Response Letter

Michael Bahn
Co-Editor-in-Chief
Biogeosciences

Dear Dr. Bahn,

Thank you very much for handling our manuscript “Initial shifts in nitrogen impact on ecosystem carbon fluxes in an alpine meadow: patterns and causes” (bg-2016-436). We are very grateful to the two reviewers for their constructive comments and suggested amendments. Their inputs have helped us improve the paper tremendously. We have carefully studied their comments, and revised our manuscript accordingly.

Here are our detailed responses to the reviews. Please note that the comments from the reviewers are in *italics* followed by our responses in **bold** text.

Sincerely,

Shuli Niu, Professor
Synthesis Research Center of Chinese Ecosystem Research Network,
Key Laboratory of Ecosystem Network Observation and Modeling,
Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101
China
Phone: 86-10-6488-8062
Fax: 86-10-6488-9399
http://sourcedb.cas.cn/sourcedb_igsnrr_cas/yw/zjrck/201303/t20130306_3787558.html

Reply to RC1

Interactive comment on “Initial shifts in nitrogen impact on ecosystem carbon fluxes in an alpine meadow: patterns and causes” by Bing Song et al.

Anonymous Referee #1

Received and published: 30 November 2016

This study addresses potential responses of different ecosystem C fluxes to gradual increases in N fertilization. The main findings of this study provide evidence that N saturation of ecosystem C fluxes can occur in a short period of time (just over 2 years since the start of the N fertilization experiment). Key findings are shown in Fig 2 where differences in NEE and ER are clear between years and along the N fertilization treatment.

Despite the results indicate that N saturation may occur at increasing N fertilization levels, the underlying mechanisms explaining why C fluxes might get saturated with N inputs are not clear. The authors suggest that decreases in NEE and ER under greater N fertilization are due to decreases in plant aboveground respiration and soil microbial respiration. Looking at Figs 3 and 4, this interpretation is not really supported by results whereby plant aboveground respiration (in 2015; Fig 4a) seems to increase rather than decrease at N8,16,32 treatments compared to N0,2,4. Similarly soil microbial respiration does not seem to decrease much under N8,16,32 treatments (Fig 3d) and actually might increase under N32 compared to N16. My point here is that although NEE and ER trends are relatively clear, the mechanisms invoked here to explain these changes are not really supported by the results. There is a problem with results interpretation here that the authors need to deal with (see my comments below).

Response: We appreciate the reviewer very much for the thoughtful comments. We address these specific comments below, and please note that our responses are bolded. We agree with the reviewer that the mechanisms should be demonstrated more clearly. Above all, we should state that the decreases of plant aboveground respiration and soil microbial respiration (R_{mic}) under the highest N addition rate were compared to that under N saturation point rather than the control treatment. We are sorry about the confused statements in the previous MS, and have explained it more clearly in the revised MS.

From the following Fig. R1k (Fig. 4e in the previous version of the MS), we can see that plant aboveground respiration decreased under N32 compared to N16. More importantly, only R_{mic} showed distinctively inverse responses to N addition rates between years, which kept increasing in 2014 (Fig. R1c) but decreasing in 2015 (Fig. R1i) along the N addition gradient. R_{mic} did decline under N32 in 2015, and soil acidity under similar N addition rate was also indicated to be the reason why R_{mic} decreased in grasslands (Chen et al., 2016; Liu et al., 2014). All these points have been clarified for better results interpretation.

Chen D, Li J, Lan Z, Hu S, Bai Y (2016) Soil acidification exerts a greater control on soil respiration than soil nitrogen availability in grasslands subjected to long-term nitrogen enrichment. Functional Ecology, 30, 658–669.

Liu W, Jiang L, Hu S, Li L, Liu L, Wan S (2014) Decoupling of soil microbes and plants with increasing anthropogenic nitrogen inputs in a temperate steppe. Soil Biology and Biochemistry, 72, 116-122.

