

Reply to the comments by Birgit Koehler

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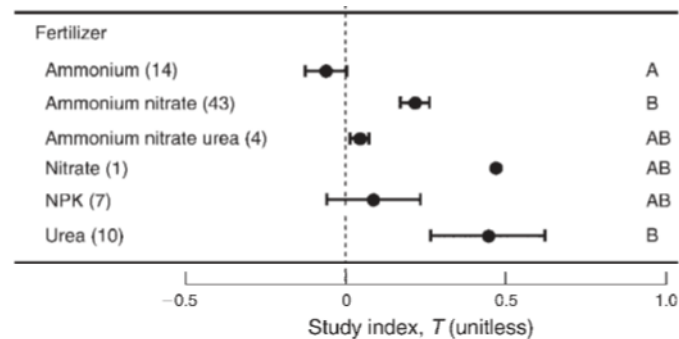
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Comment: It was a pleasure to read your very interesting discussion article. I would like to make a comment. You wrote in Sect 2.1. "We excluded experiments by applying urea and manure, because these organic N forms have limited implications for the effects of N deposition (Aronson and Helliker, 2010) as N deposition mainly occurs in forms of inorganic N (Vet et al., 2014)." Based on this statement, you excluded one of the most long-term measurements of the effects of N addition on soil CH₄ fluxes from tropical forests (4 years of data, Veldkamp et al., 2013) (as stated on P7/L5 of the discussion paper).

Reply: Thanks for your helpful comments. According to your suggestion, we have now included results of urea addition experiments in the revised manuscript. Based on an updated database with information on means and standard deviations, we have then conducted a meta-analysis to test the significance of nitrogen addition effect on soil CH₄ flux. Please see more information in our detailed reply to your specific comments.

Comment: 1) Could you please clarify more specifically where in the cited paper (Aronson and Helliker, 2010) you refer to when arguing that urea has limited implications for the effects of N deposition. When reading that paper (Aronson and Helliker, 2010), I find discussion on the form of N added on P3249-3250. The authors state there that "The effects of N addition could be similar regardless of N form used, due to the presence of microorganisms capable of rapid N transformation by variation in microbial consortia. The timing of fertilization may determine the form of N that methane-cycling microorganisms encounter in the soil more than the actual N species added. The fact that the addition of urea and ammonium nitrate were capable of increasing nitrate availability significantly in Delgado et al. (1996) underscores this point. Therefore, any conclusions of the effects of the specific N species relative to others must be highly qualified, as the form of N that results may be quite different from that added."

Reply: In the paper by Aronson and Helliker (2010), the authors showed that the effects of N fertilization on soil CH₄ uptake varied significantly with N forms (Figure R1). Specifically, the effects of urea and ammonium nitrate hardly showed an overlap (Figure R1), although the statistical analysis indicated no significant difference. 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, however, we have now included urea addition experiments in the revised manuscript in view of the fact that the effects of urea and ammonium nitrate were not significantly different.



10 Figure R1. Effects of different N forms on soil CH₄ uptake (Aronson and Helliker, 2010)

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.

15 **Comment:** In terms of the tropical N-addition study that was excluded from your review, there is detailed data on soil extractable nitrate and ammonium concentrations that shows that urea additions have chronically changed the soil inorganic N status of the studied forests (Koehler et al., 2009). Therefore, I would like to challenge your decision to exclude the study on soil CH₄ fluxes from the same sites (Veldkamp et al., 2013), based on the argument that N was added in the form of urea.

20 **Reply:** We agree with your suggestion to include studies with urea additions since the effects of urea and ammonium nitrate were not significantly different as discussed above (Aronson and Helliker, 2010). In the revised manuscript, we have updated our database by including reported results on urea addition experiments. Overall, 6 urea based experiments are included, including one from temperate forest (Geng et al., 2017), one from subtropical forest (Zhang et al., 2017), and four from tropical forest (Matson et al., 2016; Veldkamp et al., 2013; 25 Mori et al. 2017) (see more detailed references as below).

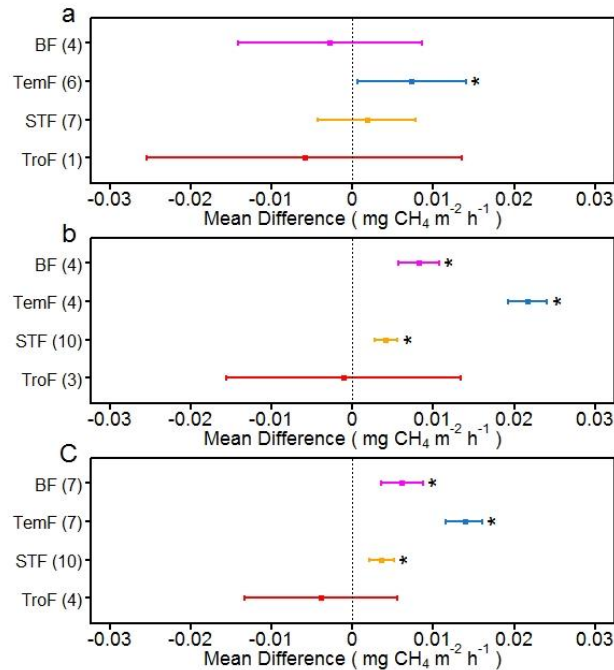
References:

