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

Ťupek et al. reply to RC1: 'Comment on egusphere-2024-3813', Anonymous Referee #1, 02 Jan 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.

The manuscript raises important questions about the effects of N fertilization on soil GHG emissions and moisture dynamics. The authors have collected unique and valuable data. The paper is mainly very clear and well written. I have read the manuscript with great interest, but I am concerned about several issues in the methodology, results, and conclusions. The data presented do not adequately support the conclusions, and there are inconsistencies between the observations and the model outputs. Below, I outline the main issues with the study followed by few detailed comments.

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.

Conflicting patterns in observations

In Supplementary Figure S2, the highest mean momentary soil moisture values appear to be in the N+ treatment during 2021–2022. However, in Figures 3F and 4B, the highest soil moisture values are in the CTR treatment. While these observations may not be strictly contradictory, they are at least unusual and require further explanation. Additionally, in 2022, the soil moisture appears lowest in N+ (S2), while in 2021 and 2023, it is approximately equal with CTR. Despite this, Figure 4B suggests there are notably more low soil moisture values (<0.2) in CTR than in N+. These discrepancies between the observations and figures need clarification.

Discrepancies (especially in extreme values) could be explained by different nature of data points. Whereas in Figure S2 data represent weekly averages at the treatment level in Figures 3F and 4B the data points are the instantaneous measurements of individual plots of the treatments.

It also seems very strange that the soil moisture has such a different dynamic in the different treatments in late July 2023, with N+ zigzagging (Fig S2). I recommend checking the dates for N+.

We checked the data in Fig. S2, and the date of measurements is correct.

To improve data interpretation, we revised Fig. 1 by adding a new panel (B) that illustrates topographic variation and distances between treatment plots.

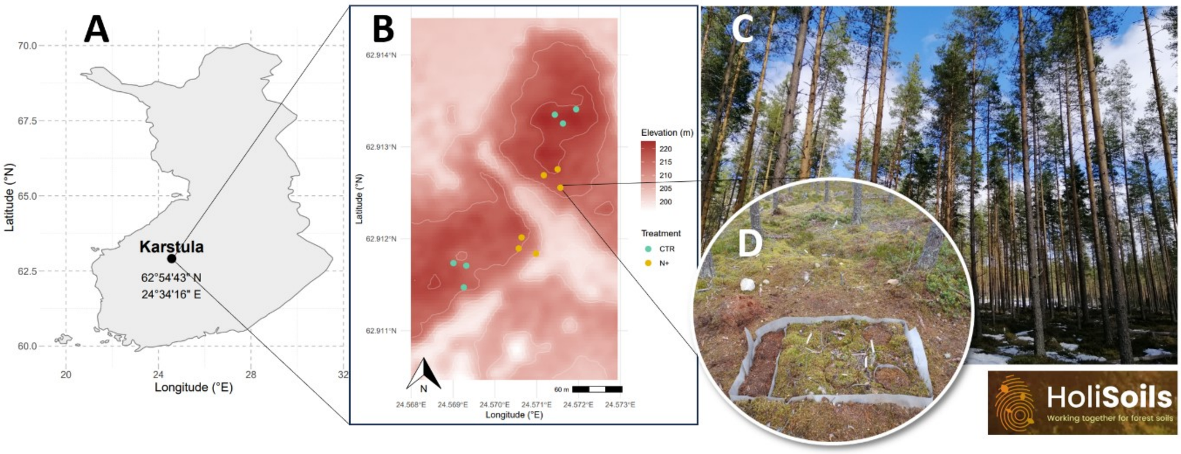

Figure 1: Geographical location of the Karstula forest study site in Finland (A); topographical variation of the study site and the location of treatment (control CTR and N-fertilized N+) plots (B), photograph of the forest stand (C); and one of six 2 x 1 m forest floor plot groups, each with four subplots used for measuring soil greenhouse gases, soil organic C, and soil temperature and moisture following the installation of a root-exclusion fabric (D).

