

We thank Reviewer 3 for taking time to give their feedback. The text in red indicates our response and the proposed modifications to the manuscript.

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 ($\delta^{13}\text{C}$) 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 CI#1 - CN proxies are robust to identify contribution of marine versus non-marine OM sources, CI#2 - marine OM is more labile than terrestrial OM and CI#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 (CI#1, CI#2, CI#3), however it corresponds to already well-established concept in biogeochemistry that does not present significant novelty.

We sincerely thank the reviewer for their time and effort in evaluating our manuscript. However, we disagree with the assessment that the study lacks sufficient novelty or relevance for publication in *Biogeosciences*. This study presents a detailed characterization of estuarine OM coupled to degradation rates in-situ and perturbed, which is in our view novel. While 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 a heavily disturbed/dredged estuarine system, which is insufficiently explored in the literature. Besides, the findings provide crucial insights into how human-induced disturbances interact with natural OM processing, with implications for carbon cycle and release. As estuarine systems facing increasing pressure of human perturbation, we believe this study represents a meaningful contribution to the field, as also noted by Reviewer 1 and 2.

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 agree that the total amount of sediment organic carbon might be in large excess relative to O₂ availability, but we want to clarify that the O₂ fluxes represent the oxygen consumption of fresh OM deposited at the sediment surface, rather than the whole sediment cores, as the oxygen penetration depth is usually a few millimeters and even less in OM-rich coastal sediments (Cai and Sayles, 1996). We will add this point to the revised manuscript.

We do not agree with the suggestion that the use of the TOC-normalized rate is an artefact. Normalization on TOC is a commonly used approach 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 us to compare OM degradation efficiency between sites with different OM compositions (more fresh algal material at site 115 while more terrestrial-derived OM at site 21A) and, furthermore, between different incubation conditions (Fig. 5e and 6b). Importantly, it highlights that caution is required when interpreting bulk OM content in terms of CO₂ release potential.

Second in importance, there is no consideration on primary production, while freshly produced algae 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.

We would like to clarify that primary production and the associated input of labile algal OM are not neglected in the manuscript. We first mentioned it in the whole-core incubation experiments, where the local OM production and transport dominate (Line 561–565). Then we expanded this aspect to the discussion of carbon release from mixed, dredged sediments (Line 619–621). We later mentioned it again in the conclusion section (Line 704–710).

Third, while the introduction suggests a study covering many sites and embracing some spatial variability, the comparison between perturbed and not perturbed 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.

The study does show a comprehensive dataset on sediment OM properties throughout the harbor: 13 sites comprising OM characterization, 6 sites dealing with reoxidation. For further context, intact core incubations were performed for two contrasting sites. Altogether, this is a very elaborate dataset that links detailed sediment (OM) properties to rates of processes, which is novel and provides new insights into the CO₂ release potential of disturbed estuarine sediments. Furthermore, this study is conducted in one of the largest estuaries and the busiest harbor of Europe, which is under the impact of substantial anthropogenic disturbance. Given the growing trend of human modifications of coastal systems, we

believe the findings are relevant and will contribute to the further understanding of human impacts on the sequestration of coastal blue carbon.

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.

This was also noticed by Reviewer 1. We will add a detailed explanation in both the Introduction and Discussion sections of the revised manuscript. In short, the open-air incubation was chosen because: (i) it provides insights into potential rates of OM mineralization of sediment exposure to oxygenated water, while limiting kinetic constraints of O₂ transportation in slurry experiments, and (ii) it tests the impact of on-land application (which is an increasing trend in sediment management practices) on carbon emission.

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

We acknowledge that freeze-drying may change the properties of the reactive OM. However, alternative methods like air-drying can also change OM characteristics, including the loss of the most labile carbon fraction during air-drying (which usually lasts a few days). Some incubation studies on soils/sediments were based on freeze-dried samples and reported limited impact of freeze-drying on carbon emissions (He et al., 2022; Wu et al., 2020). Fromin (2025) suggests that there is rarely consensus on a best practice when studying microbial processes in soils and sediments, and the appropriate methods often depend on the specific goals of the study. Given the limitations of different approaches and the aim to quantify the labile OM fraction, freeze-drying was considered the more suitable option for sample pre-treatment in our case.

References

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