

Reply to the comments by reviewer #2

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Comments: This manuscript reports on a synthesis or meta-study of studies that report the effects of N additions on soil CH₄ fluxes. The authors did a literature search using several sources, calculated a response variable, stratified results according to dose and biome, and conclude that low-level N addition increased CH₄ uptake in boreal forest while decreases occurred at high N addition levels and in all other biomes. A meta-study is only as good as the sources where the information comes from, and therefore studies used in such a meta-study should be generally accessible and peer-reviewed. Unfortunately, several of the studies used in this meta-study did not meet these criteria. I find it problematic that apart from ISI Web of Science, also Google Scholar and China National Knowledge Infrastructure are used to search for literature. To my knowledge, these two latter sources also list reports that are not peer-reviewed. I strongly suggest to ONLY use ISI as a literature source, because here only peer-reviewed sources are listed. Studies that are not peer reviewed should not be included in a synthesis. In your meta-study I suggest to exclude the following studies listed in the supplement: - two of the studies listed are a MSc thesis (Wang, 2012, Pan, 2013). Such a thesis is not considered peer-reviewed, please exclude them. - all studies that involved incubations, instead of field measurements. For example, the study by Chen et al. 2017 mentions that they did laboratory incubations. You even state in Page 3, line 16 that you only included studies that used closed static chamber technology. Apparently that is not true. There may be more studies with incubations, I did not check them all. - Please, exclude studies published in Chinese (or other non-English publications) with only an English abstract. I do not consider such studies as generally accessible. For example, the study by Hu et al., 2011, is only accessible in Chinese and there may be more in the list.

Reply: Thanks for your comments, which have helped us to improve the manuscript substantially. We fully agree that the quality of data sources is very important. In the revised manuscript, we have updated the database by a) excluding experimental results from theses without peer-review (Wang, 2012; Pan, 2013), b) excluding experimental results from laboratory incubations (only Chen et al. 2017), c) excluding an experimental study that

didn't report values of variance/standardized error (Stuedler et al., 1989), d) excluding results of experiments with plot area < 10m² or without buffer zones, and e) including more results of field experiments using urea additions. We further beg your understanding to include reports published in fully peer-reviewed Chinese journals because these publications are generally of good quality in science. Overall, we updated our database based on the criteria above and the current database includes results of 28 experiments across 22 sites (Figure R1).

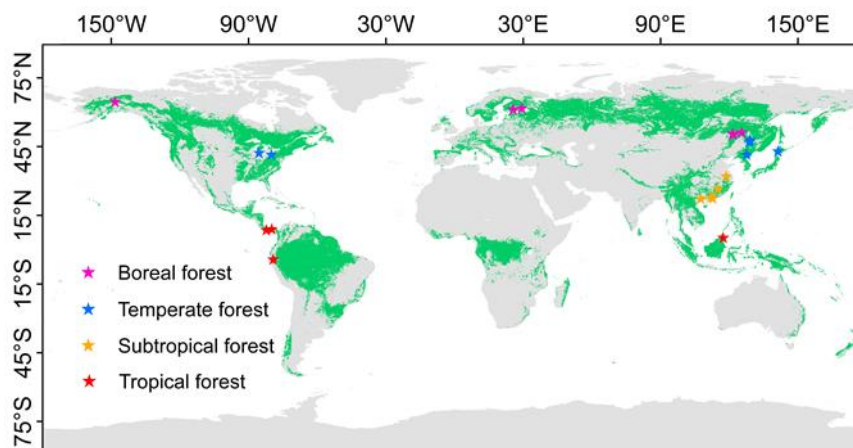


Figure R1. Geographical distribution of 28 forests receiving experimental nitrogen additions. Green shadows indicate the distribution of global forest.

10 Reference

Pan, D.R. 2013. Study on greenhouse gas emission for grassland soil below different forest soils under precipitation reduction and Nitrogen deposition in Shennongjia mountain. Gansu Agricultural University, Master thesis.

15 Stuedler, P.A., Bowden, R.D., Melillo, J.M., Aber, J.D. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soils. *Nature*, 341(6240), 314-316.

Wang, R.N. 2012. Effects of simulated atmospheric nitrogen deposition on the exchange fluxes of greenhouse gases in the temperate forest soil. Beijing Forestry University, Master thesis

Comments: You use the 60 kg N ha⁻¹ yr⁻¹ as an arbitrary cut-off between 'low' and 'high' level N addition. Did you calculate also the background N-deposition in the N additions? For example, the study by Li et al., 2015, mentions that there is a background N deposition of more than 30 kg N ha⁻¹ yr⁻¹, while the treatment is 40 kg ha⁻¹ yr⁻¹. Together this would be more than 60 kg N ha⁻¹ yr⁻¹ and the 40 kg treatment should be grouped as 'high' level

N addition. I suspect that is not how you did this and it just illustrates how arbitrary the choice of $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ is.

