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Biogeosciences Discussions

Interactive comment on "Nitrogen input ^{15}N-signatures are reflected in plant ^{15}N natural abundances of sub-tropical forests in China" *by* Geshere Abdisa Gurmesa et al.

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Response to W. W. Wessel (Referee)

General comments

This paper discusses two processes that affect the delta 15N of forests. Firstly the mixing with N deposition with a different 15N signature than the forest itself and secondly the fractionation of the 15N through different N transformation processes followed by the loss of the lighter fraction resulting in enrichment of the remaining N with 15N. The latter process is thought to happen more strongly if N availability is larger and so it is thought that a higher delta 15N is an indication of a higher N availability.

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The authors present two sets of delta 15N results of two forests in southern China: the results of the ambient situation ('control') and the results of a long term N addition experiment in the same forests. In the control experiment they find rather low delta 15N values compared to literature values. As the delta 15N value of the substantial N deposition is also rather low, they conclude that the mixing with N deposition with a low delta 15N is the dominating process in determining the 15N of the vegetation and fractionation combined with loss is not important. Secondly they discuss the effects of a long term addition of N with a higher delta 15N than that of the ambient deposition. Here they conclude that the increase in delta 15N in the vegetation is not the result of increased fractionation and loss, due to the higher N availability but that this is the result of the mixing of the added N with a high delta. Their general conclusion is that when delta 15N of forests is used to say something about N availability more attention should be given to the possible influence the delta 15N signature of the N deposition can have.

Although I do not think that the main conclusions of the authors are incorrect, I think their argument does need substantial improvement.

Response: Thanks for the constructive comments and suggestions. We have seriously addressed all the concerns. For each general and specific comment, we have provided our responses, indicating all the changes we made to the manuscript.

1. In the first place they do not make clear what delta 15N value they do expect for their forest (under ambient conditions) as a result of fractionation and loss, as described in their hypothesis i (line 101). This hypothesis i is unclear (see below) and they do not explicitly compare this hypothesis with their results. In the Discussion section they compare their results not with individual forests from the literature but with large datasets synthesized from many different forests. Why would their conclusions about their own forests not be true for the forests they cite from the literature? If not, what could be the relevant differences between their forests and those from the literature? Maybe the values calculated for southern China by Amundson et al (2003), based on MAP and

MAT can help to structure this part of the discussion?

Response: Thank you for the suggestion for using the Amundsen study to initiate the discussion part. We have strived to clarify our hypotheses and their discussion along the lines suggested. Now we have clearly indicated that we expect delta 15N value under ambient conditions to be higher for our study forest in our re-phrased hypothesis. We have also compared the hypothesis (all hypotheses) with our results. The discussion is improved by comparing our results to both individual forests from the literature (temperate forest which we missed in previous version) and large datasets synthesized from many different forests across the world.

2. At first sight it seems reasonable to consider mixing to be important in the control experiment, but this could be supported with some calculations of the effect of mixing. It seems the authors have carried out such calculations at least for some cases according to their statement in line 440, but this would be useful for this case as well.

Response: As suggested we did further calculations of the effect of mixing in the control plot using two-source mixing model (Dawson et al. 2002, cited in the manuscript), where we assumed soil and deposition N as the major N source to plants. The result showed fraction of N contributed by deposition is 60-80% in the two forests with the higher value being for the pine forest. If plants uptake such large proportion of the deposited N, it likely influence delta 15N of plants as we explained in the discussion because deposition is high at our site and it is strongly 15N-depleted. See further details below.

3. The reasoning in lines 339-356 is very difficult to follow. I will make more specific comments below.

Response: We have made substantial changes to the texts to make it clearer. For example, instead of comparing our result only to the global data in Martinelli et al. 1999, we have added more citation from individual studies from temperate forests that include data from temperate sites that have high N status. We also made it clear how our original first hypothesis was rejected by our results. See our responses to the specific comments too.

4. Concerning the N addition experiment it can be said that both the mixing process and the increased fractionation plus loss process (expected as a result of larger N availability) would lead to an increase in the delta 15N of the vegetation, so it is unclear why the authors choose that the increased delta 15N values found in the vegetation were the result of mixing and not of increased fractionation plus loss. What result of the experiment and the measurements would have led them to the other conclusion? In fact probably both mixing and fractionation plus loss contribute to some extent to the increase of delta 15N in the vegetation. Again some calculations of the mixing of the deposition might give more insight into the potential contribution of this process.

Response: We agree that fractionation combined with loss of 15N-depleted N forms can be caused by larger N availability, and it can be one of those important factors that control ecosystem delta 15N. The increase in plant delta 15N after decadal N addition can be partly explained by this fractionation plus loss processes. However, the same fractionation may not explain the tendency of decrease in soil delta 15N. When we used the added N with -0.7 delta label 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. See our response to similar question from the other referee.

