Reply to Referee #2 (Lukas Kohl) for "A Synthesis of *Sphagnum* Litterbag Experiments: Initial Leaching Losses Bias Decomposition Rate Estimates"

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Comments made by the reviewer start with a bold **Q** while our reply starts with a bold **A**. In section "Additional changes" we list additional changes we would like to incorporate in an updated version of the manuscript.

1 Reply to comments

1. **Q:** First, sincere apologies for the delays in this review. I started reviewing this paper multiple times, and tim got I got stuck while attempting to fully understand the large number of Bayesian models that the authors performed and the underlying assumptions in these models. To be honest, I'm still not sure if I understood all aspects of these models, the details of which are somewhat beyond my expertise.

   The manuscript by Teickner and coauthors reports on a reanalysis of Sphagnum litterbag experiments conducted to estimate litter decomposition rates in peatlands. They posit that the decomposition rates inferred from such experiment are overestimated if initial leaching is not taken into account. They provide a detailed analysis of how different experimental procedures may have caused particularly high or low fractions of initial leaching, and provide guidance for future litter bag experiments.

   This is a timely study of an important topic relevant to simulating carbon storage in peatlands. The study applies state of the art methods and the conclusions are well supported by the study results. The manuscript is clearly written and reads easily (well, with the exception of the underlying mathematics, but I guess that's unavoidable).

A: We thank the reviewer for their comments and questions that are useful to clarify some points we make in the manuscript. The models we use are a subset of a model that has a conceptually simple structure: It tries to estimate remaining masses in the various litterbag experiments using the decomposition model described in Frolking et al. (2001) but with initial leaching losses added:

$$m(t) = \begin{cases} m_0 & \text{if } t = 0 \\ \frac{m_0 - l_0}{(1+(\alpha-1)k_0 t)^{\frac{1}{\alpha-1}}} & \text{if } t > 0 \end{cases} \quad (1)$$

The final model is apparently complex because the studies from which we combine data can be assumed to have different parameter values (k_0, l_0, α) and we therefore use mixed effects models (Bayesian hierarchical models) to describe this variation while pooling information across the studies. This modeling approach differs to commonly used (generalized) linear mixed effects models ((G)LMM) only in two aspects, but apart from that the structure is in principle the same: First, the decomposition model equation is non-linear, and second, mixed effects are included for several parameters.

2. **Q:** I have some thoughts that could be incorporated into the manuscript, although I do not think that these are critical for the publication:

   - The authors put emphasis on how litterbag experiments can be improved to more accurately calculate mass lot rates. Over the last decade, there has been some substantial criticism of the litterbag approach (in upland studies). Catrufo et al 2015 (Nature Geosciences), for example, used uniformly isotope-labelled plant litter to study the persistence and vertical translocation of litter derived C into soil. I think a publication that focuses on how litter bag experiments best done should address the question *if* litterbag experiments are still the best approach to study decomposition rates (e.g., because isotope labelling of Sphagnum plants is not feasible).
   - Regarding the bias of l0 on k0: While reading this study, I was wondering why these are inferred at the same time using a complex model. Would is not be easier to just exclude the initial mass from the dataset, and calculate mass loss rates based on the mass from litter retrieved at different time points?
   - Finally, I have some doubt about extrapolating decomposition models fitted to data from <5 years decomposition to long time scale (up to 100 years)? Regardless if l0 is correctly quantified or not, I have doubts that decomposition over the next 95 years follows the same trends measured initially?

   A:

   - We had a look at Cotrufo et al. (2015) and the criticism against litterbag experiments is that the mesh limits mass loss due to leaching, fragmentation, and biophysical perturbation ("Litter decomposition has been conventionally measured by the litter bag method, which by inhibiting fragmentation results in an 'artificial' asymptotic value of mass remaining on the soil surface. When litter is not protected in mesh bags, it is fully exposed to biophysical perturbations that accelerate its rate of mass loss to full disappearance from the litter layer within a few years." and "The litter residue physically transferred to the mineral soil would explain the common asymptotic mass remaining found in litter bags studies, where loss of litter fragments is inhibited.").

We currently assume that this criticism does apply to *Sphagnum* litterbag experiments to a lesser extent, but we agree that such factors could also bias results and require further investigation. First, bioturbation is non-existent in many peatlands, particularly bogs. Second, we agree that it would be best to analyze to what extent meshes reduce initial leaching and this could be done with direct leaching experiments. However, we want to mention that we are aware of a study on tree leaves in terrestrial ecosystem that indicates only negligible differences in initial leaching losses when the mesh size is varied (Bokhorst and Wardle, 2013), whereas other resources cited in Cotrufo et al. (2015) do not provide direct evidence for differences in initial leaching losses due to different mesh sizes. We therefore currently assume that mesh size has a negligible effect on initial leaching losses in *Sphagnum* litterbag experiments. Third, movement of fragmented litter (e.g. due to advection) and fragmentation by small arthropods may be limited in litterbag experiments in peatlands, but also here we are not aware of systematic studies for *Sphagnum* or other plants in peatlands.

