Authors) Thank for the comment. This question has been addresed in the text.

- The sentence "The percentage of carbonates, organic matter, phosphorus and total nitrogen differed between sites, with higher values recorded for Yeste" is not well supported by Table 2. For the site factor, means and standard errors should have been indicated.

(Authors) Thank for the comment. It has been revised. We added to the graph "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*)".

- Authors should clarify the last sentence ("Under the experimental conditions, the physical and chemical variables showed a different behavior depending on the site (Yeste and Calasparra; Table 2)" highlighting the most important guidelines.

(Authors) Thank for the comment. We have added in brackets the three experimental conditions ("BT", "MAT" and "BNOT").

2.5.2 Biochemical and microbiological variables

- It could be problems with interpretation of results in Figure 1. The performed ANOVA resulted in a significant effect of site on biochemical and microbiological variables (except for Phosphatase activity), and the interaction of the two main factors (site x treatment) was not significant. However, in Figure 1 authors represented the site x treatment interaction, when the post-hoc analysis has not sense if the interaction was not significant in the ANOVA analysis. In consequence, Figure 1 appears to be testing mean separations for the interaction between site and treatment, which the ANOVA showed as being non-significant. Thus, I recommend authors modify the figure 1 according to the most important results of the ANOVA. In this sense, the results of the ANOVA will lead to a figure in which the mean values of the two sites must be represented (independently of treatment), and authors could eliminate the representation of the post-hoc test. To provide information about the measured variables a table of means and standard errors would be adequate.

- Figure 2 do not represents the site effects as the authors indicate. Site effects should be represented by mean values.

(Authors) Thank for the comments. We showed these graphs because we thought it was more clarifier. So, we can see trends in the behaviour of the properties, although they were not significantly different.

- It is hoped that soil activity slows down in the winter. Thus, lack of significant differences attributed to treatments might be due to low soil activity in winter. Discuss about this.

(Authors) Thank for the comment. We explained above the effects of seasonality.

2.5.3 Correlation analysis

- This section should be improved. The authors simply review the correlations without offer an interpretation (or explanation) of main results.
- In addition, the most important results should be highlighted, and in the discussion section an explicative paragraph about this would be welcome.

(Authors) Thank for the comments. We have considered your comment, and in discussion section have been better discussed.

2.5.4 PCA analysis

- The authors indicated that "The PCA analysis clustered the plots located in Yeste on the negative axis of PC 2 (Fig. 3)". This would be a mistake because all the plots of Yeste appear to be in the negative axe of PC1. Please, revise this sentence.

(Authors) Thank for the comments. We displayed in the x axis the PC1 and in the y axis the PC2.

- Specify whether the standardization of data has been performed (it is recommendable).
- PC1 and PC2 (especially PC1) explained a low percentage of variability. This would limit data interpretation. The authors should also indicate whether the axes have been rotated to improve observation of results.

(Authors) Thank for the comments. The data have not been standarized. The axes have not been rotated.

- As discussed for the correlation matrix, this analysis should be performed with the security that all variables are well calculated.

(Authors) Thank for the comment. All the calculated variables have been analysed again and we are sure that all the parameters were well calculated.

2.5.5 Specific comments concerning figures and tables

Table 1

- For consistence, change “plot” by “experimental condition” or “treatment”.
- Altitude is “m.a.s.l.”
- Change “Age tree” by “Mean age (years)”.
- Change “herbal vegetation” by “herbaceous vegetation”.
- Change “Macrochloa tenaccisima” by “Macrochloa tenacissima”.
- Soil type should be referenced (Taxonomic system and suborder level).
- Acronyms should be indicated.
- Change “Forest site” by “Site”, for consistency.
- Indicate n (sample data) for both soil temperature and soil moisture. The authors should indicate the sampling data in methodology section.

(Authors) Thank for the comment. Soil temperature and moisture were obtained only to characterize the study area, and we did not use these records for any other statistical analysis. Moreover, soil samples were analysed in laboratory under standard temperatures conditions (Andrés Abellán et al,. 2011)

- Delete (Winter, 2011).
- T: change by Tsoil.
- H: change for Hsoil or Soil moisture.

(Authors) Thank for the comments. These changes have been modified in the text.

Table 2

- Values of C/N ratio:
    - In general, C/N ratios calculated by authors are very high and seem be outliers). I think these values are probably wrong. In addition, C/N ratio should not be calculated in %. How authors have calculated the C/N ratio?

  (Authors) Thank you for the comment. After analyzing the dataset and values, we saw that there was an error during the conversion from the Word file to the pdf file. Colums and files were not placed in the correct position. Now, this information has been corrected and variables are in the correct units.

