Dear Editor and Reviewers,

Thank you for taking time to review our manuscript titled '*Perturbation increases source-dependent organic matter degradation rates in estuarine sediments*". Below we provide detailed responses to reviewers' comments and elaborate how each point has been incorporated into the revised manuscript.

Kind regards,
Guangnan Wu, on behalf of the coauthors

NB: Our response is given in red, also referring to the line numbers in the revised manuscript with Word's **simple markup** where the edited text can be found. Quotes of the newly edited text are indicated with "quotation marks". For ease, we mention the topic of a major comment at the start [*between parentheses in italic*] and use these when referring to other responses within our rebuttal.

Editor's comments:

Dear authors,

[*Site selection and method*ology] As you noticed, contrasted reviews were made in terms of significance for this study. You must be very carefully in the new version to clarify the differential analytical and experimental efforts made (13, 6 and 2 sites for different kind of experiments)
We added explanatory sentences at the beginning of sections 2.2–2.6 that describe the rationale behind site selection for the various analytical and experimental efforts:

- Bulk sediment analysis (Section 2.1, 2.2): "Bulk sediments were collected from 49 selected locations throughout the study area in the summer of 2021. These sites were selected from over 300 monitoring sites in the Port of Rotterdam to represent the full spectrum of depositional conditions in the main waterway and adjacent harbor areas from marine to riverine (Fig. 1)." (L159−162)
- Lipids and MOM analysis (Section 2.3, 2.4): "Sediments from 13 key locations (Fig. 1), selected to cover the full river-marine salinity transect, were used for lipids and MOM analyses." (L214−215)
- Subaerial/open-air bottle incubation (Section 2.6): "To investigate OM degradability under oxygen exposure during dredged sediment processing while avoiding oxygen supply as a limitation, open-air bottle incubations were conducted in triplicate for six sediments that covered contrasting depositional and sedimentary conditions within the research area: three marine (115, 86, NWWG-02; Fig. 1a) and three riverine (21A, B16, K1v2; Fig. 1a), with differing sediment texture (silt-rich and sand-rich) in both groups." (L297−301)
- Whole-core incubation (Section 2.5): "Triplicate intact sediment cores collected from two strongly contrasting sites (marine site 115 vs. riverine site 21A) were used for whole-core incubation. These

sites represent relatively intensively dredged marine and riverine areas, respectively, that contribute significantly to the total annual dredged sediment volume in the PoR." (L252−255)

[*O_2 fluxes*] Especially please consider limitations pointed by reviewer 3 on the interpretation of O2 fluxes maybe expanding a bit the discussion on the organic matter excess and also on the potential reoxydation rates and related CO2 fluxes while it comes to less labile organic matter.
We have added more careful consideration of the O_2 fluxes, for details please see our response to Reviewer #3's comment about [*O_2 fluxes*] below.

[*Sample processing*] In the material and methods section you should put more clearly, limitations brought by freeze-drying sediment in comparison with other methods and clearly explain why freeze drying is the most suitable in your case.
We now better justify this choice (minimal sediment alteration, reproducibility: L301−303) and discuss its (likely limited) impact (L653−657).

[*Perturbation and CH_4 escape*] If I may suggest some reading, get a look on Hulot et al (2023) and Barhdadi et al (2024) papers dealing with a decennial flood in the Loire and how this generates erosion and methane ebullition affecting benthic fluxes that are dominated by benthic advective exchanges. This is a good example of natural erosion effects that could be extrapolated to dredging effects even if it doesn't deal with the reactivity of suspended matter.
We thank the editor for this suggestion and have carefully considered the articles; we have included Hulot et al.'s work in our discussion of perturbation and CH_4 escape (L725−726).

Sincerely
Edouard

Reviewer #1:
The manuscript focuses on organic matter degradation rates in the Amsterdam harbor estuary according to strong anthropogenic influence through dredging activities, using spatial monitoring data and diverse incubation processes. The research is very interesting and meaningful for carbon cycle and greenhouse gas emission from the sediment in such impacted area. The topic of this manuscript fits well with the journal's scope, and the data collected highlighted a strong sampling effort and figures are of good quality.
However, it is important to address some issues in the manuscript before acceptance for publishment, here are some specific comments:
We sincerely thank the reviewer for their careful and constructive feedback during the first review of our manuscript.

[*Salinity and CH_4*] Line 80-82: more details in which way shifting salinity affected CH4 are needed (even if discussed in discussion, see comment “line 675-677”).

We have added information about the role of SO_4^{2-} in the microbial sediment CH_4 filter (L81−83).

Line 87: reference is missing.

Added (L89).

