nature portfolio

Peer Review File

Salt marsh litter decomposition varies more by litter type than by extent of sea-level inundation

Corresponding Author: Dr Marie ARNAUD

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Decision Letter:

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Dear Dr ARNAUD,

Your manuscript titled "Salt marsh litter quality and decomposition under sea-level rise scenarios: from leaves to fine absorptive roots" has now been seen by 3 reviewers, and we include their comments at the end of this message.
They find your study interesting and novel. Though, they also highlighted some issues that need addressing. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. In particular, we require that you demonstrate that your experimental approach and data interpretation are robust, and that you provide an in-depth discussion of your findings. Please highlight all changes in the manuscript text file.

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We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

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Best regards,

Nadine Schubert, PhD
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and also in our style and formatting guide Communications Earth & Environment formatting guide .

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- Accession codes where appropriate
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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

This is an interesting study assessing litter breakdown in a marsh organ study. I believe it is the first marsh organ study from Europe I am aware of. Very cool. While commendable, there are significant concerns regarding the experimental approach and data interpretation. I strongly advocate for a deeper engagement with existing literature on saltmarsh decomposition processes. Notably absent from consideration are pivotal studies such as Hemminga and Buth (1991, Vegetatio), which examined the decay of various plant tissue types across diverse marsh environments, including those featuring the same species utilized in this study.

It is unclear whether each litter type underwent independent replication, involving distinct samples of leaves, rhizomes, etc. from different specimen or locations within the marsh, or if all samples of a particular litter type were initially pooled and then distributed across experimental units / organ pots. In the latter scenario, statistical comparisons between litter types would be flawed due to a lack of true replication.

The authors argue that both the soil carbon balance and the vertical accretion of the system is driven by the balance of

organic matter production and organic matter decomposition. This might not be true for their study site and for minerogenic saltmarshes in general. In these contexts, accretion is primarily driven by mineral sediment inputs, with allochthonous organic carbon often comprising a substantial fraction of the long-term preserved SOC stock. Consequently, the significance of litter breakdown in the studied system may be less pronounced compared to more organogenic marsh or mangrove ecosystems elsewhere.

Given the authors' frequent reference to terrestrial studies, a more nuanced interpretation regarding the implications of litter breakdown rates for soil carbon storage and sequestration rates is expected. It is crucial to recognize that the rate of litter breakdown is not directly linked to SOC formation. Rather, emphasis should be placed on understanding the fraction of litter that stabilizes in the soil, particularly within mineral-organic complexes. For insight into this aspect, Prescott's 2010 paper (Biogeochemistry) serves as a pertinent reference.

specific comments in the pdf attached

Reviewer #2 (Remarks to the Author):

This manuscript describes a study which assesses the decomposition of different types of organic matter in a saltmarsh environment under varying inundation scenarios which simulate sea level rise. The study separated organic matter sourced from saltmarsh plants into either roots or leaves. The roots where further separated into absorptive or transportive roots and rhizomes. The study finds that absorptive roots decomposed the slowest attributed to higher lignin content despite high N content; and leaves had the fastest decomposition. This is a novel study which improves our understanding of organic matter decomposition in blue carbon systems using an approach which has not been done previously.

It is an interesting and well conducted study. I suggest some modifications to clarify some points:
• It would be useful to include information about the soil characteristics including the particle size distribution, pH and EC
• Were the litterbags which contained the different types of organic materials buried in the same mesocosm ie. one litterbag of rhizomes, one litterbag of leaves, one litter bag each of the two root types? If so, how were edge effects in each mesocosm avoided if each litterbag was buried to 0.5 cm, with each mesocosm being 20 cm in diameter?
• Duration of the experiment – why was a 6 month duration for litterbag burial chosen? It would be useful to include some justification, as the longer the experiment, the greater the amount of decomposition that will occur
• Were there periods of extreme sea levels? Was the period of measurement typical of the long term means of this site (in terms of tidal range)
• Line 275-278: unclear sentence – please clarify. It would be useful to explain the process or include supporting references in each of the processes listed here
• Line 283: while belowground litter is not a homogenous group and not uniform for decomposition, in practice and in the field, it behaves like a bulk organic matter – it would be useful to include some discussion on application of this new knowledge in terms of potential land or coastal management considerations, or carbon storage consideration
• Final conclusion point – this should include a caveat that this concluding point at this stage is relevant only for these temperate environments. The same processes and conclusions may not hold in warmer sub-tropical or tropical environments – it would be worthwhile to include some detail about this

Reviewer #3 (Remarks to the Author):

Arnaud et al. compared saltmarsh organic matter decomposition across sea-level rise (SLR) scenarios, solely based on inundation duration, and litter types, finding little influence of SLR and more significant influence by litter type. Overall, I find this manuscript well written and novel, worthy of publication within this journal. The methods are very detailed, such that this study and the statistics used to analyze this data could easily be reproduced. However, there are a few issues (one major) that should be addressed, and I would like to see further evaluation of the data such that the authors highlight from this site why assuming homogeneity in biomass decomposition leads to errors in OM estimates. I feel this would then strengthen the conclusions of the manuscript and would influence how others will look at marsh OM decomposition in the future.

One major issue I had with this manuscript was with the SLR scenarios only incorporating the inundation impact of SLR and not the potential impact of increased marsh erosion, especially since the authors make multiple statements that SLR may not significantly impact litter OM decomposition. Increased erosion would significantly alter the decomposition of the litter and SOM.

I would also consider making further implications with this study, such as what methods should be included in future marsh OM studies in order to account for differential litter decomposition. Also, if you are assuming homogeneity in decomposition, then how much would this alter belowground OM estimates? Could you provide a case study based on your samples here? This would provide some guidance as to how different OM estimates would be.

Minor

Lines 81-83 – A reference should be included here.

Line 104 – This is the first time the acronym for sea level is used, it should be defined earlier. This also goes for SLR in line 105.

Line 112 and thereon – Terminology for discussion isotopic changes should be fixed. Discussion of isotopes should involve either enriched or depleted, not higher or lower. Also, try to keep the discussion of trends in the data consistent. The results get confusing to read when the authors keep switching how they discuss their trends.

Line 113 – First time Lig:PS is used but has not been defined previously.

Table 1 – Acronyms should be defined in the table caption. Additionally, -1 in the inundation duration needs to be made a superscript.

Line 131 and thereon – These headers are quite lengthy, can they be shortened/simplified?

Figure 2 – Variable pca is hard to read. Also, why are the leaves labeled as F on on the right when they are labeled as L in Table 3?

Figure 3 – Could you incorporate the significance letters like you did in Figure 1? Also, correct the 13C and 15N superscripts.

Figure 4 – Why not use the abbreviation for the lignin indices like you do in the text and tables?

Lines 290 and 295 – Again, use depleted or enriched when discussing changes in isotopic composition.

Line 310 – ecosystems is spelled wrong.

Lines 346-354 – Could this section be re-worded to say this is simulating increased inundation resulting from SLR rather than saying these row elevations correspond to SLR scenarios? Otherwise the wording can be a little confusing as to why you place higher SL scenarios at lower elevations.

