

Interactive comment on "No long-term effect of land-use activities on soil carbon dynamics in tropical montane grasslands" *by* Viktoria Oliver et al.

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Received and published: 2 June 2017

DETAILED RESPONSE TO REFEREES

On behalf of my co-authors, I would like to thank the two anonymous referees for their thoughtful and constructive comments on our manuscript. A detailed description of how we have responded to the referees comments is provided below.

RESPONSE TO REFEREE 1

1. One critical point may be that differences in carbon stocks at Acjanaco found by this study (170? Mg C ha-1) and (253 Mg C ha-1) by a previous study (Oliveras et

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al. 2014) are substantial (larger than differences between management systems). The authors related the difference to spatial heterogeneity (L.383). If there is such a high variability, how can differences related to management differences? The work is based on a concept of different soil organic matter pools and stability. However, it is not stated which separated soil fraction correspond to which pool and stability. Therefore I cannot understand how the authors can make a statement on the effect of long-term stability of the different management systems. Moreover, as grazing is excluded only for one year before experiments started? Burning took place 6-8 years before soil sampling and grazing activity was excluded one year (?) before sampling and measurements? One main finding – as stated in the abstract (.L49- 51)- is that long-term C storage on occluded LF and HF is not impacted. What did you mean by long-term? One year? After the concept the occluded LF has a slower turnover compared to the free LF. Consequently effects of grazing may be not visible after one or two years in the occluded fraction. Or if so, what does this implicate for soil carbon dynamics? If the proportion of recalcitrant soil C increases after burning in the occluded, what are the consequences for long-term storage? Does burning favour C sequestration? Can be long-term effects gained by relatively short-term experiments? In this sense, please check title and the discussion section. The cited literature could be improved: New literature and concepts about stability of SOC could improve the manuscript, such as Schmidt et al. 2011, Nature 478, 49-56. In addition, a literature overview about density fractions is missing. E.g. one tropical study is cited for many tropical, temperate and boreal studies. L.118. The same citation is used for a generally ranking of the results. L.-401-402. Including literature about density fraction and turnover times could improve the manuscript. On the other hand general statements (management history; L.60-61) are documented with 5 citations.

Author's response: One source of variance in soil C stocks is due to differences in depths of the soil profile. All sites contained soil to a depth of 20 cm; however, beyond a depth of 20 cm, there was higher variability, with some plots containing soil while others contained parent material (i.e. regolith). Please see Tables 1 and 2 presented

here for a breakdown of total soil C stock estimates (Table 1) and a breakdown of C stock content in each soil layer (Table 2).

In Oliveras et al (2014), all C stocks were reported to 0-30 cm, and this is the main source of disagreement in one of the sites (Acjanaco). The values reported in both studies are similar for the other site (Wayqecha). However, despite these differences in means and the heterogeneity in soil C stocks, our statistical tests still indicate significant differences due to land use. That is, even with a very remarkable variability, we controlled the significance level of the tests at 5% in order to avoid Type I and type II errors, and therefore we are confident that any statistically significant differences are due to real differences arising from land use practices and not from soil heterogeneity.

With respect to some of the questions the referee raised with respect to soil C fractions the separated C fractions correspond to: a) Free LF = labile pools (1 to 5 years) b) Occluded LF = intermediate pools (+ 10 years) c) Heavy F = stable pools (centuries to millennia)

With regard to the referees' concerns about the grazing treatments, and what constitutes "long-term"; grazing was excluded for 2 years prior to sampling and measurements. By long term, we mean that carbon with long residence times (i.e. the "heavy" or mineral-associated fraction, which turns over on the timescale of centuries to millennia, was not impacted by fire or grazing. The manuscript has since been revised to clarify these points and to include new cited literature and concepts.

- When measuring the soil organic pools, the long-term effects of land use can be gained by relatively short-term experiments because burning, in theory, could have a relatively immediate impact on all the pools of carbon. Previous studies have also shown that moderate burning can favour C sequestration by incorporating charcoal deposits in the intermediate and stable pools.

- Of course a longer term study (+10 years) would be ideal but not possible in this case study. However, the findings from this study provide the first set of data on how land

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use affects different soil C pools on an understudied ecosystem. This can then provide a basis for further studies.

- Grazing has been occurring for decades on these grasslands and although the study only prevented grazing for 2 years, the soils are in a continuous dynamic state. Therefore, even though the full affect will not be seen in the more stable pools, it is interesting to see how all the pools are responding to recovery.

We thank the referee for his/her suggestion to change the title of the manuscript to provide greater specificity; we will consider altering the title for the revised version of the text.

2. L.40-42. I would suggest including only percentage of soil C and not bulk soil to improve readability.

Authors' response: "20 % of bulk soil is correct" but for clarity, the sentence has been edited to read: "20 % of the material was recovered in the free LF".

