April 07, 2016

Dear Yakov Kuzyakov,

We are very excited to have been given the opportunity to revise our manuscript, Ms. No. bg-2016-439 "**Nitrogen input ^{15}N-signatures are reflected in plant ^{15}N natural abundances of sub-tropical forests in China**". We carefully considered the comments and recommendations offered by the two
referees. Herein, we explain how we revised the paper based on those comments and recommendations.
We want to extend our appreciation for their insightful guidance.

The revision, based on the referees' collective input, includes a number of positive changes. We also got a few suggestions (including language edits) from Professor Knute Nadelhoffer; a native English speaker and a prominent researcher in the field. Based on these guidance, we:

- Edited the abstract to highlight the most important finding of the study in a more flawless language
- Revised portions of the introduction to clearly highlight knowledge gaps, and to clarify the hypotheses to be tested.
- Revised presentation of data, and added more description of the results, as requested
- We have completely revised most of the discussion to accommodate the suggestions and clarify our discussion points

We hope that these revisions improve the paper such that you and the reviewers now deem it worthy of
publication in Biogeoscience. Next, we offer detailed point-by-point responses to comments from the
referees. All line references are referring to the 'marked up version' showing major changes made to the manuscript.

Jiangming Mo (on behave of all co-authors)
Corresponding author

Response to Anonymous Referee #2

The study investigated effects of natural 15N abundance of sources in forest ecosystems on d15N 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.

Major comments

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

Response: 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 ^{15}N of 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.

To evaluate the effects of nitrogen addition, nitrogen concentration and d15N values are compered 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 replicated 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 $\delta^{15}N$, tree 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 carefully checked our wording in the results section to avoid such weakly supported statements.

In figure 2, delta 15 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 have added the data in Fig 2 into Table 4, and the *p*-values asked for are added and shown in the same way as for plants.

Specific comments

L 31, leafs ->leaves

Response: Done

L 29-30, d15N value of added nitrogen should be described in the sentence.

Response: We have added that the $\delta^{15}N$ of the added N is close to that of atmospheric N (line 23).

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 (lines 28-29). 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 used '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 already mentioned that the N addition experiment is ongoing, and it was established in July 2003. To make it clearer, we have mentioned that the N is added monthly since July 2003 (line 136).

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 (lines 176-179).

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 (lines 219-220).

L235, 'see page' should be 'soil solution.' 15N value of total inorganic N (NH4 plus NO3) should be helpful.

Response: We $\delta^{15}N$ of total inorganic N (NH4 plus NO3)' is now provided. *'see page' changed to 'soil solution' throughout the ms.*

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: We have indicated in Table and figure captions (line 260, 279 and 296) that comparison is about the control plots. We have also mentioned across the discussion when discussing difference in %N and $\delta^{15}N$ between the two forests (e.g., 407).

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 (283-288), 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: 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 $\delta^{15}N$ due to N addition go in opposite direction because the N addition of almost zero $\delta^{15}N$ (of the added N) is right between the level in plants and soil. We decide to keep the figure, since it is better in showing simultaneously the changes in both %N and $\delta^{15}N$.

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_4, that should deplete NO_3 product further to be highly negative; ii) nitrification of soil N, where NO_3 would still be depleted, but coming from $\delta^{15}N$ 2-5‰, would dilute the negative signal from i); and denitrification of NO_3 to

N_2 that would enrich the remaining NO_3. Since high rates of denitrification have be measured at the site 30 kg/ha/yr (Fang et al., 2015) this may explain why NO_3 in soil solution is only slightly depleted. This and other explanation on the observed trend in $\delta^{15}N$ in input and output fluxes is now discussed in detail (lines 300-325).

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 emphasis that the overall ecosystem ^{15}N-enrichment in tropical ecosystems due to increased N cycling rate is absent at our study site. Here we compared the ^{15}N at DHSBR to other tropical forests reviewed by several authors (e.g., Martinelli et al., 1999; Craine et al., 2009; Craine at al., 2015). We have and also re-written the discussion (lines 328-367) and clarified our wording here, including avoiding the use of the word 'overridden' as mentioned above. However, we acknowledge the difference between the two forests that support the hypothesis because the N-rich BF forests are more ^{15}N-enriched than the somewhat more 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 $\delta^{15}N$ 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. Now we have explained this in section 4.4.

L382, the contribution of fractionation process and source 15N value is not clearly known in this study.

Response: We agree that the contribution of fractionation process and ^{15}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}N signature of source N can explain the change. Again we changed the wording (see our response to the above general comment).

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

Response: 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}N source is main sole factor'. We have revised the discussion to elaborate about the effects of N addition on $\delta^{15}N$ of both plants and soils, and how this can be interpreted in terms of the importance of both fractionation and ^{15}N signature of sources to explain $\delta^{15}N$ of the studied forests (line 376-378).

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

Response: We agree with the referee that ^{15}N of total N input should be lower in the control plots. We assume that $\delta^{15}N$ of thoughfall equally affects plants in control and N-plots. Any difference between in $\delta^{15}N$ 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 draws in the direction of ^{15}N signature source input.

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

Response: This is solved by rewriting of major portions of the Discussion.

L415-416, it is difficult to conclude that source 15N is more important in PF. It is too speculative.

Response: Change in plant N content and response of plant $\delta^{15}N$ was higher in the PF than in BF, indicating that input N is more important for plant N source in the somewhat more N-limited PF. However, we have revised the part of the discussion to avoid too speculative statements (lines 400-406). Our corrected mass balance calculation showed that similar amount of (~15%) of added N is incorporated into plants pools in both forests.

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 15N 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.

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. 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, and 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 $\delta^{15}N$ value under ambient conditions to be higher for our study forest in our re-phrased hypothesis (line 102-104). We have also compared the hypothesis (all hypotheses) with our results (lines 340-342). 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 (lines 328-339).

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: We did mixing calculations in the control plot using two-source mixing model (Dawson et al. 2002, cited in the previous manuscript), and assuming soil N 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 $\delta^{15}N$ of plants as we explained in the discussion because deposition is high at our site and it is strongly ^{15}N-depleted. However, the 60-80% fractions are very high and also ignore the effects of fractionation, so we did not include them.

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 by comparing our results only to the global data in Martinelli et al. 1999, but also to other studies from temperate forests including those from temperate sites that have high N status (line 339). We also made it clear how our original first hypothesis was rejected by our results (lines 340-341). 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 ^{15}N-depleted N can be caused by larger N availability, and it can be one of those important factors that control ecosystem δ^{15}N. The increase in plant δ^{15}N after the 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 δ^{15}N. When we used the added N with -0.7 delta label as tracer for mass balance calculation (Nadelhoffer and Fry, 1994) about 15% of the added N was estimated to be taken up by the plants in both forests, 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

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 δ^{15}N between the two forests, we have mentioned that *t*-test was used as the reviewer assumed it, and the section about statistics is edited to show this (lines 195). In Table 3, we had made a copy-past mistake; the correct SE for twigs in PF is 0.05, not 0.77, and we have corrected it. Further, it is important to note (as mentioned in the table heading) that for the broad-leaved forest, 5 dominant tree species were sampled and since the species differ in %N and δ^{15}N, 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 cases added the changes in $\delta^{15}N$ 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) (lines 59).

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' (line 81).

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 includes temperate forests in general (not only N-limited forests) (lines 102-104). Similarly, the discussion is improved by adding some studies from different temperate forests that cover wider gradient of N availability including N-saturated forests (e.g., Koopmans et al., 1997; Sah & Brumme, 2003) (line 339).

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 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 $\delta^{15}N$. The preliminary data set presented in Amundson et al (2003) was from the central California coast range (Figure 4), suggested that topography could explain up to 2% variation in soil $\delta^{15}N$ at a given location. Apart from these observation used to explain within site variation in soil $\delta^{15}N$, 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 ^{15}N-depletion compared to other tropical forests. But we have mentioned this as a potential (minor) influence in the discussion (lines 364-367).

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 (lines 169).

