Interactive comment on "Livestock exclosure with consequent vegetation changes alters photo-assimilated carbon cycling in a Kobresia meadow" by J. Zou et al.

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Thank you for the comments on our manuscript in the interactive discussion and we replied to the questions and suggestions point by point.

Anonymous Referee #2

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This study investigated the impact of 6-years livestock exclosure on plant community structure and C cycling on a grassland field on Qinghai-Tibetan Plateau. Based on the plant survey, authors found livestock exclosure reduced the plant species diversity, in particular legumes and forbs. In addition, livestock exclosure significantly increased the litter production and reduced the fresh plant biomass. By applying the ^{13}C-pulse labeling technique, authors tracked the C entered the plant and soil system for 32 days.

The biggest concern I have for this study is the experiment design. Based on authors' description, there is only one fenced area (100×100 m) for the livestock exclusure treatment. All the sites for fenced treatment were chosen within this 100 x 100m area – a typical pseudo-replication. Is there any reason for not having proper replications? If experiment was based on psedo-replication, is there any information/analysis authors can provide to increase the confidence of this research finding? Apart from this issue, the grazed sites were chosen from outside the fenced area. This is a very vague description of grazed experiment sites. Were these four grazed sites from the same side of the fenced area (say all in south side of the fenced area?) or were these grazed sites from four different side of the fenced area? How far were these four grazed sites apart from each other (same for the fenced area- although they were inside this 100 x 100 m area)? Such information is important, as the results could be, or at least partly, due to the location effect (water flow, lights...).

Response: At this point, we think that our design can be accepted for making the results conclusive. Our study involves two treatments, the meadow with livestock exclosure year round and the meadow under continuous winter grazing. All the replications for the fenced treatment are dispersed at random within the 100 ×100m area, with at least 5m apart from each other. The replications for the grazed treatments situate at the north side of the fenced area, and have the same distribution with the experiment units in the fenced treatment. The experiment sites locate along a valley floor, and the two treatments are characterized by the same slope, soil type and other features. It could reduce or eliminate the effects of potential bias from intrusion sources, and increase the

confidence of this research finding.

When analysing ^{13}C in soil, authors first remove carbonates in soil. I assume the carbonates C present as a reasonable amount in relation to total organic C. How much C present in carbonates in this type of soil in general?

Response: The contents of C present in carbonates in the soil are 0.4, 3.9 and 7.6 g/kg in the layer of 0~5cm, 5~15cm and 15~30cm, respectively.

If carbonate-C is a not neglectable, why authors did not measure the ^{13}C present in inorganic form?

Response: In the plant-soil system, the plant contributes to the soil organic C pool rather than the inorganic C, moreover, there are large isotopic differences between organic and inorganic soil C, so we get rid of the inorganic C in the soil and mainly traces the change of $\delta^{13}C$ in soil orgnic C pools, instead of measuring the ^{13}C present in inorganic form.

Authors traced the ^{13}C from plant shoots, roots and soil. However, they removed the soil attached to the roots. These soils, also named rhizosphere soils, normally contains a great portion of freshly fixed C and are considered a C hotspot. Plant exudes C compounds into this narrow zone and the microbial biomass (particularly the ones utilize the plant-derived C) are normally much higher than these in the background soil. Such important C niche should not be discarded.

Response: We are in agreement with the importance of the rhizoshpere soil C pool as the reviewer mentioned, however, we didn't divided the soil into the rhizoshere soil and bulk soil in the study.

Authors mentioned that history, degrees of degradation, grazing intensity ect. are also important for assessing the impact of exclosure on grassland ecosystem function. So please provide all the relevant information in the manuscript, such as grazing intensity (rather than simply state moderate grazing in winter).

Response: We added the relevant information in the manuscript: Our experiment site is a winter grazing pasture, grazing moderately (3.51 sheep ha^{-1} $season^{-1}$) from 1 January to 30 March per year.

Why the ^{13}C is shown in the % of recovery rather than the amount of ^{13}C entered in different fractions?

Response: The amount of ^{13}C entered in different fractions is influenced by many other factors besides the treatment effect, such as the labeling method, time and so on. It is unreasonable to compare the effects of treatment using the amount of ^{13}C. However, the % of recovery participated in different fractions is statistically stable and only

influenced by the treatment effects in our study. Therefore, we use the ^{13}C data in the % of recovery rather than the amount of ^{13}C entered in different fractions.

The manuscript needs to be improved significantly for publication in terms of English (make sure the sentences are complete and clear), content and clarification. The manuscripts can also be reduced in size greatly to improve the precision. For example, on page 10 line 5-8. These two sentences can be easily combined into one. In the result section, authors do not need to repeat all the data which are already present in the table or figures. Another example is the first few sentences of discussion section 4.1 is simply repeating the results section. On page 15, line 9-18 can be removed from the discussion section, as they are either not relevant or belonging to method section.

Response: We checked the manuscript and corrected these points as the reviewer suggested, thank you!

On page 15, line 16 "we used an average $\delta^{13}C$ of the four replications to assess the dynamics and allocation of ^{13}C in the plant-soil system...". Why use average $\delta^{13}C$ of four replicates instead of use individual measured $\delta^{13}C$ to calculate the total amount of ^{13}C and recovery of ^{13}C?

