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,

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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.

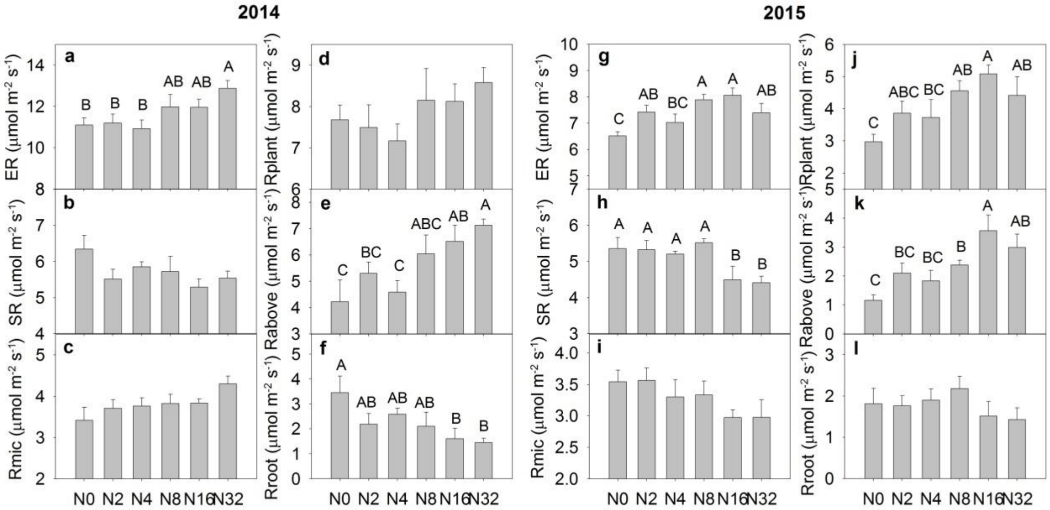

Fig. R1 Ecosystem respiration (ER) (a, g) and its components in response to the N addition gradient in 2014 and 2015 (mean ± SE, n = 5). SR: soil respiration (b, h), R_{mic}: soil microbial respiration (c, i), R_{plant}: plant respiration (d, j), R_{above}: aboveground plant respiration (e, k), R_{root}: 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 (Fig. S2), which will demonstrate our results more clearly.

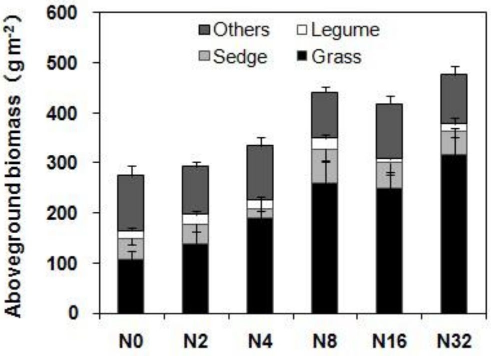

Fig. R2 Plant aboveground biomass in response to the N addition gradient in 2014. N0, N2, N4, N8, N16, N32 represent N addition rate is 0, 2, 4, 8, 16, 32 gN m^{-2} year^{-1}, respectively.

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 (Page 2, Line 14 – Page 3, Line 18), 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.

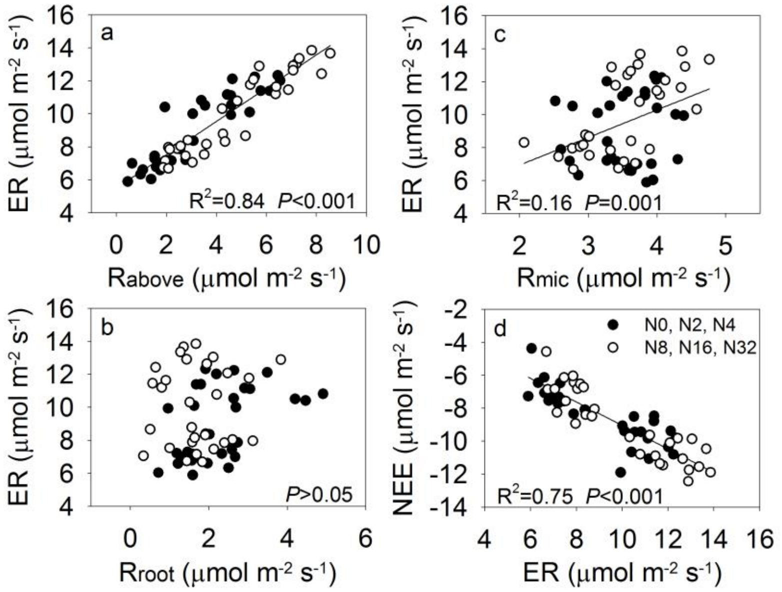

Fig. R3 Relationships between aboveground plant respiration (R_{above}), root respiration (R_{root}), soil microbial respiration (R_{mic}) and ecosystem respiration (ER) (a,b,c), ER and net ecosystem CO_2 exchange (NEE) (d) across all plots in 2014 and 2015.

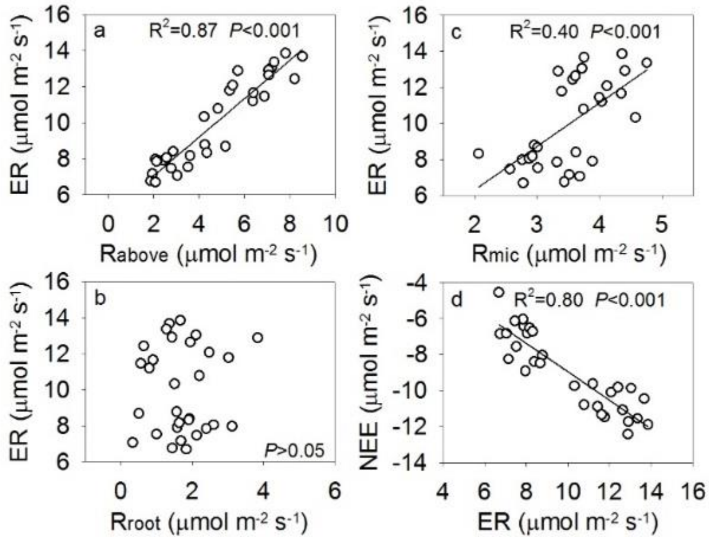

Fig. R4 Relationships between aboveground plant respiration (R_{above}), root respiration (R_{root}), soil microbial respiration (R_{mic}) and ecosystem respiration (ER) (a,b,c), ER and net ecosystem CO_2 exchange (NEE) (d) under N8, N16 and N32 in 2014 and 2015.

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 (Page 2, Line 14 – Page 3, Line 18). 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 (Page 4, Line 20). 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. S2) in the revised MS. As the reviewer suggested, we have deleted these sentences in the Results and 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 (Page 10, Line 7-12). 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.

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 tried to use piecewise analysis to calculate the breaking points and found that the method was not suitable for our data, so we used quadratic functions to calculate the threshold. The relationship between R_{mic} and ER was used to test the dependence of ER to R_{mic}, so we used a simple linear regression analysis.

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 - Fig. S2 in the revised version) 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 (Page 12, Line 9-15).

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 2, Line 9-10).

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: We have specified "the C cycle" into ecosystem C fluxes.

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 6, Line 20).

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 8, Line 3, 18).

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".

Initial shifts in nitrogen impact on ecosystem carbon fluxes in an alpine meadow: patterns and causes

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Abstract

The rising nitrogen (N) deposition could increase ecosystem net carbon (C) sequestration by stimulating plant productivity. However, how net ecosystem CO_2 exchange (NEE) and its components respond dynamically to rising N deposition is far from clear. Using a~~n~~ N addition gradient experiment (six levels: 0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$) in an alpine meadow on the Qinghai-Tibetan Plateau, we explored the responses of different ecosystem C fluxes to a~~n increasing~~ N ~~loading~~ addition gradient and revealed mechanisms underlying the dynamic responses. Results showed that NEE, ecosystem respiration (ER), and gross ecosystem production (GEP) all increased linearly with N addition rates in the first year of treatment, but shifted to N saturation responses in the second year with the highest NEE (-7.77 ± 0.48 μmol m^{-2} s^{-1}) occurring under N addition rate of 8 gN m^{-2} $year^{-1}$. The saturation responses of NEE and GEP were caused by N-induced accumulation of standing litter, which limited light availability for plant growth, under high N addition. The saturation response of ER was mainly due to decreases in ~~aboveground plant respiration and~~ soil microbial respiration under high N addition, ~~while~~ which was caused by the N-induced reduction in soil pH~~caused declines in soil microbial respiration~~. We also found that various components of ER, including aboveground plant respiration, soil respiration, root respiration, and microbial respiration, responded differentially to the N addition gradient. The results reveal temporal dynamics of N impacts and the rapid shift of ecosystem C ~~cycle~~ fluxes from N limitation to N saturation. These findings are helpful for better understanding and model projection of future terrestrial C sequestration under rising N deposition.

