Reply to Reviewer Comments for "A Synthesis of *Sphagnum* Litterbag Experiments: Initial Leaching Losses Bias Decomposition Rate Estimates"

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Contents

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We thank both reviewers for their helpful comments. Comments made by the reviewers start with a bold **Q** while our reply starts with a bold **A**. In section "Additional changes" we list additional changes included in the updated version of the manuscript.

1 Reply to Anonymous Referee 1

1.1 Reply to comments

1. **Q:** The manuscript points out additional and valuable information about the influence of leaching losses on decomposition modeling, as they can lead to notable errors over longer periods of time. From my point of view, the results from the topic under investigation underline the importance of additional research in that field. Therefore,

after carefully revising the manuscript and clearing some open questions (see further comments), I believe that the manuscript has potential to be published.

[… (see point number 2 below)]

The authors have used enough related and up-to-date works. Together with more settled works, the authors give a nice synthesis of different litterbag studies and sum up some important obstacles. However, the paragraphs are sometimes disjointed, which can make reading difficult. Also, the wording needs to be revised in some cases to be more precise. In addition, typing errors should be corrected throughout the manuscript. The number of figures and tables is ok, the layout or graphic design could be improved.

From my point of view, the present manuscript does not fulfil all requirements for being published. A precise revision, especially of the abstract, the introduction section and the discussion is needed (see comments below). After addressing these points, the manuscript has potential to be published.

A: We thank the reviewer for their comments and questions that are useful to clarify some points we make in the manuscript. In summary, the reviewer suggests the following general changes:

1. More and restructured information on the motivation for the study.
2. Extension of the discussion with explicit information how to improve litterbag studies, especially what variables to measure to get more accurate (initial) decomposition rate estimates.
3. More precise wording.
4. Improved graphic layout and design.

In general, we made the following changes to address these suggestions:

1. We addressed the related specific comments by the reviewer (see below).
2. We addressed the related specific comments by the reviewer (see below). Summarized, the aim of our manuscript is to analyze the bias in estimated mass losses due to decomposition and decomposition rates caused by ignoring initial leaching losses and to improve litterbag experiments by reducing this bias. Our analyses suggest many conceptual knowledge gaps and we see our contribution in highlighting these conceptual gaps to stimulate future experiments that analyze how important possible confounders are. To name the most important, the next experimental steps are in our opinion (1) to define sample preprocessing conditions that are considered natural, (2) to analyze how commonly applied preprocessing steps cause different initial leaching and potentially different decomposition pathways, and (3) to develop litter preprocessing methods that are similar to natural conditions and allow accurate measurement of initial dry masses. The results of these conceptual experiments give the information required to give explicit recommendations for how to improve litterbag experiments. While we state what our results imply or suggest, we refrain from more explicit recommendations because we think that these can be made only based

on the suggested conceptual experiments.
However, we agree that we could more explicitly describe the conceptual experiments we think are necessary to develop more specific guidelines for *Sphagnum* litterbag experiments. We note that the second reviewer also suggests to expand the discussion on how to improve litterbag experiments, but more in line with our aim to point to conceptual knowledge gaps and conceptual strategies to best measure decomposition rates (Kohl, 2024). We tried to incorporate the suggestions by both reviewers by expanding the discussion (please see our our reply to comment 15 by reviewer 1). If the reviewer has further suggestions or we misunderstood the suggestion, we would be thankful for further clarification.

3. We addressed the related specific comments by the reviewer (see below).
4. Unfortunately, the reviewer does not provide specific comments what could be improved in the layout or design of the figures. If the reviewer has specific comments, we are happy to consider them.

2. **Q:** The abstract section gives some background information, but the problem statement is missing. The reason why the study has been conducted should be mentioned or further elaborated to underline the importance of the present study. More background information would be necessary to understand what initial leaching is. Generally, I am a fan of short abstracts, but I would suggest including more information in the Intro and Results part of the abstract. This would increase the readers interest to read further.

