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Carbon and nitrogen dynamics in native *Leymus chinensis* grasslands along a 1000km longitudinal transect in northeast China

Biogeosciences Discuss

Dear editors,

Thank you for your positive decision on our manuscript entitled “Carbon and nitrogen dynamics in native *Leymus chinensis* grasslands along a 1000-km longitudinal transect in northeast China”. We found the 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

General Comments: The paper by Ma et al. advances a relevant topic by presenting observations of several carbon and nitrogen cycling diagnostics across a rainfall gradient. The primary interest of this data set is the synthesis of plant, soil, litter, and microbial community information, which are not typically included together in such studies.

While the data set is new, complete, and potentially useful, there are significant issues with data analysis, the conclusions drawn, and integration with the vast literature on this topic. Most importantly, I am concerned about the use of field

observations on a single day (or week) with climatology averaged over several years to address climate control on C-N cycling. Either the climate and ecosystem parameters should be collected at the same temporal scale (e.g., several years of field data) or an argument must be made that the samples collected are representative of some "average" condition. I am sure this is the case for the slow soil variables, but I anticipate substantial intra- and inter-annual variability in the plant pools, soil moisture, and soil microbiota. Further, there are some grammatical errors that obscure important content.

Thank you for your suggestions. Your comments have been fully considered in the revised MS. We have re-analyzed the data and developed new conclusions in MS. Please see the Abstract, M&M, conclusions and all Figures, Line 318-329, support information. In addition, the English language has been corrected carefully in the MS. All changes were highlighted with color.

Admittedly, this study suffers from several caveat that limit a complete understanding of how the variability of precipitation affect ecosystem C and N processes. However, these results may not alter the overall patterns of C and N dynamics along the precipitation gradient due to several reasons as follows:

1. The use of climatology averaged data with field observations on the sampling year to address climate control on C-N dynamic may not be the ideal choice, whereas the plant production was strongly correlated to mean annual precipitation (MAP) in this study. So while seasonal representations of precipitation were better predictors of plant production, they were only marginally so. Please see Support information (Appendix 1);

2. We attempt to capture the variation of plant production resulting from the climatic fluctuations and to represent the "average" condition of plant production through wide–area samplings (1-2 hm^2) in each site. Additionally, aboveground living biomass, standing litter, surface litter and root biomass were harvested by 10 – 12 randomly quadrats in mid-August 2012, which is the period of biomass attains peak value;

3. Precipitation is not distributed evenly over the growing season, and about 70 %

falls between June and August (http://cdc.cma.gov.cn; Wang & Gao, Global Ecology & Biogeography, 2003, 12: 249-259). Therefore, the measurements of soil moisture, soil respiration and net mineralization during mid-July to mid-August could also represent the "average" condition of soil moisture, soil respiration and N mineralization. However, it is a pity we do not measured soil moisture consistently during the growing season, and we will give a measurement of these data in the next step of our research;

4. Soil microbial community composition (i.e. F: B) is strongly controlled by climate history and hence un-respond to seasonal or annual climate variability (Drenovsky et al. 2010, Global Ecology & Biogeography, 2010, 19: 27-39).

We also stated these reasons in the Discussion. Please see Line 318-329. In actually, many studies also used the data of plant and soil properties in sampling year versus mean annual precipitation, such as Liu et al. (New phytologist, 2010, doi: 10.1111/j.1469-8137.2010.03388.x), Zhou et al. (Ecosystems, 2009, 12, 1369 – 1380), He et al. (Soil Biol. Biochem., 40, 2952 – 2959, 2008).

Specific Comments

1) There is no mention of precipitation in the title. This is an important piece of information that conveys the significance of the "1000 km longitudinal transect."

Thanks for your suggestion. We have revised the title. Please see Page 1, Line 1-3.