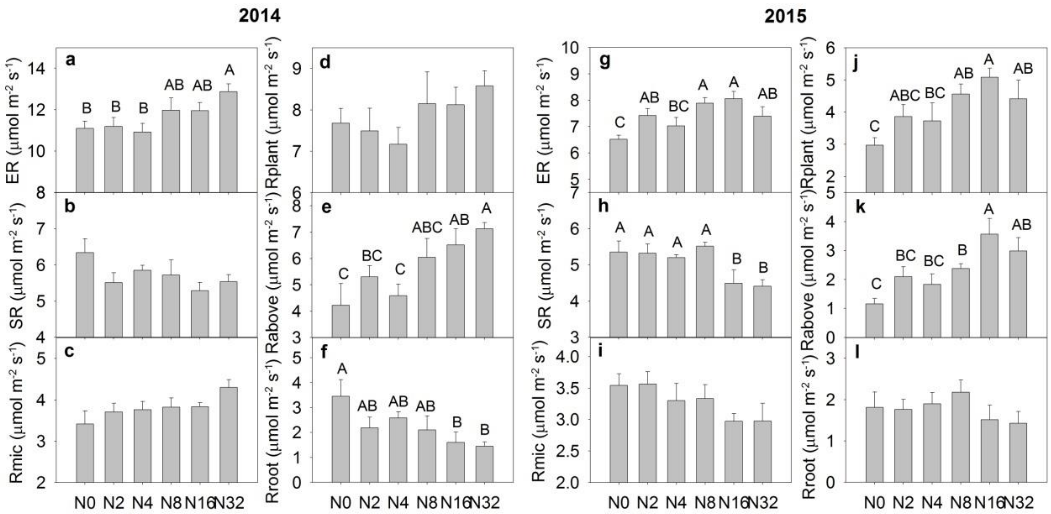

Fig. R1 Ecosystem respiration (ER) (a, g) and its components in response to the N addition gradient in 2014 and 2015 (mean ± SE, n = 5). SR: soil respiration (b, h), R_{mic}: soil microbial respiration (c, i), R_{plant}: plant respiration (d, j), R_{above}: aboveground plant respiration (e, k), R_{root}: plant root respiration (f, l). N0, N2, N4, N8, N16, N32 represent N addition rate is 0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$, respectively.

I think the authors should either better demonstrate that soil microbial respiration might play a role in mediating the N saturation effect or that other mechanisms are at play. It looks like that soil respiration in general decreases more convincingly under higher N treatments than soil microbial respiration. Also the explanation that greater standing litter might reduce plant aboveground respiration through reduced light availability makes sense but is not really supported by the results in Fig 4e for example.

Response: The decrease in soil respiration (SR) in 2015 was apparently caused by decrease in R_{mic}. The reduction of R_{mic} under high N addition level, together with low root respiration, resulted in decrease of SR in 2015. In 2014, increase of R_{mic} partly offset by the decrease of root respiration, and as a result, SR had no significant difference among N treatments.

In 2014, plant aboveground biomass (AGB) was stimulated under high N addition treatment, especially AGB of grasses (Fig. R2). In this grassland, grasses usually have higher height than other plants. The accumulation of grasses standing litter under high N addition treatment limited light condition for other plants and negatively influenced plant growth in early growing season in 2015. We have added Fig. R2 to the revised MS, which will demonstrate our results more clearly.

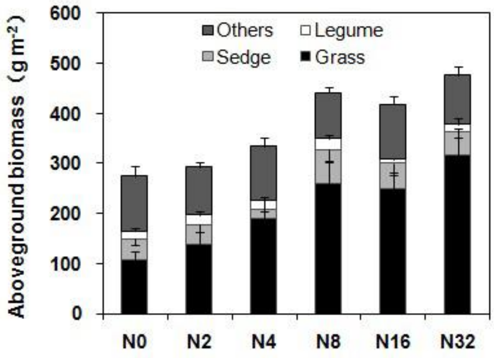

Fig. R2 Plant aboveground biomass in response to the N addition gradient in 2014. N0, N2, N4, N8, N16, N32 represent N addition rate is 0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$, respectively.

