Author Response to Referee Comment 2 (RC2)

EGUSPHERE-2026-180

Desalinization-driven deep microbial reactivation destabilizes iron-bound carbon in coastal wetland restoration

Journal relation	Biogeosciences (via EGUsphere interactive discussion)
Comment being addressed	Public reply to RC2
Document purpose	Point-by-point response with revised-text excerpts for transparency. The full revised manuscript will be submitted only through the formal revision workflow.
Response status	Prepared on behalf of all co-authors

Opening response

We sincerely thank Referee #2 for the careful, thorough, and highly constructive review of our manuscript. We greatly appreciate the referee's positive evaluation of the importance of the research question, the value of the 0–100 cm profile perspective, and the potential relevance of the PM versus DT comparison for coastal wetland restoration and blue-carbon assessment. We are encouraged that the referee considers the manuscript promising and potentially publishable after major revision.

We also thank the referee for the thoughtful suggestions regarding the experimental framing, the interpretation of deep microbial reactivation, the treatment of amino-sugar / microbial necromass statements, the transparency of carbon stock calculations, the interpretation of PLS-PM, and the internal consistency of the manuscript. These comments are highly helpful and have clarified several important points that require revision. Below, we provide a point-by-point response and indicate how we will revise the manuscript accordingly.

General comment

Reviewer comment. The manuscript is promising and potentially publishable after major revision, but the distinction between what is directly shown by the data and what is hypothesized should be sharpened, and several internal inconsistencies should be resolved.

Response. We are grateful for this overall positive and constructive assessment. We appreciate the recognition that the depth-resolved framework and the comparison of PM and DT are meaningful strengths of the study. We agree that the current manuscript needs a clearer distinction between what is directly supported by the data and what remains interpretive or hypothetical. In the revised manuscript, we will improve this distinction throughout and carefully resolve the inconsistencies identified by the referee.

Major Comment 1. Experimental framing and design clarity

Reviewer comment. The design compares invaded CK with two post-removal treatments (PM and DT), so it does not cleanly isolate mulching or tillage effects alone. In addition, the meaning of "randomized complete block design" and the relationship between Fig. 1 and the sampling layout need to be clarified.

Response. We thank the referee for this important comment and agree that the experimental framing should be stated more precisely. As the referee correctly notes, the present study compares an unremediated invaded stand (CK) with two post-removal management treatments (PM and DT). Therefore, the design does not isolate the effect of mulching or tillage alone in a strictly factorial sense; rather, it compares distinct management trajectories following invasion and, in the case of PM and DT, vegetation removal. In the revised manuscript, we will explicitly acknowledge this limitation in the Methods and Discussion and will revise the interpretation accordingly. Specifically, we will clarify that PM and DT should be understood as post-removal management treatments, whereas CK represents the invaded, unremediated reference condition. We will also revise the wording in the Introduction and Discussion to avoid implying stronger treatment-specific attribution than the design permits.

In addition, we agree that the description of the experimental layout requires clarification. We will revise the Methods and Fig. 1 caption to define the sampling design more precisely, including the number of independent plots per treatment and the relationship between the field layout and the schematic shown in Fig. 1. We will also remove or revise any wording that may have caused confusion regarding the "randomized complete block design."

> **Revised text / action in manuscript.** "Here, we compared one invaded reference condition (CK) with two post-removal management treatments, plastic mulching (PM) and deep tillage (DT), 18 months after intervention. Accordingly, the study evaluates contrasting management trajectories rather than isolating the independent effect of mulching or tillage alone in a factorial design."

Major Comment 2. Deep microbial reactivation is not fully supported by the sequencing data presented

Reviewer comment. The hypotheses focus on deep soils (30–100 cm), but the bacterial sequencing results shown are only from 0–10 cm and 10–20 cm. Therefore, the manuscript should not infer deep community composition from shallow sequencing alone.

Response. We fully agree with the referee and appreciate this important clarification. In the current manuscript, the evidence for deep-soil microbial response is based primarily on the increase in microbial biomass C (MBC) in 30–100 cm soils under PM. However, as the referee correctly points out, the bacterial community sequencing results presented in the manuscript are limited to the 0–10 cm and 10–20 cm layers. Therefore, the manuscript should not infer deep microbial community composition or taxon-specific functions directly from shallow sequencing patterns.

In the revised manuscript, we will revise the wording throughout to ensure that the interpretation is fully consistent with the measured evidence. We will replace stronger statements about "deep microbial reactivation" or deep community restructuring with more cautious formulations centered on increased deep-soil microbial biomass and potential enhancement of microbial activity under desalinized conditions. We will also explicitly acknowledge in the Discussion that direct community-level evidence from 30–100 cm is not available in the present dataset and that deeper-layer sequencing or functional assays will be needed in future work.

> **Revised text / action in manuscript.** "PM was associated with a marked increase in microbial biomass C in 30–100 cm soils, suggesting enhanced microbial activity under profile-wide desalinization. However, because bacterial community sequencing was only conducted for the 0–20 cm layers, direct evidence for deep community restructuring remains unavailable in the present study."

Major Comment 3. Amino-sugar / microbial necromass statements are not supported by the methods and results shown

Reviewer comment. The abstract and conclusion refer to amino-sugar biomarkers and microbial necromass C, but these analyses are not described and reported in the main text.