   A: We expanded the first paragraph of the abstract (old: ll. 1 to 4, new: ll. 1 to 7, track changes: ll. 1 to 8) with reasons why ignoring initial leaching losses may cause bias and larger variance of k_0 estimates, and why this bias and variance are important for understanding controls of decomposition and long-term peat accumulation:

   "Our knowledge of the magnitude and controls of *Sphagnum* decomposition rates is derived to a large extent from litterbag experiments that do not explicitly consider initial leaching losses. Previous research on vascular plants suggests that decomposition rate (k_0) estimates from litterbag experiments are biased when initial leaching losses (l_0) are ignored. In contrast, magnitudes and variability of l_0 for *Sphagnum* litterbag experiments are not well known and therefore also not the effect on *Sphagnum* k_0 estimates. As *Sphagnum* is the main peat forming species in many northern peatlands and as biases in k_0 estimates can propagate and amplify in long-term peatland models, minimizing such bias is necessary for accurate predictions of peat accumulation."

3. **Q:** L5: In the first part of the sentence, you argue that you want to test if there is a bias in k0 due to l0, while in the second part of the sentence you already argue that they do so – as your aim is to quantify error estimates.

   A: A bias is the expected difference between an estimate for a quantity and the true value of the quantity, i.e., the average error in many hypothetical repetitions. In contrast, errors in estimates refers to a difference between an estimate for a quantity and the true value of the quantity (see for example Hastie et al. (2009)). Also note

that the first part of the sentence refers to us estimating the bias (using simulated data with different amounts of initial leaching losses and a model that ignores initial leaching losses), whereas the second part refers to our analysis how initial leaching losses *increase* the variance of k_0 estimates in a model that considers initial leaching losses. Thus, both parts of the sentence in l. 5 refer to different properties of an estimate and to different analyses within our study. However, we agree that the sentence currently is ambiguous and we changed it to (old: ll. 5 to 7, new: ll. 8 to 11, track changes: ll. 9 to 12):

"We present a meta-analysis of 15 *Sphagnum* litterbag studies to estimate initial leaching losses (l_0), to analyze how much *Sphagnum* k_0 estimates are biased when the decomposition model ignores initial leaching losses, and to analyze how much the variance of k_0 estimates of k_0 estimates increases due to initial leaching losses even when they are estimated by the decomposition model."

4. **Q:** L20: the sentence is not clear to me

   A: The sentence is: "Our knowledge of the magnitude and controls of decomposition rates is derived to a large extent from litterbag experiments (Rydin et al., 2013) and these estimates inform parameters in long-term peatland models (e.g. Frolking et al. (2010))." We assume the reviewer refers to the second part, "and these estimates inform parameters in long-term peatland models (e.g. Frolking et al. (2010))." Perhaps the term "inform" causes the confusion here (if the reviewer can confirm this, this would be helpful).
   We changed this sentence to (old: ll. 19 to 21, new: ll. 23 to 25, track changes: ll. 24 to 26): "Our knowledge of the magnitude and controls of decomposition rates is derived to a large extent from litterbag experiments (Rydin et al., 2013) and these estimates are used as parameter values in long-term peatland models (e.g. Frolking et al. (2010))."

5. **Q:** L27: the sentence is not clear to me

   A: We assume the reviewer refers to the sentence starting in l. 27. This sentence is: "Finally, decomposition rate estimates are used to define parameter values in peatland models which are a major tool to analyze peat accumulation and process interactions during time ranges exceeding the duration of litterbag experiments."

   We are not sure what could be unclear here and it would be helpful if the reviewer could describe in more detail what is unclear here. As a first try, we reworded the sentence to (old: ll. 27 to 29, new: ll. 31 to 33, track changes: ll. 32 to 35): "These decomposition rate estimates are used as parameter values in long-term peatland models which allow to analyze peat accumulation and interactions of decomposition with other processes controlling peat accumulation for time ranges exceeding the duration of litterbag experiments."