    - The authors should be aware that there is a correlation between an increase in the ratio C:N and less organic matter mineralization. For example, according to Duchaufour (1984), in various types of forest soils studied, when the C:N ratio is greater than 25 mineralization is negligible. This does not occur in the forests studied.

(Authors) Thank for the comment. The values have been contrasted with other studies. Please see the previous comments related to the C/N ratio.

- Values of Carbonates (%)
    - Values are very low. The values do not correspond to an alkaline soil (pH>8).
- Available phosphorous:
    - Please, specify the units of P (available phosphorus). The units may be mg kg-1. This soil parameter must be reviewed in order to ensure accuracy.

(Authors) Thank for the comment. The values have been contrasted with other studies. Please see the previous comments related to total carbonates and available phosphorus.

- Organic matter:
    - It should be highlighted that mature forests (and especially in Yeste) have less soil organic matter than the young woodlands (thinned and unthinned forests).

(Authors) Thank you for the comment. Mature forests have less soil organic matter than the young woodlands although differences were not statistically significant.

-
    - In addition, no correlation between organic matter and plant densities (40.000 and 7.000 trees/ha) (in Calasparra and Yeste respectively).

(Authors) Thank for the comment. The plant densities described were reached inmediately after the fire event, due to the natural recruitment of the Aleppo pine. Five years later, the silvicultural treatments (thinning) were applied, reducing the density to 1600 trees/ha.

-
    - These are important results that must be discussed in more depth.

(Authors) Thank for all the comments. The discussion has been modified.

- Total N:
    - This soil parameter need be revised. Both units and values should be wrong. The values of N might be atypical for this type of forest. An explanation of these outlier values is necessary.

(Authors) Thank for the comment. The values have been contrasted with other studies. Please see the previous comment related to total carbonates and available phosphorus.

- Additional information that would be necessary:
    - n (sample data)
    - Means values for study site

(Authors) Thank for all the comments. They have been revised and modified.

Table 3

- Results

- Change “Soil respiration” by “Basal respiration (Br)”
- Change “C-Biomass” by “Microbial biomass C” (MBC)

(Authors) Thank for all the comments. They have been revised and modified.

Table 4

- Significant correlations are missing in the correlation matrix. For example, soil temperature is a relevant parameter. Why soil temperature was not included in the correlation matrix?
- This matrix should be done again including all the variables well calculated

(Authors) Thank for all the comments. We added soil temperature as a part of a characterization of the study site.

Figure 1

- Represent the units on the axe
- Change NH4+, by NH^{4+}
- Change “moles” by “mol”
- For this figure, authors can follow the guidelines indicated in the 2.5.2 section.

(Authors) Thank for all the comments. They have been revised and modified.

Figure 2

- Represent the units on the axes
- Change “Soil respiration” by “Basal respiration (Br)”

(Authors) Thank for all the comments. They have been revised and modified.

2.6 DISCUSSION

- It is necessary discuss whether values of soil activity are similar than others obtained inprevious similar research. As mentioned above, some parameters calculated should bewrong. Thus, discussion concerning relationships between N, P, C/N and soil activity, are not consistent (these parameters must be verified). To justify in part the sampling in winter, I suggest to make an review of previous research to check whether the values of enzymatic activity obtained in your experiment are representatives.

(Authors) Thank for all the comments. All the parameters have been verified.

- I don't completely agree with the following sentences:
    - Pag. 3024 (lines 2-10): “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)”.

        - The authors could recognize (for example) that for the same species, forest productivity and woodland maturity are key factors controlling soil activity.

(Authors) Thank for all the comments. In our case, we did not find statisticaly significant differences between mature and natural regenerated stands.

- Pag. 3034 (lines 12-19)"Different authors have demonstrated that higher soil temperatures and scarce soil moisture generate lower soil respiration rates, microbial biomass carbon values and dehydrogenase, phosphatase and 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, glucosidase and dehydrogenase activities"
    - The interpretation of these references is not entirely correct. Sardans et al. (2005) showed in an oak forest (Quercus ilex L.) that drought decreases soil enzyme activity in this Mediterranean woodland (the reduction of 21% of soil moisture produced by runoff and rainfall exclusion together reduced urease activity by 42–60%, protease activity by 35–45%, ⍰-glucosidase activity by 35–83% and acid phosphatase activity by 31–40%), and no significant effects were observed on alkaline phosphatase activity. In all cases, the activities of these enzymes were greater in spring than in autumn. Thus, Sardans et al. (2005) did not include the soil temperature in the trial, and theseauthors demonstrated an important seasonality in soil activity that this study has not taken into account.
    - Baldrian et al. (2010) demonstrated in a Quercus petraea forest soil that the soil moisture content positively and significantly correlated with both microbial biomass and the activity of extracellular enzymes involved in decomposition (β-glucosidase, and acid phosphatase). Thus, these authors did not study the soil temperature as factor to explain soil activity.
    - In consequence, the referenced authors have not showed that higher soil temperatures generate lower soil activity
    - On the other hand, Criquet et al. (2004) showed that acid phosphatases were negatively correlated with the temperature, but alkaline phosphatases (measured in this study) were positively correlated with soil temperature, as expected.
    - The authors emphasize that higher temperature in site 2 (Calasparra) negatively affect soil activity. However, this effect is confounded in this study, and perhaps, it is more recommendable to correlate the minor soil activity with low rainfall. In this way, authors could validate one of the formulated hypotheses. Really, soil temperature was not analyzed in the statistical analysis performed.

