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Biogeosciences
Discussions

Interactive comment on "Experimental drought induces short-term changes in soil functionality and microbial community structure after fire in a Mediterranean shrubland" *by* M. B. Hinojosa et al.

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RESPONSE TO ANONYMOUS REFEREE #1

We appreciate the comments from referee #1. We have endeavoured to respond to all the comments, which we hope it improved the understanding and potential of our results. Detailed responses are given bellow. In case of further queries, we are happy to clarify them.

REFEREE#1 GENERAL COMMENT 1 (RGC1): General comments: the paper presented by the authors addresses the interaction between fire and drought on Mediterranean soil, which is an important issue as explained in the introduction. The condition

to test the hypothesis is hence to verify the effect of fire, the effect of drought and the interaction of the two effects answering to the question if the two together led to something more than the sum of the two alone. The experimental design however is not built to support the possibility to test the interaction, or at least to conclude that it is the interaction which causes the observed variations of soil biological, chemical and physical endpoints. We miss the unburned plots under drier conditions. I see this as a limit to drive conclusions which are at least coherent with the goal of the paper, at least as introduced by the authors in their premises.

ANSWER TO GENERAL COMMENT 1 (AGC1): The main objective of our study was to test the effects of drought on different aspects of soil processes in a postfire environment. We never intended to test the interaction effect of both fire and drought. We did not intended to test the full range of effects of fire either, that is, of the various types of fires that could occur within a given fire regime (e.g., fire severity, timing of fire, etc.). We were fully aware of it and never intended to go beyond a particular type of fire, the one we experimentally produced, which, on purpose, was of high severity aiming at producing conditions similar to what would happen under prefire drought. We believe that this is correct. The investigator chooses the setup that is thought to be more appropriate for the question he/she is intending to answer. The question we chose was the one that we thought it would be more relevant in the current and future context of fires in the region. While variations in fire characteristics may also play a role in soils and the ecosystem, as it is well known, we thought that by focussing just on climate-related (drought in our case) impacts on one postfire environment would give us an idea of how these climate-related factors would affect the response of the system. Note, that our experimental approach is rather powerful to study postfire impacts of changes in precipitation since each of our plots were burned independently, so we had truly "different fires", obviously in an experimental context. If we had used a wildfire, as others have done, they would have had only a single fire, thus a non-replicated one. The literature is still full of papers using this approach, despite such limitation. Aware of our objectives, we could have simply burned the plots that were later manipulated, without needing an unburned control. However, having such an extra unburned control would permit doing an extra comparison between unburned and burned plots, both receiving normal rain, to verify burning effects alone. This comparison was secondary to our project, because general effects are well known. Yet, it would also provide additional information about the effect of fire alone under the ambient rain conditions, so the comparison with the rainfall treated plots would provide a good reference background. Therefore, we believe that our design permits answering the main question of our research, that is, the role of changes in postfire precipitation on soil processes. Note the uniqueness of our experiment since plots were treated before fire during one season and after fire, simulating the reality that, under drought conditions, more fires and more severe tend to occur. We will revise the text the make sure we do not mislead the reader, and point out which was the main objective of this research. The interaction the reviewer indicates was not our objective because such an experimental setup up was beyond our possibilities, and it was never mentioned in the text. Therefore, we will make sure that this is clear in the text.

RGC2: The second important point which weakens the study is the fact that the experiment has not been followed from the beginning. All the papers on fire experiments indicate that the most important moments are just after during the first weeks after burning, at least for nutrients dynamics, then some long terms effect can be see also later on, on biological parameters such as the ration fungi/bacteria. So it is not clear why the study does not report data from the start after burning. Also data are mentioned as personal communication but not shown, which is even weirder. Also, if the control doesn't exist for all the treatments (unburned) the other alternative (not preferred) would be to have dynamics before and after burning and not only several months after burning. There are then several unclear points which are also relevant that I explain in more details in the specific comments in attachment.