2) The abstract does not satisfactorily describe the motivation, methods, and conclusions. For example, there is no mention of the PFLA or AMF results or the redundancy analysis. Although you state ": : :likely due to the relative changes in temperature, soil arbuscular mycorrhizal fungi biomass and N availability," this sounds overly speculative, when you have data to support a more precise statement. Secondly, the second to last sentence beginning "It was concluded: : :" is not a novel conclusion, nor is it a focused description of what you observed. I would again be more precise here. Finally, I failed to understand the connection between the last sentence and your work, specifically your prediction of C and N

sinks under changing climate (which I address in other comments below).

Thanks for your suggestions.

(1) We corrected the motivation, methods, and conclusions.

(2) The data of soil fungal: bacterial biomass was used instead of soil AMF in the revised MS.

(3) We developed a novel conclusion according our observed in this study.

Please see Abstract.

3) Introduction, first paragraph: In the motivation, there seems to be some confusion between rainfall variability or "precipitation regime" (i.e., drought, extreme precipitation events) and mean annual rainfall. Your measurements and results are presented across a mean annual rainfall gradient, while the IPCC projections of increased rainfall variability are conflated with changes in mean annual rainfall. Either discuss how increased rainfall variability will change mean annual rainfall or soil water content or remove the discussion of rainfall variability – the paper can be completely motivated from the perspective of mean annual rainfall and would reduce confusion. On Page 12161, Line 12, replace "precipitation regime" with "mean annual precipitation."

Thank you for your suggestion. Your comments have been fully considered in the revised MS. Our expression is a bit vague. Therefore, we corrected these expressions in the revised MS, and replaced "precipitation regime" with "mean annual precipitation" Please see Line 26, 40, 55, 246, 269, 319, 330.

4) Page 12161, Line 18-20: The linear relation between ANPP and water availability is not always observed – see Huxman et al. (2004) and Hsu et al. (2012) for other examples.

Thank you for your suggestion. We carefully read the references reported by Huxman et al. (2004) and Hsu et al. (2012), and corrected our expression. Please see Line 57-62.

5) Page 12162, Line 25: replace "precipitation regime" with "mean annual precipitation"(also Line 28)

We replaced "precipitation regime" with "mean annual precipitation" in the revised MS. Please see Line 26, 40, 55, 246, 269, 319, 330.

6) Page 12162, Lines 8-11: References not needed for this statement.

We rewrote this section in the revised MS.

7) Page 12162, Lines 13-16: soil water availability often depends strongly on precipitation, especially in water-limited ecosystems. My opinion is that some of your references may offer insight into the mineralization-precipitation relationship, even if precipitation was not reported directly. Can you discuss this further? You might also consider linking precipitation, soil moisture, and mineralization in your results and discussion. What does it mean that soil moisture measured on a single day in July scales linearly with mean annual precipitation?

Thank you for your suggestions. Some references were added involving mineralization-soil moisture relationship, and we discussed this further in the Introduction. Please see Line 84-91. We also linked precipitation, soil moisture, and mineralization in our results by stepwise regression. Stepwise regression method could guard against over-interpreting significant multiple-correlations. Across the precipitation gradient, stepwise multiple regression analyses demonstrated that soil moisture did not significantly contribute to the variation in soil respiration and net N mineralization in *L. chinensis* grasslands across a 240 mm precipitation gradient (Table 2).

Precipitation is not distributed evenly over the growing season, and about 70 % falls between June and August (http://cdc.cma.gov.cn; Wang & Gao, Global Ecology & Biogeography, 2003, 12: 249-259). Therefore, the measurements of soil moisture in mid-July and mid-August could represent the "average" condition. However, it is a pity we do not measured soil moisture consistently during the growing season, and we

will give a measurement of these data in the next step of our research.

8) Page 12162, Lines 27-28; Page 12163, Lines 2-3; Results Section 3.3: How do you define C and N sequestration potential? In Figure 4, sequestration is the sum of shoot, root, litter, and soil (page 12167, line 19-20). Because soil C is a slow variable and intra- and inter-annual variability in litter and plant C may be important in grasslands that turnover rapidly, soil C is the best indicator of a "sequestration potential." Regardless, "sequestration potential" is a nebulous concept and therefore I would suggest only discussing carbon storage in the context of measured soil C.