(Authors) Thank you very much for all the comments. We agree with your dissertation. This part of the manuscript has been revised.

- Pag. 3035 (lines 7-14): "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 15 years after the wildfire event and the post-fire silvicultural treatment. This long-term study demonstrated that soil parameters might recover to the pre-fire levels 15 years after the fire event and thinning operations".
    - I do not understand why the authors establish a recovery period of 15 years. To make this assertion, a chronosequence should have been conducted. This sentence should be removed from the paper. I think the results not support this affirmation.

(Authors) Thank you very much for all the comments. It is true that a chronosequence study would be adequate to achieve a more complete study about when is exactly the moment where the soil can be considered recovered. We are agree that this kind of works are necessary. We are trying to enlarge the knowledge about the mid- and long-term studies of burned soil properties with this study.

Mature forests have lower values of organic matter in the two sites. Especially surprising is the result of organic matter amounts in Yeste. Although litter production should be important in the recruitment period (regeneration post-fire), the mature forest has accumulated organic matter over time. Thus, in the old woodland, the deposition of organic matter influences the soil for a longer time, and organic C pools become more important. A fraction of annual C input is always stabilized in soils. These guidelines should be discussed more widely and referencing previous studies.

(Authors) Thank you for the comment. In our case, we did not find statisticaly significant differences between mature and natural post-fire regenerated stands.

- Pag. 3035 (lines 27-29), and Pag. 3026 (lines 1-9). Discussion about the influencing effects of C:N ratio on microbiological measurements and enzymatic activities are inconsistent due to the C:N ratio calculated.

(Authors) Thank you for the comment. The C:N ratio has recalculated and the new units have been indicated in table 2. Please see previous comments related to the C:N ratio.

- It is necessary better discuss why in the site with less rainfall the urease activity is higher, but it not occur the same for enzymes related to C and phosphorus cycles.

(Authors) Thank you for the comment. Acording to Gutknecht et al. (2010), decreased N and P values are related to greater urease and phosphatase activity and higher enzyme production through soil microorganisms.

- The last paragraph is not a discussion of results (it seems an interpretation). This affirmation is not based on the main results of the experimental design. Finally, I suggest the inclusion of trials related to conifer forest soils (not only studies focusing on Quercus woodlands due to a different functioning).

(Authors) Thank you for the comment. It has been adreesed in the text.

2.7 ABSTRACT AND CONCLUSIONS

✓ Rewrite according to the revision

(Authors) Thank you for all the comments. . It has been adreesed in the text.

LITTERATURE CITTED

Aponte, C., Marañón, T. & García, L. 2010. Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry, 101, 77–92.

Bastida, F., Barberá, G. G., García, C., and Hernández, T., 2008. Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions, Appl. Soil Ecol., 38, 62–70.

Criquet, S., Ferre, E., Farnet, M., Le petit, J. 2004. Annual dynamics of phosphatase activities in an evergreen oak litter: influence of biotic and abiotic factors. Soil Biology & Biochemistry 36, 1111–1118.

Duchaufour Ph., 1984. Edafogénesis y Clasificación. Ed. Masson, S. A., Barcelona, 493 pp.Lucas-Borja, M.E., Bastida, F., Moreno, J.L., Nicolas, C., Andrés, M., López, F.R. et al. 2010.

Influence of forest cover and herbaceous vegetation on the microbiological and biochemical properties of soil under Mediterranean humid climate. European Journal of Soil Biology, 46, 273–279.

