

1 **Archaeal community composition in Holocene methane pockmarks of the Gdańsk Basin**
2 **(Baltic Sea, Poland): insights from tetraether lipids and 16S rRNA analysis**

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16 **Highlights**

17 Pockmark sediments harbour substantially higher archaeal diversity and abundance compared
18 with non-pockmark reference sediments.

19 Pockmarks appear to function as tightly coupled metabolic systems, in which various archaeal
20 groups potentially cooperate in methanogenesis, whereas non-pockmark sites contain less
21 integrated, loosely coupled sub-communities.

22 The MET4 pockmark in the Gdańsk Deep hosts the most diverse and abundant methanogen
23 community, coinciding with the highest concentrations of isoprenoid glycerol dialkyl glycerol
24 tetraether lipids (iGDGTs). However, iGDGT indices indicate mixed archaeal sources rather
25 than methanogenic lipid sources.

26 Crenarchaeol dominates the iGDGT in both pockmark and reference sediment cores, suggesting
27 strong AOA-related iGDGT synthesis. However, Nitrososphaeria-related sequences are not
28 dominant in the sedimentary archaeal community, indicating a pelagic origin of crenarchaeol.

29 **Abstract**

30 Methane pockmarks and shallow gas systems are prominent geomorphological features in the
31 Baltic Sea that act as hotspots of microbial activity. In the Gdańsk Basin, pockmarks vary in
32 gas-seepage intensity and in the extent of freshened porewater discharge, both of which
33 influence the archaeal community structure and the composition of internal methane biofilters.
34 The objective of this study was to examine the effects of methane seepage and freshened
35 porewater on the composition of archaeal communities and on the isoprenoid glycerol dialkyl
36 glycerol tetraethers (iGDGTs), membrane lipids synthesised by these communities, across the
37 gas systems examined. Additionally, the effects of these environmental factors on the use and
38 interpretation of iGDGT-based proxies under conditions of gas and water seepage were
39 assessed. The study investigates whether iGDGT patterns in these Baltic gas systems reflect
40 methane-driven processes, including anaerobic oxidation of methane (AOM) and
41 methanogenesis, porewater freshening, or pelagic contributions from ammonia-oxidising
42 archaea (AOA). The results show elevated iGDGT concentrations in pockmark cores compared
43 with reference non-pockmark cores; however, the iGDGT distribution is site-specific. Overall,
44 iGDGT concentrations are much higher at sites with reduced pockmark activity and weak
45 porewater freshening. Nevertheless, consistently low Methane Index values ($MI < 0.09$),
46 together with low GDGT-0/crenarchaeol (< 1) and low GDGT-2/cren (< 0.04) ratios, indicate
47 that the iGDGT pool lacks the typical enrichment in GDGT-1 to -3 associated with AOM,
48 suggesting no AOM imprint on the iGDGT pool. However, 16S rRNA analysis revealed that
49 the anaerobic methanotrophic archaeal lineages (ANME) consist of ANME-2b and ANME-3.
50 A strong positive correlation between OH-GDGTs and crenarchaeol, together with the
51 consistency of OH-GDGT% values with those previously reported for Baltic Sea surface
52 sediments, suggests a thaumarchaeal source of iGDGTs in the studied pockmark and reference
53 cores. The Branched and Isoprenoid Tetraether index values suggest marine archaeal GDGT
54 production. In this system, iGDGT-based proxies primarily reflect a strong pelagic presence of
55 AOA, as indicated by the dominance of crenarchaeol. This suggests that, despite the local
56 presence of methanogenic and ANME-related archaeal groups, methane-related AOM does not
57 influence the iGDGT signal due to the archaeal community structure. These findings highlight
58 the complex interplay between freshened porewater and gas seepage in shaping archaeal
59 communities, iGDGT composition, and the sedimentary record. iGDGT-based indices should
60 be used as part of a multi-proxy approach in environmental research.

61 **Keywords:** methane, pockmarks, iGDGTs, ammonia-oxidising archaea, crenarchaeol,
62 Holocene, Gdańsk Deep, Baltic Sea

64 **1 Introduction**

65 Pockmarks are concave geological structures ranging from 1 to over 100 m in width and from
66 less than 1 to 100 m in depth, formed by gas and/or water seepage from the sediments into the
67 hydrosphere (King and MacLean, 1970; Hovland and Judd, 1988). As indicators of hydraulic
68 activity, they are categorised as active or inactive depending on whether gas and/or water
69 seepage is continuous or dormant/intermittent (Hovland and Judd, 1988; Hovland et al., 2002).
70 Their seabed depressions facilitate detection through geophysical and hydroacoustic surveys,
71 making them practical proxies for investigating seepage phenomena (Hovland and Judd, 1988).
72 The formation of pockmarks requires pressure buildup in fine-grained, low-permeability
73 sediments, with methane (CH₄) the most commonly emitted component owing to its high
74 mobility (Hovland and Judd, 1988). Seepage types vary globally and include biogenic,
75 thermogenic, or hydrothermal gas seepage; groundwater; or combined gas-and-water seepage
76 (Hovland and Judd, 1988). Pockmarks predominantly form along salt-dome margins (Schmuck
77 and Paull, 1993; Taylor et al., 2000) and in dislocation zones, faults, and bedrock fractures
78 (Shaw et al., 1997). They also occur in regions of low seismicity, such as Sweden (Hovland et
79 al., 2002) and the southern Baltic Sea (Idczak et al., 2020). Glacial and post-glacial processes
80 can influence their formation by generating overpressure, as observed in the North Sea (Callow
81 et al., 2021) and the Baltic Sea (Whiticar and Werner, 1981; Whiticar, 2002; Kreuzburg et al.,
82 2023). Baltic Sea pockmarks have been documented in Eckernförde Bay and the Mecklenburg
83 Bight (Werner, 1978; Wever et al., 1998; Jensen et al., 2002; Schlüter et al., 2004; Hoffmann
84 et al., 2020; Díaz-Mendoza et al., 2023), the Stockholm Archipelago (Jakobsson et al., 2020),
85 offshore Finland (Virtasalo et al., 2019), and the Gdańsk Basin (Pimenov et al., 2010; Majewski
86 and Klusek, 2011; Brodecka et al., 2013; Jaśniewicz et al., 2019; Idczak et al., 2020; Brodecka-
87 Goluch et al., 2022). The Gdańsk Basin is the focus of this study.

88 In several Baltic settings, pockmarks have been linked to submarine groundwater discharge
89 (SGD), broadly defined as the movement of fresh, brackish, or saline groundwater into the sea
90 or ocean across the sediment-water interface. SGD encompasses both the inflow of freshened
91 groundwater and the recirculation of saline porewater or seawater, driven by hydraulic pressure
92 and density differences (Burnett et al., 2003, 2006; Moore, 2010; Taniguchi et al., 2019).
93 Evidence of an association between pockmarks and upward groundwater infiltration has been
94 documented in areas such as Eckernförde Bay (Busmann and Suess, 1998; Schlüter et al.,

