

We would like to express our sincere gratitude for additional thoughtful remarks and valuable suggestions. Below, we provide detailed responses to Reviewer 1's comments, with the corresponding revisions clearly marked in red within the manuscript.

## RESPONSE TO REVIEWER #1

*I previously reviewed this manuscript and recommended major revision. I have now evaluated the revised version (egusphere-2026-519-ATC1.pdf) together with the authors' point-by-point response. With the minor points below addressed, I consider the manuscript acceptable for Biogeosciences after minor revision.*

*Major comments:*

(1) *Downcore profiles of GDGT-derived indices (MI, RI-OH, and GDGT-0/cren) are still absent.*

**Author response 1: We have added downcore profiles of GDGT-derived indices in the supplementary material (Fig. S3).**

(2) *The validation between LOI and TOC is currently based on only two cores. How representative is this relationship for the other cores, such as MET1-BH and MET4?*

**Author response 2: The use of loss-on-ignition (LOI) as a proxy for organic matter in our study is supported by previous regional geochemical research by Łukawska-Matuszewska et al. (2014) in the area where our samples were collected. The study was based on a substantially larger dataset and showed a strong correlation ( $R^2 = 0.68$ ) between LOI and organic carbon. Findings from Łukawska-Matuszewska et al. (2014) indicated that LOI serves as an effective screening tool for assessing geochemical variability associated with organic matter content in the examined region, provided that comparable ignition conditions are applied, as in our study. This was used in our research because full-core elemental total organic carbon (TOC) data were not available for all sites. Additionally, LOI-based values were further evaluated against directly measured TOC in two representative pockmark and reference cores. Both parameters show similar variability across the sediment profile and are correlated. Consequently, the GDGT content in the sediment was analysed in relation to LOI, which was measured at all stations included in the study. Given the positive relationship between LOI and TOC in the selected cores, LOI was used as a proxy for organic matter content. It was, however, applied qualitatively at the trend level to provide geochemical context, not for biomarker normalisation.**

The reference to the regional analysis of LOI and organic carbon was added to Section 2.4, lines 281-282: “Łukawska-Matuszewska et al. (2014) previously demonstrated the applicability of LOI in the Gdańsk Basin as a proxy for sedimentary organic matter content.”

The validation was further explained in greater detail in Section 3.2:

Lines 483-489: “LOI-derived estimates of organic matter are sensitive to ignition conditions and sediment composition. Because mass loss at 550 °C may include contributions from sediment mineralogy, including inorganic carbon-bearing minerals,

**LOI-based values provide a screening-level estimate of TOC rather than an exact measurement of organic matter content. To assess whether LOI captured the main downcore stratigraphic trends in organic carbon, LOI profiles were compared with directly measured TOC in two representative cores: the MET1-MP pockmark core and the MET3 reference core (Supplementary material Fig. S2)."**

**Consistent with the regional relationship between TOC and LOI reported in the Gdańsk Basin by Łukawska-Matuszewska et al. (2014), across all paired measurements, TOC and LOI in the pockmark core MET1-MP and the reference core MET3 are significantly correlated ( $r = 0.65$ ,  $p < 0.005$ ), and linear regression explains 43% of TOC variability ( $R^2 = 0.43$ ;  $TOC = 0.612 + 0.282 \times LOI$ ). The positive correlation between LOI and TOC supports the use of LOI as a proxy for organic matter variability.**

**In Section 4.4:**

**Lines 1050-1054: "The use of LOI as an organic matter proxy is supported by previous regional geochemical research by Łukawska-Matuszewska et al. (2014), based on a substantially larger sample set, which demonstrated a strong correlation ( $R^2=0.68$ ) between LOI and organic carbon. The findings indicate that LOI is an effective screening tool for assessing geochemical variability related to organic matter."**

*(3) The manuscript currently contains 10 figures, which seems excessive. The authors may consider reducing the number of figures. For example, Fig. 2 and Fig. 3 could potentially be integrated into a single figure. In addition, some figures can be moved to supplementary files.*

