

**Preprint egusphere-2024-3813 Discussion:** Reduced microbial respiration sensitivity to soil moisture following long-term N fertilization enhances soil C retention in a boreal Scots pine forest

**Ľupek et al. reply to RC2: ['Comment on egusphere-2024-3813'](#), Anonymous Referee #2, 25 Feb 2025**

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*Authors' responses are in Italics (and in dark-blue color in the attached pdf version, which also includes revised figures and tables). Revised text is indicated by quotation marks.*

This study investigates the effects of long-term nitrogen (N) fertilization on soil heterotrophic respiration (Rh), methane (CH<sub>4</sub>) oxidation, and nitrous oxide (N<sub>2</sub>O) emissions in a boreal Scots pine forest. The results show that N fertilization increased tree biomass, litterfall, and soil organic carbon (SOC) stocks. Despite elevated Rh in fertilized plots, SOC-normalized Rh (Rh:SOC) did not differ significantly between fertilized (N+) and control (CTR) plots. N fertilization altered Rh:SOC dependencies on temperature (T) and soil water content (SWC), with N+ plots exhibiting increased temperature sensitivity and reduced SWC dependence. These shifts, combined with reduced CH<sub>4</sub> oxidation and increased N<sub>2</sub>O emissions, resulted in a net reduction in soil greenhouse gas (GHG) emissions, suggesting enhanced SOC retention under N fertilization. The findings highlight the potential of long-term N fertilization to mitigate climate warming in boreal forests by altering microbial respiration dynamics. There are some interesting findings in this study, but I have several major comments and suggestions based on the current version of manuscript. Please find them below.

*Thank you for your thorough review and constructive comments, which have helped improve our manuscript. Below, we provide detailed responses to each remark, along with corresponding revisions.*

The study reports increased Rh under N fertilization, contrasting with the widely observed suppression of soil CO<sub>2</sub> respiration by N addition. While this is an innovative discovery, further mechanistic explanations are needed. For example, how do microbial community shifts (e.g., enzyme activities, CUE, substrate quality) under N fertilization drive this response? Is this phenomenon unique to nutrient-poor boreal systems, or could it apply to other ecosystems? A broader discussion of context-dependent mechanisms is warranted.

*The response of microbial activity and substrate quality to N addition has been investigated in Karstula and detailed in Richy et al. (2024). In revised paper, we expanded discussion by highlighting following mechanisms:*

*"Nitrogen addition stimulated tree biomass production, which in turn increased carbon inputs into the soil. This enhanced carbon availability, along with increased soil nitrogen*

*concentrations, stimulated microbial activity and biopolymers degradation capabilities. This process certainly explains the observed increase in heterotrophic respiration (Rh) under N fertilization. Additionally, we observed a decline in phosphorus concentrations in N-fertilized plots compared to unfertilized plots, probably due to microorganisms mining for phosphorus to sustain their increased activity."*

The conclusions are based on a single long-term experimental site. While valuable, this limits generalizability. Are there similar responses observed in other boreal forests? The authors may need to address how site-specific factors (e.g., soil type, microbial composition, thinning history) might influence their results.

*In revised paper, we expanded discussion on generalizability of our results:*

*"While these findings likely apply to nutrient-poor boreal ecosystems, extrapolation should be done for similar stands with similar climate with caution for other ecosystems. For example, Saarsalmi et al. (2014) showed that N fertilization stimulated growth in relation to mean annual production (more in nutrient poor pine stands and less in spruce stands with higher nutrient status). Schulte-Uebbing et al. (2021) demonstrated that N addition enhance biomass carbon sequestration primarily in boreal regions, while having negative effects in tropical forests."*

*Saarsalmi, A., Smolander, A., Moilanen, M., Kukkola, M., 2014. Wood ash in boreal, low-productive pine stands on upland and peatland sites: Long-term effects on stand growth and soil properties. Forest Ecology and Management 327, 86–95.*

<https://doi.org/10.1016/j.foreco.2014.04.031>

*Schulte-Uebbing, L.F., Ros, G.H., de Vries, W., 2022. Experimental evidence shows minor contribution of nitrogen deposition to global forest carbon sequestration. Global Change Biology 28, 899–917. <https://doi.org/10.1111/gcb.15960>*

This paper mentioned global warming potential (GWP), which can be calculated from fluxes of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, but they did not calculate based on their data. It would be interesting to examine how GWP was changed under the long-term N fertilization. While reduced CH<sub>4</sub> oxidation and increased N<sub>2</sub>O emissions are noted, their combined contribution to global warming potential (GWP) is dismissed as minor. Some discussions could be added for whether long-term N fertilization could eventually offset SOC gains through cumulative N<sub>2</sub>O emissions.