Another important point is that we do not think that we currently have the expertise to provide specific recommendations on various different aspects of litterbag experiments. For example, while we agree that stable isotope labelling approaches in combination with litterbag experiments or as replacement would be useful to quantify initial leaching losses and other processes during litter decomposition, we have no own practical experience with stable isotope (^{13}C) labeling of *Sphagnum*, but we can imagine that uniform ^{13}C labeling of *Sphagnum* is difficult due to the effort necessary to grow *Sphagnum* from protonema (Heck et al., 2021), slow growth rates and uptake of carbon from other sources difficult to control (e.g. carbohydrates, carbon from methane oxidation), and we take the lack of published attempts to produce uniformly ^{13}C labeled *Sphagnum* (analyzed with a Scopus search with keywords `sphagnum AND (isotope OR 13C) AND label*`) as support for this assumption. We therefore welcome the suggestion for further improvements of *Sphagnum* litterbag experiments, but we think it is best to restrict our discussion to factors that could be used to better estimate initial leaching losses and we think we have some expertise about (please also compare with our reply to comment 15 by reviewer 1).

To expand the discussion in this direction and to emphasize our opinion that more specific experiments are needed to address conceptual knowledge gaps first, we suggest to expand section 4.3 after l. 470 as follows:

"Our results indicate that to develop more specific recommendations and standards for reporting *Sphagnum* litterbag experiments, further conceptual research with the aim to address the knowledge gaps outlined in the previous two sections is necessary. Specifically, in our opinion the next important experimental steps are (1) to define sample preprocessing conditions that are considered natural such that the decomposition process measured in litterbag experiments represents the process intended to be measured, (2) to analyze whether and how commonly applied sampling protocols (e.g. due to seasonal variations in water extractable compounds) and preprocessing steps (in particular different drying methods) cause different initial leaching losses and potentially different decomposition pathways, and (3) to develop litter preprocessing methods that are similar to natural conditions and at the same time allow accurate measurement of initial dry masses. Methods that my be helpful here are experiments similar to those conducted by Lind et al. (2022) or described in Bärlocher (1997), and a combination (or replacement) of litterbag experiments with stable isotope labeling and direct measurement of different mass fluxes (e.g., Kammer and Hagedorn (2011), Cotrufo et al. (2015)) to improve measurement accuracy and exclude additional potential confounding factors such as the long debated influence of meshes on initial leaching losses and litter fragmentation (e.g., Bokhorst and Wardle (2013))."

- Yes, this would be an adequate approach to consider initial leaching losses as suggested by our analyses. We very much sympathize with not making models unnecessarily complex. The approach suggested by the reviewer to discard the initial mass and use only the remaining masses to estimate decomposition rates would mean to explicitly consider initial leaching losses (subtracting their influence out experimentally rather than statistically) while it may be possible to use a simpler decomposition model to estimate decomposition rates.
  There are several reasons why we used a statistical approach to estimate initial leaching losses and decomposition rates in our study: First, we were interested in the magnitude and variability of initial leaching losses and their influence on other parameters. Therefore it made sense to estimate initial leaching losses. Second, due to correlated parameter errors and the limitation that available *Sphagnum* litterbag experiments do not allow to accurately separate initial leaching losses from decomposition, it was necessary to estimate initial leaching losses and decomposition rates simulataneously estimate both parameters. Third, experimentally subtracting initial leaching losses only makes sense with more than two litterbag sampling time points after the start of the experiment because otherwise nearly any deocmposition model would fit remaining masses perfectly. However, most of the available litterbag experiments have at most two smapling time points after the start of the experiment.
  Of course, when initial leaching losses are a sample preprocessing artefact that changes the decomposition process compared to more natural conditions, we would rather try to improve this aspect of the litterbag experiment first (when the aim is to estimate decomposition under natural conditions, however one defines these) before worrying about how to best consider initial leaching losses.
  To emphasize more that it may also be possible to subtract initial leaching losses out experimentally rather than statistically and discuss what properties of litterbag experiments are necessary for this, we suggest to add at l. 460: "Decom-

position rates can be estimated either by subtracting out initial leaching losses statistically (i.e., using a model similar to that used here) or experimentally (by using only remaining mass values recorded after initial leaching has occurred)."
And at l. 466: "In addition, more than two litterbag collection time points are necessary to experimentally subtract out initial leaching losses and correctly estimate decomposition rates as described in point 1. Most of the available *Sphagnum* litterbag experiments have only at most two sampling time points after the start of the experiment."

