Interactive comment on "Higher response of terrestrial plant growth to ammonium than nitrate addition" by Liming Yan et al.

Anonymous Referee #2. Received and published: 15 May 2018

My comments on this manuscript echo those of Anonymous Referee 1. The paper highlights an important issue in the interpretation of the effects of N deposition on ecosystems, especially given the different sources of ammonium and nitrate, and the changes in emissions that are expected over coming decades. In principle, a meta-analysis of the type described by this paper would be a valuable contribution to increasing our understanding of the relative effects of these two forms of nitrogen. However, I am concerned, like Anonymous Referee 1, that the way in which papers are used in this study does not provide a robust basis for the analysis.

Response: Authors really appreciate the reviewer for the positive comments and critical suggestions. Below please find our point-to-point replies, and all revisions have been made in the main text accordingly.

Of specific concern are:

Q1- *The duration of the experiment. It is not clear from the methods description how this is handled, as the methods only refer to amount of N in g m^{-2}. Is this the total N added over the course of the experiment, or the annual addition? The authors appear to include experiments of quite different lengths in their meta-analysis, but how this is taken into account in their analysis is not specified. This needs to be clarified.*

Response: The reviewer raised an important point which has usually ignored by most previous meta-analyses. We agree with the reviewer that a clear description of the experimental duration must be provided in the method. In this study, most the experiments (~90%) added the N annually (e.g., N_{add} = 10 g N m^{-2} yr^{-1}), so the response of plant growth in a certain duration of the experiment (e.g., t = 5 yrs) actually is determined by the total N amount (e.g., $N_{amount} = N_{add} * t$ = 50 g N m^{-2}). In this study, we first normalized the data with the N_{amount} (please see the equations 3-4 in the main text or below) before they were used in the meta-analysis.

In revised this version, we have clearly described how the duration of experiment was handled in the method section (Line 158-164) as:

"The meta-analysis followed the techniques described in Hedges et al., (1999), Wan et al., (2001) and Xia & Wan (2008). In this study, most the experiments (~90%) added the N annually (e.g., Nadd = 10 g N m^{-2} yr^{-1}), so the response of plant growth in a certain duration of the experiment (e.g., t = 5 yrs) actually is determined by the total N amount (e.g., Namount = N_{add} * t = 50 g N m^{-2}). Thus, we first normalized the plant growth and its variation of the N treatment as:

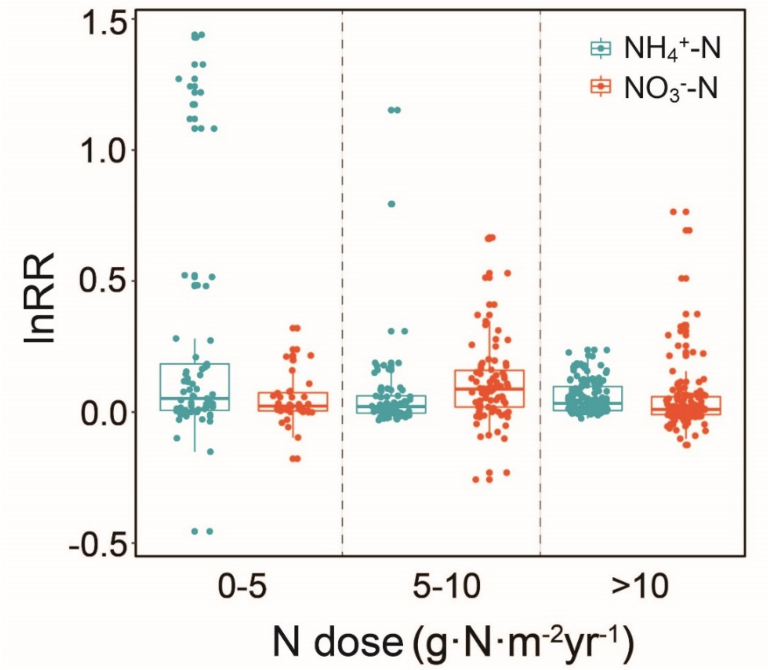
$$X'_e = X_c + \frac{X_e - X_c}{N_{amount}} \quad (3)$$

$$S'_e = S_c + \frac{S_e - S_c}{N_{amount}} \quad (4)"$$

Q2- *The dose of N added. The references listed include studies using an N addition well* *above the realistic range of atmospheric deposition, which at a global scale might be 0-50 kg ha^{-1} yr^{-1}. It is not reasonable to assume that plant growth will respond linearly (as I understand the author's analysis assumes) across a wide range of doses, and it would be informative to constrain an analysis informing deposition responses to the range of likely deposition.*