95 2004), Hanko Bay (Virtasalo et al., 2019; Purkamo et al., 2022), and the central Gulf of Gdańsk
96 (Szymczycha et al., 2016; Idczak et al., 2020). In shallow and coastal Baltic regions such as the
97 Bay of Puck, SGD is often described as the movement of recirculated seawater and its dissolved
98 constituents (Piekarek-Jankowska, 1996; Szymczycha et al., 2016). Such groundwater-
99 seawater mixing zones are commonly referred to as subterranean estuaries in permeable coastal
100 aquifers (Moore, 1999; Ruiz-González et al., 2021). However, this framework has not been
101 explicitly demonstrated for the deeper, fine-grained methane pockmarks of the central Gulf of
102 Gdańsk, where available evidence instead points to localised porewater freshening, indicated
103 by chloride (Cl⁻) and sulphate (SO₄²⁻) depletion, linked to seepage of freshened groundwater or
104 discharge of freshened porewater (Szymczycha et al., 2018; Idczak et al., 2020; Brodecka-
105 Goluch et al., 2022; Kurowski et al., 2024; Łukawska-Matuszewska and Dwornik, 2025;
106 Łukawska-Matuszewska et al., 2025). The impact of SGD discharge on pockmark sediments
107 depends on the source, chemical composition, and flow regime of the discharging fluids.
108 Nevertheless, freshened SGD and associated porewater transport can alter the distribution of
109 dissolved species, including NH₄⁺, CH₄, dissolved inorganic carbon (DIC), H₂S, Cl⁻, and SO₄²⁻
110, thereby shifting redox transition zones and influencing CH₄ cycling (Schlüter et al., 2004; Liu
111 et al., 2017; Idczak et al., 2020; O'Reilly et al., 2021; Brodecka-Goluch et al., 2022; Zhang et
112 al., 2025). In the Gdańsk Basin, pockmarks, Cl⁻ and SO₄²⁻ depletion linked to freshened
113 porewater discharge may weaken SO₄²⁻-driven anaerobic methane oxidation (S-AOM),
114 promote shallow methanogenesis, and contribute to episodic gas release into the water column.

115
116 At the sulphate-methane transition zone (SMTZ) in CH₄-rich sediments, SO₄²⁻ and CH₄ meet,
117 enabling anaerobic oxidation of methane (AOM) (Zehnder and Brock, 1980; Boetius et al.,
118 2000), mediated by sulphate-reducing bacteria (SRB) and anaerobic methanotrophic archaea
119 (ANME) (Knittel and Boetius, 2009), which limit methane emissions to the water column
120 (Reeburgh, 2007). In the Baltic Sea, a halocline at 60-80 m separates oxygenated and anoxic
121 waters, structuring key biogeochemical cycles (Kuliński et al., 2022), including an oxic-suboxic
122 nitrogen-cycling zone driven by ammonia-oxidising archaea (AOA) (Berg et al., 2015b; Jäntti
123 et al., 2018). Archaeal biomarkers for methane cycling (ANME, methanogens) and nitrification
124 (AOA), such as intact polar GDGTs (IPL-GDGTs), can be transported and preserved as core
125 isoprenoidal GDGTs (CL-iGDGTs) in sediments (Schouten et al., 2013).

126
127 Archaeal iGDGTs are membrane lipids whose structures adapt to environmental changes (De
128 Rosa et al., 1977; Huguet et al., 2006; Liu et al., 2017; Schouten et al., 2002, 2013; Sinninghe

129 Damsté et al., 2022). In marine sediments, GDGT-0 and crenarchaeol dominate, with
130 crenarchaeol produced by Thaumarchaeota (Sinninghe Damsté et al., 2022) – in current
131 nomenclature, Nitrososphaerota (Rinke et al., 2021), the main ammonia oxidisers (Berg et al.,
132 2015a, b) and iGDGT synthesisers in the Baltic Sea (Labrenz et al., 2010; Wittenborn et al.,
133 2023). Methanogens primarily produce GDGT-0, whereas methanotrophs, mainly ANME-1,
134 produce GDGT-1 to -3 (Koga et al., 1993; Pancost et al., 2001; Weijers et al., 2006; Rossel et
135 al., 2008; Blaga et al., 2009; Zhang et al., 2011; Inglis et al., 2015; Słowakiewicz et al., 2016;
136 Petrick et al., 2019). Hydroxylated-GDGTs (OH-GDGTs) are produced primarily by
137 Thaumarchaeota (Sinninghe Damsté et al., 2002; Liu et al., 2012; Kaiser and Arz, 2016; Elling
138 et al., 2017; Bale et al., 2019; Sinninghe Damsté et al., 2022), but can also be biosynthesised
139 by methanogens or ANME (Liu et al., 2012; Guan et al., 2024; Fenies et al., 2026). OH-GDGT
140 synthesis reflects cold adaptation (Liu et al., 2012) and salinity changes (Sinninghe Damsté et
141 al., 2022); OH-GDGT-0 is most abundant at high latitudes (Huguet et al., 2013; Varma et al.,
142 2024) and in Baltic Sea sediments, where culture studies have confirmed an AOA source
143 (Blainey et al., 2011; Berg et al., 2015a).

144

145 Subterranean estuaries provide a useful conceptual framework for SGD-affected systems,
146 because microbial activity in these groundwater-seawater mixing zones can modify the
147 chemical composition of groundwater before it reaches the seabed (Ruiz-González et al., 2021)
148 2021). In particular, distinct ammonia-oxidising archaea (AOA) populations occupy deeper
149 freshwater-saltwater mixing zones (Santoro et al., 2008; Rogers and Casciotti, 2010; Purkamo
150 et al., 2022; Wilson et al., 2024). These observations are relevant to archaeal lipid studies
151 because, as noted above, iGDGTs are primarily associated with Nitrososphaerota, whereas
152 GDGT-0 may also originate from methanogenic Euryarchaeota. Although direct studies of
153 GDGTs in SGD-affected marine sediments remain scarce, groundwater research indicates that
154 both CL-iGDGTs and intact polar iGDGTs (IPL-iGDGTs) are produced in situ by indigenous
155 subsurface microbial communities, and that their distributions are linked to groundwater
156 thaumarchaeal groups rather than solely to allochthonous surface inputs (Ding et al., 2018). In
157 the Baltic Sea, the synthesis of iGDGTs is strongly influenced by Nitrosopumilus-dominated
158 archaeal communities (Wittenborn et al., 2023). However, OH-GDGT distributions are highly
159 sensitive to salinity changes (Sinninghe Damsté et al., 2022). Therefore, in methane pockmark
160 sediments affected by porewater freshening, archaeal 16S rRNA and CL-iGDGT distributions
161 may reflect overlapping controls: the regional presence of Baltic AOA, CH₄-driven processes
162 including methanogenesis and methanotrophy, and an additional hydrogeochemical imprint

163 associated with porewater freshening. This makes such pockmarks a useful natural setting for
164 testing whether archaeal lipid signatures primarily reflect pelagic marine production, CH₄
165 cycling, or the influence of freshened porewater.

166

167 **2 Materials and methods**

168 **2.1 Study area**

169

170 Pockmarks in the Gdańsk Deep and the central Gulf of Gdańsk (Fig. 1) occur at water depths
171 of 1-100 m and are characterised by active gas seepage from Holocene silts and clays (Idczak
172 et al., 2020). Although the gas is predominantly biogenic (Brodecka-Goluch et al., 2022), the
173 presence of helium (up to 0.39%) (Idczak et al., 2020) and noble gases (Ne, Ar, Kr, Xe)
174 (Brodecka-Goluch et al., 2022) in samples from station MET1 suggests that these pockmarks
175 vent Middle Cambrian reservoirs, with gas migrating through sedimentary layers along faults
176 (Jaworowski et al., 2010; Idczak et al., 2020; Brodecka-Goluch et al., 2022), possibly with
177 additional contributions from the crust and mantle (Kotarba, 2010; Pokorski, 2010; Kotarba and
178 Lewan, 2013; Kotarba and Nagao, 2015).