Thanks for your suggestion. Our expression is vague. Therefore, we corrected the expression in the revised MS, and only discussed C sequestration potential in the context of measured soil C. Please see Line 305-312.

9) Page 12162, Lines 28-29: The hypothesis is not convincing or testable as stated. The first part, that precipitation is the primary driver of C and N dynamics in temperate grasslands is not completely supported by your literature review. Precipitation effects on C and N dynamics have been studied extensively (as you reference). How is your study different? How are temperate grasslands different from other ecosystems? What do you mean by "primary?" In my opinion, the second part of the hypothesis regarding soil properties and microbial communities is an important contribution of this study. I would suggest emphasizing precipitation as a driver of C-N dynamics and that your study aims at teasing apart the state variables that contribute to those dynamics across a precipitation gradient.

Thanks for your suggestion. We corrected this section and added the supporting literature in the Introduction. Please see Line 57-62. We developed a specific hypothesis and aimed at teasing apart the contributions of aboveground biomass, litter, root, soil and microbial communities to regional C and N dynamics across a 240 mm MAP gradient in *L. chinensis* grasslands. Please see Abstract, Line 54-56, 98-100.

10) Materials and Methods:

a. Climate: with respect to your interest in precipitation regimes, seasonality and the daily-scale structure of rainfall will likely affect the soil water balance and therefore soil water availability. If the climate data is at a monthly or higher frequency, I would suggest investigating how precipitation variability changes across the precipitation gradient. Again, according to the data in Huxman et al. (2004) and Hsu et al. (2012), or the theoretical studies of Porporato et al. (2003) and Daly et al. (2004), mean annual precipitation may not be the ideal choice of independent variable.

Thank you for your suggestion. Your comments have been fully considered in the revised MS. Our expression is vague, and thus we corrected these expressions in the revised MS, and replace “precipitation regime” with “mean annual precipitation”. We carefully read the studies of Huxman et al. (2004),Hsu et al. (2012), Porporato et al. (2003) and Daly et al. (2004). Admittedly, our results suffer from several caveat that limit a complete understanding of how the variability of precipitation affect ecosystem C and N processes. However, these results may not alter the overall patterns of C and N dynamics along the precipitation gradient according several reasons as follows:

1. The use of climatology averaged data with field observations on the sampling year to address climate control on C-N dynamic may not be the ideal choice, whereas the plant production was strongly correlated to mean annual precipitation (MAP) in this study. So while seasonal representations of precipitation were better predictors of plant production, they were only marginally so. Please see Support information (Appendix 1);

2. We attempt to capture the variation of plant production resulting from the climatic fluctuations and to represent the “average” condition of plant production through wide–area samplings (1-2 hm^2) in each site. Additionally, aboveground living biomass, standing litter, surface litter and root biomass were harvested by 10 – 12 randomly quadrats in mid-August 2012, which is the period of biomass attains peak value;

3. Precipitation is not distributed evenly over the growing season, and about 70 % falls between June and August (http://cdc.cma.gov.cn; Wang & Gao, Global Ecology & Biogeography, 2003, 12: 249-259). Therefore, the measurements of soil moisture, soil respiration and net mineralization during mid-July to mid-August could also represent the "average" condition of soil moisture, soil respiration and N mineralization. However, it is a pity we do not measured soil moisture consistently during the growing season, and we will give a measurement of these data in the next step of our research;

4. Soil microbial community composition (i.e. F: B) is strongly controlled by climate history and hence un-respond to seasonal or annual climate variability (Drenovsky et al. 2010, Global Ecology & Biogeography, 2010, 19: 27-39).

We also stated these reasons in the Discussion. Please see Line 318-329. In actually, many studies also used the data of plant and soil properties in sampling year versus mean annual precipitation, such as Liu et al. (New phytologist, 2010, doi: 10.1111/j.1469-8137.2010.03388.x), Zhou et al. (Ecosystems, 2009, 12, 1369 – 1380), He et al. (Soil Biol. Biochem., 40, 2952 – 2959, 2008).

b. Plant biomass and litter mass: When were the measurements obtained and why? Again, throughout the paper the connection between measurements on a single day and average climatology needs to be addressed. What is the seasonal and interannual variability of plant biomass and litter mass? Are the grass communities annual or perennial? What is the litter decomposition rate? Were your measurements obtained in the wet or dry season? Would this variability obscure your results?