Line 375 – I would imagine knowing the distance to the edge of the marsh would also be important here.

Version 1:

Decision Letter:

Dear Dr Arnaud,

Your manuscript titled "Salt marsh litter quality and decomposition under sea-level rise scenarios: from leaves to fine absorptive roots" has now been seen again by the 3 reviewers, and we include their comments at the end of this message. They agree that the manuscript has considerably improved, though Reviewer #1 has still a major concern that needs addressing. Specifically, in order to further consider your manuscript for publication we will need to be confident that the

statistical basis underlying the results is valid and robust. In addition, the two other Reviewers have identified some remaining minor issues that need your attention.

We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. Please highlight all changes in the manuscript text file.

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors adequately addressed most concerns, but they may not have fully grasped my concern regarding litter pooling. Any comparison of litter quality parameters (e.g., C/N, lignin) or decomposition rates between litter types using ANOVA (or other established statistical approaches) is, strictly considered, meaningless. This is because the most fundamental assumption of the statistical test—i.e., independence of observations—has been violated by the pooling process. For instance, what is the purpose of the ordination in Figure 2 showing low variability within litter types if all data really come from the same sample? There is only technical replication.

One option to address this point would be to focus the entire manuscript on abiotic (i.e., flooding) effects and only carefully assess and interpret litter-type effects while acknowledging the limitations. Another option would be to resample or reanalyze true replicates of the different litter types and thereby demonstrate their actual differences in composition.

Reviewer #2 (Remarks to the Author):

This clarity of this manuscript has been improved in its revisions and the authors have addressed my comments appropriately. I only have a few minor comments:
* Line 40: insert "an" before allocthonous
* Line 296: grain should be gain
* Line 298: others should be other
* lines 299-300: should read: "...such as an increase in sediment accretion, or to factors leading to an increase in organic matter accumulation..."
* Line 302: clarify what is meant by "Organic salt marsh" - does this refer to the litter or the sediment?
Line 316: replace "it is" with "they are"
Line 318-319: rewrite this sentence to avoid using contractions
Line 333-334: decomposition should refer to the litter that is sourced from the salt marshes; leave should be leaf
Line 335: delete the first "an"
Line 337: root should be roots

Reviewer #3 (Remarks to the Author):

The manuscript from Arnaud et al. has been improved considerably and I feel they have adequately addressed most of the comments/edits made by myself and the other reviewers. I only have several minor edits:

Line 115 and thereon (including tables) - you need to include units for the isotopic values

Table 2 caption - specifically mentioned standard deviations but I don't see standard deviations reported

Figure 3c - Why are there three significance letters above some of the litter types? Why not just have a and b?

Figure 4 - Since no significance letters are included, does this mean there are no significant differences? This contrasts with your table and with what you describe in the results.

Table 3 caption - you mention you use two statistical tests to look at the interaction of treatments and litter types based on whether they meet the ANOVA assumptions; however, by using two tests, you increase your chances of finding a significant differences and thus you would need to change your significance threshold to reflect this. Or you can stick to the one non-parametric test if multiple variables fail to meet the anova assumptions.

Line 262 - I think you mean low C:N here

Lines 266-270 - I understand you added this in response to one of the other reviewers but this statement feels random and unnecessary.

Line 274 - compared to which other proxies?

Line 292 and thereon - keep the use of acronyms consistent through the text (i.e. the use of SLR). Also "SLR sea-level rise" is redundant.

Line 293 - how is erosion an indirect effect?

Line 296 - elevation gain, not elevation grain

Line 297 - due to a *lack of modified rates

Line 301 - but shifting C allocation to different root functional types wouldn't help based on the data you present here, correct? If there's no difference in litter type, then why would this change anything?

Lines 308-330 - Could this all be integrated into the first discussion section? It doesn't make sense to return to the litter differences after already discussing this topic earlier.

Version 2:

Decision Letter:

Dear Dr Arnaud,

Your manuscript titled "Salt marsh litter quality and decomposition under sea-level rise scenarios: from leaves to fine absorptive roots" has now been seen by our reviewer, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment.

We therefore invite you to revise your paper one last time to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Editorial Board Member
Communications Earth & Environment

Joe Aslin
Deputy Editor,
Communications Earth & Environment
https://www.nature.com/commsenv/
Twitter: @CommsEarth

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have carefully addressed all of my remaining comments. I recommend publication of the paper.

Version 3:

Decision Letter:

Dear Dr Arnaud,

We are delighted to accept your manuscript titled "Salt marsh litter decomposition varies more by litter type than by extent of sea-level inundation" for publication in Communications Earth & Environment. Thank you for choosing to publish your interesting work with us.

We would like to encourage you to write a "Behind the paper" blog post for our Earth & Environment Community website. You will receive an invitation letter.

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Nadine Schubert
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Communications Earth & Environment

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Reviewer #1 (Remarks to the Author):

This is an interesting study assessing litter breakdown in a marsh organ study. I believe it is the first marsh organ study from Europe I am aware of. Very cool. While commendable, there are significant concerns regarding the experimental approach and data interpretation. I strongly advocate for a deeper engagement with existing literature on saltmarsh decomposition processes. Notably absent from consideration are pivotal studies such as Hemminga and Buth (1991, Vegetatio), which examined the decay of various plant tissue types across diverse marsh environments, including those featuring the same species utilized in this study.

Response: We thank the reviewer for the encouragements. We agree that this reference is central and very well appropriated to compare leaves and bulk root decay; we have now added it to support our findings at several places:

"Our results are nevertheless in accordance with previous findings showing that the above-ground organs of salt marsh plants decay faster than bulk roots[37]".

"The slower decomposition of fine absorptive roots compared to leaves could be attributed to the (high) lignin content of roots[37]"

It is unclear whether each litter type underwent independent replication, involving distinct samples of leaves, rhizomes, etc. from different specimen or locations within the marsh, or if all samples of a particular litter type were initially pooled and then distributed across experimental units / organ pots. In the latter scenario, statistical comparisons between litter types would be flawed due to a lack of true replication.

Response: We have now clarified in the revised manuscript the sampling strategy according to our objectives as this was unclear in the submitted version. We also justified our sampling approach, which was based on an initial pooling of the litter types. This has been carried out to avoid confounding effects of litter variations across different sampling locations. This approach is the most commonly used in the literature (as observed in the references compiled in the review of Ouyang et al., 2023; 2017). We focused on litter types and inundation effects and not on litter heterogeneity across sampling locations.

"We prepared 100 litterbags, including 25 filled with only leave litter, 25 only with fine absorptive root litter, 25 only with fine transportive root litter and 25 only with rhizomes litter. In each mesocosm, we buried 4 litterbags including one litterbag filled with leaf litter, one with fine absorptive root litter, one with fine transportive root litter and one with rhizome litter. We ensured that the litter collected from across our site was representative of our study area. We did not associate one location of litter collection to one mesocosm, because it could have potentially induced a confounding effect of litter location. Our focus was on testing the effects of different litter types and inundation treatments, not on litter variations within the site due to location. Nevertheless, the litter heterogeneity across our collection points was likely minimal, as all the collected litter came from *Halimione portulacoide* plants situated between the edge of the marsh and 25 meters inland, corresponding to a relatively small elevation gradient of less than 20 cm."