*Yes, our study found that in N-fertilized plots, the impact of reduced CH<sub>4</sub> oxidation and increased N<sub>2</sub>O emissions played a minor role compared to the reduction in CO<sub>2</sub> emissions.*

*For improved clarity in the revised version of the paper, we calculated the global warming potential (GWP) using the AR6 GWP-100 values (27 for CH<sub>4</sub> and 273 for N<sub>2</sub>O) and added the corresponding text to the methods and results sections along with a new table (Table 3) in revised chapter 3.6.*

Intergovernmental Panel On Climate Change (IPCC), 2023. *Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, 1st ed.* Cambridge University Press.

<https://doi.org/10.1017/9781009157896>

**Table 3: Annual global warming potential (GWP) reduction by long-term N fertilization in boreal Scots pine forest with contribution of individual greenhouse gas (GHG) fluxes (microbial respiration normalized by soil organic carbon stock  $R_{h/SOC}$ ,  $CH_4$  net oxidation, and  $N_2O$  flux net exchange) evaluated as a difference between control (CTR) and N fertilized plots (N+). Minus values indicate net GWP reduction. The GWP-100 values (27 for  $CH_4$  and 273 for  $N_2O$ ) were used for calculation of  $CO_2$ -equivalents.**

Treatment	$R_{h/SOC}$		$GWP-CO_2$		$CH_4$		$GWP-CH_4$		$N_2O$		$GWP-N_2O$		$GWP-GHG$	
	(%)		$(g CO_2 m^{-2} y^{-1})$		$(g CH_4 m^{-2} y^{-1})$		$(g CO_2-eq m^{-2} y^{-1})$		$(mg N_2O m^{-2} y^{-1})$		$(g CO_2-eq m^{-2} y^{-1})$		$(g CO_2-eq m^{-2} y^{-1})$	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
<b>CTR</b>	12.2	0.5	2214.9	90.4	-1.6	0.0	-42.9	0.5	-2.2	0.8	-0.6	0.2	2171.4	90.4
<b>N+</b>	10.3	0.3	1869.5	56.8	-1.2	0.0	-32.8	0.5	1.9	0.6	0.5	0.2	1837.3	56.8
<b>Difference</b>	-1.9	0.4	-345.4	73.6	0.4	0.0	10.1	0.5	4.2	0.7	1.1	0.2	-334.1	73.6

The increased temperature sensitivity of  $R_{h/SOC}$  in N+ plots (Fig. 4a) raises concerns about accelerated SOC loss under warming. It is interesting that the optimum temperature emerged under control but not under N+ treatment. Can you please explain why this happens? The current version only mentioned this pattern based on modeling results (lines ~210).

Regarding the emergence of a temperature optimum within the observed soil temperature range, in addition to the mechanisms discussed on lines 314–323, the temperature optimum (with limitations beyond the peak) in the control plots could partly be attributed to moisture limitation at higher temperatures.

In the preprint, our stepwise modeling approach—where temperature was fitted first, followed by temperature and moisture—may have amplified this effect. In the revised approach, we have allowed both temperature and moisture to vary simultaneously thus fitted both functions together in one model.

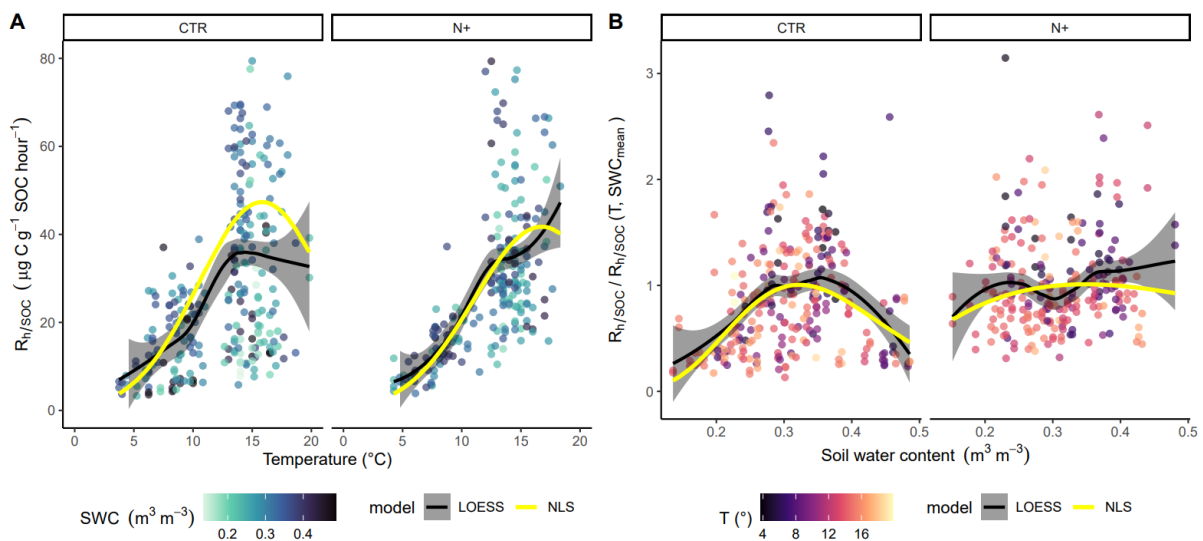
The text describing the model was revised as well as all results.

