Review 1

EGUsphere-2025-3020 Manuscript Review: Limited physical protection leads to high organic carbon reactivity in anoxic Baltic Sea sediments

Summary

To investigate the importance of mineral protection on OC reactivity in anoxic conditions, sediment cores were collected from the anoxic Baltic Sea. Their OC%, C/N ratio, $\delta^{13}C$, sulfate reduction rate, ^{210}Pb age, OC loading, and OC-FeR were determined. The downcore increase in OC% and SRR suggested intensive sulfate reduction and OC remineralization, suggesting high OC reactivity despite the anoxic conditions. High OC loading, with downcore decrease in OC/SAA, suggests inadequate mineral surface area for OC stabilization. Low abundance of OC-FeR indicated limited protection of OC by FeR minerals. In summary, the current study demonstrates that when mineral protection of OC is nearly absent, oxygen depletion alone could not decrease OC mineralization rate.

Major comments

Overall, this manuscript is well-written. The data set presented in the current study is small and simple, yet it addresses an important research question in the chemical oceanography field.

Therefore, I believe that the current manuscript contains a global significance that could be beneficial for further scientists who aim to investigate the drivers of OC preservation. Most of the methods and discussions are logical. I'd suggest this manuscript a minor revision, following my comments below.

Specific comments

Q1: Line 23-24: " Overall, these results suggest that the WGB sediments receive large amounts of OC relative to the supply of mineral particles, far exceeding the potential for OC physical protection"

This statement is true for OC/SAA; however, it might not be right for OC-FeR since the authors discussed that FeR availability is not the major factor controlling OC-FeR association (Line 267-273). Alternatively, I think the lack of OC-FeR in Baltic Sea is likely due to the separate input of FeR (from land) and OC (from freshwater/marine water column). See my comments in line 276-278.

A1: We agree with the reviewer that the Fe_R availability alone does not control the extent of OC-Fe association. However, the SSA of these sediments partly reflects that of the Fe minerals dissolved through the CBD extraction. Our previous investigations in Swedish fjords (Placitu et al., 2024) suggested that riverine inputs can deliver preformed OC-Fe to the sediments. In contrast, the WGB receives limited riverine input, and the OC pool is dominated by autochthonous marine production rather than terrestrial OM. This likely reduces the potential for stable OC-Fe association to form.

Revised text in section 3.3: “The low %OC-Fe found in WGB1 and WGB2 potentially reflects both the decoupled delivery of Fe_R and OC and the absence of substantial terrestrial inputs of Fe_R that preferentially bind terrestrial organic matter. In the WGB, Fe_R is likely to enter the Baltic Sea via surface runoff and is redistributed via sediment resuspension as described in Nilsson et al., (2021), while most OC consists of autochthonous marine production (Figure S1 in Supplementary information) which likely limits the formation of OC-Fe associations. This decoupling of OC and Fe_R inputs in WGB could explain the limited amount of OC-Fe found across the WGB sediments, supporting the hypothesis that Fe_R preferentially binds terrestrial organic matter (Dicen et al., 2019; Linkhorst et al., 2017; Riedel et al., 2013; Salvadó et al., 2015; Shields et al., 2016; Sirois et al., 2018; Wang et al., 2019; Zhao et al., 2018, 2023). »

Placitu, S., van de Velde, S. J., Hylén, A., Hall, P. O. J., Robertson, E. K., Eriksson, M., Leermakers, M., Mehta, N., and Bonneville, S.: Limited Organic Carbon Burial by the Rusty Carbon Sink in Swedish Fjord Sediments, Journal of Geophysical Research: Biogeosciences, 129, e2024JG008277, https://doi.org/10.1029/2024JG008277, 2024.

Q2: Line 74: Add "," after "(Andrén et al., 2000)".

A2: Done

Q3: Line 82: In my opinion, "particulate organic carbon (POC)" is not an appropriate terminology here. Many literatures defined POC as suspended OC particles within water column, which has not deposited and has not been incorporated into sedimentary storage yet (see reviews in Bianchi (2007)). Another set of literature defined POC as a subset of bulk sedimentary OC that is made of fragmented plant litter with low density and large particle size (e.g., Cotrufo et al., 2019). To my understanding, the current study measured OC content in bulk sediment samples. Therefore, to avoid confusion, I'd suggest the authors to change the term "particulate organic carbon (POC)" to "sedimentary organic carbon (SOC)".

Bianchi, T. S. (2007). Biogeochemistry of estuaries. Oxford University Press.

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (201S). Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience, 12(12), S8S-SS4.