179

180 The study area spans the Gdańsk Basin and includes stations MET3 and MET4 in the northern
181 Gdańsk Deep, and MET1-MP and MET1-BH in the Gulf of Gdańsk (Fig. 1). The southern
182 stations (MET1 area) receive a high input of terrestrial organic matter from the Vistula River,
183 trap plant material and organic debris, and experience moderate anthropogenic contamination,
184 resulting in elevated TOC (Idczak et al., 2020; Brodecka-Goluch et al., 2022; Łukawska-
185 Matuszewska et al., 2022; Szymczak-Żyła and Lubecki, 2022). Sedimentation rates are lower
186 in MET3 and MET4 ($\sim 0.17\text{--}0.20$ cm yr⁻¹) than at MET1 ($\sim 0.15\text{--}0.22$ cm yr⁻¹) (Szczepańska
187 and Uścińowicz, 1994; Brodecka-Goluch et al., 2022). In a previous study of pockmarks in the
188 Gdańsk Deep, Brodecka-Goluch et al. (2022) reported that methane at pockmark MET3 has
189 isotopic signatures of marine microbial gas, whereas gas venting from MET1-MP shows mixed
190 marine and terrestrial signatures, indicating the upward infiltration of freshwater. Thus,
191 methanogenic pathways vary between sites: acetoclastic at MET1-BH, hydrogenotrophic at
192 MET3, and mixed at MET1-MP (Idczak et al., 2020; Brodecka-Goluch et al., 2022). The MET1
193 pockmarks are characterised by intensive/periodic gas and freshwater seepage (Idczak et al.,
194 2020; Brodecka-Goluch et al., 2022). MET4 represents the least-studied system, located within
195 a field of multiple pockmarks (Brodecka-Goluch et al., 2020).

196

197 In the Gdańsk Basin, SGD is divided into shallow/coastal and deep/offshore components. The
198 shallow/coastal component occurs in nearshore areas and is fed by Quaternary-Cretaceous
199 coastal aquifers (e.g., Piekarek-Jankowska, 1996; Szymczycha et al., 2016, 2018). By contrast,
200 the deep/offshore component comprises artesian-type freshwater seepage with episodic
201 discharge at deep-water pockmarks such as MET1 (Szymczycha et al., 2018; Idczak et al.,
202 2020). Deep SGD originates from extensive Upper Cretaceous aquifers beneath the Gulf of
203 Gdańsk (Uścińowicz, 2011). Seepage intensity varies with hydrostatic pressure, water-column
204 processes, and fault activity (Brodecka-Goluch et al., 2022). After commercial extraction
205 ceased in 2000, natural hydrodynamic conditions returned (Uścińowicz, 2011), although the
206 extent and periodicity of deep discharge remain unquantified. SGD in the MET1 area reduces
207 sulphate and chloride concentrations with depth, thereby compressing the SMTZ to a few cm
208 below the seafloor (Idczak et al., 2020; Brodecka-Goluch et al., 2022; Łukawska-Matuszewska
209 and Dwornik, 2025). A high carbonate alkalinity flux at MET1-MP indicates substantial DIC
210 generation during anaerobic diagenesis, potentially linked to Fe(III)-AOM (Łukawska-
211 Matuszewska and Dwornik, 2025). The near-bottom chloride concentration in the Gulf of
212 Gdańsk ranges from 150 to 180 mmol L⁻¹, equivalent to ~5318-6384 mg dm⁻³ (Łukawska-
213 Matuszewska, 2016). For the Gdańsk Deep, the mean value is approximately 12 PSU,
214 equivalent to 6700-6900 mg dm⁻³ (Kapustina et al., 2026).

215

216 **2.2 Sampling at MET stations**

217

218 The research stations were divided into pockmarks (morphologically concave geological
219 structures with gas emissions to the water column and/or gas-bearing sediments with possible
220 SGD) and references (non-pockmark reference sites with gas in the sediments but without
221 emission to the water column or SGD). Pockmark stations were further classified (see
222 supplementary material Table S1) based on previous studies, including hydroacoustic and
223 geophysical investigations (Brodecka-Goluch et al., 2020, 2022; Idczak et al., 2020), as well as
224 data included in this study (SO₄²⁻ and CH₄ profiles: Fig. 2; Cl⁻ profiles: Fig. 3; echograms for
225 pockmarks MET3 and MET4: supplementary material Fig. S1), as active (gas emission to the
226 water column, influence of freshened porewater, or both: P/MET1-BH, P/MET1-MP), weakly
227 active (gas seepage and porewater freshening: P/MET4), or inactive (gas present in the
228 sediments without clear emission or freshwater seepage: P/MET3).

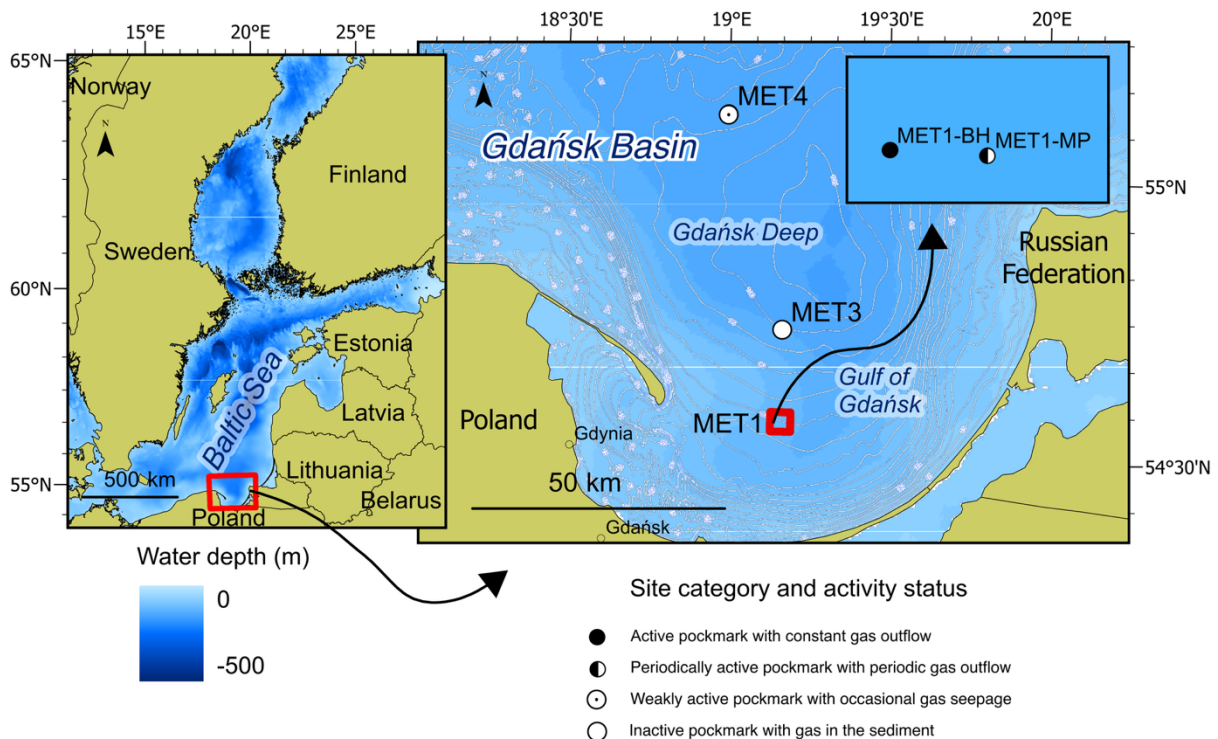
229

230 Eight 95 cm-long sediment cores were collected from the central parts of methane-seeping
 231 structures in the central Gdańsk Basin (south-eastern Baltic Sea) using a gravity corer at three
 232 areas and four study locations (MET1: MET1-MP, MET1-BH; MET3; MET4; Fig. 1,
 233 supplementary material Table S1) during a cruise aboard RV *Oceanograf* (University of
 234 Gdańsk) in October 2019. Four additional cores were collected from outside the pockmarks
 235 (~100-150 m away) as reference samples. Samples were categorised by origin: a ‘P’ prefix
 236 (P/METX; X = 1-MP, 1-BH, 3, 4) for pockmark sediments and an ‘S’ prefix (S/METX) for
 237 surrounding sediments, namely reference non-pockmark sediments.