5. I think there is something wrong with the statistical results presented in Tables 3 and 4. The tests for siginifant differences sometimes yield significant p values while the difference tested is smaller than the sum of the two standard errors. This cannot be correct. I suggest the authors provide the data and the script they have used to calculate the statistics so it becomes clear what they have done. See for example in Table 3 twigs difference between BF and PF is 0.29, while the sum of the SEs is 0.96

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Discussion paper

and p<0.01 and in Table 4 tree leaf in BF difference between control and N addition is 0.6, while the sum of the SEs is 1.1 and p<0.01. I assume two-sided tests were carried out although this was not mentioned.

Response: For the comparison of delta 15N between the two forests, we have mentioned that t-test was used as the reviewer assumed it. In Table 3, we had made a copy-past mistake; the correct SE for twigs in PF is 0.05, not 0.77. Further, it is important to note (as mentioned in the table heading) that for the broadleaf forest 5 dominant tree species were sampled and since the species differ in %N and delta 15N, species was included as a random factor in the tests using mixed ANOVA (mentioned in section 2.5); i.e. for plant compartments the N addition effects build on more than just three measurements. Thus the overlap of the SE's based plot means may not be instructive in that case.

6. I would suggest that the authors should be more careful in using the terms 15N enriched and 15N depleted and define what exactly is meant by them and relative to what (below or above zero, or relative to the delta of some other pool or flux). They use these terms many times throughout the text. See e.g. my comment below on line 402. In line 32 even the term "more enriched" is used.

Response: We have strived to clarify the wording and have in some cased added the delta changes to improve on this.

Specific comments

L25 "examined the measurement": this suggests the paper is about measurement techniques. I suggest to rephrase this.

Response: We have rephrased the sentence

L31 "leafs" the text contains many spelling errors; I suggest the authors check the text throughout for these.

Response: We have changed 'leafs' to 'leaves'. Similar spelling errors were thoroughly searched for and corrected.

L31: “old-growth forest” this forest is everywhere else described as broadleaved forest, so I would suggest to use that term here as well

Response: Corrected as suggested

L48 “recently” I think it is relevant to be more specific, so the reader knows how long this N addition has been going on. In the methods the 1990s are mentioned for DHSBR (L113).

Response: We have made it more specific, and mentioned that increase in N deposition in China has been increasing continuously since the 1980s (Liu et al. 2011).

L67 “above the atmospheric standard” I wonder whether for this criterion 0.0 0/00 is the relevant value, as atmospheric N2 is not a direct source of N for a terrestrial ecosystem.

Response: We have deleted the ‘above the atmospheric standard’ because the atmospheric N may not be direct source of N to the plants.

L81-82 “hotspots” If this is meant to be high in N deposition, I would suggest to use the latter term.

Response: We have deleted ‘hotspots’ and used ‘high N deposition’

L102 The comparison of a high N forest with temperate forests seems inconsequent. What about temperate forests with high N status?

Response: We have re-phrased our hypothesis to indicate that the comparison include temperate forests in general (not only N-limited forests. Similarly, the discussion is improved by adding some studies from different temperate forests that cover wider gradient of N availability including N-saturated forests (Koopmans et al. 1997).

L103 second hypothesis: I wonder which results could lead to the rejection of this hypothesis, given the experimental conditions. The first part of this hypothesis seems

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Discussion paper

not very challenging and the second part is not very specific.

Response: We have separated the hypothesis into two (see our response to your comment on 393). We have referred each of our three hypotheses in the discussion and explained if they are confirmed or rejected by our results.

L114 “steep slopes” Amundson et al (2003) have suggested that under these circumstances delta 15N might be lower (see their paragraph [26])

Response: We are aware that topography can have a significant influence on the landscape-scale patterns of plant and soil δ15N. The preliminary data set presented in Amundson et al (2003) was from the central California coast range (Figure 4), suggested that up to 2% variation in soil δ15N at a given location. Apart from these observation used to explain within site variation in soil δ15N, we found no study that compared sites with different topography. We do not believe this steep slope at DHSBR is important factor to explain the distinct 15N-depletion compared to other tropical forests. But we have mentioned this as a potential (minor) influence in the discussion.

L182 “including a dry period” If the authors mean that there were not any water samples in Dec and Jan because of a lack of precipitation then please state this.

Response: We have indicated that these dry months are the period when there were not any water samples because of a lack of precipitation.

L186-187 A collector with an area of 8000 cm2 seems extremely large. Is this a correct value?

Response: The 0.8m2 was a total interception area for the five collectors. We have corrected it.

L215 “plant species as a random factor” Apparently this is not the case for the pine forest, which contains only one dominant species (L159).