AGC2: As indicated above, our main interest was to determine the effect of changes in precipitation after fire in the soil. As it happens, the plots were burned at the end

of summer (September 23th, 2009). Rainfall reduction treatments continued until the end of October (only one month and just for the SD+ treatment). Sampling at that time would have been confusing as HC+ and MD+ treatments would have similar rainfall pattern. After October, all plots received the same rainfall, and later, in early spring, it is when the plots started to be differentiated again by modifying the rainfall. Thus, sampling at the end of spring is when we thought that the largest effects of drought would be found in burned soils, because before that it was too early, and afterwards, due to the summer, most process would recede, and differences would most likely disappear. So we chose the approximate moment that we thought would produce the largest differences in the system. If no differences would occur at this time, it is unlikely that we would have seen any significant changes before. We acknowledge that a greater sampling is always desired, but given the limitations in space in these experiments, one has to choose when it is more appropriate to use the limited area available for a destructive sampling. In relation to the comment regarding data on the effect of fire alone, without rainfall manipulation, some of this information was obtained in a set of reserve plots that was burned following the completion of the experimental burning of our manipulative plots. This information has been published recently by Karhu et al. 2014, and it will be mentioned properly in the revised version of the manuscript. In the first version of our paper, these data are mentioned as personal communication because the manuscript written by Karhu et al. was not published by the time we submitted it.

REFEREE#1 SPECIFIC COMMENT 1 (RC1): I would like to see a graph with the annual average rainfall values (the multiannual mean of total monthly precipitation of each month of the year) of historical rainfall which the authors have used to set this level of rainfall (1948-2006) plotted together with the total monthly mean of the environmental control.

ANSWER TO SPECIFIC COMMENT 1 (AC1): The background information, that is, the long-term precipitation (1948-2006) used as reference can be seen, plotted as accumulated rainfall on biweekly periods, in Parra et al. (2012). The actual trends of the historical control mimic, with minimal differences, the long- term trend (see again Parra et al. 2012). So this information can largely be derived from Fig. 2A. However, we are happy to include a figure with the data indicated for greater clarity.

RC2: The choice of reducing the rainfall during the summer is due to climatic trend evidence or simply to the willingness to see a harshening of summer aridity? Isn't already the summer in Spain sufficiently dry to limit microbial activity in the way we are discussing? Are the wetter months like winter, autumn, spring any important for the discussion? Does a reduction in rainfall occur also during these months in the climatic trend of climate changes in Spain?

AC2: Projections for high emission scenarios for the region result in reduced total precipitation and changes in temporal patterns, whereby precipitation tends to concentrate towards the winter months (Christensen et al. 2007, 2013). That is, a lengthened summer season is projected. Consistent with this, we set an experimentation that used such projections as main reference, by reducing total precipitation to 450 mm (percentile 8, MD) and 325 (percentile 2, SD), and extended the drought period from 2 months (HC) to 5 (MD) and 7 (SD), respectively, expanding such period more towards spring and autumn. This information was provided in Parra et al. (2012), but adding additional explanations would allow the reader to better understand this piece of research without consulting that paper. Therefore, we will add additional information in the revised text to clarify this.

RC3: It is not clear the experimental set up. From the description it seems that only the environmental control was split in two to have a comparison between burned and unburned. It is not clear to me then how can we compare the effect of drought and burning on soil endpoints if I do not have for the different treatments the control unburned. How can I be sure that what I see is due to the interaction and not simply to more extreme drought conditions? The authors themselves indicate that their objective is: we hypothesise that drought conditions after fire will reduce: : :..". How can I know if a reduction in microbial biomass for example in burned and drier condition compared

with undisturbed is due simply to one factor or to combination of both? Do I have an unburned very dry plot to say if it already affects BM?

AC3: We must refer to the previous explanations regarding the motivations of the experiment (the answer to general comment 1, i.e. AGC1). Again, our focus was the effect of changes in precipitation on the postfire soil processes. As we have indicated earlier, the comparison of burning effect alone, that is, the two sets of plots that received the same natural precipitation (EC+ vs EC-), was done as a secondary objective. Yet this permitted putting into perspective fire effects in a natural context with the other rainfall manipulation effects.

RC4: The combination of fire and drought would have been surely more interesting starting measurements after fire in 2009 as the peak of mineralization occurs just after fire and most of it might be already fading away after a whole autumn, winter, spring, when rainfall is higher. Could the author comment on this and justify their choice?