Response: We agree with the reviewer that the dose of N addition in most experiments is much higher than the realistic range of atmospheric deposition. As shown by the following figure, we further separated the field N experiments into three groups in terms of the dose of N addition (i.e., 0-5, 5-10 and >10 g m^{-2} yr^{-1}). We found the highest response of plant growth to NH_4^+-N addition was under the dose of 0-5 g m^{-2} yr^{-1}, while that to NO_3^--N addition occurred with the N amount of 5-10 g m^{-2} yr^{-1}. Thus, considering the reality of N deposition rate (i.e., 0-5 g m^{-2} yr^{-1}), plant could be more sensitive to NH_4^+-N than NO_3^--N addition. In this revised version, we have added this figure as Fig. 7, and added sentences to discuss this important point on Lines 364-370 as:

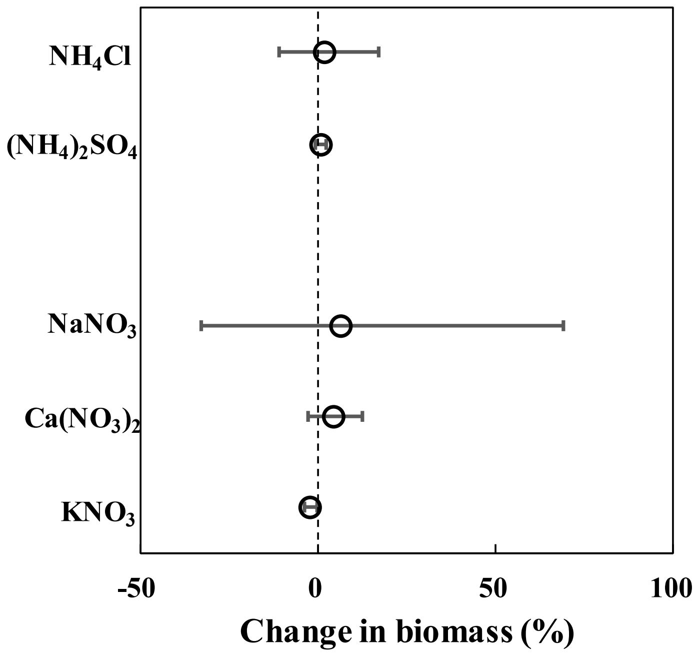
> "6. The dose of N addition in most experiments in this study is above the realistic range of atmospheric deposition (e.g., 0-5 g m^{-2} yr^{-1}). When separated the field N experiments into three groups in terms of the dose of N addition (i.e., 0-5, 5-10 and >10 g m^{-2} yr^{-1}), we found that the highest response of plant growth to NH_4^+-N addition was under the dose of 0-5 g m^{-2} yr^{-1}, while that to NO_3^--N addition occurred with the dose of 5-10 g m^{-2} yr^{-1} (Fig. 7). Thus, considering the reality of N deposition rate (e.g., 0-5 g m^{-2} yr^{-1}), plant could be more sensitive to NH_4^+-N than NO_3^--N addition."



Q3- *The counter-ion used. Studies with ammonium, for example, might use sulphate, chloride, phosphate etc. and the study design needs to ensure that any growth responses are only due to the ammonium itself. However, having checked references cited as studying ammonium responses in the paper's reference list, I am not convinced this is the case. I expected, for example that all the studies labelled as NH_4 would apply this* *with an inorganic anion, but they seem to include studies applying fertiliser with a mix of nutrients with N in the form of NH_4. The authors need to include full details of the chemical form of the applied N in all cases, and to ensure this is included as a factor in met-analysis.*

Response: The reviewer is right that neither NH_4^+-N nor NO_3^--N could be added without an inorganic anion or cation. This is also one major reason to use NH_4NO_3 in previous experimental studies. In this revised version, we have done two improvements to highlight this issue. First, as suggested by the reviewer, we have added the detailed information of the chemical form of applied N in all studies in the supplementary Table S1. Second, we analyzed the impacts of different inorganic anions or cations. As shown by the following figure, there was no significant difference between the anions or cations (both $P > 0.05$). This figure has also been added as the supplementary Fig. S3 in this revised version. We also added a few sentences to raise this point as one limitation of this study on Lines 386-389:

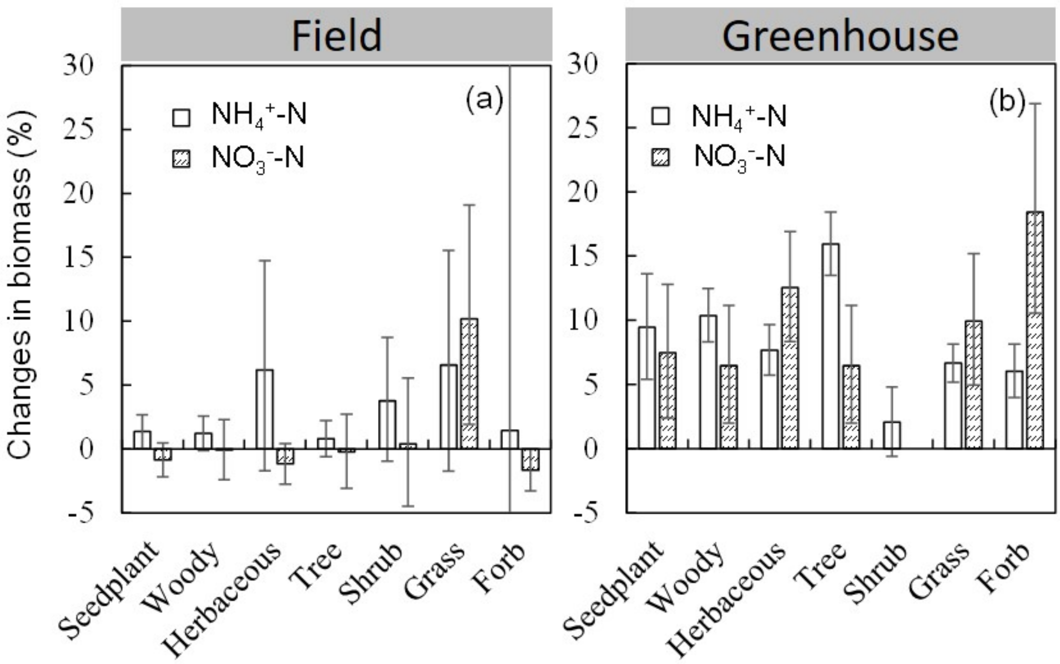
> "Lastly, neither NH_4^+-N nor NO_3^--N could be added in the experiments without an inorganic anion or cation. Although no difference was detected between anions or cations (Supporting information Figure S3), their effects could be included in the NH_4^+-N or NO_3^--N addition in this study."



Q4- *It is clear from the sub-sample of papers included in the meta-analysis that I examined that the studies vary from pot experiments in a greenhouse using seedlings to application to mixed plant communities under field conditions. It is very unlikely that growth responses are going to be the same under these very different conditions and very different plant growth stages. The authors need to focus on field studies of established plants, to provide a clear guide to likely responses under field conditions. If other studies under controlled environments are to be included, this needs to included as a separate factor in the meta-analysis.*

Response: The reviewer raised an important issue which has been neglected by the previous syntheses. As shown by a recent work by Poorter *et al.* (2016; New Phytol. 212: 838-855), there are many differences between plants growing in greenhouse and the field. As suggested by the reviewer, we divided the experiments into two groups, including "field" and "greenhouse". As shown by the following figure, the response patterns in the "field" experiments are similar with those presented in Fig. 4. That is, plant growth was more enhanced by significantly enhanced by NH_4^+-N than NO_3^--N addition (panel a). However, greater responses were found for plants growing in greenhouse (panel b). These results are important to explain the general patterns shown by Fig. 4, so we added this figure as supporting Fig. S1 in this revised version. We also have added sentences in the "Results" and "Discussion" sections to highlight the issue raised by the reviewer as:

