Review Report 1

Recommendation: Major revision

This manuscript addresses an important and timely question in coastal wetland restoration: whether commonly used *Spartina alterniflora* management practices have unintended consequences for deep soil carbon stability. The study is potentially valuable because it goes beyond the surface soil and examines a 0–100 cm profile, compares two restoration interventions (plastic mulching and deep tillage), and attempts to integrate hydro-salinity, reactive Fe fractions, microbial biomass, bacterial community composition, and path modeling into a mechanistic framework. The topic is relevant to restoration ecology, blue carbon accounting, and post-invasion management.

Overall, I find the manuscript promising and potentially publishable after major revision. The comparison between PM and DT is interesting, and the depth-explicit perspective is a clear strength. However, the current version has several important issues related to experimental interpretation, internal consistency, and the strength of mechanistic inference. In particular, I think the authors need to more carefully separate what is directly shown by the data from what is hypothesized, and they need to resolve several inconsistencies between the abstract, methods, results, figures, and interpretation.

Response:

We sincerely thank Reviewer for the constructive and detailed comments. We appreciate the reviewer's positive assessment of the depth-resolved framework and the comparison between plastic mulching and deep tillage. The comments helped us clarify the experimental framing, strengthen the evidence chain, reduce overinterpretation, and improve the internal consistency of the manuscript. We have revised the manuscript substantially, and our point-by-point responses are provided below.

My first major concern is the experimental framing. The study compares CK, PM, and DT 18 months after treatment, but CK is described as an unremediated invaded stand, whereas PM and DT both involve prior vegetation removal and subsequent intervention. As a result, the present design does not cleanly isolate the effect of mulching or tillage alone; rather, it combines invasive plant removal and post-removal management. This should be acknowledged more explicitly in the interpretation. Relatedly, the methods describe a "randomized complete block design," but the blocks are not clearly defined in the text. The figure caption also refers to "three sampling sites," whereas the methods describe five replicate plots per treatment. The experimental layout therefore needs to be clarified much more precisely.

Response:

We thank the reviewer for this important comment. We agree that our original description of the experimental design was imprecise. In the revised manuscript, we removed the term "randomized complete block design" and now describe the experiment as a comparative field design including three field management states: CK, unremediated *S. alterniflora* stands; PM, vegetation removal followed by plastic mulching; and DT, vegetation removal followed by deep tillage.We also clarified the hierarchical sampling structure. Within each management state, five independent 20 m × 20 m sampling plots were established as plot-level replicates, resulting in 15 plots in total. Within each plot, five soil cores were collected and composited by depth, yielding

75 composite soil samples.

We further acknowledge that the design compares integrated field management states rather than fully isolated single-factor effects. This limitation is now stated in both the Methods and Discussion. Specifically, we note that contrasts between CK and the two managed treatments include both vegetation removal and subsequent management effects. Because vegetation removal was common to PM and DT, their comparison remains useful for evaluating divergent belowground responses to two post-removal management strategies.We also revised the Fig. 1 caption to avoid the misleading phrase "three sampling sites." The caption now states that the map shows representative areas for the three field management states and that the five replicate plots within each management state are not individually shown.

My second major concern is that the manuscript's strongest mechanistic claims about **deep microbial reactivation** are not fully supported by the microbial community data presented. The hypotheses explicitly focus on deep soils (30–100 cm), yet the bacterial sequencing results shown in the paper are only for the 0–10 cm and 10–20 cm layers. The discussion nevertheless uses these surface/subsurface patterns to infer the identity and role of deep microbial groups under PM. In its current form, the manuscript demonstrates increased deep-soil MBC under PM, but it does not directly demonstrate how bacterial community structure changed in the deep layers themselves. This gap should be acknowledged much more clearly, and the mechanistic language should be softened.