Also in relation to results interpretation, the authors need to acknowledge that variability in their findings could be related to their very short-term study, which may not capture key changes in NEE and ER and the underlying mechanisms involved. I would expect that it will take 3-5 years of N fertilization to better clarify these.

Response: Thanks for the critical comments. Ecosystem C fluxes may respond to N addition in different ways during different stages and the underlying mechanisms may also change, just as the N saturation theory stated. Although it is better to take a long-term study to clarify the underlying mechanisms, we believe that our study found the early response signals of changes in ecosystem C fluxes under N addition and revealed the potential mechanisms at early stage.

Overall, the manuscript needs a thorough editing in relation to sentence structure and language especially abstract and introduction but in general all throughout the manuscript.

Response: Thanks for pointing this out. We have tightened some paragraphs in the Introduction as the Referee #2 suggested, and carefully edited the sentence structure and language throughout the MS.

Discussion

I am not sure whether the explanation that: "The N saturation responses of ER and thus NEE are mainly caused by the decrease of aboveground plant respiration and soil microbial respiration under high N addition treatments in 2015" (page 11, lines 8-9), is well supported by the results. If I look at Fig. 4e I see an increase in aboveground plant respiration (i.e. Rabove) in 2015 under the N16 treatment and a slight decrease under the N32 treatment, which is however still higher than the N8 treatment. What I can see is an overall decrease of Rabove across all treatments in 2015 when compared to 2014. Even the 'assumed' decreases in soil microbial respiration are not clear in Fig. 3d, actually it looks like that Rmic almost increases between N16 and N32.

Page 11, lines 9-11. I might agree with the statement that: "The decrease of *aboveground plant respiration under N32 treatment is primarily due to that N addition stimulated plant growth and thus standing litter accumulation after plant senescence (Fig. S1)", but again this is not clear from the results shown. Fig. S1 might provide evidence of litter accumulation but is this the only treatment (N32), which was associated with an increase of plant litter? What about N16?*

Response: Thanks for the reviewer's critical comments. Please see our first and second responses above. Fig. R1 and Fig. R2 explained our results more clearly.

Again on pag. 11, lines 17-19, the authors suggest that: "The relationships between ER and soil microbial respiration (Fig. 6c) indicate that the decrease of microbial respiration contributes to the reduction of ER under high N addition rates in 2015", which is not really what is shown in Fig. 6c. This figure shows an overall positive relationship between Rmic and ER but this has not to do with increases in N addition rates. The role of N fertilization here is not clear mainly because there is no distinction between N treatments (al points are the same). The authors should show where the high N-addition-treatment points are positioned in this graph to make their explanation convincing.

Response: We thank the reviewer very much for the thoughtful comments. We would replace Fig. 6 by Fig. R3 in the new draft of our MS. In Fig. R3, open circles indicate the variables under high N addition rates. We further explored the relationships between these variables only under high N addition rates (N8, N16, N32) and found that the coefficients were larger (Fig. R4), which could make the explanation more convincing as the reviewer pointed out.