The authors argue that both the soil carbon balance and the vertical accretion of the system is driven by the balance of organic matter production and organic matter decomposition. This might not be true for their study site and for minerogenic saltmarshes in general. In these contexts, accretion is primarily driven by mineral sediment inputs, with allochthonous organic carbon often comprising a substantial fraction of the long-term preserved SOC stock. Consequently, the significance of litter breakdown in the studied system may be less pronounced compared to more organnic marsh or mangrove ecosystems elsewhere.

Response: We agree with the reviewer that organic salt marsh SOC stock includes a large portion of dead root materials; therefore, an alteration of root production and decomposition will disproportionally modify their soil surface elevations and resilience to sea level rise. In minerogenic salt marshes, that hold more mineral soils (like in our site), the elevation change is dominated by sediment inputs, but here as well, a change in soil surface elevation might occur if root production and decay are altered, notably under global changes.

We have now added in the method the total organic content of our soil and added clarification of this point in the discussion as follow:

"The total organic carbon content was 4% between the 0-10 cm depth in the sediment revealing the minerogenic nature of our site (unpublished results of Amann et al.)."

"Organic salt marsh includes a large portion of dead root materials; therefore, an alteration of root production and decomposition will disproportionally modify their soil surface elevations and resilience to sea level rise[48]. In salt marshes with more mineral soils, like in our site, the current elevation change is dominated by sediment inputs[49], but a change in soil surface elevation might occur if root production and decay are altered, notably under global changes[50], such accelerated sea level rise[46,51]."

Given the authors' frequent reference to terrestrial studies, a more nuanced interpretation regarding the implications of litter breakdown rates for soil carbon storage and sequestration rates is expected. It is crucial to recognize that the rate of litter breakdown is not directly linked to SOC formation. Rather, emphasis should be placed on understanding the fraction of litter that stabilizes in the soil, particularly within mineral-organic complexes. For insight into this aspect, Prescott's 2010 paper (Biogeochemistry) serves as a pertinent reference.

Response: We have now been through all the text and have corrected it to be more nuanced and accurate on the processes that we have investigated. We agree with the reviewer, that the soil organic matter is only partly composed of litter - that undergoes processes of decomposition and transformation into stabilised compounds-, as well as allochthonous inputs, microbial residues and other dead organic materials. During decomposition, the litter is first broken down into simpler compounds by microorganisms like bacteria, fungi, and other soil organisms. These microorganisms consume the litter releasing enzymes that break down complex molecules into simpler ones. After these initial processes, the organic material undergoes further transformation, leading to the formation of stabilised organic matter compounds though organo-mineral interactions. However, our study does not encompass this aspect. We have now made it clear throughout our manuscript.

In addition of the requested changes, we have added:

"Here, we define decomposition as all biological processes contributing to organic matter (OM) mass loss and transformation, in addition to leaching from litter only[60], and not consider stabilization processes that occurs in later phase."

We also discussed it in the discussion:

"Further efforts, should consider processes of OM stabilization in sediments, such as the association of OM with minerals[53]. Indeed, the litter is first broken down into simpler compounds by microorganisms like bacteria, fungi, and other soil organisms. The decomposition process results in a processing of most of the litter inputs to the soil[53]. The decomposition products may then undergoes process of stabilization, which was not addressed by our study."

specific comments in the pdf attached. To ease the answering of the comments, I have extracted the comments of the PDF and added reference to the lines in which the comments where added.

Abstract:

L25: Wrong species name. It is either Halimione portulacoides or Atriplex portulacoides

Response: Corrected to *Halimione portulacoides*. We also added in the introduction "*Atriplex portulacoides*" in bracket for clarity.

L26: Unclear what this means, please rephrase

Response: We have now reformulated as follow: "The OM decomposition rate varied only between the inundation treatments with the longest and shortest durations"

L30: this study is focussed on litter decomposition it cannot provide insight into SLR on effects on other OM pools such SOM

Response: We have now replaced by litter decomposition.

Introduction

L38-39: A number of recent studies have shown that particularly in minerogenic systems, such as the site your study was conducted, allochthonous OM inputs play a larger role for long-term C burial. Compare van de Broek et al. 2018 GCB

Response: Thanks for this very interesting study. We have now modified the introduction as follow:

A salt marsh C sink is largely a balance between primary production and the decomposition of autochthonous plant litter, as well as the inputs of C from allochthonous source (especially in mineral salt marshes)[3,4].

L42: please specify, do you refer to accelerated SLR here?

Response: Very good points! We have added "accelerated SLR" throughout the manuscript.

L43: Studies by Mueller et al. 2016 GCB and 2018 Biogeosciences on SLR effects should be considered here

Response: Thanks, we have now added those references.

L47: Other factor too. How about more higher sulfate availability (Morrissey et al. 2014 GCB) or changes in plant root activity controling priming effects (Mueller et al. 2016 GCB)?

Response: Agree, now modified as:

"Inundation might reduce OM degradation due to oxygen limitations, higher sulphate availability or changes in plant root activity[10-15]."

L65: Of litter not of OM in general.

Response: now replaced with litter.

L82: please provide support for this statement

Response: We have now provided a reference as follow: "We focused on the impact of accelerated sea-level rise represented by the inundation duration and frequency on the decomposition of leaf litter and contrasting root functional types, which are the main contributors to autochthonous blue carbon accumulating in salt marshes[4]."

Reference: [4]Alongi, D. M. Carbon Balance in Salt Marsh and Mangrove Ecosystems: A Global Synthesis. Journal of Marine Science and Engineering 8, 767 (2020).

L92: again, accelerated SLR?

Response: Very good points! We have added "accelerated SLR" throughout the manuscript.

Results

L118: Table 1: here is more detail needed. Does this refer the soil? If so, to which soil depth?

Response: We have now clarified in the text below the table, as well as on the Method section:

Note: [1]measured on four random dates across all mesocosms and measured as volumetric water content in a cylinder of sediment of 3 cm in diameter and 6 cm in length, n = 100. [2]measured at the marsh organ between May 4, 2021 and October 20, 2021. Please see Methods section for more information.

Method:
"We measured the volumetric water content of the soil using an ML3 ThetaProbe (± 1%, DeltaT Devices; Cambridge, UK) across all of the mesocosms at four random times (n = 100). The ML3 ThetaProbe measures the volumetric water content in a cylinder of sediment 3 cm in diameter and 6 cm in length."

L144: Figure 1: Please make clearer in which direction inundation duration increases. Also, instead of testing for diffs across 5 categories, I suggest to conduct regression analysis using inundation duration as a continuous predictor.

Response: We have modified the figure (see below). We have done a regression analysis and added the result, yet from the Figure below, we can observe that a linear regression will mask the heterogeneity of the processes – indeed between the SLR60 to SLI the mass loss is levelled off and then increased again. We have now added:

"We did a linear regression between mass loss and inundation as a continuous variable (as h d^{-1})."