Response: The total amount of ^{13}C is calculated by the equation: ^{13}C amount (mg m^{-2}) = ^{13}C excess (at %) · ^{13}C pool size (g m^{-2}) · 10. There are two independent variables determining the amount. These two variables may vague the variations between the treatments due to the probable large intra-group variations. Also, it has been suggested that the pulse labeling tended to cause less homogeneous ^{13}C distribution compared with natural labeling. Therefore, we use average $\delta^{13}C$ of four replicates instead of using individual measured $\delta^{13}C$ to calculate the total amount of ^{13}C and recovery of ^{13}C, in order to discover the treatment effects.

Discussion part needs to improve significantly (go deeper instead of touch the surface). Authors compared the findings with other studies, but did not discuss the importance and impact of such findings to the ecosystems. In addition, in many places, authors provided the possible support for the observed results without further explanation.

Response: We appreciated the comment as the reviewer mentioned and improved it.

For instance, on page 15 line 5, authors mentioned that nutrient level may be another factor affecting the plant community. Is there any data or data from other relevant studies to support this hypothesis?

Response: We were sorry that we could not provide more data or data from other relevant studies to support it. It was an idea. We deleted it in the manuscript, thank you!

I felt very strange that the ^{13}C in roots was so much lower than the ^{13}C in soil. As I understand, C allocated from atmospheric CO_2 to belowground is mainly through plant roots, apart from a

small portion of C can be directly fixed by autotrophic microbes from $^{13}CO_2$. Authors only simply stated that "the finding is consistent with another study using stable C labeling (hafner et al., 2012)". Then authors explained the plants were in the flowing stage and most of C may be attributed to produce the seeds. This explains low ^{13}C in roots. But where the large amounts of ^{13}C in soil come from? I would like to read more to understand this unusual finding.

Response: In our study, we investigated the new assimilated ^{13}C allocation in several carbon pools of the plant-soil system, including shoot, root, soil and soil respiration. The ^{13}C allocation in roots was 4-8 times lower than that in soil at the end of the chase period. We indicated that the roots provided a rapid transport and much ^{13}C fluxed into soil as root exudate. Also, it was supported by the slowly and gradually increasing of ^{13}C in roots during the chase period.

On page 18, line 3-4, "suggesting that more ^{13}C is allocated into roots in the fenced grassland". From figure 3, the difference is not significant. Also on page 18, line 5-6 "there was less ^{13}C migration into soils under exclosure". However, from page 11 line 9-10, authors stated that more ^{13}C entered plant-soil system in fenced compared to control grazed plots (495 mg vs. 370 mg). Simple calculation showed that more ^{13}C amount present in soil in fenced than grazed plot. I think authors want to say is the "less portion of total fixed ^{13}C by plants migrated into soil in fenced plot compared to grazed plot." Please be precise on describing and making conclusions. But again, why not use amount of ^{13}C data? Why use recovery %?

Response: We agreed with the comment as the reviewer mentioned. Indeed, there were some mistakes with the description of the conclusions, and we corrected it. Due to the pulse labeling method, we think that using recovery % rather than amount of ^{13}C could reveal the treatment effects on carbon cycling in the plant-soil system.

In the conclusion section, authors stated on line 6 "There were relationships between the variations of vegetation community structure and C cycling". From the manuscript, I did not find any data to support this sentence. Authors did discuss the possible links between these two parts, although. Without further supporting evidence, please reword this sentence accordingly. Do the C% in shoot and roots among four types of vegetation differ a lot? Is it possible to find some links by the shifting the biomass of different types to the C cycling? Just an idea.

Response: Our results indicated livestock exclosure decreased the shoots biomass and plant richness compared with the grazing management. Moreover, our data from stable isotope analysis showed that exclosure decreased the photo-assimilated ^{13}C cycling rate in plant-soil system. Several researches have demonstrated that shoot biomass and plant richness had effects on C sequestration and cycling. Higher shoot biomass were beneficial to C transport belowground. Plant richness was related to shoot biomass by using diverse sources including light, nitrogen and so on. Also, it could stimulate the activity of microorganism in soil to promote C turnover. Based on our results, we could

suggest livestock exclosure altered the photo-assimilated ^{13}C cycling through the vegetation change.

Table 1: why not present the SD for the species richness?

Response: In the method, we defined the species richness as the sum of species number found in the four quadrates at each treatment site. As a discrete variable, we think it is not suitable to present the SD for the species richness.

What is the vegetation cover (%)- which is never be mentioned in the manuscript.

Response: We were sorry not to mention the vegetation cover (%) in the manuscript. We added the contents about the vegetation cover in the manuscript. Thank you!

Table 2: Which sample day is the presented C stock data based on?

Response: The carbon stock data presented in Table 2 are the average carbon stocks during the whole 32-day chase period. The carbon stocks (g m^{-2}) of the different pools in the plant-soil systems were assumed to be constant during the chase period (in Discussion 4.2 on page 15 line 14-16).

Figure 1 can be removed, as it did not add any extra information to the manuscript.

Response: Thank you, we removed it.