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

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 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.

1. **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.

Table 1. Total C stocks 0-30 and 0-20 cm for Wayqecha and Acjanaco.

Site	Land use	Total C stock 0-30 cm (Mg C ha^{-1})	Total C stock 0-20 cm (Mg C ha^{-1})
Acjanaco	**Grazed burnt**	136 ± 30	117 ± 17
	Non grazed burnt	182 ± 24	170 ± 12
	Grazed non burnt	144 ± 16	130 ± 8
	Non grazed non burnt	238 ± 33	166 ± 22
	Average	175 ± 17	146 ± 10
Wayqecha	**Grazed burnt**	123 ± 10	107 ± 8
	Non grazed burnt	175 ± 47	131 ± 18
	Grazed non burnt	126 ± 24	125 ± 25
	Non grazed non burnt	140 ± 31	125 ± 26
	Average	150 ± 15	122 ± 9

Table 2. Soil organic carbon (SOC) stocks down the soil profile Wayqecha and Acjanaco.

Land use	Depth (cm)	Acjanaco SOC (Mg C ha^{-1})	Wayqecha SOC (Mg C ha^{-1})
Grazed burnt	0-5	40.91 ± 6.45	40.03 ± 1.66
	5-10	32.74 ± 4.37	26.64 ± 1.56
	10-20	43.08 ± 13.42	40.80 ± 4.99
	20-30	57.61 ±	15.98 ± 3.18
Non grazed burnt	0-5	53.50 ± 4.54	40.33 ± 3.29
	5-10	40.93 ± 4.67	27.14 ± 5.73
	10-20	76.04 ± 3.69	63.35 ± 21.21
	20-30	35.44 ±	44.39 ± 29.46
Grazed non burnt	0-5	41.37 ± 3.16	41.29 ± 11.46
	5-10	34.65 ± 6.59	41.27 ± 9.76
	10-20	53.74 ± 15.98	42.03 ± 5.13
	20-30	44.15 ±	3.03 ±
Non grazed non burnt	0-5	40.66 ± 8,28	38.70 ± 5.27
	5-10	44.38 ± 5.39	31.00 ± 3.64
	10-20	81.44 ± 23.98	55.39 ± 17.27
	20-30	71.59 ± 13.37	14.75 ± 4.43

All values are given with 1 standard error of the mean (n = 3). Except for at 20-30 cm for Acjanaco grazed burnt, grazed non burnt and Wayqecha grazed non burnt where only one sample could be taken.

With respect to some of the questions the referee raised with respect to soil C fractions the separated C fractions correspond to:

1) Free LF = labile pools (1 to 5 years)
2) Occluded LF = intermediate pools (+ 10 years)
3) 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 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.

Author's response:

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: ~ 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 soil is descripted 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 CO_2 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.

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 re-written 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 pseudo-replication 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 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 setting-up 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 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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Davies, G. M., and Gray, A.: Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring), Ecology and Evolution, 5, 5295-5304, 10.1002/ece3.1782, 2015.

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Pennock, D. J.: Designing field studies in soil science, Canadian Journal of Soil Science, 84, 1-10, 10.4141/S03-039, 2004.

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1. Title page

No long-term effect of land-use activities on soil carbon dynamics in tropical montane grasslands

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Running title: Tropical montane grassland soil carbon dynamics

Keywords: Andean montane grasslands, soil respiration, fire, grazing, puna, soil carbon, land-use activities, soil density fractionation.

2. Abstract

Montane tropical soils are a large carbon (C) reservoir, acting as both a source and a sink of CO_2. Enhanced CO_2 emissions originate, in large part, from the decomposition and losses of soil organic matter (SOM) following anthropogenic disturbances. Therefore, quantitative knowledge of the stabilization and decomposition of SOM is necessary in order to understand, assess and predict the impact of land management in the tropics. In particular, labile SOM is an early and sensitive indicator of how SOM responds to changes in land use and management practices, which could have major implications for long term carbon storage and rising atmospheric CO_2 concentrations. The aim of this study was to investigate the impacts of grazing and fire history on soil C dynamics in the Peruvian montane grasslands; an understudied ecosystem, which covers approximately a quarter of the land area in Peru. A density fractionation method was used to quantify the labile and stable organic matter pools, along with soil CO_2 flux and decomposition measurements. Grazing and burning together significantly increased soil CO_2 fluxes and decomposition rates and reduced temperature as a driver. Although there was no significant effect of land use on total soil C stocks, the combination of burning and grazing decreased the proportion of C in the free LF, especially at the lower depths (10-20 and 20-30 cm). In the control soils, 20 % of the material recovered was in the free LF, which contained 30 % of the soil C content. In comparison, the burnt-grazed soil, had the smallest recovery of the free LF (10 %) and a significantly lower C content (14 %). The burnt soils had a much higher proportion of C in the occluded LF (12%) compared to the not-burnt soils (7%) and there was no significant difference among the treatments in the heavy F (~ 70%). The synergistic effect of burning and grazing caused changes to the soil C dynamics. CO_2 fluxes were increased and the dominant temperature driver was obscured by some other process, such as changes in plant C and N allocation. In addition, the free LF was negatively affected when these two anthropogenic activities took place on the same site. Most likely a result of reduced detritus being incorporated into the soil. A positive finding from this study is that the total soil C stocks were not significantly affected and the long term (+10 years) C storage in the occluded LF and heavy F were not negatively impacted. Possibly this is because of low intensity fire, fire-resilient grasses and the grazing pressure is below the threshold to cause severe degradation.

Commented [OV1]: Reviewers comment: L.40-42. I would suggest including only percentage of soil C and not bulk soil to improve readability

20 % of bulk soil is correct but text changed to: “20 % of the material was recovered in the free LF”

Commented [OV2]: Reviewers comment: L.46-47: As autotropic respiration was not measured, I would omit these speculations in the abstract.

Autotrophic respiration removed from text

Commented [OV3]: Reviewers comment: L. 49-51: Please specify what you mean by long-term

~ 10+ years included in text

3. Introduction

High altitudinal montane grasslands (3200 - 4500 m a.s.l) account for a major proportion of land cover in the Andes, particularly in Peru, where they make-up approximately 25 % of land cover (Feeley and Silman 2010). 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 (Luteyn 1992). 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 (Sarmiento and Frolich 2002). To some extent, this natural system is tolerant of these management practices (Ramsay 1992). However, in recent years, it has become apparent that the combination of global warming and the considerable pressure from agricultural expansion have resulted in increased fire occurrence and subsequent destruction of tropical montane cloud forest (Cochrane and Ryan 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 (Di Pasquale *et al.* 2008).

