Response to Reviewer Comments for Manuscript

Carbon dioxide release driven by organic carbon in minerogenic salt marshes

egusphere-2025-4621

We would like to thank the reviewers for their thoughtful comments and feedback. We revised the manuscript thoroughly and the changes are provided here: comments by the editor and reviewer in normal front, our reply (author comments) in *italic* front, and the Revised/added text in green. All line numbers in the revised text refer to the revised manuscript.

Editorial support team: Section "CRediT authorship contribution statement": please use initials for the names of the authors.

Author comments: *As request by the editorial support team, we have revised the "CRediT authorship contribution statement" so that the author names are listed using initials instead of full names.*

CRediT authorship contribution statement

NK: Investigation, Methodology, Formal Analysis, Visualization, Writing – Original Draft Preparation, Conceptualization. FR: Investigation, Writing – Review & Editing. LJK: Methodology, Writing – Review & Editing. RK: Methodology, Writing – Review & Editing. AK: Writing – Review & Editing, Funding Acquisition. PJ: Conceptualization, Funding Acquisition, Supervision, Project Administration, Writing – Review & Editing.

Reviewer 1:

Reviewer comment 1

This manuscript presents a well-designed and timely study investigating controls on organic carbon (OC) decomposition and CO_2 release in minerogenic salt marsh sediments of the Wadden Sea. The work addresses an important gap in the blue carbon literature, which has historically focused on peat-dominated North American marshes. The combination of detailed geochemical characterization with an in situ organic carbon manipulation experiment is innovative and provides new insight into the relative roles of electron acceptors and OC quality in regulating greenhouse gas dynamics.

The manuscript is generally well written, logically structured, and well-grounded in relevant biogeochemical literature. The conclusions are consequential and should interest the coastal biogeochemistry, blue carbon, and wetland modelling communities.

Author comments: *Thank you for the positive feedback!*

Reviewer comment 3

However, several aspects of the experimental design, interpretation, statistics, and framing require clarification or strengthening before publication. Some assumptions remain insufficiently justified, and a few conclusions appear stronger than the presented evidence supports.

The conclusion that electron acceptor availability, particularly sulphate, does not limit organic carbon (OC) decomposition is not fully supported by the data presented. First, the study relies solely on concentration profiles of sulphate, Fe(II), and O_2, but concentrations alone cannot indicate whether terminal electron acceptors (TEAs) were saturating or limiting for microbial respiration; resolving TEA limitation requires information on rates of sulphate and iron reduction or O_2 fluxes, which were not measured. Additionally, the OC addition experiment demonstrates stimulation of CO_2 production by acetate but does not distinguish whether this reflects true OC limitation or an unquantified shift in TEA-dependent respiration pathways, since no sulphate or iron-reduction rate measurements were performed to assess potential changes in electron acceptor turnover. To strengthen the conclusion, the authors should either moderate their claim or provide additional justification such as discussion of expected TEA turnover dynamics in minerogenic marshes, reaction–transport considerations, or clear reasoning as to why concentration data alone can rule out TEA limitation. As written, the conclusion overstates what can be confidently inferred from the available evidence and should acknowledge these uncertainties.

Author comments: *The reviewer brings up an important and valid point. It is true that our conclusion in only based on concentrations and no O_2, Fe(III), SO_4^- turnover rates were determined. Based on different lines of evidence, we concluded that TEAs likely did not limit OC decomposition: First, SO_4^{2-} remained high throughout the sampled depth and the sulfate:chloride ratio stayed stable (only a slight decrease was observed in the intertidal flat). This indicates no strong SO_4^{2-} depletion with depth. We considered whether the lack of observed decrease was due to continuous resupply of SO_4^{2-} with infiltrating tidal water. However, the fine particle size of the sediment suggests that tidal water does not percolate completely through the sediment, even over multiple tidal cycles. Thus, we should have been able to observe SO_4^{2-} consumption due to microbial reduction if occurring.*

Second, the OC addition experiment showed a clear response due to the addition of labile OC (acetate). We agree that these results cannot exclude potential shifts in TEA respiration pathways; however, the rapid increase of CO_2 release after addition of acetate (in some injection cycles already 1.5h after addition) combined with no depletion of SO_4^{2-} or observed changes in TEA availability suggest that microorganisms capable of utilizing acetate were already present in the sediment, and is consistent with electron donor limitation rather than electron acceptor limitation.

Third, porewater and solid-phase respiration end products e.g., Fe(II) and/or sulfide levels give evidence that Fe(III) and SO_4^{2-} reduction was occurring. This suggests that these electron

acceptors were available and utilized. Furthermore, the high SO_4^{2-} concentrations may suppress methane (CH_4) formation. The absence of CH_4 therefore indicates that electron acceptors were not depleted, as a thermodynamical more favorable electron acceptor was still available.

In summary, we suggest that there are indications of an electron donor limitation. We agree that the absence of rate measurement introduces uncertainty, and we will add text in the conclusions (line 727-730) describing that our data do not fully exclude TEA depletion but are consistent for OC limitation, in addition to softening statements at different points in the text.

Revised/added text (line numbers in revised manuscript):

Line 22-24: Overall, we found that the microbially mediated CO_2 release was likely limited by OC availability and composition, and electron acceptor availability was unlikely to be the primary limiting factor, as evidenced by the presence of aqueous sulfate (SO_4^{2-}) at all tested depths and the lack of detectable CH_4.

Line 590-592: Collectively, due to the occurrence of Fe(III) reduction (especially in the upper sediment layers) and the availability of SO_4^{2-} throughout the sediment, we suggest that electron acceptor availability likely did not limit microbial OC decomposition in our study.

Line 599-601: Based on the availability of electron acceptors (e.g., SO_4^{2-}) at all depths and the lack of detectable CH_4, we hypothesize that at our field site and other comparable coastal sites, OC is likely the constraint on microbially mediated CO_2 release and that electron acceptors are likely not a limiting factor.

Line 727-730: We caution here that we did not directly measure TEA reduction rates. Future studies should investigate turnover rates, potentially utilizing isotopes to confirm this finding. Overall, our results indicate that the OC composition, rather than the concentration alone, controlled CO_2 release in both succession zones. This suggests that OC composition likely plays a limiting role in microbially mediated CO_2 release from minerogenic salt marshes.

Reviewer comment 3

The interpretation that methanogenesis is absent, and therefore that electron acceptors are non-limiting, relies heavily on the lack of detected CH_4 in porewater and flux measurements. However, several alternative explanations for CH_4 absence are not considered. Methane produced at depth can be rapidly consumed by anaerobic oxidation of methane (AOM), or physically flushed from the sediment via tidal exchange before detection. In addition, the manuscript does not report detection limits for dissolved CH_4 or flux measurements, making it unclear whether low but non-zero methanogenesis could have gone undetected. To strengthen the argument, the authors should discuss these potential CH_4 sink processes and provide analytical detection limits or moderate the conclusion to reflect these uncertainties, as the current data cannot rule out methanogenesis or CH_4 cycling within the system.

Author comments: *The reviewer raises an excellent point about the lack of methane. We agree that we did not discuss potential reasons for this lack of CH_4 in the porewater in the original manuscript. The reviewer is right - the absence of detectable CH_4 alone cannot definitively exclude methanogenesis within the sediment. We examined different lines of reasoning to determine if CH_4 was being produced and then consumed at the depths we investigated.*

First, we observed no CH_4 in the porewater over depth (until 50 cm) over multiple field campaigns (in this study as well a previous study at this site (Kubeneck et al., 2025)). We also conducted measurements of emitted CH_4 using headspace chambers from the sediment in a separate experiment in 2022, with resulting CH_4 concentrations similar to those in ambient air (Table S3). Second, anaerobic oxidation of methane (AOM) with SO_4^{2-} requires a 1:1 stoichiometry; this implies that SO_4^{2-} should show a decrease with depth if CH_4 was being oxidized. In the case of the pioneer marsh, we saw no change in the SO_4^{2-} concentration over depth, while in the case of the tidal flat, a slight decrease (< 5%) could be observed (Figure 2). Some studies conducted at the Wadden Sea have detected CH_4; however, CH_4 was present only at depths where SO_4^{2-} was largely depleted (Røy et al., 2008; Wu et al., 2015). *Hence, we suggest that methanogenesis, and consequently AOM, if any, may could occur at depths below 50 cm in the intertidal flat, provided that labile OM is available at that depth.*

Although we did not measure CH_4 in any of our measurement campaigns, we acknowledge that it is possible that some CH_4 was physically flushed out by tides. Furthermore, we thank the reviewer for the suggestion about reporting the detection limits for CH_4.

We would like to note here that our ongoing work focuses on characterizing the microbial community in the sediments, which will provide further insight into the question of methanogenesis.

We will revise the manuscript by adding the detection limit and discussing possible reasons for the absence of detected CH_4.