A related issue is the handling of microbial necromass and amino sugars. The abstract and conclusion state that "amino-sugar biomarkers indicated coherent shifts in microbial necromass C with Fe–OC dynamics," suggesting an important line of evidence for the paper's interpretation. However, in the methods and main results provided here, I could not find a dedicated amino-sugar methods section, analytical description, or corresponding results presentation. If these data are central to the argument, they need to be fully described and reported. If not, these statements should be removed or greatly reduced.

Response:

We agree with the reviewer. In the revised manuscript, we substantially softened the interpretation of deep microbial reactivation and aligned the discussion with the measured variables. We now interpret the deep-soil response primarily as an increase in microbial biomass C, rather than as direct evidence of deep-layer bacterial community restructuring or functional activation.

We explicitly state in the Discussion that bacterial sequencing was conducted only for the 0–10 and 10–20 cm layers. Therefore, the increase in MBC in the 30–100 cm layers should be interpreted as evidence of increased microbial biomass rather than direct evidence of changes in deep-layer bacterial community composition or function.

We also revised terms such as "reactivate deep-soil microbial communities," "activated deep microbiome," and "metabolic awakening" to more cautious expressions such as "increased deep-soil microbial biomass," "was associated with," and "may have reduced osmotic limitation." In addition, we removed statements that inferred deep microbial taxa from the shallow sequencing data and added a future research statement calling for depth-resolved sequencing, metagenomics, and activity assays.

About the microbial necromass and amino sugars we thank the reviewer for identifying this issue. We agree that the previous statement regarding amino-sugar biomarkers was unsupported in the current manuscript. Because amino-sugar biomarkers were not described in the Methods and were not reported in the Results, we removed this line of evidence from the Abstract and Conclusion. The original sentence referring to amino-sugar biomarkers and microbial necromass C has been deleted. The revised manuscript now states more cautiously that the coupled declines in Feo and Fe–OC suggest a potential weakening of Fe-mediated protection of subsoil C under rapid desalinization.

My third major concern is the carbon stock calculation and the interpretation of net carbon loss. The methods state that SOC stocks were calculated using both fixed-depth and equivalent soil mass approaches, but only the fixed-depth equation is shown, and the results section mainly discusses SOCS-FD. If bulk density differs among treatments, the ESM approach becomes especially important and should either be reported clearly or removed from the methods. In addition, SOC is estimated by loss-on-ignition whereas TC is measured by elemental analysis; the manuscript would benefit from a stronger justification of why LOI-based SOC is appropriate for these estuarine wetland soils and how the authors ensured comparability between the different C metrics. A supplementary worked example of the stock calculation would also improve transparency.

Response:

We agree with the reviewer and have revised the carbon stock calculation section. We removed the statement that both fixed-depth and equivalent soil mass approaches were used. The revised Methods now state that SOC stocks were calculated using the fixed-depth approach. We also explicitly note that, because management practices, particularly deep tillage, may alter bulk density and soil mass, these estimates should be interpreted as fixed-depth comparisons rather than equivalent-soil-mass-corrected stock estimates. We also added clarification regarding the use of LOI-derived SOC. Specifically, we now state that LOI-derived SOC was used as an operational estimate of organic C and interpreted separately from elemental-analyzer-derived TC. We therefore treat TC and SOC as complementary C metrics rather than directly interchangeable measurements.

My fourth major concern is that the PLS-PM analysis is interpreted too strongly as evidence of causality. The authors use PLS-PM to argue that PM altered physicochemical stress, destabilized reactive Fe pools, and thereby amplified microbial decomposition and SOC/Fe-OC loss. This is a reasonable conceptual model, but the data remain observational within a single post-treatment sampling campaign. The discussion itself acknowledges that there are no pore-water chemistry data, no direct GHG fluxes, and no direct measurements of iron-reducing activity, so the present evidence is not sufficient to distinguish microbial mineralization from leaching/export or redistribution processes. I therefore encourage the authors to treat the path models as inferential and heuristic rather than strongly causal.