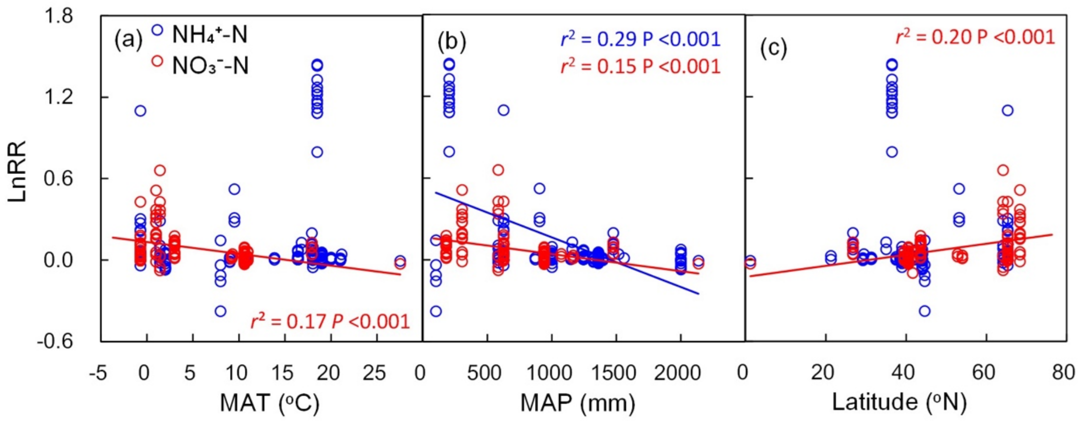
> Line 226-231: "It should be noted that these response patterns were calculated on all available studies, including experiments which were conducted in both greenhouses and the field. As shown by the supporting information Figure S1, the general patterns of Fig. 4a were similar with that across the field experiments. However, greater responses of plant growth to both NH_4^+-N and NO_3^--N addition were found across the greenhouse experiments."
>
> Line 353-357: "4, The different response patterns between greenhouse and field experiments (Supporting information Figure S1) suggests that the general plant responses to N addition in this meta-analysis (i.e., Fig. 4) could be larger than the natural ecosystems. More research efforts exploring the differences and similarities between plants growing in the greenhouse and field are needed (e.g., Poorter *et al.*, 2016).



Q5- *As clearly argued by Referee 1, plant growth responses to N can be constrained by a range of other environmental factors, such as climate and water availability. Soil characteristics are also likely to critical in determining responses to added nitrate and ammonium. These factors need to be considered in the authors' analysis.*

Response: Based on the field experiments, we have analyzed the dependences of plant response upon environmental factors, including mean annual temperature (MAT), mean annual precipitation (MAP) and latitude. As shown by the following figure, MAT and latitude only affected plant growth response to NO_3^--N addition, while MAP negatively influenced plant growth responses to NH_4^+-N and NO_3^--N addition. These patterns collectively suggest that plants growing in colder and drier regions are more sensitive to NH_4^+-N and NO_3^--N addition than that growing in warmer and wetter regions. This finding is inconsistent with the response patterns of net primary productivity to N addition at the global scale (LeBauer & Treseder, 2008). We have added this figure as Fig. 6 in this revised version, and added a few sentences to discuss the difference between these patterns and those in the previous studies on Lines 358-363 as:

> "5. Mean annual temperature (MAT) and latitude only affected plant growth response to NO_3^--N addition, while mean annual precipitation (MAP) negatively influenced plant growth responses to NH_4^+-N and NO_3^--N addition (Fig. 6). These patterns collectively suggest that plants growing in colder and drier regions are more sensitive to NH_4^+-N and NO_3^--N addition than that growing in warmer and wetter regions. This finding is inconsistent with the response patterns of net primary productivity to N addition at the global scale (LeBauer & Treseder, 2008)."



Q6- *It is also surprising to me that several relatively recent long-term field experiments designed to compare the effects of ammonium and nitrate directly are not included in the papers used in the analysis. These include studies of an ombrotropic mire (Sheppard et al., 2014, mentioned in the intro but not included in the meta-analysis), of a Tibetan alpine meadow (Song et al. (2015) and maquis vegetation (Dias et al., 2017). Since these studies directly compare the growth and ecological effects of the two forms of nitrogen, they seem a more important source of information than a comparison of independent studies of the two forms which may be biased by the many differences in experimental design which are not accounted for in the authors' analysis.*

Response: As pointed out by the reviewer, long-term studies with inter-comparison between ammonium and nitrate addition are more direct to answer the question in our study. We have checked the references which suggested by the reviewer. Below are the reasons for why they were excluded in our analysis:

- Song & Yu (2015) conducted an 8-yr N fertilization experiment to examine the effect of long-term N addition on ecosystem stability its driving mechanisms. They found the form of N (ammonium and nitrate) did not affect the stability. In their Fig. 1c, no difference in the responses of community biomass to ammonium and nitrate addition were detected. We didn't include the data from this experiment because our study only included species-level data. However, their results have been discussed in this revised version on Lines 301-306 as:

> "Furthermore, NH_4^+-N addition show a higher stimulation on plant growth but a lower variability among plant functional types than NO_3^--N addition (Fig. 4), contrasting the result of Song & Yu (2015), which observed the synchrony among functional groups were not affected by N form after 8-year fertilization in an alpine meadow on the Tibetan Plateau. Our result suggests that future N deposition with more NH_4^+-N input will more evenly enhance the growth of different plant functional types."