Revised/added text:

Line 24: … at all tested depths and the lack of detectable CH_4.

Line 312-313: In both the pioneer marsh and intertidal flat, no CH_4 release, neither as fluxes or in the porewater up to a depth of 50 cm, was detected (detection limit: 0.28 and 0.53 ppm respectively; Table S3).

Line 381-832: In all treatments and the control, no CH_4 as a flux was detected (lower than detection limit (0.28 ppm); Table S3).

Line 492-493: Methane was not detected in the fluxes of any treatment in the intertidal flat plots (lower than detection limit (0.28 ppm); Table S3).

Line 579-592: We examined different possible explanations for the lack of detected CH_4 as we could not entirely exclude that CH_4 was produced further down in the sediment and oxidized via anaerobic methane oxidation (AOM), as observed in some coastal wetlands (Capooci et al., 2024; La et al., 2022; Wang et al., 2019), or by lateral transport to surrounding tidal channels (Trifunovic et al., 2020). We did not measure any CH_4 as a efflux or in the porewater over multiple field campaigns, similar to a study conducted at the same study site by Kubeneck et al. (2025). Furthermore, the absence of an observed decrease in SO_4^{2-} concentration, particularly in the pioneer marsh, suggest a lack of AOM until 50 cm, as CH_4 and SO_4^{2-} are consumed in a 1:1 stochiometric ratio during sulfate AOM. The few other studies that have detected CH_4 in the Wadden Sea were at depths where SO_4^{2-} was largely depleted (Røy et al., 2008; Wu et al., 2015), which is not the case in our study. Thus, our results indicate that CH_4 production and consumption is unlikely until 50 cm, and could occur, if at all, below these depths. Further analysis using microbial analysis and/or CH_4 injection experiments is needed to fully exclude methanogenesis and AOM at lower depths.

SI: Detection limit of CH_4 (Table S3):

Table S3. Detection limit of CH_4 for the porewater samples (2022) and for the fluxes (2023). *Headspace gas of chambers was not exchanged before measurement. Thus, ambient air was present in the samples as well (concentration ambient air 4.27 ± 0.28 ppm). No linear response of CH_4 was observed and changes within the incubation time in some cases were below detection limit. Not available (n.a.).

	Detection limit	Range of measured samples
CH_4 flux (2023)	0.28 ppm	3.78 – 7.72 ppm *
CH_4 porewater (2022)	0.53 ppm	n.a. (all below detection limit)

Reviewer comment 4

The OC manipulation experiment provides valuable insight into short-term microbial responses to labile and complex carbon additions. However, each injection cycle spans only 48 hours and represents an instantaneous, high-concentration OC pulse. Natural OC inputs, such as root exudation, plant litter deposition, or episodic eutrophication, occur over much longer and more variable temporal scales, and microbial communities may respond differently to varying OC inputs. To avoid over-extrapolating short-term dynamics to ecosystem scale processes, the manuscript should more explicitly discuss how these pulse-style experiments relate to natural OC supply regimes and the extent to which the observed responses can/cannot be generalized to longer-term carbon cycling or environmental change scenarios.

Author comments: *Thank you for this valuable point. We agree that OC inputs of natural events (e.g., eutrophication or root exudation) have a lower OC input compared to concentrations used in this experiment and last longer than 48 h (e.g., root exudation over a growing period). The high concentrations over a short incubation time (48 h) were intentionally chosen to test in a mechanistic, process-oriented framework, if OC addition can stimulate microbially mediated CO_2 release from minerogenic salt marshes. Our goal was not to simulate OC supply at natural levels but rather to analyze the system response to increased labile and complex OC availability. High OC concentrations were chosen to minimize the effect of dilution and physical flushing due to tidal exchange which potentially obscure the biogeochemical response. We acknowledge that there might be differences in the carbon cycle response between short-term, high concentration OC inputs and sustained, low OC inputs. To address this limitation and avoid over-extrapolation, we added a paragraph in the discussion addressing short-term, high concentration OC inputs to natural OC supply (line 657-669). Moreover, we will discuss the extent to which our findings can be transferred to long-term C cycling in minerogenic salt marshes.*

Revised/added text:

Line 657-669: Furthermore, it is important to note that the OC concentrations used in this experiment are higher than those expected for naturally occurring OC inputs, such as root exudates, which are typically released at lower concentrations with a continuous input. Thus, upscaling the enhanced CO_2 fluxes measured in our study might result in overestimation of CO_2 release from minerogenic salt marshes. Our findings rather reveal, on a process level, that the addition of labile OC stimulates microbially mediated CO_2 release. Enhanced CO_2 release from the acetate amended plots was measured at nearly all sampling time points (1.5, 24, and 48 h) without a clear trend, while the concentration of the inert tracer showed a slight decrease over the same period (Fig. S3) – indicating slight dilution and flushing of the injected OC. This suggest that the elevated CO_2 release was driven by enhanced availability of labile OC independently of its concentration. These findings allow us to generalize that the system is likely limited by labile OC availability, regardless of the concentration; however, further work should quantify how the magnitude of CO_2 promotion corresponds to OC concentration, particularly under low, naturally sustained OC input rates. In conclusion, we can reliably predict

the direction of increased OC inputs to minerogenic salt marshes, but further studies are needed to predict the specific long-term magnitude of changes in the carbon cycle in these ecosystems.

Reviewer comment 5

Lines 40-42: This statement is very general. Authors should mention *which* climate-driven processes, such as temperature, sea-level rise, vegetation shifts, storm frequency, are most relevant to OC turnover in minerogenic marshes. This would help frame the specific hypothesis tested later in the manuscript.

Author comments: *We see the reviewer's point and added a few scenarios as examples.*

Revised/added text:

Line 41-43: Therefore, understanding carbon turnover in coastal wetlands is crucial for predicting how these ecosystems will respond to climate change, such as temperature increase, sea-level rise, and eutrophication of coastal waters.

Reviewer comment 6

Lines 56-59: I suggest clarifying why minerogenic sediments might differ from organogenic ones here. For example, in TOC content, mineral surface area, or porewater exchange, would better justify the hypothesis. This distinction is central to the paper, but it's currently presented it in broad terms.

Reviewer comment 7

Lines 64-68: This is a key knowledge gap the study is addressing. I would suggest explaining in more detail examples of the work in European Saltmarshes with respect to TEA turnover and not GHG emissions.

Reviewer comment 8

Lines 59-61: I suggest rewording this text to focus on how previous work has focused on locations that are biogeochemically different to the current study and mention the geographic locations as an aside. Currently this text could be misinterpreted as implying that previous work is geographically narrow or uninformative. Explaining how the U.S. sites differ biogeochemically (peat-dominated, high TOC, strongly reducing conditions) would prevent oversimplification and more clearly position the present study as filling a genuine gap.

Author comments: *Thank you for the comments related to differences between organogenic and minerogenic salt marshes. Since these comments are related, we combined the responses to all three. We acknowledge your feedback and will refine the descriptions of the differences.*

Also, adding detailed examples with respect to TEAs is a valid point regarding the key question of if the system is limited by electron donor or acceptors.

Revised/added text:

Line 61-64: Past studies on OC dynamics (including GHG release) in salt marshes have largely concentrated on the eastern coast of the US (Capooci et al., 2024; Kostka et al., 2002; Lowe et al., 2000; Seyfferth et al., 2020), which is dominated by organogenic peat marshes. Organogenic marshes are generally low energy, microtidal wetlands, characterized by a high organic matter deposition via autochthonous pathways that results in high TOC contents (Logemann et al., 2025).

Line 67-74: European salt marshes, in contrast, are primarily minerogenic, i.e., contain high fractions of mineral sediment due to high sedimentation rates, resulting in comparably lower TOC content (Nolte et al., 2013). Studies conducted in European salt marshes have focused on the TEA turnover (e.g., SO_4^{2-}-respiration rates) and not GHG emissions (Bosselmann et al., 2003; de Beer et al., 2005; van Erk et al., 2023). These studies showed that O_2 penetrates down the sediment, Fe(III) is available, and SO_4^{2-} reduction occurs. Hence, these studies have provided indirect links between belowground biogeochemistry, especially in the context of available TEAs, and the release of GHGs in minerogenic salt marshes; however, a direct investigation of the determining factor(s) of OC degradation from these ecosystems is missing.

Reviewer comment 9

Lines 91-95: I suggest including quantitative information here. The current phrasing provides qualitative differences but lacks quantitative information (inundation duration, frequency, elevation relative to MHW). Hydrology strongly influences redox conditions and solute transport and providing explicit values or ranges would help readers assess how representative and comparable the two zones are.