**Author response 3: We have reduced the number of figures by moving one figure to the supplementary material (Fig. S2, Comparison of loss-on-ignition values (LOI%) and directly measured total organic carbon (TOC wt%) in two representative sediment cores...) and by integrating Fig. 2 and Fig. 3 into a single figure, now Fig. 2.**

*(4) The Highlights and Abstract still partly overstate the evidence for tightly coupled ammonia oxidation cooperation, compared with the more cautious interpretation presented in the main text. In general, highlights should consist of concise bullet points, each expressed as a single sentence. For example, the third and fourth highlights should be rephrased more cautiously.*

**Author response 4: We agree with the reviewer. The highlights are corrected as follows:**

**Lines 17-23: „Pockmark sediments harbour substantially higher archaeal diversity and abundance than non-pockmark reference sediments.**

**The P/MET4 pockmark in the Gdańsk Deep hosts the most diverse and abundant methanogen community, coinciding with the highest concentrations of isoprenoid glycerol dialkyl glycerol tetraether lipids (iGDGTs).**

**Crenarchaeol dominates the iGDGT pool in both pockmark and reference sediment cores, indicating strong Nitrososphaeria-related iGDGT synthesis in the water column."**

**The Abstract was clarified; the last sentence was removed and rewritten as follows:**

**Lines 52-55: „These findings highlight the complex interplay between freshened porewater and gas seepage in shaping archaeal communities, and the role of ammonia-oxidising Nitrososphaeria in controlling iGDGT composition and the sedimentary record.”**

*Minor comments:*

*Line 802: MDS and NMDS are not consistently used, please use one.*

**Author response 5: It was corrected to MDS, as follows:**

**Lines 805-806: “MDS shows significant separation (PERMANOVA,  $p = 0.003$ )...”**

*Line 814: ANME-2a/2b and ANME-2a-2b are misused, please use consistently.*

**Author response 6: ANME-2a-2b and ANME-2a/2b were indeed misused, and the mistake has now been corrected to ANME-2a-2b, since the aim was to identify the family group (in accordance with the taxonomy originally produced by the reference database used in our bioinformatic workflow). Additionally, to avoid confusion, we ensured the taxonomic levels are more clearly indicated, as in:**

**Lines 873-877: “This is most evident at lower taxonomic levels, where ANME-2b (genus-level distribution; supplementary material Fig. S7a,b), ANME-2a-2b, Methanosarcinaceae, Methanosaetaceae, Methanomicrobiaceae (family-level distribution; Fig. 8e; supplementary material Fig. S6), and other CH<sub>4</sub>-associated taxa are enriched in selected pockmark horizons.”**

*Line 831: “no GDGTs were detected” seems not correctly expressed because GDGTs are central to the study and it should be detected in marine sediments. Please rephrase.*

**This oversight was introduced during the iterative rounds of our revisions. We appreciate the careful reading and have corrected the mistake in the revised version:**

**Lines 832-833: “Archaeoglobaceae form a peripheral node and does not link to any GDGTs in pockmarks.”**

*Line 827: Hadarchaeales should be Hadarchaeales*

**It was corrected to Hadarchaeales, as follows:**

**Lines 827-829: “The second community is dominated by ammonia-oxidising Nitrosopumilaceae and clusters with Hadarchaeales”**

*Line 1009: Nitrossopaheria should be Nitrososphaera*

**It was corrected to Nitrososphaeria, in accordance with the taxonomy originally produced by the reference database used in our bioinformatic workflow and nomenclature by Rinked et al. (2021), as follows:**

**Lines 1008-1011: “This decoupling suggests that the bulk GDGT pool integrates in situ archaeal production, contributions from sedimentary lineages such as**

**Methanomicrobia, Methanosarcinia, Thermoplasmata, and possibly others and uncultivated archaea, together with pelagic input of Nitrososphaeria-derived crenarchaeol.”**

*Line 1143: RI-OH and RI-OH, second index should be RI-OH’*

**It was corrected to RI-OH’, as follows:**

**Lines 1200-1202: “Average OH-GDGT% values align with those reported for Baltic Sea surface sediments (Sinninghe Damsté et al., 2022), and RI-OH and RI-OH’ values are within the Baltic/Skagerrak Surface sediment ranges (Sinninghe Damsté et al., 2022).”**