1 Introduction

Anthropogenic reactive nitrogen (N) inputs to the terrestrial biosphere has increased more than threefold over the past century and is predicted to increase further (Lamarque et al., 2005; Galloway et al., 2008). Because of the strong coupling of ecosystem carbon (C) and N cycles, excess N deposition could have significant impacts on ecosystem C cycle (LeBauer and Treseder, 2008; Liu and Greaver, 2010; Lu et al., 2011). Ecosystem net C sequestration is ~~usually~~ predicted to increase or have no significant change under rising N deposition (Nadelhoffer et al., 1999; Magnani et al., 2007; Reay et al., 2008; Niu et al. 2010; Lu et al., 2011; Fernandez-Martinez et al., 2014). However, we have limited understanding on the dynamics ~~of~~ N ~~regulations~~ responses of C sequestration in terrestrial ecosystems, which is crucial for model projection of future terrestrial C cycle under rising N deposition (Reay et al., 2008).

Although N addition generally enhances plant growth~~,~~ and ecosystem net primary productivity (NPP)~~, and plant C pool size according to~~ based on global syntheses of N addition experiments (LeBauer and Treseder, 2008; Xia and Wan, 2008; Lu et al., 2011), ~~N~~ the responses of ecosystem C fluxes vary with N loading rates (Liu and Greaver, 2010; Lu et al., 2011). According to N saturation hypothesis, NPP is assumed to slowly increase with N addition rates first, then get at the maximum value at N saturation point and finally decline with further increase of N input (Aber et al., 1989; Lovett and Goodale, 2011). During this process, ecosystem shifts from a N limited, a N intermediate, to a N saturation stage as N deposition increases. Similarly, the response of net ecosystem CO_2 exchange (NEE) to rising N deposition is likely nonlinear (Tian et al., 2016), because its components of gross ecosystem production (GEP) and ecosystem respiration (ER) may respond nonlinearly to increasing N loading rates (Fleischer et al., 2013; Gomez-Casanovas et al., 2016). In the N limited stage, low rates of N addition could stimulate ecosystem productivity (Aber et al., 1989), GEP (Fleischer et al., 2013; Gomez-Casanovas et al., 2016), and ER (Hasselquist et al., 2012; Zhu et al., 2016), while in the N saturation stage, high doses of N addition could have negative effects on GEP and ER (Treseder, 2008; Janssens et al., 2010; Maaroufi et al., 2015).

Moreover, ER can be divided into aboveground plant respiration, belowground plant respiration (root respiration), and soil microbial respiration. These components of ER could be affected by plant aboveground biomass, root biomass, soil organic matter and microbial biomass C, which may respond variously to N addition (Phillips and Fahey, 2007; Hasselquist et al., 2012). For example, root respiration would be enhanced or not significantly changed under N addition, while soil microbial respiration may be suppressed by N addition (Zhou et al., 2014). The different responses of various components of ER to N addition will also consequently change NEE response. Nevertheless, there is limited knowledge on how various components of NEE respond differentially to N addition gradient. ~~Low rates of N addition could stimulate ecosystem productivity (Aber et al., 1989) and soil respiration (Hasselquist et al., 2012; Zhu et al., 2016), but high doses of N addition could have negative effects on soil respiration and microbial biomass (Treseder, 2008; Janssens et al., 2010; Maaroufi et al., 2015). Therefore, we need multi-levels of N loading to explicitly quantify the dynamic responses of ecosystem C cycle to increasing N loading. According to N saturation theory, NPP was assumed to slowly increase with N addition rates first, then get at the maximum value at N saturation point and finally decline with further increase of N input (Aber~~

~~et al., 1989; Lovett and Goodale, 2011). However, other C cycle processes have not yet been well examined against N saturation concept.~~ In addition, the N responses of ecosystem C fluxes may shift with time because of changes in plant community structure and other limiting factors (Niu et al., 2010). We don’t know ~~the~~ yet at which time ~~when~~ ecosystem C fluxes ~~cycle~~ get~~s~~ N saturated ~~yet~~under increasing N input. The mechanisms underlying ~~why~~ the saturation response of C fluxes ~~get saturated with N inputs~~ are ~~also~~ even far from clear, which hinders us from accurately predicting the C cycle in response to rising N deposition.

~~Large uncertainty also remains for the direction, magnitude, and underlying mechanisms of different C flux components in response to increasing N loading. Net ecosystem~~ ~~CO_2~~ ~~exchange (NEE) is the balance between gross ecosystem production (GEP) and ecosystem respiration (ER) (Chapin III et al., 2011). Limited number of previous studies demonstrated that NEE had a positive (Niu et al., 2010; Huff et al., 2015) or no significant response (Bubier et al., 2007; Harpole et al., 2007) to N addition. The insignificant changes in NEE may be due to the incongruent responses of GEP and ER to N addition. GEP is determined by plant photosynthesis, while ER can be divided into aboveground plant respiration, belowground plant respiration (root respiration), and soil microbial respiration. These components of ER could be affected by plant aboveground biomass, root biomass, soil organic matter and microbial biomass C, respectively, and may respond variously to N addition (Phillips and Fahey, 2007; Hasselquist et al., 2012). For example, root respiration would be enhanced or not significantly changed under N addition, while soil microbial respiration may be suppressed by N addition (Zhou et al., 2014). The different responses of various components of ecosystem C cycle to N addition will consequently change NEE. Nevertheless, there is still limited knowledge on how various components of NEE respond differentially~~

~~to N addition gradient.~~

In this study, we explored the responses of various ecosystem C cycle processes to a~~n~~ N addition gradient in an alpine meadow on the Qinghai-Tibetan Plateau. The Qinghai-Tibetan Plateau has an area of 2.5 million km^2 with alpine meadow covering 35 % of this area, and it is sensitive to environmental change and human activities (Chen et al., 2013). The objectives of this study were to explore how different components of ecosystem C fluxes respond to increasing N loading gradient. Specifically, we addressed the following questions: (i) how do NEE and its components respond to N addition gradient in the alpine meadow? (ii) whether various C cycle processes can get N saturated? If so, at which N addition level they are saturated and how do the responses shift with time? and (iii) what are the mechanisms underlying N saturation responses of different C cycle processes?

2 Materials and methods

2.1 Study site

The study site is located in an alpine meadow in Hongyuan County, Sichuan Province, China, which is on the eastern Qinghai-Tibetan Plateau (32°48′ N, 102°33′ E). The altitude is ~3500 m. Long-term (1961-2013) mean annual precipitation is 747 mm with approximately 80 % occurring in May to September. Long-term mean annual temperature is 1.5 ℃ with monthly mean temperature ranging from -9.7 ℃ in January to 11.1 ℃ in July. The dominant species in this alpine meadow are *Deschampsia caespitosa* (Linn.) Beauv., *Kobresia setchwanensis* Hand. -Mazz., *Carex schneideri* Nelmes, and *Anemone rivularis* Buch.-Ham.. The vegetation cover of this grassland is over 90 %. The soil in the study site is classified as Mat Cry-gelic Cambisol according to the Chinese classification, with surface soil bulk density being

0.89 g cm^{-3}. The soil organic C content and total N content are 37 gC kg^{-1} and 3.5 gN kg^{-1}, respectively. The background N deposition is ranging from 0.87 to 1.38 gN m^{-2} $year^{-1}$ on the eastern Qinghai-Tibetan Plateau, and the natural N deposition rate in China is ranging from 0.11 to 6.35 gN m^{-2} $year^{-1}$ (Lü and Tian, 2007).

2.2 Experimental design

We conducted a~~n~~ N addition experiment with six levels of N addition rate (0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$) in early 2014. The six N treatments were represented by N0 (control), N2, N4, N8, N16, and N32, respectively. The treatments were randomly assigned with five replications, so there were totally 30 plots. Each plot was 8 × 8 m, and the distance between any two adjacent plots was 3 m. The N addition treatments started from May, 2014. In 2014 and 2015, N was applied by hand as NH_4NO_3 (>99 %) every month from May to September (i.e. during the growing season) before rainfall. In order to distribute dry NH_4NO_3 evenly in the plots, we mixed dry NH_4NO_3 with enough amounts of soil to apply.