Author comments: *Unfortunately, we were not able to determine specific reliable information about the elevation relative to MHW. We could find general information that the water depth of the intertidal flat during inundation is 1 m below the MHW and for the pioneer marsh 0.5 m below MHW, however, no data for the specific site. Thus, we decided to only include inundation duration.*

Revised/added text:

Line 101-105: In general, both the pioneer marsh and intertidal flat are inundated daily during high tide, with the pioneer marsh experiencing less and shorter inundation (< 3h fully inundated) compared to the intertidal flat (> 3h fully inundated) (de Vlas et al., 2013). During a spring tide, which occurs twice a month, the magnitude of high and low tide is amplified leading to stronger exposure of the pioneer marsh to tides (Gao, 2019; Kvale, 2006).

Reviewer comment 10

Lines 111-115: Were actions taken to reduce compaction? Push-core sampling can cause compression or smearing, especially in fine-grained sediments. This text would benefit from a short note on steps taken to minimize disturbance.

Author comments: *We see the reviewer's concern and thus added a more detailed description of the push core sampling approach. As we are familiar with the concern of compaction, we have attempted to decrease this effect by using open push cores and only capping them after the cores were pushed into the sediment. Further, we sampled sediment/porewater not from the edges where there was the potential for smearing but rather from the middle which was likely undisturbed.*

Revised/added text:

Line 123-126: To minimize compression, we used open push cores and only capped them when the core liner was fully in the sediment. Furthermore, the inside wall of the cores was plain and clean to smoothly insert the core liner in the sediment. To further minimize disturbance, the sampled cores were immediately closed, vertically transported, and stored in the dark.

Line 128-129: We made sure to not take sediment or porewater samples at the edges but rather from the middle of the cores, where the sediment is likely undisturbed.

Reviewer comment 11

Lines 175-177: It would be useful for the readers to know the chamber volume here.

Author comments: *We added the chamber volume (3000 cm^3).*

Revised/added text:

Line 195-196: Gas sampling was conducted using an opaque, static, non-flow gas chamber made of polypropylene (chamber volume 3000 cm^2).

Reviewer comment 12

Line 195: Should be 'inner diameter of 2.5 cm, and a length of 10 cm'

Author comments: *Thank you for this comment - changed.*

Revised/added text:

Line 214-216: For this, push cores (inner diameter of 2.5 cm, and a length of 10 cm) were taken from the middle of each plot at the same positions as the porewater samples and immediately frozen until further analysis.

Reviewer comment 13

Lines 246-248: This section lacks detail for the reader to appreciate what numerical analysis was carried out. I appreciate all the details of statistical analysis is provided in the supplement, but some of the important information should be provided within the main body of the text. As a minimum, a summary of the statistical workflow should be included in the main text.

Author comments: *We expanded a short version of the applied tests and kept the detailed explanation in the SI.*

Revised/added text:

Line 267-276: For statistical analysis RStudio (R version R-4.4.3) was used. The significance level for all tests was set at $p < 0.05$. Normal distribution of the data and homogeneity of variances were tested by Shapiro-Wilk test and Levene test, respectively. Correlations between parameters was tested with the relevant tests (Pearson's correlation test or Spearman's rank correlation test depending on the normality of the data). Statistical differences between two groups were tested with a t-test and for more than two groups with a one-way Analysis of Variance (ANOVA) or Kruskal-Wallis rank sum test. For differences in the CO_2 release, a linear mixed model was applied. More details on the chosen tests and model are given in Supplement, S1.7. We reported the p-value in the text; further relevant statistical test results and parameters are shown in the corresponding sections in the SI. The variability of the geochemistry analysis is represented by the standard deviation of triplicates/duplicates. For the in situ experiment, the variability is reflected in the standard error of triplicates. For duplicate analyses, variability reflects the range of the two samples.

Reviewer comment 14

Line 315: 'for the' is repeated

Author comments: *Thank you for the comment. We removed the duplication.*

Revised/added text:

Line 345-346: Here, the mean residual fraction was 38.2 ± 4.8 % for the acetate treatment and 37.3 ± 3.6 % for the humic acid treatment.

Reviewer comment 15

Line 341: 'Complimentarily' is not an appropriate word here. Something like 'Similarly' or 'In comparison' should be used

Author comments: *Thanks, we replaced it with "Similarly".*

Revised/added text:

Line 374-376: Similarly, the cumulative CO_2 emissions from the acetate treated plots were the highest while the emissions from the humic acid and control plots were in a similar range for all four injection cycles (Fig. 4b).

Reviewer comment 16

Line 496: Typo 'decreasing to 0mM below.'

Author comments: *Thank you for the comment. The original sentence was confusing, so we have rephrased it and added the O_2 concentrations to provide context and clarify what the 0 mM in the original sentence refers to.*

Revised/added text:

Line 537-540: Based on microsensor measurements during low tide, we observed O_2 concentrations in the top 2 mm decreasing with depth, from 131.02 ± 26.49 to 0.18 ± 0.12 µmol L^{-1} in the pioneer marsh, and in the intertidal flat from 155.17 ± 12.71 to 0.62 ± 1.10 µmol L^{-1}, reaching 0 µM below that depth.

Reviewer comment 17

Line 503: I suggest rewording 'We speculate'. Speculation is not something that's encouraged in scientific work.

Author comments: *Thanks, we exchanged it with "suggest".*

Revised/added text:

Line 546-547: We suggest that the lack of O_2 penetration beyond 2 mm at our study site results from the presence of fine particles.

Reviewer comment 18

Line 623: should be 'led' instead of 'lead'

Author comments: *We have corrected this to "led".*

Revised/added text:

Line 690-692: The functional gene analysis provided further evidence for this, as SRB were present (absolute gene copy numbers in Supplement, Fig. S7); however, none of the treatments led to an increase in their metabolic activity compared to the control (Fig. 6).

Reviewer comment 19

Line 627: 'was much higher' should be 'were much higher'

Author comments: *We have now corrected this to "were".*

Revised/added text:

Line 695: … concentrations and availability of SO_4^{2-} were much higher.

Reviewer comment 20

Figures 4-8: Statistically significant differences should be denoted on the figures. It is common practice to place italics letters above bars in such comparison to show statistical groupings. This makes it easier for the reader to very quickly determine the statistical differences, if any are present.

Author comments: *Thank you for this point. We have now tried to strike a balance between readability and statistical information on the plots by only denoting the significant differences in the bar plots (Figure 5b and 8). We did not indicate statistical significance in the line graph of the CO_2 plot (Figure 4a), as this would have decreased figure clarity and significance was not consistently observed across all cycles. The key observation was that the acetate treatment showed consistently higher CO_2 release compared to humic acid and control, although differences were not significant at all time points and injection cycles, likely due to high variability among replicates reflecting natural in situ heterogeneity. We therefore did not denote statistical significance to avoid misinterpretation of the results. No statistically significant between treatments and control was measured for the bar plots of the cumulative CO_2 release (Figure 4b), likely due to the variability of the fluxes that resulted in variable cumulative CO_2 emissions. We added a line (line 378-381) explaining the potential absence of statistical differences in the cumulative CO_2 release. For Figure 7a/b (CO_2 release from the intertidal flat), no statistical analyses were applied due to some missing values caused by nonlinear CO_2 release during the incubation time of gas sampling. Therefore, we decided to not apply statistical comparisons and thus rephrased it accordingly (line 486-492) and will remove Table S12. Figure 6 already had significance denoted.*

Revised/added text:

Line 378-381: No statistical differences were measured between the cumulative CO_2 emission of the acetate treated plots and the control or humic acid treatment. Overall, these differences were smaller than those seen at individual CO_2 fluxes at specific time points (Fig. 4a), likely due to high variability in fluxes that resulted in variable cumulative CO_2 emissions.

Line 486-492: Figure 7a presents the CO_2 release from the intertidal flat over three injection cycles 1.5, 24, and 48 h post injection. Acetate treated plots released the highest CO_2 in all three injection cycles compared to the humic acid and the control plots. Similar to the pioneer marsh, no strong differences were observed between humic acid treated plots and the control plots. Consistently, the maximum cumulative CO_2 emissions were observed in the acetate treated plots (Fig. 7b). Due to nonlinearity of CO_2 release over the incubation time of gas sampling, some data points are missing; therefore, statistical comparison of CO_2 release

between treatments and the control was not done. Nevertheless, plots amended with acetate consistently showed higher CO_2 releases across all injection cycles.

