BGD-10-9493-2013

Effects of belowground litter addition, increased precipitation and clipping on soil carbon and nitrogen mineralization in a temperate steppe *Biogeosciences Discuss*

Dear editors,

Thank you for your positive decision on our manuscript entitled "Effects of belowground litter addition, increased precipitation and clipping on soil carbon and nitrogen mineralization in a temperate steppe". We found comments from reviewers are very constructive and helpful in improving the quality of this manuscript. We revised and partly re-wrote the manuscript based on comments and suggestions given by the reviewers. All changes were highlighted with color. The English language has also been improved carefully.

Below you will find our responses to the reviewers. We appreciate your consideration of our manuscript. Sincerely,

Linna Ma, Renzhong Wang

Responses to reviewer:

Referee #1

The present study deals with carbon and nitrogen dynamics in steppe grassland soils as affected by driving factors. The stated purpose is to evaluate the effects of 3 treatments: 2 changes in C inputs (either through direct incorporation of litter into the top soil or by clipping of aboveground biomass) and changes in precipitation (simulated with irrigation), and possible interactions. While the study does address a relevant topic for the study of ecosystem biogeochemistry (i.e. the dynamics of C and N in soils under simulated changes in precipitation and C inputs), and involves a large amount of measurements and a reasonable experimental design, it has a number of major flaws. 1. The first and most important is the lack of clear hypotheses to be tested, with only the objective of finding effects of the mentioned driving factors on C and N mineralization but based on no specific mechanism.

Thank you for your suggestion. Your comments have been fully considered in the revised MS. We developed a specific hypothesis about belowground particulate litter addition, increased precipitation, clipping (reduced belowground photosynthates allocation) and their might interact on soil C and N mineralization based on the specific mechanism. Please see Line 89 - 95, 101 - 105.

2. A rather large amount of soil biological and chemical properties are measured and correlated with mineralization rates. Because there are no clear questions, all variables are included in several multiple linear correlations which (almost necessarily) result in some significant models that relate the spatial variability of fluxes with mostly soil moisture and microbial community properties. These correlations are interpreted as causative and the treatment effects explained through them.

Thank you for your suggestion. We proposed a specific hypothesis based on the specific mechanism. Therefore, all the relate variables in this study were necessarily. We used the method of stepwise regression to guard against over-interpreting significant multiple-correlations. Stepwise regression combines forward selection and backward elimination. At each step, the best remaining variable is added, provided it passes the significant at 5 % criterion, then all variables currently in the regression are checked to see if any can be removed, using the greater than 10 % significance criterion. The process continues until no more variables are added or removed. It is not guaranteed to find the best subset of independents but it will find a subset close to the best. If several independent variables are existed higher correlations, which may contribute to overfitting or spurious correlation, stepwise regression can delete one or more correlated independent variables to lest including complexity such as interactions. Therefore, stepwise regression would not result in some significant

models. Please see the revised MS.

3. While a relationship between microbial properties and respiration is expected, they may both be the consequence of changes in other underlying driver factors such as SOM quality and quantity, or changes in soil physical characteristics. It should be noted that incorporation of litter in the study will change soil density and water retention characteristics (and its effects on respiration) and these are not well considered in the study.

Thank you for your suggestion. We have measured soil organic matter quantity (soil light organic matter and heavy organic matter content). Please see Fig. 3.

In this study, we expected to add the particulate litter to the upper soil layers without drastically damaging the root systems and soil physical characteristics. For this purpose, we carefully used sharp forks to loosen the surface soil (10 cm), and gradually and homogeneously added litter to the soil in the 0 - 10 cm layer. The soil pores were carefully filled with soil and gently compacted by hand. To create consistent soil disturbance across treatments, the plots with no particulate litter addition were processed in the same manner as the plots that received particulate litter addition. Therefore, soil physical characteristics such as soil water retention were not change significantly in this experiment (data not shown). However, it is a pity we do not measured soil density. We will give a measurement of these data in the next step of our research.