Commented [OV4]: Reviewers comment: L.58-65: How often are these grasslands burnt? Every 10-20 years, once for pasture establishment? How important is burning for these systems?

This paragraph now includes a bit more information about burning in puna systems

Previous research in these Andean montane grasslands have measured large soil C stores, (Gibbon *et al.* 2010; Oliveras *et al.* 2014b). However, despite the concern on the effects of land management practices, there are very few studies on soil C dynamics in this tropical region of the Peruvian Andes. It is particularly unclear how land management affects the soil C dynamics and sequestration potential under the influence of grazing and burning. For example, (Oliveras *et al.* 2014b) , found that grazing and fire in montane grasslands resulted in decreased net primary productivity, but there were no differences between these two disturbances. Studies in other montane grasslands have found that an increase in the frequency of fire events can reduce the amount of soil organic matter (SOM) in the top soil (Knicker 2007), or it may increase the biomass growth period afterwards, causing more detritus to accumulate in the upper soil layers (Ojima *et al.* 1994).

Commented [OV5]: Reviewers comment: L.69: What do you mean by soil C balances?

Changes from ‘balance’ to ‘dynamics’

SOM influences many soil functions and occupies a key position in the global C cycle (Lal 2004). It is a highly heterogeneous and dynamic composite of organic molecules (such as: polysaccharides, lignin, aliphatic biopolymers, tannins, lipids, proteins and aminosugars) derived from progressively decomposed plant, animal and microbial material (Zimmermann *et al.* 2007a; Totsche *et al.* 2010).

The turnover of SOM is a balance between the inputs of material into the soil (e.g., above and belowground litter, dissolved organic C) and the rate of SOM decomposition. The rate of decomposition is a consequence of complex interactions and interdependence between the organic matter and its environment. This includes: biochemical recalcitrance (compound chemistry), physical protection (adsorption of SOM to reactive surfaces of mineral particles and the physical protection within aggregates) (Six and Jastrow 2002), climate (temperature, water availability), soil acidity, soil redox state (Raich and Schlesinger 1992; Kirschbaum 1995; Stockmann *et al.* 2013) and, functional composition of the soil microbial community (Fierer 2007; Allison 2012). More recently, it has been considered that C stability is mainly dependent on its biotic and abiotic environment, rather than the molecular structures of C inputs (Schmidt *et al.* 2011).

In order to understand soil C dynamics, a variety of measureable C pools have been identified within SOM according to biological stability, decomposition rate and turnover time (Krull, Baldock and Skjemstad 2003; Trumbore 2009; Stockmann *et al.* 2013). Specifically, SOM can be classified into three significant pools: active, recalcitrant and inert (Trumbore 1993; Bol *et al.* 2009). The active (also termed the labile) pool contains a high C concentration and is composed of physically available and chemically mineralizable plant material (sugars and amino acids) (Zou *et al.* 2005; Petrokofsky *et al.* 2012). Consequently, it is less stable and plays an essential role in the short-term nutrient cycles, with a turnover ranging from days to a few years (Wander 2004).

The resistant pools (also known as intermediate, slow recalcitrant or refractory) (Krull, Baldock and Skjemstad 2003) contain physically and chemically transformed material residing on and within the surface of clay and silt minerals. The combination of physically protected and biochemically recalcitrant SOM (alkyl and lignin-derived aromatic C) (Coleman and Jenkinson 1996; Petrokofsky *et al.* 2012) causes this C pool to have a turnover on decadal timescales (Six *et al.* 2002). This pool is important for long-term C sequestration, sorption, cation exchange capacity and soil water-holding capacity (Wander 2004).

The inert pool (passive pool) contains highly carbonized organic material resistant to microbial mineralisation and has a turnover time of decades to thousands of years. Charcoal or black C tends to reside in this pool and is considered to have a recalcitrant structure due to its high degree of aromaticity, which causes it to have an estimated residence time of 5000 to 10 000 years (Derenne and Largeau 2001). Although this pool has a low C concentration, it is the largest and conceptually unaffected by land-management or climate, making it the most stable and relevant for long-term C storage (Falloon and Smith 2000). It is also central to the stabilization of humus and soil aggregation (Brodowski *et al.* 2006).

Land-use change and land management studies have found that even when the bulk soil C does not appear to be affected, the distribution of SOM pools may change due to their differing sensitivities to environmental forcing or external perturbation (Zimmermann *et al.* 2007a). It is commonly accepted that the labile pools are the most sensitive to changes in vegetation management and are identified as an indicator of soil quality changes in the short-term (Kennedy and Papendick 1995; Islam and Weil 2000). However, while several studies have found the labile pool to be more sensitive to land management (Conant *et al.* 2011; Wang and Wang 2011), others have found no discernible effect on pool size (Leifeld and Kögel-Knabner 2005). For instance, labile pools can either increase (Poeplau and Don 2013) or decrease, depending on the magnitude of C inputs (e.g. roots, litter fall) or the level of grazing intensity (Figueiredo, Resck and Carneiro 2010).

Quantification of different SOM pools and how they respond to land management is important for understanding C dynamics and their relative role in the global C cycle (Trumbore 1997; Bayer *et al.* 2001). SOM turnover models use conceptual SOM pools but now it is possible to substitute these pools with measurable fractions of SOC (Skjemstad *et al.* 2004; Zimmermann *et al.* 2007b). The separation of these pools has both originated and led to many methods of soil fractionation, including: physical (size, density, aggregation) and chemical (solubility, mineralogy). Density fractionation has been very successful at assessing the short and long-term dynamics of soil C storage (Christensen 2001; Marín-Spiotta *et al.* 2008; Marin-Spiotta *et al.* 2009; Mueller and Koegel-Knabner 2009). This procedure is based on the application of several disaggregating treatments, dispersion, followed by density separations using organic solutions or inorganic salts (von Lützow *et al.* 2007) and represents a variety of pools that are related to microbial function based on the location within the soil matrix and degree of association with minerals (Krull, Baldock and Skjemstad 2003; Trumbore 2009). Six et al., 2002 used sodium polytungstate (SPT) to isolate light and heavy fractions of SOM because of its high viscosity at high concentrations. This method was later adapted by (Marin-Spiotta *et al.* 2009) and (Mueller and Koegel-Knabner 2009) to separate SOM pools into three distinct fractions: the free light fraction (active pool), occluded light fraction (resistant pool) and heavy fraction (inert pool).