A3: We agree with the proposed change and have replaced "particulate organic carbon (POC)" with "sedimentary organic carbon (SOC)" throughout the manuscript.

Q4: Line 150: What's about OC mineralization by reduction of NO_3^- to NH_4^+, or reduction of Fe^{3+} to Fe^{2+}?

A4: In persistently anoxic sediments, the contribution of denitrification, DNRA, manganese and iron reduction to organic carbon mineralization is negligible compared to sulphate reduction, given the dominance of sulphate in the porewater geochemistry. If these pathways were quantitatively important, the total reactivity would be even higher than our estimates, meaning our current mineralization rates are likely underestimated.

We included an additional sentence in the main text "These sediments are anoxic, unaffected by bioturbation, and sulphate SO_4^{2-} is not depleted, so one can assume that all OC mineralization results from sulphate reduction. Contributions from denitrification, DNRA, manganese and iron reduction are negligible; however, it is important to note that the reported rates may slightly underestimate total reactivity"

Q5: Line 164-165: "...inputs, reflected in the $\delta^{13}C$ signature and C/N ratios of these sediments, highlighting the presence of 'fresh' material"

$\delta^{13}C$ and C/N ratios are proxies of OC sources, not their freshness. I do agree with the authors that the principal sources of OC in WGB cores are freshwater and marine planktons.

However, these proxies do not reflect whether these OC are made of 'fresh' plankton-derived OC, or 'degraded' plankton-derived OC. To determine whether these OC are fresh or degraded, the authors need additional proxies such as $\Delta^{14}C$ (age of OC) or biomarkers such as stanol/sterol ratios (higher stanol reflected more degraded OC).

A5: We agree with the comment, the word fresh was modified to ‘’autochthonous freshwater and marine material’’

Q6: Line 182-183: To my understanding, Katsev and Crowe (2015) calculated k-values from changes in concentration of C with depth and time, using the equation $k = -\frac{U}{C}\frac{dC}{dX}$. In contrast, the current study calculated k-values from SRR, through the measurement of 35S radiotracer during laboratory incubation (Van de Velde et al., 2023). Is it possible that the difference between these two methodologies resulted in the shift of data points away from the $\log_{10}$k-$\log_{10}$t anoxic trendline?

A6: We disagree with the reviewer on that point. Katsev and Crowe (2015) specify clearly that the use of burial velocity (U=dx/dt) is when dating of the sediment was not available. Where dating was available (which is our case here), they explicitly mention that k was directly calculated as $k= -C^{-1}$ dC/dt. As such, Katsev and Crowe dataset already contains k values derived from both expressions.

Q7 Was there any possible process that could interfere with the measurement of ^{35}S post-incubation (e.g., partitioning of consumed $^{35}SO_4^{2-}$ to H_2S (g) and FeS_2 (solid))?

A7: The use of radioactive ^{35}S tracer (Jørgensen, 1978) is a well-established method to quantify sulphate reduction rates in sediments. To terminate incubations, zinc acetate (ZnAc) is added, which immediately precipitates all dissolved sulphide (including radiolabeled products) as insoluble ZnS. This prevents loss of volatile H_2S and simultaneously halts microbial activity. Reduced sulphur species that have already reacted further into solid phases—such as FeS, FeS_2 and elemental sulphur—are subsequently recovered using the chromium reduction method (Canfield et al., 1984), in which these species are quantitatively converted to H_2S and trapped again as ZnS (by adding ZnAc). All the ZnS formed is then titrated ensuring that both dissolved and solid-phase pools of reduced S are accounted for. Therefore, while partitioning of reduced sulphur into multiple pools does occur, the combined use of ZnAc fixation and chromium reduction ensures reliable SRR quantification.

Jørgensen BB. 1978 A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments: I. measurement with radiotracer techniques. Geomicrobiol. J. 1, 11-27. (doi:10.1080/01490457809377721)

Canfield DE, Raiswell R, Westrich JT, Reaves CM, Berner RA. 1986 The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54, 149-155. (doi:10.1016/0009-2541(86)90078-1)

Q8: Was there any possible mechanism that can reduce or recycle $^{35}SO_4^{2-}$ without the need to consume OC?

A8: There are potentially other processes e.g. chemolithoautotrophic sulphur oxidizers (using reduced sulphur with nitrate) or the various disproportionation reactions that might potentially interfere with the $^{35}SO_4^{2-}$ incubations (without OC oxidation) identified in Baltic Sea sediments. However, we consider these metabolisms to be negligible relative to sulphate reduction. Indeed, van de Velde (2023) modelling shows a very good agreement between measured and modelled SO_4^{2-} and DIC concentrations in WGB cores suggesting that sulphate reduction alone account for most of the OC mineralization and SO_4^{2-} concentration variation at depth.