Response:

We thank the reviewer for this important suggestion. We have revised the manuscript to avoid treating PLS-PM as direct causal evidence. In the Methods, we changed the description of PLS-PM to state that it was used to explore hypothesized direct and indirect associations among management treatments, soil physicochemical properties, reactive Fe pools, microbial variables, and C fractions. In the Results and Discussion, we replaced causal wording such as "directly destabilized," "drove," "fueled," and "amplified microbial decomposition" with more cautious wording such as "was associated with," "covaried with," and "was consistent with the hypothesized pathway." We now explicitly state that the path relationships should be interpreted as exploratory associations rather than direct causal evidence. We also added discussion of alternative mechanisms, including microbial mineralization, dissolved Fe–organic export, and within-profile redistribution, and clarified that these cannot be distinguished without direct measurements of pore-water DOC, Fe(II)/Fe(III) speciation, CO_2/CH_4 fluxes, and lateral hydrological export.

The manuscript also contains several internal inconsistencies that should be corrected before further consideration. For example, the methods describe figure 2h as MBC and 2i as MBN, but the results text refers to deep-soil MBC increases in Fig. 2i. Likewise, the text discussing Fed and Fep appears to cite figure panels inconsistently relative to the figure caption. In the alpha-diversity paragraph, the text states that PM reduced Shannon and Chao1 "($P < 0.05$)" but then says "although no significant differences were detected," which is contradictory. I also suggest revising some of the more dramatic wording, such as "catastrophic collapse" and "failure of the Iron Gate," to better match the tone expected in a research article.

Response:

We carefully checked and corrected the figure references, variable labels, and significance statements throughout the manuscript. MBC is now consistently cited as Fig. 2h and MBN as Fig. 2i. Fe-related variables are now cited according to the revised Fig. 3 order: Fe–OC, SOCS-FD, Fep, Fed, Feo, and f_{Fe_OC}. We also corrected the alpha-diversity statement. The revised text now states that alpha diversity metrics tended to be lower under PM, but the differences were not statistically significant. In addition, we removed overly dramatic phrases such as "catastrophic collapse" and "failure of the Iron Gate." These have been replaced by more measured wording, such as "potential weakening of Fe-mediated C protection" and "reduced Fe-associated C protection." Once again, we thank the experts for their valuable review comments. Once again, we thank the experts for their valuable review comments.

Despite these concerns, I think the manuscript has several strengths. The depth-resolved framework is important, the comparison of PM and DT is potentially informative for management, and the authors are asking a meaningful question about whether restoration practices may mobilize legacy subsoil C. With substantial revision, particularly in clarifying design, tightening the evidence chain, and improving internal consistency, this study could make a useful contribution.

Specific suggestions for revision

1. Clarify the experimental design and the meaning of "randomized complete block design," including how many independent plots were sampled and how the layout in Fig. 1 corresponds to the methods.

Response: Revised as described above. The Methods now describe a comparative field design with three management states and five independent 20 m × 20 m plot-level replicates per management state.

2. Explicitly acknowledge that the design compares invaded CK with two post-removal

management treatments, which limits strict attribution of treatment-specific effects.

Response: We added this limitation in both the Methods and Discussion.

3. Revise the deep microbial interpretation so that it is based on measured evidence; avoid inferring deep community composition from shallow sequencing alone.

Response: We now base the deep microbial interpretation on measured MBC and explicitly state that deep-layer community composition was not directly measured.

4. Either fully describe and report the amino-sugar/necromass analysis or remove those claims from the abstract and conclusion.

Response: We removed the unsupported amino-sugar and microbial necromass statements from the Abstract and Conclusion.

5. Report the equivalent soil mass approach if it was actually used, and provide more transparent stock calculations.

Response: We now report only the fixed-depth approach and clarify that these are fixed-depth comparisons rather than ESM-corrected estimates.

