

## ***Interactive comment on “Nitrogen input <sup>15</sup>N-signatures are reflected in plant <sup>15</sup>N natural abundances of sub-tropical forests in China” by Geshere Abdisa Gurmesa et al.***

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Response to Anonymous Referee #2

The study investigated effects of natural <sup>15</sup>N abundance of sources in forest ecosystems on δ<sup>15</sup>N value in two different types of forest ecosystems receiving relatively high nitrogen deposition in China. The study is valuable because there are very few long term nitrogen addition experiments in the area. The theme of the study is suitable for Biogeosciences. However there are some problems and manuscript should be revised. Response: Thanks for the constructive comments and suggestions that were very useful to improve our manuscript. We have revised our manuscript by implementing those suggested changes and/or by adding more explanation to clarify our points.

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Major comments

There are two processes explaining nitrogen isotope ratio; <sup>15</sup>N of sources and fractionation processes. Authors discuss the relative contribution of these factors. Authors concluded that source <sup>15</sup>N is more important than fractionation in the study. However, it is very difficult to separate these two processes. Authors stressed the importance of source <sup>15</sup>N too much. Description of the manuscript should be revised substantially.

Response: We highlight the importance of <sup>15</sup>N signature of sources because of three reasons: First, plants are more <sup>15</sup>N-depleted at our study site than seen in global data for tropical forests (Martinelli et al. 1999 and others cited in the manuscript (e.g., Nardoto et al. (2014). According to these studies tropical forests are <sup>15</sup>N-enriched because of increased N cycling rates due to high N availability. Since we found that deposition N is not only high, but also strongly <sup>15</sup>N-depleted, we argued that <sup>15</sup>N of sources add an imprint on top of the effects from fractionation processes. Second, we found that experimental addition of N (with δ<sup>15</sup>N different from that of the plants) moved the δ<sup>15</sup>N of ecosystem pools (plant and soil) toward the <sup>15</sup>N signature of the added N, pointing to the importance of <sup>15</sup>N of sources for ecosystem δ<sup>15</sup>N. Third, when we used the added N with -0.7 δ as tracer for mass balance calculation (Dawson et al. 2002), about 20% of the added N was estimated to be taken up by the plants in the BF. This was close to the estimated fate (24% to plants) of a stronger tracer (Gurmesa et al. 2016) and thus hint that the input N is substantially incorporated into plants although they over all do not increase the uptake in BF. Finally, a recent paper (Perakis et al. 2015) also highlighted the importance of the <sup>15</sup>N of source N, although it was from N-fixation. We agree that it is difficult to separate the contribution of the two processes. To address this concern, we have revisited our text and conclusions to moderate and clarify the statements on the effect of <sup>15</sup>N sources, not to oversell the point. Probably our use of the word 'override' is part of overstating the case. In the revision we use 'dominates effects of fractionation' and keep mentioning also the fractionation signal.

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To evaluate the effects of nitrogen addition, nitrogen concentration and  $\delta^{15}\text{N}$  values are compared between the control and nitrogen added plots. There only three replication in each treatment and statistical power is very low. Because of this weakness care should be taken when the authors discuss the non-significant results. For example, nitrogen concentration of tree leaves at the pine forest was greater in the N added plot at 10 percent level in table 3. When considering the small number of replication, it is difficult to conclude that there is no significant effect of nitrogen addition.

Response: Lack of enough replication is the common limitation of N addition as well as isotope studies. Not many studies have used three true replicate plots for this kind of studies in tropical forests. We agree that the statistics analysis is not very strong to make strong conclusion. Note however, that for the broadleaf forest 5 dominant tree species were sampled and since the species differ in %N and  $\delta^{15}\text{N}$  species was included as a random factor in the tests; i.e. for plant compartments the N addition effects build on more than just three determinations. We have checked our wording in the results section carefully to avoid such weakly supported statements.