Line 606: no () for the reference. Dauwe et al. (2001)

Line 642: Zander et al. (2022)

Spelling and grammar were checked and corrected in the revised manuscript.

Line 89-92: yes, but give examples of naturally and anthropogenically induced sediment disturbance.

Added (L92−93).

Line 111: avoid terms like “our”. Here use “the”. To be corrected throughout the manuscript (e. g. “our study” replaced by “the present study”…).

Line 139: “our”?

Corrections were made throughout the revised manuscript.

Line 122-123: out of context here…

We modified the paragraph to accommodate and highlighted the significance of sediment reuse (L121−127).

[*Subaerial bottle incubation experiment*] Line 138-139: representative of dredged sediment conditioned on land? Is this conditioning the major process for dredged sediment? Line 151 mentioned sediment relocated in the sea. More explanations are needed to justify the choice of these open-air incubations.

In our revision, we emphasized the increased interest in sediment reuse rather than relocation to sea, including subaerial applications (L121−123) and specifically linked the bottle incubation experiment to this (L138-139). In section 4.4, (L710-720), we further elaborated on the implications of subaerial sediment applications.

Line 151-152: The rates shown are per year?

Yes; added (L154).

Line 155-156: how many cores replicates for each location?

Clarification was added. Bulk sediments were collected using a single sediment core (L162), while triplicate cores were used for the incubation of intact sediments (L252).

Line 242-243: Even evident for the author, precise the reason to have 20 cm of overlying water.

We added an explanation to the revised manuscript (L255−259).

Line 285: Why 37 days incubation period were chosen?

Within the scope and constraints of the project, we focused on short-term CO_2 emissions and terminated the experiment when rates stabilized at low levels after about one month. This is now explained in L309−311.

Line 581: Short range of these different values is needed.

Added (L610).

Line 675-677: include the concept of SMTZ (sulfate-methane transition zone)

Added (L723).

Line 681: “depending on”

Changed (L730).

Line 687: I don't know if the conclusion needs to be so precise about the results of the study…

We shortened the conclusion by removing unnecessary details in the revised manuscript (L737−750)

Additional comments: where are the dredging locations on Fig. 1? Dredging affects the upstream and downstream areas of the estuary in a similar way?

Dredging is ubiquitous and heterogeneous in the harbor area and not easily captured in a simple map, with largest volumes removed from the more recent and downstream (marine) port area (L151−158). We mention the relatively large contribution from areas around sites 115 and, to a lesser extent, site 21 (L253−255).

Please give some details on this subject. What would be the consequences of such an imbalance, according to quantity of sediment dredged in each zone?

More intensive dredging occurs in the marine area, therefore dredging predominantly perturbs sediment with relatively labile OM (L664−666).

What about the sediment relocated in the sea in terms of potential carbon mineralization processes?

Discussion was added in the revised manuscript, detailing the different fates of sedimentary C under marine and subaerial conditions (L710−720).

And finally CO2/CH4 effluxes toward the atmosphere?

Ultimately the efflux to the atmosphere is what matters and we hence appreciated this comment. However, gas exchange between the sea surface and the atmosphere was beyond the scope of this study. The relationship between our results and implications for estuarine CO_2 and CH_4 release into the atmosphere are mentioned in L611−614 and L722−729.

Reviewer #2:

"Perturbation increases source-dependent organic matter degradation rates in estuarine sediments" by Guangnan Wu et al. is a comprehensive and well-executed study investigating the chemical characteristics of sediment organic matter in Rotterdam harbour and the potential response of sediment carbon stocks to human disturbances (i.e. dredging). The study employs a range of techniques in the basic characterization of samples along a salinity transect (bulk sediment C/N, del13C, BIT index and pyrolysis-GCMS analysis of macromolecular organic matter) followed by a set of incubation experiments (both classical whole-core and homogenized sediment subaerial set-ups) to show how remineralization processes respond to disturbance. The data is of high quality and is generally processed and interpreted well, with a high degree of integration between the various lines of evidence. Overall, the outcome is convincing and should be published. However, I outline below one key issue related to the data processing that requires more careful consideration in the text, and several minor comments that should be addressed.

We sincerely thank the reviewer for their positive recommendation as well as the constructive feedback on our manuscript.