We also revised text in results by including following sentence:

" High variation in soil moisture between CTR and N+ plots (located on average 122 m apart) could be attributed to the measured topsoil humus layer being highly affected by microscale variations of vertical and lateral water flows due to variable microtopography and tree canopy openings (Fig. 1B)."

Conflicting results by the observations and the model

The authors themselves state based on the observations that 'Rh showed sensitivity to T and SWC, rising with warmer conditions and declining in dry periods, then recovering after rewetting events (Fig. S2).'

To improve the clarity in temporal patterns between treatments the text in results was revised:

"R_h showed sensitivity to T and SWC, generally rising with warmer conditions and declining in dry periods, then recovering after rewetting events (Fig. S2). However, this pattern was more pronounced in CTR than in N+ plots."

Then, in the results and discussion they state that 'Soil moisture effects on Rh/SOC were only observed in control (CTR) plots' and 'In our N-fertilized plots, Rh/SOC was largely independent of soil moisture.'

With the added sentence above, this now aligns with the message in the results.

These latter ones arise from the model that is fitted to the growing season (Apr-Oct) data and then applied for the whole year. These estimates are presented without any estimates of uncertainty, which undermines its reliability.

To enhance model reliability, we replaced the stepwise fitting approach with a single model where both temperature and moisture vary simultaneously (see details below).

In the revised text, we have included standard errors (SE) in the presented values in the abstract, updated Chapter 3.6 with a new table of annual GHG and GWP estimates, and revised Chapter 4.3.

Additionally, we provide SE and p-values for estimated parameters in Table 1, goodness-of-fit statistics (R^2, RMSE, MBE, MAE) in Table 2, and standard deviations (SD) for cross-validation goodness-of-fit statistics (SD of R^2, RMSE, and MAE) in Table S1. Error bars (SE) are also included in Figure 6.

I see that the main issue behind this is that the models presented in the study do not perform well, especially during the dry year of 2021 (Figure 5). In the figure, for example, the observed soil moisture data for N+ in 2021 show a significant mid-season drop, which is as substantial, if not even larger than the drop in CTR. However, the model fails to capture this, and based on the model, the authors conclude that there is no soil moisture effect. In other words, the main conclusion of the whole paper appears to be unsupported by the observational data and raises questions about the validity of the model. The poor performance might arise from the model structure but also that there are significantly fewer data points for the N+ treatment under very wet conditions (>0.45 SWC, just two observations). Similarly, under dry conditions (<0.2), there are notably more measurements for the CTR treatment than for the N+ treatment. This limited dataset could easily skew the soil moisture response curve.

Given your remark, to have more confidence in the model, we revised our stepwise modeling approach—where temperature was fitted first, followed by temperature and moisture—which may have impacted the observed temperature optimum in control and hindered relations to moisture in N fertilized plots. In the revised model, we fitted the full temperature and moisture model in one step where we have allowed both temperature and moisture vary simultaneously. Thus, in the revised paper we evaluate only temperature-moisture model, as both variables are correlated and impact microbial respiration simultaneously.

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 β₁ and β₂ 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."

However, 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 below).