Revised figures:

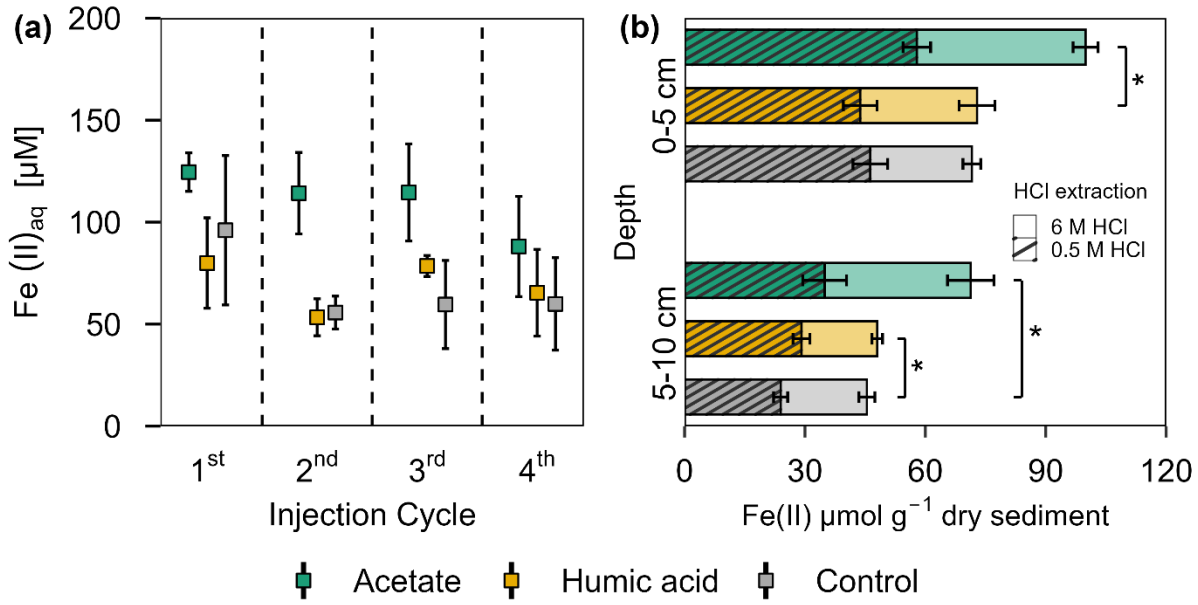


*Figure 5b: Line 408-409: … Significance is denoted for the 0.5 M HCl extraction. Statistical details are given in the SI (Table S10), significance level p < 0.05 *.*

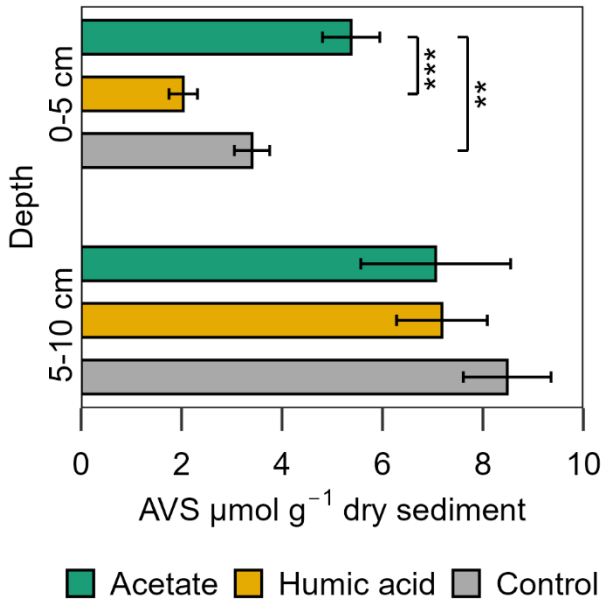


*Figure 8: Line 510-512: …Significance is denoted for the upper sediment layer (0-5 cm), deeper layer no statistically significant difference occurred. Statistical details are given in the SI (Table S13), significance level p < 0.05 *, p < 0.01 **, and p < 0.001 ***.*

References

Bosselmann, K., Böttcher, M.E., Billerbeck, M., Walpersdorf, E., Theune, A., Huettel, M., Jørgensen, B.B., 2003. Iron-Sulfur-Manganese Dynamics in Intertidal Surface Sediments of the North Sea. Berichte - Forschungszentrum Terramare. 32–35.

Capooci, M., Seyfferth, A.L., Tobias, C., Wozniak, A.S., Hedgpeth, A., Bowen, M., Biddle, J.F., McFarlane, K.J., Vargas, R., 2024. High methane concentrations in tidal salt marsh soils: Where does the methane go? Global Change Biology 30, 1–19. https://doi.org/10.1111/gcb.17050

de Beer, D., Wenzhöfer, F., Ferdelman, T.G., Boehme, S.E., Huettel, M., Van Beusekom, J.E.E., Böttcher, M.E., Musat, N., Dubilier, N., 2005. Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. Limnology and Oceanography 50, 113–127. https://doi.org/10.4319/lo.2005.50.1.0113

de Vlas, J., Mandema, F., Nolte, S., van Klink, R., Esselink, P., 2013. Nature conservation of salt marshes. The influence of grazing on biodiversity, PUCCIMAR report 09. ed, PUCCIMAR report 09. It Fryske Gea, Olterterp.

Gao, S., 2019. Chapter 10: Geomorphology and Sedimentology of Tidal Flats, in: In: Coastal Wetlands (Second Edition). Elsevier, pp. 359–381. https://doi.org/10.1016/B978-0-444-63893-9.00010-1

Kostka, J.E., Gribsholt, B., Petrie, E., Dalton, D., Skelton, H., Kristensen, E., 2002. The rates and pathways of carbon oxidation in bioturbated saltmarsh sediments. Limnology and Oceanography 47, 230–240. https://doi.org/10.4319/lo.2002.47.1.0230

Kubeneck, L.J., Rothwell, K.A., Notini, L., ThomasArrigo, L.K., Schulz, K., Fantappiè, G., Joshi, P., Huthwelker, T., Kretzschmar, R., 2025. In Situ Vivianite Formation in Intertidal Sediments: Ferrihydrite-Adsorbed P Triggers Vivianite Formation. Environmental Science & Technology 59, 523–532. https://doi.org/10.1021/acs.est.4c10710

Kvale, E.P., 2006. The origin of neap–spring tidal cycles. Marine Geology 235, 5–18. https://doi.org/10.1016/j.margeo.2006.10.001

La, W., Han, X., Liu, C.-Q., Ding, H., Liu, M., Sun, F., Li, S., Lang, Y., 2022. Sulfate concentrations affect sulfate reduction pathways and methane consumption in coastal wetlands. Water Research 217, 118441. https://doi.org/10.1016/j.watres.2022.118441

Logemann, E.L., Goesele, C., Jensen, K., Mueller, P., 2025. Soil Organic Carbon Stocks of German Salt Marshes: A Comparative Study Along Low- and High-Energy Coastlines. JGR Biogeosciences 130, e2025JG008797. https://doi.org/10.1029/2025JG008797

Lowe, K.L., Dichristina, T.J., Roychoudhury, A.N., Van Cappellen, P., 2000. Microbiological and Geochemical Characterization of Microbial Fe(III) Reduction in Salt Marsh Sediments. Geomicrobiology Journal 17, 163–178. https://doi.org/10.1080/01490450050023836

Nolte, S., Koppenaal, E.C., Esselink, P., Dijkema, K.S., Schuerch, M., De Groot, A.V., Bakker, J.P., Temmerman, S., 2013. Measuring sedimentation in tidal marshes: a review on methods and their applicability in biogeomorphological studies. Journal of Coastal Conservation 17, 301–325. https://doi.org/10.1007/s11852-013-0238-3

Røy, H., Lee, J.S., Jansen, S., De Beer, D., 2008. Tide-driven deep pore-water flow in intertidal sand flats. Limnology & Oceanography 53, 1521–1530. https://doi.org/10.4319/lo.2008.53.4.1521

Schlesinger, W.H., Bernhardt, E.S., 2013. Chapter 7: Wetland Ecosystems, in: Biogeochemistry (3rd Edition). Elsevier, Waltham, MA, USA, pp. 233–274. https://doi.org/10.1016/B978-0-12-385874-0.00007-8

Seyfferth, A.L., Bothfeld, F., Vargas, R., Stuckey, J.W., Wang, J., Kearns, K., Michael, H.A., Guimond, J., Yu, X., Sparks, D.L., 2020. Spatial and temporal heterogeneity of geochemical controls on carbon cycling in a tidal salt marsh. Geochimica et Cosmochimica Acta 282, 1–18. https://doi.org/10.1016/j.gca.2020.05.013

Trifunovic, B., Vázquez-Lule, A., Capooci, M., Seyfferth, A.L., Moffat, C., Vargas, R., 2020. Carbon Dioxide and Methane Emissions From A Temperate Salt Marsh Tidal Creek. Journal of Geophysical Research: Biogeosciences 125, e2019JG005558. https://doi.org/10.1029/2019JG005558

van Erk, M.R., Bourceau, O.M., Moncada, C., Basu, S., Hansel, C.M., De Beer, D., 2023. Reactive oxygen species affect the potential for mineralization processes in permeable intertidal flats. Nature Communications 14, 938. https://doi.org/10.1038/s41467-023-35818-4

Wang, Jiaqi, Hua, M., Cai, C., Hu, J., Wang, Junren, Yang, H., Ma, F., Qian, H., Zheng, P., Hu, B., 2019. Spatial-Temporal Pattern of Sulfate-Dependent Anaerobic Methane Oxidation in an Intertidal Zone of the East China Sea. Applied and Environmental Microbiology 85, 1–15. https://doi.org/10.1128/AEM.02638-18

Wu, C.S., Røy, H., De Beer, D., 2015. Methanogenesis in sediments of an intertidal sand flat in the Wadden Sea. Estuarine, Coastal and Shelf Science 164, 39–45. https://doi.org/10.1016/j.ecss.2015.06.031

Reviewer 2:

Reviewer comment 1

This manuscript presents a comprehensive in situ investigation of organic carbon (OC) turnover and CO_2 release in minerogenic salt marsh sediments of the Wadden Sea. By integrating porewater and solid-phase geochemistry, microbial functional analyses, and an OC manipulation experiment, the authors address an important and timely question: whether OC decomposition in salt marshes is primarily limited by electron acceptor availability or by OC availability and composition.