AC4: We believe that, on comparative terms, the peak effects of drought in our post-fire environment would be seen more clearly in spring 2010, not in autumn after fire (2009). Keep in mind that the plots were burned at the end of summer (September 23th, 2009) and the rainfall reduction treatments continued until the end of October. Thus, after fire, both historical control (HC+) and moderate drought (MD+) plots would have a very similar precipitation pattern as for the MD+ the rainfall exclusion would be effective just until September 30th, 2009. In this sense, sampling at autumn would have been confusing, because we could not detect differences in moderate drought treatments (MD+). The effect of drought in a post-fire environment could have been detected only in SD+ treatment, which had a month of rainfall reduction after fire. We discussed this in part also in a previous comment (see the answer to general comment 2, i.e. AGC2).

RC5: The dates of sampling should be specified also to understand how they fit compared with the period of induced drought during the discussion of results (right at the beginning, after xx days, at the end:)

AC5: We are happy to provide all the dates in the revised version.

RC6: The extracellular enzymes tend to be quite resilient to environmental conditions compared to intracellular enzymes (like ATPases or Dehydrogenases, etc) and might not be representative of the microbial dynamics during "periods" of unfavourable conditions if they are transient and not perennial (like in a desert). Could the author discuss and justify their choice of microbial endpoint indicators compared with other available in the range of possible endpoints?

AC6: Intracellular enzyme activities, as the ones proposed by the reviewer#1 (for example, dehydrogenase), are processes that occur in every viable microbial cell and they are measured to determine overall potential microbiological activity of soil. However, soil enzyme assays (including enzyme assays developed to detect general microbial activity, as dehydrogenase) often require the addition of a surrogate substrate and, as a result, the assay determines the potential enzymatic activity and not the actual level of activity in the sample. Thus, in order to evaluate general soil microbial activity we measured soil respiration rather than any intracellular enzyme activity. In addition, these intracellular enzymes that correlate closely with microbial activity, sometimes may be less suitable to predict seasonal changes or dynamics in soil quality because they would reflect recent exceptional effects that may be transitory (as for example, due to a single rainfall event before the sampling day in summer). Thus, extracellular enzymes that remain adsorbed and are more resistant to proteolysis, thermal and chemical denaturation, may be better indicators for integrative seasonal changes as the ones considered in our study (Dick & et al., 1996). In general, hydrolytic enzymes, as the ones analysed in our study, are good choices as soil functionality indicators because organic residue-decomposition organisms are probably the mayor contributors to soil enzyme activity (Dick, R.P., 1994). Hydrolytic enzyme activities as phosphatases, β-glucosidase and arilsulfatase are thought to play critical roles in soil nutrient cycle. Thus, we used them in our study as indicators of the metabolic capacity of the soil (i.e.

soil functionality), and the potential of these soils to liberate these elements from soil organic matter (Nannipieri et al. 2002). We chose them because they are among the soil enzyme activities most reported in the literature. We have tried to interpret carefully the role of enzyme activities measured in this study, and in the use of these measurements as indicators of soil functionality along the discussion of our results, taking into account both advantages and disadvantages of their use. We will revise the text to make sure these concepts are well reflected, with appropriate background literature.

RC7: Why the authors measured C mineralization and not N mineralization as well given that one of their objectives is to see if the availability of minerals is reduced in response to draught and fire?

AC7: In order to evaluate changes in the availability of N as a response of fire and the joint effect of fire and drought, the labile concentrations of N-NO_3^- and N-NH_4^+ were measured. N mineralization was not measured specifically because previously to our work another research group was involved in the study of soil processes related with N cycling. Unfortunately, these studies could not continue due to funding limitations.

RC8: Why the authors used a derived measure of microbial biomass (from ELFA) and not a direct official measure of BM