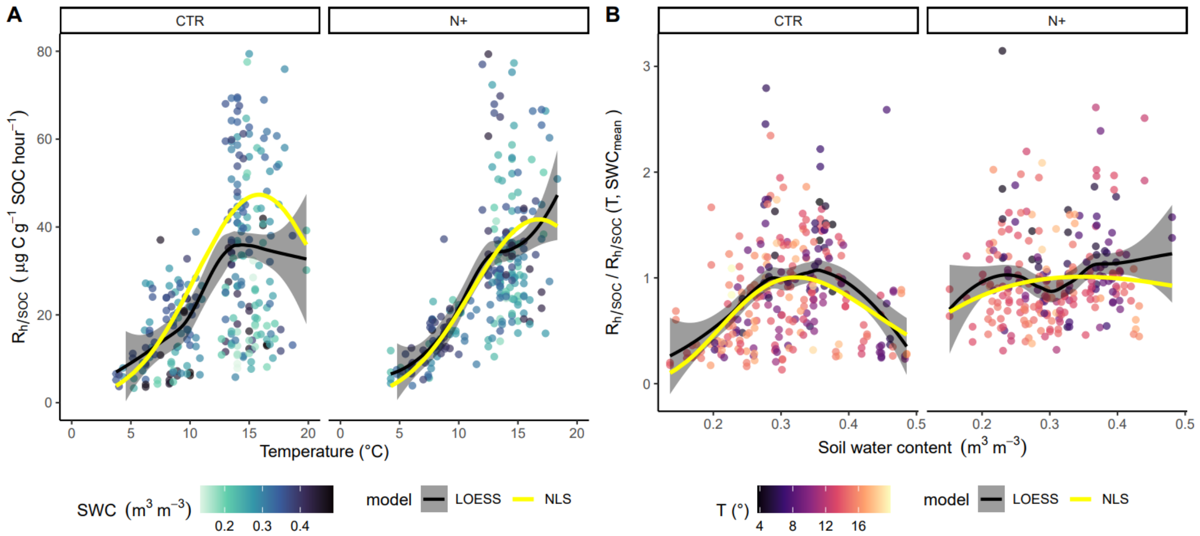

Figure 4: (A) Dependence of soil microbial respiration normalized by soil organic carbon ($R_{h/SOC}$, μg C g⁻¹ SOC h⁻¹) on soil temperature at 5 cm depth (T, °C). (B) Ratio of measured $R_{h/SOC}$ to modeled $R_{h/SOC}(T,SWC_{mean})$ as a function of volumetric water content (SWC, m³ m⁻³) at 5 cm depth. 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.

The model performance for the N+ plots simulations improved for year 2021, being able to better reconstruct reduction of respiration during the summer drought event (see updated Fig. 5 below).

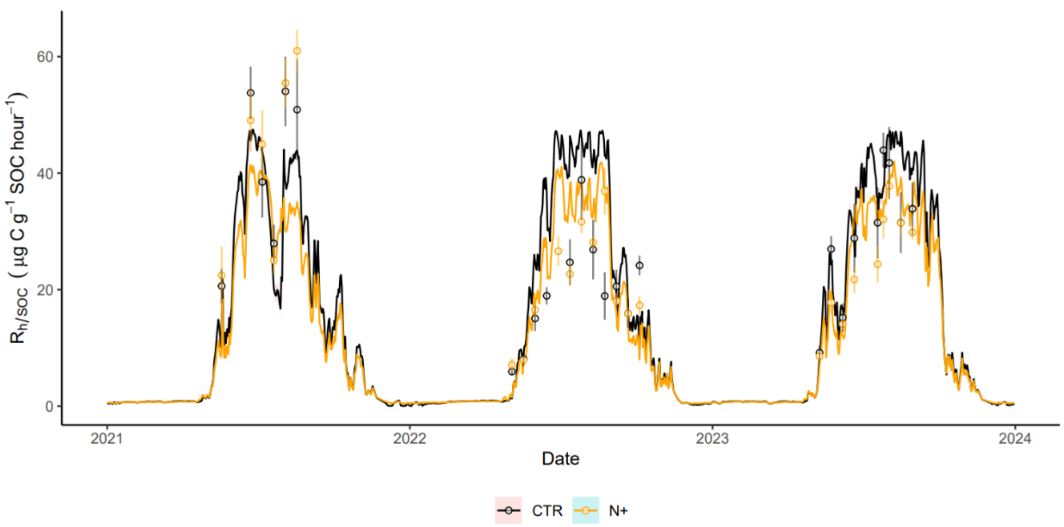

Figure 5: Time series of daily mean $R_{h/SOC}$ (μg C g⁻¹ SOC h⁻¹) in CTR and N+ plots, with measurements shown as points (error bars indicate SE) and model estimates as lines (ribbons indicate SE).