The aim of this study is to gain further mechanistic insights into the impact of land-use management on soil C losses and different SOM fractions in Peruvian montane grasslands. In order to investigate the effects of burning and grazing on soil C stocks, we took advantage of an ongoing burning/grazing study that was established in July-August 2010 (Oliveras *et al.* 2014b). The specific objectives of this study were to:

a. Quantify and compare the effect of fire history and grazing on total SOC stocks and the three main SOM pools (free light fraction, occluded light fraction and heavy fraction) at different soil depths down to 30 cm;
b. Quantify differences in soil respiration and decomposition rates on historically burnt and grazed sites;
c. Evaluate the role of soil temperature and soil moisture in regulating soil respiration.

4. Material and methods

4.1 Site descriptions

The undulating terrain in the Peruvian montane grassland is commonly used by the local communities for extensive cattle grazing and although the study area is in the National Park, burning and grazing still occasionally takes place. This study included two sites that were identified as being burnt in 2003 (Wayqecha) and 2005 (Acjanaco) (Fig 1). The site at Wayqecha is located at approximately 3085 m a.s.l. in Wayqecha Biological Station (13°18´S, 71°58´W), where the mean annual precipitation is 1560 mm and mean annual air temperature is 11.8 °C. The site at Acjanaco (13°17'S, 71°63´W), is located on the Manu national park boarder at 3400 m a.s.l and has a mean annual precipitation of 760 mm and

Commented [OV6]: Reviewers comment: L.92-101: see general comment on new literature on SOC stability and ecosystem properties (e.g. Schmidt et al. 2011)
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.

Literature review rewritten to include new literature on density fractions and turnover times

Commented [OV7]: Reviewers comment: L.124/L: Which particle-sizes were separated? Where are the results?

Particle size fractionation removed

Commented [OV8]: Reviewers comment: L.133: please specify different management systems
L.133-134: please specify labile and stable OM pools

Text changed to include management systems and OM pools

Commented [OV9]: Reviewers comment: L.135-137: Which environmental drivers do you mean except soil temperature and VWC? Please specify the objective

Text changed to include soil moisture and temperature mean annual air temperature 6.8 °C (Girardin *et al.* 2010) (Table 2). The wet season runs from October to March and there are more noticeable variations in diurnal temperatures than seasonal differences (Zimmermann *et al.* 2009). Grass species composition are similar on both sites (*Calamagrostis longearistata, Scirpus rigidus and Festuca dolichophylla)* (Oliveras *et al.* 2014a). The soils are classified as Umbrisols and are typically only 30 cm deep with a thick acidic organic rich A layer overlying a thin stony B/C horizons and no O horizon (Gibbon *et al.* 2010) (soil characteristics shown Table 1). The sites are predominantly on Palaeozoic (~450 Ma) meta-sedimentary mudstones (~80 %) (Carlotto *et al.* 1996)

4.2 Experimental design

The sites were set up in a factorial design in July-August 2010 to investigate the effects of fire (burnt, not-burnt) and grazing (grazed, not-grazed) on soil C stocks, soil C fractions and soil respiration. The two sites (Acjanaco and Wayqecha) were selected to include a burnt and unburnt area no more than 200 m apart, which were then split into two subplots (2 x 2 m); one with fencing, constructed 2 years prior to sampling, to stop cattle grazing and one left unfenced. Each site contained a factorial combination of the two treatments i.e. burnt-not grazed; burnt-grazed; not burnt-grazed; and not burnt-not grazed (Fig. 2). The fire at Acjanaco was in 2005 and before that, this area had not been burnt since the mid-70s. The more recent fire occurred in Wayqecha in 2003, and there is no information about the disturbance history before 2003.

Commented [OV10]: Reviewers comment: L.163-164. please add information: How long were these sites were grazed / not grazed and about fire frequency.

The known fire history was included in the text

4.3 Soil respiration and environmental measurements

On each plot, four permanent PVC chamber bases (diameter 20 cm, height 10 cm) were deployed randomly for the measurement of soil surface CO_2 fluxes, which took place morning and afternoon at two monthly intervals from July 2011 to July 2012. Soil respiration measurements were quantified using a static flux chamber technique with a Vaisala CARBOCAP® carbon dioxide probe and temperature sensor fitted inside a PVC cylindrical chamber (diameter 20 cm, height 20 cm), covered with a gas tight lid. The rate of CO_2 accumulation was measured every 30 seconds for 3 minutes by placing the chamber on the fixed chamber base with a gas tight rubber seal. Simultaneously, air temperature and atmospheric pressure were measured, using a type K thermocouple (Omega Engineering Ltd., UK) and Garmin GPSmap 60CSx (Garmin Ltd., USA).

Flux rates were calculated in R 3.0.2 (R_Core_Team, 2012) using the *HMR* package (Pedersen, Petersen and Schelde 2010) by plotting the headspace concentration (ppm) against time (minutes) for each collar, which gave a linear or non-linear regression, depending on the best fit.

Commented [OV11]: Reviewers comment: 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?

Annual emissions removed

In addition, soil temperature (at 5 cm and 10 cm depth) and soil moisture (at 10 cm depth) were simultaneously measured in three locations adjacent to the collars using a ML2x ThetaProbe equipped with 12 cm rods (Delta-T Ltd., UK) and type K thermocouples (Omega Engineering Ltd., Manchester, UK).

4.4 Soil sampling and analysis

Soil sampling: 50 g soil samples were taken in July 2012 with six replicates at 0-5, 5-10, 10-20 and 20-30 cm depths on each site. In many instances, the soil depths were shallow before reaching the bedrock, so samples were only taken at 20-30 cm where possible. Soil samples were air-dried and sieved with a 2 mm mesh sieve before being shipped to the University of St Andrews for all further analysis (Brown and Lugo 1982).

Bulk density: soil bulk density was determined by the soil core method (Klute 1986). Undisturbed soil cores (30 cm^3) were taken from three soil pits at 0-10, 10-20 and 20-30 cm. The samples were dried at 105 °C for 48 hours and bulk density was estimated as the mass of oven-dry soil divided by the core volume.