*Line 1154: Fig. 6 should be Fig. 11. Nanoarchaeota–GDGT correlation is cited as Fig. 6; this should refer to the correlation network (Fig. 11).*

**We have updated the figure reference. As we reduced the number of figures in the main text, the old Fig. 11 is now Fig. 9:**

**Lines 1211-1212: “Nanoarchaeota may also possess GDGTs, previously attributed to their biological hosts (Zeng et al., 2022), which could explain their correlation with GDGTs (Fig. 9).”**

## **References**

Rinke, C., Chuvochina, M., Mussig, A. J., Chaumeil, P.-A., Davín, A. A., Waite, D. W., Whitman, W. B., Parks, D. H., and Hugenholtz, P.: A standardized archaeal taxonomy for the Genome Taxonomy Database, *Nature Microbiology*, 6, 946–959, <https://doi.org/10.1038/s41564-021-00918-8>, 2021.

**We would like to express our sincere gratitude for additional thoughtful remarks and valuable suggestions. Below, we provide detailed responses to Reviewer 2's comments, with the corresponding revisions clearly marked in red within the manuscript.**

## **RESPONSE TO REVIEWER #2**

*With the addition of the porewater data the manuscript improved considerably. The extensive dataset including iGDGT, archaeal community composition and porewater data provides a useful foundation for intercomparison. Especially the incorporation of methane seepage, freshened SGD, and reference sites makes this an interesting dataset, accounting for a variety of environmental factors within marine sediments in the Baltic Sea. More improvement could be achieved by presenting the findings in a more concise and clear manner, as some of the take-away messages tend to get buried by other information. A recommendation could be to identify the most important messages of the manuscript, and critically assess which parts of the manuscript actively contribute to building these arguments. Data, figures or text that is not directly relevant could perhaps be moved to the Supplementary Material.*

### *General comments*

*It could be worth it to also plot the sulphate normalized to the chloride concentration (e.g.,  $SO_4/Cl$ ) to account for the dilution affect due to porewater freshening (as a check). The downcore decrease in sulphate namely is both due to mixing with the freshwater that is low in sulphate, and consumption through for example sulphate reduction. By not normalizing against Cl, you cannot differentiate between the two effects. This also might affect where you place your SMTZ, and thus influence the porewater chemistry and archaeal community composition mismatch.*

**Author response 1: To determine whether the observed downcore decrease in sulphate resulted primarily from microbial sulphate consumption or from dilution with freshened porewater, we checked whether plotting sulphate normalised to the chloride concentration would alter the shape of the profile and/or shift the position of the estimated sulphate-methane interface (SMI), indicating the need to apply a correction for salinity-driven dilution.**

**We can see that, regardless of whether we normalise the data or use the raw concentrations, the shape of the profile remains unchanged. The porewater profiles of methane and sulphate intersect at the same depth, so normalisation also does not change the depth at which the estimated SMI occurs.**

**The calculations were carried out as follows:**

$$SO_4^{2-}_{norm} = SO_4^{2-}_{pw} \times (Cl_{bw} / Cl_{pw})$$

**where  $SO_4^{2-}_{pw}$  and  $Cl_{pw}$  are concentrations measured in the same sample and**

**$Cl_{bw}$  is the concentration in the bottom water (reference).**

**This preserves the  $SO_4^{2-}/Cl$  ratio expected for conservative mixing and highlights any  $SO_4^{2-}$  deficit or excess due to reactions. Multiplying by  $Cl_{bw}$  (reference value) corrects**

the measured  $\text{SO}_4^{2-}$  to the reference salinity, so any remaining differences indicate non-conservative behaviour (e.g., microbial sulphate reduction).

The shape of the profiles remains the same for  $\text{SO}_4^{2-}$  (before normalisation) and for  $\text{SO}_4^{2-}/\text{Cl}^-$ , as shown in Fig. 1 and 2. We have also produced graphs for  $\text{CH}_4$  and  $\text{SO}_4^{2-}$ . In one figure, we have included the normalised  $\text{SO}_4^{2-}$  and the raw  $\text{SO}_4^{2-}$  before normalisation to check whether the SMI depth changes (Fig. 3).