- We fully agree that the assumption that existing decomposition models allow to correctly extrapolate decomposition rates to even 20 years or longer is not well tested. The problem is that the assumption is generally not easy to test and that there are, to our knowledge, currently no better approaches to model long-term decomposition rates, which is why the assumption, in combination with litterbag experiments, is used in long-term peatland models (e.g. Frolking et al. (2001), Bauer (2004), Heijmans et al. (2008), Heinemeyer et al. (2010), Morris et al. (2012), Chaudhary et al. (2018), Bona et al. (2020)).
  As described here and elsewhere (Frolking et al. (2001), Clymo et al. (1998)), there are attempts to incorporate an assumed slow down of decomposition rates into these peatland models, but such a slow down is difficult to estimate based on litterbag data and peat core data due to short time periods and various sources of errors. We think that progress here may be possible when the accuracy of decomposition rate estimates increases and we hope our study contributes to this aim. As our analysis does not allow to draw conclusions how one could use litterbag experiments to analyze long-term decomposition processes, we prefer not to further discuss this aspect in our manuscript. However, we agree that it might be helpful to highlight this problem and we suggest to add to the end of section 4.3:

  "Also with regard to refining decomposition rate parameter values in long-term peatland models, more research is necessary, in particular to understand the slow down of decomposition rates when litter chemistry changes during decomposition. As discussed in previous studies Frolking et al. (2001) and shown here, current litterbag experiments do not allow to estimate such a slow down. Therefore, more precise decomposition rate estimates are a necessary but not sufficient condition for addressing this problem."

2 Additional changes

1. l. 45: We will change "Available estimates from direct measurement and few two-pool litterbag experiments …" to "Available estimates from direct measurement and few litterbag experiments …" because some of the studies do not explicitly consider two pools whem modeling decomposition.

2. l. 107: We will change "… the Holocene Peatland Model (Frolking et al., 2010), one of the peatland models studied in many studies." to "the Holocene Peatland

Model (Frolking et al., 2010), one of the most widely applied and tested peatland models."

3. l. 293: We will change "The overestimation of k_0 when ignoring initial leaching losses becomes however …" to "However, the overestimation of k_0 when ignoring initial leaching losses becomes …"

4. l. 345: We will change "In the following paragraphs we suggest what caused small initial leaching losses in these studies." to "In the following paragraphs we suggest causes for small initial leaching losses in these studies."

5. l. 396 to 398: We will change "… whether initial leaching losses differ between studies which discard capitula, which use whole plants, or which use stem parts of different length, as can be expected from previous studies and the observation that already senesced or decomposed *Sphagnum* litter has smaller initial leaching losses …" to "… whether initial leaching losses differ between studies that discard capitula, that use whole plants, or that use stem parts of different length, as can be expected from previous studies and the observation that already senesced or decomposed *Sphagnum* litter has smaller initial leaching losses …"

6. l. 398: We will change "Ssystematic" to "Systematic".

7. l. 406: W ewill change "Relevance of considering leaching losses in litterbag experiments" to "Relevance of considering initial leaching losses in litterbag experiments".

8. l. 462: We will change "samples" to "sampled".

9. l. 464: We will change "temperal" to "temporal".

10. l. 490: We will change "The data used in this study is derived from Teickner and Knorr (2024a)." to "The data used in this study are derived from Teickner and Knorr (2024a)."

11. l. 497: We will add "We thank Cristian Estop-Aragonés for helpful comments that improved an earlier version of this manuscript."

12. In the caption of Fig. 4 in the main text we will change "Remaining masses predicted by the model ignoring initial leaching losses minus remaining masses with the simulation model …" to "Remaining masses predicted by the model ignoring initial leaching losses minus the simulated remaining masses (considering different amounts of initial leaching losses) …"

13. In the caption of Fig. 5 in the main text we will state explicitly that the shown values do not include data from Bengtsson et al. (2017).

14. In supporting information S1, l. 43 to 45 we gave the wrong estimate for initial leaching losses in the fen in Moore et al. (2007). The corrected sentence is: "Samples in the pond had the lowest initial leaching losses (on average -1 percent of the initial mass) and samples in the fen the largest (on average 14 percent of the initial mass)."

15. In the formulas in the supporting information we changed “inv_logit” to “logit^{-1}” to make the formula consistent with the main text.

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