Soil fractionation: Soils C fractions were separated using a method developed by (Marín-Spiotta *et al.* 2008) and (Mueller and Koegel-Knabner 2009). This method is useful for separating SOM based on the location within the soil matrix and the degree of association with minerals. Prior to the experiment, a sub-sample of soil was taken for moisture correction. The air-dried soil material (15 g) was sieved in a 2mm mesh sieve to remove any living roots and larger organic material and was then saturated with 60 mL sodium polytungstate solution (NaPT, Na_6 [$H_2W_{12}O_{40}$], Sometu-Germany) at a density of 1.85g/mL and centrifuged for 45 minutes at 3600 rpm and allowed to settle overnight. The floating free light fraction (free LF) was aspirated via a pump and rinsed with 500 mL of deionised water through a 0.4 µm

Commented [OV12]: Reviewers comment: 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

Clarification that any living roots were removed from the soil was included in the text polycarbonate filter (Whatman Nuclepore Track Etch Membrane) to remove residual NaPT. The remaining slurry was further saturated with 60 mL sodium polytungstate solution (1.4 g cm^{-3}), mixed using a benchtop mixer (Mixer/Vortexer - BM1000) for 1 minute at 3200 rpm and dispersed ultrasonically (N10318 Sonix VCX500 sonicator Vibra-cell ultrasonic processor) for 3 min at 70 % pulse for a total input of 200 J/mL. Centrifugation (45 minutes at 3600 rpm) was used to separate the occluded light fraction (occluded LF) from the mineral residue and allowed to sit overnight to achieve further separation by flotation of organic debris and settling of clay particles in solution. The occluded LF was then aspirated via a pump and rinsed. In order to remove the NaPT from the heavy fraction (heavy F), deionised water was mixed with the material and centrifuged for 15 minutes at 4000 rpm 5 times. All fractions were oven dried at 100 °C overnight, weighed and physically ground to a fine powder before C analysis and isotope analysis. The recovery of the soil C density fractions was 96 %.

Commented [OV13]: Reviewers comment: 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 = ?%

Soil recovery included in text

Carbon analysis: bulk soils were ground and homogenised using a grinding mill (Planetary Mono Mill PULVERISETTE) in preparation for C analysis at the University of St Andrews laboratories using a Finnegan Delta plus XP gas source mass spectrometer coupled to an elemental analyser (EA-IRMS).

Decomposition estimates: A decomposition experiment was set up as an additional estimate of soil organic matter mineralisation, using birch wood sticks as a common substrate. Five sticks were placed in a mesh bag with three 2 cm holes cut into each bag to allow accessibility for both microfauna and fauna. In July 2011, eighteen bags were buried at 10 cm depth in groups of six, in close proximity on each plot (Fig. 2). Three bags, one from each group, was collected every two months. The sticks were weighed before the experiment started and again after collection, once they were air dried, to determine mass loss. The rate of decomposition was then calculated from the slope of a linear regression with time against mass loss.

4.5 Statistical analysis

Statistical analyses were conducted in R version 3.0.2 (R_Core_Team, 2012). Outliers were observed by visual inspection of the boxplots where points outside of the hinges (third quartile) were removed and the data were checked for normal distributions. The CO_2 flux and volumetric water content (VWC) data were not normally distributed and therefore log transformed prior to parametric statistical analysis. Linear mixed effect models were conducted to identify any relationships between the environmental variables and soil characteristics with soil CO_2 fluxes for each site, individually. In this respect, mixed model restricted maximum likelihood analysis (REML) were computed using the *lme4* package (Bates *et al.* 2014) to include random intercepts for each collar and for the effect of grazing nested within the burnt sites. Analysis of variance (ANOVA) and Tukey's Honest Significant Different (HSD) post hoc test were used to examine statistically significant differences between means of the environmental data among the sites. Linear regression analysis was used on the decomposition data and tested to identify any relationships with the soil CO_2 fluxes. Differences in soil C between the areas were analysed using a one-way ANOVA and TukeyHSD post-hoc test, after testing for normality and homogeneity of variances.

5. Results

5.1 Soil respiration and environmental drivers

The overall annual CO_2 mean for the pooled data set, including all types of land management, was 1.39 ± 0.05 µmol m^{-2} s^{-1}. The combination of grazing and burning significantly increased soil CO_2 fluxes at Wayqecha (2003) but not at Acjanaco (Fig 2). Regardless of land use, the plots at Wayqecha (2003) had greater variability and overall higher mean annual soil temperature (15 °C) and CO_2 flux (1.34 ± 0.09 µmol m^{-2} s^{-1}) compared to the sites in Acjanaco (2005) (12 °C and 0.79 ± 0.03 µmol m^{-2} s^{-1}) (Table 2). The highest measured temperatures and CO_2 fluxes at Wayqecha were synchronously recorded during July-11, November-12 and March-12, whereas at Acjanaco the changes in CO_2 flux with season and temperature were less pronounced.

Commented [OV14]: Reviewers comment: L.261-262: Does at Acjanaco grazing and burning significantly increase soil CO2 fluxes? From Figure 2, I do not get the impression.

Text changed to clarify that we mean grazing and burning significantly increase soil CO2 fluxes at wayqecha, not Acjanaco.

Season (which run from October to March), soil and air temperature were the main drivers of soil respiration (*p*-values = 0.031, 9.3 x 10^{-7} and 0.0001, respectively), with higher temperatures having a positive effect on soil CO_2 fluxes. However, when analyzing the grazed-burnt plots at both Wayqecha and Acjanaco, there was no relationship between CO_2 fluxes and temperature or any of the other environmental variables measured.

Commented [OV15]: Reviewers comment: L.269: How is season defined? By soil temperature and VWC? Are soil temperature and air temperature not strongly correlated?

Season was defined in the site descriptions section

5.2 Decomposition rates

The decomposition of the birch wood sticks was slow, with an overall average weight loss of ~ 20 % in one year. Grazing alone appeared to slightly increase the rate of decomposition when all the data were pooled together (grazed: y = 104.53 + -4.23x, R^2 = 0.98, not grazed: y = 103.63 + -3.11, R^2 0.94), but burning alone did not affect decomposition rate (burnt: y = 103.34 + -3.57, R^2 = 0.96, not burnt: y = 104.82 + -3.76x, R^2 = 0.97) (Fig 3). Site-specific differences were observed for decomposition rates; for example, decomposition was generally faster at Wayqecha compared to Acjanaco. In particular, the grazed - not burnt plot at Wayqecha showed the fastest overall rate of decomposition (y = 101.98 + -0.19x, R^2 = 0.77) and the not grazed - not burnt plots (controls) had the slowest decomposition rates (Fig 3) on both sites.