6. **Q:** L36: "in reality…" delete

   A: We deleted "in reality" in l. 36.

7. **Q:** L 41:" and came to the conclusion" concluded that…

   A: Thank you for this suggestion, we changed this sentence as suggested.

8. **Q:** L65: leach

   A: We changed "leach" to "leaching".

9. **Q:** L65: it is unclear which plants will be investigated. Do you focus on Sphagnum species or include others? Especially as you mention other, also vascular plants and lichens afterwards (L 75ff)

   A: We changed l. 65 to from "What is the magnitude of initial leaching losses in and their variability between species and studies?" to "What is the magnitude of initial leaching losses in *Sphagnum* litterbag experiments and their variability between species and studies?" to explicitly state that we analyze only *Sphagnum* litterbag experiments (old: l. 65, new: ll. 69 to 70, track changes: ll. 72 to 73).

10. **Q:** L69: again, litterbag experiments in general or Sphagnum litter only?

    A: We changed the beginning of the sentence in l. 69 from "To address these questions, we first simulate litterbag experiments" to: "To address these questions, we first simulate *Sphagnum* litterbag experiments" to explicitly state that we analyze only *Sphagnum* litterbag experiments (old: l. 69, new: l. 74, track changes: l. 77).

11. **Q:** L147: why only 15% not 18% as mentioned above?

    A: There was no special reason to use 15 mass-% as upper bound for the simulation other than that this value is near the largest estimate for initial leaching losses that can be directly derived from previous studies. The conclusions of our simulation will not change if we also include an initial leaching loss of 18 mass-% or larger because already for an initial leaching loss of 15 mass-% the difference in estimated vs true decomposition rates was largest and also the difference in remaining mass of peat accumulated up to a certain time (see Fig. 4). That said, we understand the desire to use consistent value ranges throughout the manuscript and therefore now also include an initial leaching loss of 18 mass-% in the simulation. An updated version of Fig. 4 in the manuscript is shown in Fig. 1.