238

239 Onboard, the 95 cm cores for biomarker analysis were sectioned into a top 0-5 cm interval and
 240 subsequent 10 cm intervals (hereafter referred to as horizons) for the remainder of the cores,
 241 yielding 72 samples for geochemical analysis (some bottom samples are missing). Samples for
 242 microbial analysis were subsampled at a slightly coarser 10-cm resolution from the top (1),
 243 mid-depth (5), and bottom (7, 10) intervals using a sterile spatula.

244



245

246 Fig. 1. Map of the study area in the south-eastern Baltic Sea, the Gdańsk Basin. The main panel shows
 247 the Gdańsk Basin, including the Gdańsk Deep and the Gulf of Gdańsk. Symbols indicate the category
 248 and activity status of the investigated pockmark sites (tagged with a ‘P’ prefix) with respect to venting
 249 status, based on MBES and SBES observations collected during the present and previous research

250 campaigns in 2019-2025. Reference stations (labelled as surrounding stations and tagged with an “S”
251 prefix) are referred to as non-pockmark cores or reference cores. They are positioned approximately
252 100-150 m from the corresponding pockmark margins on morphologically regular seafloor lacking
253 pockmark depressions; they are not shown separately on the map due to the map’s scale. Additional
254 data, including CH₄/SO₄²⁻ profiles and Cl⁻ profiles, are presented in Figures 2 and 3, respectively. More
255 detailed data, for example, concerning porewater freshening, are available in the supplementary material
256 (Table S1). Map source: Eurostat/GISCO, 2024, scale 1.

257

258 **2.3 Porewater sampling**

259

260 Porewater samples were collected under anoxic conditions from intact, hermetically sealed
261 sediment cores using Rhizon® samplers. Sediment subsamples for CH₄ determination were
262 collected immediately after core retrieval. Sampling was performed through pre-drilled holes
263 in the core liners using 3 mL syringes (with the Luer tip removed), and the material was
264 immediately transferred into 20 mL vials in accordance with the protocol described by
265 Jørgensen et al. (2001). Chloride and SO₄²⁻ concentrations in porewater were measured by high-
266 performance ion chromatography (Metrohm 850 Professional IC) with analytical precision
267 better than 3%. Methane concentrations were determined by the standard headspace technique
268 using a gas chromatograph (Perkin-Elmer) equipped with a flame ionisation detector (FID) and
269 an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm), with helium as the carrier gas. The
270 method detection limit (LOD) was 0.2 μmol dm⁻³. Measured CH₄ concentrations were corrected
271 for sediment porosity. Porosity for each sample was calculated from water and organic matter
272 contents (LOI; see below) using the equations of Engvall (1978) and Carman and Jonsson
273 (1991).

274

275 **2.4 LOI analysis**

276

277 The organic matter content was assessed by gravimetric loss-on-ignition (LOI) after
278 dehydration (105 °C for 24 h) and dry combustion (550 °C for 6 h). Because LOI-derived
279 organic matter estimates are sensitive to ignition conditions and sediment composition, and
280 because mass loss at 550°C may include contributions from sediment mineralogy, including
281 inorganic carbon-bearing minerals, LOI-based values provide only a screening-level estimate
282 of total organic carbon (TOC) rather than an exact measurement. Therefore, LOI-based values
283 were evaluated against directly measured TOC for two representative cores. TOC content in

284 sediments was measured using a CHNS autoanalyser (Perkin Elmer 2400) following Parsons
285 et al. (1984). For TOC analysis, samples were acidified with 1 M HCl to remove inorganic
286 carbon, following Hedges and Stern (1984). Due to the positive relationship between LOI and
287 TOC in the selected cores from two study sites ($n = 17$; $R^2 = 0.43$; $p < 0.005$), LOI was used as
288 a high-resolution proxy for organic matter content, i.e., qualitatively (at the trend level) to
289 provide geochemical context rather than for biomarker normalisation.

290

291 **2.5 Tetraether lipid extraction and analysis**

292 All freeze-dried samples were ground with a mortar and pestle. Approximately 1 g of each
293 sample was extracted with dichloromethane:methanol (DCM:MeOH, 2:1, v/v) in an ultrasonic
294 water bath. Total lipid extracts (TLEs) were chromatographed on a silica gel column with n-
295 hexane and methanol as eluents to obtain apolar and polar fractions, respectively.

296 The polar fraction containing core GDGTs was re-dissolved in hexane/isopropanol (99:1, v/v),
297 spiked with a known amount of an internal standard (a C₄₆ glycerol trialkyl glycerol tetraether;
298 Huguet et al., 2006), and filtered through a 0.45 μm polytetrafluoroethylene syringe filter.
299 GDGTs were analysed at Utrecht University using an ultra-high-performance liquid
300 chromatograph (UHPLC; Agilent 1260 Infinity) coupled to an Agilent 6130 single-quadrupole
301 mass detector, following the method proposed by Hopmans et al. (2016). Quantification was
302 performed by manually integrating the peak areas of the protonated ions ($[\text{M}+\text{H}]^+$) in
303 ChemStation software (B.04.03), and the results were compared with those of the internal
304 standard. Selected ion monitoring (SIM) was used to detect and identify GDGTs. The target
305 ions were m/z 1302, 1300, 1298, 1296 and 1292 for iGDGTs and OH-GDGTs.

306 Absolute GDGT concentrations were calculated relative to the C₄₆ internal standard and
307 sediment dry weight, following established GDGT quantification approaches (Huguet et al.,
308 2006, 2010), and are therefore reported as semi-quantitative concentrations (Bijl et al., 2025).
309 Derived fractional abundances and GDGT-based indices were calculated from the integrated
310 peak areas. Raw peak areas, derived fractional abundances and indices, and semi-quantitative
311 absolute concentrations are reported in accordance with the GDGT data-reporting
312 recommendations of Bijl et al. (2025).

313 Each sediment horizon was analysed once. Replicate analyses were not available for individual
314 horizons. Therefore, sample-specific analytical uncertainty could not be estimated, and error

315 bars are not shown in the downcore plots. Method uncertainty includes peak integration,
316 instrumental response, extraction/processing effects, and the semi-quantitative nature of C₄₆-
317 based concentration estimates (Bijl et al., 2025).

318 **2.6 DNA isolation, sequencing, and data analysis**

319 Genomic DNA from the sediment samples was isolated using the EURx kit for complex
320 matrices (Soil DNA Purification Kit, no. E3570, EURX Ltd., Poland). The protocol requires
321 mechanical homogenisation to release cells from the sediment matrix. The isolated genomic
322 DNA was subjected to metabarcoding analysis. Sequencing of the hypervariable V3–V4 region
323 of the 16S rRNA gene was outsourced to GENOMED S.A. (Warsaw, Poland). Specific primer
324 sequences (developed by Zymo Research, CA, USA) were used to amplify the selected region
325 and prepare libraries (341F: CCTACGGGDGGCWGCAG, CCTAYGGGGYGWCWGCAG;
326 806R: GACTACNVGGGTMTCTAATCC).