The detail method and information were added in the M&M. Please see Line 128-131 and our responses to COMMET 10 (a). However, it is a pity we do not collected the data of seasonal and inter-annual variability of plant biomass and litter mass, litter decomposition rate in this study.

c. Page 12165, Lines 4-6 and Page 12167, Lines 5-7: Why did you not report the

ratio of fungal to bacterial PLFA?

The contribution of soil fungal: bacterial PLFA to ecosystem C and N dynamics were reported in Results and Discussion. Please see Line 205-206, 282-289, Abstract.

11) Results, Section 3.1: If the canopy is closed and the roots and soil moisture are shallow, I do not find it surprising that root biomass did not change across the climate gradient. Indeed, on page 12169, you note that this is "consistent with those of studies on effects of increased precipitation on root biomass at local and regional scales." Also, in Figure 2c, the root biomass looks more non-linear than any of the models claimed to be quadratic.

Thank you for your suggestion. We corrected the expression in Discussion. Please see Line 265-266. We thoroughly re-plotted all the figures in the MS. Please see Figure 2.

12) Results, Section 3.3: How was C and N mineralization measured? This is not covered in the methods. Also, from Figure 4, the quadratic relationships are not well supported (also page 12169, lines 27-19). How do linear models perform with this dataset? Does the quadratic model provide any additional explanatory power? You may also try a segmented regression.

Thank you for your suggestion. The methods of soil respiration and N mineralization were added in M&M. Please see Line 152-165. We thoroughly re-plotted all the figures in the revised MS. The samples were averaged for each site and plotted the mean and standard deviation in the Figures. Additionally, segmented linear regression, also known as piecewise regression is a method in regression analysis in which the independent variable is partitioned into intervals and a separate line segment in these regions. The boundaries between the segments are breakpoints. In the determination of the most suitable trend, statistical tests must be performed to ensure that this trend is reliable (significant). When no significant breakpoint can be detected, one must fall back on a regression without breakpoint. The regression analyses were performed using the packages for R (R Development Core Team, USA). The AIC criterion was used to select a best model for each data set. We fit a linear or segmented linear model

to each data set using least squares regression. Please see Line 176-178, all Figures.

13) Page 12170, Lines 27-29: This sentence is difficult to understand.

Therefore, we deleted this sentence in the revised MS.

14) Page 12171, Lines 1-3: I don't see a saturation in heavy fractions of C or N in Figures 3H and 3J.

Our expression was wrong in the old MS. We deleted this sentence in the revised MS.

15) Page 12171, Lines 15-22: These conclusions are not very well supported by the data or your analysis. How would changes in global precipitation regimes strongly affect ecosystem C and N dynamics? Again, what is your definition of "precipitation regime?" Why do the "grasslands of northeast China exhibit tremendous potential for enhancing C and N sequestration at the regional scale?" You haven't discussed how precipitation is expected to change in this region or how sequestration could be enhanced. Further, the conclusions that precipitation, temperature, etc. play a role in ecosystem C and N dynamics is not new and needs to be focused to represent the specific conclusions drawn from your dataset.

Please see our response to COMMENT 3 and 10 (a). We re-analyzed the data and partly rewrote conclusions in the revised MS. In addition, we developed a novel conclusion drawn from our dataset in this study. Please see Line 336-339, 40-43.

Technical Corrections

1) Abstract, first line: The words "unprecedented" and "profoundly" are not quantitative and rather strong – consider replacing or omitting.

We deleted this sentence in the revised MS.

2) Abstract, line 4: what do you mean by "complexity in precipitation?"

We deleted this sentence in the revised MS.

3) Abstract, line 19: remove the word "on" after "influence"

The word "on" was deleted in the MS. Please see Line 40.