3. L.46-47: As autotropic respiration was not measured, I would omit these speculations in the abstract.

Authors' response: Autotrophic respiration has been omitted.

4. L. 49-51: Please specify what you mean by long-term

Authors' response: \sim 10 + years.

5. L.58-65: How often are these grasslands burnt? Every 10-20 years, once for pasture establishment? How important is burning for these systems?

Authors' response: Manuscript has been changed to include more details about burning in this region: "Every year, especially in the dry season, large areas of these grasslands are burned to support traditional cattle grazing, which has been apparent since the early 1500s (Ellenberg, 1979; Balslev and Luteyn, 1992; Molinillo and Monasterio, 1997). Fires for agricultural clearing and maintenance of these highly productive forage grasses is of considerable importance in these ecosystems and for the livelihood of the local people (Ellenberg 1958; Janzen 1973; Balser and Wixon, 2009). Evidence of fire scars and charcoal deposits along the forest-puna tree line demonstrate a gradual encroachment into the adjacent tropical montane cloud forest (Lægaard 1992)."

6. L.69: What do you mean by soil C balances?

Authors' response: C balances has been changed to C dynamics.

7. L.92-101: see general comment on new literature on SOC stability and ecosystem properties (e.g. Schmidt et al. 2011)

Authors' response: We thank the referee for the suggested reference. The manuscript has now been improved with a detailed literature review including more information about density fractions and turnover times.

8. L.124/L: Which particle-sizes were separated? Where are the results?

Authors' response: Particle-size has been omitted. The method only included density fractionation.

9. L.133: please specify different management systems 10. L.133-134: please specify labile and stable OM pools

Authors' response: Manuscript changed to: "Evaluate the effect of fire history and grazing on the free LF, occluded LF and heavy F soil carbon pools"

11. L.135-137: Which environmental drivers do you mean except soil temperature and VWC? Please specify the objective

Authors' response: Manuscript changed to: "Quantify differences in soil respiration and evaluate the role of soil temperature and soil moisture in regulating soil respiration."

12. Table 1: I would like to have the given information (BD, pH C:N, Soil C) at least for both sites and different depth (and management system). For me it is not clear which

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soil is described in Table 1.

Authors' response: A table has been included to include the soil characteristics for site and at two soil depths (0-10, 10-20 cm).

13. L.163-164. please add information: How long were these sites were grazed / not grazed and about fire frequency.

Authors' response: The fire at Acjanaco was in 2005 and before that, this area had not been burnt since the mid-70s. The most recent fire occurred in Wayqecha in 2003, and we do not have information about the disturbance history before 2003. We also do not have information about the grazing history.

14. L.185: How were the bi-monthly measurements extrapolated to gain annual emissions? What is the uncertainty of the annual emission? The annual emission is only based on 6 measurement days – without information on soil temperature course of the year. Soil respiration is driven by soil temperature (L270), but measurements only included day measurements at a very low frequency. What do you want to express with the annual emission rates?

Authors' response: Reviewer made a valid point and the calculations have been edited to average rather than annual emissions.

15. L.203-208: Does the free LF included (living) roots or were they sorted out before? (This would have major implications for the yield of free LF), see also comment L.299

Authors' response: Methods section edited to include: "The air-dried material was sieved in a 2 mm mesh sieve to remove any living roots and larger organic material and was then saturated...."

16. L.203-223: I am missing information about soil C recovery in density fractions: bulk soil measured = 100%, sum of soil C in density fractions = ?%

Authors' response: The recovery of the soil C density fractions was 96 %, which has

now been included in the manuscript

17. L.261-262: Does at Acjanaco grazing and burning significantly increase soil CO2 fluxes? From Figure 2, I do not get the impression.

Authors' response: The reviewer is correct in stating that burning and grazing did not significantly increase CO2 fluxes at Acjanaco. The sentence has been reworded to: "However, this was only noticeable at Wayqecha (2003) and not at Acjanaco (2005) (Fig 2)."

18. L.269: How is season defined? By soil temperature and VWC? Are soil temperature and air temperature not strongly correlated?

Authors' response: The wet season runs from October to March, which has been cited in other studies for this region and is defined by precipitation. For the linear mixed model, season was included as a categorical variable. Correlation was checked for soil and air temperature but were not strongly correlated.

19. L. 299: belowground carbon stock = soil carbon stock + living roots? Authors' response: The belowground carbon stock does not include living roots. Subheading changed to "Soil C stocks".