- Sheppard et al. (2014) reported the interesting responses of relative cover changes among species to ammonium and nitrate addition in an ombrotrophic bog. They found some species (e.g., *Calluna vulgaris*) preferred for ammonium N while others (e.g., *Hypnum jutlandicum*) for nitrate N. We didn't include this study in our analysis because the data were reported with coverage rather than dry mass (or biomass). However, we also cited this work as the evidence for the species-specific preferences for ammonium and nitrate on Lines 309-312 as:

> "However, within an ecosystem, the preference for ammonium or nitrate could varies among species. For example, Sheppard et al. (2014) reported the relative cover changes of some species in an ombrotrophic bog (e.g., *Calluna vulgaris*) preferred for ammonium N while others (e.g., *Hypnum jutlandicum*) for nitrate N."

- Dias et al. (2017) used a 7-yr N fertilization experiment to study the effect of N deposition on a Mediterranean maquis community. In their Table 1, they reported the responses of leaf traits to different N treatments. There are two reasons we didn't include these data. First, this study includes three N treatments, including 40A (i.e., 40 kg ha^{-1} yr^{-1} NH_4^+-N as a 1:1 mixture of NH_4Cl and $(NH_4)_2SO_4$), 40AN (i.e., 20 kg ha^{-1} yr^{-1} NH_4^+-N and 20 kg ha^{-1} yr^{-1} NO_3^--N as NH_4NO_3) and 80AN (i.e., 40 kg ha^{-1} yr^{-1} NH_4^+-N and 40 kg ha^{-1} yr^{-1} NO_3^--N as NH_4NO_3). Their results reflect the difference between NH_4^+-N and NH_4NO_3 rather between NH_4^+-N and NO_3^--N. Their finding of no difference between NH_4^+-N and NH_4NO_3 is consistent with the finding in our study. Second, only leaf trait (i.e., mg $leaf^{-1}$) data were reported in the Table 1, and the total leaf biomass were not provided in this study.

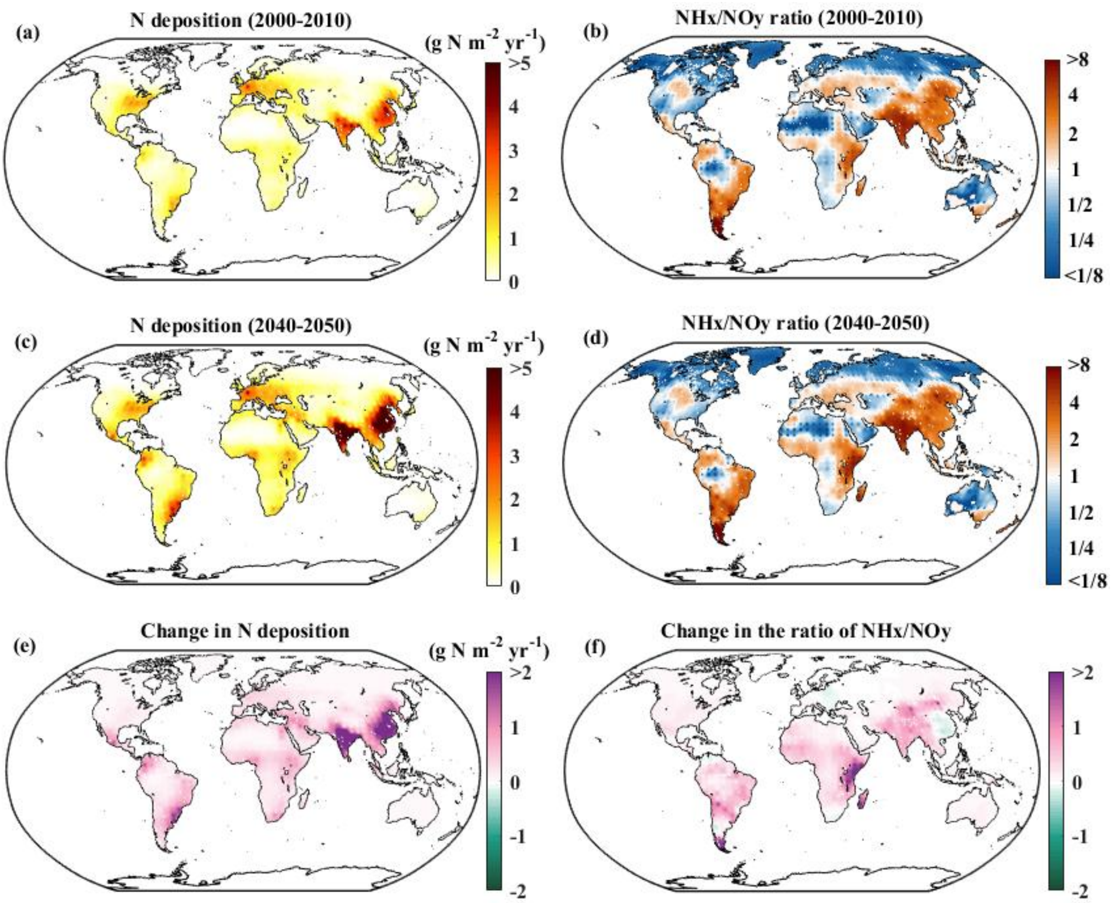
Q7- *The authors also present in Figure 2 the global trends of the ratio of ammonium to nitrate over the period 2010-2100. However, the description of how these trends were derived is very limited, referring both to the use of environmental driver sets and the use of linear regression. The latter approach seems questionable since the trends in emissions of NH_4^+ and NO_3^- over this period are unlikely to be linear, and the authors make no clear statement of the scenario assumptions used to derive these future trends.*