4) Line 25: "covering" should be replaced with "cover"

The word "covering" was replaced with "cover". Please see Line 48.

5) Page 12162, Lines 5-6, Lines 7-8, Lines 25-27: these sentences need to be rewritten.

These sentences have rewritten in the revised MS. Please see Line 77-81, 98-100.

6) Pager 12166, Line 15: "were all linearly increased" should be "all linearly increased"

We deleted this sentence in the revised MS.

7) Page 12166, Line 26: "long" should be "along" (and page 12170, line 29)

The word "long" was replaced with "along". Please see Line 229.

8) Page 12167, Line 6: what do you mean by "style?"

The sentence has been rewritten in the revised MS.

9) Page 12168, Line 24: "drives to impact" should be reworded

The sentence has been rewritten in the revised MS. Please see Line 246.

10) Page 12171, Lines 15-16: This sentence needs to be reworded

This sentence was reworded in the revised MS. Please see Line 330.

References

Huxman et al., 2004, Nature: doi:10.1038/nature02561

Hsu et al., 2012, Global Change Biology: doi:10.1111/j.1365-2486.2012.02687.x

Porporato et al., 2003, Advances in Water Resources: doi:10.1016/S0309-1708(02)00094-5

Daly et al., 2004, Journal of Hydrometeorology: doi:10.1175/1525-7541(2004)005<0559:CDOPTA>2.0.CO;2

Thank you for your suggestion. We carefully read these references and added in the revised MS. Please see References.

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

Referee #2

Reviewer Comments

The ms titled "Carbon and nitrogen dynamics in native Leymus chinensis grasslands along a 1000km longitudinal transect in northeast China" by Ma et al., presents an interesting dataset of belowground and aboveground measurements of plant and soil parameters that may be of use to the general scientific community interested in how those vary with changes in mean annual precipitation. In terms of scientific significance, the ms can be ranked as good; the scientific quality and presentation qualities are fair. The ms does need more work before it can be accepted for publication.

Major comments:

1. Soil C and N mineralization are shown in results and discussed, yet in the methods there is no mention of how the data was gathered.

The methods of soil respiration and N mineralization were added in M&M. Please see Line 152-165.

2. The data is presented on per area basis, have the authors considered the data on a per mass basis and if so, how do the trends compare then?

Thank you for your suggestion. We presented the data of plant and litter N content on a per mass basis in Figure 2, and compared the trends in the Results and Discussion. Please see Line 290-297.

3. The study focuses on variability in below and above ground parameters along a precipitation gradient, based on mean annual precipitation amounts, however, across the same sites there is also a 5.5 degree C difference in mean annual temperature. The two climate variables are highly correlated, which was at times mentioned in the manuscript, yet it was not clear how any temperature effects were accounted for before any conclusions about precipitation regimes were reached. I see you have at least 3 sites with the same MAP and different MAT (Table 1), perhaps you can use those for a more detailed, closer examination of any effects of MAT on your measured parameters and use results from that analysis to infer MAT-MAP confounding effects.

Thank you for your suggestion. In this study, there is also a 5.5 degree difference in mean annual temperature, which likely confounded the precipitation effect on ecosystem C and N cycles. However, stepwise multiple regression analyses demonstrated that MAT did not significantly contribute to the variation in C and N sequestration, but mean monthly temperature significantly impact soil respiration and net N mineralization in *L. chinensis* grasslands across a large-scale precipitation gradient (Table 2). The detail content was reported in Results and Discussion. Please see Line 274-289, 238-243, Table 2.

Given that the regional regressions with MAT accounted for less of the C and N dynamics than did MAP, we think our focus on precipitation as the major climatic control is justified.

4. Table 2, mentioned on page 12168, is missing.

Table 2 was supplied in the revised MS.

5. Have there been similar studies in other grasslands (even if not completely

measuring all of the parameters you have measured), which can be used to put your results into a broader context? It seems most of your references are from studies conducted along the same North East China Transect (NECT).

Thank you for your suggestion. We compared our study with some similar studies in other grasslands which could put our results into a broader context. The added references were highlighted with color in the revised MS. Please see References.