The study is methodologically sound, data-rich, and clearly written. The focus on European minerogenic marshes is particularly valuable given the predominance of organogenic systems in the literature. The main conclusion, that OC composition and availability dominate over electron acceptor availability in controlling CO_2 release, is compelling, but in several places overstated and would benefit from more nuanced framing.

Overall recommendation: Major revision.

Author comments: *Thank you for the positive feedback and the comments to improve the study.*

Reviewer comment 2

Abstract: Lines 22–26

The Abstract concludes that microbial CO_2 release was not limited by electron acceptor availability, based on sulfate presence and absence of CH_4. This conclusion is strong relative to the evidence presented.

Suggestion to rephrase to indicate the relative importance rather than absolute:

"Electron acceptor availability was unlikely to be the primary limiting factor under the conditions studied".

Author comments: *The reviewer brings up an important and valid point. We conclude based on different lines of evidence that TEA likely did not limit OC decomposition, which we will explain in more detail as responses to the other comments below. We appreciate the suggestion to rephrase the statement in the abstract to emphasize relative importance rather than an absolute conclusion.*

Revised/added text:

Line 22-24: Overall, we found that the microbially mediated CO_2 release was likely limited by OC availability and composition, and electron acceptor availability was unlikely to be the primary limiting factor, as evidenced by the presence of aqueous sulfate (SO_4^{2-}) at all tested depths and the lack of detectable CH_4.

Introduction

Reviewer comment 3

Line 29 rephrase to “at the interface between land and the open sea”

Author comments: *Rephrased.*

Revised/added text:

Line 30: Vegetated coastal wetlands, located at the interface between land and the open sea,…

Reviewer comment 4

The Introduction is well written and provides a thorough background but does not clearly state the hypotheses for the study.

It is also unclear whether differences between pioneer marsh and intertidal flat were hypothesized a priori.

Suggestions: Explicitly state hypotheses at the end of the Introduction and clarify whether spatial contrasts are expected mechanistically or are primarily comparative.

Author comments: *We thank the reviewer for this important suggestion. We now explicitly state our hypotheses at the end of the introduction and shortly clarify the differences between both zones (pioneer marsh and intertidal flat). A more detailed description of these two zones is given in the Material and Methods (line 98-103).*

Revised/added text:

Line 81-87: Building on those results, we conducted an in situ manipulation experiment investigating the impact of two contrasting OC sources (acetate, humic acid) on GHG emissions. We hypothesized that (i) the high energy and sediment inputs in minerogenic salt marshes result in low TOC supply and high TEA availability. (ii) We further hypothesize that this leads to the likely limitation of electron donor and not acceptor on OC decomposition and (iii) the composition of OC plays a more important role than the concentration in CO_2 release from a minerogenic salt marsh. These hypotheses were tested in two successional zones of a salt marsh, a pioneer marsh with sparse pioneer vegetation and a non-vegetated intertidal flat.

Methods:

Reviewer comment 5

The experimental section and the OC manipulation experiment is well designed and described in details. But the ecological relevance of the injected OC concentrations requires a bit

clarification. Also explicitly state that the experiment targets short-term process responses, not long-term carbon budgets.

Author comments: *We thank the reviewer for raising this important point. We agree that the ecological relevance of this experiment should be clarified. We have therefore added an explanation how both labile and complex OC addition reflects realistic scenarios in the Materials and Methods (line 152-157). Furthermore, we explicitly mention that our experiment was a short-term OC addition experiment in the Materials and Methods (line 191-192). We agree that OC inputs of natural events (e.g., eutrophication or root exudation) have a lower OC input compared to concentrations used in this experiment and last longer than 48 h (e.g., root exudation over a growing period). The high concentrations over a short incubation time (48 h) were intentionally chosen to test in a mechanistic, process-oriented framework, if OC addition can stimulate microbially mediated CO_2 release from minerogenic salt marshes. Our goal was not to simulate OC supply at natural levels but rather to analyze the system response to increased labile and complex OC availability. High OC concentrations were chosen to minimize the effect of dilution and physical flushing due to tidal exchange which potentially obscure the biogeochemical response. We acknowledge that there might be differences in the carbon cycle response between short-term, high concentration OC inputs and sustained, low OC inputs. To address this limitation and avoid over-extrapolation, we added a paragraph in the discussion addressing short-term, high concentration OC inputs to natural OC supply (line 657-669). Moreover, we will discuss the extent to which our findings can be transferred to long-term C cycling in minerogenic salt marshes.*

Revised/added text:

Line 152-157: Studying both labile and complex OC additions is ecologically relevant as salt marshes receive OC from multiple sources (Temmink et al., 2022). Eutrophication of coastal waters and/or root exudates can supply readily degradable OC to salt marshes, while increased organic matter load in rivers can deliver more complex OC compounds to salt marshes. The applied OC compounds in our study, therefore, represent environmental scenarios and allows us to investigate how these OC sources influence GHG release under realistic conditions.

Line 191-192: The applied approach allowed us to assess short-term OC process response in minerogenic salt marshes, rather than long-term responses.

Line 657-669: Furthermore, it is important to note that OC concentration used in this experiment are higher than those expected for naturally occurring OC inputs, such as root exudates, which are typically released at lower concentrations with a continuous input. Thus, upscaling the enhanced CO_2 fluxes measured in our study might result in overestimation of CO_2 release from minerogenic salt marshes. Our findings rather reveal, on a process level, that the addition of labile OC stimulates microbially mediated CO_2 release. Enhanced CO_2 release from the acetate amended plots was measured at nearly all sampling time points (1.5, 24, and 48 h) without a clear trend, while the concentration of the inert tracer showed a slight decrease over the same period (Fig. S3) – indicating slight dilution and flushing of the injected

OC. This suggest that the elevated CO_2 release was driven by enhanced availability of labile OC independently of its concentration. These findings allow us to generalize that the system is likely limited by labile OC availability, regardless of the concentration; however, further work should quantify how the magnitude of CO_2 promotion corresponds to OC concentration, particularly under low, naturally sustained OC input rates. In conclusion, we can reliably predict the direction of increased OC inputs to minerogenic salt marshes, but further studies are needed to predict the specific long-term magnitude of changes in the carbon cycle in these ecosystems.

Results:

Reviewer comment 6

Several statements in the Results section interpret mechanisms rather than reporting observations.

My suggestion restricts the results to direction and magnitude of change, statistical significance and variability observed, and move the mechanistic interpretation to the Discussion section.

Author comments: *We thank the reviewer for this comment and carefully revised the results section to ensure its focus on results (magnitude, statistical significance, and variability observed). Specifically, we shortened explanatory text (line 294), moved explanations to the Material and Methods section (line 414-417 moved to line 243-245) or deleted it (line 431 as already stated in Material and Methods). We removed interpretations (line 441 and line 522) or shortened them (line 530-533) and, if not already stated, moved them to the corresponding discussion section. We did not identify additional statements in the results that we believe are mechanistic interpretations. Furthermore, we decided to keep introductory sentences at the beginning of some paragraphs to remind the reader of the purpose of the measurement. We think that these sentences provide necessary contextual information without interpreting the results mechanically, e.g., in line 289-290 "Porewater and solid phase measurements from the push and microsensor cores analysis show availability of electron acceptors (O_2, Fe(III), and SO_4^{2-}) over depth in both the pioneer marsh and intertidal flat (Fig. 2)." And in line 321-322 "Bromide was used in the in situ experiment as an inert tracer to test the washing out of the injection solution from the experimental plot over the sampling time of one injection cycle (48 h).".*

Revised/added text:

Line 294: ~~We measured concentrations of Fe(II) as an indicator of Fe(III) reduction.~~ Aqueous Fe(II) (as an indicator of Fe(III) reduction) showed a decreasing tend in both zones, with…

Line 414-417 moved to line 243-245: We acknowledge that the weaker acid extraction extracted Fe(II) from carbonates and sulfides in addition to iron (oxyhydro)oxides. We

therefore used this approach to determine the crystallinity of iron minerals and call it poorly (extracted by 0.5 M HCl) and higher (extracted by 6 M HCl) crystalline iron minerals (and not (oxyhydr)oxides).