2.3 Ecosystem C cycle properties and soil pH measurement

Ecosystem C fluxes were measured using a transparent static chamber (0.5 × 0.5 × 0.5 m) attached to an infrared gas analyzer (LI-6400XT; LI-COR Environmental, Lincoln, Nebraska, USA) in the field. During each measurement, the chamber was positioned over a square steel frame, which was permanently inserted into soil and offered a flat base for the chamber. Inside the chamber, two electric fans were mounted in order to mix the chamber atmosphere. The measurements were conducted twice per month on clear, sunny days from May to September in 2014 and 2015. Nine consecutive recordings of CO_2

concentration were taken on each base at 10-second intervals. CO_2 flux rates were determined from the time-courses of the concentrations to calculate net ecosystem CO_2 exchange (NEE). After the measurement of NEE, the chamber was covered by an opaque cloth and the CO_2 measurement was repeated. As the second measurement eliminated light, the CO_2 flux value obtained represented ecosystem respiration (ER). Gross ecosystem production (GEP) was calculated as the difference between NEE and ER. Negative or positive NEE and GEP values represent net C uptake or release, respectively. The detailed methods have also been described in Niu et al. (2008) and Niu et al. (2013).

Soil respiration (SR) was assessed following the measurement of NEE and ER. It was also measured with LI-6400XT attaching a soil CO_2 flux chamber (991 cm^3 in total volume; LI-6400-09; LI-COR Environmental, Lincoln, Nebraska, USA). A PVC collar (10.5 cm in diameter and 5 cm in height) was permanently installed 2-3 cm into the soil. The soil respiration chamber attached to LI-6400XT was placed on each PVC collar for 1-2 minutes to measure SR. Living plants inside the collars were removed regularly by hand to eliminate aboveground plant respiration. Soil heterotrophic respiration (i.e. soil microbial respiration, R_{mic}) was measured using the same method as soil respiration. Differently, the PVC collar was 40 cm in height and installed 36-38 cm into the soil. As >90 % of plant roots were distributing in the topsoil (0-20 cm), 40-cm-long PVC collars could cut off old plant roots and prevented new roots from growing inside the collars. Plants in the collars were completely removed by hand to exclude C supply. The experiment was conducted in early 2014 and the measurements of CO_2 fluxes above these 40-cm-long PVC collars began in late July in 2014, leaving enough time for the remaining plant roots inside the collars to die. Thus CO_2 fluxes in those deep collars represented R_{mic}. The method was same to Wan et al. (2005) and Zhou et al. (2007). Root respiration (R_{root}) was calculated by value of SR minus

R_{mic}. Aboveground plant respiration (R_{above}) was calculated by ER minus SR, and ecosystem plant respiration (R_{plant}) was calculated as the difference between ER and R_{mic}. All the measurements of ecosystem C fluxes were simultaneous~~contemporaneous~~.

Soil samples were collected from the topsoil (0-10 cm) of the 30 plots on August 15, 2014 and August 14, 2015. Two soil cores (8 cm in diameter and 10 cm in depth) were taken at least 1 m from the edge in each plot, and then completely mixed to get a composite sample. The soil samples were sieved by a 2 mm mesh and then were air-dried for chemical analysis. Soil pH was determined with a glass electrode in a 1:2.5 soil:water solution (w/v).

2.4 Statistical analysis

Repeated-measures ANOVA (RMANOVA) was used to examine N addition effects on each ecosystem C flux over the growing season in 2014 and 2015. When we evaluate N addition effects on the different components of ER and their proportions, we averaged their values across the year and then used one-way ANOVA to test the differences among treatments. To test the response pattern of ecosystem C cycle properties to the N addition gradient, we fitted the response parameter to linear or quadratic functions which had the highest R^2. Simple linear regression analyses were used to evaluate relationships of ER with its components and NEE across the two years. $\triangle R_{mic}$ and $\triangle$pH were calculated by data in different N addition treatments minus data in the control treatment. All data were tested for normal distribution before statistical analysis. The a posteriori comparisons were performed by DUNCAN test, and the effects were considered to be significantly different if $P<0.05$. All statistical analyses were conducted with SAS V.8.1 software (SAS Institute Inc., Cary, North Carolina, USA).

3 Results

3.1 NEE and its components in response to N addition gradient

Net ecosystem CO_2 exchange (NEE) varied throughout the growing seasons in both 2014 and 2015. The maximum rates of net CO_2 uptake (indicated by large negative values of NEE) occurred in July in both years (Fig. 1a,d). N addition had a significant impact on NEE in 2014 (P=0.020) and a marginally significant effect in 2015 (P=0.059) (Table 1). ~~Annual~~ M~~m~~ean NEE across months had different responses to the N addition gradient between the two years (Fig. 1a,d). It increased linearly with N addition rates in 2014 (Fig. 2a), but shifted to a saturating response with N addition rates in 2015 (Fig. 2d). The largest NEE was -7.77 ± 0.48 μmol m^{-2} s^{-1} under 8 gN m^{-2} $year^{-1}$ addition rate (N8) in 2015.

The N addition gradient had significant effects on ER (P=0.033 and 0.006, respectively) and GEP (P=0.002 and 0.038, respectively) in both 2014 and 2015 (Table 1). Similar to NEE, both ER and GEP showed linear responses to N addition rates in 2014 but shifted to saturation responses in 2015 (Fig. 2). On average, ER was enhanced by 0.9-16.1 % in 2014 and 7.9-23.7 % in 2015 under different N addition treatments. GEP was increased by 2.4-19.2 % in 2014 and 6.7-20.5 % in 2015 under different N addition levels, with maximal values being -24.40 ± 0.48 μmol m^{-2} s^{-1} under 32 gN m^{-2} $year^{-1}$ in 2014 and -15.38 ± 0.72 μmol m^{-2} s^{-1} under 16 gN m^{-2} $year^{-1}$ in 2015 (Fig. 2).

3.2 Components of ecosystem respiration in response to N addition gradient

We divided ER into aboveground plant respiration (R_{above}), soil respiration (SR), root respiration (R_{root}), and microbial respiration (R_{mic}), and found that different ER components showed diverse responses to N addition gradient. M~~Annual m~~ean SR across months was not significantly changed by N addition gradient in 2014 (Table 1; Fig. 3). However, in 2015, it ranged from 4.98 ± 0.33 μmol m^{-2} s^{-1} to 6.23 ± 0.23 μmol m^{-2} s^{-1} under different N addition levels, with significant reduction under high N addition levels of 16 and 32 gN m^{-2} year^{-1} (P=0.010; Fig. 3). Additionally, the relationship between SR and N addition rates was not significant in 2014 (Fig. 3a), while SR leveled off under high N addition rates in 2015 (Fig. 3c). Interestingly, R_{mic} increased linearly with N addition rates in 2014 (Fig. 3b), while it decreased with N addition rates in 2015 (Fig. 3d). Comparing among various components of ER, only R_{mic} showed distinctively inverse responses to N addition rates between years~~, which kept increasing in 2014 but decreasing in 2015 along the N addition gradient (Fig. 3)~~. All other components of ER generally showed similar response tendency between two years (Fig. 3a,3c,4). R_{above} increased with increasing N addition rates in 2014 (Fig. 4b) but got the maximum value at N16 in 2015 (Fig. 4e). By contrast, R_{root} decreased with increasing N addition rates in 2014 (Fig. 4c), while it had no statistically significant response to N addition gradient in 2015 (Fig. 4f). ~~Comparing among various components of ER, only R_{mic} showed distinctively inverse responses to N addition rates between years, which kept increasing in 2014 but decreasing in 2015 along the N addition gradient (Fig. 3). All other components of ER generally showed similar response tendency between two years (Fig. 3a,3c,4).~~

In addition, the proportions of different efflux components to ER differed in response to N addition gradient between years (Fig. 5). The proportions of R_{above} to ER kept increasing with N addition rates in 2014 but got saturated at N16 in 2015 (Fig. 5a,d). The proportions of R_{root} to ER ranged from 31.90 ± 6.69 % in N0 plots to 11.18 ± 1.28 % in N32 plots in 2014 (Fig. 5b), but was not significantly different among N addition levels in 2015 (Table 1; Fig. 5e). In 2014, the contributions of R_{mic} to ER did not significantly change under N addition treatments (Table 1; Fig. 5c), whereas they declined along the N addition gradient in 2015 (Fig. 5f).