Response: Mixed model ANOVA was where plant species used as a random factor was

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Discussion paper

used for compartments with mean from several species (canopy tree in BF and understory vegetation in both forests. For canopy layer in PF, only one dominant species, a simple t-test was used. We have clarified this in the manuscript.

L233 Table 2 . At first sight it looks like leaching losses have lower deltas than the deposition, indicating the occurrence of fractionation and loss of N with low deltas, thus increasing the 15N content of the remaining N. However, as deposition is dominated by NH4, while leaching is dominated by NO3, this is not the case. Calculating a weighted average delta 15N for all chemical species in all fluxes may show this. This can support the argument that fractionation plus loss is not evident from this budget, although it is of course incomplete. Are here not any values for the added N plots?

Response: Using delta 15N data in Table 2 and the concentration data presented in Fig. S1, we calculated the weighted average delta 15N for total dissolved inorganic N (DIN). The data (presented in revised Table 3) showed that soil solution ass slightly higher deltas 15N than the deposition in both forests. We have two samples for the added N plots but only in the broadleaved forest (BF), and it shows even higher delta 15N∼ -2.8‰ the values in the control plot (-5.7‰ Table 2). Since nitrate is enriched in soil solution, it may appear to show no evidence for fractionation and loss of N with low deltas. However, this argument may not be true if denitrification (reported at our site) dominates nitrification because the two processes have antagonistic effect on delta 15N of the nitrate. The nitrate measured in the soil solution also comes from nitrification of soil ammonium, which can still be enriched compared to the nitrate in deposition N. These points are now added to the discussion about delta 15N of the water samples (also see our response to comments on L329-332 by the other referee).

L233 Table 2 In the text it is stated that runoff was measured only in one plot per treatment (line 192), so how can there be an average of three measurements for runoff here?

Response: The runoff was collected in one plot per treatment, but at three different points, which we used as replicate. Since these are not true replicates, we have removed the SE and sample size.

L251 Fig.1 Are these samples that were taken monthly between Sept and Feb (4 samples) with 3 replicates, in total 12? I suggest to explain this in the caption. Again how was this for runoff (see my previous remark on Table 2)? What could be the cause for the variation found? Is there no substantial time delay between the moment of deposition and the moment the deposited N reaches the subsoil or the runoff?

Response: Yes, these are samples that were taken monthly between Sept and Feb (4 samples) with 3 replicates, making total of 12 samples. For the surface runoff, sampling was done only in one plots, but at three points, which were shown in the graph. We have explained this in the caption.

L261-263 "N concentration of N pool weighted average plant pools calculated per plot". The reader is referred to Table 3, but in there are only N concentrations of individual pools.

Response: This sentence is now deleted because it was repeated. Data on effects of N addition on N pool weighted average plant pools was correctly presented later and appropriately referred to Fig 2 (which was Fig 3 in the previous version).

L295 Fig.2 I suggest to increase the size of the symbols in the legend so the different patterns used are more easily recognized. This is also a problem in the supplement figure.

Response: Information in Fig 2 is now included in Table 4 based on a comment from another reviewer. We have increased the size of the symbols in all other figurers including the supplement figure.

L307 "decrease as expected" It is true that the delta of the N input into the forest is still lower than the delta of the soil, but the addition has substantially increased the delta of the total N input, so one might as well expect an increase in the soil delta as a result of this.

Response: Our expectation was that added N has lower delta 15N than the soil, and its incorporation into the soil N pools would lower the soil delta 15N. The only way the added N can increase soil delta is if it caused substantial fractionation that results in loss of 15N-depleted N form and enrichment of soil N pool. This was not proved to be the case at our study site.

L325 “other regions” please specify which regions are meant.

Response: Other regions mean those in Germany (Freyer 1978) and Chesapeake Bay (Russel et al., 1998). However, we have no evidence for 15N-depleted deposition N in these regions, and the two cited papers are also old studies. So we have deleted this part of the sentence and explained our finding in relation to other relevant studies.

L337 “surprisingly” I suggest the authors clarify what they expect here.

Response: Our expectation, as stated in our first hypothesis, was enrichment of leaf $\delta 15N$ at our study cite that is higher than the average leaf $\delta 15N$ observed for temperate forests on global scale.

L342-345 This remark on the enrichment factor seems misplaced here, as nowhere else in the paper something is said about the enrichment factor. It is also unclear to me why this would support the previously mentioned hypothesis.

Response: We agree with this point, and have deleted the sentence.

L345 “rejects this hypothesis” Which result precisely makes the authors decide to reject? Do the authors reject the full hypothesis or only mean that the increase in delta 15N simply does not happen? Nothing is said about hypothesis i from the introduction. I would suggest to refer to this hypothesis as well, although it needs to be rephrased, as I mentioned earlier.

