REVIEW REPORT

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

J. Hedo, M. E. Lucas- Borja, C. Wic, M. Andrés Abellán, and J. de Las Heras

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.

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 and organic 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.

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).

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.

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).

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.

2. SPECIFIC COMMENTS

2.1. INTRODUCTION SECTION

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

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

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.
- 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 was conducted. Sampling without controlling the plant cover could generate a confounded effect and no significant conclusions can be drawn.
- 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
- 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.
- The data collection was carried out in 2011 (1994 + 17 = 2011)

2.4. STATISTICAL ANALYSIS

- Why the authors have not considered random effects?
- Interactions of factors that have been considered should be indicated.
- The study of correlation between variables should be included in this section.

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

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.

- 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.

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.

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.
- 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.
- As discussed for the correlation matrix, this analysis should be performed with the security that all variables are 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
- Delete ~~(Winter, 2011).~~
- T: change by Tsoil.
- H: change for Hsoil or Soil moisture.

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?
    - 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.
- 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.
- 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).
    - In addition, no correlation between organic matter and plant densities (40.000 and 7.000 trees/ha) (in Calasparra and Yeste respectively).
    - These are important results that must be discussed in more depth.
- 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.
- Additional information that would be necessary:
    - n (sample data)
    - Means values for study site

Table 3:

- Result**s**
- Change "Soil respiration" by "Basal respiration (Br)"
- Change "C-Biomass" by "Microbial biomass C" (MBC)

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.

Figure 1:

- Represent the units on the axes

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

Figure 2:

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

2.6. DISCUSSION

- It is necessary discuss whether values of soil activity are similar than others obtained in previous similar research. As mentioned above, some parameters calculated should be wrong. 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.
- 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.
    - 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%, β-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 these

authors 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.

o 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.

- 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.
- 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.
- 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.

- 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).

2.7. ABSTRACT AND CONCLUSIONS

- ✓ Rewrite according to the revision

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.

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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.

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