3.3 Causes for the N saturation responses of ecosystem C fluxes

In order to examine the causes for the N saturation responses of ER in 2015, we examined the relationship between ER and its various components. The results showed that ER had significantly positive correlation with R_{above} and R_{mic} (Fig. 6a,c) but not with R_{root} (Fig. 6b). Moreover, NEE closely correlated with ER (Fig. 6d). ~~The findings indicated that the saturation response of R_{above} and the declined response of R_{mic} in combination contributed to N saturation response of ER and the consequent saturation response of NEE in 2015.~~ We further explored the causes for decreasing R_{mic} with N addition in 2015 and found that N addition significantly reduced soil pH in 2015 (Fig. 7a). N-induced reduction in soil microbial respiration (ΔR_{mic}) was positively dependent on N-induced reduction in soil pH (ΔpH) in 2015 (Fig. 7b), but not in 2014. ~~The decreased R_{above} at high N addition rates was attributed to the accumulated standing litter mass and thus less light condition under high N addition treatments (Fig. S1).~~

4 Discussion

4.1 Nitrogen saturation responses of ecosystem C fluxes and the causes

Our ~~findings~~ results showed that ecosystem C fluxes (NEE, ER, and GEP) in this alpine meadow ~~had linear responses in the first year but shifted to saturation responses in the second year (Fig. 2,3). The linear responses in the first year suggest N limitation for ecosystem C cycle in this alpine meadow. The saturation responses in the second year indicate N demands for ecosystem C fluxes may get saturated~~

~~under high N addition rates and will decrease further with more N addition.~~were in a N limitation stage in 2014; but in 2015, they were in the limitation stage at low N addition rates and shifted to the saturation stage at high N addition rates. ~~Being~~ The findings confirm~~consistent with~~ the N saturation hypothesis proposed for the response of NPP to N addition (Aber et al., 1998; Aber et al., 1989; Lovett and Goodale, 2011)~~, NEE also showed three stages of response to N gradient in the second year, in which net C sequestration increased first with N addition levels, then saturated under N addition rate of approximately 8 gN m^{-2} year^{-1}, and eventually decreased with any higher N addition rates~~. Beyond that, this study provides more comprehensive evidence for various ecosystem C fluxes and more details on the dynamic N responses. Most previous N addition studies used only one level of N addition and found that NEE showed a positive (Niu et al., 2010; Huff et al., 2015) or no significant response (Harpole et al., 2007; Bubier et al., 2007) to N addition. One level of N addition could not give solid assessment and quantification of ecosystem responses to N addition. By using a~~n~~ N addition gradient experiment, this study comprehensively showed the ~~dynamic~~ saturation responses of NEE and its components to different N loading rates.

The N saturation response of NEE in 2015 was mainly attributed to the saturation responses of ER and GEP (Fig. 2), while ~~The~~ the N saturation response~~s~~ of ER ~~and thus NEE are~~ was ~~mainly~~ likely caused by the ~~decrease~~ saturation response of aboveground plant respiration and decreasing soil microbial respiration ~~under high~~along the N addition gradient~~treatments in 2015~~. The decrease of aboveground plant respiration under N32 treatment ~~is~~ was primarily due to that N addition stimulated plant growth and thus standing litter accumulation after plant senescence (Fig. S1-S2). In 2014, plant aboveground biomass (AGB) was stimulated under high N addition treatment, especially AGB of grasses (Fig. S2). In this grassland, grasses usually have higher height than other plants. The accumulation of grasses standing litter under N32 treatment limited light condition for other plants and negatively influenced plant growth in the early growing season in 2015. ~~The greater standing litter mass could reduce light availability for plant growth under N32 treatment in the second year.~~ Therefore, GEP and NEE ~~decreased~~ did not keep increasing at the highest N addition rate, leading to N saturation response. The N-induced light limitation for plant growth was also observed in other ecosystems, like temperate grassland (Niu et al., 2010; Kim and Henry, 2013). Moreover, our results showed that most components of ER had similar response patterns between the two years except soil microbial respiration that increased in 2014 but decreased in 2015 along with N addition rates. 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. Thus, we propose that soil microbial respiration might play a key role in mediating the N saturation effects for ER and thus NEE, which is not reported in previous studies. The decline of microbial respiration under high N addition conditions was primarily due to the N-induced reduction in soil pH (Fig. 7). Previous study suggested soil pH was the most important driver for responses of microbes under high N addition rates (Liu et al., 2014; Song et al., 2014; Chen et al., 2016). N addition can lead to soil acidification and bring negative impacts on soil microbial growth and activities (Liu et al., 2014; Tian et al., 2016). In this study, the decreased soil pH may cause toxicity effects on microbial activity (Treseder, 2008; Zhou et al., 2012) and thus reduces microbial respiration after two years of N addition.

4.2 The time and N threshold for the saturation responses

Our findings demonstrate that N responses of ecosystem C fluxes shifted from linear response to saturation response over the two years of treatments (Fig. 2). A recent study revealed that ecosystem C fluxes exhibited saturating responses to N addition during two consecutive measurement years in a temperate grassland (Tian et al., 2016). However, their measurement was conducted after ten years of N addition treatments (similar N addition rates with our study), so it did not capture the early response signals of ecosystem C exchange. Results of another N addition gradient experiment carried out in three marsh ecosystems showed that aboveground plant biomass increased linearly with N addition rates after seven months of treatment, but showed saturating responses after 14 months of N addition (Vivanco et al., 2015). Taken together with our results, it suggests that N saturation of ecosystem C fluxes ~~may~~ might happen within couple years of N input. The different responses between years in this study are not likely due to climate differences, because temperature and precipitation were not significantly different between 2014 and 2015. We acknowledge that our findings are just based on the short-term study, while long-time experiment may capture more robust patterns on N saturation and the underlying mechanisms, but the findings of the initial shift of N responses are helpful to better understand the dynamics of ecosystem in response to external N input~~very quickly~~.

The N saturation threshold for ecosystem C fluxes of this alpine meadow is approximately 8 gN m^{-2} year^{-1}. This level is much higher than that in an alpine steppe on the Qinghai-Tibetan Plateau (Liu et al., 2013). In Liu et al.'s study, biomass N concentration, soil N_2O flux, N-uptake efficiency and N-use efficiency showed saturating responses at N addition rate of 4 gN m^{-2} year^{-1}. The discrepancy is probably caused by different precipitation at the two sites. The precipitation is 747 mm in our study site and is 415 mm in their study site. The lower precipitation may constrain ecosystem's response to N addition in Liu et al. (2013). Likewise, the N saturation load ~~for~~ in our alpine meadow is higher than that ~~for~~ in an alpine dry meadow in Colorado (Bowman et al., 2006) and is comparable with that in a temperate steppe of Eurasian grasslands ~~with~~ which found a ~~saturable~~ saturation N addition rate of approximately 10.5 gN m^{-2} year^{-1} (Bai et al., 2010). The higher saturation levels indicate that this alpine meadow is more limited by N comparing with other resources. Furthermore, the N critical load for causing changes in ecosystem C cycle processes is around 2 gN m^{-2} year^{-1} in this alpine meadow. In the first year, ecosystem C exchanges were not significantly different between N0 and N2 treatments, but C fluxes were greater in N2 plots than that in N0 plots in the second year (Fig. 1). This threshold for triggering changes in ecosystem C fluxes ~~changes~~ is comparable to that in another alpine meadow on the mid-south of the Tibetan Plateau (Zong et al., 2016). ~~Observed~~ Considering that atmospheric wet N deposition is ranging from 0.87 to 1.38 gN m^{-2} year^{-1} on the eastern Qinghai-Tibetan Plateau (Lü and Tian, 2007~~).~~), ~~Our~~ our estimate on N critical load suggests that ecosystem C cycle ~~would~~ may 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.

4.3 Diverse responses of C flux components to N addition gradient

The components of ER showed diverse responses to the N addition gradient (Fig. 4,5). For example, in 2014, aboveground plant respiration and its proportion to ER increased, but belowground plant respiration and its proportion to ER decreased with N addition amounts. Microbial respiration decreased and its proportion to ER did not change with the N addition gradient. To our knowledge, ~~there was~~ no previous study ~~examining~~ examined different components of ER in response to N addition gradient. ~~Previous~~ Some studies conducted in alpine grassland demonstrated that N addition had no significant effects on ER (Jiang et al., 2013; Gong et al., 2014), since aboveground biomass did not respond to N addition in their studies.

In this study, greater plant growth and aboveground biomass under N addition enhanced aboveground plant respiration and ~~then~~ thus stimulated ER. The lack of N effect on soil respiration (SR) in 2014 may be attributed to the counteractive responses of soil microbial respiration and root respiration to N addition. In the first year, N addition ameliorated the nutrient limitation for microbes, thus soil microbial activity and biomass increased in short term (Treseder, 2008) and subsequently stimulated microbial respiration (Peng et al., 2011). On the other hand, N addition could reduce belowground biomass allocation (Haynes and Gower, 1995), leading to decrease in root respiration. The increase of soil microbial respiration partly offsets the decrease of root respiration. As a result, SR had no significant difference among N treatments in the first year. However, in the second year, soil microbial respiration declined under high N addition levels, in combination with the low root respiration, resulting in decreases of SR under N16 and N32 treatments. This decrease in SR was also observed in other ecosystems under long-term or high levels of N addition (Yan et al., 2010; Zhou and Zhang, 2014; Maaroufi et al., 2015). In summary, these results indicate that ER and its components could respond to N addition gradient in different ways.