Sardans, J. and Peñuelas, J.: Drought decreases soil enzyme activity in a Mediterranean holmoak forest. Soil Biology & Biochemistry, 37, 455–461, 2005.Waring, R. & Running, S. 1998. Forest Ecosystems. Analysis at Multiples Scales. Academic Press, San Diego, CA

LITTERATURE CITTED IN THE REVISION

Andrés Abellan, C. Wic Baena, F.A. García Morote, M.I. Picazo Cordoba, D. Candel Pérez, M.E. Lucas-Borja 2011. Influence of the soil storage method on soil enzymatic activities. Forest Systems 20(3), 379-388

Bastida F, Barberá GG, García C, Hernández T. 2008. Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Applied Soil Ecology 38: 62-70

Bastida F, Moreno JL, Hernández T, García C. 2006. Microbiological degradation soil index in a semiarid climate. Soil Biology & Biochemistry 38: 3463-3473

Guerra Delgado, A. C.S.I.C. 1968. Leyenda del Mapa de Suelos de España (1:1.000.000). Inst. Nac. Edafol. CSIC, Madrid

Gutknecht JLM, Henry HAL, Balser TC. 2010. Inter-annual variation in soil extra-cellular enzyme activity in response to simulated global change and filn disturbance. Pedobiologia 53: 283–293

Hedo J, Lucas-Borja ME, Wic C, Andrés Abellán M, de las Heras J. 2014. Soil respiration, microbial biomass and ratios (metabolic quotient and MBC/TOC) as quality soil indicators in burnt and unburnt Aleppo pine forest soils. Journal of Forests 1(2): 20-28

Lucas-Borja ME, Bastida F, Moreno JL, Nicolás C, Andrés M, López FR. 2010. The effects of human trampling on the microbiological properties of soil and vegetation in Mediterranean Mountain areas. Land Degradation & Development 22: 383–394

Lucas-Borja ME, Candel D, Jindo K, Moreno JL, Andrés M, Bastida F. 2011. Soil microbial community structure and activity in monospecific and mixed forest stands, under Mediterranean humid conditions. Plant and Soil 354: 359–370

Lucas-Borja, M.E., Candel, D., López-Serrano, F.R., Andrés, M., Bastida, F. 2012. Altitude-related factors but not Pinus community exert a dominant role over chemical and microbiological properties of a Mediterranean humid soil. European Journal of Soil Science 63: 641-549.

Lucas-Borja, M.E., Wic-Baena, C., Moreno, J.L., Dadi, T., García, C., Andrés-Abellán, M. 2011. Microbial activity in soils under fast-growing Paulownia (Paulownia elongata x fortunei) plantations in Mediterranean areas. Applied Soil Ecology 51: 42-51

Wic-Baena C, Andrés-Abellán M, Lucas-Borja ME, Martínez-García E, García-Morote FA, Rubio E, López-Serrano FR. 2013. Thinning and recovery effects on soil properties in two sites of a Mediterranean forest, in Cuenca Mountain (South-eastern of Spain) Forest Ecology and Management 308: 223-230

Wu, J.P., Liu, Z.F., Sun, Y.X., Zhou, L.X., Lin, Y.B., Fu, S.L. 2013. Introduced Eucalyptus urophylla plantations change the composition of the soil microbial community in subtropical China. Land Degradation & Development 24: 400-406

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

Javier Hedo[1], Manuel Esteban Lucas-Borja[2], Consuelo Wic[2], Manuela Andrés Abellán[2] and Jorge de Las Heras[1]

[1] {Department of Plant Production and Agricultural Technology} School of Advanced Agricultural Engineering, Castilla La Mancha University. Campus Universitario s/n, CP 02071, Albacete (Spain). Tel: 0034 967599200; Fax: 0034 967599238.

[2] {Department of Agroforestry Technology and Science and Genetics} School of Advanced Agricultural Engineering, Castilla La Mancha University. Campus Universitario s/n, CP 02071, Albacete (Spain). Tel: 0034 967599200; Fax: 0034 967599238.

Correspondence to: J. (Javier.Hedo@gmail.com)

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. Thus, the long-term consequences and post-fire silvicultural management in the form of thinning have a significant effect on the site recovery after fire. Moreover, significant site variation was generally seen in soil enzyme activities and microbiological parameters. 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.

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). Also, increasing runoff and surface erosion rates have been exposed (Alegre-Prats et al. 2013). 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). 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 (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) there are no significant differences among treatment (burned and thinned plots), control (burned and no thinned plots) and mature (unburned and unthinned plots) because of the soil/vegetation recovery capacity. 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.

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. All the plots were selected in areas with a low slope (< 5%).

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. 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 also 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). 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). In relation to the experimental treatments, enzymatic activities presented similar values in the "BT", "MAT" and "BNOT" plots (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 also among the microbiological variables, such as 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. 2008). 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) recently showed that decreased N and P results in greater urease and phosphatase activity and higher enzyme production through soil microorganisms. 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.