To improve the interpretation of results we revised the text in discussion accordingly:

"Due to differences in carbon stocks between treatments, decomposition rates expressed as R_h are not directly comparable between CTR and N+. Therefore, drawing conclusions on respiration rate differences required normalizing respiration by SOC. However, high variability in momentary $R_{h/SOC}$ measurements prevented definitive conclusions, as the mean $R_{h/SOC}$ values were not statistically different between CTR and N+.

In contrast, model parameters describing functional dependencies on soil moisture were statistically significant for CTR but not for N+. However, neither the CTR nor N+ models showed bias (Table 2 and S1). Differences in functional forms between CTR and N+ (Fig. 3) led to lower annual respiration estimates for N+ compared to CTR (Fig. 6)."

Based on the model results, we conclude that respiration sensitivity to soil moisture was reduced in N+ compared to CTR.

To address your remark that respiration in N+ was also reduced during severe drought, we revised the title to: 'Long-term nitrogen fertilization alters microbial respiration sensitivity to temperature and moisture, potentially enhancing soil carbon retention in a boreal Scots pine forest.'

Misleading conclusions about GHG emissions

The authors conclude: 'Our results also suggest a net reduction in soil GHG emissions with long-term N fertilization.'

However, this is only supported by the poor model and not by the observational data, which show increased Rh (with no difference in Rh/SOC), reduced CH4 sink and increased N2O emissions in the fertilized treatment compared to the control. These observations indicate that the net impact of N fertilization on soil GHG emissions may be neutral or even negative. To see the relative importance of CH4 and N2O, it would be useful for the reader to see all fluxes as CO2 equivalents.

The inability to compare Rh means directly (due to SOC differences between treatments) as well as the need for model in annual upscaling of Rh/SOC (due to T, SWC seasonality) was explained previously. However, to increase confidence in Rh/SOC model results in the revised paper we improved the model fitting to allow temperature and moisture vary simultaneously resulting in better match between the model estimates and measurements (Fig. 5). We also clarified in conclusions reliability of the model by adding following sentence:

"Although the models showed relatively large mean residuals when evaluated against individual measurements, their mean bias errors were close to zero (Table 2)."

In the preprint, we mention the contribution of different processes to CO_2 emissions reduction potential after long-term N fertilization in abstract L27-29 and in discussion L349-353. In the revised text, we also help the reader to visualize the relative contributions of individual processes in CO_2-equivalents as GWP-100 potentials by detailing these results into a new table (Table 3) in revised chapter 3.6.

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 (g CO_2 m^{-2} y^{-1})		CH_4 (g CH_4 m^{-2} y^{-1})		GWP-CH_4 (g CO_2-eq m^{-2} y^{-1})		N_2O (mg N_2O m^{-2} y^{-1})		GWP-N_2O (g CO_2-eq m^{-2} y^{-1})		GWP-GHG (g CO_2-eq m^{-2} y^{-1})	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
CTR	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
N+	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
Difference	-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**

Issues with Methods

The methods section contains critical gaps that limit the reproducibility and reliability of the study:

The manuscript does not describe the equations or models used for tree biomass in the methods but refer to Lehtonen et al. in the Fig 2 caption. The methods section should be improved here.

The Fig2a caption was revied "(A) Estimated tree biomass and litterfall from 1980 to 2020 forest tree stands inventory measurements."

The biomass equations and litter turnover rates models citing Repola (2009) and Lehtonen et al. (2016) were detailed in chapter "2.2.1 Tree inventory and litterfall".

The manuscript does not include any estimates of uncertainty for their main result that is the decreased (modelled) Rh/SOC after fertilization.

In revised paper we added standard error (SE) in presented values of abstract, and updated Chapters 3.6 and 4.3. The SE of main results are also provided in Fig. 6.