20. L.300-305: and L381-383. Comparison of soil carbon stocks of Acjanco from different studies Oliveras et al. 2017 submitted and Oliveras et al. 2014): If there is a high spatial variability (170 vs 253) how can be differences found at the different sites (grazedungrazed- burned-not burned) traced back to differences in management and not also to spatial variability? Please check carbon stock 152 vs 170. I have difficulties to account the number of replicates of soil C sampling (from design description I got the impression of 4 replicates, Table 2 : n=3. Eventually a small graphic with sampling design would help to understand the experimental design.

Authors' response: Please refer to authors' response 1. A diagram showing the sampling design has now been included.

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21. L. 362. As heterotrophic respiration is not measured: may enhances.., as it is a speculation

Authors' response: Manuscript edited to include "May enhance..."

22. L 364: Is the N loss reflected by different C/N ration in soil?

Authors' response: C/N ratio wasn't mentioned in the stated study but this sentence can be taken out to avoid any confusion.

23. L.376: It would be nice to have a range of soil C stocks found in montane grassland soils Authors' response: The manuscript has been edited to include a range of soil C stocks found in montane grasslands.

24. L.385-L399. There was no effect of burning on total soil C and no significant effect of grazing on total soil C. However grazing had a more negative effect on total soil C. please clarify.

Authors' response: The wording in the manuscript has been changed to clarify that there was no significant effect of either burning or grazing but that grazing had a more negative effect than burning on total soil C.

25. L.401-411: Please expand literature and discussion. In addition, please check the number cited (10%) and carefully consider the land use type. I do not understand L 403-404. It would be nice to have the range of free LF found in tropical soils in order to rank and interpret the gained results (L 403-406).

Authors' response: Manuscript has been edited to include a range of free LF found in tropical soils and a discussion with more cited literature.

26. L.413-420: Does this mean that burning favours long-term stabilisation of soil C as charcoal? It is it is stated (L49-51), that the long term storage in the occluded fraction was not negatively impacted, but has a positive effect?

Authors' response: Soil carbon and charcoal carbon stocks and their dynamics in the

soil profile after fire is limited but some previous studies on burning have suggested that charcoal contributes to the slow carbon pools in soils, so this was one potential explanation for why we saw a positive effect in the occluded LF.

RESPONSE TO REFEREE 2

27. It is an interesting study but my major concern is about the experimental design. There is no random plot or site selection.

Authors' response: With all due respect to the referee, this is an incorrect interpretation of our experimental design. Treatment plots were set-up according to a randomised block design (L128) (see Oliveras et al. 2014, Table 1). We acknowledge that the manuscript may have not explained this clearly enough, and we have therefore rewritten this part to clarify this point.

28. Hence, there is no true replicate in the whole study. This makes it very difficult or even impossible to interpret the results in an appropriate way.

Authors' response: The key criticism that the referee raises here is that our study is an example of a pseudo-replicated experiment, and that the study may therefore be invalid. However, we respectfully disagree with this perspective, and present three counter-arguments to this criticism here. There is a long-standing and well-established debate in the soil science and ecological literature about whether or not pseudoreplication in field experiments invalidates them (Davies and Gray, 2015;Hurlbert, 1984;Schank and Koehnle, 2009;Pennock, 2004). The consensus that has emerged from this 30-year old debate is that pseudo-replication alone does not invalidate an experiment (Davies and Gray, 2015;Hurlbert, 1984;Schank and Koehnle, 2009;Pennock, 2004).

First, provided that the experimental design allows for appropriate interspersion of experimental treatments, the problem of pseudo-replication can be ameliorated by ensuring quasi-independence of experimental treatments from each other by dispersing

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them in space and time (Hurlbert, 1984). We were mindful of this concern in designing our experiment, and achieved appropriate interspersion of our treatments by settingup the experiment according to a randomised block design (Oliveras et al 2014). This is one of the approaches recommended by statisticians for ameliorating the effects of pseudo-replication (see point 27) (Hurlbert, 1984).

Second, scientists have argued that even if a study is pseudo-replicated, this does not mean, a priori, that these studies are invalid or fundamentally flawed. This is particularly true for experiments where practical circumstances do not allow for the implementation of a fully controlled and replicated study (Davies and Gray, 2015;Hurlbert, 1984). For example, many natural disturbances (e.g. fire, landslides, storm events, volcanic eruptions, pest outbreaks) are often difficult to predict and almost impossible to replicate, particularly at large spatial scales (Davies and Gray, 2015;Schank and Koehnle, 2009). Likewise, many anthropogenic disturbances (e.g. biomass burning, clear-felling, hydraulic mining, peatland drainage) may be difficult to simulate at realistically large spatial scales, due to constraints imposed by time and resources (Davies and Gray, 2015;Schank and Koehnle, 2009). For example, Davies and Gray (2015) and Schank and Koehnle (2009) assert that provide precautions are taken in the design of experiments and analysis of the data (Hurlbert, 1984), these types of disturbance- or landscape-scale experiments are still interpretable and valid. This line of argumentation is particularly salient for the work we have presented here. Because the research was conducted in Manu National Park, where burning is prohibited by park authorities, we were unable to conduct large-scale controlled burns to simulate the effects of wildfire. Therefore, our only recourse - knowing that burning is an important disturbance in these high elevation ecosystems - was to select study sites that burned naturally, accounting as far as possible for the effects of differences in key pedogenic factors (i.e. parent material, time since disturbance, relief/topography, climate, and biota). To account for differences in site age (i.e. time since burning) in our study sites, we incorporated time as a factor in our mixed effects modelling. Moreover, there is already a scientific publication about grassland productivity where this exact experimental designed was used (Oliveras et al 2014) and the reviewers at that time did not have any concerns on this matter.