4. Although ST and SM where measured in the field, the mineralization measurements where done in the lab and it is not clear what moisture and temperature are being related to these and why a T and M model was not applied to estimate their effects throughout the year in the field. It is also not clear when and how often SCM was measured

Thank you for your suggestion. In the field, it is hard to estimate soil C mineralization rate (microbial respiration), because soil respiration measured in situ included plant root respiration and microbial respiration. Therefore, C mineralization could only be

measured in the lab with short-term incubation, this method could also reflect potential C mineralization rate at different sampling times during the growing seasons. Other studies also used the same method to measure soil C mineralization such as Zhang et al. (2005, Global Change Biology 15: 1544 - 1556). In addition, the measurement of soil N mineralization was conducted in situ incubation, thus we established models among soil C mineralization (and N mineralization) and soil temperature and moisture were reasonable. However, it is a pity we do not consistent recorded soil temperature and moisture throughout the whole year using dataloggers. We will give a measurement of these data in the next step of our research. We also added some information about measurement of soil temperature and moisture (0-10cm). Please see Line 175-177.

5. Apart from a descriptive analysis the study does a poor job at explaining the actual underlying connections between factors. The large number of factors included without an a-priori hypothesis linking them makes the interpretations in the discussion very weak and speculative. The lack of relevant results is noticed in the closing paragraph, which states that effects were observed but gives no further conclusion.

Your comments have been fully considered in the revised MS. We developed a specific hypothesis based on the specific mechanism and explained the underlying connections between factors. Please see Introduction, Line 89 - 95, 101 - 105.

6. The English of the manuscript still requires considerable efforts to be understood. Many grammatical mistakes are found and many passages need revising. I recommend that the paper is revised properly by a native speaker before the next submission.

Thanks for your suggestions. The English language has been improved in the MS.

7. Also some terms were not defined (light and heavy organic matter) and some are confusing (e.g. SOM input is used to refer to addition of litter to topsoil, but

clipping will also change below-ground SOM inputs). In general, the connection of ideas throughout the text should follow a more rigorous logic.

Your comments have been fully considered in the revised MS. We developed a specific hypothesis about belowground litter addition, increased precipitation, clipping (reduced belowground photosynthates allocation) and their might interact on soil C and N mineralization based on the specific mechanism. Therefore, the connection of ideas throughout the MS followed a rigorous logic in the revised MS. Please see Line 89 - 95, 101 - 105. In addition, some not defined and confused terms were clarified in the revised MS. Please see Line 313 - 314.

8. The large number of bar plots is non informative.

We added some information and checked all the figures carefully in the revised MS.

9. The study finally shows that changes in C input and precipitation have an effect. This is not at all new nor unexpected. They also show significant interaction effects. However, there is finally no convincing argument explaining these. E.g. moisture and temperature conditions in the field at different depths were likely changed with both clipping and SOM addition. These conditions throughout the soil profile, which are main drivers of SOM decomposition, could alone be responsible for the observed interactions, but this more detailed ST and SM information is not given. ST and SM being known as the major drivers of SR should be measured in detail at several depths in such type of field experiments. Thanks for your constructive suggestion. Admittedly, soil moisture and temperature are the main drivers of C and N mineralization, and we also found that all the concurrent seasonal variations of soil temperature and moisture significantly contributed to the temporal fluctuations of soil C and N mineralization in this study. However, in spatial scales, we considered responses of soil C and N mineralization to changes in spatial location of different treatments and replications and excluded the influence of sampling time. These results showed that soil net C and N mineralization rates only correlated with soil microbial biomass, soil moisture, the ratio of fungal to

bacterial PLFAs and arbuscular mycorrhizal fungi PLFAs. Therefore, the effects of soil temperature and moisture on soil C and N mineralization may not the most important drivers at spatial scales. Other studies also found similar results such as Xia et al. (2009, Global Change Biology 15: 1544 - 1556), and Xu and Wan (2008, Soil Biology Biochemistry 40: 679 - 687). Moreover, soil moisture in belowground litter addition and increased precipitation under the clipped plots were similar to those in the unclipped, thus soil moisture could not be responsible for the observed interactions between clipping and belowground litter addition (and increased precipitation).

It is unfortunate that soil moisture and temperature data in the field at deeper depths (10-20cm, 20-30cm) were not be recorded at a finer temporal scale. We only measured soil temperature and moisture (0-10cm) during the time of sampling. We rewrote this section in the revised MS. Please Line 175 - 177. We will give a measurement of these data in the next step of our research.

10. I would suggest that the authors focus on specific mechanisms relating their treatments with the response variables and derive hypotheses that can be tested with available or new data, and that they avoid over-interpreting significant multiple-correlations that are common in soils.

Thanks for your suggestions. The old MS was lack of clear hypotheses and based on no specific mechanism. In the revised MS, your comments have been fully considered. We developed a specific hypothesis about belowground litter addition, increased precipitation, clipping (reduced belowground photosynthates allocation) and their might interact on soil C and N mineralization based on the specific mechanism. Please see Line 89 - 95, 101 - 105.