Decomposition was not a strong overall predictor for CO_2 fluxes for the pooled dataset, although there were some strong correlations between these two variables at specific study sites. For example, there was a strong relationship between decomposition and soil CO_2 fluxes at Acjanaco (y = 0.38 + -0.18x, R^2 = 0.99) (i.e. faster mass loss = higher soil respiration), whereas at Wayqecha, this relationship was weak (y = 1.56 + 0.06x, R^2 = 0.07). Land-use did not appear to influence the decomposition rate-soil CO_2 flux relationship.

5.3 Soil C stocks

Commented [OV16]: Reviewers comment: L. 299: belowground carbon stock = soil carbon stock + living roots?

Change to: “Soil C stocks”

Grazing, burning and the combination of burning and grazing did not significantly alter total soil C at any depth down to 30 cm on either of the sites (Table 3). The overall sum of all the measured depths showed signs of a decrease in C stocks on the grazed soils, from 189 ± 32 Mg C ha^{-1} on the undisturbed sites to 130 ± 20 Mg C ha^{-1} on the grazed-burnt sites, but this was not statistically significant at the $P < 0.05$ level. On average, Acjanaco (2003) had higher C stocks (175 ± 17 Mg C ha^{-1}) compared to Wayqecha (2005) (150 ± 15 Mg C ha^{-1}).

The pooled dataset demonstrated that these soils have a notably large free LF (~20 %). When looking at the different treatments and averaging the data across the soil profile (0-30 cm), burning and grazing had a significant negative effect on the proportion of C in the free LF (Table 4). The free LF in the control soils made 20 % of the bulk soil mass and 30 % of the soil C content compared to the burnt-grazed soils, which had the smallest recovery of free LF (10

%) and had significantly lower C content (14 %). However, when analysing the depths individually, there was only a significant loss of C in the free LF at 10-20 and 20-30 cm depth, with a reduction of ~ 16 % (Fig 4). When analysing the two sites separately, the burnt- grazed soils at Wayqecha had a significantly smaller proportion of C in the free LF at 0-5 cm (*p*-value = 0.002), whereas at Acjanaco there were no significant differences among the land uses.

The occluded LF appeared to be positively affected by burning in comparison to grazing, with burnt soils displaying a significant increase in the occluded LF. For example, when pooling the data from across different soil depths (0-30 cm), for the two sites combined, the burnt soils had a much higher proportion of C in the occluded LF (12 %) compared to the not-burnt soils (7 %). There were no significant differences among the treatments in the heavy F, with an average of ~ 70 %.

6. Discussion

6.1 Soil respiration and decomposition rates

In this study, soil CO_2 fluxes ranged from 2.35 to 3.82 to Mg C ha^{-1} yr^{-1}, which is in the lower range (0.7 – 14.8 Mg C ha^{-1} yr^{-1}) of other high elevation montane grassland studies (Cao *et al.* 2004; Geng *et al.* 2012; Muñoz, Faz and Zornoza 2013; Fu *et al.* 2014) and corroborates prior work by Oliveras et al., 2014 (3.4 - 3.7 Mg C ha^{-1} yr^{-1}). The absence of a seasonal trend in temperature and moisture has also been noted in other studies from the same region (Girardin *et al.* 2010; Teh *et al.* 2014).

Higher soil respiration and faster decomposition rates were consistently measured on the plots at Wayqecha (burnt in 2003) than at Acjanaco (2005), which is in keeping with Oliveras *et al.,* 2014. These site-specific differences may not be a reflection of the age of burning but rather Acjanaco being at a slightly higher elevation and on average 4 °C cooler. Despite the variance in mean annual temperature, the two sites both showed a positive correlation between temperature and soil respiration. Interestingly though, the decomposition rates at Acjanaco correlated with the CO_2 fluxes, suggesting that decay was a good predictor of CO_2 flux. This was in contrast to the lower elevation site in Wayqecha, where CO_2 fluxes did not correlate with decomposition rates, implying that autotrophic respiration or other environmental factors may have had a stronger influence on soil respiration.

Burning alone or grazing alone enhanced soil respiration and decomposition rates when these land management practices were considered separately, with soil temperature identified as the main environmental driver in each of these treatment types. However, when plots had been exposed to both burning and grazing together, soil temperature no longer correlated well with soil respiration. The combination of burning and grazing also produced higher soil respiration rates than the two treatments independently. While this pattern has been identified before in other studies (Ward *et al.* 2007), the drivers of this increase are less well understood, and the influence of grazing and burning have been known to have confounding effects (Michelsen *et al.* 2004). One potential explanation is that burning and grazing together act synergistically, and may obscure the influence of temperature due to the action of other complex processes or drivers, such as changes in plant C allocation and autotrophic respiration following the effects of the two combined disturbances. For example, studies have found that when foliage is cut, photosynthate and other resources are allocated to the growth of new shoots rather than to the roots (Schmitt, Pausch and Kuzyakov 2013), causing a decline in root respiration (García-Oliva, Sanford and Kelly 1999). The resulting root death may enhances heterotrophic microbial activity, counteracting the effects of reduced root respiration.

Commented [OV17]: Reviewers comment: L. 362. As heterotrophic respiration is not measured: may enhances.., as it is a speculation

'may enhance' included in text

Alternatively, burning can cause significant losses of N due to combustion, and grasses may compensate for increased N limitation by increasing their allocation to roots, thereby increasing root respiration and potentially promoting enhanced belowground C cycling (Johnson and Matchett 2001). Some evidence was found for this type of response in prior work; Oliveras *et al.,* 2014, found higher below and above-ground C stocks in undisturbed soils. While overall net primary productivity (NPP) was higher on undisturbed sites, NPP belowground was greater with grazing and fire, suggesting a shift in plant allocation patterns after these disturbances.

6.2 Belowground C stocks

Overall, large total SOC stocks were measured in these montane grasslands (123 – 238 Mg C ha^{-1}), which is in keeping with other high elevation grassland studies and are probably attributable to low temperatures and wet conditions causing slow mineralisation of SOM and turnover rates. For example, in the Qinghai-Tibetan Plateau grasslands and páramo grasslands of the Colombian, Ecuadorian and Peruvian Andes, total SOC stocks can range between 80 – 250 Mg C ha^{-1} (Hofstede 1995; Zimmermann *et al.* 2010; Farley *et al.* 2012; Li *et al.* 2013; Muñoz, Faz and Zornoza 2013; Oliveras *et al.* 2014b).