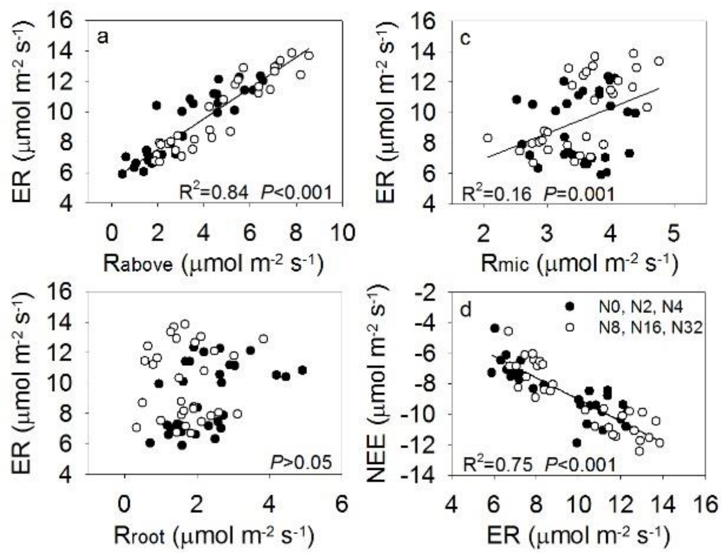

Fig. R3 Relationships between aboveground plant respiration (R_{above}), root respiration (R_{root}), soil microbial respiration (R_{mic}) and ecosystem respiration (ER) (a,b,c), ER and net ecosystem CO_2 exchange (NEE) (d) across all plots in 2014 and 2015.

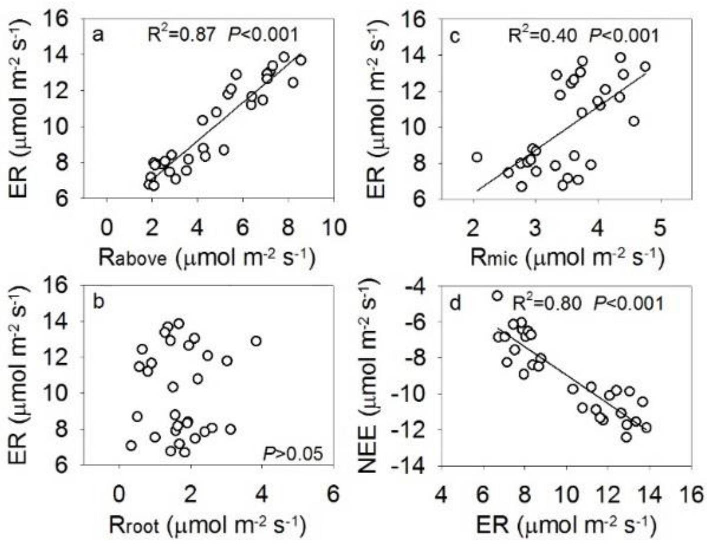

Fig. R4 Relationships between aboveground plant respiration (R_{above}), root respiration (R_{root}), soil microbial respiration (R_{mic}) and ecosystem respiration (ER) (a,b,c), ER and net ecosystem CO_2 exchange (NEE) (d) under N8, N16 and N32 in 2014 and 2015.

Page 13, lines 16-21. This section does not provide a clear view of some potential mechanisms involved in the N saturation effect. I think the authors need either to make a more convincing case for a reduction of soil microbial respiration under N additions. Conclusions need to be rewritten after a better interpretation of key results.

Response: Thanks for the valuable suggestions. We have made a clear interpretation based on your comments and the corresponding results in the new draft.

Reply to RC2

Anonymous Referee #2

Received and published: 8 December 2016

Song and co-authors investigate how changes in N deposition affect the net CO_2 sink or source strength of an alpine meadow, and study the mechanisms that govern changes in CO_2 processes. They measure NEE and ER, soil and microbial respiration and estimate aboveground plant and root respiration in plots across a N addition gradient. I would like to highlight that only a few field experiments have studied this topic using multiple N addition rates, and that these studies are important to understand whether the sink strength of grasslands will saturate at future N deposition rates. Because N deposition is predicted to change during this century and we don't fully understand how it will impact CO_2 processes in terrestrial ecosystems, the topic is of global importance and within the scope of Biogeosciences.

Response: We greatly appreciate the reviewer for the positive comments.

My main concerns are related with how the ms is written, how some of the data is interpreted, and with the fact that some conclusions are not justified by the results. I believe that the ms would benefit if the authors could tighten some paragraphs in the Introduction. In my opinion the second and third paragraph of the introduction lack of direction and intent, and they are somehow repetitive. I think the authors should start this paragraph explaining that the response of NEE to N deposition is likely nonlinear, and that depending on how N affects the main components determining NEE (i.e. GEP and ER), ecosystems will transition from a N limited to a N saturation stage. For instance, some articles showed that GPP and NEP do not respond linearly to changes in N as ecosystems shift to a N saturation stage (e.g. Flescher et al., 2013, DOI: 10.1002/gbc.20026; Gomez et al., 2016, DOI: 10.1111/gcb.13187). Then they could explain how changes in N affect these main components that determine NEP through changes in processes such as plant and root biomass.