We also used the method of stepwise regression to guard against over-interpreting significant multiple-correlations. Stepwise regression combines forward selection and backward elimination. At each step, the best remaining variable is added, provided it passes the significant at 5 % criterion, then all variables currently in the regression are checked to see if any can be removed, using the greater than 10% significance

criterion. The process continues until no more variables are added or removed. It is not guaranteed to find the best subset of independents but it will find a subset close to the best. If the several independent variables are existed higher correlations, which may contribute to overfitting or spurious correlation, stepwise regression can delete one or more correlated independent variables to lest including complexity such as interactions.

Some specific comments:

1. L 28 Change 'SOM input' to some other expression throughout the manuscript, more specific to the treatment (e.g. litter addition). SOM input is too vague and can refer to above or below-ground inputs.

Thanks for your suggestions. We corrected this expression in the revised MS. Please see the revised MS.

2. L 28 Do not use the expression 'SOM inputs (or increased precipitation)' as the two are very different factors even if they showed similar effects. They are not interchangeable so should not be 'or' but rather 'and'.

Thanks for your suggestions. We corrected these expressions in the revised MS. Please see the revised MS.

3. L30 The first sentences here sound too repetitive.

The sentences were rewritten in the revised MS. Please see Line 26-31.

4. L34 year, and L35 NNR was not defined L37- 38 again parenthesis problem.

We rewrote this sentence in the revised MS. Please see Line 34-40.

5. L79-80 Sentence is not clear.

We deleted this sentence in the revised MS.

6. L101-104 Both question 1 and 2 are the basically the same.

We rewrote this section in the revised MS. Please see Line 101-105.

We appreciate your constructive comments and suggestions that have helped us improve this manuscript.

Referee #2

===== General comments =====

This ms describes a manipulative field experiment conducted in a temperate grassland steppe, in which organic matter input (clipping and direct incorporation into soil) and precipitation were the main factors. The authors examined the resulting effects on soil C and N mineralization and microbial community structure. This is an interesting subject, the approach taken has novel aspects, and in general is appropriate for Biogeosciences.

1. There are a number of significant problems, however. First, the clipping portion of the experimental design is quite problematic: (a) it's not part of the randomized block design, and this fact needs to be made clear, and (b) it's not replicated! Or, rather, it's pseudo-replicated, but given the very small spatial scale of the experiment, this obviously raises questions about whether you can draw *any* inferences about the effect of clipping. At a minimum, you need to demonstrate that these two areas were identical (in biomass, etc.) before the experiment began.

Thanks for your suggestions. (a) Our expression is a bit vague. Therefore, we rewrote this section in the revised MS. Please see Line 121-129. (b) The experimental area was divided into two sites. The one was natural site, and the other was clipping site. Therefore, we only have two clipping levels: natural condition and clipping.

Twenty-four 2×2 m² plots were established in each site. Every 24 plots were based on a randomized block design and exposed to ambient, belowground particulate litter addition, increased precipitation and combination of particulate litter addition and increased precipitation. Overall, the experiment used a block-nested design. We also demonstrated that these two sites were identical before the experiment began. Please see Line 123-124.

2. Second, I'm concerned that there's some results overlap with papers published in PLOS ONE last year (Ma et al., both cited in the ms). For example, the results about SOM effects on soil temperature and moisture, as well as PLFA ratios, appeared in the "Soil Microbial Properties and Plant Growth Responses" 2012 paper.

Thanks for your suggestions. We have deleted meteorological data (Fig. 1) which have already presented in our PLOS ONE paper (Ma et al. 2012).

In fact, the proportion of results same to our PLOS ONE paper was very small. In this BGD manuscript, we presented soil microclimate, soil total PLFAs, the ratio of fungal to bacterial PLFAs in both clipped and unclipped plots, whereas we only showed soil microclimate, the ratio of fungal to bacterial PLFAs in unclipped plots in our PLOS ONE paper. Because the results of soil microclimate, the ratio of fungal to bacterial PLFAs and total PLFAs were very useful to explain the variations of soil C and N mineralization, we want to present these results in the revised MS. Meanwhile, the same results were marked the source in Figure legends (Figure 2A, C; Figure 4A, K) in the revised MS. There are other reasons for present these results in the revised MS. The main emphasis of BGD-MS is very different from our PLOS ONE paper. This experiment want to compare the effects of belowground litter addition and increased precipitation on soil C and N mineralization under clipped (reducing belowground photosynthates allocation) and natural conditions, because most of the temperate steppes were heavily grazing and repeatedly harvesting (reducing belowground photosynthates allocation) in our study area. Moreover, we enlarged complexity of this experimental design, including water and two changes in C inputs.