In figure 2,  $\delta^{15}\text{N}$  in soil seems different between the control and nitrogen added plots, p-value should be shown for each soil layer and total soil as shown for plant compartments. Authors should describe the limitation of the study about statistical analysis and careful interpretations are required.

Response: We agree to add the data in Fig 2 into Table 4 so that the p-values asked for can be added and shown in the same way as for plants.

Specific comments

L 31, leafs ->leaves

Response: Done

L 29-30,  $\delta^{15}\text{N}$  value of added nitrogen should be described in the sentence.

Response: We have added that the  $\delta^{15}\text{N}$  of the added N is close to that of atmospheric N

spheric N

L37, "plant N% was unchanged. . .," nitrogen concentration was marginally increased in pine leaves and significantly in understory vegetation in the pine forest.

Response: We have revised the sentence, indicating the directions (tendency) of changes though it is not significant. We agree that the term 'unchanged' may not be appropriate for the said reason.

L39, "the signal from the input may override," 'override' is not a proper word in the situation. Fractionation is also an important process for explaining the difference between plant and soil and between soil depths.

Response: In the revision we use 'dominates the signal' instead of 'override' and keep mentioning also the fractionation effects (see our response above).

L137, duration of nitrogen addition should be clearly shown.

Response: We have mentioned that the N addition experiment was established in July 2003, and that it is ongoing now. We have also mentioned that the N is added monthly.

L192-193, information of surface runoff is not sufficient. What is the size of the barrier? How did you collect the water samples?

Response: We have provided more information on how we sampled the surface runoff using the steep slope nature of the plots.

L229-230, p-value of statistical analysis should be shown in table 2.

Response: No significant difference was detected between the two forests. This information is now included in the table caption.

L235, 'see page' should be 'soil solution.'  $\delta^{15}\text{N}$  value of total inorganic N ( $\text{NH}_4$  plus  $\text{NO}_3$ ) should be helpful.

Response: The change is made as suggested.  $\delta^{15}\text{N}$  value of total inorganic N ( $\text{NH}_4$

plus NO<sub>3</sub>)' is also provided.

L238, section title should be revised. It would be "effects of forest types."

Response: Revised as suggested.

L239-240, the information about earlier study should be described in discussion.

Response : Mention of earlier study moved to the discussion.

L240-241, clearly indicate that this comparison is about the control plots.

Response: Now we have clearly indicated that this comparison is about the control plots in the discussion.

L245-247, p-value should be shown. Information about fig 2 should be included in table 4 as shown for table 3.

Response: The data in Fig.2 is now included into Table 4 as suggested.

L263, p-value of statistical analysis should be described.

Response: The p-values were already given, but at another place. Hence, we deleted this part.

L295-299, information of the graphs should be included in table 4 and statistical analysis for N addition should be shown.

Response: Changes implemented as suggested.

L312-316 Figure 3, Information of the graphs should be presented in tables as shown in table 3 and 4. The effects of nitrogen addition should be indicated. Nitrogen concentration and d15N of whole ecosystem (plant plus soil) would be helpful.

Response: As suggested we added the data to the tables as well. We did not give the combined plant+soil values because i) the soil pool is large and will dominate; ii) the direction of change in delta 15N due to N addition go in opposite direction because the

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N addition of almost zero delta 15N (of the added N) is right between the level in plants and soil.

L329-332, Mean value of d15N of soil solution is much lower than throughfall or precipitation. Is there any reason for this difference?