Line 431 removed: ~~Overall, a decreasing trend from higher contents in the upper 5 cm to lower contents in the deeper layer (5-10 cm) was notable for all treatments and crystallinities.~~

Line 441 removed: The impact of the added OC on the bacterial community was analysed by qPCR. ~~to quantify the total bacterial abundance (bacterial 16S rRNA gene copy numbers), the abundances of Geobacter spp. as an indicator for Fe(III) reduction, and the dsrA gene as an indicator for sulfate-reducing bacteria (SRB) in the pioneer marsh.~~

Line 522 removed: ~~Overall, an increase for all treatments and the control in the AVS content with depth was measured.~~

Line 530-533 shortened: No significant increase in the RNA-based copy numbers of *dsrA* genes in both depths (Fig. S12c) were observed; however, we detected slightly higher RNA-based dsrA gene copies in the acetate treatments (0.33 ± 0.06 log2FC) compared to the control in the upper layer.

Reviewer comment 7

Lines 285–286; 345

The absence of detectable CH_4 is an important result. Report the CH_4 detection limit and also clarify whether CH_4 was assessed both in porewater and as surface fluxes.

Author comments: *Thank you for the comment regarding the CH_4 detection limit. We will revise the manuscript to include the detection limit and clarify whether it refers to porewater or fluxes.*

Revised/added text:

Line 24: … at all tested depths and the lack of detectable CH_4.

Line 312-313: In both the pioneer marsh and intertidal flat, no CH_4 release, neither as fluxes nor in the porewater up to a depth of 50 cm, was detected (detection limit: 0.28 and 0.53 ppm respectively; Table S3).

Line 381-382: In all treatments and the control, no CH_4 release as a flux was detected (lower than detection limit (0.28 ppm); Table S3).

Line 492-493: Methane was not detected in the effluxes of any treatment in the intertidal flat plots (lower than detection limit (0.28 ppm); Table S3).

SI: Detection limit of CH_4 (Table S3):

Table S3. Detection limit of CH_4 for the porewater samples (2022) and for the fluxes (2023). *Headspace gas of chambers was not exchanged before measurement. Thus, ambient air was present in the samples as well (concentration ambient air 4.27 ± 0.28 ppm). No linear response of CH_4 was observed and changes within the incubation time in some cases were below detection limit. Not available (n.a.).

	Detection limit	Range of measured samples
CH_4 flux (2023)	0.28 ppm	3.78 – 7.72 ppm *
CH_4 porewater (2022)	0.53 ppm	n.a. (all below detection limit)

Reviewer comment 8

Lines 294-307

The bromide tracer convincingly demonstrates limited physical washout, but the definition of residual fraction is not immediately clear. Provide a simple equation defining residual fraction in methods or results rather than Supplement.

Line 305–307: Simplify wording of residual fraction definition.

Author comments: *Thank you for the feedback regarding the term "residual fraction". We are aware that this is not a common term, so providing a simple equation defining it may help the reader. We avoided using the term recovery fraction because our measurements reflect the concentration (Br^- or DOC) remaining in the experimental plots, not the total recovered. To avoid confusion, we will add a clear definition in the results and provide an equation.*

Revised/added text:

Line 332-337: Here, residual fraction is defined as the ratio between the Br^- concentration measured in the porewater 48 h post injection and the expected total Br^- concentration in an experimental cylinder (Eq. (1); details in S1.4). The expected total Br^- concentration includes both the native Br^- and the added Br^- during the experiment (expected Br^-) after accounting for dilution in the sediment. Details on the calculation of the Br^- residual fraction are provided in Supplement, S1.4.

$$\frac{Br^-_{concentration\ at\ the\ end\ of\ an\ injection\ cycle}}{Br^-_{expected}} = residual\ fraction \qquad (1)$$

Line 338-340: The residual fraction of DOC is defined in the same way as for Br^-, representing the proportion of measured DOC after 48 h to the expected DOC (native DOC + added acetate/humic acid) and was calculated analogously to Br^-, with DOC concentrations used instead (Eq. (1) and S1.4).

Discussion:

Reviewer comment 9

The Discussion repeatedly concludes that electron acceptor availability did not limit OC decomposition (Lines 494–538; 545–548).

The authors move from:

“Fe(II) is present / increases

To

“Electron acceptor availability did not limit OC decomposition”

The paragraph (Lines 514–527) is well written and appropriate. However, elevated Fe(II) may indicate enhanced reduction rates rather than absence of limitation. So elevated Fe(II) tells that iron reduction is happening faster now. It does not prove that iron was never limiting or constraining the system. The authors conclude that electron acceptors are not limiting (strong statement) and OC alone controls decomposition (very strong statement).

Authors need to be cautious about this and rephrase and moderate the interpretation throughout the manuscript to emphasize that OC availability and composition dominated process rates under the conditions studied, rather than concluding that electron acceptor availability was generally non-limiting.

Author comments: *The reviewer brings up an important and valid point. It is true that our conclusion in only based on concentrations and did not directly test that Fe(III) or SO_4^{2-} was limiting or constraining the system using turnover rates.*

We first address the point about iron reduction: we agree that the porewater Fe(II) concentrations we observed with depth (lines 557-570) do not necessarily mean that Fe(III) is not limiting, but rather that there is some Fe(III) reduction occurring. We chose not to make changes specifically at this point in the text since we address the general point about the TEA limitation below.

Based on different lines of evidence, we concluded that TEAs likely did not limit OC decomposition: First, SO_4^{2-} remained high throughout the sampled depth and the sulfate:chloride ratio stayed stable (only a slight decrease was observed in the intertidal flat). This indicates no strong SO_4^{2-} depletion with depth. We considered whether the lack of observed decrease was due to continuous resupply of SO_4^{2-} with infiltrating tidal water. However, the fine particle size of the sediment suggests that tidal water does not percolate completely through the sediment, even over multiple tidal cycles. Thus, we should have been able to observe SO_4^{2-} consumption due to microbial reduction if occurring.

Second, the OC addition experiment showed a clear response due to the addition of labile OC (acetate). We agree that these results cannot exclude potential shifts in TEA respiration pathways; however, the rapid increase of CO_2 release after addition of acetate (in some

injection cycles already 1.5h after addition) combined with no depletion of SO_4^{2-} or observed changes in TEA availability suggest that microorganisms capable of utilizing acetate were already present in the sediment, and is consistent with electron donor limitation rather than electron acceptor limitation.

Third, porewater and solid-phase respiration end products e.g., Fe(II) and/or sulfide levels give evidence that Fe(III) and SO_4^{2-} reduction was occurring. This suggests that these electron acceptors were available and utilized. Furthermore, the high SO_4^{2-} concentrations may suppress methane (CH_4) formation. The absence of CH_4 therefore indicates that electron acceptors were not depleted, as a thermodynamical more favorable electron acceptor was still available.

In summary, we suggest that there are indications of an electron donor limitation. We agree that the absence of rate measurement introduces uncertainty, and we will add a paragraph in the conclusion (line 727-730) describing that our data do not fully exclude TEA depletion but are consistent for OC limitation, in addition to softening the conclusions.

Revised/added text:

Line 590-592: Collectively, due to the occurrence of Fe(III) reduction (especially in the upper sediment layers) and the availability of SO_4^{2-} throughout the sediment, we suggest that electron acceptor availability likely did not limit microbial OC decomposition in our study.

Line 599-601: Based on the availability of electron acceptors (e.g., SO_4^{2-}) at all depths and the lack of CH_4, we hypothesize that at our field site and other comparable coastal sites, OC is likely the constraint on microbially mediated CO_2 release and that electron acceptors are likely not a limiting factor.

Line 727-730: We caution here that we did not directly measure TEA reduction rates. Future studies should investigate turnover rates, potentially utilizing isotopes to confirm this finding. Overall, our results indicate that the OC composition, rather than the concentration alone, controlled CO_2 release in both succession zones. This suggests that OC composition likely plays a limiting role in microbially mediated CO_2 release from minerogenic salt marshes.

Reviewer comment 10

Statistical reporting and methods are briefly described, but replication and model structure are not fully transparent. State sample sizes per treatment zone, and injection cycle, also specify whether error bars represent SD or SE.