- 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.
- Geng, J., Cheng, S., Fang, H., Yu, G., Li, X., Si, G., et al. (2017). Soil nitrate accumulation explains the nonlinear
5 responses of soil CO₂ and CH₄ fluxes to nitrogen addition in a temperate needle-broadleaved mixed forest. *Ecological Indicators*, 79, 28-36.
- 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.
- Mori, T., Imai, N., Yokoyama, D., Mukai, M., & Kitayama, K. (2017). Effects of selective logging and
10 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.
- Zhang, K., Zheng, H., Chen, F., Li, R., Yang, M., Ouyang, Z., et al. (2017). Impact of nitrogen fertilization on
15 soil–Atmosphere greenhouse gas exchanges in eucalypt plantations with different soil characteristics in southern China. *PLoS One*, 12(2), e0172142.

Comment: 2) In that same study that you excluded from your review (Veldkamp et al., 2013), N addition did not decrease soil CH₄ uptake, which is in conflict with the conclusion you made in your discussion article (e.g.
20 abstract “However, high-level N addition significantly decreased growing-season soil CH₄ uptake across boreal, temperate, and subtropical forests.”). N-addition to the tropical forest sites in Panama that you excluded from your review (Veldkamp et al., 2013) did not affect soil CH₄ fluxes.

Reply: Thanks for your comments. We have realized that speculating on the effects of N additions in tropical forests based on results from subtropical forests is not appropriate. In the revised manuscript, we have updated
25 our database by including information of means and standard deviations and tested the significance of nitrogen addition effect on soil CH₄ flux by conducting a meta-analysis in R software with metaphor package (Viechtbauer, 2010). We used a mean difference (Flux_{treatment}-Flux_{control}, being the difference of mean growing-season soil CH₄ fluxes between the treatment plots and control plots) as the effect size to evaluate the effect of N additions. Based on 4 experiments from tropical forest (Veldkamp et al., 2013; Matson et al., 2016; Mori et al.,
30 2017), we show that simulated N deposition (<60 kg N⁻¹ yr⁻¹) and high-level N addition (<60 kg N⁻¹ yr⁻¹) both had no significant effect on soil CH₄ uptake (Figure R2a&b). Overall, no significant effect of N additions on soil CH₄

uptake was indeed found in tropical forest, neither at low or high N additions (Figure R2c). We have revised the manuscript accordingly.



- 5 Figure R2. The mean difference (and 95% confidence intervals) of growing-season soil CH₄ flux to a) low-level N addition (<60 kg N⁻¹ yr⁻¹), b) high-level N fertilization (> 60 kg N⁻¹ 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 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.
- 15 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.

Comment: I suggest that you include that detailed study on the effects of N-addition on CH₄ fluxes from tropical forest soils, rather than excluding it with the argument that “the results based on N additions by using urea shed limited lights on the effect of N deposition” (P7/L5-6). Further information on detailed and long-term soil CH₄ profiles (Koehler et al., 2012) may assist in discussing that study in the context of your review, and in discussing why the N-addition effects on CH₄ fluxes in these sites may differ from the overall results you found in your review.

Reply: Thanks for your suggestion. As in our reply above, we have conducted a reanalysis by including experiments with urea additions in tropical forest. Based on limited sample size, our results indeed indicate that N additions have no significant effects on soil CH₄ fluxes in tropical forest (Fig. R2). As N deposition in many tropical regions is expected to increase in the future (Lamarque et al., 2013), further efforts are needed to evaluate the effect of N deposition on soil CH₄ flux in tropical forest by conducting extra N addition experiments with low-level additions of inorganic N. We have also revised the result section and cited Koehler et al. (2009 & 2012) when discussing the potential mechanisms in tropical forest.

Reference

Lamarque, J.F., Dentener, F., McConnell, J., Ro, C.-U., Shaw, M., Vet, R., Bergmann, D., Cameron-Smith, P., Dalsoren, S., Doherty, R., Faluvegi, G., Ghan, S. J., Josse, B., Lee, Y. H., MacKenzie, I. A., Plummer, D., Shindell, D. T., Skeie, R. B., Stevenson, D. S., Strode, S., Zeng, G., Curran, M., Dahl-Jensen, D., Das, S., Fritzsche, D., and Nolan, M. 2013. Multi-model mean nitrogen and sulfur deposition from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): evaluation of historical and projected future changes, *Atmos. Chem. Phys.*, 13, 7997-8018.

Yours sincerely,

Birgit Koehler

References

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

Koehler B., Corre M.D., Veldkamp E., Wullaert H., Wright S.J.: Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biology* 15(8), 2049-2066, 2009

- Koehler B., Corre M.D., Steger K., Well R., Zehe E., Sueta J.P., Veldkamp E.: An indepth look into a tropical lowland forest soil: nitrogen-addition effects on the contents of N₂O, CO₂ and CH₄ and N₂O isotopic signatures down to 2-m depth. *Biogeochemistry* 111, 695-713, 2012; Erratum *Biogeochemistry* 111, 715-717, doi: 10.1007/s10533-012-9780-6, 2012
- 5 Veldkamp E., Koehler B., Corre M.D.: Indications of nitrogen-limited methane uptake in tropical forest soils. *Biogeosciences* 10, 5367-5379, 2013