Commented [OV18]: Reviewers comment: L.376: It would be nice to have a range of soil C stocks found in montane grassland soils

A range of C stocks from montane grasslands has been included in the text

Soil C stocks were higher at Acjanaco than at Wayqecha. This is in agreement with Oliveras *et al.,* 2014, although the Acjanaco sites in this previous study were higher (253 compared to 175 Mg C ha^{-1} reported here), perhaps reflecting within site spatial heterogeneity. There was no significant effect of either burning or grazing but grazing had a more negative effect than burning on the total soil C stocks. This negligible effect of burning may be a consequence of low intensity fires, fire-resilient grasses, and potentially low fuel loads at the time of burning (Knicker 2007). Grassland fires on slopes can move very quickly, so even when intense, the transfer of heat to the soil is less damaging due to low residence times (Rollins, Cohen and Durig 1993). As a result, surface temperatures do not typically exceed 100 °C or 50 °C at 5 cm depth (Campbell *et al.* 1995), and organic matter can only be fully volatilized between 200 and 315 °C (Knicker 2007). Even if the soils were dry at the time of burning which is possible during the dry season, then belowground temperatures would rise very slowly because of the insulating properties of air-filled pores, which curtail heat transfer belowground (Neary *et al.* 1999).

Commented [OV19]: Reviewers comment: 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.

Text changed to clarify that we mean both burning and grazing had no significant effect on total soil C

Grazing on the other hand, had a more negative impact on total SOC content than burning but there was not a significant loss of total soil C. One explanation is that the grazing pressure in these sites may have been below the threshold required to cause severe degradation, supporting previous studies in the Peruvian Andes, where they also found no significant effect of grazing or burning on total SOC stocks (Gibbon *et al.* 2010; Oliveras *et al.* 2014b).

Overall, the free LF was larger than in other tropical systems (30 % of total soil C). By comparison, studies in Equador, Brazil and Puerto Rico found the free LF ranged from only 4-12 % of total soil C content (Paul, Veldkamp and Flessa 2008; Marin-Spiotta *et al.* 2009; Potes *et al.* 2012). However, it is difficult to compare the results of this study to other tropical fractionation studies because in general, most field sites are in tropical lowland pastures where soil C stocks tend to be lower. When comparing to other high elevational studies, for example, in permafrost meadow ecosystems in the Tibetan Qinghai Province, results are similar, with the free LF making up 27 % of the total soil C stocks (Dörfer *et al.* 2013).

Commented [OV20]: Reviewers comment: L.401-411: Please expand literature. In addition, please check the number cited (10%) and carefully consider the land use type.
It would be nice to have the range of free LF found in tropical soils in order torank and interpret the gained results (L 403-406).

10 % and the type of land use is correct.
Additional free LF data from other studies has been included for comparison

Grazing had a negative impact on the free LF. As grazing is known for reducing aboveground biomass (Johnson and Matchett 2001; Gibbon *et al.* 2010), a lower incorporation of detritus into the soil is not surprising and has been observed in other grazing studies (Figueiredo, Resck and Carneiro 2010; Cao *et al.* 2013). The effects of grazing on the free LF were most pronounced when grazing and burning occurred together, in which case, the free LF showed the most pronounced declines. To our knowledge there are also no other studies assessing the impact of grazing and burning on soil C fractions in high altitude tropical grasslands.

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. In this study, the significant positive effect of burning on the occluded LF may be the results of charcoal particles (from burning) becoming incorporated into the occluded LF. Charcoal, because of its low density, tends to reside in the lighter fractions (Cadisch *et al.* 1996; Glaser *et al.* 2000; Sollins *et al.* 2006), despite its recalcitrance. Because the fires took place almost ten years ago, the charcoal may no longer be resident the free LF but may have become occluded into soil micro-aggregates due to its high sorptive capacity (Qayyum *et al.* 2014). Once incorporated into micro-aggregates, charcoal can be maintained for centuries after fire (Zackrisson, Nilsson and Wardle 1996).

Commented [OV21]: Reviewers comment: 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?

Question answered in authors response and text changed to include a sentence on long-term effects in short-term experiments

7. Conclusions

This study highlights the complexities of how land management can affect soil C dynamics in montane tropical grasslands. The results suggest that montane grasslands are resilient to soil

C losses under moderate intensity land use. Total C stocks appeared unaffected by burning and grazing, although a change was observed in the distribution of soil C across different soil C fractions, with burning leading to a significant reduction in the free LF pool and an enhancement of the occluded LF pool. Most specifically, our study shows that land management affected the magnitude and drivers of soil respiration and decomposition. Individually, burning and grazing alone increased soil CO_2 fluxes, which was apparently driven by shifts in soil temperature. However, the combined effect of burning and grazing together interacted synergistically, leading to enhanced soil respiration rates, while simultaneously obscuring the role of temperature and other environmental drivers, potentially due to changes in patterns of plant C and N allocations.

8. Acknowledgements

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9. Authorship

V. Oliver designed the study, conducted the fieldwork, statistical data analysis and wrote the manuscript. I. Oliveras designed the study, provided supervision and contributed to writing the manuscript. J. Kala and R. Lever conducted fieldwork and laboratory analysis. Y. A. Teh obtained funding for the work, provided supervision for the whole study and contributed to writing the manuscript.

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Figure 1 Map illustrating the two sites in the high elevation montane grassland (circles). The green area represents the Manu National Park.