Author comments: *We thank the reviewer for this comment. We expanded the statistical description in the main manuscript (line 267-276). Details on model structure are given in the SI (S1.7 Statistical analysis; response variable was CO_2, fixed effect was treatment and time after injection, random effect was treatment replicates). Additionally, we added the sample size per treatment and injection cycle in the figure captions (Figure 2-8). The figure caption also*

now specifies whether the error bars are standard error or standard deviation. Due to some missing samples values for the CO_2 release in the intertidal flat resulting from nonlinear CO_2 release during the incubation time of gas sampling, we decided to not apply statistical comparisons and thus rephrased it accordingly (line 486-492) and will remove Table S12.

In the figure caption of Figure 2, the order was slightly changed, and the corresponding sub-panel number (a-e) was added. For Figure 3, details on samples size for each treatment and injection cycle were added. The caption of Figure 4 (line 358-366) has been revised, and information in line 361 and 362 was replaced with more detailed information in line 364-366, similar to figure caption of Figure 7 (line 479-485). For all other figure captions, details about injection cycle and treatment were added. We tried to add sufficient information to provide clarity while avoiding confusion for the reader. Thus, the general sample size is reported. Additional information to figures and statistical information tables has also been added in the Supporting Information (SI).

Revised/added text:

Line 267-276: For statistical analysis RStudio (R version R-4.4.3) was used. The significance level for all tests was set at $p < 0.05$. Normal distribution of the data and homogeneity of variances were tested by Shapiro-Wilk test and Levene test, respectively. Correlations between parameters was tested with the relevant tests (Pearson's correlation test or Spearman's rank correlation test depending on the normality of the data). Statistical differences between two groups were tested with a t-test and for more than two groups with a one-way Analysis of Variance (ANOVA) or Kruskal-Wallis rank sum test. For differences in the CO_2 release, a linear mixed model was applied. More details on the chosen tests and model are given in Supplement, S1.7. We reported the p-value in the text; further relevant statistical test results and parameters are shown in the corresponding sections in the SI. The variability of the geochemistry analysis is represented by the standard deviation of triplicates/duplicates. For the in situ experiment, the variability is reflected in the standard error of triplicates. For duplicate analyses, variability reflects the range of the two samples.

Line 486-492: Figure 7a presents the CO_2 release from the intertidal flat over three injection cycles 1.5, 24, and 48 h post injection. Acetate treated plots released the highest CO_2 in all three injection cycles compared to the humic acid and the control plots. Similar to the pioneer marsh, no strong differences were observed between humic acid treated plots and the control plots. Consistently, the maximum cumulative CO_2 emissions were observed in the acetate treated plots (Fig. 7b). Due to nonlinearity of CO_2 release over the incubation time of gas sampling, some data points are missing; therefore, statistical comparison of CO_2 release between treatments and the control was not done. Nevertheless, plots amended with acetate consistently showed higher CO_2 releases across all injection cycles.

Figure captions:

Figure 2

Line 285-288: (e) Total organic carbon (TOC) in the sediment. For (b-d), push cores were taken in triplicates in both zones to a depth of 25 cm in 2023. Duplicate push cores for (e) the TOC were sampled in 2022. For all sub-figures, markers denote mean ± standard deviation (due to limited sample mass, some depth values only show mean and the range of two samples, or only a single value). All cores were sampled during low tide.

Figure 3

Line 353-354: Markers represent the mean of the triplicates, with error bars indicating the corresponding standard error for treatments and control in both zones for DOC and Br^- across all injection cycles.

Figure 4

Line 361 and 362 replaced by additional details in line 364-366: For (a/b), markers represent mean ± standard error of triplicates for all treatments and the control across injection cycles. For the 1st and 3rd injection cycle for the acetate treatment (both 1.5 h values) were based on duplicate measurements, which is thus also the case for the (b) cumulative CO_2 emission of these cycles.

Figure 5

Line 403: (a): Triplicates for each treatment and control for each injection cycle were collected and mean ± standard error is shown.

Line 409-410: (5b): For each treatment and control, each spatial triplicate (n = 3) was analyzed in triplicate (total n = 9) for each depth (0-5 and 5-10 cm); results are presented as mean ± standard error.

Figure 6

Line 466-468: Sample sizes include triplicates of each treatment and control at both depths, represented as mean ± standard error (exception of duplicate measurements for 16s RNA-based humic substances (5-10 cm) and 16s RNA-based control (0-5 cm)).

Figure 7:

Line 479-485: For (a/b), markers represent the mean ± standard error of triplicates for all treatments and the control across injection cycles, except where missing values for CO_2 release occurred due to nonlinear CO_2 release during the gas sampling incubation time. (a) duplicate measurements are reflected for the 1st injection cycle for the control (1.5 and 24 h), for the 2nd injection cycle for the acetate treatment and control (48 h), and the 3rd injection cycle for the acetate treatment and control (1.5, 24, and 48 h). Single measurement values are shown for the control in the 1st (48 h) and 2nd (1.5 h) injection cycle. For (b), cumulative CO_2 emissions, the acetate treatment shows duplicate measurements for the 2nd and 3rd injection and for the control, only single values are reflected.

Figure 8:

Line 512-513: Each spatial triplicate (n=3) was analyzed in triplicates (total n = 9) for each treatment and the control at both depths; results are presented as mean ± standard error.

Figures in Supporting information:

Figure S3: …Markers represent the mean ± range of duplicates for each time point and treatment/control for each succession zone. The ranges are smaller than the markers; hence, they are not visible.

Figure S4: …Data are shown as mean ± standard error of triplicates for each treatment/control and injection cycle.

Figure S5: … (a/b) Data are shown as mean ± standard error of triplicates for each treatment/control and injection cycle.

Figure S6: … (a) Data are shown as mean ± standard error of triplicates for each treatment/control and injection cycle. … (b) Each spatial triplicate (n = 3) was analyzed in duplicates (total n = 6) for each treatment/control and both depths; results are presented as mean ± standard error.

Figure S7: … Samples size compress triplicates of each treatment/control and both depths; represented as mean ± standard error (exception of duplicate measurements for 16s RNA-based humic substances (5-10 cm) and 16s RNA-based control (0-5 cm)).

Figure S8: … (a/b) Data are shown as mean ± standard error of triplicates for each treatment/control and injection cycle.

Figure S9: … Data are shown as mean ± standard error of triplicates for each treatment/control and injection cycle.

Figure S10: … (a) Triplicates for each treatment and control for each injection cycle were collected and mean ± standard error is shown… (b) Each spatial triplicate (n = 3) was analyzed in triplicates (total n = 9) for both depths; results are presented as mean ± standard error.

Figure S11: … Markers show mean ± standard error of triplicates for each treatment/control and injection cycle.

Figure S12: …Sample sizes include triplicates, represented as mean ± standard error for each treatment/control and both depths.

Figure S13: … Samples size compress triplicates, represented as mean ± standard error for each treatment/ control and both depths.

Added samples size to statistical details given in the Supporting information:

Table S5: … Pioneer marsh samples size including values across all injection cycles for DOC comparison: acetate: n = 12 and humic acid n: = 11, and for Br^- comparison: acetate: n = 12, humic acid: n = 10, and control: n = 10. Sample size for the intertidal flat (across all injection cycles) for Br^- comparison: acetate: n = 8, humic acid: n = 9, and control: n = 9.

Table S6: … Samples size for each time point and each treatment and control for each injection cycle was 3 (n = 3) with exception for the acetate (1.5 h) in the 1^{st} and 3^{rd} injection cycle.

Table S7: … Samples size acetate (n = 34), humic acid (n = 36), and control (n = 36).

Table S8: … Sample size includes values across all injection cycles for acetate, humic acid, and control (each n = 12).

Table S9: … Data are presented as mean ± standard error of triplicates (n = 3) for each treatment and the control at both depths.

Table S10: … Sample size includes triplicate measurements (n = 3) for each injection cycle for acetate, humic acid, and control, resulting in a total samples size of n = 12 per treatment/control.

Table S11: … Samples size for each treatment and the control contained spatial triplicates (n = 3), which were analyzed in triplicates (total n = 9) for each depth (0-5 and 5-10). Exceptions: 6 M HCl extraction at 0-5 cm, control: two spatial triplicates were only analyzed in duplicates (n = 8), same as for one spatial acetate replicate (n = 8) at 5-10 cm.

Table S12: …Sample sizes include triplicates (n = 3) of each treatment/control and depth.

Table S13: … Each spatial triplicate (n = 3) was analyzed in triplicates (total n = 9) for each treatment and the control for both depths. Exceptions for acetate (0-5 and 5-10 cm): one spatial triplicate was only analyzed in duplicates (total n = 8) for both depths.

Table S14: … Each spatial triplicate (n = 3) was analyzed for each treatment/control and both depths.

Conclusion:

Reviewer comment 11

The Conclusions are strong but occasionally extend beyond the scope of the experiment.

Emphasize that findings reflect short-term OC inputs and reduce a bit to sharpen the conclusion.