Key issue:

[*Calculation OM-derived DIC fluxes*] In processing the results of the whole-core incubations, ammonium fluxes are used to estimate the fraction of the DIC flux that is derived from organic matter remineralization (results shown in Fig. 5e). This calculation assumes Redfield stocihiometry for the degrading material, i.e. ammonium and DIC from remineralization are released in the ratio 106:16. This may be valid for the marine end-member site 115 but not necessarily for riverine end-member site 21A. Considering the bulk sediment C/N ratio data it is likely that site 21A releases more DIC per mole ammonium, even if the most reactive fraction of the sedimentary organic matter is relatively nitrogen rich. I suggest that this uncertainty is somehow included in the calculations, for example by presenting additional bars in Fig. 5e or a summary table. This issue is important because the quantification of remineralization-derived DIC production in the whole-core incubations is used later in the study when comparing with rates in the subaerial incubations, ie. assessing by how much dredging of sediment onto land would stimulate remineralization.

We thank the reviewer for pointing out the uncertainty regarding the OM-driven DIC flux, which we estimate by using the DIN flux and the Redfield C/N ratio. The uncertainty introduced by assuming Redfield was likely limited: the bulk C/N ratios in the surface sediment were 7.2 (115) and 9.9 (21A)—the somewhat elevated C/N at site 21 will likely have had little effect, because as the reviewer mentions the C/N of the reactive, likely N-rich, OM degrading during short-term core incubations will be lower than the bulk C/N.

However, the reviewer's comment did highlight for us that our approach did not account for anaerobic N loss which can be substantial in estuarine sediment (e.g. Seitzinger, 1988) and would cause a decoupling between DIN production from OM degradation in the sediment and the (comparatively low) DIN efflux. Using the suppressed DIN efflux and Redfield then underestimates the OM-derived DIC efflux, and consequently overestimates the DIC flux associated with e.g. $CaCO_3$ dissolution. In light of the importance of the DIC flux in evaluating the difference in OM degradation rates under submarine and subaerial conditions, we now include additional data, i.e. (1) the measured total alkalinity (TA) flux and (2) the SO_4^{2-} reduction rate as TA source as calculated from the porewater SO_4^{2-} gradient in the uppermost sediment. We arrive at a more robust estimation of the OM-derived DIC flux as an indicator of the OM degradation rate, mentioned in L415−418 in the revised manuscript and detailed in the SI. This approach and inclusion of the TA data also address a later comment by Reviewer #2 about $CaCO_3$ dissolution rates. Our new approach reduces the contribution of $CaCO_3$ dissolution (which is likely low in organic-rich coastal sediment; Krumins et al., 2013) and decreases the boost in OM degradation rates between in-situ and subaerial OM degradation rates (from 3–7 to 2–3; incorporated in L33, L630, 712, 743), but not to an extent that affects the overall conclusions of the work.

Minor comments:

Line 81-82: No need to highlight CH4 specifically. The point is valid for the balance between any given set of remineralization pathways.

Revised as detailed in the response to Reviewer #1's comment about [*Salinity and CH_4*].

Line 84-87: Consider rewording the sentence ("estuaries" appears to be used in both a general and a specific way in the same sentence) and add the reference.

Changed (L87−88).

Line 88: Should be "penetration depth".

Line 99: Make sure to use brackets correctly.

Line 123: Should be "dredged sediment".

Line 137-139: Should be "bottle incubations"

Line 139: Should be "Our results show..." or "We show..."

Line 141: Should be "the properties of OM influence..."

Line 213: Remove "an"

Line 383: Should be "overlying water".

Line 438: Should be "thus" not "this"

Line 478: Should be "may explain the observation that".

Line 607: Check the formatting of the citation.

Line 620: Should be "10 times faster than..."

Changed. We checked and corrected spelling and grammar in the revised manuscript.

Line 162: Use "end-members" or similar, rather than "realm"
Changed (L169).

Line 220-229: It would be useful for a reader trying to reproduce this method to know how the HF was disposed of during the protocol. Is it evaporated at some stage? This is a dangerous chemical and lab protocols need to be carefully designed.
We added the disposal of HF in the revised manuscript (L234, 237).

Line 229: Clarify that "MOM" here refers to the residue of the previous steps.
Changed (L239−240).

Line 283: It is not clear what is meant by "60% water-filled volume". It is difficult to estimate the porosity of freeze-dried and homogenized sediment after re-wetting due to changes in grain size distribution during these processing steps.
We added an assumption in the revised manuscript that porosity remained the same after sediment rewetting (L306−307).

Line 345: "Asterisk/stars" not "asteroids". Also unclear what the arrows in the plot indicate, should they be pointed in the opposite direction to link the numerical labels to the 2D fields in the x-y plot?
Corrected in Fig. 2 and its caption.

Line 245: Should be Table S3, not S2.
Corrected (L376).

Fig. 6: Please add the information about "marine" vs. "riverine" stations to the plot or legend.
Added. See Fig. 6.