“NLS regression was used to extrapolate  $R_{h/SOC}$  to continuous hourly data and to upscale  $R_{h/SOC}$  to the annual level. The combined  $T$  and  $SWC$  dependency of  $R_{h/SOC}$  was modeled by multiplying a Gaussian  $T$  function as described in Tuomi et al. (2008) with a Ricker function for  $SWC$  (Bolker, 2008) (Eq. 1):

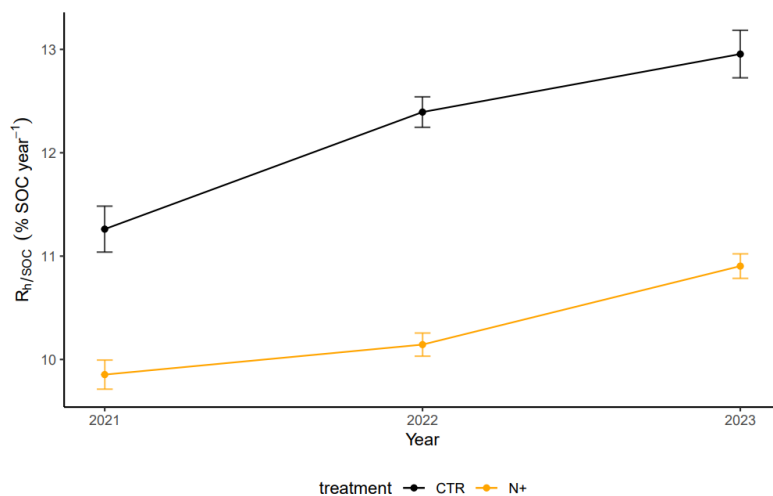
$$R_{h/SOC}(T, SWC) = e^{(\beta_1 T + \beta_2 T^2)} (a SWC e^{(-b SWC)})^c, \quad (1)$$

where  $\beta_1$  and  $\beta_2$  are parameters controlling the exponential  $T$  response, and parameters  $a$  determine the initial slope,  $b$  the post-optimal decline, and  $c$  the peak height of SWC response.”

The results of the revised model (the shape of the temperature and moisture responses) are close to those from the preprint (see updated Fig. 4 and 6 below, and Table 3). However, the temperature optimum with the revised model was observed for both CTR and N+ plots at 15.8 °C and 16.8 °C, respectively.



**Figure 4:** (A) Dependence of soil microbial respiration normalized by soil organic carbon ( $R_{h/SOC}$ ,  $\mu\text{g C g}^{-1} \text{SOC h}^{-1}$ ) on soil temperature at 5 cm depth ( $T$ ,  $^{\circ}\text{C}$ ). (B) Ratio of measured  $R_{h/SOC}$  to modeled  $R_{h/SOC}(T, SWC_{mean})$  as a function of volumetric water content (SWC,  $\text{m}^3 \text{m}^{-3}$ ) at 5 cm depth. Panels display results separately for control (CTR) and N-fertilized (N+) plots. Shading of turquoise points in (A) reflects varying SWC, while shading of red points in (B) corresponds to variation in  $T$ . Black lines indicate local polynomial regression (LOESS) fits with gray ribbons showing 95% confidence intervals; yellow lines represent nonlinear least square (NLS) regression model fits.



**Figure 6:** Annual  $R_{h/SOC}$  (% SOC per year) estimated with NLS models driven by combined temperature and moisture ( $R_{h/SOC}(T, SWC)$ , Eq. 1) in CTR and N+ plots, using hourly  $T$  and SWC data for model inputs (Fig. S1).