6. Tone down causal language around PLS-PM and around unmeasured mechanisms such as Fe reduction, mineralization, and lateral export.

Response: We revised PLS-PM and mechanism-related language throughout the manuscript and now treat the models as exploratory and inferential.

7. Carefully check all figure references, variable labels, and contradictory significance statements.

Response: We corrected the Fig. 2h/2i, Fe-related panel references, and alpha-diversity significance statement. We also revised figure captions and variable labels.

We sincerely thank Reviewer 1 for the thoughtful and constructive comments, which substantially improved the clarity, rigor, and balance of the manuscript.

Review Report 2

Manuscript Title: Desalinization-driven deep microbial reactivation destabilizes iron-bound carbon in coastal wetland restoration **Journal:** EGUsphere (Preprint)

General Comments

This manuscript investigates the deep-soil biogeochemical responses to *Spartina alterniflora* eradication, comparing plastic mulching (PM) and deep tillage (DT) over an 18-month period. The study's evaluation of the 0-100 cm soil profile addresses a relevant gap in coastal wetland restoration assessments, which often restrict their focus to the top 30 cm. The finding that PM-induced desalinization increases deep-soil microbial biomass and destabilizes iron-bound organic carbon (Fe-OC) provides useful mechanistic insights into subsoil carbon dynamics. Overall, the manuscript presents valuable data. However, several areas require further clarification and deeper discussion before it can be recommended for publication.

Response: We sincerely thank Reviewer 3 for the constructive comments and for recognizing the value of the 0–100 cm soil profile assessment. The comments helped us strengthen the discussion of C–N coupling, clarify the fate of destabilized C, and better define the temporal scope of our 18-month study. We have revised the manuscript accordingly.

Major Comments

1. **Coupling of Carbon and Sediment Nitrogen Transformations** The manuscript details the depletion of soil carbon pools, noting that PM reduced total carbon (TC) by 19-35% and soil organic carbon (SOC) by 34-65%. Concurrently, the results indicate a significant restructuring of the nitrogen pools, with PM causing a 51%-74% loss in total nitrogen (TN) alongside altered distributions of ammonium and nitrate (Figure 2e-g). Given the stoichiometric coupling of carbon and nitrogen cycles in coastal wetland sediments, the impact of *Spartina alterniflora* management on sediment nitrogen transformation warrants more detailed discussion. Elaborating on how these shifts in inorganic nitrogen availability might regulate or interact with the deep microbial reactivation would strengthen the mechanistic interpretation of the observed decomposition dynamics.

Response: We thank the reviewer for this important suggestion. We added a new Discussion section entitled "Coupled C and N responses under post-removal management." In this section, we discuss the concurrent declines in TC, SOC, and TN under PM and the altered vertical distributions of NH_4^+ and NO_3^-. We now interpret these changes as evidence that C depletion was accompanied by broader sediment nutrient reorganization. We also discuss how changes in inorganic N availability may influence microbial biomass and organic matter decomposition by modifying microbial nutrient demand, C:N balance, and resource acquisition strategies. At the same time, we clearly acknowledge that rates of N mineralization, nitrification, denitrification, DNRA, and microbial assimilation were not measured. Therefore, the N responses are interpreted as coupled indicators of sediment biogeochemical reorganization rather than direct evidence of altered N cycling processes.

2. **The Fate of Destabilized Carbon** The study reports a net soil C decline of 65 ± 12 Mg C ha^{-1} over 18 months under the PM treatment. As acknowledged in Section 4.2, the absence of pore water chemistry data makes it difficult to definitively partition the fate of the lost Fe-OC between complete microbial mineralization to greenhouse gases (CO_2 / CH_4) and the lateral export of dissolved Fe(II)-organic complexes via leaching. Consequently, references to "climate impacts" or "carbon emissions" in the introduction and abstract should be carefully moderated. Framing the results strictly around carbon "destabilization and potential loss" will align the conclusions more closely with the measured variables and avoid overextrapolating the findings.