Response: Thanks for raising this issue. We have added a discussion of the fractionation pathways including i) nitrification of deposition NH<sub>4</sub>, that should deplete NO<sub>3</sub> product further to be highly negative; ii) nitrification of soil N, where NO<sub>3</sub> would still be depleted, but coming from delta 2-5, would dilute the negative signal from i); and denitrification of NO<sub>3</sub> to N<sub>2</sub> that would enrich the remaining NO<sub>3</sub>. Since high rates of denitrification have been measured at the site 30 kg/ha/yr (Fang et al. 2015) this may explain why NO<sub>3</sub> in soil solution is only slightly depleted. L345-347, when you compare the d15N value between BF and PF, pine forest had lower d15N. The positive correlation between N availability and leaf 15N still exists within the area. Therefore, it is difficult to conclude the results reject the hypothesis. Response: The reviewer has raised good point here. We want to emphasize that the overall ecosystem 15N-enrichment in tropical ecosystems due to increased N cycling rate is absent at our study site. Here we compared the 15N at DHSBR to other tropical forests reviewed by several authors (e.g., Martinelli et al., 1999; Craine et al., 2009; Craine et al., 2015). We have clarified our wording here, including the use of the word 'overridden' as mentioned above. We acknowledge the difference between the two forests that support the hypothesis because the N-rich BF forests are more 15N-enriched than the relatively N-limited PF.

L362-375, description is only about BF. Is there any comment on PF?

Response: We did not focus on the PF because we observed no clear pattern in soil delta 15N with soil depth. We believed this to be due to the effects of erosion and soil mixing caused by human disturbances until recent years in the PF. This was explained under section 4.2 (previous section). Now we have explained this under this section to

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address the concern by the reviewer.

L382, the contribution of fractionation process and source  $^{15}\text{N}$  value is not clearly known in this study.

Response: We agree that the contribution of fractionation process and  $^{15}\text{N}$  signature of source cannot be separated using the current data. The statement was not meant to rule out fractionation but to show that the  $^{15}\text{N}$  signature of source N can explain the change. Again we changed the wording.

L392-397, it is difficult to understand. N addition possibly decreases the fractionation during n mineralization and may increase plant  $^{15}\text{N}$ . It is difficult to conclude that  $^{15}\text{N}$  source is main sole factor.

Response: We added more text here to explain the figure; hope it helped. The globally established knowledge is that increased N input increases fractionation, and we do not have evidence that N addition might have reduced fractionation during mineralization. However, we agree that fractionation can still be important contributing factor and we had no intention to boldly say that ' $^{15}\text{N}$  source is main sole factor'. We have revised the discussion to elaborate this.

L398-403, the results are based on non-significant results. It is very difficult to conclude the decrease is due to  $^{15}\text{N}$  of added N. Because N input by throughfall has lower value than added N,  $^{15}\text{N}$  of total N input should be lower in the control plots. I thought the description is not correct.

Response: We agree that ' $^{15}\text{N}$  of total N input should be lower in the control plots'. We assume that delta  $^{15}\text{N}$  of throughfall equally affects plants in control and N-plots equally. Any difference between in delta  $^{15}\text{N}$  is likely due to the effect of the N addition. Obviously the effect of N addition was not significant due the large soil N pool that is less responsive to contemporary N input manipulation. But, the observed trend supports or explanation on the importance of  $^{15}\text{N}$  signature source input.

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L405-442, the section should be moved to just before the previous section 4.2.

Response: We have moved the section previous section 4.2.

L415-416, it is difficult to conclude that source  $^{15}\text{N}$  is more important in PF. It is too speculative.

Response: We have indication that incorporation of the added N is high in the PF. Change in plant N content was higher in the PF, indicating the input N has more effect on plants in the PF than in BF. Based on these observations, we argued that input N is more important for plant N source in the relatively N-limited PF. Our conclusion on the response of delta  $^{15}\text{N}$  is correct. We have revised the sentence to make it not too speculative that is not supported by data. We connected our statement to the result of the isotope mixing calculations that were further down in line 440-442.

L436-439, description about N addition should not be described in this section.

Response: We were not sure what the reviewer meant here. But this part of the text was changed also in response to the other reviewer.

L445-455, it is difficult to conclude that  $^{15}\text{N}$  of source is more important than fractionation process. The contribution of fractionation is still also important factor. Conclusion should be revised substantially.

Response: We have revised the conclusion with careful wording as also detailed in our response to the general comments above.

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