Reply: Thanks for your comments. We defined the threshold of N deposition versus high-level N addition based on a global assessment of N deposition by the World Meteorological Organization (WMO) Global Atmosphere Watch programme (GAW) (Vet et al., 2014). This assessment shows a range of N deposition in various regions of the world, from $1\text{--}62 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Specifically, the maximum level of N deposition occurs in eastern and southern China (Vet et al., 2014). We thus used $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as a threshold for a possible maximum of N deposition, in order to distinguish it from N fertilization with extremely high levels of N additions. In the revised manuscript, we have conducted a meta-analysis (Viechtbauer, 2010) by using background N deposition as a moderator. We used a mean difference ($\text{Flux}_{\text{treatment}} - \text{Flux}_{\text{control}}$, the difference of mean growing-season soil CH_4 fluxes between the treatment plots and control plots) as the effect size to evaluate the effect of N additions. Including background N deposition to N addition is normally not done as the N addition effect is compared to a control receiving the same deposition and addition may lead to erroneous interpretations (e.g Hetterlingh et al., 2015). The standard approach is to assess the impact of background deposition as a moderator of the N addition effects (see e.g. Granath et al., 2014; Midolo et al., 2018). We did so and our results indicate that background N deposition has a significant influence on the effect of N additions ($p=0.0006$). However, since average N deposition varied significantly across global forest biomes, showing a trend from subtropical forest ($14.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) > temperate forest ($7.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and tropical forest ($7.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) > boreal forest ($1.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), this hinders us to separate the effects of background N deposition from the effect of forest biomes. We have revised the manuscript to avoid any misunderstanding.

Reference

- Aronson, E.L., Helliker, B.R. 2010. Methane flux in non - wetland soils in response to nitrogen addition: a meta-analysis. *Ecology*, 91(11), 3242-3251.
- 25 Cleveland, C. C., Houlton, B. Z., Smith, W. K., Marklein, A. R., Reed, S. C., Parton, W., et al. 2013. Patterns of new versus recycled primary production in the terrestrial biosphere. *Proceedings of the National Academy of Sciences*, 110(31), 12733-12737.
- Granath, G., Limpens, J., Posch, M., Műcher, S., de Vries, W.. 2014. Spatio-temporal trends of nitrogen deposition and climate effects on Sphagnum productivity in European peatlands. *Environmental Pollution* 30 187, 73-80.

- Hettelingh, J-P., Stevens, C., Posch, M., Bobbink, R., de Vries, W. 2015. Assessing the impacts of nitrogen deposition on plant species richness in Europe. In W. de Vries, J-P. Hettelingh & M. Posch (eds) Critical Loads and Dynamic Risk Assessments: Nitrogen, Acidity and Metals in Terrestrial and Aquatic Ecosystems, Environmental Pollution Volume 25, Springer ISSN 1566-0745, 573-586.
- 5 Liu, L.L., and Greaver, T.L. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO₂ sink may be largely offset by stimulated N₂O and CH₄ emission. Ecology Letters, 12, 1103–1117.
- Midolo, G., Alkemade, R., Schipper, A.M., Ben fez-López, A., Perring, M.P., de Vries, W. 2018. Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis. Global Ecology and Biogeography, 28(3), 398-413.
- 10 Schwede, D.B., Simpson, D., Tan, J., Fu, J., Dentener, F., Du, E., and De Vries, W.2018. Spatial variation of modelled total, dry and wet nitrogen deposition to forests at global scale. Environmental Pollution, 243, 1287-1301.
- Vet, R., Artz, R.S., Carou, S., Shaw, M., Ro, C.U., Aas, W. et al. 2014.A global assessment of precipitation chemistry and deposition of sulfur, nitrogen, sea salt, base cations, organic acids, acidity and pH, and phosphorus, Atmospheric Environment, 93, 3–100.
- 15 Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor package. Journal of Statistical Software, 36(3), 1-48.

Comments: You did not mention any other criteria for inclusion or exclusion of studies. However, I think you should define what you consider a sufficiently large plot and a sufficiently wide buffer zone between treatments. Also, were all studies having true replicates? This kind of important information on the quality of studies is completely ignored in your manuscript.