AC8: A number of static methodologies are available to estimate soil microbial biomass size. These methods include microscopic direct counts or chemical (muramic acid) content for bacteria and chitin or ergosterol for fungi, for example. Physiological methods for estimating total microbial biomass are widely use. These include fumigation-incubation and substrate-induced respiratory response. Biochemical analysis have also been used for biomass determination; these include, for example, arginine ammonification and the amount of ATP present in soil. Each methods has its own advantages and disadvantages, but each is able to indicate differences among soils. To our knowledge, no single method has emerged that accurately measures the microbial biomass of a given soil. The use of the amount of soil fatty acids, although as an indirect approach, is advantageous because it is a rapid and relatively inexpensive way of assessing both the composition and biomass of soil microbial communities in soils. Soil fatty acid analysis is an efficient way for rapidly screening whether the fungal or bacterial components of the soil have been affected by a treatment. The conversion factors established to estimate fungal and bacterial biomass-C from soil fatty acids amounts show good correlation with other methods of determining the biomass of these groups (Klamer and Baath, 2004; Frostegard and Baath, 1996). In addition, it should be mention that ELFAs have been used as a measure of general microbial biomass in previous works; for example: Dennis et al.(2013), Sun et al. (2011), Hopkins et al. (2008) Gregory et al. (2007).

RC9: The author state that the used one-way ANOVA to test the effects of burning and rainfall pattern at each sampling event. In theory when the influence of two interacting factors is analysed a 2 way anova should be used. The fact that one-way was used is due to the design which indeed doesn't allow testing the interaction, which gives less strength to the premise of the study.

AC9: The plot layout followed a block design, for which we had 4 blocks, each with 5 treatments. The statistical test we first carried out was set to evaluate whether blocks had significant effects or not. As it happens, block-effects were never significant. Thus we chose to use a one-way ANOVA to test our results, which we believe is correct. This does not permit to test the interaction that the reviewer indicates, and we never did test such interaction. Fire alone effects were tested independently, because this was more meaningful than including it in an overall single test with a posteriori contrast. We will clarify this in the revised version to avoid any misinterpretation.

RC10. The fact that the authors did not find significant differences in soil water content in summer and in winter brings us back to comment 2. I assume that summer in Spain is very arid and so it is difficult to reduce significantly the rainfall in such a period. I assume also that most of the rainfall occurs between late autumn up to early spring, centred on winter. Thus again if the sheltering systems works from May to October (at

its best) it is clear that no differences could be seen in winter. So in order to understand how meaningful is the experimental set up a satisfactory answer to point 2 should be given and summarized in the paper somewhere to justify the choice made.

AC10: Yes, the reviewer is correct. In fact, that was the purpose of the experiment. We hope that our response to general comments 2 (AGC2) improve the understanding and potential of our results. We will try to be sure that this point is clear in the revised version of the paper.

RC11: I think that physic-chemical parameters could be discussed in one single paragraph, idem for biological analysis, given that especially for the former points are few in time and not that much can be discussed.

AC11: We are happy to reduce the discussion, focussing on the main objective of this research as indicated above.

RC12: Mineral N: it is not always true that ammonium N was lower in burned plots. The same is true for NO3. Moreover it should be underlined that both NH4 and NO3 were very low both in burned and unburned plots (EC). I don't see any clear reverted effect in winter for the drought treatments for NO3, they all seem to flat down.

AC12: According to the results obtained in the repeated measures analysis of variance (ANOVA), soil ammonium concentration significantly decreased as a consequence of fire under ambient rainfall. The results of this statistical test are shown in Table 1. However, when we tested these differences in each sampling time, we can see that this reduction is significant in both spring and winter 2010 (Fig. 2a). In any case, we will rewrite the sentences describing these results in order to avoid any misunderstanding. In addition, following the suggestion, it will be underlined that both NH_4^+ and NO_3^- were very low both in burned and unburned plots (EC). In relation to soil nitrate we mentioned that "burned soils under drought treatments (MD+ and SD+) showed, on average, 8.5, 3.5 and 5.5 fold more nitrate than the historical control (HC+) treatment in spring, summer and autumn, respectively. Nevertheless, these effects of drought on soil nitrate concentration were reverted in winter (Fig. 2b, Table 1)". Taking into account that drought produced an increase of soil nitrate concentration in burned plots (MD+ and SD+) in comparison to the burned historical control (HC+), we considered that in winter this effect was reverted because nitrate values were the same for drought treatments and the historical control (low levels). In order to avoid any misunderstanding this sentence will be rewritten in the revised text.

RC13: The samples used for C mineralization where incubated at the field conditions of water content? I mean as they were sampled without adjustment of water content reflecting the field conditions or all at the same water content as typically done for potential measurements? It is not specified in the methods basically are you testing the potential or effective mineralization?