Response: We agree with the reviewer. We revised the Abstract, Introduction, Discussion, and Conclusion to avoid overinterpreting C stock declines as confirmed atmospheric C emissions. Terms such as "net C loss," "carbon emissions," and "climate impacts" were replaced or moderated using phrases such as "potential C stock loss," "apparent fixed-depth C stock decline," and "potential C destabilization." We also revised the Discussion to explicitly state that, because we did not measure pore-water DOC, Fe(II)/Fe(III) speciation, CO_2 or CH_4 fluxes, or lateral hydrological export, we cannot determine whether the observed Fe–OC decline resulted primarily from microbial mineralization, dissolved export, or within-profile redistribution. We removed the regional CO_2 -equivalent extrapolation and the comparison with emissions from a mid-sized city. The revised manuscript now frames the result as potential C destabilization rather than confirmed emissions.

3. **Temporal Limitations** The 18-month timeframe captures the initial perturbation responses to the restoration interventions. Ecological restoration is a long-term process, and it remains uncertain whether these soil carbon losses will stabilize, decelerate, or continue over time. This temporal limitation should be explicitly stated earlier in the abstract and introduction to establish

appropriate scientific expectations for the temporal scope of the findings.

Response: We thank the reviewer for this helpful comment. We now state more clearly that the study represents an 18-month field assessment and captures the initial response to post-removal management. We added this temporal framing in the Abstract and Introduction, and expanded it in the Discussion section on limitations and future research. Specifically, we now state that multi-year monitoring is needed to determine whether the observed C stock declines stabilize, decelerate, or reverse as vegetation recovery, hydrological conditions, and sediment biogeochemistry develop over time. This revision clarifies the temporal scope of our findings and avoids implying a long-term trajectory from a single 18-month sampling campaign.

Minor Comments

- **Abstract Clarification:** The abstract states that "Amino-sugar biomarkers indicated coherent shifts in microbial necromass C with Fe-OC dynamics". However, the methodology and results for these amino-sugar biomarkers are absent from the main text and methods section. Please include the relevant analytical protocols and data, or remove this statement if it falls outside the scope of the current manuscript.
- **Response:** We thank the reviewers for pointing out these important issues. We agree that the previous abstract included an unsupported statement regarding amino-sugar biomarkers and microbial necromass C. Because amino-sugar biomarkers were not described in the Methods and were not reported in the Results, we have removed this statement from the Abstract and Conclusion to avoid introducing an unsupported line of evidence.
- **Carbon Stock Calculations:** Section 2.5 mentions using both fixed-depth (FD) and equivalent soil mass (ESM) approaches to calculate SOC stocks. While the FD formula is provided (Equation 2), the results section must explicitly specify which method is being utilized when discussing the carbon stock depletions. Bulk density changes resulting from treatments like deep tillage (DT) can significantly skew interpretations if only fixed-depth calculations are applied without clear context.
- **Response:** We revised Section 2.5 to state that SOC stocks were calculated using the fixed-depth approach. We removed the statement that the equivalent soil mass approach was used. We also added a caution that the resulting stock estimates should be interpreted as fixed-depth comparisons rather than equivalent-soil-mass-corrected estimates, particularly because deep tillage may alter bulk density and soil mass.
- **Figure Clarity:** Ensure the resolution of Figure 1 is sufficient in the final production stage so that the text detailing the core schematic and measurement purposes is clearly legible.
- **Response:** We thank the reviewer for this comment. We revised the Fig. 1 caption to clarify the sampling design and will ensure that the final submitted figure has sufficient resolution for all text, schematic elements, and sampling information to be clearly legible. The caption now also clarifies that the map shows representative management areas and that the five replicate plots within each management state are not individually shown. We sincerely thank Reviewer 3 for the valuable comments, which helped us improve the mechanistic balance, temporal framing, and management relevance of the manuscript.