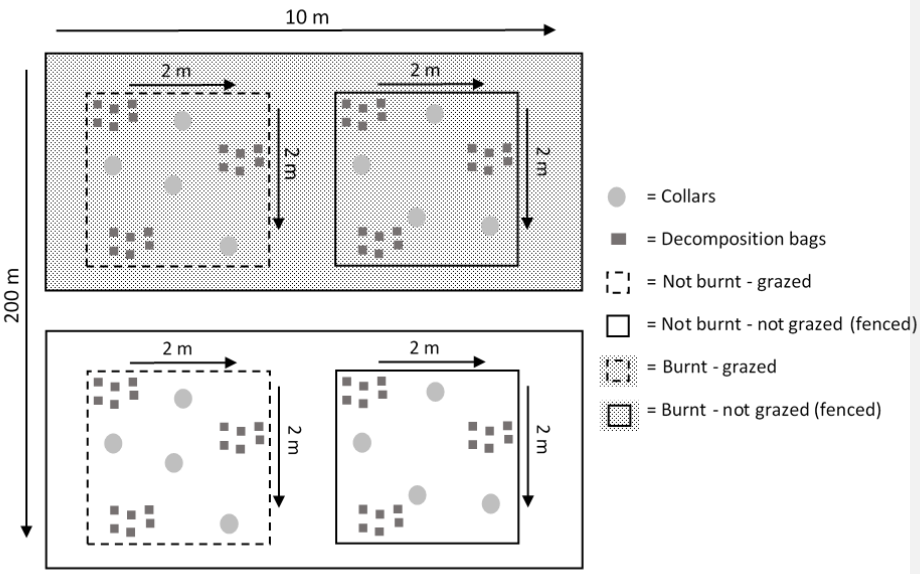


Figure 2 Schematic diagram illustrating the set-up of the plots. This experimental design was established at both Acjanaco and Wayqecha. Soils from three pits in each plot were collected for analysis.

Commented [OV22]: Diagram showing the plot set-up included

Table 1 Soil description for each land management at Wayqecha and Acjanaco (mineral soil particle size taken from (Diem *et al.* 2017 - *submitted to Biogeosciences*).

Site	Land use	Bulk density (g cm^{-3})		pH	Mineral soil particle size		
					Sand	Silt	Clay
		0-10 cm	10-20 cm	0-10 cm	0-10 cm		
Wayqecha (2003)	Grazed - burnt	0.45 ± 0.03	0.37 ± 0.05	4.3 ± 0.2			
	Not grazed - burnt	0.25 ± 0.13	0.47 ± 0.03	4.1 ± 0.1			
	Grazed - not burnt	0.43 ± 0.01	0.61 ± 0.10	4.3 ± 0.1			
	Not grazed - not burnt	0.30 ± 0.07	0.46 ± 0.05	4.5 ± 0.2	43.0 ± 3.2	54.4 ± 3.0	2.6 ± 0.2
Acjanaco (2005)	Grazed - burnt	0.41 ± 0.03	0.47 ± 0.05	4.8 ± 0.2			
	Not grazed - burnt	0.40 ± 0.02	0.45 ± 0.06	4.4 ± 0.2			
	Grazed - not burnt	0.34 ± 0.03	0.35 ± 0.03	4.1 ± 0.1			
	Not grazed - not burnt	0.36 ± 0.06	0.48 ± 0.13	5.0 ± 0.3			

Commented [OV23]: Reviewers comment: 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 soil is descripted in Table 1.

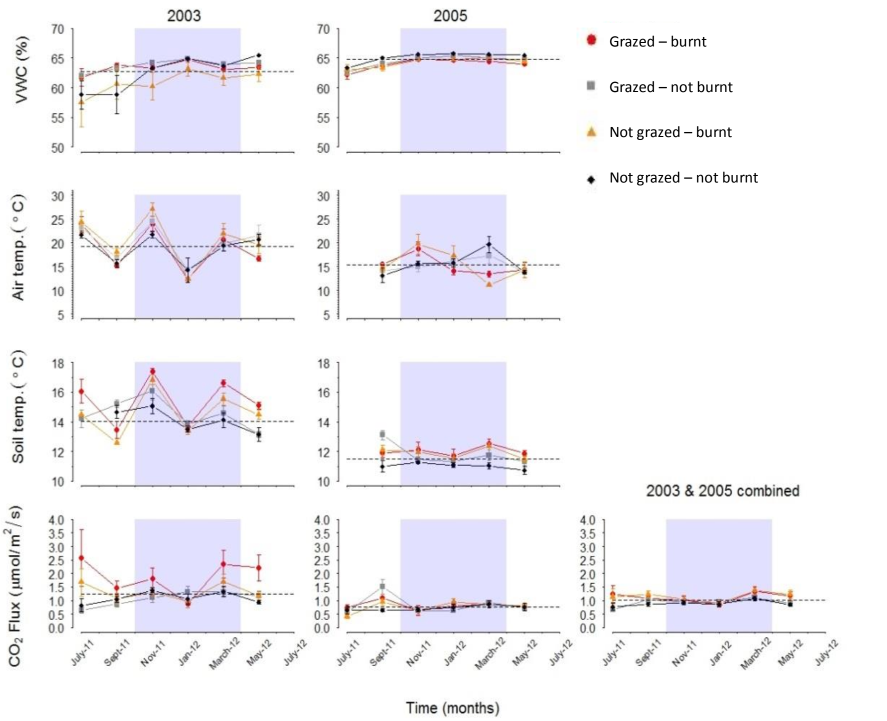
More information on soil properties included for each plot

Table 2 Annual and seasonal mean soil temperature, VWC and CO_2 flux for Wayqecha and Acjanaco for each land management system. Different letters down the columns represent significant differences between sites.