In relation to fire and post-fire silvicultural treatments, soil moisture and temperature showed no significant differences in the “BT”, “MAT” and “BNOT” plots, thus may explain in a large part 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 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 did not diminish 6 years after thinning.

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. (2010, 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. 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. 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 the effect of thinning treatments and forest site in order to preserve soil quality under the adaptative forest management context.

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References

Alegre Prats, S., Cortizo Malvar, M., Simões Vieira, D.C., MacDonald, L., Keizer, J.J.: Effectiveness of hydromulching to reduce runoff and erosion in a recently burnt pine plantation in central Portugal. Land. Degrad. Dev, DOI: 10.1002/ldr.2236. 2013.

Allué, J.L.: Atlas Fitoclimático de España. Taxonomías. Instituto Nacional de Investigaciones Agrarias. Ministerio de Agricultura, Pesca y Alimentación, Madrid. 1990.

Almagro, M., López, J., Querejeta, J.I., Martínez-Mena, M.: Temperature dependence of soil CO2 efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem. Soil. Biol. Biochem, 41, 594-605, 2009.

Andrés Abellán, M., Wic-Baena, C., García Morote, F., Picazo Córdoba, M., Candel Pérez, D., Lucas-Borja, M.: Influence of the soil storage method on soil enzymatic activities. Forest Syst, 20, 379-388, 2011.

Armas, C.M., Santana, B., Mora, J.L., Notario, J.S., Arbelo, C.D., Rodríguez-Rodríguez, A.: A biological quality index for volcanic Andisols and Aridisols (Canary Islands, Spain): variations related to the ecosystem development. Sci. Total Environ, 378, 238–244, 2007.

Aznar, J.M., González-Pérez, J.A., Badía, D., Martí, C. At what depth are the properties of a gypseous forest topsoil affected by burning? Land Degrad. Dev, DOI: 10.1002/ldr.2258. 2013.

Bååth, E., Anderson, T.H.: Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil. Biol. Biochem, 35, 955–963, 2003.

Baldrian, P., Merhautová, V., Petránková, M., Cajthaml, T., Šnajdr, J.: Distribution of microbial biomass and activity of extracellular enzymes in a hardwood forest soil reflect soil moisture content. Appl. Soil. Ecol, 46, 177–182, 2010.

Bastida, F., Barberá, G.G., García, C., Hernández, T.: Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Appl. Soil. Ecol, 38, 62-70. 2008.

Boydak, M., Dirik, H., Çalıkoğlu, M.: Biology and silviculture of Turkish red pine *Pinus brutia* Ten. Laser Ofset Matbaa Tesisleri San. Tic. Ltd. Sti., Ankara. 2006.

Brandt, A.J., de Kroon, H., Reynolds, H.L., Burns, J.H.: Soil heterogeneity generated by plant-soil feedbacks has implications for species recruitment and coexistence. J. Ecol, 101, 277–286, 2013.

Bremner, J.M.: Nitrogen availability indexes. In: Black CA. (ed.) Methods of Soil Analysis. American Society of Agronomy. Part 2. Agronomy 9, pp.1324–1345, 1965.

Câmara Ferreira, A.C., Carvalho Leite, L.F., Ferreira de Araújo, A.S., Eisenhauer, N.: Land-use type effects on soil organic carbon and microbial properties in a semi-arid region of northeast Brazil. Land. Degrad. Dev, DOI: 10.1002/ldr.2282, 2014.

Cerdà, A. & Lasanta, A.: Long-term erosional responses after fire in the Central Spanish Pyrenees: 1. Water and sediment yield. Catena, 60, 59-80, 2005.

Criquet, S., Ferre, E., Farnet, A.M., Le Petit, J.: Annual dynamics of phosphatase activities in an evergreen oak litter: Influence of biotic and abiotic factors. Soil. Biol. Biochem, 36: 1111–1118, 2004.

De las Heras, J., Moya, D., Vega, J.A., Daskalakou, E., Vallejo, R., Grigoriadis, N., Tsitsoni, T., Baeza, J., Valdecantos A, Fernandez C, Espelta J, Fernandes P. 2012. Post-Fire Management of Serotinous Pine Forests. In: Moreira F, Arianotsou M, Corona P, De las Heras J. (eds.) Post-Fire Management and Restoration of Southern European Forests. Managing Forest Ecosystems 24, Springer pp. 151-170.

Dick, R.P., Breakwell, D.P., Turco, R.F.: Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran JW, Jones AJ (eds.) Methods for assessing soil quality pp. 247-271, 1996.

Espelta, J.M., Verkaik, I., Eugenio, M., Lloret, F.: Recurrent wildfires constrain long-term reproduction ability in *Pinus halepensis* Mill. Int. J. Wildland. Fire, 17, 579–585, 2008.