Response: Thank the reviewer very much for the constructive comments and suggestions. We have revised the MS as suggested and made our points more clearly. Specifically, we tighten the introduction by merging the second and third paragraph in the Introduction. The paragraph starts with explaining that the response of NEE to N deposition is likely nonlinear, which depends on how N affects the main components. Then we illustrate how ecosystems may transfer from a N limited to a N saturation stage with increasing N input. We have explained how changes in N affect these main components that determine NEE through changes in ecological processes. The references of Flescher et al. 2013 and Gomez et al. 2016 have been cited in the revised MS.

The authors added six levels of N. However, ecosystems are receiving natural rates of N deposition. Thus, I think it is important to state in Material and Methods that these experimental N rates are imposed to naturally occurring N deposition. In addition, could the authors explain why they use dry N addition treatments instead of wet?

Response: The natural N deposition rate in Chinese grasslands has been added in Material and Methods. Because the study site has high precipitation, we applied the N fertilizer when it was raining, which can make the N fertilizer dissolved and avoid additional water application. It is sound to determine only the N effects.

Just for clarity, I recommend the authors not to present results from Figure 4 until they have presented all results from Figure 3 (page 9, lines 5-13).
I am not sure I agree with the statement that 'the saturation response of Rabove and the declined response of Rmic in combination contributed to N saturation response of ER and the consequent saturation response of NEE in 2015' (page 10 lines 5-7)'. I think that if ER saturates as N increases, NEE would only saturate if GEP saturates. In addition, this statement should be in Discussion rather than Results.

Response: Thanks for the valuable suggestions. We have modified these

statements in the revised MS. The reviewer is right. Fig. 2f in the MS showed that GEP also reached saturation and had similar response to the N addition gradient as NEE.

Page 10, line 8-9. It is not clear to me if increased pH reduction as N increases, reduces Rmic in 2014. In both 2014 and 2015, pH decreases as N increases. Are changes in pH in 2014 affecting Rmic?

Response: In 2014, changes in soil pH did not significantly affect R_{mic}.

Page 10, line 10. I don't think the authors should conclude that decreased Rabove as N increased was attributed to the accumulated standing litter mass and thus less light condition under high N addition treatments' based on a photo rather than data. In addition, this statement should not be presented in Results but in discussion.

Response: We have added data of plant aboveground biomass (Fig. R2) in the revised MS. As the reviewer suggested, we have presented these statements in the Discussion.

Page 10, line 15. 'Our findings showed that ecosystem C fluxes (NEE, ER, and GEP) had linear responses in the first year but shifted to saturation responses in the second year'. Please rephrase this sentence using specific language. Based on the authors results, these processes are in the limitation stage in 2014; in 2015, they are in the limitation stage at low rates and at rates at or above 20 g N m^{-2} $year^{-1}$ they shift to the saturation or declining stage.
The paragraph at the end of page 10, beginning of page 11 is repetitive. The first few sentences (line 15-19) are providing the same information than the last sentences (line 20-23). Please tighten the writing.
The authors state that 'saturated under N addition rate of approximately 8 gN m^{-2} $year^{-1}$' (page 11, line 1). I think the authors are fitting thresholds 'by-eye' although there are many statistical methods that can be used to calculate thresholds.

Response: Thanks for the thoughtful comments. We have rewritten these sentences. We stated the N saturation threshold was approximately 8 gN m^{-2} $year^{-1}$ based on our N addition treatments. As limited N addition rates were applied, we think it should be cautioned to calculate a certain threshold. In the revised manuscript, we used statistical method to detect the threshold.