Fig. 7: What do the stipple vs. solid lines indicate? Also show which sites are "marine" and "riverine".
In the revised manuscript, solid lines were used for all subplots, and site information was also added in Fig. 7.

Line 451: This is a valid consideration rather than a confounding factor.
Changed (L485).

Line 517: It is not unusual that soil OM contains similar chemical signatures to the higher plants that grow and decay in them. It is perhaps misleading to state that the correlation between BIT and the EMMM results implies low contribution of vegetation input, rather it should be stated that, as you conclude later, the plant signals are transferred via the soils (where they also pick up the BIT signal).

Because terrestrial OM pools are indeed not easily differentiated, we simplified the paragraph on proxy comparison (Fig. 8) (L546-554).

Line 551-552: The comparison with ocean margin trends is not useful here as the reactivity gradients in near-shore areas is more likely to be controlled by terrestrial inputs than by water-depth fractionation of sinking OM.
Based on the various reviews, we revised section 4.2, including removing the reference to pelagic water-depth trends, see L577 ff.

Line 564: Remove "ancient". There is no information about age of the material so this is not relevant.
Removed.

Line 567-568: Interpretation of carbonate dissolution needs to be expanded. Is there evidence for this in the data or literature?
As detailed above (see Reviewer #2's comment about [*Calculation OM-derived DIC fluxes*]), we have modified the calculation of the OM-derived DIC flux which also affected the estimation of DIC from $CaCO_3$ dissolution.

Line 577: The higher CH4 flux in a whole core incubation at a more freshwater site can be due to the higher position in the sediment column of the SMTZ. See e.g. the diagram in Fig. 7 of https://esd.copernicus.org/articles/13/633/2022/esd-13-633-2022.html.
We incorporated the SMTZ-shift into the text (L605).

Line 633: Also refer to Keil et al. (1994).
Added (L671).

Line 677-678: Separate the statements about global AOM estimates and dredging impacts, these are not obviously connected.
This section has been revised based on this and other reviewer comments and statements have been separated (L722-734).

Reviewer #3:

The manuscript of G. Wu et al. investigates the increase of organic matter (OM) degradation produced by anthropogenic perturbations such as dredging activity. The method stands on the elemental (C/N) and isotopic (δ13C) characterization of organic matter of 49 sites from the Port of Rotterdam. Molecular characterization was done on 13 of the 49 sites, reoxydation experiments on 6 sites and in situ OM remineralization have been estimated on 2 sites. The authors conclude from their studies that Cl#1 - CN proxies are robust to identify contribution of marine versus non-marine OM sources, Cl#2 - marine

OM is more labile than terrestrial OM and Cl#3-aerobic conditions accelerates OM remineralization compared to natural (mainly anaerobic) conditions.

From my point of view, this manuscript is not reaching the quality requirement to get published in BG: the rare new knowledges brought by this manuscript are only site specific. In the conclusion, the authors underline more general concepts (Cl#1, Cl#2, Cl#3), however it corresponds to already well-established concept in biogeochemistry that does not present significant novelty.

We thank the reviewer for their time and effort in evaluating our manuscript. Reviewer #3's assessment about the quality and significance of the work strongly contrasts with evaluations by Reviewer #1 and #2, who highlighted the importance and high quality of the work. Uniquely, our work combines detailed sediment and OM characterization with incubation of intact and disturbed sediments to link CO_2 emission potential to OM properties (rather than content) and biogeochemical processes as function of environmental conditions. Whereas some of the broader concepts discussed (e.g. selective preservation, aerobic mineralization) are indeed established in biogeochemistry, the contribution of this study lies in applying and contextualizing these concepts within the complex settings of a heavily perturbed estuarine system, which is understudied in existing literature. In addition, the findings provide crucial insights into how human-induced disturbances interact with natural OM processing, with implications for carbon cycling. Such knowledge is also essential for informed policy making on processing dredging waste. As estuarine systems face increasing pressure of human perturbation, we believe this study represents a meaningful contribution to the field, as also noted by both Reviewer #1 and #2.

[*O_2 fluxes*] In addition, a detailed reading of the manuscript reveal that the scientific hypothesis defended along the manuscript (that perturbation increases source-dependent organic matter degradation rates in estuarine sediment) is not properly argued. While the source characterization of the OM received large analytical efforts, and a valid interpretation supported by some modelisation, four others critical step of the demonstration seems not strong enough. First, and most important, the demonstration of a source and composition dependence of OM degradation rate is not convincing. The authors build their hypothesis from the literature and a reasonable intuition but that is in complete opposition with the dataset presented. Indeed, the two sites, selected because they present very contrasted OM origin and composition, present very similar O2 fluxes that is interpreted -reasonably- as a similar intensity of remineralization rate. This result should better be used to underline a limit of the observations from the literature. Instead, the authors use an artefact (normalization by the TOC) to build a contrast between the two stations while the relation between quantity of OM and remineralization rate have not been presented. It seems more rational to conclude from their dataset that OM quantity does not play an important role in deposit, probably because it is in large excess.