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

Response: Corrected to show that $0.8m^2$ was a total interception area for the five collectors (173).

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 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 (lines 195-198).

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 $\delta^{15}N$ data in Table 2 and the concentration data presented in Fig. S1 (now Table S1), we calculated the weighted average $\delta^{15}N$ for total dissolved inorganic N (DIN). The data (presented in revised Table 3) showed that soil

solution ass slightly higher $\delta^{15}N$ than the deposition in both forests. We have two samples for the added N plots but only in the broad-leaved forest (BF), and it shows even higher $\delta^{15}N$ (~ -2.8‰) than 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 $\delta^{15}N$. However, this argument may not be true if denitrification (reported at our site) dominates nitrification because the two processes have antagonistic effect on $\delta^{15}N$ 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 $\delta^{15}N$ of the water samples (lines 316-325). 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 (line 179). We have indicated in Table 2 footnote that the SE for runoff SE is for pseudo-replicates within one plot (line 222).

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. However, we decided to only mean from the pseudo-replicates for the four months. We have explained this in the caption line 238-239).

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 misplaced. 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) (lines 283-288).

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: Since look at the effect of the N addition we the decrease (from control to N-plot) can only be an effect of the addition, so the $\delta^{15}N$ of the total N input should not be relevant here.

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 ^{15}N-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 an enrichment of leaf $\delta^{15}N$ at our study site that was higher than the average leaf $\delta^{15}N$ 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 $\delta^{15}N$ compared pre-dominantly N-limited temperate forests due to increased fractionation combined with loss of ^{15}N-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 (lines 340-341). We have also re-structured the paragraphs, and re-phrased the hypothesis to make it brief and direct (lines 102-104).

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 (lines 348).

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), and we have edited the sentence to show this (lines 345-347). However, we do not believe that our concluding statement in lines 454-455

in previous version (now in lines 435-436) is different from our explanation here. Our conclusion emphasizes the importance of considering ^{15}N signature of input N when interpreting $\delta^{15}N$ of ecosystems as a proxy for ecosystem N cycling, and we have edited the sentence to make it clear (lines 435-436).

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 (lines 390-391).

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 $\delta^{15}N$ towards the ^{15}N signature of the added N due to its incorporation into ecosystem pools, and

iii) response of $\delta^{15}N$ to N addition would differ between the two forests due to differences in their initial N status and N cycling rates. The statement is now related to the hypothesis number (ii) (lines 394-396).

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 $\delta^{15}N$, and we have included it in our explanation of the increase in plant $\delta^{15}N$ after the decadal N addition (376-378). However, increased fractionation plus loss of depleted N may not explain the decrease in soil $\delta^{15}N$ (although it was not significant) caused by the N addition. The plausible explanation for the changes in $\delta^{15}N$ in both plants and soils is the effect of an imprint from the ^{15}N signature of the added N. We have revised the statement with clearer explanation indicating how the importance of ^{15}N signature of sources outweighs that of fractionation in soil (lines 389-393).

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 '^{15}N-depleted' relative to $\delta^{15}N$ of the bulk soil. We have clarified this as a relative comparison, and presented the $\delta^{15}N$ for the added N and plants (line 378).

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 N as a tracer since its ^{15}N signature differs from the ambient deposition N as well as that of both plants and soil. For the calculation to be meaningful, it requires that $\delta^{15}N$ significant differ between the control and the N-plots, thus the calculation can only be done for the plant pool. We have cut the long text on this in the previous version to make it brief and easy to understand, have included the assumptions that were made in the calculation (line 379-388).

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 ^{15}N-depleted deposition and thus may also have low $\delta^{15}N$ in plants (written as 'an imprint from ^{15}N-depleted N deposition' in previous version). We have added some words to make it clearer (line 429).

L447 "further confirmed" see my remark on L396

Response: We have explained why we focused on the importance of ^{15}N signature of the input N. See our response to the above comments on line 396. We have made slight changes in our wording (e.g., used 'support' instead of 'confirmed') not to overstate the conclusion (lines 429-432).

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), ^{15}N signature of the N deposition differs sufficiently from the $\delta^{15}N$ 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 avoid wording that indicate fractionation is not important at all (line 434). We are not sure if the reviewer hints that generalization should be including the cause that he mentioned. We have also included that the importance increase with significantly elevated N deposition which is widespread in many regions (lines 435-436).

Nitrogen input ^{15}N-signatures are reflected in plant ^{15}N natural abundances of sub-tropical forests in China

Geshere Abdisa Gurmesa [1,2,3], Xiankai Lu[1], Per Gundersen[2], Yunting Fang[4], Qinggong Mao[1], Chen Hao[5], Jiangming Mo[1]

[1] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems and Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[2] Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark
[3] Sino-Danish Center for Education and Research, Aarhus, Denmark
[4] State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China
[5] Huanjiang Observation and Research Station for Karst Ecosystem, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Correspondance to: Jiangming Mo (mojm@scib.ac.cn)

Abstract. Natural abundance of ^{15}N ($\delta^{15}N$) in plants and soils can provide time-integrated information related to nitrogen (N) cycling within ecosystems, but it has not been well tested in warm and humid sub-tropical forests. In this study, we used ecosystem $\delta^{15}N$ to assess effects of increased N deposition on N cycling in an old-growth broad-leaved forest and a secondary pine forest in a high N deposition area in southern China. We measured $\delta^{15}N$ of inorganic N in input and output fluxes under ambient N deposition, and N concentration (%N) and $\delta^{15}N$ of major ecosystem compartments under ambient deposition and after decadal N addition at 50 kg N $ha^{-1}yr^{-1}$ that has a $\delta^{15}N$ of 0-0.7‰. Our results showed that the total inorganic N in deposition was ^{15}N-depleted (-10 ‰) mainly due to high input of strongly ^{15}N-depleted NH_4^+-N. Plant leaves in both forests were also ^{15}N-depleted (-4 to -6 ‰). The broad-leaved forest had higher plant and soil %N, and was more ^{15}N-enriched in most ecosystem compartments relative to the pine forest. Nitrogen addition did not significantly affect %N in the broad-leaved forest, indicating that the ecosystem pools are already N-rich. However, %N was marginally increased in pine leaves and significantly in understory vegetation in the pine forest. Soil $\delta^{15}N$ was not changed significantly by the N addition in either forest. However, the N addition significantly increased the $\delta^{15}N$ of plants toward the ^{15}N signature of the added N, indicating incorporation of added N into plants. Thus, plant $\delta^{15}N$ was more sensitive to ecosystem N input manipulation than %N in these N-rich sub-tropical forests. We interpret the depleted $\delta^{15}N$ of plants as an imprint from the high and ^{15}N-depleted N deposition N that may dominate the effects of fractionation that are observed in most warm and humid forests. Fractionation during the steps of N cycling could explain the difference between negative $\delta^{15}N$ in plants and positive $\delta^{15}N$ in soils, and the increase in soil $\delta^{15}N$ with depths. Nevertheless, interpretation of ecosystem $\delta^{15}N$ from high N deposition regions needs to include data on the deposition ^{15}N signal.

Key words: Natural ^{15}N abundance, N addition, N deposition, sub-tropical, China

1 Introduction

Nitrogen (N) deposition onto terrestrial ecosystems has dramatically increased due to anthropogenic activities (Galloway, 2005) and since the 1980s the increase has been particularly strong in China including in the warm and humid regions (Liu et al. 2011). Nitrogen deposition that exceeds plant and microbial demand may increase nutrient leaching and soil acidification (Lu et al., 2014), and potentially causes nutritional imbalances in vegetation (Schulze, 1989). Studies of fates and process responses to increased N deposition using coordinated N addition experiments in temperate and boreal forests show that the effects of increased N deposition largely depend on the initial N status of the forests (Gundersen et al., 1998; Hyvönen et al., 2008). Accordingly, N limited forests often show a growth response to added N and retain most of the deposited N, whereas N saturated forests subjected to N deposition often lose considerable N through leaching and denitrification. Although some studies from (sub) tropical regions also suggest that N leaching from tropical forests is related to the initial N status of the forests (Chen and Mulder, 2007; Fang et al., 2009), observations thus far are not conclusive, especially in regions that are subjected to increased anthropogenic N deposition (Townsend et al., 2011).