Regardless of which data we use, the SMI remains at the same depth, confirming that no correction is needed and that new graphs do not need to be added to the publication.

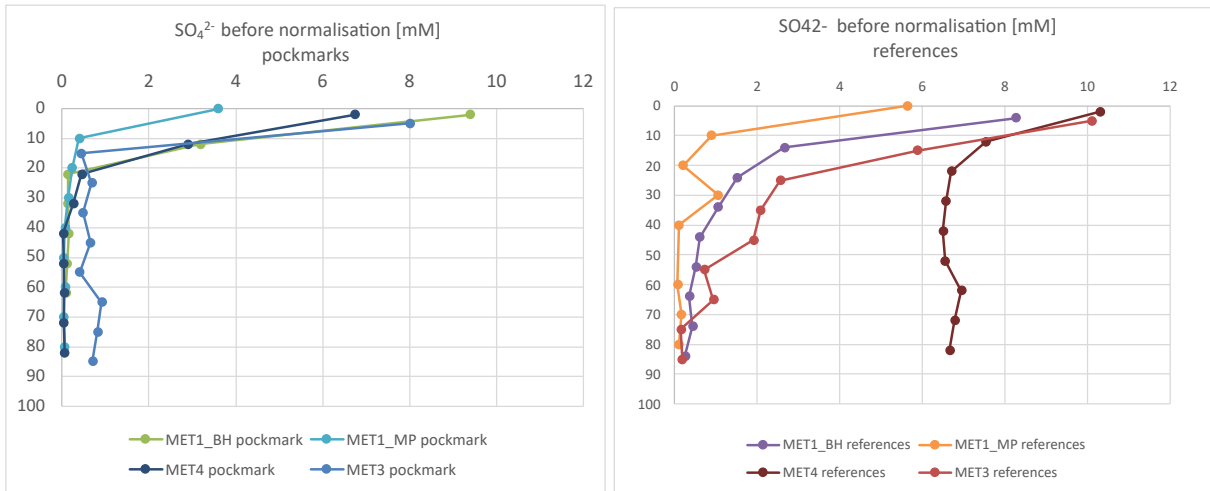


Figure 1. Plots showing porewater concentrations of sulphates in pockmarks (left) and references (right) before normalisation.

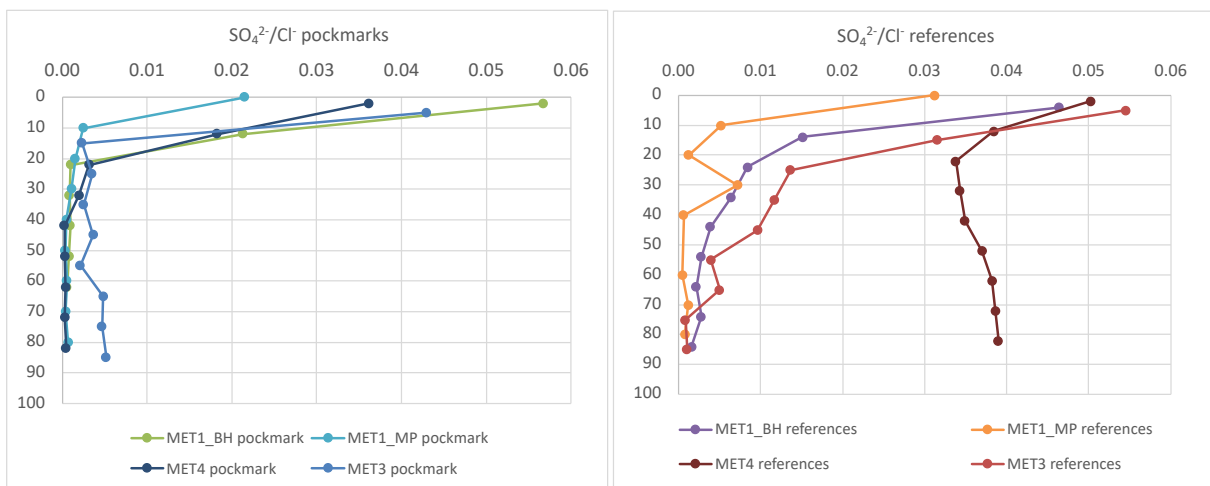


Figure 2. Plots showing porewater concentration of sulphate normalised to the chloride concentration in pockmarks (left) and references (right).