Response. We sincerely thank the referee for identifying this inconsistency. We agree completely. The statements concerning amino-sugar biomarkers and microbial necromass C were inadvertently retained from an earlier manuscript draft and are not sufficiently supported by the methods and results presented in the current version.

To ensure full consistency between the Abstract, Methods, Results, and Conclusion, we will remove these statements from the revised manuscript. The revised version will focus only on the variables that are actually described and analyzed in the study, including soil physicochemical properties, carbon and nitrogen pools, iron fractions, Fe-bound organic carbon, microbial biomass, and bacterial community structure.

> **Revised text / action in manuscript.** Action taken: The amino-sugar / microbial necromass statements will be deleted from the Abstract and Conclusion.

Major Comment 4. Carbon stock calculation and transparency

Reviewer comment. The manuscript mentions both fixed-depth (FD) and equivalent soil mass (ESM) approaches, but only the FD equation is shown and the results mainly discuss SOCS-FD. In addition, SOC is estimated by LOI whereas TC is measured by elemental analysis; the rationale and transparency should be improved.

Response. We thank the referee for this very helpful comment. The stock values discussed in the current manuscript are based on the fixed-depth (FD) approach. We agree that the wording in the Methods was not sufficiently precise and may have incorrectly implied that both FD and ESM calculations were fully reported and interpreted in the manuscript.

In the revised manuscript, we will revise Section 2.5 to state explicitly that the stock values presented in the main text are FD-based, and we will remove wording that implies otherwise unless the ESM results are fully reported. We will also improve the description of the stock calculation procedure to make it more transparent.

Regarding the use of LOI-derived SOC and elemental-analyzer-derived TC, we agree that this distinction should be explained more clearly. In the revised manuscript, we will clarify that LOI-derived SOC was used for depth-integrated SOC stock estimation, whereas TC determined by elemental analysis was used to characterize overall carbon-pool changes. We will also add a supplementary worked example of the stock calculation to improve transparency.

> **Revised text / action in manuscript.** “The depth-integrated SOC stock values discussed in this study are based on the fixed-depth approach. We have revised the Methods to clarify this analytical basis and added a supplementary worked example to improve transparency in stock calculation.”

Major Comment 5. PLS-PM is interpreted too strongly as evidence of causality

Reviewer comment. The PLS-PM framework should be treated as inferential and heuristic, not as strong causal evidence, especially given the absence of pore-water chemistry, GHG fluxes, and direct Fe-reducing activity measurements.

Response. We fully agree with the referee. Our intent was to use PLS-PM as a framework for summarizing the covariance structure among restoration treatment, physicochemical conditions, reactive iron pools, microbial indicators, and carbon fractions. However, we acknowledge that in several parts of the manuscript the interpretation became overly causal.

In the revised manuscript, we will moderate the language throughout and present the PLS-PM results more clearly as inferential, heuristic, and conceptual support for the proposed linkages, rather than as direct proof of causality. We will also revise the Discussion to make the study limitations more explicit, particularly the lack of pore-water chemistry, direct greenhouse gas flux measurements, and direct evidence for iron-reducing activity. In addition, we will more clearly acknowledge that the observed carbon depletion could reflect a combination of mineralization, leaching/export, and/or redistribution processes.

> **Revised text / action in manuscript.** "The PLS-PM results should be interpreted as an inferential framework describing associations among desalinization, reactive iron pools, microbial indicators, and carbon fractions, rather than as direct evidence of causal process pathways."

Major Comment 6. Internal inconsistencies and overly dramatic wording

Reviewer comment. There are inconsistencies in figure references, variable labels, and significance statements, and some wording is too dramatic for a research article.

Response. We thank the referee for identifying these issues. We agree and will conduct a thorough editorial revision of the manuscript to correct all internal inconsistencies. Specifically, we will correct the figure references for MBC/MBN in Fig. 2, re-check the panel citations for Fed/Fep/Feo, and revise the alpha-diversity description so that the reported significance is internally consistent. We will also carefully revise the tone of the manuscript and remove overly dramatic phrases such as "catastrophic collapse" and "failure of the Iron Gate," replacing them with more neutral scientific wording.

> **Revised text / action in manuscript.** Action taken: All figure references, variable labels, significance statements, and overly dramatic wording will be systematically corrected in the revised manuscript.

Response to the referee's summary recommendations

In the revised manuscript, we will:

- clarify the experimental design and revise the wording around the "randomized complete block design";
- explicitly acknowledge that the study compares one invaded reference with two post-removal management treatments;
- restrict deep microbial interpretation to what is directly supported by measured evidence;
- remove the amino-sugar / microbial necromass claims from the Abstract and Conclusion;
- clarify the analytical basis of the stock calculations and improve transparency;
- moderate causal language related to PLS-PM and unmeasured mechanisms; and
- carefully check all figure references, variable labels, and significance statements.

Closing statement

Once again, we sincerely thank Referee #2 for this careful and constructive review. The comments have been highly valuable in helping us improve the clarity, rigor, and internal consistency of the manuscript. We believe that these revisions will substantially strengthen the study.

Sincerely,
On behalf of all co-authors