"When treated as a continuous variable, the inundation treatment (as h d^{-1}) was not linearly related to the mass loss (r^2 = 0.005; p= 0.5)."

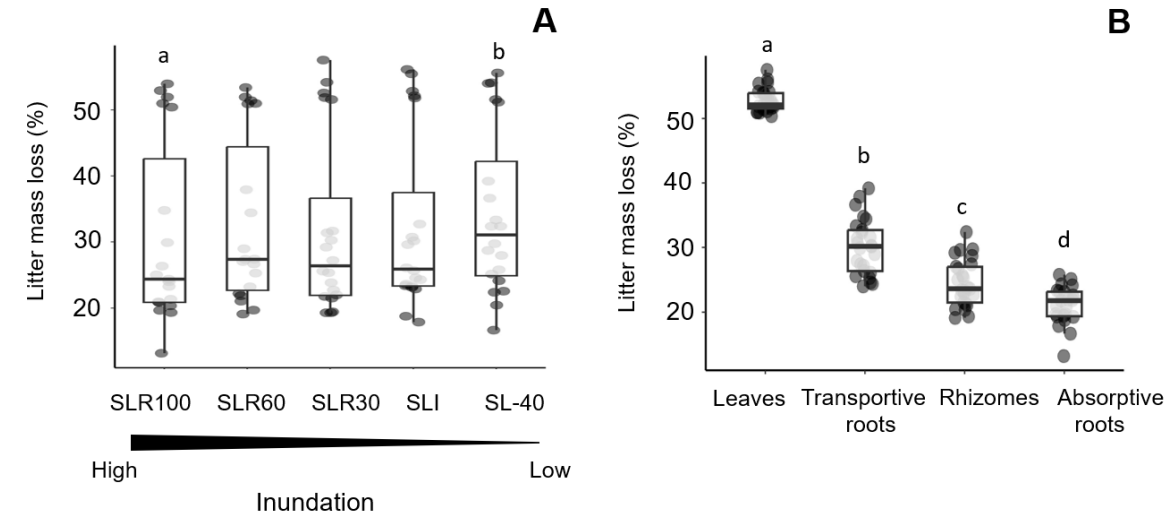

Figure 1. Response of the litter mass loss (%) to inundation regimes (A) and litter types (B) for *Halimione portulacoides* over the 170-day measurement period.

L165: lipids have a lower NOSC than lignins and are therefore more stable under anoxic conditions (Keiluweit et al. 2016 Biogeochemistry). Still they decomposed faster in your study. this is something you may want to discuss.

Response: We have now added in the discussion:

"Based on the thermodynamic driving force for the oxidation of OM as a function of nominal oxidation state of carbon (NOSC), the lipids should be more preserved than lignin under anaerobic conditions[38], which contrasts with our finding as leaves decomposed the fastest though mostly composed of lipids."

L196: Figure3: It is uncommon to use percentage change in C and N isotopes. Please refer to the common delta notations

Response: Here the percentage change is more meaningful to compare the degradation stage across litters, as each litter has different initial stable isotope ratios. Nevertheless, we have included the raw isotope value in supplementary table for reader who might want to have them.

L217: it is unclear what the statistical tests are

Response: We have now added in the legend the statistical tests we have used.

L228: I refer the authors to work by Hemminga and Buth 1991 (Vegetatio) and other studies by Hemminga conducted in salt marshes of Belgium and the Netherlands. They already describe that plant tissue type/part is the strongest predictor for decay rate. They even looked at lignin contents in a bunch of common species including H. portulacoides.

Response: Here we compare fine absorptive roots and leaves, yet Hemminga and Buth (1991) did not separate the roots by their function, therefore the reference is not appropriated here. Nevertheless, we agree with the reviewer that this reference is very appropriated to compare leaves and bulk root decays and have now added it at several places:

"Our results are nevertheless in accordance with previous finding showing that the above-ground organs of salt marsh plants decay faster than bulk roots[37]".

"The slower decomposition of fine absorptive roots compared to leaves could be attributed to the (high) lignin content of roots[37]"

L248: but not lipids

Response: We have now removed this sentence and replaced as follow:

"Based on the thermodynamic driving force for the oxidation of OM as a function of nominal oxidation state of carbon (NOSC), the lipids should be more preserved than lignin under anaerobic conditions[38], which contrasts with our finding as leaves decomposed the fastest though mostly composed of lipids."

L263: what about temperature artefacts?

Response: We have now added the following in the experimental design limitations:

The impact of shading on soil temperature may also affect litter decomposition in the experiments. To mitigate shading effects and ensure consistent sunlight exposure for each row, we positioned the elevation platform so that it faced southward[48].

L275: your study only investigates litter breakdown not OM decay in general

Response: We have now replaced OM by litter.

Conclusion/Discussion

L318: cn is not a biochemical parameter?

Response: We have now removed "biogeochemical parameter".

L320: litter decomposition does not capture stabilization, neither SOM decay

Response: We have now replaced OM decomposition by litter decomposition for clarity. Thanks.

L336: What are OM contents of the soil. This data would be needed to assess if organic matter input and stabilization is at all a relevant accretion driver of your marsh system. Furthermore, del13C and soil C/N should be used to assess the relative importance of autochthonous vs. allochthonous OM inputs to the soil system. This is needed to justify some of your implications stated above

L344: are autochthonous inputs at all relevant in minerogenic marshes? Compare Allen 2000 but also Reef et al. 2017 GCB.

Both comments above have been grouped together for the answer. Response: We did not assess all those soil properties unfortunately and there is no data available in the literature on it in our soil. Yet, from the field work and unpublished data from colleague, we could observe that while we had a large volume of surface soil occupied by roots, the site is mineral (4% of total organic carbon in 0-10 cm depth). Therefore, we have amended our method and discussion as follow:

"The total organic carbon content was 4% between the 0-10 cm depth in the sediment revealing the minerogenic nature of our site (unpublished results of Amann et al.)."

"Organic salt marsh includes a large portion of dead root materials; therefore, an alteration of root production and decomposition will disproportionally modify their soil surface elevations and resilience to sea level rise[48]. In salt marshes with more mineral soils, like in our site, the current elevation change is dominated by sediment inputs[49], but a change in soil surface elevation might occur if root production and decay are altered, notably under global changes[50], such accelerated sea level rise[46,51]."

L369: Was the litter collected from these cores kept separated and each marsh organ pot or treatment combination received an independent replicate or were litter materials mixed and then distributed acorss the pots? In the latter case, you would not work with statistically independent replicates for each litter type and you are not able to statistically compare litter types which each other.

Response: We have now clarified the sampling strategy as it was unclearly written.