AC13: In order to test soil C mineralization, the soils samples were incubated at field conditions in terms of water content (i.e. no adjustment of water content was carried out, apart of maintaining the original soil moisture along the whole incubation). The only optimal environmental variable that was controlled was temperature. Thus, effective C mineralization at optimum temperature was what we measured. We appreciate this comment and we will change the wording to clarify this.

RC14: Fig. 5. Looking at figure 5 can we say that "further drought would have a negative effect on burned soil"? can you say that? Can you exclude that further drought would not have a negative effect on BM anyway, even if the soil is not burned? I don't think so as you miss the unburned drier condition. So which indication can we give here? AC14: In the manuscript we say "Under natural rainfall, soil microbial biomass was significantly lower in burned soils (EC-) than in the unburned ones (EC+) ($P<0.05$), showing a reduction of 25% and 40 % in spring 2010 and 2011, respectively (Fig. 5). Soil microbial biomass was further reduced in the burned soils as a consequence of drought ($P<0.05$), with no significant differences between MD+ and SD+ treatments, being this decrease of 27% and 35% in spring 2010 and 2011, respectively, with respect to the historical control (HC+) (Fig. 5)." In these sentences, our purpose was

not to refer the interactions that you indicate, as we could not test this. We just wanted to describe the effect of drought on the burned soils. Of course, a comparison with unburned soil was also made for the relevant ambient control treatment. To avoid any misunderstanding, in the revised version we will modify the text to make this clear.

RC15: Fig 6 is not clear and tables A1 and A2 do not help to have a picture of which microbial groups are most affected. Something more explanatory and ecological would help.

AC15: The interpretation of soil fatty acids profiles as indicative of different groups of organisms or indicating physiological state of the microorganisms is not straightforward. There are two approaches to analyse these data. One approach relies on using the whole soil fatty acid profile pattern, filtered through a multivariate statistical technique. Because such tools aim at reducing data set complexity, at identifying major patterns and putative causal factors, they certainly find many applications in microbial ecology. Nonmetric multidimensional scaling (NMS) is generally efficient at identifying underlying gradients and at representing relationships based on various types of distance measures. Not surprisingly, NMS has found also an increasing number of applications in microbial ecology. Thus, the results obtained using this multivariate analysis of soil fatty acids profiles in our study are a powerful way of exploring and summarizing the data. In this sense, Fig. 6 represents the ordination diagram resulting from NMS analysis, and Table A2 give further details about the correlation between single fatty acids and the ordination axes. This is a rather classical type of multivariate analysis. However, it should be noted that in NMS ordination the proximity between objects corresponds to their similarity, but the ordination distances do not correspond to the original distances among objects. Thus, the PERMANOVA analysis was used to test for significant differences between the means of two or more groups of multivariate quantitative data (i.e. fatty acid profiles in our case). Again, this is a common ecological approach. The results of this test are shown in Table A1. With this way of analysing the data the main question that was answered was: have there been changes in the soil microbial community structure (as a whole) due to the implemented treatments? The second approach to analyse these type of data involves trying to elucidate the treatment effects on specific groups of microorganisms, assuming that certain fatty acids are markers for a particular group or at least indicative of changes in that group. The presence of indicator fatty acids unique to certain taxa is inferred from pure culture studies. In this sense, the effect of the studied treatments in the microbial groups is shown in Tables 2 and 3 of our paper. We believe that both approaches render significant ecological information about the groups involved, with a reference to their potential functionality, and their dynamics through time.

RC16: It is not clear why the authors discuss the dynamics of nutrient immediately after fire, which indeed is the most interesting phase when really most of the nutrients can be leached and so a lower rainfall input would make the difference, but then they do not start the study immediately after fire and data are not shown and is cited as personal communication. I don't think this make sense.

AC16: We refer to our previous comment concerning the reasons for the temporal pattern of sampling. The reference to data as personal communication will be updated now and clarified as appropriate in the revised version.

RC17: The authors continue to mention their results as immediately after fire (spring summer 2010) when actually the immediately after fire, technically speaking are the first weeks after burning, in autumn 2009.

AC17: We agree "immediately" could be misinterpreted, even if, very often, the first year after fire is what is commonly referred to by this. So, technically speaking a more correct wording would be "shortly after fire". This will be changed in the new version of the paper.

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