5 Conclusions

Based on a field N addition gradient experiment, this study tested N saturation theory against multiple C cycle processes and found that ecosystem C fluxes of NEE, GEP, and ER shifted from linear responses to saturation responses over two years of N addition. The saturation responses of NEE and ER were mainly caused by N-induced decreases in aboveground plant respiration and soil microbial respiration under high N addition rates. Furthermore, N-induced reduction in soil pH was the main mechanism underlying declines in microbial respiration under high N addition. The N critical load for causing ecosystem C fluxes changes and the N saturation threshold in this alpine meadow were 2 and 8 gN m^{-2} $year^{-1}$, respectively. We also revealed that various components of ER, including aboveground plant respiration, soil respiration, root respiration, and microbial respiration, responded differentially to N addition gradient. The findings suggest that the C cycle processes have differential responses to N addition between aboveground and belowground plant parts, and between plants and microbes. Our findings provide experimental evidences for the dynamic N responses of ecosystem C cycle, which is helpful for parameterizing biogeochemical models and guiding ecosystem management in light of future increasing N deposition.

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References

Aber, J. D., Nadelhoffer, K. J., Steudler, P., and Melillo, J. M.: Nitrogen saturation in northern forest ecosystems, BioScience, 39, 378-286, 1989.

Aber, J. D., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited, BioScience, 48, 921-934, doi: 10.2307/1313296, 1998.

Bai, Y., Wu, J., Clark, C. M., Naeem, S., Pan, Q., Huang, J., Zhang, L., and Han, X.: Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from Inner Mongolia Grasslands, Global Change Biology, 16, 358-372, doi: 10.1111/j.1365-2486.2009.01950.x, 2010.

Bowman, W. D., Gartner, J. R., Holland, K., and Wiedermann, M.: Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: Are we there yet?, Ecological Applications, 16, 1183-1193, 2006.

Bubier, J. L., Moore, T. R., and Bledzki, L. A.: Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog, Global Change Biology, 13, 1168-1186, doi: 10.1111/j.1365-2486.2007.01346.x, 2007.

Chapin III, F. S., Matson, P. A., and Vitousek, P.: Principles of terrestrial ecosystem ecology, Springer, 2011.

Chen, D., Li, J., Lan, Z., Hu, S., and Bai, Y.: 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, 2016.

Chen, H., Zhu, Q., Peng, C., Wu, N., Wang, Y., Fang, X., Gao, Y., Zhu, D., Yang, G., and Tian, J.: The impacts of climate change and human activities on biogeochemical cycles on the Qinghai - Tibetan Plateau, Global Change Biology, 19, 2940-2955, 2013.

Fernandez-Martinez, M., Vicca, S., Janssens, I. A., Sardans, J., Luyssaert, S., Campioli, M., Chapin, F. S., Ciais, P., Malhi, Y., Obersteiner, M., Papale, D., Piao, S. L., Reichstein, M., Roda, F., and Penuelas, J.: Nutrient availability as the key regulator of global forest carbon balance, Nature Climate Change, 4, 471-476, doi: 10.1038/Nclimate2177, 2014.

Fleischer, K., Rebel, K. T., van der Molen, M. K., Erisman, J. W., Wassen, M. J., van Loon, E. E., Montagnani, L., Gough, C. M., Herbst, M., Janssens, I. A., Gianelle, D., and Dolman, A. J.: The contribution of nitrogen deposition to the photosynthetic capacity of forests, Global Biogeochemical Cycles, 27, 187-199, doi: 10.1002/gbc.20026, 2013.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., and Sutton, M. A.: Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions, Science, 320, 889-892, doi: 10.1126/science.1136674, 2008.

Gomez-Casanovas, N., Hudiburg, T. W., Bernacchi, C. J., Parton, W. J., and Delucia, E. H.: Nitrogen deposition and greenhouse gas emissions from grasslands: Uncertainties and future directions, Global Change Biology, 22, 1348-1360, doi: 10.1111/gcb.13187, 2016.

Gong, Y., Mohammat, A., Liu, X., Li, K., Christie, P., Fang, F., Song, W., Chang, Y., Han, W., and Lü, X.: Response of carbon dioxide emissions to sheep grazing and N application in an alpine grassland-Part 1: Effect of sheep grazing, Biogeosciences, 11, 1743-1750, 2014.

Harpole, W. S., Potts, D. L., and Suding, K. N.: Ecosystem responses to water and nitrogen amendment in a California grassland, Global Change Biology, 13, 2341-2348, doi: 10.1111/j.1365-2486.2007.01447.x, 2007.

Hasselquist, N. J., Metcalfe, D. B., and Högberg, P.: Contrasting effects of low and high nitrogen additions on soil CO_2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest, Global Change Biology, 18, 3596-3605, 2012.

Haynes, B. E., and Gower, S. T.: Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin, Tree physiology, 15, 317-325, 1995.

Huff, L. M., Potts, D. L., and Hamerlynck, E. P.: Ecosystem CO_2 exchange in response to nitrogen and phosphorus addition in a restored, temperate Grassland, The American Midland Naturalist, 173, 73-87, 2015.

Janssens, I., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A. J., Grace, J., and Matteucci, G.: Reduction of forest soil respiration in response to nitrogen deposition, Nature Geoscience, 3, 315-322, 2010.

Jiang, J., Zong, N., Song, M., Shi, P., Ma, W., Fu, G., Shen, Z., Zhang, X., and Ouyang, H.: Responses of ecosystem respiration and its components to fertilization in an alpine meadow on the Tibetan Plateau, European Journal of Soil Biology, 56, 101-106, 2013.

Kim, M. K., and Henry, H. A.: Net ecosystem CO_2 exchange and plant biomass responses to warming and N addition in a grass-dominated system during two years of net CO_2 efflux, Plant and Soil, 371, 409-421, 2013.

Lü, C. Q., and Tian, H. Q.: Spatial and temporal patterns of nitrogen deposition in China: synthesis of observational data, Journal of Geophysical Research: Atmospheres (1984–2012), 112, 2007.

Lamarque, J. F., Kiehl, J. T., Brasseur, G. P., Butler, T., Cameron-Smith, P., Collins, W. D., Collins, W. J., Granier, C., Hauglustaine, D., Hess, P. G., Holland, E. A., Horowitz, L., Lawrence, M. G., McKenna, D., Merilees, P., Prather, M. J., Rasch, P. J., Rotman, D., Shindell, D., and Thornton, P.: Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition, Journal of Geophysical Research-Atmospheres, 110, Artn D19303, doi: 10.1029/2005jd005825, 2005.

LeBauer, D. S., and Treseder, K. K.: Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed, Ecology, 89, 371-379, doi: 10.1890/06-2057.1, 2008.

Liu, L. L., and Greaver, T. L.: A global perspective on belowground carbon dynamics under nitrogen enrichment, Ecology Letters, 13, 819-828, doi: 10.1111/j.1461-0248.2010.01482.x, 2010.

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

Liu, Y. W., Xu, X. L., Wei, D., Wang, Y. H., and Wang, Y. S.: Plant and soil responses of an alpine steppe on the Tibetan Plateau to multi-level nitrogen addition, Plant and Soil, 373, 515-529, 2013.

Lovett, G. M., and Goodale, C. L.: A new conceptual model of nitrogen saturation based on experimental nitrogen addition to an oak forest, Ecosystems, 14, 615-631, doi: 10.1007/s10021-011-9432-z, 2011.

Lu, M., Zhou, X. H., Luo, Y. Q., Yang, Y. H., Fang, C. M., Chen, J. K., and Li, B.: Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis, Agriculture Ecosystems & Environment, 140, 234-244, doi: 10.1016/j.agee.2010.12.010, 2011.

Maaroufi, N. I., Nordin, A., Hasselquist, N. J., Bach, L. H., Palmqvist, K., and Gundale, M. J.: Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils, Global Change Biology, 21, 3169-3180, 2015.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P. G., and Kolari, P.: The human footprint in the carbon cycle of temperate and boreal forests, Nature, 447, 849-851, 2007.

Nadelhoffer, K. J., Emmett, B. A., Gundersen, P., Kjonaas, O. J., Koopmans, C. J., Schleppi, P., Tietema, A., and Wright, R. F.: Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests, Nature, 398, 145-148, doi: 10.1038/18205, 1999.

Niu, S., Wu, M., Han, Y., Xia, J., Li, L., and Wan, S.: Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe, New Phytologist, 177, 209-219, 2008.

Niu, S., Sherry, R. A., Zhou, X., and Luo, Y.: Ecosystem carbon fluxes in response to warming and clipping in a tallgrass prairie, Ecosystems, 16, 948-961, 2013.

Niu, S. L., Wu, M. Y., Han, Y., Xia, J. Y., Zhang, Z., Yang, H. J., and Wan, S. Q.: Nitrogen effects on net ecosystem carbon exchange in a temperate steppe, Global Change Biology, 16, 144-155, doi: 10.1111/j.1365-2486.2009.01894.x, 2010.