I believe that the presentation of the idea that 'The N saturation responses of ER and thus NEE are mainly caused by the decrease of aboveground plant respiration and soil microbial respiration under high N addition treatments in 2015' (page 11) is not justified by their results. Above 15 g m^{-2} $year^{-1}$ NEE reaches a transition threshold and it starts declining. At this stage, further N additions do not seem to be affecting Rmic (Fig. 3), and Rabove declines just slightly at N rates at 32 g m^{-2} $year^{-1}$. I think the *authors should consider fitting thresholds using statistical methods; this way the breaking points would be accurate and the trend of each line could be calculated. Perhaps the data that could justify this statement is in Fig. 6c. However, I think that the authors should be cautious drawing this conclusion because Rmic and RE are intrinsically correlated (i.e. Rmic is a component of RE). The authors should calculate the self-correlation coefficient instead of a simple coefficient of determination. Please see Vickers et al., 2009 (http://dx.doi.org/10.1016/j.agrformet.2009.03.009) for more information on this statistical approach. The same applies to Rabove and Rroot, and RE; Rabove and Rroot are components of RE.*

Response: Thank the reviewer very much for the critical comments and valuable suggestions. Based on the reviewer's suggestion, we have used statistical method to calculate the breaking points. We have also tried to calculate the self-correlation coefficient between components as suggested.

I couldn't find plant growth or standing litter biomass data that supported the statement 'The decrease of aboveground plant respiration under N32 treatment is primarily due to that N addition stimulated plant growth and thus standing litter accumulation after plant senescence' (page 11). Therefore, I am not sure this statement is justified by the authors' results. The same applies to page 14, lines 2-4.

Response: Thanks for the comments. We have added a figure (Fig. R2) to justify the results.

I think that caution should be used when presenting the idea that 'Taken together with our results, it suggests that N saturation of ecosystem C fluxes may happen very quickly.' I agree with the authors that a plausible explanation could be that the net CO_2 sink strength of this system saturated after 2 years of treatment. However, another plausible explanation that should be acknowledged is that differences in climate between 2014 and 2015 could explain variations in the response of C fluxes to N addition. For instance, if 2015 was drier than 2014, N demands for plant growth would be met faster.

Response: Thanks for the suggestion. We totally agree with the reviewer and have refined the statement by more clearly justifying the results.

I am not sure I agree with 'Our estimate on N critical load suggests that ecosystem C cycle would be largely affected under future N deposition scenarios and ecosystem may sequester more C from the atmosphere in the alpine meadow of Qinghai-Tibetan Plateau.' because the authors conducted a 2-year study in which several levels of N were added and to present this idea I believe they would need a long-term study.

Response: We agree with the reviewer. Changes in C sequestration under increasing N deposition might need longer time to study. We have deleted the sentence.

Minor comments

Page 2, line 9 – I am not sure that I agree with the statement that 'ecosystem net C sequestration is usually predicted to increase under rising N deposition'. Some articles suggest that net C sequestration will increase and others show that it will decrease. See for instance Naddelhoffer et al. 1999 (doi:10.1038/18205). Please rephrase.

Response: Thanks for the valuable suggestion. The reviewer is correct. We have modified the sentence.

Page 3, line 5 – I am not sure that 'the C cycle gets saturated', I think I would rather prefer if the authors refer to the specific process that is saturated (e.g. the C sink strength saturates). Please rephrase.

Response: Greet suggestion! We have specified "the C cycle" into ecosystem productivity.

Page 7, line 2 – I think the authors mean 'simultaneous' rather than 'contemporaneous'. Please clarify.

Response: The reviewer is correct! We have clarified "simultaneous" as suggested.

Page 8, line 6 – I think that the authors mean 'monthly mean NEE' rather than 'annual mean NEE'. Please rephrase throughout the ms.

Response: Thanks. We have rephrased the term throughout the MS as suggested.

Page 11, line 5 – 'a N addition gradient experiment' rather than 'an N addition experiment'.

Response: Thank the reviewer for bringing it up. We have changed into "a N addition gradient experiment".