Fluxes were measured over only 3 minutes using a large (21 L) chamber. Considering the low flux rates of CH4 and N2O, it is doubtful whether this short measurement duration is sufficient for reliable estimates. It might be though but makes me worry if the equipment used is sensitive enough to detect such low fluxes. Of course, it is not the volume that is important here, but the area, but this is not given. Based on the chamber description in the manuscript, no one could repeat it.

Other

The diameter of measurement plots (30 cm) thus of the chamber is mentioned in the preprint on line 112. We revised the text by including dimension of 30 cm diameter in chamber description.

To improve the confidence on the precision of the GHG measurements, we revised the paper by adding description of measurement method detection limits:

"The CH_4 and N_2O concentrations were measured during 3 min intervals with 5 second averaging at the 0.25 ppb precision for CH_4 and 0.20 ppb precision for N_2O. The minimum detectable flux of measurements estimated using the formula by Parkin et al., (2012) was 0.0238 μg m^{-2} h^{-1} for CH_4 and 0.0524 μg m^{-2} h^{-1} for N_2O.

Parkin, T.B., Venterea, R.T., Hargreaves, S.K., 2012. Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. J. Environ. Qual. 41, 705–715." https://doi.org/10.2134/jeq2011.0394

We clarified in results that:

"The method detection limits were smaller than SE of mean CH_4 and N_2O fluxes."

The authors state in the introduction: 'Moreover, full accounting of GHG emissions should include emissions associated with N fertilizer production.' However, they do not include these emissions in their own analysis and conclusions.

The associated emissions with fertilizers productions were accounted for according to Osorio-Tejada et al. (2022) and presented in chapter 4.3 lines 351-353.

We revised methos by adding:

"The associated emissions with fertilizers productions were accounted for according to Osorio-Tejada et al. (2022). We estimated the CO_2 emissions associated with six nitrogen fertilization events, which occurred once per decade between 1960 and 2020. The applied nitrogen fertilization rate was 180 kg N ha^{-1} per event. Converting this to ammonia (NH_3) using the molecular weight ratio of NH_3 to N (17.031/14.007) resulted in an estimated 218.86 kg NH_3 ha^{-1} per fertilization event. Given an emission factor of 2.96 kg CO_2 per kg NH_3, this corresponds to 647.93 kg CO_2 ha^{-1} per event. Over six fertilization events spanning 60 years,

the annualized CO_2 emission was calculated as 64.79 kg CO_2 ha^{-1} yr^{-1}, equivalent to approximately 6.5 g CO_2 m^{-2} yr^{-1}."

Osorio-Tejada, J., Tran, N.N., Hessel, V., 2022. Techno-environmental assessment of small-scale Haber-Bosch and plasma-assisted ammonia supply chains. Science of The Total Environment 826, 154162. https://doi.org/10.1016/j.scitotenv.2022.154162

Although CH4 and N2O fluxes are a central part of one of the hypotheses, those are not well motivated and the discussion does not address these at all.

We revised the hypothesizes "We hypothesized that (i) increased soil nitrogen availability would enhance soil organic carbon (SOC) accumulation and heterotrophic respiration (R_h) due to greater biomass growth and litter inputs, while SOC-normalized R_h ($R_{h/SOC}$) would decline due to reduced microbial nitrogen demand; and (ii) nitrogen fertilization would alter CH_4 oxidation and increase N_2O emissions compared to N-limited soils, reflecting shifts in microbial activity and substrate availability."

Reasoning for the hypothesis (ii) is detailed on lines 46-49. We discussed these findings shortly in Chapter 4.3 lines 349 -351 in relations to studies by Maljanen et al., (2006), and Öquist et al., (2024).

We revised text in chapter 4.3 by adding: "Although, the CH_4 and N_2O fluxes need consideration due larger GWP than CO2 and potentially large N2O fluxes after fertilization, the CH4 and N2O fluxes observed in our study were very close to zero thus showed negligible contribution to total forest soil GHG emissions."