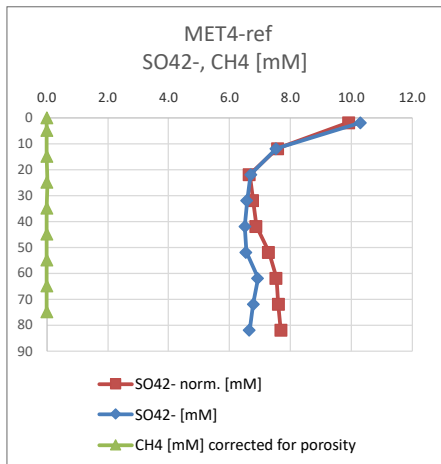
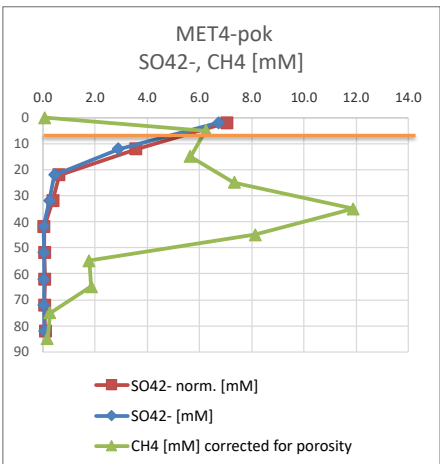
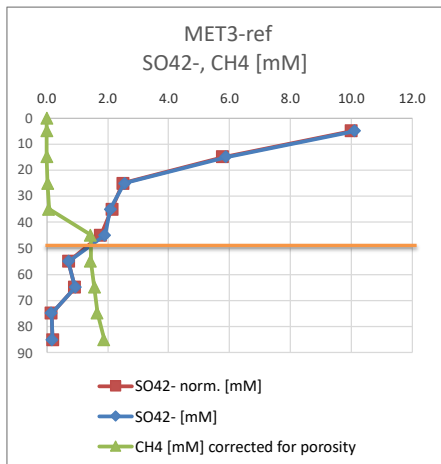
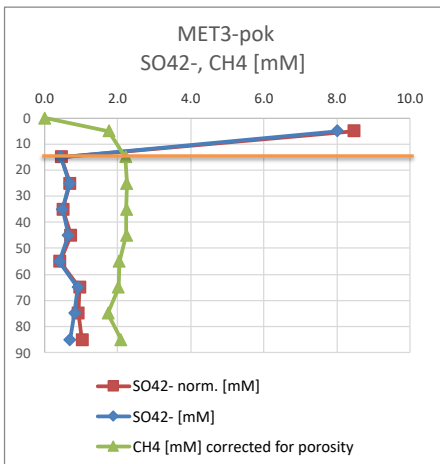
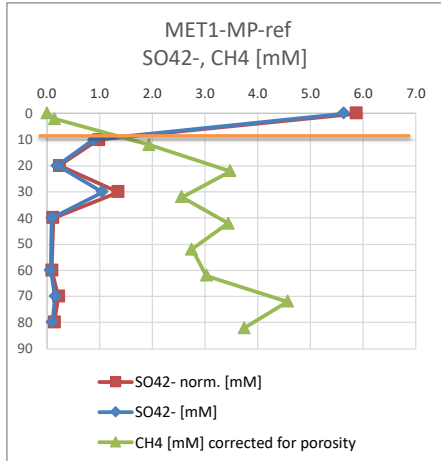
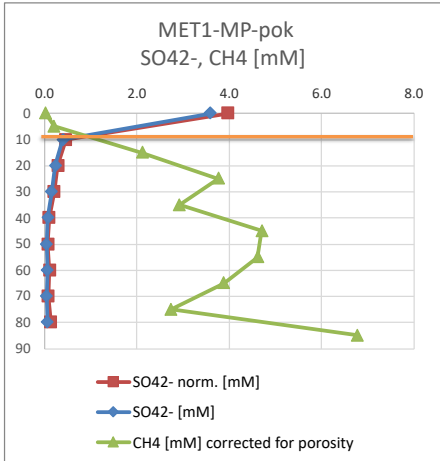
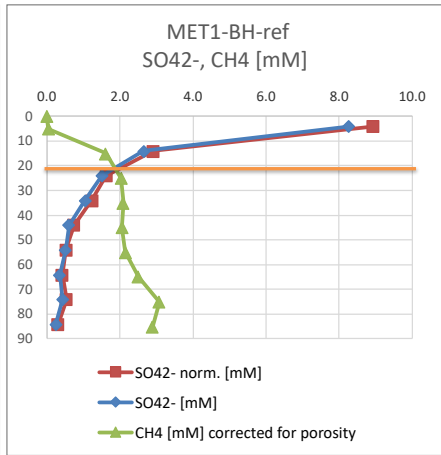
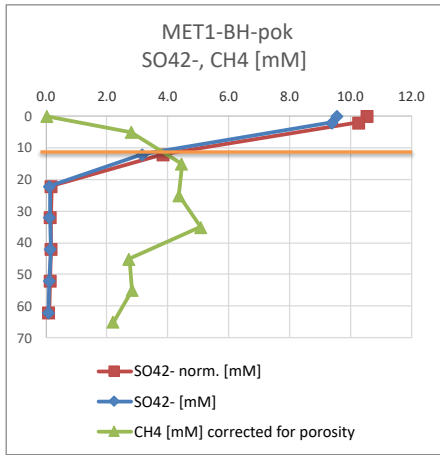