Furthermore, if a trend in the ratio is to be useful interpreted, the reader needs to know what total N deposition is in these areas (the ratio will be much more relevant where deposition is high) and what the baseline ratio in 2010 is on from which these trends are based (e.g. a trend to a lower ratio, with nitrate dominance, might be from a baseline of high ammonium dominance or a baseline already with nitrate dominance. If the authors wish to include this analysis, it needs much more detail and context than is provided in the current version of the paper.

Response: Authors thank the reviewer for the great suggestions. According to the reviewer's suggestion, we have replaced the original Fig. 2 with the below one in this revised version. We avoid using the linear regression analysis to interpret the trends of N deposition. Instead, we mapped the total N deposition and its NH_x/NO_y ratio over 2000-2010 (panel a, b) and 2040-2050 (panel c, d) as well as their changes between the two periods (panels e and f). As shown by the figure, the eastern Asia had the highest N deposition rate (panel a, c), which was dominated by NH_x deposition (panel b, d). The increase in N deposition is also the fastest in the eastern Asia, but the NH_x/NO_y ratio in this region is decreasing (panel f). We have updated these methods and results in this revised version as:

> Lines 137-147 "Then, we used global N deposition dataset which was gridded to 0.5°×0.5° spatial resolution from the Multi-scale Synthesis and Terrestrial Model Intercomparison Project (MsTMIP) (https://daac.ornl.gov/cgi-bin/dsviewer.pl?ds_id=1220) (Huntzinger et al., 2013; Wei et al., 2015). In this database, the simulation of annual nitrogen deposition was based on the assumption that the temporal trends of NH_x and NO_y depositions were consistent with those of NH_3 and NO_x emissions between 1890 and 1990 (Dentener, 2006), and nitrogen deposition increased linearly over the time periods of 1860-1890 and 1990-2050. Following these assumptions and the methods of Tian et al. (2010), the total N deposition and its NH_x/NO_y ratio was calculated at each 0.5°×0.5° grid in each year from 2000 to 2050. Their annual average during the period of 2000-2010 and 2040-2050 were compared and the results were shown in Fig. 2"

> Lines 204-209 "As shown by the Fig. 2, the eastern Asia had the highest N deposition rate (panel a, c), which was dominated by NH_x deposition (panel b, d). The increase in N deposition is also the fastest in the eastern Asia (panel e). The fast increase in global NH_x/NO_y in the future was consistent with that derived from the MsTMIP environmental data (Fig. 2f). However, the trend of NH_x/NO_y was decreasing in some regions, such as the southeastern China and western Europe (Fig. 2f)."


(a)
N deposition (2000-2010)
(g N m-2 yr-1)
>5
4
3
2
1
0
(b)
NHx/NOy ratio (2000-2010)
>8
4
2
1
1/2
1/4
<1/8
(c)
N deposition (2040-2050)
(g N m-2 yr-1)
>5
4
3
2
1
0
(d)
NHx/NOy ratio (2040-2050)
>8
4
2
1
1/2
1/4
<1/8
(e)
Change in N deposition
(g N m-2 yr-1)
>2
1
0
-1
-2
(f)
Change in the ratio of NHx/NOy
>2
1
0
-1
-2