The natural abundance of ^{15}N ($\delta^{15}N$) in leaves and other ecosystem compartments is relatively easy to measure and may provide time-integrated information about N cycling in ecosystems (Handley and Raven, 1992; Robinson, 2001). Differences in $\delta^{15}N$ between ecosystem compartments and among ecosystems result from isotopic fractionation during each of the many steps of the N cycle. In particular, N losses through leaching and denitrification lead to preferential losses of the lighter ^{14}N forms whereas compounds with isotopically heaver ^{15}N are retained in the N pools or further cycled in the ecosystem (Högberg, 1997). Recent advances in the interpretation of $\delta^{15}N$ variation among ecosystems based on the compilation and analysis of global data on foliar and soil $\delta^{15}N$ have revealed general global patterns in relation to climate and N availability (Martinelli et al., 1999; Amundsen et al., 2003; Craine et al., 2009; 2015a, b). Foliar $\delta^{15}N$ values are generally elevated under N rich conditions, i.e. increasing leaf $\delta^{15}N$ with increasing leaf N concentration and higher leaf $\delta^{15}N$ in warmer climates (Craine et al., 2009). Tropical forests, which are often N-rich, have higher foliar $\delta^{15}N$ than temperate forests (Martinelli et al., 1999). However, global analyses contain almost no data from eastern Asia, including sub-tropical regions of China now receiving high N deposition (Fang et al., 2011a).

The influence of increased N deposition on $\delta^{15}N$ levels is not well known. For example, even though plant $\delta^{15}N$ could increase with N deposition (Emmett et al., 1998), it may not be the case across all regions where not only ecosystem N status but also a region-specific ^{15}N signature of deposited N may influence ecosystem $\delta^{15}N$ (Fang et al., 2011b; Pardo et al., 2006). Moreover, interpretation of ecosystem $\delta^{15}N$ is hampered by the uncertainties in $\delta^{15}N$ of plant N sources, the magnitude of isotopic fractionations during N transformation processes, and the complex behavior of ^{15}N in soils and plants (Robinson, 2001).

Plant leaf and soil $\delta^{15}N$ are most commonly used to assess N status and changes in N cycling rates, but other ecosystem pools are neglected or rarely measured. The turnover times of N pools vary among different ecosystem compartments, and thus their $\delta^{15}N$ values may respond differently to specific disturbances. For example, within plant compartments, small active N pools such as leaves reflect recent N cycling whereas the larger N pools such as wood or soil might reflect long-term changes in N cycling (Craine et al., 2015a). Nevertheless, reports of $\delta^{15}N$ values in all major ecosystem pools are rare (e.g. Liu, 1995), emphasizing the need for more rigorous studies to provide complete $\delta^{15}N$ patterns in the leaf-to-soil continuum, and their response to N input manipulation, especially in the tropical forests.

We evaluated $\delta^{15}N$ values of sub-tropical forests, and their responses to increased N deposition using long-term N addition experimental plots established in 2003 in an old-growth broad-leaved forest and a pine plantation forest in the Dinghushan Biosphere Reserve in southern China (Mo et al., 2006). The old-growth forest is more N-rich, and has less N retention capacity than the pine forest (Fang et al., 2006). Nitrogen addition studies in these forests documented that increased N input causes increased N leaching (Fang et al., 2008, 2009), N_2O emission (Zhang et al., 2008) and soil acidification (Lu et al., 2014). Here, our objectives are (1) to compare $\delta^{15}N$ values of ecosystem compartments across the leaf-to-soil continuum in the two forests, and (2) to assess responses of $\delta^{15}N$ in major ecosystem pools to decadal N addition in the two forests. We hypothesized that i) $\delta^{15}N$ values of plants and soil in these forests would follow the global patterns predicted from climate and thus be higher in these sub-tropical forests than in those reported for temperate forests, ii) N addition would change plant and soil $\delta^{15}N$ towards the ^{15}N signature of the added N due to its incorporation into ecosystem pools, and iii) response of $\delta^{15}N$ to N addition would differ between the two forests due to differences in their initial N status and N cycling rates.

2 Methods

2.1 Study site

The study was conducted in the Dinghushan Biosphere Reserve (DHSBR) in the Guangdong province, southern China (112°33' E and 23°10' N) with typical sub-tropical monsoon climate. Mean annual temperature (MAT) and mean annual precipitation (MAP) are 22.2 C° and 1927 mm, respectively. The reserve has experienced high rates of atmospheric N deposition (21-38 kg N ha^{-1} yr^{-1} as inorganic N in bulk precipitation) since 1990's (Fang et al., 2008). In 2009 to 2010, total wet N deposition was 34.4 kg N ha^{-1} yr^{-1} (Lu et al., 2013). We used two common forest types that grow on the relatively steep slopes in the reserve; an old-growth broad-leaved forest (hereafter named as BF) and a pine plantation forest (hereafter named as PF) (Mo et al., 2006). The BF is a regional climax mixed broad-leaved forest, which has been protected for at least the last 400 years with minimum human disturbances (Shen et al., 1999). The PF was planted after a clear-cut of the original climax forest in the 1930s and has been subjected to human disturbances such as litter and shrub harvesting until the recent past (Mo et al., 2005).

Table 1. Selected characteristics of the mineral soil (0-10 cm) in the two forest types. Data on soil bulk density, total P and extractable NH_4^+-N are obtained from Fang et al. (2006). Values given in parenthesis indicate SE (n = 3).

Parameters	Broad-leaved forest (BF)	Pine forest (PF)
Bulk density (g cm^{-3})	0.9 (0.03)	1.3 (0.03)
pH (H_2O)	3.8 (0.02)	4.0 (0.04)
C concentration (%)	3.8 (0.80)	1.8 (0.03)
N concentration (%)	0.3 (0.04)	0.1 (0.01)
C/N ratio	13.6 (0.9)	13.9 (0.7)
Total P (mg kg^{-1})	59 (3)	43 (3)
Extractable NH_4^+-N (mg kg^{-1})	2.1	3.3
Extractable NO_3^--N (mg kg^{-1})	12.7	2.6

The major canopy species in the BF are *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*, *Cryptocarya chinensis*, and *Syzygium rehderianum* and the most common understory species is *Hemigramma decurrins*. *Pinus massoniana* and *Dicranopteris dichotoma* are the dominant tree and understory species in the PF, respectively.

No N-fixing tree species were found in the plots. The soil in the reserve is classified as Lateritic Red Earth (Oxisol) formed from Devonian sandstone and shale with a thin layer of forest floor litter (0.5-3.0 cm), but the soil depth is variable ranging from 30 cm in the PF to more than 60 cm in the BF. Probably due to erosion after the clear-cut and the continued human disturbance the PF had lower total soil carbon, N and phosphorus (P) content than the BF (Table 1).

2.2 Experimental design

We used an ongoing N addition experiment established in both forests in July 2003 (Mo et al., 2006). The experimental plots used for this study consist of control plots and N addition treatment at 50 kg N ha^{-1} yr^{-1} (hereafter named as N-plots) each with three replicates in both forests. Each plot is 10 m x 20 m with at least a 10 m wide buffer strip to the next plot. In the N-plots, NH_4NO_3 is mixed with 20 L of water, and is added monthly since July 2003 below the canopy using a backpack sprayer, whereas the control plots received equivalent 20 L water with no fertilizer. The added N has $\delta^{15}N$ of about -3 ‰ on NH_4^+-N and about 1.8 ‰ on NO_3^--N, with $\delta^{15}N$ of NH_4NO_3 being -0.7 ‰.