"We prepared 100 litterbags, including 25 filled with only leave litter, 25 only with fine absorptive root litter, 25 only with fine transportive root litter and 25 only with rhizomes litter. In each mesocom, we buried 4 litterbags including one litterbag filled with leaf litter, one with fine absorptive root litter, one with fine transportive root litter and one with rhizome litter. We ensured that the litter collected from across our site was representative of our study area. We did not associate one location of litter collection to one mesocosm, because it could have potentially induced a confounding effect of litter location. Our focus was on testing the effects of different litter types and inundation treatments, not on litter variations within the site due to location. Nevertheless, the litter heterogeneity across our collection points was likely minimal, as all the collected litter came from *Halimione portulacoide* plants situated between the edge of the marsh and 25 meters inland, corresponding to a relatively small elevation gradient of less than 20 cm."

Reviewer #2 (Remarks to the Author):

This manuscript describes a study which assesses the decomposition of different types of organic matter in a saltmarsh environment under varying inundation scenarios which simulate sea level rise. The study separated organic matter sourced from saltmarsh plants into either roots or leaves. The roots where further separated into absorptive or transportive roots and rhizomes. The study finds that absorptive roots decomposed the slowest attributed to higher lignin content despite high N content; and leaves had the fastest decomposition. This is a novel study which improves our understanding of organic matter decomposition in blue carbon systems using an approach which has not been done previously.

Response: We thanks the reviewer for their encouraging comments!

It is an interesting and well conducted study. I suggest some modifications to clarify some points:
• It would be useful to include information about the soil characteristics including the particle size distribution, pH and EC

Response: We have now added the particle size. Unfortunately, we did not measure the soil pH and the electrical conductivity. Nevertheless, we have added the salinity of the water.

"The water salinity is around 32 ppt[54]. The sediments are dominated by silt (77%), followed by clay (12%) and sand (10%) at the surface and sand dominated in the deep sediment layers (unpublished results of Amann *et al.).*"

• Were the litterbags which contained the different types of organic materials buried in the same mesocosm ie. one litterbag of rhizomes, one litterbag of leaves, one litter bag each of the two root types? If so, how were edge effects in each mesocosm avoided if each litterbag was buried to 0.5 cm, with each mesocosm being 20 cm in diameter?

Response: Yes, in each mesocosm we buried four litter bags having one type of litter each. The litterbags were placed vertically in the sediments, and were not thick (may-be 0.5cm), so there was plenty of space between the litterbags and the mesocosms edge.

We have now clarified in the method section:

"We prepared 100 litterbags, including 25 filled with only leave litter, 25 only with fine absorptive root litter, 25 only with fine transportive root litter and 25 only with rhizomes litter. In each mesocosm, we buried 4 litterbags including one litterbag filled with leaf litter, one with fine absorptive root litter, one with fine transportive root litter and one with rhizome litter. "

"In each mesocosm, we buried four litter bags having one type of litter each. The litterbags were placed vertically in the sediments, and were not thick (< 1 cm), so there was plenty of space between the litter bags and the mesocosms edge."

• Duration of the experiment – why was a 6 month duration for litterbag burial chosen? It would be useful to include some justification, as the longer the experiment, the greater the amount of decomposition that will occur

Response: We choose the most active months for decomposition due to the limited duration of the funding to conduct this experiment. Nevertheless, our incubation time was sufficient to address our research questions. We have stated again the limitation in the method in the experimental part as follow:

"Our incubation time was limited and only represents the first phase of OM decomposition."

• Were there periods of extreme sea levels? Was the period of measurement typical of the long term means of this site (in terms of tidal range)

Response: There were no periods of extreme sea levels at the studied site during our measurement periods from 2020 to 2022 and in 2021, after classical successive patterns of spring and ebbing tide periods along the years. Tidal waters immersed the site only during a quarter of time during flooding tides in spring and autumn. Water height differences between 2020, 2021 (sampling period) and 2022 remained below 15 cm. The period of measurement in 2021 was therefore typical of previous years in terms of tidal range at the site. We have now added in this description in the method as follow:

"The period of measurement in 2021 was typical of previous years in terms of tidal range at the site[54]. There were no periods of extreme sea levels at the studied site during our measurement periods from 2020 to 2022 and in 2021 after classical successive patterns of spring and ebbing tide periods along the years[54] (based also on unpublished measurements). Tidal waters immersed the site only during a quarter of time during flooding tides in spring and autumn[54]. Water height differences between 2020, 2021 (sampling period) and 2022 remained below 15 cm[54] (based also on unpublished measurements)."

• Line 275-278: unclear sentence – please clarify. It would be useful to explain the process or include supporting references in each of the processes listed here

Response: Thanks for pointing that out. We have now clarified this part as follow:

"Our results imply that, with predicted sea-level rise, elevation grain through carbon accrual[5] is unlikely due to a modified OM decomposition rate. A potential elevation gain and resistance of the studied salt marsh to sea-level rise may therefore be restricted to others factors, such as an increase of sediment accretions, or to factors leading to an increase of organic matter accumulation, such as an increase in root production and life-expectancy, or a shift of C allocation to root functional types decomposing at a slower rate (i.e. fine absorptive roots)[45–47]."

• Line 283: while belowground litter is not a homogenous group and not uniform for decomposition, in practice and in the field, it behaves like a bulk organic matter – it would be useful to include some discussion on application of this new knowledge in terms of potential land or coastal management considerations, or carbon storage consideration

Response: We have now added some discussion about this topic:

"This implies that extrapolating litter decomposition from one single type of litter, often done with leaves as it is easier to collect, might not be representative of the overall bulk soil litter decomposition. Therefore, to accurately quantify the litter inputs to soil that are part of the carbon stock, the production, mortality, and decomposition of each litter type should be quantified."

"Global decomposition rate of salt marshes have been recently estimated to be $5.9 \pm 0.5 \times 10^{-3}$ d^{-1} using exclusively leaf litter measurements[17]. If we assume equal inputs of all litter types to the soil, using only the decomposition rate of leaves would artificially inflate the global decomposition rate by 1.7 times. Further litter decomposition studies should therefore incubate separately fine roots by orders or functions (i.e. absorptive vs transportive), and separate them from rhizomes and leaves to be accurate. Further, to accurately assess belowground organic matter dynamics, it's critical to determine decomposition, mortality, and production rates[44] for all litter types in salt marshes. This approach will help identify and quantify organic matter inputs that may persist longer in the sediments of organic salt marshes and/or may become stabilized in mineral salt marshes."

• Final conclusion point – this should include a caveat that this concluding point at this stage is relevant only for these temperate environments. The same processes and conclusions may not hold in warmer sub-tropical or tropical environments – it would be worthwhile to include some detail about this

Response: Actually, a study showed similar results for mangroves in the tropics. The tropical and subtropical salt marshes have nevertheless not been studied. We have now added those points in the discussion section as follow:

"Similar results have been found in mangroves[23], but so far, no such investigations have been carried out in tropical and subtropical salt marshes."

Reviewer #3 (Remarks to the Author):

Arnaud et al. compared saltmarsh organic matter decomposition across sea-level rise (SLR) scenarios, solely based on inundation duration, and litter types, finding little influence of SLR and more significant influence by litter type. Overall, I find this manuscript well written and novel, worthy of publication within this journal. The methods are very detailed, such that this study and the statistics used to analyze this data could easily be reproduced.

Response: We thanks the reviewer for their encouraging comments!