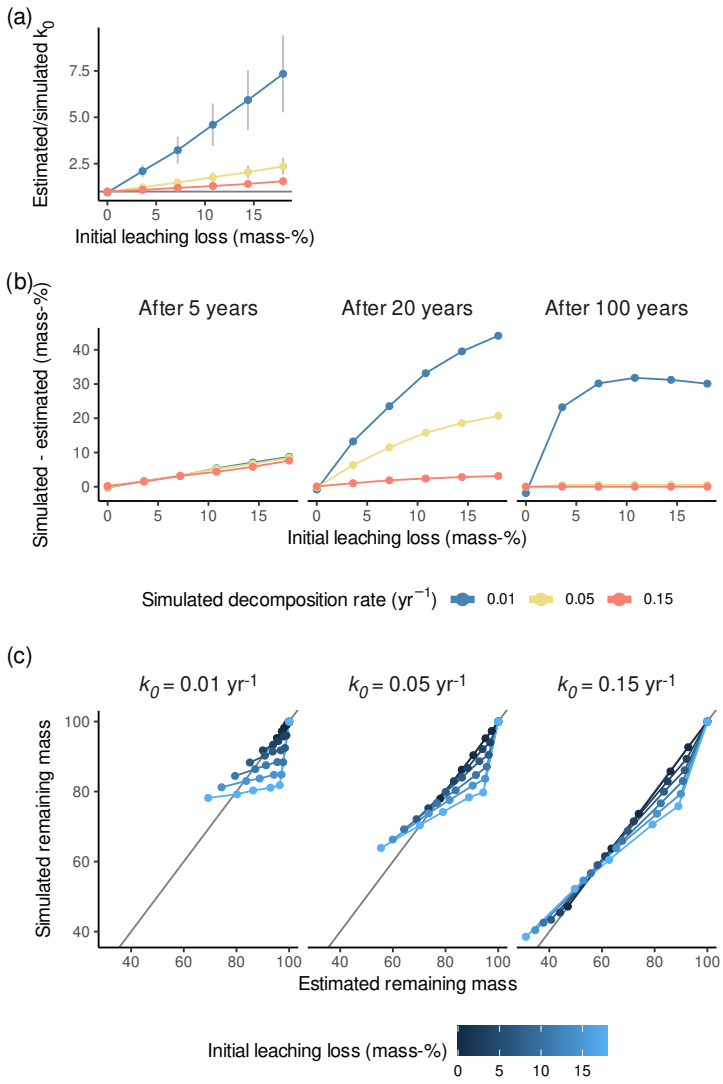

Figure 1: Results of the simulation experiment. (a) Estimated divided by simulated decomposition rates (k_0) versus simulated initial leaching losses (l_0) for the three simulated decomposition rates. Error bars are standard errors. The horizontal grey line represents a ratio of estimated to simulated decomposition rates of 1. (b) Remaining masses predicted by the model ignoring initial leaching losses minus the simulated remaining masses (considering different amounts of initial leaching losses), either after 5, 20, or 100 years of decomposition. Positive values mean that with k_0 estimated while ignoring initial leaching losses remaining masses are underestimated. (c) Simulated remaining masses versus remaining masses predicted by the model ignoring initial leaching losses for the three simulated decomposition rates and the simulated litterbag retrieval times.

12. **Q:** L154f: check typos

    A: We changed the paragraph starting in l. 154 from "These simulated masses were then used to fit the model ignoring initial leach loss (equation (2)) using nonlinear

least squares regression regression to obtain estimated average and standard deviation for k_0, as is often done in litterbag experiments. We compared these values to the decomposition rate values that were used to simulate the data." to "These simulated masses were then used to fit the model ignoring initial leaching losses (equation (2)) using non-linear least squares regression to estimate the average and standard deviation for k_0, as is often done in litterbag experiments. We compared these values to the decomposition rate values that were used to simulate the data." to correct the typos (old: ll. 154 to 156, new: ll. 159 to 161, track changes: ll. 165 to 168).

13. **Q:** Figure 4 (a): what does the grey horizontal line indicate?

    A: We thank the reviewer for pointing this out. The horizontal grey line represents a ratio of estimated to simulated decomposition rates of 1 and alignment of the computed ratios with this line indicates unbiased estimates for k_0. We updated the caption of Fig. 4 (a) in the manuscript as shown for Fig. 1 in our reply here.

14. **Q:** L355: "for several hours", what was the time span? Within 48h? or less?

    A: The comment refers to the sentence (old: l. 353 to 356): "Castells et al. (2005) used fresh *Sphagnum* plants in their study where they quantified only small initial leaching losses. Bartsch and Moore (1985) air-dried their samples for only 24 to 48 h, Schipperges and Rydin (1998) (Fig. 2 and 3) have shown that *Sphagna* can survive drying for several hours if the water content does not decrease too much. Therefore, the *Sphagnum* plants may have not been completely dead which reduces initial leaching losses".

    In Schipperges and Rydin (1998), it is stated that "There was no recovery of net photosynthesis by any of the species when rewetting followed complete desiccation, after either 2 or 4 d of dry storage" and "When the mosses were dried only until they reached their compensation water content (Expt 1a) there was some recovery of *S. magellanicum* and *S. cuspidatum* (see e.g. *S. cuspidatum*, Fig. 3)." According to Fig. 3 in Schipperges and Rydin (1998), the described rewatering occurred after about 9 to 12 hours. Thus, the *Sphagnum* plants died in the experiments in Schipperges and Rydin (1998) within a time range of about 12 hours to 2 to 4 days.