2.3 Sampling and analysis of plant and soil pools

In both forests, major ecosystem compartments including leaves, twigs, branches, bark and wood of canopy trees, leaves of understory vegetation, fine roots, and 0-30 cm mineral soil were sampled in January 2013 to determine their N concentration (%) and $\delta^{15}N$ (‰). A branch per dominant tree species per plot was cut from the height reached using a pole pruner (c. 7-8 m) taking advantage of the steep slope, and was separated into leaves, twigs and small branches. Bark samples were cutoff the dominate trees at breast height using a knife. After removing the bark, wood cores were sampled using an increment borer and separated visually into sapwood (usually the outer 2-3cm recent wood) and older wood (heartwood). Dominant understory plant species were cut with a knife and kept separate for each species. A total of seven tree species in the canopy/sub-canopy layer and five plant species in the understory layer (young trees, shrubs, herbs and liana) of the BF were sampled. In the PF, the dominant pine tree and five species in the understory layer were sampled. Mineral soil samples were taken using an auger (5.1 cm in diameter) and were divided into three layers (0-10, 10-20, 20-30 cm). Two soil cores were sampled and pooled together to form one composite sample for each depth per plot. Living fine roots were hand-sorted from the soil samples for each depth, but were combined to one composite sample for the whole profile (0-30 cm) because the amount of fine roots in each depth were too small to grind and analyze separately. Litterfall was collected monthly during July-September 2012 and was pooled together to make one composite sample per plot. The litter was sorted in the laboratory into leaf and others (branches, fruits, flowers, barks), but only leaf values are reported.

All plant and soil samples were oven-dried at 70 ^oC, and ground to a fine and homogeneous powder. Mineral soils were sieved (2mm mesh) to remove non-soil materials, air-dried at room temperatures and milled to fine powder. Subsamples were dried at 105 °C, and all results are reported on 105 °C basis. Based on their approximate %N, about 4-5 mg of the samples were weighed into tin capsules, and $\delta^{15}N$ and N concentration of the samples were determined simultaneously on an isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd.) coupled to an automatic, online elemental analyzer (vario ISOTOPE cube). An internal standard needle sample from temperate forests, which has been analysed in multiple runs at several laboratories, was used to check reproducibility of the $\delta^{15}N$ determination. We analyzed %N and $\delta^{15}N$ separately for each dominant tree species per plot, but compartment mean values are reported. Natural abundance $\delta^{15}N$ in samples was reported in per mil (‰) relative to the ^{15}N content of atmospheric N_2.

2.4 Sampling and analysis of water samples

Precipitation, throughfall, surface runoff and soil solution were sampled monthly from September 2012 to February 2013 (dry December and January, where there were not enough precipitation to generate water samples) in the control plots to assess the $\delta^{15}N$ of N input and output in the two forests under ambient N deposition. Bulk precipitation was collected at an open area close to the experimental site using an open glass funnel (12 cm in diameter), connected to a 5 L sampling bottle with polypropylene tubes. Throughfall was collected by PVC pipes at five random points within each plot (with a total intercept area of 0.8 m^2) at about 1.3m above the ground in each forest. Each collector was connected to two 50L buckets with polypropylene tubes. Soil solutions from 20cm depth (seepage water) were obtained using two zero tension tray lysimeters (755 cm^2 per tray) installed in each plot. Each lysimeter was connected to a 20 L bottle using the steep slope of the sites to facilitate sampling. In both forests, one selected plot for each treatment was delimited hydrologically by placing stable plastic materials and low cement barriers around them. The cement barriers (covered by the plastic material) on the downslope side of these plots were constructed to enable the sampling of the surface runoff in three sections, which were then used as pseudo-replicates.

Natural ^{15}N abundances of NH_4^+-N and NO_3^--N in water samples were analyzed after chemical conversion to nitrous oxide (N_2O). The NH_4^+-N was initially oxidized to nitrite (NO_2^-) by hypobromite (BrO^-) and the NO_2^- is then quantitatively converted into N_2O by hydroxylamine (NH_2OH) under strongly acidic conditions (Liu et al., 2014). Similarly, a series of chemical reactions of vanadium (III) chloride (VCl_3) and sodium azide under acidic conditions was used to convert NO_3^--N into N_2O (Lachouani et al., 2010). The produced N_2O was subsequently analysed for ^{15}N abundance by a purge-and-trap coupled with an isotope ratio mass spectrometer (PT-IRMS) (Liu et al., 2014).

2.5 Calculations and Statistics

To evaluate effects of decadal N addition on the whole ecosystem (plant and soil) %N and $\delta^{15}N$, we determined N pool weighted plot means of %N and $\delta^{15}N$ using N pools for each compartment and tree species contribution quantified in Gurmesa et al. (2016). We excluded the heartwood and sapwood pools in the plant pool calculations for two reasons; first the low %N in wood samples caused larger uncertainties on the $\delta^{15}N$ determinations, and secondly heartwood and a major part of the sapwood were formed prior to the initiation of the N addition treatment. We expect the later to be the explanation that particular heartwoods showed opposite effects of N addition compared to all other compartments.

Differences between the two forests in plot mean %N and $\delta^{15}N$ of the different ecosystem compartments and N pool weighted plot means in control plots were analysed using *t*-tests. The effect of N addition treatment on %N and $\delta^{15}N$ of each tree compartments in the BF and understory leaf in both forests was analyzed using mixed model ANOVA with treatment as explanatory factor and plant species as a random factor because plant species differed significantly in both parameters (Gurmesa, 2016). All other tests of treatment effects on %N and $\delta^{15}N$ was analysed using simple *t*-test on plot means.

3 Results

3.1 Concentration and $\delta^{15}N$ of NH_4^+-N and NO_3^--N in water samples

Dissolved NH_4^+-N in water samples in both input (precipitation and throughfall) and output fluxes (surface runoff and soil solution) were ^{15}N-depleted (negative $\delta^{15}N$) in both forests (Table 2). The $\delta^{15}N$ of NO_3^--N was ^{15}N-enriched in precipitation and throughfall, and became ^{15}N-depleted in surface runoff and soil solution. However, for dissolved inorganic N (DIN) the concentration weighted $\delta^{15}N$ (calculated based on data in Table 2 and concentration data in Table S1) were ^{15}N-depleted but slightly increased from precipitation input to soil solution. Mean $\delta^{15}N$ of both NH_4^+-N and NO_3^--N in input and output fluxes did not significantly differ between the two forests. The temporal variation in $\delta^{15}N$ was large (-28 to 2 ‰) for NH_4^+-N but minor (2 to 5 ‰) for NO_3^--N (Fig. 1b, d, x-axis). The $\delta^{15}N$ of NH_4^+-N in surface runoff and soil solution were significantly and positively related to the variation in $\delta^{15}N$ of NH_4^+-N in throughfall in both forests (Fig 1a, b), but the correlation was not significant for NO_3^--N (Fig 1c, d).

Table 2. $\delta^{15}N$ (‰) of NH_4^+-N, NO_3^--N and dissolved inorganic N (DIN) in bulk precipitation, throughfall, surface runoff and soil solution at 20cm depth in control plots from September 2012 to February 2013. Numbers in parenthesis for precipitation, throughfall and soil solution indicate standard error of the mean (SE) (n = 3). For all water fluxes, no significant difference in $\delta^{15}N$ of both NH_4^+-N and NO_3^--N was detected between the two forests.

Fluxes	Broad-leaved forest (BF)			Pine forest (PF)		
	NH_4^+-N	NO_3^--N	DIN	NH_4^+-N	NO_3^--N	DIN
Precipitation[a]	-16.6	4.1	-9.9	-16.6	4.1	-9.9
Throughfall	-15.2 (2.3)	3.6 (0.2)	-7.9 (1.2)	-15.5 (1.8)	2.8 (0.3)	-9.9 (0.5)
Surface runoff[b]	-13.1(1.7)	-1.9 (0.6)	-6.2 (1.0)	-9.7 (1.0)	-1.5 (0.6)	-5.4 (0.1)
Soil solution	-22.6 (0.9)	-0.9 (1.3)	-5.7 (0.7)	-21.3 (2.3)	-0.9 (0.2)	-7.3 (1.1)

[a] Precipitation was collected at open area within the reserve, and was assumed to be the same for both forests.