The title and main findings revolve around soil moisture dependency, yet this was not one of the original hypotheses or a focus in the introduction. This shift in focus feels post-hoc, as if it were added after analyzing the data and models, rather than being a central research question from the start. For that reason, the story does not seem to hold together.

The title in preprint reflects the main findings supporting hypothesis (i) expected changes in respiration and soil C stock after fertilization.

Considering your remark that during severe drought period observed respiration of N+ was also reduced, we revised the tittle: "Long-term nitrogen fertilization alters microbial respiration sensitivity to temperature and moisture, potentially enhancing soil carbon retention in a boreal Scots pine forest".

Detailed comments, by line number

15: Carbon (C) is usually written out in full the first time it is mentioned, as was done for nitrogen (N) even though we all know it.

We changed C to carbon.

30-32: The conclusion seems overly broad, given that just one upland forest was studied. Your site was originally a very poor Scots pine forest, but you generalize your conclusions to all forest types. What about peatland forests? Do you know, even for your own site, what, for example, the N2O fluxes were just after the fertilisation events or in earlier phases of the rotation?

To narrow down the concluding sentence of the abstract we replaced "boreal forest" by "boreal Scots pine forests on mineral soils" and in revised text of conclusions chapter we replaced "boreal Scots pine ecosystems" by "boreal Scots pine forests on mineral soils".

73-74: These seem like very nice references, but I'm not sure that they are both conducted in the actual boreal zone and represent the entire boreal zone?

Yes, these studies were conducted in Southern Finland and Estonia, within the boreal and hemi-boreal zones, representing conditions of the southern boreal region. The sentence was revised. "In southern boreal region's Scots pine forests on well-drained mineral soils, …"

L81: The introduction lacks any reasoning/motivation for such a hypothesis.

Reasoning for the hypothesis is detailed on lines 46-49.

L89: Even if you follow the silvicultural practices in principle, there can be a lot of variation in practice. So for the sake of repeatability I would add some details on the harvests, like how much basal area was reduced or something like that.

The sentence was revised to include information on basal area:

"*The stand underwent thinning in 1990 (reducing 16.2% and 26.5% of basal area (BA) for CTR and N+, respectively), and 2015 (reducing 36.7 % and 40.1% of BA for CTR and N+, respectively).*"

111: Please be more specific and use dates instead of growing seasons.

In revised text we replaced the growing seasons by exact dates.

112: I don't understand. Did you take measurements from two individual points within each of three or six plots, which you refer to as a group? If you had two points, is that a group or a pair? How close were the groups to each other? Are they independent? How do you take into account in the statistical analyses that the two points are close and probably not independent? Please clarify the description of the overall setup including what was the distance between the points and groups and treatments.

In revised text we reformulated "12 plot groups (two 30 cm diameter plots per group; n=6 per treatment)." to "12 plots (6 plots or 3 pairs per treatment). Plots in each pair were located 30 cm apart (Fig. 1c) and CTR and N pairs were on average 122 m apart (Fig. 1b)."

Location of the plots resulted from the experimental setup of the fertilization treatments. In the revised text we clarified:

"As the single plot area was relatively large (706 cm2), we considered 2 plots pair to be representative of the trenched area (Fig. 1c) and 3 pairs to be representative of the spatial variation of the treatment."

114: Why do you use both Rh and R_h for heterotrophic respiration here and elsewhere? It gives a slightly unfinished impression.

R_h is correct and in revised text we corrected Rh in all instances.

116-119: This paragraph seems to be the earlier or at least less complete version of the following one, please combine these sections to avoid redundancy.

In revised text the lines 116 – 119 were combined with lines 121-129.

116&122: Don't you need to write down the manufacturer's details anymore?

In revised text we added manufacturer's details "(LICOR, Lincoln, NE, USA)".

131 Depth should be given

L131 mentions depth "at 5 cm depth"

132 end date is missing

In revised text the end date was added "until end of December 2023"

142 You have not yet introduced CTR and N+

in revised text the abbreviations were explained at the first instance