Fernández, C., Vega, J. A., Jiménez, E., Vieira, D. C. S., Merino, A., Ferreiro, A., Fonturbel, T.: Seeding and mulching + seeding effects on post-fire runoff, soil erosion and species diversity in Galicia (NW Spain). Land. Degrad. Dev, 23, 150- 156. DOI 10.1002/ldr.1064, 2012.

García, C., Hernández, T., Costa, F., Ceccanti, B., Masciandaro, G.: The dehydrogenase activity of soil as an ecological marker in processes of perturbed system regeneration. In: Gallardo-Lancho, J. (Ed.) Proceedings of the XI International Symposium of Environmental Biochemistry. CSIC, Salamanca, España, pp. 89–100, 1993.

García, C., Gil, F., Hernández, M.T., Trasar, C.: Técnicas de Análisis de Parámetros Bioquímicos en Suelos. Mundi-Prensa, Madrid. 2003.

García-Orenes, F., Guerrero, C., Roldán, A.,Mataix-Solera, J., Cerdà, A., Campoy, M., Zornoza, R., Bárcenas, G., Caravaca. F.: Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. Soil. Till. Res, 109 (2), 110-115. 10.1016/j.still.2010.05.005, 2010.

Guerra Delgado, A. C.S.I.C. (1968). Leyenda del Mapa de Suelos de España (1:1.000.000). Inst. Nac. Edafol. CSIC, Madrid.

Grady, K.C., Hart, S.C.: Influences of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: a retrospective study. For. Ecol. Manag, 234, 123–135, 2006.

Guénon, R., Vennetier, M., Dupuy, N., Roussos, S., Pailler, A., Gros, R.: Trends in recovery of Mediterranean soil chemical properties and microbial activities after infrequent and frequent wildfires. Land. Degrad. Dev, 24: 115-128. DOI 10.1002/ldr.1109, 2013.

Guitián, F., Carballas, T.: Técnicas de análisis de suelos. Pico Sacro, Santiago de Compostela, 1976.

Gutknecht, J.L.M., Henry, H.A.L., Balser, T.C.: Inter-annual variation in soil extra-cellular enzyme activity in response to simulated global change and fire disturbance. Pedobiologia, 53, 283-293, 2010.

Fterich, A., Mahdhi, M., Mars, M.: The effects of Acacia tortilis subsp. raddiana, soil texture and soil depth on soil microbial and biochemical characteristics in arid zones of Tunisia. Land. Degrad. Dev, 25 (2): 143–152. DOI: 10.1002/ldr.1154, 2014.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E.: Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil. Biol. Biochem, 38, 2565–2575, 2006.

Irvine, J., Law, B.E., Hibbard, K.A.: Postfire carbon pools and fluxes in semiarid ponderosa pine in Central Oregon. Glob. Change Biol, 13, 1748–1760, 2007.

Kandeler, E., Stemmer, M., Klimanek, E.: Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil. Biol. Biochem, 31, 261-273, 1999.

Kumar, J.D., Sharma, G.D., Mishra, R.R.: Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. Soil. Biol. Biochem, 24, 761-767, 1992.

Lasanta, A., Cerdà, A.: Long-term erosional responses after fire in the Central Spanish Pyrenees: 2. Solute release. Catena, 60, 80-101, 2005.

Leone, V., Borghetti, M., Saracino, A.: Ecology of post-fire recovery in *Pinus halepensis* in southern Italy In: Trabaud, L (ed.) Life and Environment in Mediterranean Ecosystems. WIT Press, Southampton. pp. 129–154, 2000.

Lucas-Borja, M.E., Bastida, F., Moreno, J.L., Nicolás, C., Andrés, M., López, F.R.: The effects of human trampling on the microbiological properties of soil and vegetation in Mediterranean Mountain areas. Land. Degrad. Dev, 22, 383–394, 2010.

Lucas-Borja, M.E., Candel, D., Jindo, K., Moreno, J.L., Andrés, M., Bastida, F.: Soil microbial community structure and activity in monospecific and mixed forest stands, under Mediterranean humid conditions. Plant. Soil, 354, 359-370, 2011.

Lucas-Borja, M.E., Candel, D., López-Serrano, F.R., Andrés, M., Bastida, F.: Altitude-related factors but not *Pinus* community exert a dominant role over chemical and microbiological properties of a Mediterranean humid soil. Eur. J. Soil. Sci, 63, 541-549, 2012.

Mabuhay, J.A., Nobukazu, N., Horikoshi, T.: Microbial biomass and abundance after forest fire in pine forests in Japan. Ecol. Res, 18, 431-441, 2003.