Reply: Thanks for your suggestions. In the revised manuscript, we have updated the database by a) excluding experimental results from theses without peer-reviews, b) excluding experimental results from laboratory incubations, c) excluding an experimental study that didn't report values of standard deviation/standardized error (no information for replicates), d) excluding results of experiments with plot area < 10m² or without buffer zones. We have also recorded information of replicates, plot area, buffer zone, and variance/standard deviation in our updated database. In the revised method section, we have included more detailed information for the criteria of data collection.

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Comments: I found it very adventurous that you excluded the only peer-reviewed study conducted in tropical ecosystems (Veldkamp et al., 2013) with the argument that urea as an organic N form has ‘limited implications for the effects of N deposition’, then cite Aronson and Helliker, (2010) as the source for this statement (page 3, line 20), and later lament that there are no studies conducted in tropical areas and then even extrapolate the results from subtropical forests to the tropics. -First of all, while urea is strictly spoken an organic N source, it is quickly hydrolysed ($\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3$) after application and the gaseous NH_3 reacts with water to form ammonium (NH_4^+). Only on soils with a high pH there are significant losses through volatilization. -Second, in the paper by Aronson & Helliker (2010), I did not find any statement that urea has limited implications for the effects of N deposition. In contrast, they also analysed studies with urea additions. They found no difference between Urea and other pure N fertilizers. They also concluded that ‘any conclusions of the effects of specific N species relative to others must be highly qualified, as the form of N that results may be quite different from that added’. Therefore, to quote the Aronson & Helliker (2010) paper as the source why studies that add urea should be excluded is misleading. -Third, ignoring the only tropical study and later filling up the gap with studies from subtropical areas is very adventurous. Especially since the study conducted in the tropics did not following the hypothesized trend across biomes.

Reply: Thanks for your suggestions. We fully agree that it is not appropriate to exclude N addition experiments using urea in tropical forests. In the paper by Aronson and Helliker (2010), the authors showed that the effects of N fertilization on soil CH_4 uptake did vary significantly with N forms (Figure R2). Specifically, the effects of urea and ammonium nitrate showed slight overlap, although the statistical analysis indicated no significant difference (Figure R2). This result motivated us to exclude experiments with urea additions in our previous manuscript, because a) N deposition mainly occurs in inorganic N forms, and b) urea might be less capable to indicate the effects of N deposition. As suggested by you and other reviewers, we have updated our database by searching urea addition experiments in the literature in view of the fact that the effects of urea and ammonium nitrate were not significantly different. As a result, four experiments from tropical forest were included in our updated database (Veldkamp et al., 2013; Matson et al., 2016; Mori et al., 2017).

Using a meta-analysis in R software with metaphor package (Viechtbauer, 2010), we show that low-level N addition ($<60 \text{ kg N}^{-1} \text{ yr}^{-1}$, in the range comparable to N deposition) and high-level N fertilization ($>60 \text{ kg N}^{-1} \text{ yr}^{-1}$) both had no significant effect on soil CH_4 uptake (Figure R3a&b). Overall, no significant effect of N additions on soil CH_4 uptake was found in tropical forest (Figure R3c). We have revised the manuscript accordingly.

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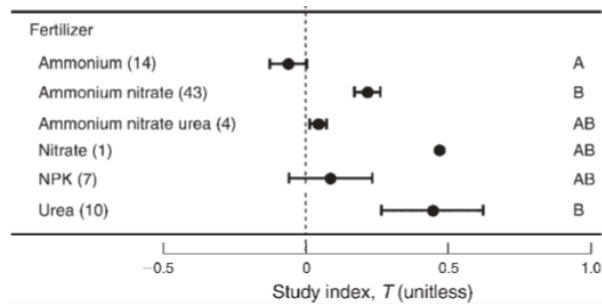
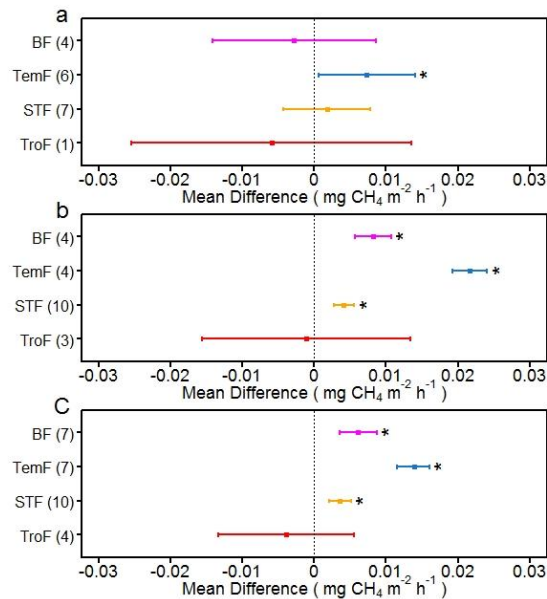