Author comments: *We thank the reviewer for this valid comment. We agree that the original conclusion was too strong, as electron acceptors limitation was not explicitly tested and short-term OC inputs was not mentioned. We have revised the conclusion accordingly, clarified its limitation and acknowledged that this should be tested in further studies. Additionally, we state*

that our results are short-term OC inputs responses. The revised/added text (line 727-730) are already mentioned above in response to the reviewer comment that "electron acceptor availability did not limit OC decomposition".

Revised/added text:

Line 727-730: We caution here that we did not directly measure TEA reduction rates. Future studies should investigate turnover rates, potentially utilizing isotopes to confirm this finding. Overall, our results indicate that the OC composition, rather than the concentration alone, controlled CO_2 release in both succession zones. This suggests that OC composition likely plays a limiting role in microbially mediated CO_2 release from minerogenic salt marshes.

Line 740-741: The results of this in situ study contribute to our understanding of short-term carbon dynamics in minerogenic temperate salt marshes.

Line 747-749: Further, the in situ experiment simulated a potential increase of short-term OC inputs to the ecosystem, reflecting scenarios associated with climate change such as inundation of previously unflooded areas due to sea level rise and storm surges or eutrophication.

Line 751-754: Our study thus provides valuable insight into the consequences of such short-term scenarios for GHG release and highlights that the input of labile OC (e.g., primary production during eutrophication, root exudates) into the sediment of a minerogenic salt marsh results in higher CO_2 releases.

<u>Minor Comments:</u>

Reviewer comment 12

Line 31–36: Global carbon burial statistics could be shortened.

Author comments: *We decided to leave the section as it is, as we believe it is important to emphasize the role of vegetated coastal areas in global carbon cycles in order to motivate the need for studies on OC dynamics.*

Reviewer comment 13

Line 305–307: Simplify wording of residual fraction definition.

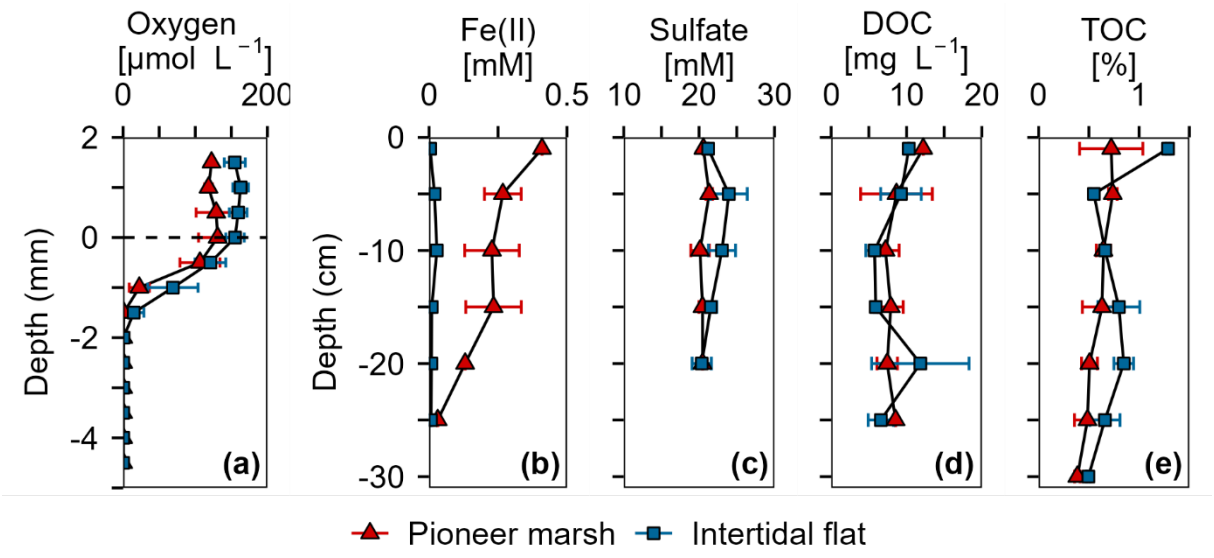
Author comments: *Thanks for this relevant comment. We simplified the definition and provided a simple equation (see response above).*

Reviewer comment 14

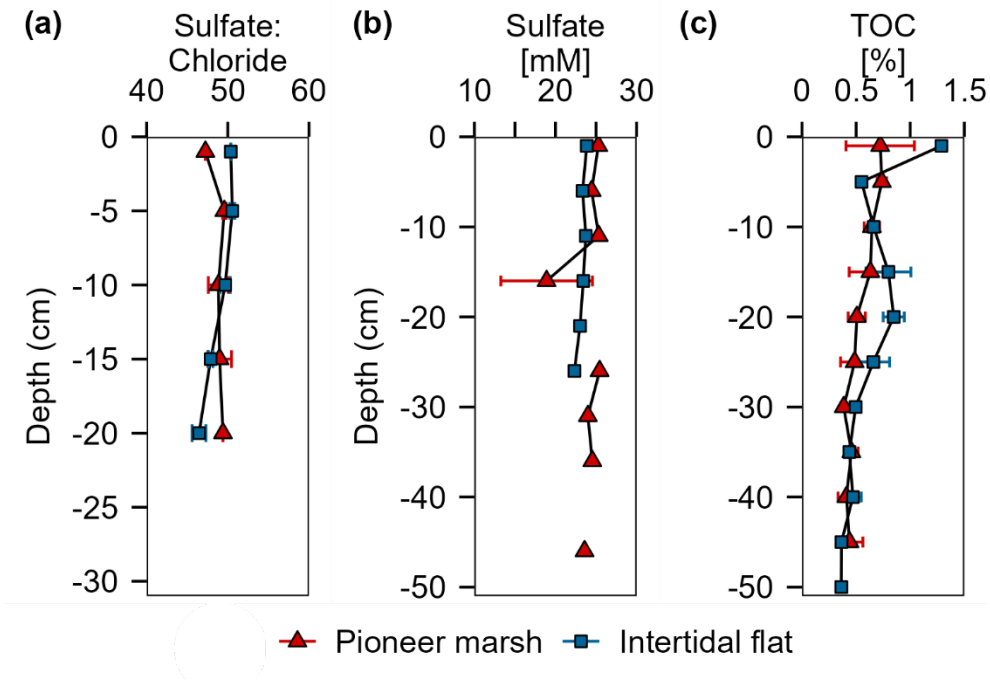
Ensure consistent color coding for treatments across all figures.

Author comments: *Thank you for this comment. We carefully checked the color coding across all figures and found no inconsistencies among treatments. However, in Figure 2 (geochemical analysis), the intertidal flat was presented in the same gray color as the control in the in situ experimental figures. To improve visual differentiation, we changed the color of the intertidal flat from grey to blue in Figure 2 and in Figure S2 (in the SI).*

Revised/added text:



Line 280-281: Figure 2. Overview of porewater and sediment biogeochemistry in terms of electron acceptors (O_2, Fe(III), SO_4^{2-}) and electron donor (DOC, TOC) from in situ push cores in the pioneer marsh (red triangles) and intertidal flat (blue squares).



SI: Figure S2. Porewater and sediment biogeochemistry in terms of electron acceptor (SO_4^{2-}) and donor (organic carbon) from in situ push cores in the pioneer marsh (red triangles) and intertidal flat (blue squares).

Reviewer comment 14

Line 381: Avoid reflexive phrasing such as “we are aware that”.

Author comments: *Thanks, we replaced it with “We acknowledge”. Since this sentence was moved to the Material and Method section, we made the change there.*

Line 243-244: We acknowledge that the weaker acid extraction extracted Fe(II) from carbonates and sulfides in addition to iron (oxyhydro)oxides.

Recommendation:

Reviewer: This manuscript is a **strong and valuable contribution** to coastal biogeochemistry specially to carbon cycling in minerogenic salt marshes. With moderated claims regarding electron acceptor limitation, clearer hypothesis framing, and improved separation of Results and Discussion, it should be suitable for publication.

Author comments: *We thank the reviewer for this positive feedback! We have responded to each comment and made corresponding changes, and the manuscript has been improved accordingly.*

References

Poffenbarger, H.J., Needelman, B.A., Megonigal, J.P., 2011. *Wetlands* 31, 831–842.

Sanders-DeMott, R., Eagle, M.J., Kroeger, K.D., Wang, F., Brooks, T.W., O'Keefe Suttles, J.A., Nick, S.K., Mann, A.G., Tang, J., 2022. *Global Change Biology* 28, 4539–4557.

Temmink, R., J.M., Lamers, L.P.M., Angelini, C., Bouma, T.J., Fritz, C., van de Koppel, J., Lexmond, R., Rietkerk, M., Silliman, B.R., Joosten, H., van der Heide, T., 2022. *Science* 376, 1–7.