Mataix-Solera, J., Guerrero, C., García-Orenes, F., Bárcenas, G.M., Torres, M.P., Bárcenas, M.: Forest Fire Effects on Soil Microbiology. In: Cerdá A, Robichaud PR (eds.) Fire Effects on Soils and Restoration Strategies Enfield. New Hampshire pp. 133-175, 2009.

Merilä, P., Smolander, A., Strömmer, R.: Soil nitrogen transformations along a primary succession transect on the land-uplift coast in western Finland. Soil. Biol. Biochem, 34, 373–385, 2002.

Moya, D., De las Heras, J., López-Serrano, F., Leone, V.: A post-fire management model to improve Aleppo pine forest resilience. In: De las Heras J, Brebbia CA, Viegas D, Leone V (eds.) Modelling, monitoring and management of forest fires I. WittPress, Southhampton pp. 311-319, 2008.

Muscolo, A., Sidari, M., Mercurio, R. Influence of gap size on organic matter decomposition, microbial biomass and nutrient cycle in Calabrian pine (Pinus laricio, Poiret) stands. For. Ecol. Manage. 242, 412-418, 2007.

Nannipieri, P., Grego, S., Ceccanti, B.: Ecological significance of the biological activity in soil. In: Bollag JM, Stotzky G (eds.) Soil Biochemistry, vol 6. Marcel Dekker: New York pp. 293–355, 1990.

Olsen, S.R., Sommers, L.E.: Phosphorus. In: Page AL, Miller RH, Keeney DR (eds) Methods of Soil Analysis. Chemical and Microbiological Properties, 2nd ed. American Society of Agronomy, Madison. pp. 403-427, 1982.

Rivas Martínez S.: Memoria del mapa de series de vegetación de España (1:400.000). ICONA. Ministerio de Agricultura, Pesca y Alimentación, Madrid. 1987.

Rutigliano, F.A., Ascoli, R.D., De Santo, A.V. Soil microbial metabolism and nutrient status in a Mediterranean area as affected by plant cover. Soil Biol. Biochem. 36, 1719-1729, 2004.

Sardans, J., Peñuelas, J.: Drought decreases soil enzyme activity in a Mediterranean holm oak forest. Soil. Biol. Biochem, 37, 455–461, 2005.

Schmitz, M.F., Atauri, J.A., de Pablo, C.L., Martín de Agar, P., Rescia, A.J., Pineda, F.D.: Changes in land use in Northern Spain: effects of forestry management on soil conservation. For. Ecol.Manag, 109, 137-150, 1998.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H.: Stoichiometry of soil enzyme activity at global scale. Ecol. Lett, 11: 1252-1264, 2008.

Smith, L.J., Papendick, R.I.: Soil organic matter dynamics and crop residue management. In: Metting B (ed.) Soil Microbial Ecology, Marcel Dekker, New York, pp. 65-94, 1993.

Tabatabai, M.A., Bremner, J.M.: Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil. Biol. Biochem, 1: 301-307, 1969.

Tabatabai, M.A.: Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R. (eds.) Methods of analysis, part 2, 2nd ed. Agronomy 9, 389-396, 1982.

Trasar-Cepeda, C., Leirós, C., Gil-Sotres, F., Seoane, S.: Towards a biochemical quality index for soils: an expression relating several biological and biochemical properties. Biol. Fert. Soils, 26, 100–106, 1998.

Úbeda, X., Outeiro, L.: Physical and chemical effects offire on soil. In Fire Effects on Soils and Restoration Strategies, Cerdà A, Robichaud PR (eds). Science Publishers. CRC Press: Boca Raton, F; 105–133, 2009.

Van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J., Wardle, D.A.: Plant-soil feedback: the past, the present and future challenges. J. Ecol, 101, 265-276, 2013.

Vance, E.D., Brookes, P.C., Jenkinson, D.S.: An extraction method for measuring soil microbial biomass. Soil. Biol. Biochem, 19, 703-707, 1987.

Wic-Baena, C., Andrés-Abellán, M., Lucas-Borja, M.E., Martínez-García, E., García-Morote, F.A., Rubio, E., López-Serrano, F.R.: Thinning and recovery effects on soil properties in two sites of a Mediterranean forest, in Cuenca Mountain (South-eastern of Spain) For. Ecol. Manag, 308, 223-230, 2013.

Yeomans, J., Bremner, J.M.: A rapid and precise method for routine determination of organic carbon in soil. Commun. Soil. Sci. Plan, 19, 1467-1476, 1989.

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**

[a] Significant correlations; ns: non-significant; *P≤0.05; **P≤0.01; ***P≤0.001

[b] **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.