Figure R2. Effects of different N forms on soil CH_4 uptake (Aronson and Helliker, 2010)



- 5 Figure R3. The mean difference (and 95% confidence intervals) of growing-season soil CH_4 flux to a) low-level N addition ($<60 \text{ kg N}^{-1} \text{ yr}^{-1}$), b) high-level N fertilization ($>60 \text{ kg N}^{-1} \text{ yr}^{-1}$) and c) all N treatments in boreal (BF), temperate (TemF), subtropical (STF), and tropical forest (TroF), respectively. The asterisk (*) indicates a significant effect ($p<0.05$).

Reference:

- 10 Aronson, E.L., Helliker, B.R. 2010. Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. *Ecology*, 91(11), 3242-3251.
- Matson, A. L., Corre, M. D., Veldkamp, E. 2016. Canopy soil greenhouse gas dynamics in response to indirect fertilization across an elevation gradient of tropical montane forests. *Biotropica*, 49(2), 153-159.

Mori, T., Imai, N., Yokoyama, D., Mukai, M., Kitayama, K. 2017. Effects of selective logging and application of phosphorus and nitrogen on fluxes of CO₂, CH₄ and N₂O in lowland tropical rainforests of borneo. *Journal of Tropical Forest Science*, 248-256.

Veldkamp, E., Koehler, B., Corre, M. D. 2013. Indications of nitrogen-limited methane uptake in tropical forest soils. *Biogeosciences*, 10(8), 5367-5379.

Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, 36(3), 1-48.

Comments: The hypothesis to be tested (Page 2, line 21 and further) is weak and based on incomplete assumptions. You simply assume that N availability increases from boreal to tropical biomes, which is not true. If you read publications by Vitousek more carefully you will see that the main factor is not the biome but how heavily weathered soils are. More than half of the tropics is located on soils that are not heavily weathered (e.g. montane forests) and N availability is expected to be as low as other biomes where young, less weathered soils dominate.

Reply: Thanks for your comments. Nitrogen availability is internally driven by mineralization and externally from biological N fixation and N deposition. Recently, the role of N inputs from bedrock weathering is increasingly recognized, but the role of this N source for uptake from shallow soil depth is very limited. Previous studies have shown that the sum of N inputs from biological N fixation and N deposition dominate the external N input and these inputs increase from boreal forest to tropical forests (Cleveland et al., 1999&2013; Du and De Vries, 2018). Moreover, the rate of N mineralization also shows an increase from boreal forest to tropical forest (Cleveland et al., 2013; Deng et al., 2018). Overall, we think that the increase in N availability from boreal forest to tropical forest has been well recognized in literature.

Reference

Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O., et al. 1999. Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems. *Global Biogeochemical Cycles*, 13(2), 623-645.

Cleveland, C. C., Houlton, B. Z., Smith, W. K., Marklein, A. R., Reed, S. C., Parton, W., et al. 2013. Patterns of new versus recycled primary production in the terrestrial biosphere. *Proceedings of the National Academy of Sciences*, 110(31), 12733-12737.

Du, E., De Vries, W. 2018. Nitrogen-induced new net primary production and carbon sequestration in global forests, *Environmental Pollution*, 242, 1476–1487.

Deng, M., Liu, L., Jiang, L., Liu, W., Wang, X., Li, S., et al. 2018. Ecosystem scale trade-off in nitrogen acquisition pathways. *Nature Ecology & Evolution*, 2(11), 1724.

Comments: You group all forest ecosystems together and make no difference between natural forest and plantations or managed forests. Tree plantations typically have significant growth rates and are almost always N limited, also in tropical and subtropical conditions. Ignoring this may lead to wrong conclusions.

Reply: This is a good point. We have checked our database and include information of natural forest or plantations. We found that only 10 of 28 forests (1/7 boreal, 3/7 temperate and 6/10 subtropical) were plantations. This doesn't allow us to test the difference between plantations and natural forests for each forest biome due to uneven small sample size. As the database is the most updated from literature, our results represent the best knowledge we can derive at this stage. In the revised manuscript, we have discussed the uncertainties possibly due to the different N status of natural forest and plantations. We have also labelled natural forests and plantations in the revised summary table for the database.

Comments: In summary, this synthesis is poorly conducted. There are studies included that are not peer-reviewed and there were no quality criteria for the studies that were included. The hypothesis is weak and based on incomplete assumptions. The distinction between 'high level' and 'low level' N addition is arbitrary and the background N deposition is ignored. Finally, I could not find any objective reason why studies where urea was added were excluded. Given these weaknesses of this synthesis I strongly doubt the validity of the conclusions and I recommend not to publish this manuscript in Biogeosciences.