Commented [OV24]: Annual emissions removed

Site / land use	Soil temp. (°C) at 5 cm	VWC (%) at 5 cm	CO_2 flux ($\mu mol\ m^{-2}\ s^{-1}$)
Wayqecha (2003)	14.7 ± 0.1	62.3 ± 0.4	1.31 ± 0.09
Grazed – burnt	15.3 ± 0.3[a]	63.4 ± 0.3[ab]	1.88 ± 0.23[a]
Grazed - not burnt	14.5 ± 0.2[ab]	63.8 ± 0.2[ab]	1.07 ± 0.07[b]
Not grazed - burnt	14.6 ± 0.3[ab]	60.9 ± 1.0[c]	0.99 ± 0.08[bc]
Not grazed - not burnt	14.1 ± 0.2[b]	62.5 ± 0.8[bc]	1.10 ± 0.07[ab]
Dry season	14.1 ± 0.2	61.4 ± 0.8	1.35 ± 0.16
Wet season	15.1 ± 0.20	63.8 ± 0.3	1.31 ± 0.10
Minimum	11.6	29.9	0.22
Maximum	18	65.8	8.33
Acjanaco (2005)	11.6 ± 0.1	64. 5 ± 0.1	0.91 ± 0.03
Grazed – burnt	12.0 ± 0.2[c]	64.0 ± 0.2[ab]	0.82 ± 0.05[bc]
Grazed - not burnt	11.5 ± 0.2[cd]	64.5 ± 0.2[ab]	0.84 ± 0.07[bc]
Not grazed - burnt	11.9 ± 0.1[cd]	64.2 ± 0.2[ab]	0.77 ± 0.05[c]
Not grazed - not burnt	10.8 ± 0.1[d]	65.1 ± 0.2[a]	0.72 ± 0.05[c]
Dry season	11.6 ± 0.1	63.8 ± 0.2	0.81 ± 0.04
Wet season	11.7 ± 0.1	65.1 ± 0.1	0.74 ± 0.03
Minimum	9.5	57.1	0.09
Maximum	13.7	67.7	2.69
GRAZED – BURNT	13.8 ± 0.2[a]	63.7 ± 0.2[a]	1.35 ± 0.13[a]
GRAZED – NOT BURNT	13.2 ± 0.2[a]	64.1 ± 0.1[a]	0.95 ± 0.05[b]
NOT GRAZED – BURNT	13.3 ± 0.2[a]	62.6 ± 0.5[a]	0.88 ± 0.05[b]
NOT GRAZED – NOT BURNT	12.6 ± 0.2[a]	63.8 ± 0.4[a]	0.91 ± 0.05[b]

Table 3. Mean soil C content (Mg C ha^{-1}) for each depth and total C stocks (0-30 and 0-20 cm) on all the land management systems. Different letters down the columns within each depth represent significant differences among sites. All values are given with 1 standard error of the mean (n = 3).



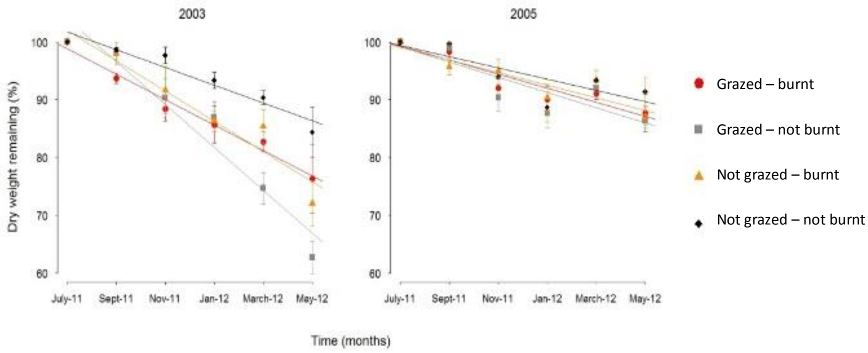


Figure 4 Mass losses (%) of sticks from the decomposition experiment (*n* = 3) on two burnt sites (2003 = Wayqecha and 2005 = Acjanaco) with grazed subplots and control plots.

Table 4 Mean mass recovery of density fractions and proportion of total C residing in the three density fractions (%) from the total soil profile (0-30 cm). Different letters down the columns represent significant differences.

	Free LF		Occluded LF		Heavy F	
	Fraction of total C (%)	**Mass of soil recovered (%)**	**Fraction of total C (%)**	**Mass of soil recovered (%)**	**Fraction of total C (%)**	**Mass of soil recovered (%)**
Grazed - burnt	14.0 ± 5.3^{b}	9.9 ± 3.6^{a}	10.8 ± 2.6^{ab}	9.8 ± 3.4^{ab}	76.0 ± 8.0^{a}	78.4 ± 7.2^{a}
Not grazed - burnt	19.7 ± 8.3^{ab}	15.1 ± 8.5^{a}	14.2 ± 2.5^{a}	11.3 ± 4.7^{a}	66.1 ± 10.5^{a}	76.6 ± 8.3^{a}
Grazed - not burnt	22.7 ± 13.3^{ab}	16.2 ± 8.5^{a}	8.9 ± 2.1^{bc}	5.3 ± 1.6^{bc}	68.3 ± 14.0^{a}	76.7 ± 8.1^{a}
Not grazed - not burnt	30.0 ± 5.7^{a}	19.5 ± 5.5^{a}	5.2 ± 0.8^{c}	4.3 ± 0.7^{c}	64.7 ± 6.1^{a}	69.7 ± 5.8^{a}

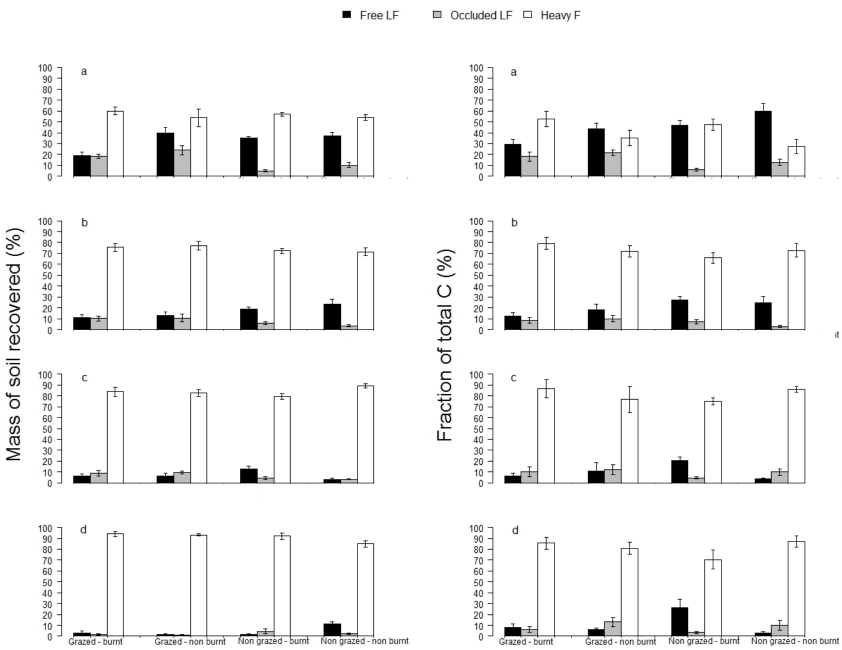


Figure 5 Mass of soil recovered in the three density fractions (%) on the four left bar plots and the proportion of total C residing in the three density fractions (%) on the four right bar plots for the different land uses (a = 0-5 cm, b = 5-10 cm, c = 10-20 cm, d = 20-30 cm). Error bars indicate 1 standard error of the mean (*n* = 6).