    We decided not to give a specific time range because the time without water supply is only one control of the water loss that presumably leads to the plants dying. Controls of the evapotranspiration rate (e.g. temperature, advection of air, geometry of the sample etc.) and the size of *Sphagnum* fragments thus will have an important influence on the water loss and absolute water stock of a *Sphagnum* fragment at a specific time. Thus, differences in drying times can be easily explained away by differences in drying conditions and *Sphagnum* fragment size at the moment. As indicated in the manuscript, more research is needed to support our hypothesis that initial leaching losses from dried-to-death *Sphagnum* fragments are larger than initial leaching losses from dried-but-living *Sphagnum* fragments and this requires more research on how water content controls when *Sphagnum* fragments die.

15. **Q:** L442ff: the aim is to improve litterbag experiments in the field, and you point

out that the collection of litterbags shortly after the start of the experiment is necessary. Could you also give a suggestion which parameters should be measured to describe initial decomposition rates? Could that also help to make future studies more comparable?

A: We assume that the reviewer defines "initial decomposition rates" in line with our definitions for decomposition (as depolymerization and possibly mineralization of litter material) and the time range considered as initial (i.e., up to three weeks, see l. 33). Thus, we assume the reviewer asks what variables to measure to accurately estimate depolymerization rates during the first days to approximately three weeks and whether accurate estimates of such initial decomposition rates could make future studies more comparable.

We think that an affirmative answer to this question has as necessary condition that litter preprocessing and litterbag experiments itself can be standardized such that initial leaching can be accurately measured, and it also depends on how large initial leaching losses are during litterbag experiments. If initial leaching losses are large, say larger than 5 mass-%, we currently assume that the mass loss due to depolymerization during the first three weeks would be negligible. This would make separation of initial leaching losses from decomposition easy in practice since the error one makes in assuming all initial mass loss are leaching losses would be small. It would also indicate that measuring initial decomposition would not make future studies more comparable because of the difficulty to accurately measure the small mass fluxes identifiable as losses due to depolymerization. If initial leaching losses are small, it would not any more be a small error to assume all initial mass losses are initial leaching losses, but we still assume that depolymerization losses are so small that they are hard to quantify and we currently do not think that this would help to make litterbag experiments comparable. If initial mass fluxes from both processes are not negligible, this would require to differentiate mass losses from both processes which would require more sophisticated strategies than simple litterbag experiments, for example using stable isotope labels, as brought up by Lukas Kohl in his review (Kohl, 2024), or addition of antibiotics to suppress enzymatically catalyzed depolymerization (e.g., Coulson and Butterfield (1978)).

We suggest that the first step in future research on this topic should be to check that litterbag experiments are representative for the sample preprocessing and decomposition process under natural conditions (see section 4.2 in our manuscript) because we assume that in most cases decomposition rate estimates are useful only when they are representative for the decomposition process under natural conditions. To this end, one has to define what usually happens to *Sphagnum*, i.e., what the natural conditions are, during the transition from a living plant to litter that gets decomposed, and how sample preprocessing steps in litterbag experiments differ from that. In addition, one has to estimate how sample preprocessing steps — in particular the drying — control enzymatic activities and microbial growth in litter samples. If any of these differs in an ecologically relevant magnitude this would imply that at least some preprocessing steps make litterbag experiments unrepresentative for decomposition under

natural conditions. The aim should be to adjust preprocessing steps such that the process measured is representative for decomposition under natural conditions and it is nevertheless possible to accurately estimate initial dry masses.

If initial leaching losses (as defined in our study) are negligible under these conditions (as e.g. suggested by Coulson and Butterfield (1978)), initial decomposition rates could be directly measured. As mentioned above, we assume that the initial time period is too short to expect that the presumably small mass differences can be accurately quantified by measuring mass differences of litter bags because import of material from the matrix probably is a large error source. However, one could use stable isotope labeled litter and use gas flux and pore water measurements to estimate initial decomposition rates under these conditions, as mentioned above.