We include further discussion about the similarity in short-term O_2 fluxes and the underlying reasons (similar processes acting at the sediment-water interface) in the revised manuscript (specifically L591–592. We further agree that sedimentary organic carbon turnover in the core incubation experiments might to some extent depend on O_2 availability (L593–594). We have kept the TOC-normalized rates in

the manuscript; this approach is commonly used to assess the relative reactivity of OM (e.g. Freitas et al., 2025; Zander et al., 2020, 2022), especially when TOC content differs considerably between sites (in this case 2.2 wt.% and 5 wt.%). The TOC-normalization allows comparing OM degradation rates between sites with different OM compositions (more fresh algal material at site 115 compared to the more refractory terrestrial-derived OM at site 21A) and, furthermore, between different incubation conditions (Fig. 5d and 6b).

[*Algae-derived organic matter*] Second in importance, there is no consideration on primary production, while freshly produced algea are probably more labile than any other carbon source. In other word the local recycling of carbon between photosynthesis and respiration is neglected in this manuscript while it is probably very important in this very shallow environment.
Algal material and its degradation plays a central role in the manuscript. We now explicitly address the role of freshly produced algae in controlling short-term O2 fluxes (L591−592). Study of benthic primary productivity as O_2 source was not part of this study, but likely insignificant in these highly turbid waters.

[*Site selection and methodology*] Third, while the introduction suggests a study covering many sites and embracing some spatial variability, the comparison between perturbated and not perturbated sediment is done only on 2 sediment, which seems not enough to support conclusions that could be generalized to others estuaries, and thus limits the interest of the results.
We have detailed the rationale for site selection for the various analyses and experiments, please see our response to the editor's comment about [*Site selection and methodology*]. We note that this study incorporates a range of analyses and experiments for integrated insight into OM properties and degradation rates; within the scope of the project, it was only feasible to perform whole-core incubations with sediments from two contrasting and important dredging sites.

The open-air bottle experiments based on six sediments further supported the observed relationship from whole-core incubation: marine sediment OM was more reactive/biodegradable than riverine sediment OM. Therefore, we believe that our work does reflect a general relationship between OM composition and CO_2 release potential in human-impacted estuaries (e.g. the Elbe estuary; Zander et al., 2022), but the spatial distribution of the more degradable OM (e.g. algal material) may differ between systems, now also mentioned in the Implications (L695−700)

[*Subaerial bottle incubation experiment*] Fourth, the methodology of perturbation does not seem adapted to an estuarine environment, since it corresponds to a not water-saturated sediment more adapted to mimic soil aeration than resuspension induced by dredging activities.
Please see our response to the editor's comment about experimental approach for the [*Subaerial bottle incubation experiment*].

[*Sample processing*] Additionally, the dry freezing of the sediment before incubation would certainly modify the properties of the reactive organic matter – which have not been tested.

Please see our response to the editor's comment about [*Sample processing*] above.

References

Freitas, N. L., Walter Anthony, K., Lenz, J., Porras, R. C., and Torn, M. S.: Substantial and overlooked greenhouse gas emissions from deep Arctic lake sediment, Nat Geosci, https://doi.org/10.1038/s41561-024-01614-y, 2025.

Krumins, V., Gehlen, M., Arndt, S., Van Cappellen, P., and Regnier, P.: Dissolved inorganic carbon and alkalinity fluxes from coastal marine sediments: Model estimates for different shelf environments and sensitivity to global change, Biogeosciences, 10, 371–398, https://doi.org/10.5194/bg-10-371-2013, 2013.

Seitzinger, S. P.: Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance, Limnol Oceanogr, 33, 702–724, https://doi.org/10.4319/lo.1988.33.4part2.0702, 1988.

Zander, F., Heimovaara, T., and Gebert, J.: Spatial variability of organic matter degradability in tidal Elbe sediments, J Soils Sediments, 20, 2573–2587, https://doi.org/10.1007/s11368-020-02569-4, 2020.

Zander, F., Groengroeft, A., Eschenbach, A., Heimovaara, T. J., and Gebert, J.: Organic matter pools in sediments of the tidal Elbe river, Limnologica, 96, 125997, https://doi.org/10.1016/j.limno.2022.125997, 2022.