Figure 3. Plots showing porewater concentrations of sulphates after normalisation, sulphates before normalisation, and methane in pockmarks (left) and references (right).

### *Specific comments*

#1 Lines 58-59: “These findings highlight the complex interplay between freshened porewater and gas seepage in shaping archaeal communities, iGDGT composition, and the sedimentary record.” In the sentences leading up to this one, the conclusion is that pelagic AOA is more important for the iGDGT composition. However, in this more conclusive sentence that does not seem to be represented as only freshened porewater and gas seepage are highlighted as environmental controls?

**Author response 2: It has been corrected:**

**Lines 52-55: “These findings highlight the complex interplay between freshened porewater and gas seepage in shaping archaeal communities, and the role of ammonia-oxidising Nitrososphaeria in controlling iGDGT composition and the sedimentary record.”**

#2 Line 98: “In shallow and coastal Baltic regions such as the Bay of Puck, SGD is often described as the movement of recirculated seawater and its dissolved constituents (Piekarek-Jankowska, 1996; Szymczycha et al., 2016).” However, the Szymczycha et al., 2016 paper describes very freshened porewater at their SGD sites, how does this connect to the recirculated seawater?

**Author response 3: The terminology used was incorrect. Although submarine groundwater discharge can encompass both freshened groundwater and recirculated seawater, Szymczycha et al. (2016) primarily describe the mixing of groundwater and seawater within the subterranean estuary in the Bay of Puck. This fragment has been replaced:**

**Lines 92-100: „Evidence of an association between pockmarks and upward groundwater infiltration has been documented in areas such as Eckernförde Bay (Busmann and Suess, 1998; Schlüter et al., 2004), Hanko Bay (Virtasalo et al., 2019; Purkamo et al., 2022), and the central Gulf of Gdańsk (Szymczycha et al., 2016; Idczak et al., 2020). In the Gdańsk Basin area (Fig. 1), deep-water, fine-grained pockmarks are associated with localised porewater freshening, indicated by chloride (Cl<sup>-</sup>) and sulphate (SO<sub>4</sub><sup>2-</sup>) depletion, linked to seepage of freshened groundwater or discharge of freshened porewater (Szymczycha et al., 2018; Idczak et al., 2020; Brodecka-Goluch et al., 2022; Kurowski et al., 2024; Łukawska-Matuszewska and Dwornik, 2025; Łukawska-Matuszewska et al., 2025). “**

#3 Lines 112-114: “In the Gdańsk Basin, pockmarks, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> depletion linked to freshened porewater discharge may weaken SO<sub>4</sub><sup>2-</sup>--driven anaerobic methane oxidation (S-AOM), promote shallow methanogenesis, and contribute to episodic gas release into the water column.” Do you have a reference for this?