If initial leaching losses are not negligible under natural conditions, the next step would be to develop a model that accurately describes the controls of initial leaching losses and thus also the magnitudes of initial leaching losses. Here, experiments similar to those described in Lind et al. (2022) may be useful, as well as stable isotope labelling experiments.

We note that this is only a very rough outline and we do not attempt to provide a full plan for how to design litterbag experiments, simply because there are many open questions and uncertainties. A step-wise approach with adaptions as new information becomes available is required.

Our suggestions to improve litterbag experiments are therefore mainly of conceptual nature: We point out what experiments would be required to check that decomposition rate estimates align with the natural decomposition process, what experiments would likely show important differences in sample pre-treatment on initial leaching, and that litterbag experiments need to be designed to accurately estimate and differentiate initial leaching from decomposition if they should provide useful information on effects of some treatment on decomposition to parameter values for peatland models. One step to accurately estimate and differentiate initial leaching from decomposition is to sample one batch of litterbags shortly after the start of the litterbag experiments.

To provide these information in our manuscript, we expanded section 4.3 after l. 470 as follows (new: ll. 478 to 495, track changes: ll. 490 to 507):

"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)).
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."

We are not sure whether this addresses the reviewer's question, but if there are additional suggestions the reviewer would like to discuss or ideas the reviewer has why measuring initial decomposition rates would be useful, we are happy to discuss these.

16. **Q:** Could you sum up the take home message? How can initial leaching effects be prevented in further studies?

    Regarding the take home messages, these can be summarized as follows:

    1. Initial leaching losses in *Sphagnum* litterbag experiments should not be assumed negligible in general.

    2. Initial leaching losses in *Sphagnum* litterbag experiments may vary in dependency of environmental conditions and sample pre-treatment, particularly between drying procedures.

    3. Therefore, if the aim is to quantify mass losses due to decomposition, *Sphagnum* litterbag experiments should explicitly estimate initial leaching losses to make sure estimates for decomposition as mass loss or decomposition rates are not confounded with these initial leaching losses. Moreover, future research needs to address whether sample pre-treatment makes the decomposition process in litterbag experiments unrepresentative for natural conditions and suggest adjustments to allow litterbag experiments to measure decomposition under natural conditions if necessary.

    We think that all of these points appear in the conclusions section of the manuscript, but we agree that this section can be improved to better summarize key points of our study. We therefore changed the conclusions section (please see our reply to comment 17 of reviewer 1).

    Regarding the second question — How can initial leaching effects be prevented in further studies? — We think that our reply to comment 15 by reviewer 1 does address this question. We cannot give an explicit recipe to design litterbag experiments now because a series of conceptual experiments is necessary first to define natural conditions

for sample preprocessing, to identify preprocessing strategies that align with these conditions, to estimate initial leaching losses and the factors controlling them, and finally to analyze whether this leads to reproducible results and how precise decomposition rates can be measured then.

17. **Q:** General notes:

    - Abbreviation C carbon
    - Check spelling and typesetting
    - Air-dried/ air dried, keep it uniform
    - One-pool/one pool
    - Check tables and figures
    - Your discussion points out clearly, that many studies lack in information about the methodology, including sample preparation, corrections and calculations. This could also be included in the Conclusion.

    A:

    - The abbreviation for carbon (C) appears for the first time in l. 432, but is not defined there. We replaced this abbreviation by "carbon" (old: l. 432, new: l. 434, track changes: l. 446).
    - We corrected the typos pointed out by the reviewer and also combined some paragraphs in the methods and results sections, as suggested by the reviewer.
    - We changed all occurrences of "air dried" to "air-dried" in the text.
    - We changed all occurrences of "one-pool" to "one pool" in the text.
    - Apart from comment 13 by reviewer 1 we are not aware of explicit recommendations what to change in tables or figures. If the reviewer has further specific comments in addition to comment 13 we would like to consider them.
    - We thank the reviewer for this suggestion. While this is also a result of our study, we think that it is less important within the scope of our study and would not fit well within the conclusions without distracting from our core results. We think that our expanded discussion and conclusions imply that special attention should be paid to any aspects of sample collection, sample pre-processing, and experimental design that could have an influence on initial leaching losses. We changed the conclusions section to pronounce this aspect more (old: ll. 472 to 489, new: ll. 497 to 513, track changes: ll. 509 to 533):