However, there are a few issues (one major) that should be addressed, and I would like to see further evaluation of the data such that the authors highlight from this site why assuming homogeneity in biomass decomposition leads to errors in OM estimates. I feel this would then strengthen the conclusions of the manuscript and would influence how others will look at marsh OM decomposition in the future. One major issue I had with this manuscript was with the SLR scenarios only incorporating the inundation impact of SLR and not the potential impact of increased marsh erosion, especially since the authors make multiple statements that SLR may not significantly impact litter OM decomposition. Increased erosion would significantly alter the decomposition of the litter and SOM.

Response: We agree that we studied only the impact of increased inundation, and not if sea level rise would accelerate erosion leading to a transfer of the OM to the open sea. We have now made this important point in the abstract, introduction, method and discussion:

Abstract:

"We compared salt marsh OM decomposition and quality across simulated sea-level scenarios **(by altering the duration and frequency of inundation)"**

introduction:

"SLR might increase the inundation duration and frequency, as well as induce coastal erosion[7]."

We have also discussed this point in the limitation of our study (method):

"Lastly, we measured the OM decomposition rate only and not the potential losses occurring through erosion, although SLR can lead to an increase of salt marsh erosion, and thus loss of OM[9]."

Finally, we have also highlighted in the discussion:

"The predicted accelerated SLR sea-level rise might nevertheless induce a loss of OM through in-direct effect such as salt marsh sediment erosion[9]".

I would also consider making further implications with this study, such as what methods should be included in future marsh OM studies in order to account for differential litter decomposition. Also, if you are assuming homogeneity in decomposition, then how much would this alter belowground OM estimates? Could you provide a case study based on your samples here? This would provide some guidance as to how different OM estimates would be.

Response: We thanks the reviewer for those suggestions. We have now added some methodological guidance and estimates. Yet, those estimates might not be fully accurate, as we do not know the proportion of each litter types to the soil.

"Global decomposition rate of salt marshes have been recently estimated to be $5.9 \pm 0.5 \times 10^{-3}$ d^{-1} using exclusively leave litter measurements[17]. If we assume an equal inputs of all litter types to the soil, using only the decomposition rate of leaves would artificially inflate the global decomposition rate by 1.7 times. Further litter decomposition studies should therefore incubate separately fine root by orders or functions (i.e. absorptive vs transportive), and separate them from rhizomes and leaves to be accurate. Further, to accurately assess belowground organic matter dynamics, it's critical to determine decomposition, mortality, and production rates[44] for all litter types in salt marshes. This approach will help identify and quantify organic matter inputs that may persist longer in the sediments and may become stabilized."

Minor

Lines 81-83 – A reference should be included here.

Response: We have now provided a reference as follow: "We focused on the impact of accelerated sea-level rise represented by the inundation duration and frequency on the decomposition of leaf litter and contrasting root functional types, which are the main contributors to autochthonous blue carbon accumulating in salt marshes[16]."

Reference: [16]Alongi, D. M. Carbon Balance in Salt Marsh and Mangrove Ecosystems: A Global Synthesis. Journal of Marine Science and Engineering 8, 767 (2020).

Line 104 – This is the first time the acronym for sea level is used, it should be defined earlier. This also goes for SLR in line 105.

Response: We have now defined the acronym early in the text and replaced everywhere sea level rise by SLR.

Line 112 and thereon – Terminology for discussion isotopic changes should be fixed. Discussion of isotopes should involve either enriched or depleted, not higher or lower. Also, try to keep the discussion of trends in the data consistent. The results get confusing to read when the authors keep switching how they discuss their trends.

Response: The isotope terminology has been fixed by using enriched or depleted when using raw data of the $\delta^{13}C$. We have now rewritten part of the results to make them consistent.

Line 113 – First time Lig:PS is used but has not been defined previously.

Response: We have now defined the acronym early in the text as follow:

"The loss of both C and N is also widely reported after decomposition, and analyses of stable isotope composition and lignin ratios (lignin-to-polysaccharide (LigPS)…"

Table 1 – Acronyms should be defined in the table caption. Additionally, -1 in the inundation duration needs to be made a superscript.

Response: We have now defined the acronyms and corrected the superscript as follow:

"Note: [1]measured on four random dates across all mesocosms and measured as volumetric water content in a cylinder of sediment of 3 cm in diameter and 6 cm in length, n = 100.

[2]measured at the marsh organ between May 4, 2021 and October 20, 2021. h d^{-1} is hour per day. SLI is the mean altitude of our site. SLR30 is the simulated SLR under SSP1-2.6. SLI60 is the simulated SLR under SSP3-7. SLR100 is the simulated SLR above predictions, and SL-40 represents a lower level of inundation than the mean inundation of our site. Please see Methods section for more information."

Line 131 and thereon – These headers are quite lengthy, can they be shortened/simplified?

Response: We have now shortened all the headers.

Figure 2 – Variable pca is hard to read. Also, why are the leaves labeled as F on on the right when they are labeled as L in Table 3?

Response: We have now labelled the leaves as L in the PCA. The variables are indeed hard to read, because they are so numerous. Enlarging them won't help, so we have now added a general name within the group at the top of them to make it clearer. We have also added in figure legend a reference to an appendix table, where all the pyrolysis products are given in details.

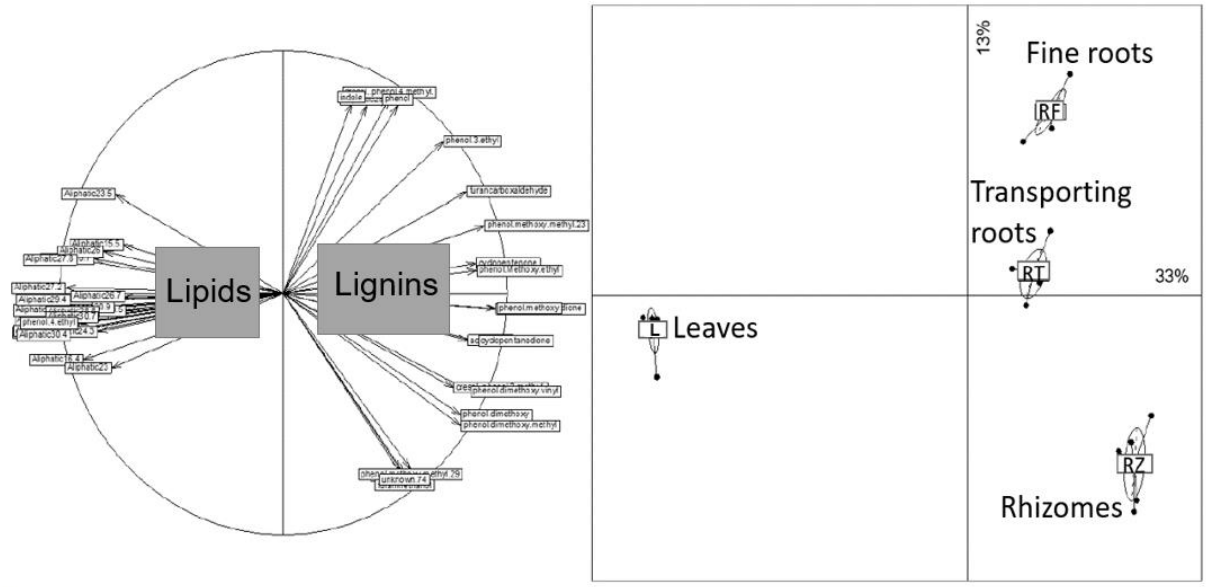

Figure 2. PCA from the results of the pyrolysis/GC/MS of the different types of OM for Halimione portulacoides. For a detailed lists of the pyrolysis products used in the PCA, please see Table SI2.