Response: The hypothesis was that tropical forests that have high soil N availability due to increased N deposition have higher delta 15N compared pre-dominantly N-limited temperate forests due to increased fractionation combined with loss of 15N-depleted N in tropical forests. Since we did not observe such ecosystem enrichment at our site, which also has high N deposition, we concluded that the hypothesis is rejected. To make it clearer, we have re-phrased the whole sentence, and indicated why our result rejects this hypothesis. We have also re-structured the paragraphs, and re-phrased the hypothesis to make it brief and direct.

L347 “other depleting factors” I think “other” should be removed as the previously mentioned process is an enriching factor.

Response: The sentence is re-phrased in connection with our response to the above comment on line 345.

L348-349 “in other Chinese forests with high N deposition” Why only or especially in Chinese forests? And would this not depend on the delta 15N value of the N deposition? Maybe the authors have the literature in mind they mention in lines 323Â . If that is the case they should refer explicitly to these results. The authors make a different and more general statement in lines 454-455.

Response: Yes, we refer to those for which we already cited references (Fang et al., 2011a; Wang et al., 2014; line 334 not 323). We have explicitly clarified this. However, we do not believe that our concluding statement in lines 454-455 is different from our explanation here. In lines 454-455, we tried to emphasis the importance of considering 15N signature of input N when interpreting delta 15N of ecosystems as a proxy for ecosystem N cycling.

L381 “It was interpreted” I suppose this was done by the references mentioned just before this sentence. To make this clearer to the reader I suggest to change the sentence from passive into active voice.

Response: We have changed the sentence into active voice.

L393 “in line with our second hypothesis” This can only be true for the first part of this hypothesis

Response: We have separated the hypothesis into two, and wrote it as: ii) N addition would change plant and soil delta 15N towards the 15N signature of the added N due to its incorporation into ecosystem pools, and iii) response of delta 15N to N addition would differ between the two forests due to their difference in their initial N status and N cycling rates. The statement is now related to the second hypothesis.

L396-397 “it shows again” I disagree. From these results one could argue as well that it is the result of increased N availability resulting in increased fractionation plus loss of depleted N.

Response: We understand why the reviewer disagree with the points we made. For plants, fractionation can partly explain the increase in delta 15N, and we have included it in our explanation of the increase in plant delta 15N after the decadal N addition. However, increased fractionation plus loss of depleted N may not explain the decrease in soil delta 15N (although it was not significant) caused by the N addition. The plausible explanation for the changes in delta 15N in both plants and soils is the effect of an imprint from the 15N signature of the added N.

L402 “also after addition of 15N depleted N” In the experiment by the authors the N added was 15N enriched (at least compared to the ambient N deposition).

Response: We referred the added N as ‘15N-depleted’ relative to delta 15N of the bulk soil. This is clarified now.

L440 “calculations based on an isotope mixing model” I would suggest to add some information on how this was calculated and which simplifying assumptions were made in the calculation.

Response: Our wording is not precise enough here, we did a mass balance calculation that uses and assume the added fertilizer as a tracer since its 15N signature differs from the ambient as well as from both plants and soil. To be meaningful it also requires that significant differences in signatures are observed between the control and the N addition treatment, thus the calculation can only be done for the plant pool. In a previous version we had a long section on this that we now see we had cut the text to be too brief. We have included the assumptions and added more explanations in the text. Including also that for the BF forest the result match that of a tracer experiment that we have published in a separate paper.

L445 "in humid tropical forests of southern China" why would this be true for all these forests, not just for the forest investigated? Possibly because of the delta 15N value of the deposition there (see line 323)? Then the authors should refer to this. Would the region differ in this respect from other regions in the world?

Response: We have already mentioned that forests (including those at our study site and others reported in the references we cited) receive 15N-depleted deposition and thus may also have low delta 15N in plants (written as 'an imprint from 15N-depleted N deposition' in previous version). We have added some words to make it clearer.

L447 "further confirmed" see my remark on L396

Response: We have explained why we focused on the importance of 15N signature of the input N. See our response to the above comments on line 396.

L452 "more important" this is only the case if the 15N signature of the N deposition differs sufficiently from the delta 15N of the ecosystem, and the N deposition is sufficiently large. If that is not the case the mixing probably would not dominate the fractionation plus loss of depleted N.

Response: As shown in our data (Table 2, Table 4), 15N signature of the N deposition differs sufficiently from the delta 15N of the ecosystem. Total N deposition at our site was measured to be 51 kg N per year during 2013-2014, which is sufficiently large. Thus, our conclusion is reasonably sound. We have re-phrased our conclusion to

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Discussion paper

avoid wording that indicate fractionation is not important at all. We are not sure if the reviewer hints that generalization should be including the cause that he mentioned. We have included that the importance increase with significantly elevated N deposition which is widespread in many regions.

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