**Author response 4: It has been supported by reference to Idczak et al. (2020) as follows:**

**Lines 106-109: “In the pockmarks of Gdańsk Basin, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> depletion linked to freshened porewater discharge may weaken SO<sub>4</sub><sup>2-</sup>-driven anaerobic methane oxidation (S-AOM), promote shallow methanogenesis, and contribute to episodic gas release into the water column (Idczak et al., 2020).”**

*#4 Line 440: How were the SMTZ midpoints and ranges defined? The MET3 estimated SMTZ interval in Figure 2 seems for example very thin to me when compared with the sulphate and methane gradients that change over a much broader depth. Furthermore, I would suggest to use consistent units for the methane, sulphate and chloride (that is; mM), but I leave that choice to the authors.*

**It has been simplified, and SMTZ is now replaced by SMI (sulphate-methane interface), defined by the intersection of porewater concentrations of sulphate and methane, as follows:**

**435-445: “For the purpose of this study, the SMI is defined as the layer within the sediment at which the porewater concentrations of SO<sub>4</sub><sup>2-</sup> and CH<sub>4</sub> are equal. At this interface, AOM couples SO<sub>4</sub><sup>2-</sup> reduction to CH<sub>4</sub> oxidation, consuming most of the CH<sub>4</sub> and SO<sub>4</sub><sup>2-</sup> and producing low concentrations of both. The depth of SMI, as well as the differences between pockmark and reference sites, vary by location, with SMI in pockmarks located closer to the sediment surface (Fig. 2). In pockmark sediments, the SMI was located 5–15 cm below the seafloor, whereas in non-pockmark sediments it was 10–50 cm below the seafloor. At MET1-BH and MET3, the SMI in the pockmarks was shallower than at the reference stations; at MET1-MP, the SMI in the pockmark was similarly shallow to that at the reference site; at MET4, no SMI was detected within the sampled interval at the non-pockmark site, while the pockmark exhibited a shallow SMI.”**

**Since our study only identified the porewater concentrations of sulphate and methane, and not other geochemical indicators, such as rates of sulphate reduction or anaerobic oxidation of methane, the introductory part was also rewritten and a more simplistic perspective on the SMI was presented, as follows:**

**Lines 111-115: „At the sulphate-methane interface (SMI), where the subseafloor intersection of downward-diffusing sulphate and upward-diffusing CH<sub>4</sub> occurs, ascending CH<sub>4</sub> is consumed in AOM (Zehnder and Brock, 1980; Boetius et al., 2000). It is mediated by sulphate-reducing bacteria (SRB) and anaerobic methanotrophic archaea (ANME) (Knittel and Boetius, 2009), which limit CH<sub>4</sub> emissions to the water column (Reeburgh, 2007).”**

*#5 Line 992: “Lipid preservation could also be a side effect of the less ebullitive nature of methane seepage in pockmarks P/MET3 and P/MET4: the kinetics and episodicity of CH<sub>4</sub> supply and freshened porewater discharge, which influence redox zonation.” This sentence and the causal relation are unclear to me. Can it be specifically made clear how more consistent CH<sub>4</sub> supply/less episodicity as typically associated with ebullition change the redox zonation?*

**Author response 6: The paragraph was rewritten and clarified as follows:**

**Lines 1067-1077: „ Lipid preservation could potentially be a side effect of the less ebullitive nature of methane seepage in pockmarks P/MET3 and P/MET4, thereby**

enhancing CH<sub>4</sub> accumulation over time: the kinetics and episodicity of CH<sub>4</sub> supply to the seabed and of freshened porewater discharge are reduced in pockmarks P/MET3 and P/MET4 compared with those reported in previous studies (Idczak et al., 2020; Brodecka-Goluch et al., 2022; Łukawska-Matuszewska and Dwornik, 2025; Rzepa et al., 2026) for more hydrologically complex pockmarks of the MET1 area. Additionally, present-day dissolved CH<sub>4</sub> and SO<sub>4</sub><sup>2-</sup> profiles may not fully capture the temporal geochemical variability of this environment. As reported by Treude et al. (2005), the spatial and temporal heterogeneity caused by gas ebullition allows methanogens and sulphate reducers to coexist in Eckernförde Bay, feeding the shallow SMI, but also enables CH<sub>4</sub> to escape into the overlying water column, bypassing the microbial barrier.”