Reply: Thanks again for your helpful comments. First, we have updated the manuscript by a) excluding experimental results from theses, b) excluding experimental results from laboratory incubations, c) excluding an experimental study that didn't report values of standard deviations/standardized error, d) excluding results of experiments with plot area < 10m² or without buffer zones, and e) including more results of field experiments using urea additions. More detailed information for standard data collection has also been included in the revised section on data and method. Second, we have included more detailed evidence from literature for the gradients of N availability across forest biomes, both internally driven by mineralization and externally from biological N fixation and N deposition. This underpins our hypothesis that the effect of N additions likely varies across forest biomes due to a shift in N availability. Third, the threshold of maximum N deposition versus high-level N addition is defined based on a global assessment of N deposition by the World Meteorological Organization (WMO) Global Atmosphere Watch programme (GAW) (Vet et al., 2014), which shows a range of N deposition

in various regions of the world, from 1~62 kg N ha⁻¹ yr⁻¹. We thus used 60 kg N ha⁻¹ yr⁻¹ as a threshold for a possible maximum of N deposition, in order to distinguish it from N fertilization with extremely high levels of N additions. Fourth, we have updated our database by including urea addition experiments and conducted a reanalysis using meta-analysis. Finally, we included background N deposition as a moderator of N addition effects. The renewed analysis indicates that only temperate forest shows a significant response ratio to low-level N addition, while high-level N fertilization results in significant response ratios across boreal, temperate and subtropical forests (Fig. R4). In view of the fact that N deposition is generally low in global forest ecosystems and rarely exceeding a maximum of 60 kg N ha⁻¹ yr⁻¹ (Vet et al., 2014; Schwede et al., 2018), our results imply that separating the effects of N deposition and high-level N fertilization is necessary to avoid overestimate the effects of global N deposition. However, this has never been considered by existing meta-analyses at a global scale (Liu and Greaver, 2009; Aronson and Helliker, 2010). Overall, we believe that the main concerns have been well addressed in the revised manuscript.

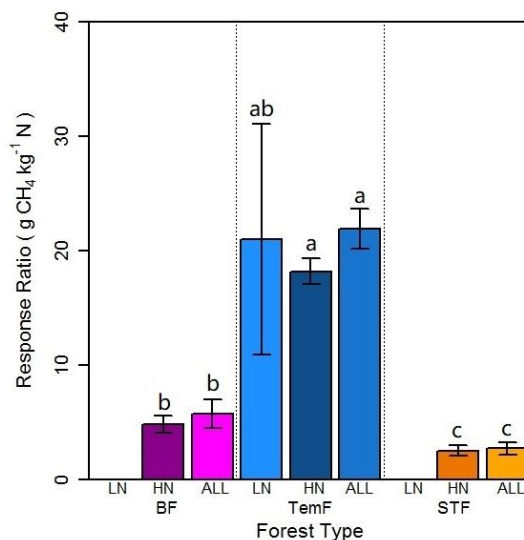


Figure R4. The response ratio of growing-season soil CH₄ flux to low-level N addition (LN, <60 kg N⁻¹ yr⁻¹), high-level N fertilization (HN, > 60 kg N⁻¹ yr⁻¹) and all N treatments (ALL) in boreal (BF), temperate (TemF) and subtropical forest (STF), respectively. Response ratio of insignificant effect (N treatments in tropical forest and LN in boreal and subtropical forest) is not shown.

Reference

Aronson, E.L., Helliker, B.R. 2010. Methane flux in non - wetland soils in response to nitrogen addition: a meta-analysis. *Ecology*, 91(11), 3242-3251.

Liu, L.L., and Greaver, T.L. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO₂ sink may be largely offset by stimulated N₂O and CH₄ emission. *Ecology Letters*, 12, 1103–1117.

Schwede, D.B., Simpson, D., Tan, J., Fu, J., Dentener, F., Du, E., and De Vries, W. 2018. Spatial variation of modelled total, dry and wet nitrogen deposition to forests at global scale. *Environmental Pollution*, 243, 1287-1301.

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Vet, R., Artz, R.S., Carou, S., Shaw, M., Ro, C.U., Aas, W. et al. 2014. A global assessment of precipitation chemistry and deposition of sulfur, nitrogen, sea salt, base cations, organic acids, acidity and pH, and phosphorus, *Atmospheric Environment*, 93, 3–100.