327 PCR was performed using Q5 Hot Start High-Fidelity 2× Master Mix, with reaction conditions
328 in accordance with the manufacturer’s recommendations. Sequencing was performed on a
329 MiSeq sequencer using paired-end (PE) technology (2 × 300 nt) with Illumina’s v3 kit. FASTQ
330 files were processed with *fastp* (v. 0.23.2) (Chen et al., 2018) to improve raw sequence quality
331 by trimming adapters, filtering low-quality reads, and removing artefacts. The sequences were
332 further analysed using Kraken2 (Wood et al., 2019) following the protocol described by Lu et
333 al. (2022). The SILVA database (v. 138) was used for taxonomic assignment (Quast et al.,
334 2013). Bracken was then applied to the Kraken2 reports, set to the genus level with a threshold
335 of five (Lu et al., 2017). The resulting data were transformed before analysis. To address zero
336 values, the data were imputed using the R package *Compositions* (v. 1.4.0.1) (Palarea-
337 Albaladejo and Martín-Fernández, 2015). The centred log-ratio (clr) transformation was then
338 applied using the *Compositions* package for R (v. 2.05; Aitchison, 1982; Quinn et al., 2019; van
339 den Boogaart et al., 2024).

340 Taxonomic names are reported according to the database classification used in the
341 bioinformatic workflow. The Deep Sea Euryarchaeotic Group (DSEG) is therefore retained
342 when returned by the database, although this lineage is classified within Thermoplasmatota in
343 newer phylogenetic classifications (Rinke et al., 2021).

344

345 **2.7 Statistical analysis and data visualisation**

346 Multidimensional analyses, correlograms, and hierarchical analyses were conducted using R (R
347 Core Team, 2023). RStudio 2025.05.0+496 “Mariposa Orchid” and R version 4.3.3 (2024-02-
348 29) on the x86_64-apple-darwin20 (64-bit) platform were used for all analyses. A heatmap of
349 the archaeal community (at the class level) with cluster analysis was generated using the
350 Heatmap function in the *ComplexHeatmap* package. To examine the grouping of samples
351 between pockmark and reference sites, Classical Multidimensional Scaling (MDS; principal
352 coordinates analysis) was performed using the `dist` function (`dist_matrix <- dist(data), method`
353 `= "euclidean"`) and the `cmdscale` function (`mds <- cmdscale(dist_matrix)`) from the *stats*
354 package. The MDS results were visualised using the *ggplot2* and *ggrepel* packages (Wickham,
355 2016).

356 Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using
357 `adonis2` (`permutations = 999, method = "bray"`) in the *vegan* package. Principal Component
358 Analysis (PCA) was conducted using the *FactoMineR* package, and the results were visualised
359 with `fviz_pca_var` from the *factoextra* package. A correlation network illustrating relationships
360 between Archaea (at the family taxonomic level) and GDGTs was constructed using `cor` from
361 the *stats* package; `graph_from_adjacency_matrix` (`mode = "undirected", weighted = TRUE,`
362 `diag = FALSE`) from the *igraph* package; `mutate_as_tbl` from the *tidygraph* package; and
363 *ggraph* for visualisation. Community structure was identified using `group_louvain` (multilevel
364 optimisation of modularity via `igraph::cluster_louvain()`), which implements the multi-level
365 modularity optimisation algorithm described by Blondel et al. (2008). Cross-correlations
366 between iGDGT and OH-GDGT concentrations were calculated in R using `stats::cor.test()` with
367 Pearson correlation. Concentrations were \log_{10} -transformed ($\log_{10}[x + 10^{-6}]$) before correlation
368 analysis to reduce right skew. Correlations were computed using pairwise complete
369 observations. To correct for multiple testing, *p*-values were adjusted using the Benjamini–
370 Hochberg false discovery rate (FDR) procedure.

371 2.8 Calculation of indices

372 The GDGT-0/crenarchaeol ratio was calculated to assess potential contributions to iGDGT
373 production from methanogens (although GDGT-0 is not exclusive to them) and ammonia-
374 oxidising archaea (Blaga et al., 2009). Values > 2 have been proposed to indicate a substantial
375 methanogenic input (Blaga et al., 2009; Schouten et al., 2013; Zell et al., 2014).

376 $\text{GDGT-0/cren} = [\text{GDGT-0}] / [\text{crenarchaeol}]$

377 The GDGT-2/crenarchaeol (GDGT-2/cren) index is used as an additional screening tool to
378 assess a potential AOM contribution (Weijers et al., 2011). An elevated GDGT-2/cren ratio
379 suggests increased synthesis of GDGT-2 within the SMTZ, likely from methanotrophic
380 Euryarchaeota (Pancost et al., 2001; Wakeham et al., 2003; Stadnitskaia et al., 2005).

$$381 \text{ GDGT-2/cren} = [\text{GDGT-2}] / [\text{crenarchaeol}]$$

382 The Methane Index (MI) is based on GDGT-1 to GDGT-3 and crenarchaeol, and reflects the
383 balance between methanotrophic Euryarchaeota and Nitrososphaeria, whether planktonic or
384 benthic (Zhang et al., 2011). GDGTs associated with methanotrophs — mainly GDGT-1 to
385 GDGT-3 (Pancost et al., 2001; Zhang et al., 2011) — are primarily produced by ANME-1
386 (Rossel et al., 2008). The MI ranges from 0 to 1, with higher values (>0.3-0.5) indicating a
387 greater relative contribution of GDGT-1 to GDGT-3 than of crenarchaeol and cren', and are
388 linked to high methane fluxes (Kim and Zhang, 2023). The MI, as defined by Zhang et al.
389 (2011), was calculated as follows:

$$390 \text{ MI} = [\text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3}] / [\text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3} + \text{cren} + \text{cren}']$$

391 The Branched and Isoprenoid Tetraether index (BIT) estimates the relative contribution of
392 branched GDGTs to the combined pool of branched GDGTs and crenarchaeol, following
393 Hopmans et al. (2004), and serves as an indicator of terrestrial organic matter input into a marine
394 environment. However, brGDGTs may also be produced in situ in aquatic and sedimentary
395 environments (Peterse et al., 2009; Dearing Crampton-Flood et al., 2019 and references
396 therein). The BIT index ranges from 0 to 1, with higher values indicating a greater relative
397 contribution of brGDGTs compared with crenarchaeol:

$$398 \text{ BIT} = [\text{brGDGT-Ia} + \text{II-a} + \text{IIIa}] / [\text{br-GDGT-Ia} + \text{IIa} + \text{IIIa} + \text{cren}]$$

399 The percentage of OH-GDGTs reflects the relative contribution of hydroxylated iGDGTs to the
400 total iGDGT pool and indicates an increased contribution from OH-GDGT-producing archaea
401 and/or adaptation to low temperature and salinity. The index was calculated following Huguet
402 et al. (2013):

$$403 \text{ OH-GDGT\%} = \frac{\Sigma[\text{OH-GDGT-0} + \text{OH-GDGT-1} + \text{OH-GDGT-2}]}{\{\Sigma[\text{OH-GDGT-0} + \text{OH-}]} \\ 404 \text{ GDGT-1} + \text{OH-GDGT-2}] + \Sigma[\text{GDGT-0} + \text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3} + \text{cren} + \text{cren}']\} \times \\ 405 100$$