Q9: Was there any new production of OC due to sulfate respiration (e.g., production of new microbial biomass) so that changes in sedimentary OC content does not directly follow 2:1 stoichiometry (Equation 4)?

A9: Sulphate reducers can divert a small fraction of the consumed OC into anabolic reactions and new biomass, but this C assimilation is typically very low, on the order of a few percent max. of C substrate depending on the compounds (Stolyar et al., 2007; Londry & Des Marais, 2003; Pellerin et al.,2020). Thus, this assimilated fraction does not alter significantly the 2:1 stoichiometry.

Stolyar, S., Van Dien, S., Hillesland, K. L., Pinel, N., Lie, T. J., Leigh, J. A., & Stahl, D. A. (2007). *Metabolic modeling of a mutualistic microbial community*. *Molecular Systems Biology*, 3:92. https://doi.org/10.1038/msb4100131

Pellerin, A., Antler, G., Marietou, A., Turchyn, A. V., & Jørgensen, B. B. (2020). *The effect of temperature on sulfur and oxygen isotope fractionation by sulfate reducing bacteria (Desulfococcus multivorans)*. *FEMS Microbiology Letters*, 367(9), Article fnaa061. https://doi.org/10.1093/femsle/fnaa061 (Pure)

Londry, K. L., Jahnke, L. L., & Des Marais, D. J. (2004). *Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria*. *Applied and Environmental Microbiology*, 70(2), 745-751. https://doi.org/10.1128/AEM.70.2.745-751.2004

Q10: Have the authors tried calculating k-values from changes in OC concentration with depth and time (using Katsev and Crowe (2015) method), without using SRR? Did they yield the same or different k-values?

A10: van de Velde et al., 2023 modelled OC respiration rate at these sites using the downcore OC profile (as in Katsev and Crowe, 2015) and with SO_4^{2-} reduction rate and showed that they agree well (see Fig. 3 in van de Velde, 2023).

Q11: Line 206: add ")" to "...Mayer, 1994b))".

A11: Done.

Q12: Line 225 (Figure 2): Since the paragraph below discussed changes in OC loading with depth, I'd suggest the authors to improve this figure by showing the depth information on the plot. The authors may adjust the color of data point to represent depth (e.g., lighter color = surficial, darker color = deep), or they may just simply label the depths above every data point.

A12: We agree with this suggestion and improved the Figure 4 following the recommendation of the reviewer, i.e. with a colour code in each data point denoting depth.

Q13: Line 240: Apart from intense OC mineralization, is there any alternative mechanism that potentially resulted in downcore decrease in OC loading? For example, was there any temporal change in mineral input to the Baltic sea? Was there any historical record of increasing light availability or increasing nutrient input to the Baltic sea which could accelerate the production of autochthonous OC in recent years? Increasing OC production rate could result in higher OC: SSA in younger surficial sediment layers, compared to the older deeper sediments.

A13: The reviewer is right that there has been an acceleration of the production of autochthonous OC in large parts of the Baltic Sea in recent decades due to eutrophication. Indeed, the Baltic Sea has experienced major eutrophication during the 20th century (e.g., Conley et al. 2009), linked to nutrient enrichment, which likely increased autochthonous OC production and could contribute to higher OC:SSA values in more recent sediments relative to deeper, older layers. While resolving these temporal dynamics in WGB directly is out of the scope of the study, the observed downcore decline in OC:SSA is consistent with progressive degradation and mineralization of OC, as also suggested by high sulphate reduction rates.

Revised text: "The very high OC loadings observed in the WGB surface sediments most likely reflect a combination of intense autochthonous OC inputs, stimulated by eutrophication, together with a limited role of mineral surfaces in constraining OC accumulation." [...] "Enhanced nutrient loading and primary production in the Baltic Sea during the 20th century (Conley et al., 2009) likely contributed to these elevated OC:SSA values in surficial sediments, as increased deposition of autochthonous OC on particle surfaces was not supported by a proportional increase in mineral inputs."

Q14: Line 276-278: "The lack of major rivers in this part of the Baltic Sea could explain the limited amount of OC-Fe found across the WGB sediments, supporting the hypothesis that FeR preferentially binds terrestrial organic matter"

It's not that FeR preferentially binds with terrestrial or marine OC. The literature in this list observed that FeR is preferentially associated with terrestrial "vascular plant" OC, due to the abundance of reactive functional groups (such as phenols and carboxylic) in vascular plant materials. In the current study, molecular structure of OC is not the main factor controlling whether FeR will preferentially bind with terrestrial or marine OC. Vascular plant-OC is nearly absent in Baltic sea sediments while the majority of OC in is made of marine or freshwater POC (Fig. S1).