[b] The indicated SE is for pseudo-replicates within one plot.

3.2 Effects of forest type

As expected based on the differences in disturbance regime, the BF is more N-rich than PF. Nitrogen concentrations of plant compartments were significantly higher in the BF than in the PF, except in leaves of canopy trees, litter-fall and fine roots for which the difference was marginally significant (Table 3). Soil %N was significantly higher in the BF at all depths (Table 3).

Most plant compartments are ^{15}N-depleted with understory and tree leaves, twigs and branches being most ^{15}N-depleted (below -4 ‰) whereas bark and sapwood were less ^{15}N-depleted within each forest (Table 4). The $\delta^{15}N$ of all plant compartments differ significantly between the two forests with the PF being more ^{15}N-depleted than the BF (Table 4). Soil $\delta^{15}N$ did not show significant difference between the two forests at any depth (Table 4).

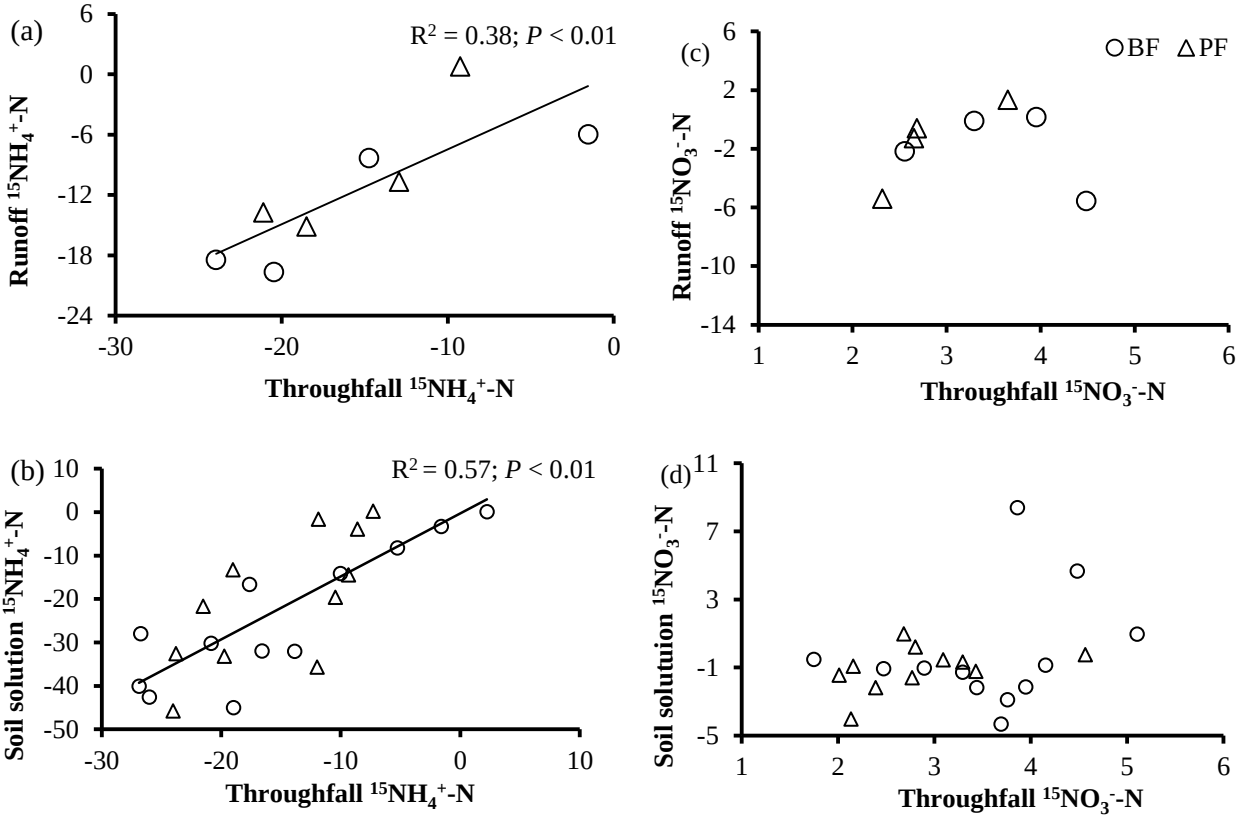

Figure 1. Correlation between $\delta^{15}N$ (‰) of NH_4^+-N in throughfall and that of NH_4^+-N in surface runoff (a), and soil solution (b), and correlation between $\delta^{15}N$ of NO_3^--N in throughfall and that of NO_3^--N in surface runoff (c), and soil solution (d). For throughfall and soil solution, $\delta^{15}N$ were from samples taken monthly between September and February in each of the 3 plots. For surface runoff, samples were only from one plot. No significant effect of forest type was detected; thus the regression line shown was based on data from both forests.

When compared based on N pool weighted plot mean, the two forests differed significantly in plant %N and $\delta^{15}N$ (Fig. 2a). For the soil, the two forests also differed significantly in N pool weighted plot mean %N, with the BF having the higher value, but not in N pool weighted plot mean $\delta^{15}N$ (Fig. 2b).

3.3 Effects of N addition on %N and $\delta^{15}N$

Nitrogen concentrations in all measured plant and soil compartments were not significantly affected by N addition in the BF, except in the sapwood (Table 3). In the PF, mean %N values were greater in most plant compartments on fertilized plots, but the change was significant only in leaves of understory plants, whereas soil %N was unchanged (Table 3).

Plant $\delta^{15}N$ was negative in both control and N-plots in both forests, but N addition significantly increased the $\delta^{15}N$ of most plant compartments (Table 4). The changes were more pronounced in the small active plant pools such as leaves of trees and understory plants.

Table 3. Mean %N of different ecosystem pools in the broad-leaved (BF) and pine forests (PF). Values in parenthesis indicate SE of plot means (n = 3). Within each forest type p-values for the effect of N addition are shown. The last column shows p-values for a difference between the ambient plots of the two forests using t-test. Bolded p-values indicate significant difference.

Compartment	Broad-leaved forest (BF)			Pine forest (PF)			Forest type effect
	Control	N addition	p-values	Control	N addition	p-values	p-values
Plants							
Tree leaf	1.71 (0.19)	1.69 (0.19)	0.48[§]	1.44 (0.11)	1.68 (0. 28)	0.16	0.12
Twig	1.28 (0.19)	1.17 (0.23)	0.59 [§]	0.99 (0.05)	0.97 (0.08)	0.79	**0.01**
Branch	0.86 (0.15)	0.81 (0.16)	0.13 [§]	0.58 (0.05)	0.60 (0.06)	0.85	**0.03**
Bark	0.71 (0.16)	0.7 (0.16)	0.55 [§]	0.57 (0.02)	0.61 (0.05)	0.53	**0.01**
Sapwood	0.27 (0.07)	0.3 (0.07)	**<0.01** [§]	0.18 (0.02)	0.11 (0.02)	0.07	**0.03**
Heartwood	0.16 (0.04)	0.16 (0.03)	0.28 [§]	0.06 (0.00)	0.09 (0.03)	0.35	**<0.01**
Understory leaves	2.04 (0.02)	1.98 (0.17)	0.09 [§]	1.61 (0.41)	1.77 (0.40)	**<0.01** [§]	**<0.01**
Fine root	1.4 (0.16)	1.81 (0.17)	0.15	0.87 (0.13)	0.96 (0.04)	0.58	0.06
Litter-fall	1.56 (0.05)	1.48 (0.06)	0.45	1. 39 (0.04)	1.72 (0.09)	0.06	0.09
Soil							
0-10 cm	0.27 (0.04)	0.28 (0.01)	0.83	0.13 (0.01)	0.12 (0.01)	0.39	**0.03**
10-20 cm	0.18 (0.01)	0.19 (0.01)	0.59	0.07 (0.00)	0.06 (0.00)	0.37	**<0.01**
20-30 cm	0.12 (0.00)	0.14 (0.00)	0.14	0.06 (0.00)	0.05 (0.00)	0.18	**<0.01**

[§] Due to significant differences between the sampled tree or understory plant species the effect of N addition was tested in a mixed model ANOVA with species as random factor.