406 The ring indices of hydroxylated tetraethers (RI-OH and RI-OH') quantify the degree of
407 cyclisation, that is, the number of cyclopentane rings in the molecules. This number increases
408 with temperature and decreases with salinity (Sinninghe Damsté et al., 2022). The RI-OH' is
409 more sensitive in cold regions (Varma et al., 2024). The indices were calculated following Lü
410 et al. (2015):

$$411 \text{ RI-OH} = \{[\text{OH-GDGT-1}] + 2 \times [\text{OH-GDGT-2}]\} / \{[\text{OH-GDGT-1}] + [\text{OH-GDGT-2}]\}$$

$$412 \text{ RI-OH}' = \{[\text{OH-GDGT-1}] + 2 \times [\text{OH-GDGT-2}]\} / \Sigma[\text{OH-GDGTs}]$$

413 **3 Results**

414 **3.1 Porewater profiles of SO_4^{2-} , CH_4 , and Cl^-**

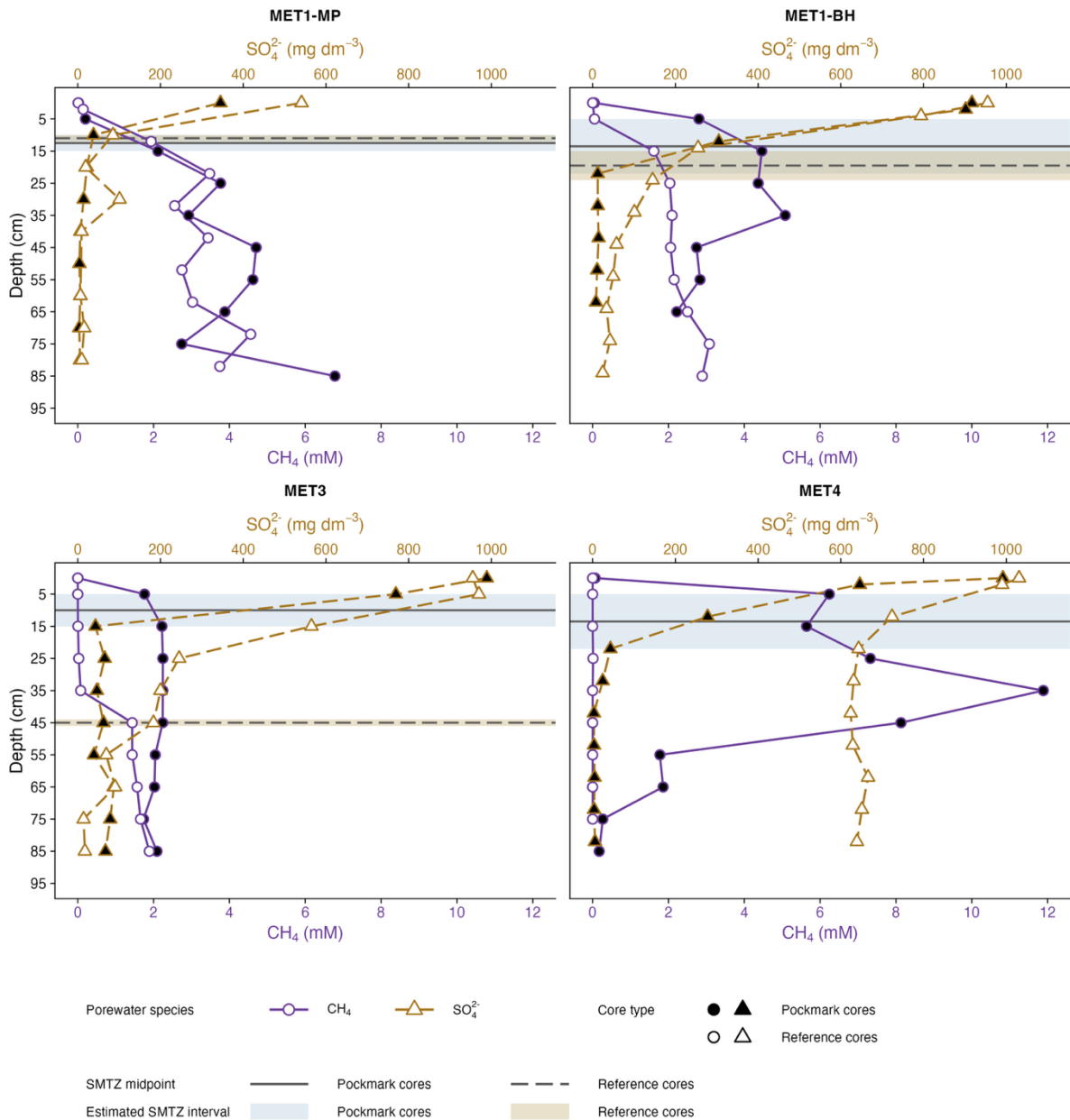
415

416 Pockmarks across all sites show greater sulphate depletion, with a mean SO_4^{2-} concentration
417 $1.5\times$ lower than in non-pockmarks (244.4 ± 371.0 SD mg dm⁻³ vs 377.5 ± 364.4 SD mg dm⁻³,
418 respectively, Fig. 2). Although SO_4^{2-} concentrations span similar value ranges in pockmarks
419 and non-pockmarks ($3.1\text{--}992$ mg dm⁻³ vs $6.6\text{--}1031$ mg dm⁻³, respectively), their median values
420 differ substantially by a factor of ~ 5 (40.9 vs 200 mg dm⁻³, respectively). Pockmarks also show
421 SO_4^{2-} depletion occurring closer to the surface, meaning the depth at which SO_4^{2-} first drops
422 below 100 mg dm⁻³ (at 17.3 ± 5.9 cm in pockmark cores compared with 36.3 ± 23.5 cm in
423 reference cores) and 50 mg dm⁻³ (17.3 ± 5.9 cm in pockmark cores compared with 497.7 ± 27.8
424 cm in reference cores) is 2 and $3\times$ shallower, respectively. Pockmarks show mean CH_4
425 concentrations 2.3-fold higher than at reference sites (3.1 ± 2.6 mM vs 1.3 ± 1.4 mM, mean \pm
426 SD; Fig. 2). Median CH_4 concentrations were also higher in pockmark-cores (2.24 vs 1.44 mM),
427 reflecting the influence of particularly high CH_4 concentrations in selected pockmark horizons.
428 MET4 exhibits the most extreme values across the examined sites and the greatest differences
429 between pockmark and reference cores; CH_4 reached the highest values in the pockmark core,
430 whereas the reference core remained close to $\text{CH}_4\text{-free}$. By contrast, MET3 shows the least
431 differentiation between pockmark and reference cores and has lower overall methane
432 concentrations.