Climatic change and human disturbance are happening in concert with one another so that ecosystems are experiencing these changes simultaneously. Although we have enabled a better understanding of how soil C and N mineralization may respond to any one factor, understanding how multiple factors interact with each other to influence soil C and N mineralization responses are still limited.

3. Third, the figures are not very clear or imaginative. They mirror the results, actually–a great mass of data all plotted together, without a consistent theme or story. (See other reviewer's comment about lack of clear hypotheses.) Thanks for your suggestions. We thoroughly re-plotted all the figures in the MS. Please see all the Figures.

The old MS was lack of clear hypotheses and based on no specific mechanism. In the revised MS, your comments have been fully considered. We developed a specific hypothesis about belowground litter addition, increased precipitation, clipping (reduced belowground photosynthates allocation) and their might interact, based on the specific mechanism. Please see Line 89 - 95, 101 - 105.

4. Finally, English quality is mixed; generally good, but there are consistent errors throughout which makes reading distracting and at times the meaning unclear.

Thanks for your suggestions. The English language has been improved in the MS.

===== Specific comments =====

1. Page 9494, lines 13-19: somewhat confusing; break up sentence and clarify if possible

Our expression is a bit vague. We rewrote this sentence in the revised MS. Please see Line 35-40.

2. P. 9496, l. 13: evidence doesn't claim, people do

This is an uncompleted comment.

We have revised this sentence. Please see Please see Line 76-80. In fact, this evidence was claimed in Kaiser et al. (2011, Ecology).

3. P. 9497, l. 22-: so the clipping treatment (i) isn't part of the randomized block and (ii) is pseudo-replicated, not truly replicated

Thanks for your suggestions. Our expression is vague. Therefore, we rewrote this section in the revised MS. Please see Line 121-129.

4. P. 9501, l. 22-: I'm a little unclear how these stepwise linear analyses were performed, clarify if possible. Given the number of factors being tested, how did you guard against overfitting / spurious correlations?

The best-known method to guard against overfitting or spurious correlation is stepwise regression. Stepwise regression combines forward selection and backward elimination. At each step, the best remaining variable is added, provided it passes the significant at 5 % criterion, then all variables currently in the regression are checked to see if any can be removed, using the greater than 10 % significance criterion. The process continues until no more variables are added or removed. It is not guaranteed to find the best subset of independents but it will find a subset close to the best.

If the several independent variables are existed higher correlations, which may contribute to overfitting or spurious correlation, stepwise regression can delete one or more correlated independent variables to lest including complexity such as interactions.

5. P. 9502, l. 3-15 as well as PLFAs: some of these results have already been presented in your PLOS ONE paper

Thanks for your suggestions. We have deleted meteorological data which have already presented in PLOS ONE paper and Please see the answer of *General comments 2*.

6. P. 9504, l. 12-16: move to discussion

We have moved this section to discussion in the revised MS. Please see Line 392-395.

7. P. 9509, l. 3: what long-term implications? Be specific

In this sentence, our expression is a bit vague. Therefore, we deleted the word "long-term" in the revised MS.

8. Table 1: this table would be much more informative with d.f. and F values included; you could indicate significance by shading/underlining

Thanks for your suggestions. Table 1 has been rewritten with more information. Because all the d.f.= 1 (the results from the repeated measurement ANOVAs) and the space were too narrow, we do not added d.f. in Table 1. We indicated significance in bold and italic. Please see Table 1.

9. Table 2: give unit for all variables

The units for all variables were added in Table 2.

10. Figures 3-5: bar chart overload. Bar charts make comparisons tough between dates, and are generally a poor use of space I think. Consider re-plotting at least some of these data, being more imaginative (e.g. box-and-whisker plots; point plots; etc) and focusing on most interesting comparisons.

Thanks for your constructive suggestion. We thoroughly re-plotted all the figures in the MS. Please see all the Figures.

11. Figure 4: what are June-3, Aug-3, Sep-3 values? Poor labeling generally Thank you. We have corrected Fig. 4. Please see Fig. 4.

12. Figure 5: is bottom-right of x axis mislabeled? Should those be Jun-2, Aug-2, Sep-2?

We have corrected Fig. 5. Please see Fig. 5.

We appreciate these helpful comments and suggestions that have helped us improve the quality of our paper.