However, effect of N addition on $\delta^{15}N$ was inconsistent in the wood parts (Table 4). For heartwood, the effects were significant, but in different directions than in other plant pools for both forests. Due to low %N and challenges in grinding of wood samples it was difficult to get reliable $\delta^{15}N$ results for these samples. Also much of the sampled wood was formed prior to the treatment and thus, no further evaluation was done for the wood samples. Nitrogen addition did not cause significant effects on $\delta^{15}N$ of litter-fall and fine roots. In the BF, there was no correlation between leaf %N and $\delta^{15}N$, but a positive correlation was found for the PF as both %N and $\delta^{15}N$ tended to increase in parallel due to N addition (data not shown).

Nitrogen addition tended to decrease soil $\delta^{15}N$ in the BF at all depths, but with no significant changes in any layer (Table 4). In the PF, soil δ15N was unchanged by N addition (Table 4).

Table 4. Mean $\delta^{15}N$ (‰) of plant pools in the broad-leaved (BF) and pine forests (PF). Values in parenthesis indicate SE of plot means (n = 3). Within each forest type p-values for the effect of N addition is shown. The last column shows p-values for differences between the ambient plots of the two forests using t-test. Bolded p-values indicate significant differences.

Sample type	Broad-leaved forest (BF)			Pine forest (PF)			Forest type effect
	Control	N addition	p-values	Control	N addition	p-values	p-values
Tree leaf	-4.0 (0.5)	-3.4 (0.6)	**0.02** §	-5.4 (0.1)	-3.5 (0.3)	**0.01**	**<0.01**
Twigs	-4.3 (0.8)	-3.8 (0.9)	0.09 §	-5.7 (0.1)	-4.0 (0.3)	**0.02**	**<0.01**
Branches	-4.6 (0.4)	-4.1 (0.3)	**<0.01** §	-5.7 (0.2)	-4.1(0.6)	0.12	**0.03**
Bark	-2.8 (0.8)	-2.4 (0.6)	0.05 §	-4.0 (0.4)	-2.6 (0.2)	**0.03**	0.06
Sapwood	-1.9 (0.5)	-1.8 (0.3)	0.51 §	-0.9 (0.4)	1.8 (1.6)	0.23	0.09
Heartwood	-1.6 (0.9)	-2.3 (0.9)	**0.05** §	3.2 (0.8)	-0.71 (1)	**0.04**	**0.03**
Understory leaves	-3.6 (0.9)	-2.2 (1.1)	**<0.01** §	-5.6 (0.5)	-3.54 (0)	**<0.01** §	**0.01**
Fine root	-2.8 (0.6)	-1.7 (0.8)	0.33	-5.1 (0.5)	-3.6 (0.3)	0.08	**0.04**
Litter-fall	-3.9 (0.1)	-3.9 (0.1)	0.98	-4.8 (0.2)	-4.0 (0.3)	0.11	**0.04**
Soil							
0-10 cm	2.2 (0.4)	1.6 (0.6)	0.46	2.6 (0.8)	2.3 (0.4)	0.69	0.63
10-20 cm	4.0 (0.3)	3.2 (0.2)	0.09	4.1 (1.4)	4.4 (0.3)	0.88	0.93
20-30 cm	5.4 (0.3)	4.8 (0.5)	0.39	3.3 (1.4)	4.0 (0.2)	0.68	0.26

§ Due to significant differences between the sampled tree or understory plant species the effect of N addition was tested in a mixed model ANOVA with species as random factor.

In summary, the effect of added N on pool weighted plot mean plant %N was not significant in either BF (p = 0.86) or in PF (p = 0.25) more pronounced in the PF (Fig. 2a). However, weighted plot mean plant $\delta^{15}N$ were significantly increased in both forests (p = 0.04 for BF and p = 0.03 for PF) by the N addition. In the soil, where the N pool is obviously larger than in the plants, the effect of the N addition on weighted average %N was not significant in both forests (Fig. 2b). The direction of change in soil $\delta^{15}N$ was a decrease as expected with incorporation of the added N ($\delta^{15}N$= -0.7 ‰), but the change was again not significant (Fig. 2b).

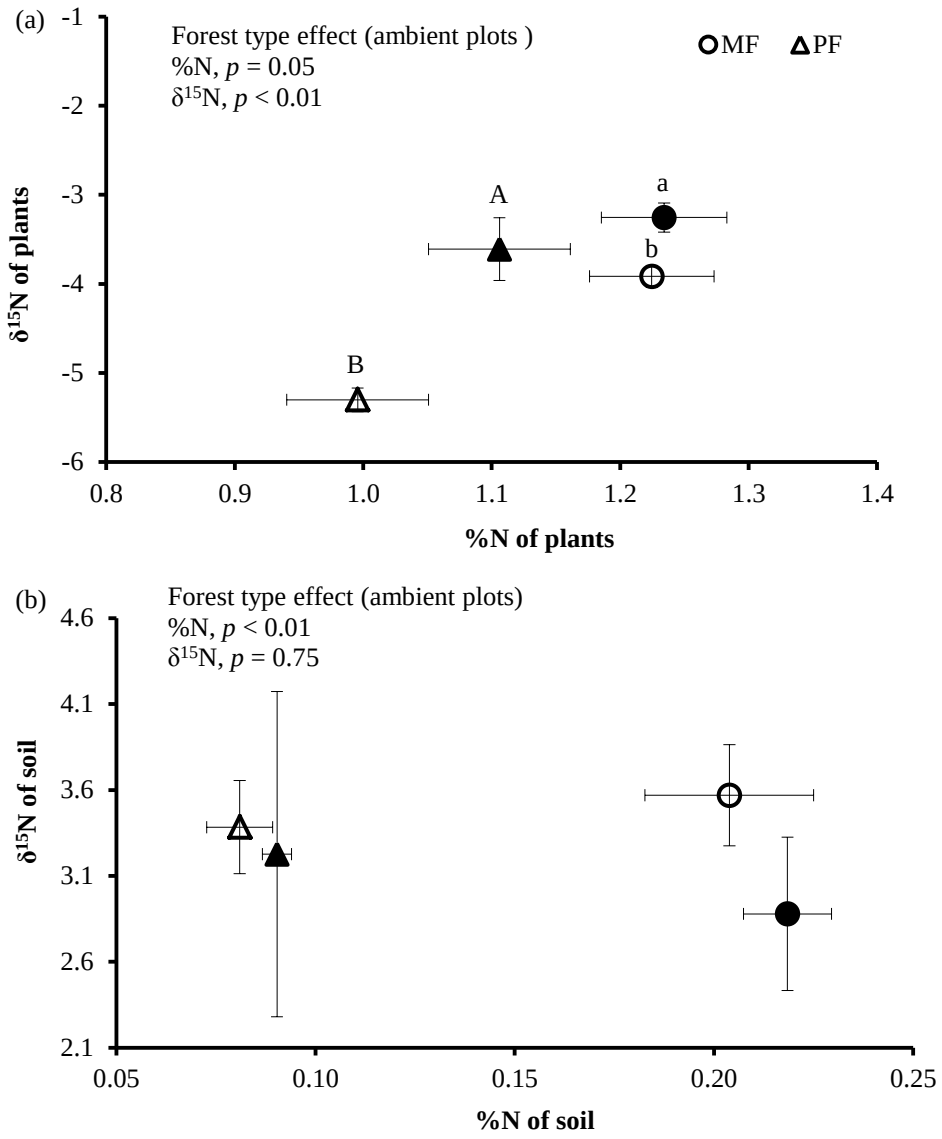

Figure 2. Overall effect of N addition on plot average weighted %N and $\delta^{15}N$ of plants (a), and soil (b) for broad-leaved forest (○) and pine plantation (Δ). Error bars indicate SE of plot means (n = 3). Open symbols indicate control plots and closed symbols indicate N-plots. In (a), significant effects of N addition within forest type is indicated by different letters; lowercase for BF and uppercase for PF. The p-values shown in the upper right corners are tests for differences in %N and $\delta^{15}N$ between the two forests (ambient plots).