Minor comments:

Page 1, Line 15: change "Nutrient availability effects microbial respiration kinetics" to "Nutrient availability affects microbial respiration kinetics."

*changed as suggested*

Page 4, Line 75: Clarify the source of GWP values (23 for CH<sub>4</sub>, 296 for N<sub>2</sub>O). Are these IPCC AR5 or AR6 values? Update citation if necessary.

*GWP values (on Page 4, Line 75) were from Ramaswamy et al. (2019)*

*Ramaswamy, V. et al., 2019: Radiative Forcing of Climate: The Historical Evolution of the Radiative Forcing Concept, the Forcing Agents and their Quantification, and Applications. Meteorological Monographs, 59, 14.1–14.101, doi:10.1175/amsmonographs-d-19-0001.1.*

*We revised version we used AR6 GWP-100 values (27 for CH<sub>4</sub>, 273 for N<sub>2</sub>O) and updated the citation.*

*Intergovernmental Panel On Climate Change (Ipc), 2023. Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, 1st ed. Cambridge University Press.*

*<https://doi.org/10.1017/9781009157896>*

Page 5, Line 95: Provide details on measurement protocols (e.g., instruments used, precision) for tree diameter, height, and crown base height.

*In revised text we updated the tree measurement protocols accordingly:*

*"In each CTR and N+ plot, the breast-height diameter (d1.3) of all trees has been measured using a caliper (±1 mm precision) once per decade since 1960, as well as after the 2015 thinning. Additionally, in a permanent subset of trees representing various size categories, tree height and crown base height have been recorded using a hypsometer (precision ~0.5–1 m)."*

Page 6, Line 125: Elaborate on how chamber headspace linearity was monitored. Was a threshold R<sup>2</sup> value used to accept/reject flux calculations?

*We revised text accordingly: "..., and linearity was monitored visually during the measurements to accept only fluxes with increasing trends in CO<sub>2</sub> concentration evolution."*

Page 8, Line 185: Report ANOVA statistics (F-value, degrees of freedom, p-value) for Rh differences between N+ and CTR.

*We added ANOVA statistics (F-value, degrees of freedom, p-value):*

*“Pairwise ANOVA showed that mean annual soil microbial  $R_h$  ( $\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) was significantly higher in N+ ( $0.58 \pm 0.01 \text{ SE}$ ) than in CTR plots ( $0.46 \pm 0.01 \text{ SE}$ ) (F-value 15.96, degrees of freedom 449, p-value  $8.92\text{e-}05$ ) (Fig. 3a).”*

Page 10, Line 220: Add  $R^2$  values or confidence intervals to Table 1/Table 2 to quantify model explanatory power.

*We added  $R^2$  values to Table 2. Please note, that with one improved model where both temperature and moisture vary simultaneously, there was no reason for AIC and BIC model comparison statistics.*

**Table 2: Goodness-of-fit statistics for NLS models based combined temperature and SWC (Eq. 1): proportion of explained variance ( $R^2$ ), root mean square error (RMSE), mean bias error (MBE), and mean absolute error (MAE). RMSE, MBE and MAE in  $\mu\text{g C g}^{-1} \text{ SOC h}^{-1}$ .**

Treatment	$R^2$	RMSE	MBE	MAE
$\mu\text{g C g}^{-1} \text{ SOC h}^{-1}$				
CTR	0.41	15.55	-0.33	11.42
N+	0.40	13.36	-0.48	9.28

Page 14, Line 280: Expand the discussion on why N fertilization reduced SWC sensitivity (e.g., microbial adaptation, substrate shifts).

*We reformulated the Line 280 on why N fertilization reduced SWC sensitivity:*

*“In our study, soil microbial respiration responded to N fertilization with a reduced sensitivity to soil moisture (Fig. 4b), suggesting a potential mechanism (e.g., microbial adaptation, substrate shifts) for enhanced carbon retention in fertilized plots which are discussed in detail in following chapters.”*

Page 16, Line 340: Suggest specific model improvements (e.g., incorporating nonlinear N effects on T/SWC modifiers) to address current limitations.

*Thank you for request to elaborate this interesting point. We added following sentences into the discussion:*

*“Incorporating nonlinear nitrogen effects on temperature and soil moisture modifiers depends on the model's structure. In soil carbon-only models like Yasso, updating these modifiers with a larger dataset that includes nitrogen deposition gradients and soil organic carbon stocks could improve performance. Conversely, in soil carbon-nitrogen models that already account for SOC-N interactions, existing functional relationships should be re-evaluated, considering their interactions with environmental modifiers.*