6. You keep highlighting the relationship between total plant biomass and MAP – this relationship is clearly driven by above ground biomass only, given that belowground biomass you have shown to be constant across the gradient. So is that not redundant information? Similarly reporting the S:R ratio or C:N ratios, when you already show the components of each?

Thank you for your suggestion. We deleted the data of total plant biomass and S:R ratio in the revised MS. However, soil C: N was used to explain our results, and thus conserved in the MS.

7. Table 1 shows you sampled 18 sites, yet on the graphs the lines of points are at best 11-12. From MAP data it does look like some may overlap, perhaps you could plot the data more clearly. Why not average the samples for each site and plot the mean and standard deviation (given at all sites the sampling was equal). This would reduce the scatter and focus your patters; any overlapping data points can be given a different symbol and actually be seen; the standard deviation can also be shown as "error bars" if you want to highlight the spatial variability within each site (which in itself could be further investigated and compared among the sites – are they comparable when accounting for this spatial variability?).

Thank you for your suggestion. We thoroughly re-plotted all the figures in the MS. Please see all the Figures. Four sampling sites were deleted because of the similar mean annual precipitation across the precipitation gradient. We averaged the samples for each site and plotted the mean and standard deviation in the Figures.

8. I am not convinced that your data fits a quadratic relationship (Fig. 3, c) – why/how did you arrive at that conclusion? Linearity is also questionable in the given scatter of points of the other figures in Fig 2 and 3 – perhaps fit means.

Thank you for your suggestion. We thoroughly re-analyzed the data and re-plotted all the figures in the revised MS. The samples were averaged for each site and plotted the mean and standard deviation in the Figures. Additionally, segmented linear regression, also known as piecewise regression is a method in regression analysis in which the independent variable is partitioned into intervals and a separate line segment in these regions. The boundaries between the segments are breakpoints. In the determination of the most suitable trend, statistical tests must be performed to ensure that this trend is reliable (significant). When no significant breakpoint can be detected, one must fall back on a regression without breakpoint. The regression analyses were performed using the packages for R (R Development Core Team, USA). The AIC criterion was used to select a best model for each data set. We fit a linear or segmented linear model to each data set using least squares regression. Please see the Figures.

Minor comments:

1. It is more acceptable to write species names in italics (ex. *Leymus chinensis*)

We wrote the species names in italics in the revised MS.

2. Check your reference list – there are references on that list, which are not in the text (ex. McCulley et al 2005; Olsson et al 1999; Sayer 2006)

Thank you. The reference list was carefully checked in the revised MS.

3. Grammatical clarity can be improved. (ex. it is not clear what is mean by the following?: " ... heavy fractions of C and N followed by steady phases with MAP..." (line 25, p.12165) or "Related research ...have demonstrated that the responses of soil C and N content to increased precipitation represent increases... and no changes... at local and regional scales in the temperate grasslands."

(lines23-27, page 12169) or lines 1-4 on page 12170, etc)

The English language has been corrected carefully in the MS. All changes were highlighted with color. Please see 274-288.

4. title suggestion: "Carbon and nitrogen dynamics of a native grassland along a 1000km longitudinal precipitation gradient in northeast China" ? or discuss C and N dynamics in light of MAT too.

Thanks for your suggestion. We have revised the title. Please see Page 1.

5. why was data not shown for fungal/bacterial biomass (line 6, page 12166 – that that was already described in more details in one of your previous papers on the sites and dataset, then please cite the paper)

The contribution of soil fungal: bacterial PLFA to ecosystem C and N dynamics were reported in Results and Discussion. Please see Line 205-206, 282-289, Abstract.

6. How is "soil microbial biomass" is an environmental factor? – line 11, page 12167.

The soil microbial biomass is not an environmental factor. Therefore, we deleted this sentence in the revised MS.

7. "The relative reduction in soil N availability in mesic sites..." – relative to what? (lines 19 - 20, page 12169)

We rewrote this sentence in the revised MS. Please see Line 287.

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