Peng, Q., Dong, Y., Qi, Y., Xiao, S., He, Y., and Ma, T.: Effects of nitrogen fertilization on soil respiration in temperate grassland in Inner Mongolia, China, Environmental Earth Sciences, 62, 1163-1171, 2011.

Phillips, R. P., and Fahey, T. J.: Fertilization effects on fineroot biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soils, New Phytologist, 176, 655-664, 2007.

Reay, D. S., Dentener, F., Smith, P., Grace, J., and Feely, R. A.: Global nitrogen deposition and carbon sinks, Nature Geoscience, 1, 430-437, 2008.

Song, B., Niu, S. L., Li, L. H., Zhang, L. X., and Yu, G. R.: Soil carbon fractions in grasslands respond differently to various levels of nitrogen enrichments, Plant and Soil, 384, 401-412, 2014.

Tian, D. S., Niu, S. L., Pan, Q. M., Ren, T. T., Chen, S. Q., Bai, Y. F., and Han, X. G.: Nonlinear responses of ecosystem carbon fluxes and water-use efficiency to nitrogen addition in Inner Mongolia grassland, Functional Ecology, 30, 490-499, doi: 10.1111/1365-2435.12513, 2016.

Treseder, K. K.: Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies, Ecology Letters, 11, 1111-1120, doi: 10.1111/j.1461-0248.2008.01230.x, 2008.

Vivanco, L., Irvine, I. C., and Martiny, J. B. H.: Nonlinear responses in salt marsh functioning to increased nitrogen addition, Ecology, 96, 936-947, doi: 10.1890/13-1983.1, 2015.

Wan, S., Hui, D., Wallace, L., and Luo, Y.: Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie, Global Biogeochemical Cycles, 19, 2005.

Xia, J. Y., and Wan, S. Q.: Global response patterns of terrestrial plant species to nitrogen addition, New Phytologist, 179, 428-439, doi: 10.1111/j.1469-8137.2008.02488.x, 2008.

Yan, L., Chen, S., Huang, J., and Lin, G.: Differential responses of auto- and heterotrophic soil respiration to water and nitrogen addition in a semiarid temperate steppe, Global Change Biology, 16, 2345-2357, 2010.

Zhou, L. Y., Zhou, X. H., Zhang, B. C., Lu, M., Luo, Y. Q., Liu, L. L., and Li, B.: Different responses of soil respiration and its components to nitrogen addition among biomes: A meta-analysis, Global Change Biology, 20, 2332-2343, doi: 10.1111/Gcb.12490, 2014.

Zhou, X., Wan, S., and Luo, Y.: Source components and interannual variability of soil CO_2 efflux under experimental warming and clipping in a grassland ecosystem, Global Change Biology, 13, 761-775, 2007.

Zhou, X., Zhang, Y., and Downing, A.: Non-linear response of microbial activity across a gradient of nitrogen addition to a soil from the Gurbantunggut Desert, northwestern China, Soil Biology and

Biochemistry, 47, 67-77, 2012.

Zhou, X., and Zhang, Y.: Seasonal pattern of soil respiration and gradual changing effects of nitrogen addition in a soil of the Gurbantunggut Desert, northwestern China, Atmospheric Environment, 85, 187-194, 2014.

Zhu, C., Ma, Y. P., Wu, H. H., Sun, T., La Pierre, K. J., Sun, Z. W., and Yu, Q.: Divergent Effects of nitrogen addition on soil respiration in a semiarid grassland, Scientific Reports, 6, doi: 10.1038/srep33541, 2016.

Zong, N., Shi, P., Song, M., Zhang, X., Jiang, J., and Chai, X.: Nitrogen critical loads for an alpine meadow ecosystem on the Tibetan Plateau, Environmental management, 57, 531-542, doi: 10.1007/s00267-015-0626-6, 2016.

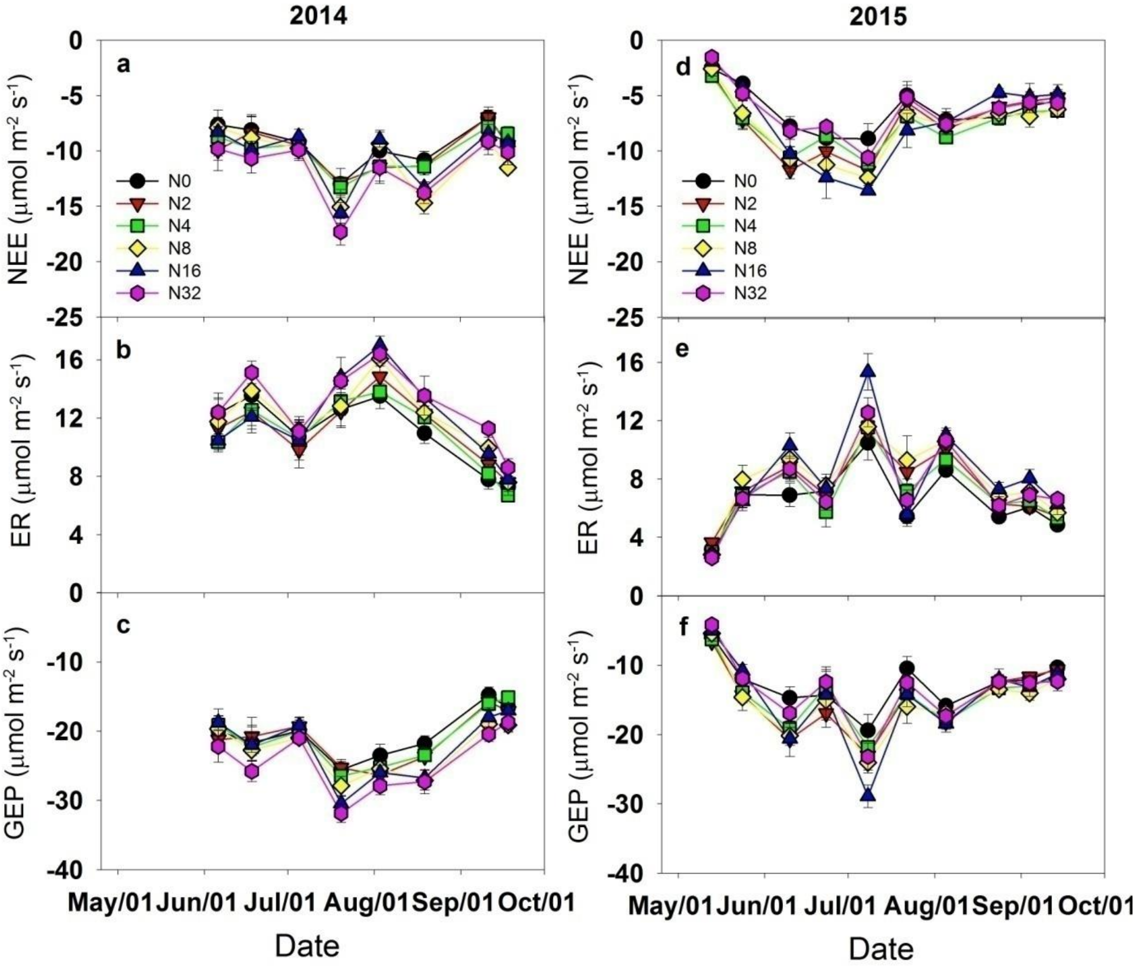


Figure 1. Seasonal dynamics of net ecosystem CO_2 exchange (NEE) (a, d), ecosystem respiration (ER) (b, e), and gross ecosystem production (GEP) (c, f) in 2014 and 2015. N0, N2, N4, N8, N16, N32 represent N addition rate of 0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$, respectively.