Hence, the input of FeR and OC to the Baltic may occur through separate processes. For example, land-derived FeR may enter the Baltic Sea via overland flow while POC may be formed in-situ within water column. In this case, pre-formed FeR was unable to react with newly formed OC.

This leads to an important discussion that "while allochthonous land-derived OC-FeR can survive anoxic conditions in marine sediments over millennia (Faust et al., 2021), the anoxic conditions inhibit autochthonous formation of new OC-FeR, even though the Baltic Sea received excessive input of both FeR and OC from separate mechanisms".

A14 : We thank the reviewer for pointing out the need to clarify the factors controlling OC-Fe associations in the Baltic Sea sediments. We agree that the molecular structure of OC is not the main factor controlling whether FeR will preferentially bind with terrestrial or marine OC, since vascular plant-derived OC is nearly absent and the majority of OC in Baltic sediments is marine SOC (Fig. S1). In response to the comment, we revised the text (see below) to clarify that the limited OC-Fe observed in the WGB sediments potentially reflects the decoupled delivery of Fe_R and OC. Specifically, Fe_R may be delivered via land-derived inputs (e.g., runoff and sediment resuspension), while SOC is largely produced *in situ* in the water column. This explanation aligns with previous observations that allochthonous OC-Fe complexes can survive over millennia, while autochthonous formation of OC-Fe is limited under anoxia (Faust et al., 2021). We believe this revision addresses the reviewer’s concern and provides a more comprehensive interpretation of the mechanisms controlling OC-Fe formation in the Baltic Sea sediments.

Revised text: “The low %OC-Fe found in WGB1 and WGB2 potentially reflects both the decoupled delivery of Fe_R and OC and the absence of substantial terrestrial inputs of Fe_R that preferentially bind terrestrial organic matter. In the WGB, Fe_R likely enter the Baltic Sea via surface runoff and is redistributed via sediment resuspension, while most OC consists of autochthonous marine production (Fig. S1 in Supplementary information) which likely limit the formation of OC-Fe associations. The decoupling of OC and Fe_R inputs in WGB could explain the limited amount of OC-Fe found across the WGB sediments, supporting the hypothesis that Fe_R preferentially binds terrestrial organic matter (Dicen et al., 2019; Linkhorst et al., 2017; Riedel et al., 2013; Salvadó et al., 2015; Shields et al., 2016; Sirois et al., 2018; Wang et al., 2019; Zhao et al., 2018, 2023).

Q15: Line 283: Replace "clay" with "mineral surface". There is no direct measurement of clay-bound OC in the current study.

A15: Agree, corrected.

Q16: Line 285-286: "Hence, our results suggest that limited physical protection reduces the importance of O_2 for OC mineralization, as OC remains easily accessible for microbes, in this case, sulphate reducers"

Linguistically, I think "limited physical protection reduces the importance of O_2 for OC mineralization" is not an appropriate statement. I think O_2 is still an important factor for OC mineralization in the condition of limited physical protection. In other words, unprotected OC will be degraded faster in the aerobic conditions (due to the ease of electron transfer etc.), compared to the anaerobic conditions.

I'd suggest the authors to restructure this sentence to "the absence of O_2 does not necessarily result in deceleration of OC decomposition, as we observed high SRR in anoxic Baltic Sea sediments. This significant loss of OC in anaerobic condition is likely due to limited physical protection of OC by minerals".

A16: We thank the reviewer for this clarification. We agree that our initial phrasing could be misinterpreted as suggesting that O_2 is not important for OC mineralization, which is not what we intended. As the reviewer points out, oxygen remains the most efficient electron acceptor and OC will be degraded faster under oxic than anoxic conditions. Our intended message was that, in the WGB sediments, the absence of O_2 does not necessarily slow down OC decomposition, since high SRR indicate that “unprotected” OC is still readily available for anaerobic degradation. We revised the ending of the conclusion accordingly:

Revised text:” Together with consistently high sulphate reduction rates across the basin, these results suggest that anoxic conditions in the WGB do not necessarily result in a slowdown of OC mineralization. Instead, the significant degradation of OC under anaerobic conditions is likely facilitated by the limited physical protection of OC by mineral surfaces, which keeps a large portion of OC accessible to sulphate reducers. ”