4 Discussions

4.1 $\delta^{15}N$ of N in deposition and soil solution

Deposition N (bulk precipitation and throughfall) was ^{15}N-depleted in NH_4^+-N and ^{15}N-enriched in NO_3^--N (Table 2), but since NH_4^+-N is the dominating N form (Table S1) DIN deposition is ^{15}N-depleted (-10 to -8 ‰) as also previously reported in the region (Zhang et al., 2008; Koba et al., 2012). The source of the NH_4^+-N is likely NH_3 emissions from activities in the intensively used agricultural land surrounding DHSBR. Agricultural NH_3 emissions are usually ^{15}N-depleted (Bauer et al., 2000). The source of the NO_3^--N contribution may originate from NO_x produced by coal combustion in mega-cities in the Guangdong province.

The low $\delta^{15}N$ of NH_4^+-N in the soil solution of both forest resemble that in precipitation and throughfall (Table 2), and it is likely due to transport of ^{15}N-depleted throughfall N through macrospores as supported by the positive relationship between $\delta^{15}N$ of NH_4^+-N in soil solution and that in throughfall (Fig. 1b). The further ^{15}N-depletion of NH_4^+-N (6 to 7 ‰) from throughfall to soil solution may occur by preferential retention of the heavier ^{15}N isotope by cation exchange on soil surfaces (e.g. Karamanos and Rennie, 1978), although preferential nitrification of the lighter isotope could work in the opposite direction. This fractionation effect of nitrification (leaving the substrate NH_4^+-N ^{15}N-enriched and the product NO_3^--N ^{15}N-depleted (Högberg, 1997)) may explain the relative ^{15}N-enrichment of NH_4^+-N (2 to 6 ‰) from throughfall to surface runoff in both forests (Table 2). A contribution of NO_3^--N from nitrification of ^{15}N-depleted throughfall NH_4^+-N during surface runoff passing through the biological active litter layer may also explain the 4 to 6 ‰ ^{15}N-depletion of NO_3^--N from throughfall to surface runoff (Table 2).

While NO_3^--N is the dominant N-form in soil solution (Table S1) and the N leaching fluxes are almost as large as the N inputs by deposition in both forests (Fang et al., 2009), nitrification is an important process in the soils at DHSBR. However, as soil solution NO_3^--N was ^{15}N-enriched (-1 ‰) relative to the ^{15}N-depleted throughfall NH_4^+-N (-15 ‰) this cannot be the main substrate for nitrification in the soil. Also the relative narrow temporal variation of $\delta^{15}N$ for soil solution NO_3^--N (Fig. 1d) indicate dominance of a substrate for nitrification with stable $\delta^{15}N$ content such as soil organic N and/or adsorbed NH_4^+. On the other hand, gaseous losses of ^{15}N-depleted N by denitrification would ^{15}N-enrich soil N as well as soil solution NO_3^--N (Houlton et al., 2006). For the BF, denitrification N losses have been estimated to be as high as 2.6 kg N ha^{-1} yr^{-1} as N_2O (Zhang et al., 2008) and 30 kg N ha^{-1} yr^{-1} as N_2 (Fang et al., 2015). This may explain why DIN in soil solution is slightly ^{15}N-enriched relative to the DIN input (bulk precipitation or throughfall), despite the apparent importance of fractionation via nitrification in the soils of both forest.

4.2 $\delta^{15}N$ of plants and soil under ambient condition

Climate is important in regulating global patterns of $\delta^{15}N$ in plants and soils (Amundson et al., 2003; Craine et al., 2009; 2015b). Based on the relationships between plant and soil $\delta^{15}N$ and climate parameters (MAT and MAP) established by Amundson et al. (2003), the expected $\delta^{15}N$ at DHSBR are 0.4 ‰ for plants and 5.2 ‰ for the top 10cm soil. In a global synthesis for forests Martinelli et al. (1999) reported an average leaf $\delta^{15}N$ at 3.7 ± 3.5 ‰ for tropical forests and a major recent survey across Amazonas observed similar ^{15}N-enriched leaf $\delta^{15}N$ levels (3.1 ± 2.3 ‰) (Nardoto et al., 2014). For tropical forest soils Martinelli et al. (1999) reported 9.3 ± 1.8 ‰ for the top 10cm. However, the observed leaf $\delta^{15}N$ at DHSBR were much lower, between -4 ‰ and -6 ‰ for the two forests (Table 4). Similar low leaf $\delta^{15}N$ (-2 to -5 ‰) were found in other (sub) tropical forest in eastern Asia (Fang et al., 2011a; Wang et al., 2014; Kitayama and Iwamoto, 2001). The top 10 cm soil $\delta^{15}N$ at DHSBR (2.2 to 2.6 ‰, Table 4) were again lower than expected from local climate or observed in tropical forest. Apparently, the ecosystem $\delta^{15}N$ values at DHSBR are more close to the values reported for temperate forests by Martinelli et al. (1999) for leaves (-2.8 ± 1.8 ‰) and for soil (1.6 ± 3.6 ‰) as well as those reported from N-saturated temperate forests (Koopmans et al. 1997; Sah & Brumme, 2003).

Thus, our results reject our first hypothesis that ecosystem $\delta^{15}N$ at DHSBR would compare with other observations from warm and humid climates; also DHSBR forests were not more ^{15}N-enriched than temperate forests. Martinelli et al. (1999) discussed reasons for the ^{15}N-enrichment of tropical ecosystems (relative to temperate forest) and concluded it could result from open N cycles in tropical forests, with fractionation during microbial activities resulting in losses of isotopically light ^{14}N forms which leave isotopically heavy N to cycle internally within tropical

ecosystems. Despite noticeable fractionation processes in the soil at DHSBR (section 4.1) and high N availability leading to considerable N losses, there is no evident ecosystem ^{15}N-enrichment at DHSBR or in other Chinese forests with high N deposition (Fang et al., 2011a; Wang et al., 2014).

We suspect this phenomenon to be an imprint from the high and ^{15}N-depleted N deposition (Table 2). The ^{15}N signature of deposition N can alter plant $\delta^{15}N$ by direct uptake in the canopy and by altering the signature of available N in the soil (Craine et al., 2015a) (as it is noticeable for NH_4^+-N in soil solution; Fig 1b). A similar mechanism involving preferential uptake of particularly ^{15}N depleted NH_4^+-N could also explain the occurrence of ^{15}N-depleted plants in tropical rainforests in southern China (Wang et al., 2014). Such influence of deposition N can be region-specific as shown for some forests in Europe that appear to follow a different trajectory for increasing leaf $\delta^{15}N$ with N deposition than forests in USA (Pardo et al., 2006).

The conclusion that plant $\delta^{15}N$ is influenced by the ^{15}N-depleted N deposition is further supported by the result that tree ring $\delta^{15}N$ of *Pinus massoniana* at DHSBR (sampled nearby the PF plots) decreased from 2 ‰ in the 1960s to -1 ‰ in the late 1990s, and that the decrease was found to coincide with the increasing deposition of ^{15}N-depleted N over the last 50 years (Sun et al., 2010). In line with that long-lived plant compartments (bark and wood) were less ^{15}N-depleted than short-lived compartments (leaves, twigs and branches) in both forests (Table 4).

The lower soil $\delta^{15}N$ in DHSBR relative to the global average for tropical forest soils may in part also be an imprint from the ^{15}N-depleted N deposition. However, with an N-pool at ~2400 kg N ha^{-1} (equal to more than 60 years of N deposition) alone in the top 10 cm (Gurmesa et al., 2016), the influence should be minor compared to that in short-lived plant compartments that holds an N-pool an order of magnitude less.

The steep slopes at DHSBR may contribute slightly to lower the soil $\delta^{15}N$, because steeper slopes promote non-fractionating erosional losses of soil organic matter and decrease the residence time of soil N compared to forests on more gentle slopes, that on the other hand may have more fractionation from denitrification due to greater soil moisture (Amundson et al., 2003; Hilton et al., 2013; Perakis et al., 2015).