*#6 Lines 1023-1039 in section 4.5 Porewater freshening and potential alternative methane oxidation pathways: This is all very useful background information, but I miss a bit the link and bridge to your data and results. Do you also see any of these processes reflected in your results? Or are there any discrepancies with previously published literature?*

**Author response 7:** The whole section was removed and replaced by a brief, passing mention as follows:

**Lines 991-998:** „Kurowski et al. (2024) analysed the MET1-BH pockmark and suggested that iron-dependent anaerobic oxidation of methane (Fe-AOM) serves as an alternative pathway when sulphate is limited in the MET1 area. The reduced SO<sub>4</sub><sup>2-</sup> availability in porewaters of CH<sub>4</sub>-rich Baltic Sea sediments appears to shift the microbial community towards utilising iron (oxyhydr)oxides as alternative oxidants for AOM (Egger et al., 2017). These iron (oxyhydr)oxides are common in the Gulf of Gdańsk (Kurowski et al., 2024). As indicated by higher concentrations of dissolved Fe<sup>2+</sup> and Mn<sup>2+</sup> and increased precipitation of authigenic carbonates, AOM in the MET1 area may be coupled to iron reduction (Rzepa et al., 2026).”

*#7 Lines 1205-1210 in Conclusion: Perhaps add a more specific sentence on the implications of your findings for using iGDGTs as proxies for methane seepage and SGD.*

**Author response 8:** The Conclusions section was revised, and the implications are now more specific:

**Lines 1271-1288:** „Our study defines the conditions and limitations under which iGDGTs can be interpreted in Baltic Sea pockmarks. Local CH<sub>4</sub>-driven processes do not fundamentally alter the sedimentary tetraether lipid record, which instead reflects the broader marine environmental signal. The clearest combined biomarker, geochemical, and microbiological evidence for methane cycling, observed mostly at pockmark P/MET4, indicates increased iGDGT concentrations, elevated CH<sub>4</sub>, depleted SO<sub>4</sub><sup>2-</sup>, and shallow SMI, along with enrichment of ANME-2b, ANME-3, hydrogenotrophic methanogens, and where Methanosarcina, Methanosaeta, and Methanoregula co-occur. However, the iGDGT distribution in the weakly active pockmark P/MET4 is similar to that of the adjacent reference sediments, probably indicating a pelagic AOA source. The

**inactive pockmark P/MET3 also shows increased accumulation of archaeal tetraether lipids compared with the reference core. As in pockmark P/MET4, the iGDGT distribution is primarily associated with water-column processes of pelagic AOA rather than with processes driven by CH<sub>4</sub>-cycling communities in the sediment, as evidenced by the dominance of crenarchaeol. Active MET1 pockmarks also show iGDGT distributions similar to those of adjacent reference cores, so the differences between pockmarks lie in the concentration of iGDGTs rather than in their distribution. Therefore, the concentration profiles of iGDGT and OH-GDGT could perhaps serve as a proxy for primary productivity in the Baltic Sea.”**

*Technical corrections*

*# 1 In “Highlights” line 27: abbreviation AOA is not introduced.*

**Author response 9: The ‘Highlights’ section was rewritten and clarified, without the AOA abbreviation, as follows:**

**Line 17-23: „Pockmark sediments harbour substantially higher archaeal diversity and abundance than non-pockmark reference sediments.**

**The P/MET4 pockmark in the Gdańsk Deep hosts the most diverse and abundant methanogen community, coinciding with the highest concentrations of isoprenoid glycerol dialkyl glycerol tetraether lipids (iGDGTs).**

**Crenarchaeol dominates the iGDGT pool in both pockmark and reference sediment cores, indicating strong Nitrososphaeria-related iGDGT synthesis in the water column.”**