Figure 3 – Could you incorporate the significance letters like you did in Figure 1? Also, correct the 13C and 15N superscripts.

Response: We have now added the significance letters. Superscripts have now been corrected as follow:

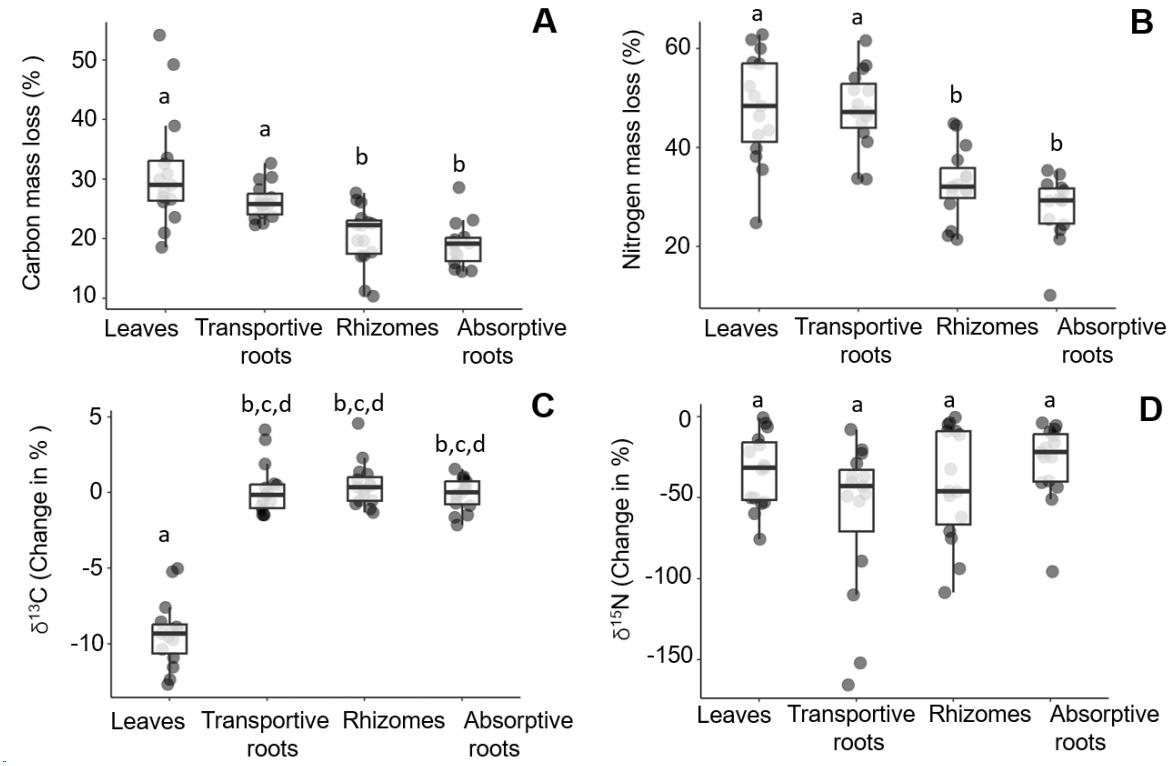

Figure 4 – Why not use the abbreviation for the lignin indices like you do in the text and tables?

Response: I have now used the abbreviations as suggested.

Lines 290 and 295 – Again, use depleted or enriched when discussing changes in isotopic composition.

Response: Now fixed.

Line 310 – ecosystems is spelled wrong.

Response: Thanks for pointing that, it is now corrected.

Lines 346-354 – Could this section be re-worded to say this is simulating increased inundation resulting from SLR rather than saying these row elevations correspond to SLR scenarios? Otherwise the wording can be a little confusing as to why you place higher SL scenarios at lower elevations.

Response: Thanks, we have now clarified as follow:

"We designed five elevations: the first mesocosm row was set at an elevation of 130 cm, corresponding to the mean altitude of our site (referred to as "SLI"), and the mean inundation of our site. The second mesocosm row was positioned 30 cm lower than the SLI, simulating increased inundation resulting from the projected SLR under SSP1-2.6 (low emission scenarios)[57]. The third mesocosm row was placed 60 cm lower than the SLI, simulating

increased inundation resulting from the projected SLR corresponding to the upper limit of the SSP3-7.0 (high emission scenarios)[57]. The fourth mesocosm row was established 100 cm lower than the SLI, simulating increased inundation resulting from the worst-case scenarios of SLR under SSP5-8.5.

Line 375 – I would imagine knowing the distance to the edge of the marsh would also be important here.

Response: Thanks, we have now added the distance to the edge of the marsh.

"All the collected litter came from *Halimione portulacoide* plants situated between the edge of the marsh and 25 meters inland, corresponding to a relatively small elevation gradient of less than 20 cm."

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors adequately addressed most concerns, but they may not have fully grasped my concern regarding litter pooling. Any comparison of litter quality parameters (e.g., C/N, lignin) or decomposition rates between litter types using ANOVA (or other established statistical approaches) is, strictly considered, meaningless. This is because the most fundamental assumption of the statistical test—i.e., independence of observations—has been violated by the pooling process. For instance, what is the purpose of the ordination in Figure 2 showing low variability within litter types if all data really come from the same sample? There is only technical replication.

One option to address this point would be to focus the entire manuscript on abiotic (i.e., flooding) effects and only carefully assess and interpret litter-type effects while acknowledging the limitations. Another option would be to resample or reanalyze true replicates of the different litter types and thereby demonstrate their actual differences in composition.

We thank the reviewer for their comments that have improved the accuracy of the manuscript. Below, we detail our response addressing their concerns.

We decided to follow the first option proposed by the reviewer. We have now carefully assessed and interpreted litter-type effects while acknowledging the limitations throughout the manuscript. In particular, we have added a paragraph on the method to justify our approach and highlight its potential limitations (e.g., a reduction of variability associated with litter quality location across the replicates). We have now explicitly stated our pooling method in the introduction and the results sections to be as transparent as possible for the reader. We have also carefully presented our results throughout the results section, including in figures and tables. We have now discussed the limitations and advantages of this method in the discussion section as well. We purposefully did not associate one plant from one location to one mesocosm because it could have potentially introduced a confounding effect of litter quality due to location. This pooling has been reported as important to "ensure there are no biases in root properties, for example, in relation to collection depth or edaphic conditions", for example linked to collection location (Halbritter et al., 2020). As we intended to test the influence of the edaphic environment (inundation) too, the incubated litter should be as homogenous as possible. Nevertheless, we agree that this pooling process might have homogenized the samples. We have modified the following:

- Added our method and its limitations in the **introduction section** as follows:

"For the comparison of litter types mass loss and chemistry, we used a recommended pooling method[34] to avoid bias due to plant location, which might have reduced the variability between replicate of each litter type. As we intended to test the influence of the edaphic environment (inundation), the incubated litter should be as homogenous as possible."