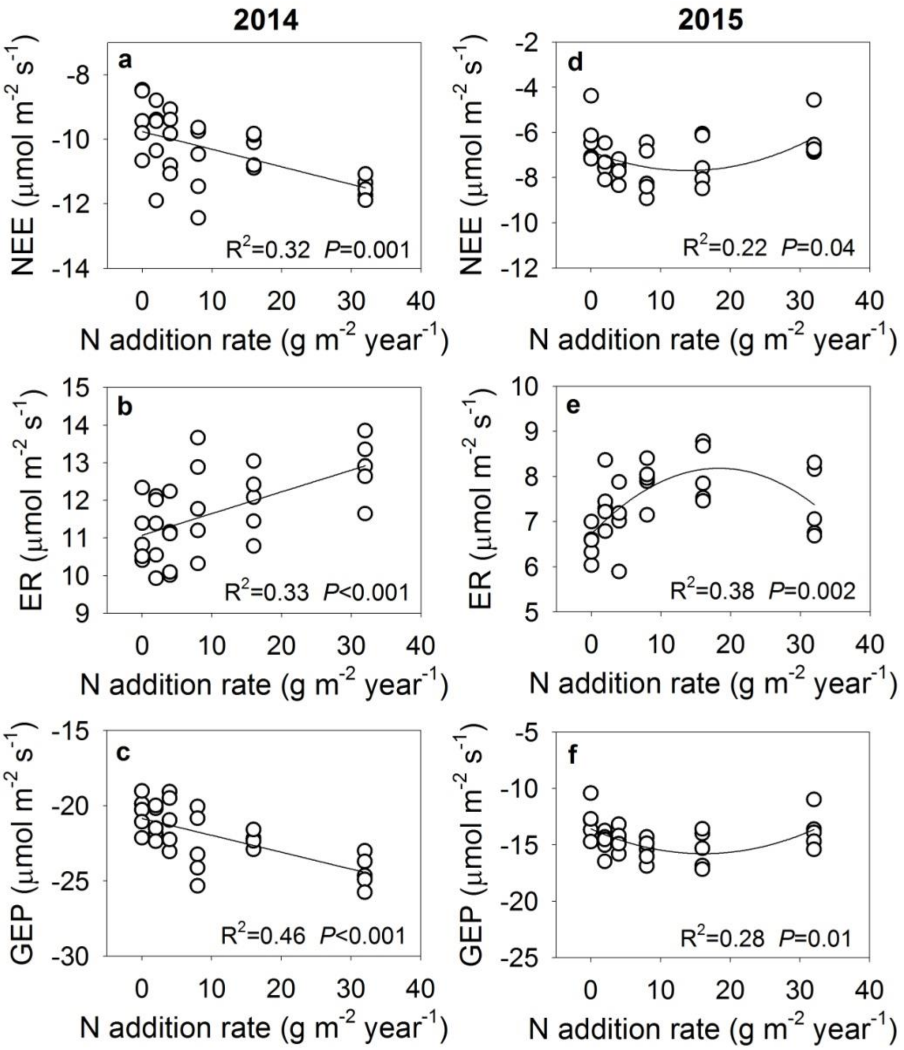


Figure 2. Relationships between N addition rate and net ecosystem CO_2 exchange (NEE) (a, d), ecosystem respiration (ER) (b, e), and gross ecosystem production (GEP) (c, f) in 2014 and 2015.

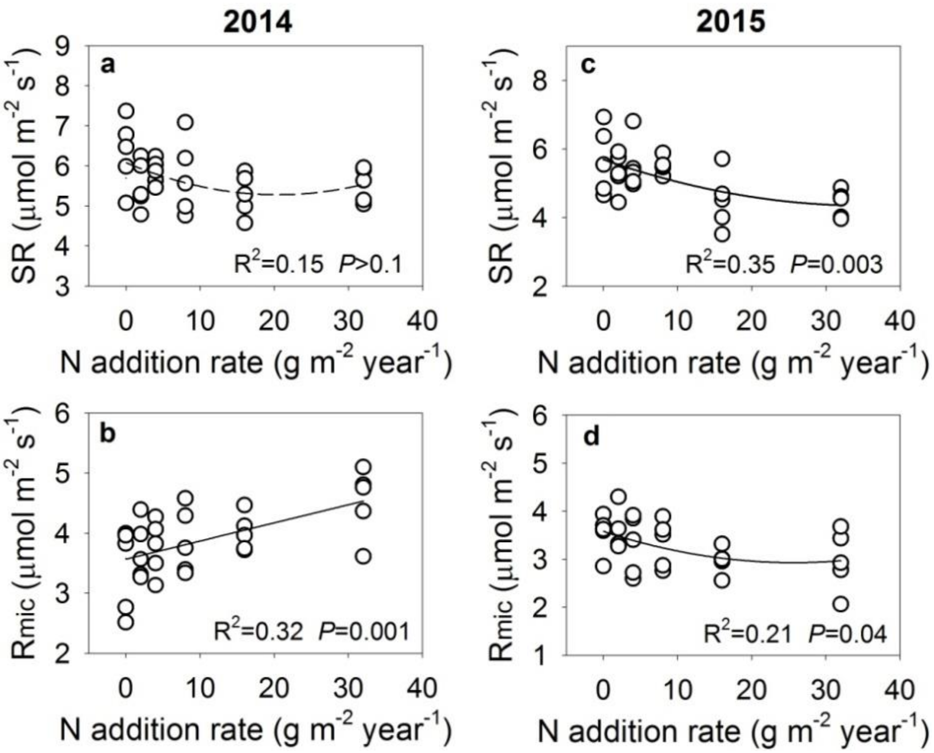


Figure 3. Relationships between N addition rate and soil respiration (SR) (a, c), and soil microbial respiration (R_{mic}) (b, d) in 2014 and 2015.

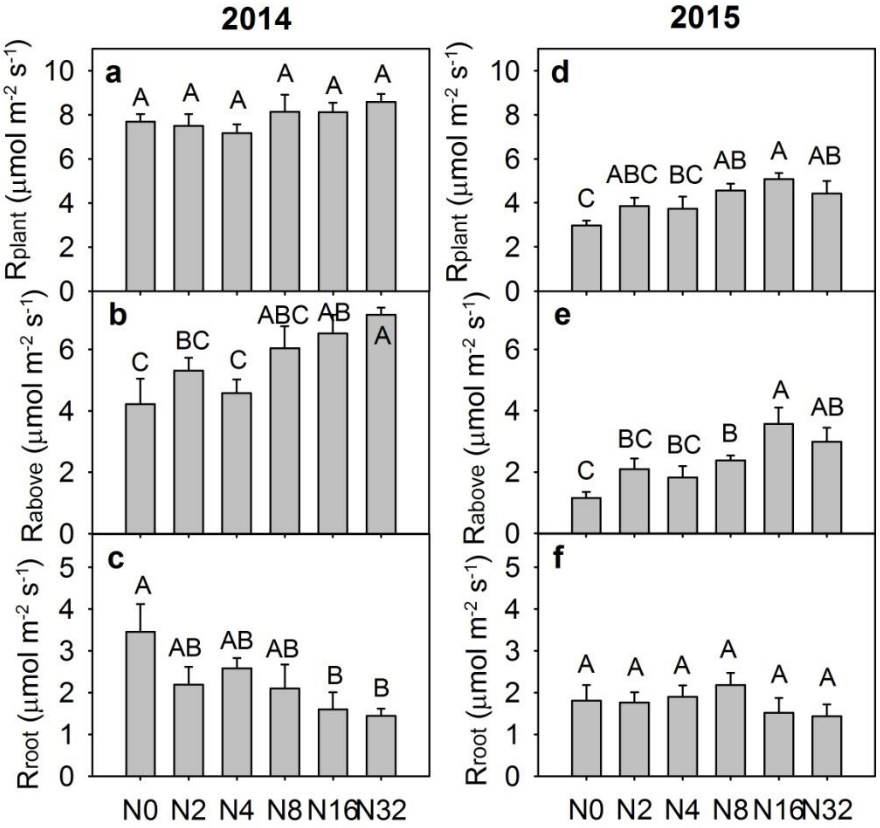


Figure 4. Plant respiration and its components in response to the N addition gradient in 2014 and 2015 (mean ± SE, n = 5). R_{plant}: plant respiration (a, d), R_{above}: aboveground plant respiration (b, e), R_{root}: plant root respiration (c, f). N0, N2, N4, N8, N16, N32 represent N addition rate of 0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$, respectively.