4.3 Effects of N addition on $\delta^{15}N$

Nitrogen addition increases N availability and is thus expected to increase plant $\delta^{15}N$ as a result of fractionation during N uptake and cycling, as discussed above. Several N addition experiments in temperate forests indeed observed this effect (Högberg et al., 2011; Högberg et al., 2014; Korontzi et al., 2000; McNulty et al., 2005; Näsholm et al., 1997). Accordingly, plant $\delta^{15}N$ in both forests at DHSBR were increased by N addition (Table 4, Fig 2a). The changes in $\delta^{15}N$ occurred in small and short-lived plant compartments (e.g. leaves, roots) that are responsive to contemporary N input manipulation (Fang et al., 2006; Johannisson and Hogberg, 1994; Pardo et al., 2002) compared to the large, long-lived and less responsive compartments (e.g. bark and wood). Such changes in plant $\delta^{15}N$ could be a result of fractionation processes, but alternatively it may originate from uptake and incorperation of the added N fertilizer, that had an enriched ^{15}N signature (-0.7 ‰) relative to $\delta^{15}N$ of the plants (e.g. -4 to -6 ‰ in leaves).

Assuming fractionation effects are minor, the decadal N addition with -0.7 ‰ $\delta^{15}N$ can be viewed as a tracer addition, since it differs from the abundance in the major ecosystem pools. Based on a ^{15}N mass balance calculation (Nadelhoffer and Fry, 1994), and using the control plots as reference, the fraction of added N that was incorporated into plants could be estimate (Table S2). Since the calculation relies on the difference in $\delta^{15}N$ between the control and the N-plots in the target pool, it is only meaningful when this difference is significant. Thus, the fraction of added N

incorporated could only be estimated for the total plant N pool, but not for the soil (Fig. 2). The results showed that ~15 % of the total 500 kg N ha^{-1} added over a decade was incorporated into plant pools in both forests. For BF this was less than the estimated fate (24 % to plants) of a stronger tracer (Gurmesa et al., 2016). Nevertheless, it indicates substantial incorporation of input N into plants in BF even though the N addition did not increase the net uptake in the forest, i.e. no change in %N in plant compartments at BF.

For soils, N addition tended to decrease $\delta^{15}N$, opposite to results in other long-term experimental N addition (Högberg, 1991; Högberg et al., 1996, 2011) where soil $\delta^{15}N$ increased after addition of N. The authors explained that the increase was the result of fertilizer-induced fractionation due to increased N transformation rates. In our study, fractionation may also occure, but with the decreasing tendency of soil $\delta^{15}N$ indicates incorporation of the isotopically lighter added N (relative to the soil) is likely as discussed by Högberg et al. (2014).

The result supports our second hypothesis that the added N is incorporated into the ecosystem N pools with plant (and soil) $\delta^{15}N$ changing slowly toward the ^{15}N signature of the decadal N addition. This again highlights the importance of the ^{15}N signature of input N in controlling ecosystem $\delta^{15}N$.

4.4 Effects of forest type

As expected from previous studies, the BF is more N-rich than the PF as indicated by higher %N in major ecosystem pools in BF (Table 3). Accordingly, plant %N in short-lived compartments (and in the pool weighted plant pools) did not respond to the decadal N addition in BF, whereas plant %N in PF tended to increase, though only significantly in understory plants (Table 3, Fig. 2a). In the BF, the plant tissues were apparently saturated with N, while the PF still could retain part of the addition (Fang et al., 2009). Most plant compartments in BF are more ^{15}N-enriched than the PF (Table 4) and the change in plant $\delta^{15}N$ after decadal N addition was most pronounced in PF (Fig. 2a). This again could hint a difference in N status, where the larger changes in plant $\delta^{15}N$ in the PF indicate larger incorporation of added N into plants in PF than in BF in agreement with our third hypothesis.

The difference under ambient conditions may in part be related to higher N cycling rates and subsequent losses of the lighter ^{14}N in the BF through fractionating processes, and subsequent plant uptake of ^{15}N-enriched soil N (Magill et al., 2000; Zhang et al., 2008; Nadelhoffer and Fry, 1994). On the other hand, leave $\delta^{15}N$ in PF can be more affected by ^{15}N-depleted deposition as the forest is still expanding in biomass and has lower N availability, thus it might depend more on the ^{15}N-depleted atmospheric N input than the BF does. An additional explanation could be that the PF is dominated by *Pinus massoniana*, which has ectomycorrhizal fungi whereas majority of the plants in the BF have arbuscular mycorrhizal association (Gurmesa, 2016), and ectomycorrhizal plants are found to be more ^{15}N-depleted than arbuscular mycorrhizal plants (Craine et al., 2009; 2015a).

Soil $\delta^{15}N$ did not significantly differ between the BF and PF (Table 4; Fig. 2b), although we expected soil to be more ^{15}N-enriched in the BF than in the PF. Soil $\delta^{15}N$ are reported to increase with organic matter age (Bauer et al., 2000), and we expect soil organic matter of the top soil to be older in the BF, because this layer might have been lost by erosion in the PF as it could be noted from the lower C, N and P concentration (Table 1), and lack of depth pattern of soil $\delta^{15}N$ in the PF (Fig 2b). A common feature in soil profiles is ^{15}N-enrichment with soil depth (Bauer et al., 2000; Emmet et al., 1998; Koba et al., 2010; Boeckx et al., 2005) as observed in the undisturbed BF, but not in the disturbed PF (Table 4). The absence of a ^{15}N-enrichment profile may again be an effect of erosion and soil mixing from human disturbances that may shape soil N and $\delta^{15}N$ patterns over ecosystem succession (Perakis et al., 2015). The ^{15}N-

enrichment with depth is known to occur as a result of fractionation followed by removal of lighter ^{14}N by plants, microbes, or through leaching following decomposition, whilst the ^{15}N-enriched N fraction is transported and accumulated at deeper soil profile (Högberg et al., 2011; Hobbie and Högberg, 2012; Nadelhoffer et al., 1988).

5 Conclusion

We show that forests at DHSBR (and other humid tropical forests of southern China) are likely ^{15}N-depleted due to imprints from ^{15}N-depleted N deposition, particularly NH_4^+-N in the region. This effect of the input N (deposition) ^{15}N signature was further supported by our observation that $\delta^{15}N$ of plants (and soil) were changed toward the ^{15}N signature of added fertilizer N, which also shows that fertilizer additions are incorporated into forest N pools even at high N availability. We found that broad-leaved forests and early successional forests differ in their %N and $\delta^{15}N$, and accordingly differ in their response to increased N input. The significant changes in plant $\delta^{15}N$ toward the $\delta^{15}N$ value of the added N observed in both forests indicate that the ^{15}N signature of incoming N could dominate the effects from fractionation during the steps of N cycling. Thus, the ^{15}N imprint of increased N deposition should be considered in using ecosystem $\delta^{15}N$ to interpret ecosystem N cycling characteristics, particularly in regions with high N emissions.

Authors' contribution: Gundersen P. and Mo J. conceived and designed the experiments. Gurmesa A.G., Lu X., Mao Q. and Zhou K. performed the data acquisition. Gurmesa A.G. analyzed the data. Gurmesa A.G. and Gundersen P. wrote the manuscript. Lu X. and Mo J. commented and edited the article.

Conflicts of interest: The authors declare that they have no conflict of interest.

Acknowledgment

This study was initiated under the Sino-Danish Centre for Education and Research (SDC), and supported by the National Basic Research Program of China (2014CB954400), and the National Natural Science Foundation of China (No. 41473112; 31370498), and the SDC. We would like to thank the State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, the Chinese Academy of Sciences (No. LFSE 2013-13) for analyzing the water samples. We also wish to thank Lijie Deng and Xiaoping Pan for their skillful assistance in field and laboratory works. We also thank Knute Nadelhoffer, Wim Wessel and an anonymous referee for their helpful comments on earlier version of the manuscript.

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