433

434 Sulphate and CH_4 show clear, opposing depth trends across all four study locations, except at
435 non-methane MET4, where CH_4 is absent, and sulphate is high throughout the profile (Fig. 2).
436 SO_4^{2-} is initially high in surface sediments and declines with depth, whereas CH_4 shows the
437 opposite pattern. Based on the definition of the SMTZ as the depth where porewater SO_4^{2-} and

438 CH₄ concentrations oppose each other, this pattern may indicate the SMTZ location. However,
 439 the magnitude and depth of these changes, as well as differences between pockmark and
 440 reference sites, vary by location. Midpoints ranged from 10–15 cm in pockmark sediments and
 441 10–45 cm in non-pockmark sediments, suggesting a shallower SMTZ in pockmarks than in
 442 reference non-pockmarks of MET1-BH and MET3, a similarly shallow SMTZ in MET1-MP,
 443 and no SMTZ in the sampled interval of non-pockmark MET4.
 444



445
 446 Fig. 2. Profiles of sulphate and methane differ in absolute concentrations across sites. The bands
 447 representing the SMTZ width in the sulphate and methane profile plots were defined by two criteria:
 448 where sulphate declines to $\leq 20\%$ of its surface value, and where methane becomes detectably present,
 449 defined as $\geq 20\%$ of the maximum value in each profile (with a minimum threshold of 0.1 mM). Dashed

450 (reference cores) and solid (pockmark cores) horizontal lines indicate the midpoint of the estimated
451 SMTZ.

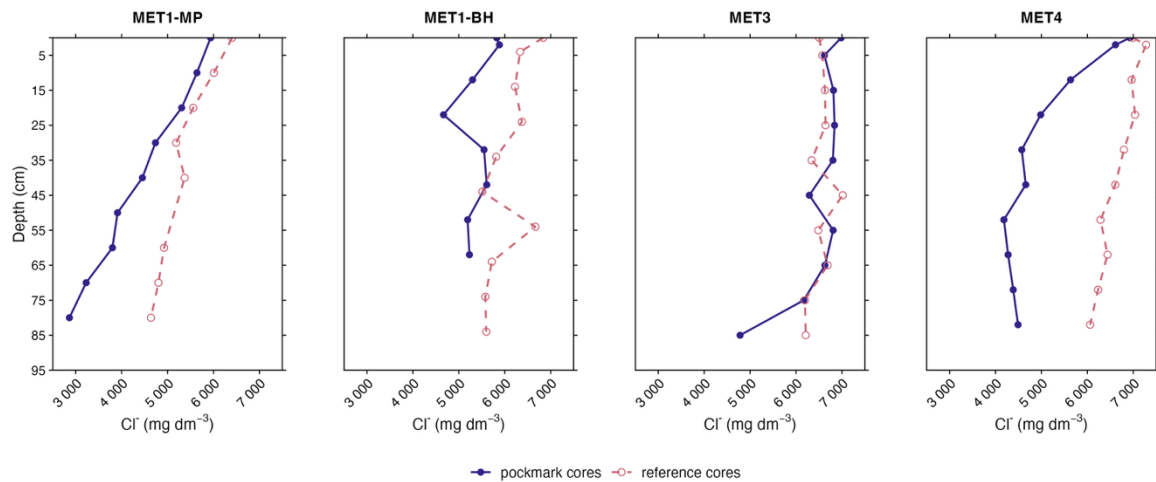
452

453 Chloride porewater profiles differed among the investigated sites, with generally lower
454 concentrations in pockmark cores than in reference cores (Fig. 3). Across the sediment-depth
455 intervals, Cl^- concentrations averaged $6198 \pm 645 \text{ mg dm}^{-3}$ in reference cores and 5367 ± 1089
456 mg dm^{-3} in pockmark cores.

457

458 The strongest contrast between the pockmark and reference cores is evident at MET4 (Fig. 3).
459 This contrast reflects a pronounced downcore Cl^- depletion in the MET4 pockmark core (from
460 6927 mg dm^{-3} at the surface to $4182\text{-}4490 \text{ mg dm}^{-3}$ below 50 cm depth), compared with the
461 reference core, which shows consistently higher and steady chloride concentrations ($6060\text{-}7279$
462 mg dm^{-3}). This pattern indicates freshwater enrichment within the MET4 pockmark core
463 relative to the adjacent reference core. However, the lowest concentrations occur in the MET1-
464 MP pockmark core, where Cl^- decreases steadily with depth (from 5938 mg dm^{-3} at the surface
465 to 2863 mg dm^{-3} at the bottom of the core). Whereas Cl^- concentrations also decrease with depth
466 in the reference core of MET1-MP, they remain higher than in the MET1-MP pockmark core
467 (Fig. 3). In the MET1-BH pockmark core, Cl^- concentrations are lower than in the reference
468 core throughout the sediment profile, but the pattern is irregular (a dip to 4667 mg dm^{-3} at 20
469 cm and a recovery to $\sim 5200\text{-}5600 \text{ mg dm}^{-3}$ below). In the MET1-BH reference core, Cl^-
470 concentrations are higher and more variable ($5512\text{-}6838 \text{ mg dm}^{-3}$). At MET3, the pockmark
471 and reference cores are largely similar across the depth profile and remain among the highest
472 Cl^- levels in the dataset. The main difference appears in the deepest MET3 pockmark sample,
473 where the chloride concentration drops (4781 mg dm^{-3} at 85 cm), whereas the reference core
474 remains stable ($6192\text{-}7015 \text{ mg dm}^{-3}$) (Fig. 3).

475



476

477 Fig. 3. Depth profiles of porewater chloride (Cl^-) at MET1-MP, MET1-BH, MET3, and MET4, ranging
 478 from 2863 to 7279 mg dm^{-3} . The profiles consistently show higher chloride concentrations in reference
 479 sediments than in pockmark sediments. The extent and shape of these profiles vary among sites. The
 480 most distinct separation between pockmark and reference profiles occurs at MET4 and MET1-MP,
 481 whereas MET3 shows substantial overlap. Depth is given in cm below sea floor (bsf).