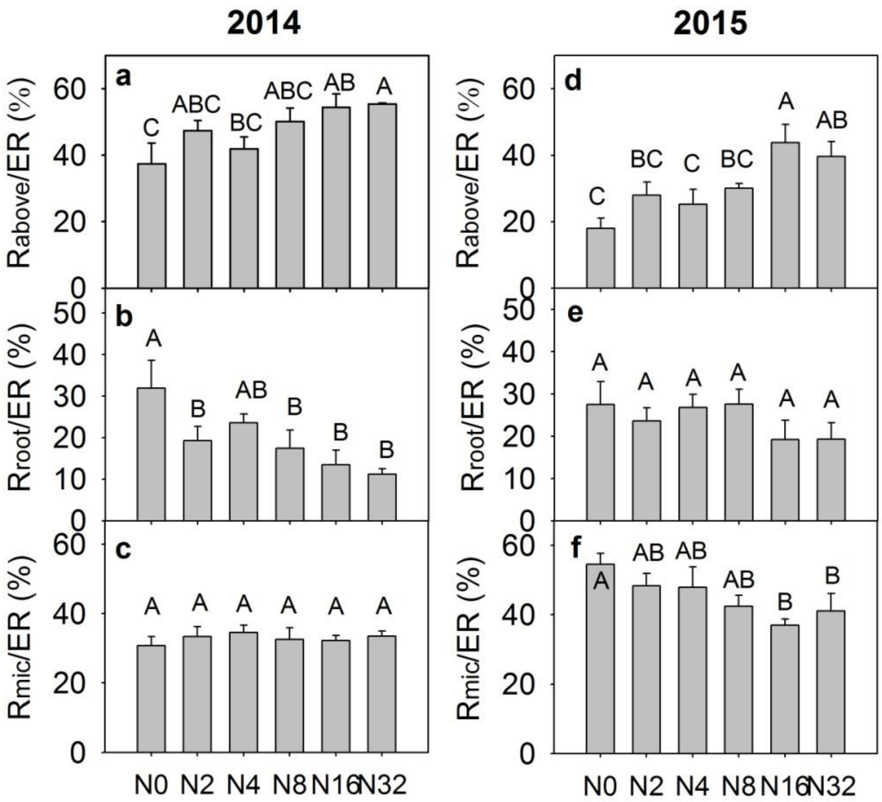
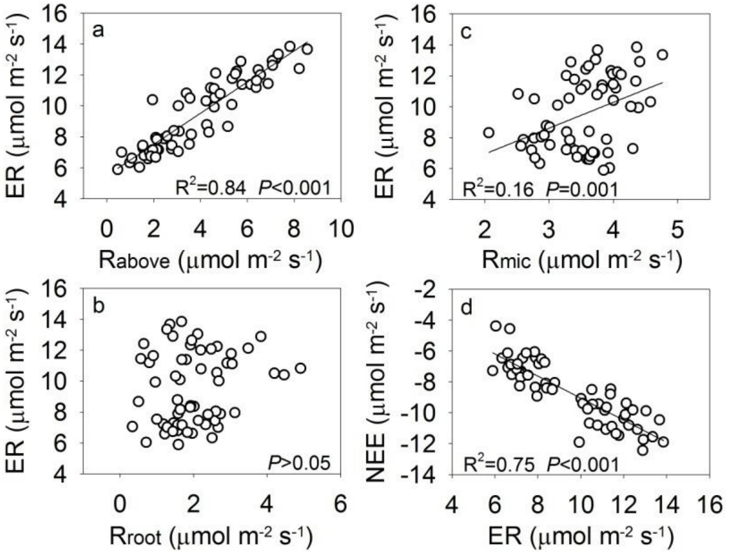


Figure 5. The contributions of different source components to ecosystem respiration (ER) in response to the N addition gradient in 2014 and 2015 (mean ± SE, n = 5). R_{above}: aboveground plant respiration, R_{root}: plant root respiration, R_{mic}: soil microbial respiration. N0, N2, N4, N8, N16, N32 represent N addition rate of 0, 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$, respectively.



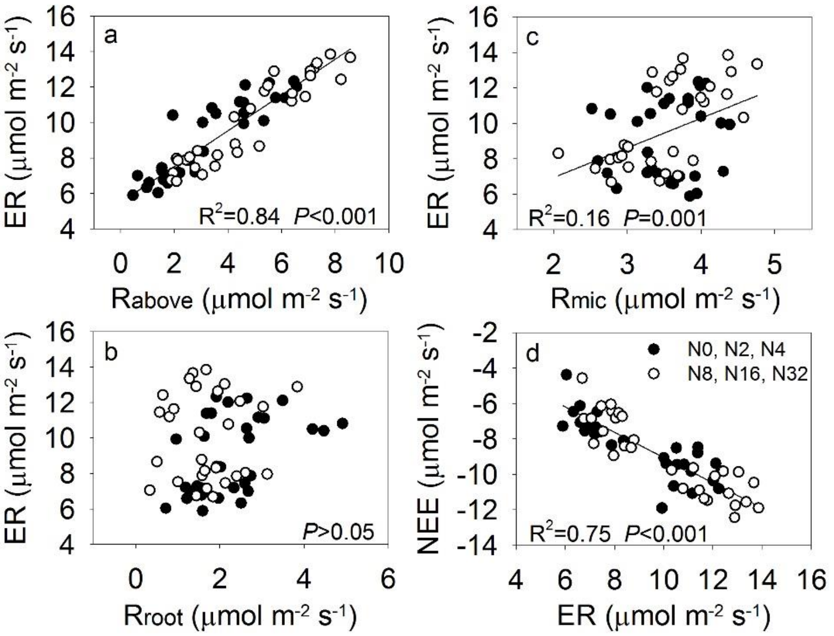


Figure 6. Relationships between aboveground plant respiration (R_{above}), root respiration (R_{root}), soil microbial respiration (R_{mic}) and ecosystem respiration (ER) (a,b,c), ER and net ecosystem CO_2 exchange(NEE) (d) across all plots in 2014 and 2015.

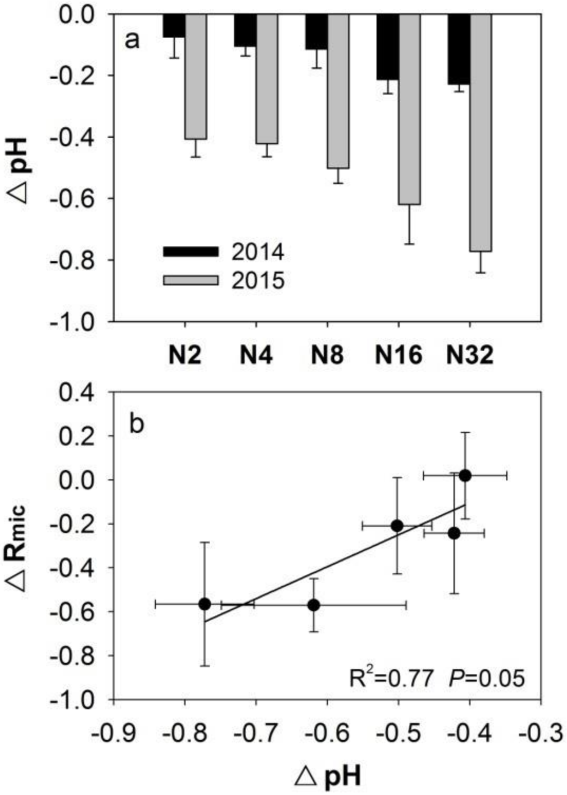


Figure 7. N-induced changes in soil pH (∆pH) (a) (mean ± SE, n = 5) and the dependence of N-induced changes in soil microbial respiration (∆R_{mic}) on N-induced changes in soil pH (∆pH) in 2015 (b). N2, N4, N8, N16, N32 represent N addition rate of 2, 4, 8, 16, 32 gN m^{-2} $year^{-1}$, respectively.

Table 1. Results (F and *P* values) of one-way ANOVA on the effects of nitrogen addition on ecosystem C fluxes in 2014 and 2015. NEE: net ecosystem CO_2 exchange, ER: ecosystem respiration, GEP: gross ecosystem production, SR: soil respiration, R_{mic}: soil microbial respiration, R_{plant}: plant respiration, R_{above}: aboveground plant respiration, R_{root}: plant root respiration.

	df	NEE		ER		GEP		SR		R_{mic}	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
2014	5	3.35	0.020	2.95	0.033	5.37	0.002	1.56	0.209	1.49	0.246
2015	5	2.50	0.059	4.35	0.006	2.83	0.038	3.94	0.010	1.40	0.259

	df	R_{plant}		R_{above}		R_{root}		R_{above}/ER		R_{root}/ER		R_{mic}/ER	
		F	*P*	F	*P*	F	*P*	F	F	F	*P*	F	*P*
2014	5	1.06	0.409	3.84	0.011	2.64	0.049	3.08	0.027	3.56	0.015	0.28	0.919
2015	5	3.25	0.022	5.38	0.002	0.78	0.573	5.54	0.002	0.97	0.456	2.46	0.062

Initial shifts in nitrogen impact on ecosystem carbon fluxes in an alpine meadow: patterns and causes

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Supplementary Material

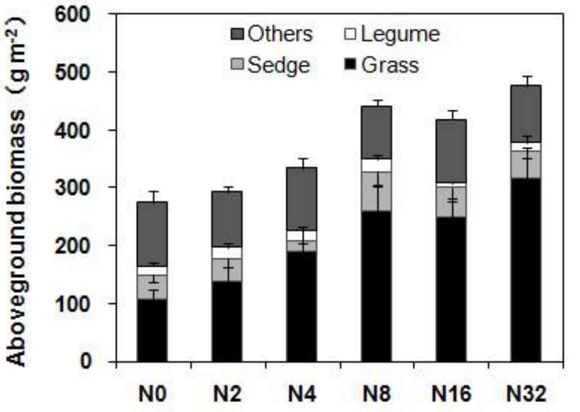

Figure S1. Plant aboveground biomass in response to the N addition gradient in 2014. N0, N2, N4, N8, N16, N32 represent N addition rate is 0, 2, 4, 8, 16, 32 gN m^{-2} year^{-1}, respectively.

~~**Figure S1.** N-induced accumulation of standing litter in high N addition plots (photos were taken on June 13).~~

~~**Figure S1**~~ **Figure S2.** N-induced accumulation of standing litter in high N addition plots (photos were taken on June 13).