      "Simulations, estimated initial leaching losses from 15 litterbag studies, and error analysis suggest that decomposition rates are overestimated if initial leaching losses are ignored. With average initial leaching loss magnitudes as reported in previous studies and as estimated here (3 to 18 mass-%), this implies an overestimation of remaining masses up to several tens of percent during decades of decomposition.
      Our estimates indicate that initial leaching losses > 5 mass-% are not uncommon and vary as much within species as overall, somewhat contradictory to the results

of many previous studies measuring small initial leaching losses from *Sphagnum*. This may be explained by pre-treatment of litter — even only air-drying — which may increase initial leaching losses compared to fresh Sphagnum and may cause large intra- and inter-study variation in initial leaching losses for the same species, similar to what has been observed for leaves from trees.
We therefore suggest that a correct estimation of mass losses due to decomposition and of decomposition rates in *Sphagnum* litterbag experiments requires to explicitly estimate initial leaching losses.
Our analyses also suggest that future *Sphagnum* litterbag experiments should sample a batch of litterbags few days to weeks after the start of the experiment because this allows a more accurate estimation of both initial leaching losses and decomposition rates than is possible with currently available data, especially in experiments with small decomposition rates.
Finally, if differences in sampling protocols (e.g. seasonal variations in contents of water extractable compounds) and drying procedures (even only air-drying) cause different amounts of initial leaching and change relative amounts of leaching of inhibiting or facilitating compounds, this may make litterbag experiments with large initial leaching losses caused by pre-treatment unrepresentative for decomposition under natural conditions where our results suggest less initial leaching losses."

2 Reply to Referee 2 (Lukas Kohl)

2.1 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 expanded section 4.3 after l. 470 as follows (new: ll. 478 to 495, track changes: ll. 490 to 507):

"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 to 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 added at l. 460 (new: ll. 462 to 464, track changes: ll. 474 to 476): "Decomposition 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 (new: ll. 470 to 473, track changes: ll. 482 to 485): "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 added to the end of section 4.3 (new: ll. 491 to 495, track changes: ll. 503 to 507):

  "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."

3 Additional changes

1. (old: l. 45, new: l. 49, track changes: l. 52): We changed "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. (old: l. 107, new: l. 113, track changes: ll. 117 to 118): We changed "… 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. (old: l. 293, new: l. 297, track changes: l. 306): We changed "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. (old: l. 345, new: l. 349, track changes: ll. 368 to 369): We changed "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. (old: ll. 396 to 398, new: ll. 398 to 400, track changes: ll. 409 to 411): We changed "… 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. (old: l. 398, new: l. 400, track changes: ll. 411 to 412): We changed "Ssystematic" to "Systematic".

7. (old: l. 406, new: l. 408, track changes: l. 420): We changed "Relevance of considering leaching losses in litterbag experiments" to "Relevance of considering initial leaching losses in litterbag experiments".

8. (old: l. 462, new: l. 466, track changes: l. 478): We changed "samples" to "sampled".

9. (old: l. 464, new: l. 468, track changes: l. 480): We changed "temperal" to "temporal".

10. (old: ll. 490 to 491, new: ll. 514 to 515, track changes: ll. 534 to 535): We changed "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. (old: l. 497, new: ll. 521 to 522, track changes: ll. 541 to 542): We added "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 changed "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 now 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.

16. In the supporting information in l. 180, we changed "improved $\hat{R}$" to "rank-normalized $\hat{R}$".

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