Third, mindful of the potential problems posed by pseudo-replication for interpreting the data, we implemented a sampling design where we quantified key process-based variables (e.g. decomposition rate, temperature, moisture), in order to deepen our mechanistic understanding of how soil C stocks were linked to the factors that regulate their turnover and loss. We also implemented some degree of control in our management studies, by installing grazing exclosures. While these measures do not negate the issue of pseudo-replication per se, they establishes the mechanistic relationship between soil C stocks and their control variables (e.g. grazing, organic matter decay rate, temperature, moisture), enabling us to establish if disturbance (fire) and land management practices (presence or absence of cattle) were linked to underlying shifts in control variables.

29. Unfortunately, results are mainly analysed/described based on pooled data (P9, L260-261; P10, L280-284, L292, L308-315; P11, L319-324;) derived from two different sites with significant site-specific differences and differences in fire history (e.g. P10,L284; P11,L338; P5,L145). Then, this information even gets lost throughout discussion and conclusions (e.g. P12, L347-352; P13, L385-386, L395-396; P14, L413-420).

Authors' response: With all due respect to the referee, this is a misapprehension of how we approached our data analysis. In order to account for the potential effects arising from different aged sites, we included time since burning as a variable in our mixed effects model. Moreover, we included key environmental variables (e.g. temperature, moisture) and site properties (e.g. organic matter decay rate) as co-variates, to take into account the role of underlying site differences in modulating soil C stocks. The only reason the two sites were not discussed separately in parts of the text is because the mixed effects model indicated that there was no significant difference arising from different times since burning; however, we want to emphasise that for the statistical

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analyses themselves, the data were not pooled but always included time since burning as an independent variable. The revised manuscript will be altered to better clarify this point.

P1,L3: Title is too general.

Authors' response: We thank the referee for his/her suggestion to change the title of the manuscript to provide greater specificity; we will consider altering the title for the revised version of the text.

30. P1,L32: . . . impacts of burning but not of fire history. Oliver et al. have not studied effects of past fire frequency or intensity on soil C dynamics but rather differences in soil C dynamics at two sites 8/9 years and 6/7 years, respectively, after a burning event.

Authors' response: Manuscript has been changed to "impacts of burning..."

31. P6,L162: Explain "puna areas".

Authors' response: Changed to: "Both puna sites were selected".

32. P6,L162: Do you have more information about the "unburnt" grassland area. I guess that this "control" grassland area has been burnt as well in the past. Are there potential differences between both "control" sites?

Authors' response: We do not have information on the "unburnt" areas. Only that they have not been burnt since the late 70s. Potentially there are differences between the "control" sites in their burning history.

33. P5,L132: grazing and burnt plots.

Authors' response: Text has been changes to "grazing and burnt plots".

34. P5,L133-134: Please explain the connection between labile and stable organic matter pools with your quantified soil C content in free light, occluded and heavy fractions more in detail! What is what?

Authors' response: Labile pool = freeLF, Stable pools = occluded LF and heavy F. A more detailed literature review on soil fractionation has been included in the manuscript.

35. P5, L135-L137: Please do not pool the data among sites but rather describe/interpret the site-specific patterns.

Authors' response: A description of each site has now been included as well as the pooled data.

36. P6, L159-170: A figure presenting the spatial distribution of the plots at both sites would be great.

Authors' response: A figure showing the spatial distribution of the plots has been included.

37. P8,L234: Please explain "proximity". Did the bags cover the whole area? What was the distance between buried bags?

Authors' response: The decomposition experiment was done in triplicate on each plot, with 6 bags buried no more than 30 cm apart for each experiment. The 3 decomposition experiments were randomly located within each plot to cover the heterogeneity on the plot.

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Pseudoreplication is a Pseudoproblem, Journal of Comparative Psychology, 123, 421-433, 10.1037/a0013579, 2009.

Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/bg-2017-113/bg-2017-113-AC1supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-113, 2017.