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

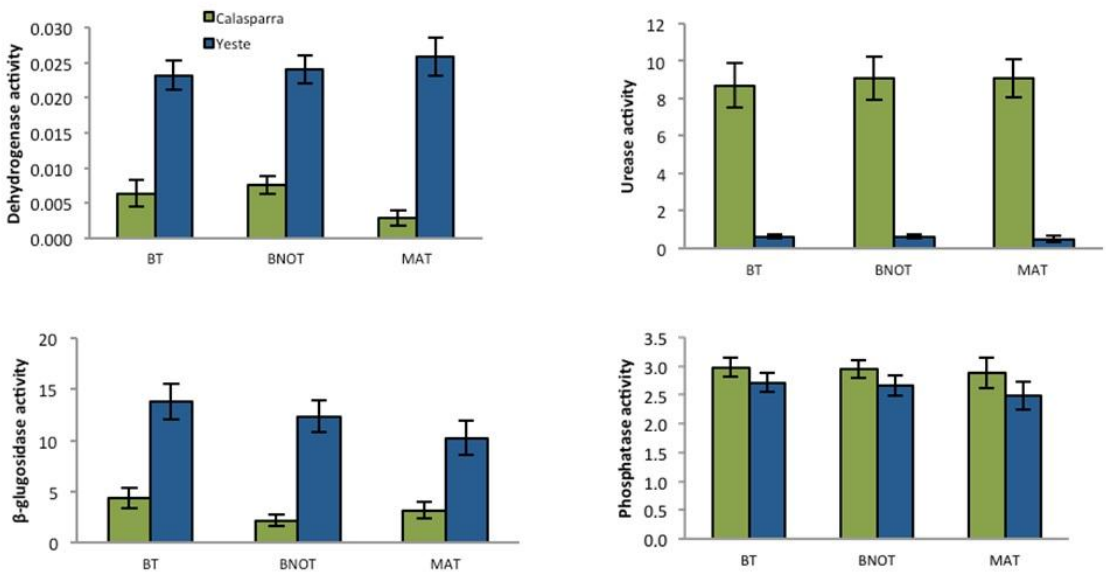

Figure 1. Dehydrogenase activity (µg (INTF) g^{-1} soil $hour^{-1}$), β-glucosidase activity (µmol PNP g^{-1} dry soil $hour^{-1}$), phosphatase activity (µmol PNP g^{-1} dry soil $hour^{-1}$) and urease activity (µmol $N\text{-}NH_4^+$ g^{-1} dry soil $hour^{-1}$) in relation to the experimental site.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

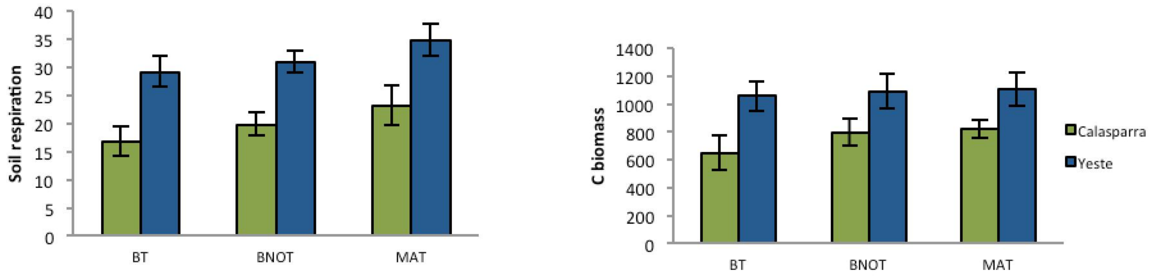

Figure 2. Basal soil respiration (mg CO2 kg^{-1} soil) and microbial biomass carbon (mg kg^{-1}) in relation to the experimental site. Error bars are the LSD intervals at P <0.05.

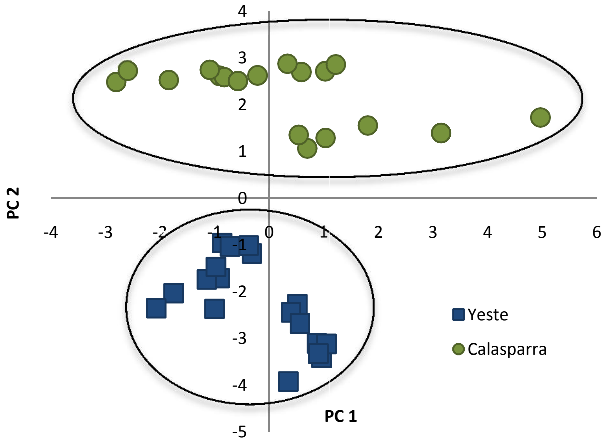

Figure 3. Principal components analysis of the experimental sites Yeste and Calasparra