- In **the results section**:

We have now removed Figure 2. We have modified the title of Table 2 to state the limitations of our approach as follows:

"The data are based on triplicate analyses of each pooled sample of the litter type, which might have reduced the variability within each litter type and precluded firm conclusions about the results."

We have now clearly indicated that our results are based on pooled samples throughout the *Mass loss* and *Change of elemental composition, stable C and N isotope composition, and lignin ratios* sections of the results (see text highlighted in yellow)

We have now been very cautious in reporting the results of our analysis by modifying the title of Figure 1 as follows:

"The litter mass loss for each litter type is based on pooled litters from different locations, which might have reduced the variability across replicates."

We have also now modified the titles of Figure 2 and 3 by adding the following statement:

"The chemistry results for each litter type are based on pooled litters from different locations, which might have reduced the variability across replicates."

We have also now modified the title of Table 3 by adding the following statement:

"The statistical results per litter type are based on replicate made of pooled litters from different locations, which might have reduced the variability across the replicates."

- **In the discussion section**, we have now been very cautious about our results by stating:

"It is worthwhile to note that our findings on litter mass loss and chemistry across different types of litter, as well as findings from other studies, are often based on replicates made from pooled litters from different individuals. The use of standardized or pooled litter has been recommended to limit any bias due to litter quality as confounding factor[34,42]."

- **In the methods section**, we have now provided the rationale for our approach.

We purposefully did not associate one plant from one location to one mesocosm, as this could have potentially introduced a confounding effect of litter quality due to location. The approach of using standardized or pooled material in decomposition studies has been highly recommended in methodological papers to avoid such biases[34,42] and has been employed in many decomposition studies in saltmarshes[54] and in studies comparing different types of litter. Nevertheless, this approach reduces the variability across replicates, and this limitation of our study should be kept in mind when interpreting our results.

Reviewer #2 (Remarks to the Author):

This clarity of this manuscript has been improved in its revisions and the authors have addressed my comments appropriately.

We thank the reviewer for their constructive feedback, which has greatly helped us improve the manuscript.

I only have a few minor comments:
* Line 40: insert "an" before allocthonous

Response: We have now corrected the sentence; it should have been in plural form. Thanks for pointing that out!

* Line 296: grain should be gain

Response: corrected, thanks.

* Line 298: others should be other

Response: corrected, thanks.

* lines 299-300: should read: "...such as an increase in sediment accretion, or to factors leading to an increase in organic matter accumulation..."

Response: corrected, thanks.

* Line 302: clarify what is meant by "Organic salt marsh" - does this refer to the litter or the sediment?

Response: We have now clarified as follows:

“Organic salt marsh sediments include a large portion of…”

Line 316: replace "it is" with "they are"

Response: corrected, thanks.

Line 318-319: rewrite this sentence to avoid using contractions

Response: We have now rewritten the sentence as follows:

“The Lig:PS proxy strongly increased upon the decomposition of fine transportive roots and, to a lesser extent, of rhizome and fine absorptive roots. This indicates an increase in the lignin concentration of the litter during decomposition, consistent with findings for litter decomposition in terrestrial environments.”

Line 333-334: decomposition should refer to the litter that is sourced from the salt marshes; leave should be leaf

Response: corrected, thanks.

Line 335: delete the first "an"

Response: corrected, thanks.

Line 337: root should be roots

Response: corrected, thanks.

Reviewer #3 (Remarks to the Author):

The manuscript from Arnaud et al. has been improved considerably and I feel they have adequately addressed most of the comments/edits made by myself and the other reviewers.

We thank the reviewer for their constructive feedback, which has greatly helped us improve the manuscript.

Line 115 and thereon (including tables) - you need to include units for the isotopic values

Response: done, thanks.

Table 2 caption - specifically mentioned standard deviations but I don't see standard deviations reported

Response: We have now corrected this inconsistency.

Figure 3c - Why are there three significance letters above some of the litter types? Why not just have a and b?

Response: We have now corrected this error.

Figure 4 - Since no significance letters are included, does this mean there are no significant differences? This contrasts with your table and with what you describe in the results.

Response: We have now added the significance letters.

Table 3 caption - you mention you use two statistical tests to look at the interaction of treatments and litter types based on whether they meet the ANOVA assumptions; however, by using two tests, you increase your chances of finding a significant differences and thus you would need to change your significance threshold to reflect this. Or you can stick to the one non-parametric test if multiple variables fail to meet the anova assumptions.

Response: We have now used only the one non-parametric test – the results are similar. We have modified the legend accordingly.

Line 262 - I think you mean low C:N here

Response: We have now corrected this error.

Lines 266-270 - I understand you added this in response to one of the other reviewers but this statement feels random and unnecessary.

Response: We have now improved the flow of the sentence that it feels less random and unnecessary as follows:

“The lipids are believed to be more preserved than lignin under anaerobic conditions, which contrasts with our finding that leaves decomposed the fastest, despite being mostly composed of lipids.”

Line 274 - compared to which other proxies?

Response: We have now reformulated the sentence as follows:

“These differences might be related to the lignin composition, as the guaiacyl/syringyl lignin ratio (LigG:LigS proxy) was associated with the lower decomposition rate of fine absorptive roots compared to rhizomes and fine transportive roots.”

Line 292 and thereon - keep the use of acronyms consistent through the text (i.e. the use of SLR). Also "SLR sea-level rise" is redundant.

Response: corrected, thanks.

Line 293 - how is erosion an indirect effect?

Response: We have now corrected as follows:

“The predicted accelerated SLR might nevertheless induce a loss of OM through indirect effect, such as salt marsh sediment losses by erosion, which could otherwise retain OM[9].”

Line 296 - elevation gain, not elevation grain

Response: corrected, thanks.

Line 297 - due to a *lack of modified rates

Response: corrected, thanks.

Line 301 - but shifting C allocation to different root functional types wouldn't help based on the data you present here, correct? If there's no difference in litter type, then why would this change anything?

Response: I have now clarified as follows:

“If mangroves allocate more carbon to fine absorptive roots, these roots will constitute a larger proportion of the litter. Since fine absorptive roots decay slowly, their relative dominance in the litter will result in a slower overall decay rate, regardless of the level of inundation.”

Lines 308-330 - Could this all be integrated into the first discussion section? It doesn't make sense to return to the litter differences after already discussing this topic earlier.

Response: We agree that returning to the topic of litter differences at the end of the discussion is not ideal. We have now added this paragraph after the section on the "Effect of litter type on belowground OM decomposition rates." We did not integrate it into the first paragraph because it does not address the same topic precisely, and combining them would result in an overly long paragraph that mixes different subjects.