482

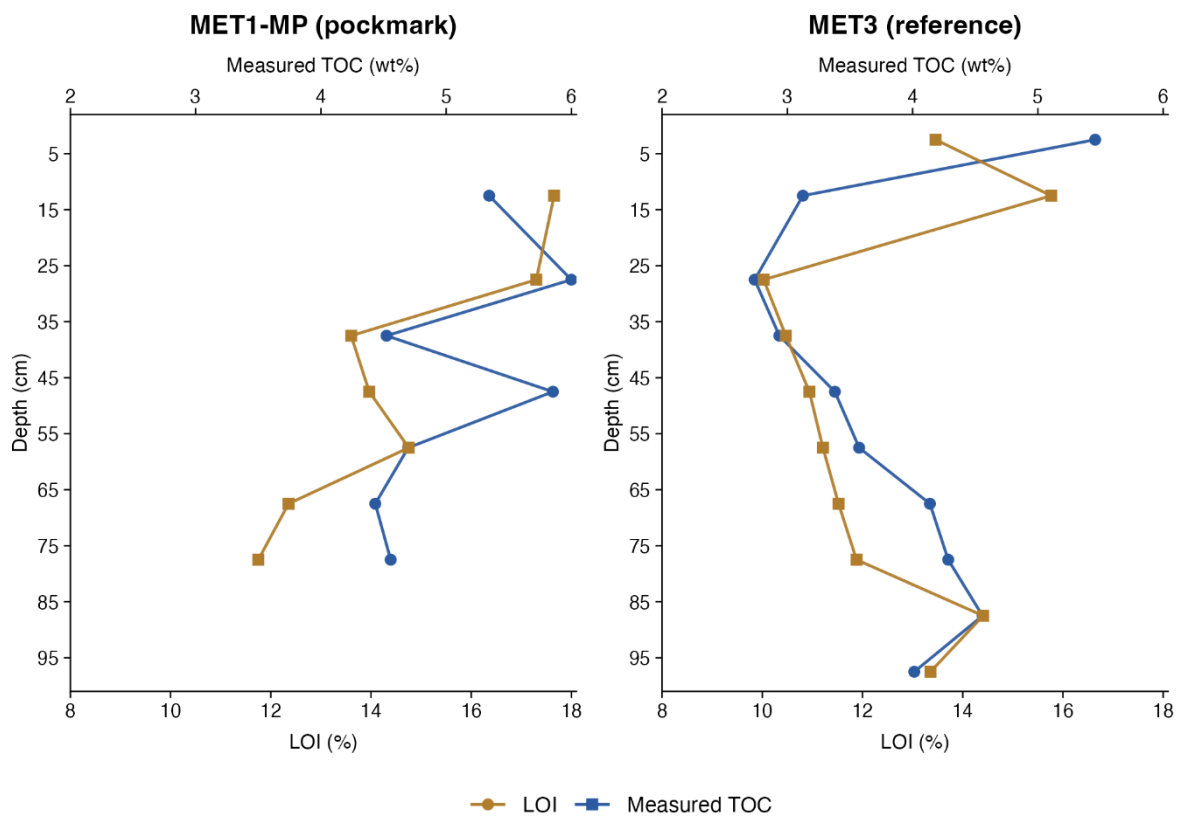
483 3.2 LOI

484

485 To assess whether LOI captured the main downcore stratigraphic trends in organic carbon, LOI
 486 profiles were compared with directly measured TOC in two representative cores: the MET1-
 487 MP pockmark core and the MET3 reference core (Fig. 4). Across these samples, LOI and
 488 measured TOC showed a positive relationship ($n = 17$, $R^2 = 0.43$, $p = 0.004$), supporting the use
 489 of LOI as a proxy for organic matter variability. In both cores, intervals of increased LOI
 490 consistently coincide with higher measured TOC, demonstrating that LOI reliably reflects
 491 variations in organic matter content, despite differences in absolute values.

492

493 In the MET1-MP pockmark core, both LOI and measured TOC are relatively elevated in the
 494 upper to middle part of the core and generally decrease below ~ 45 -55 cm depth (Fig. 4). This
 495 indicates a downcore decline in bulk organic matter content below the mid-core interval. In the
 496 MET3 reference core, measured TOC is highest in the shallowest interval and decreases through
 497 the upper-middle section of the core, while LOI follows a comparable vertical pattern (Fig. 4).
 498 Therefore, LOI is used below as a qualitative, high-resolution proxy for bulk organic matter
 499 trends in the studied cores, providing a sedimentary and geochemical context for the GDGT
 500 and microbial distributions.



502

503 Fig. 4. Comparison of loss-on-ignition values (LOI%, lower x-axis) and directly measured total organic
 504 carbon (TOC wt%, upper x-axis) in two representative sediment cores from the south-eastern Baltic Sea:
 505 the MET1-MP pockmark core from the Gulf of Gdańsk and the MET3 reference core from the Gdańsk
 506 Deep. Both parameters are plotted against sediment depth (cm bsf). This comparison was used to assess
 507 whether LOI captures the main stratigraphic variability in organic matter content. Across both cores,
 508 LOI and measured TOC show a positive correlation ($n = 17$, $R^2 = 0.43$, $p = 0.004$), supporting the use
 509 of LOI as a qualitative, trend-level proxy for variability in bulk organic matter. Although sample-scale
 510 offsets occur between LOI and TOC, the profiles broadly reproduce comparable downcore patterns.

511

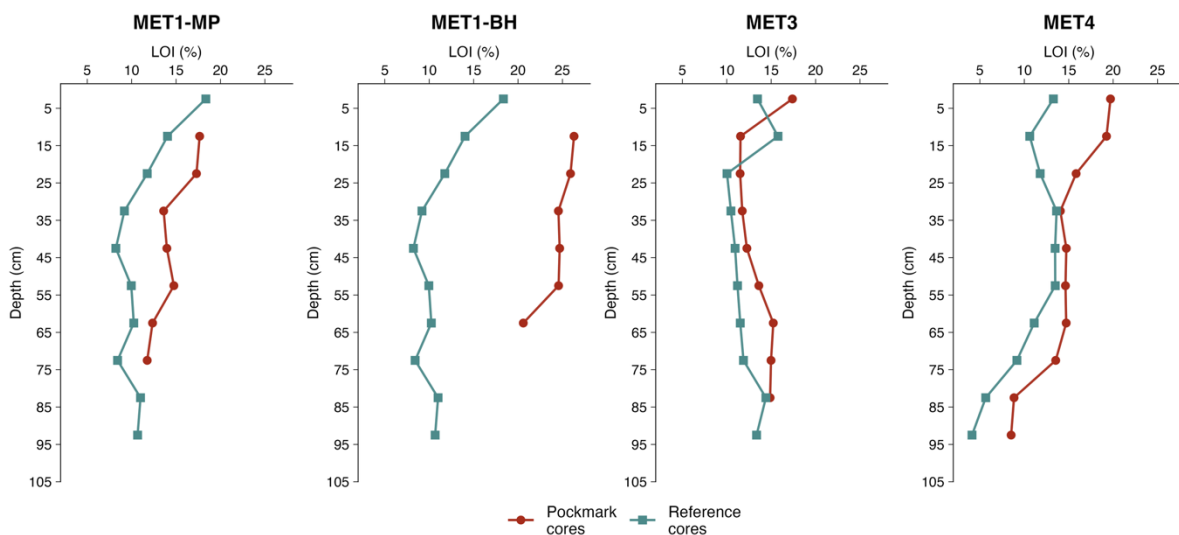
512 LOI values show site-specific and downcore variability, with varying degrees of contrast
 513 between pockmark and reference cores, indicating differences in bulk organic matter content
 514 and relative enrichment in pockmark cores, especially pronounced in MET1-BH (Fig. 5). In the
 515 MET1 area, the same reference core was used for comparison with the MET1-MP and MET1-
 516 BH pockmarks. LOI values were elevated in selected pockmark cores compared with reference
 517 cores, particularly in MET1-BH and MET1-MP, with LOI values on average approximately
 518 2.3-fold higher in the MET1-BH pockmark core and 1.4-fold higher in the MET1-MP
 519 pockmark core relative to the shared MET1 reference core. The strongest enrichment occurs at

520 MET1-BH, where pockmark LOI values are about 1.9-3.0 times higher than the corresponding
521 reference values. In contrast, pockmark MET3 shows only a weak average difference of ~1.1-
522 fold, with depth-wise ratios ranging from 0.7 to 1.3, indicating that LOI values are broadly
523 overlapping between pockmark and reference cores.

524

525 At MET4, pockmark LOI values were on average ca. 1.4-fold higher than in the reference core,
526 with the relative difference increasing downcore and reaching approximately 2.1-fold in the
527 deepest interval of the profile, where the reference core shows low LOI values of ~4% (Fig. 5).
528 The LOI results, used as a proxy for organic matter content, indicate that organic matter
529 accumulation is site-specific and should not be generalised across all pockmarks.

530



531

532 Fig. 5. Downcore variation of loss-on-ignition (LOI%) in sediment cores collected from the Gulf of
533 Gdańsk (MET1-MP, MET1-BH) and the Gdańsk Deep (MET3, MET4), south-eastern Baltic Sea. LOI
534 values are plotted against depth bsf (cm) and serve as a qualitative, screening-level proxy for variability
535 in bulk organic matter. Pockmark sediments are compared with adjacent reference (non-pockmark)
536 sediment cores at each site; for the MET1-MP and MET1-BH pockmarks, the same reference core is
537 used. LOI values are elevated in the pockmark cores at MET1-MP, MET1-BH, and MET4, with the
538 strongest enrichment observed at MET1-BH. By contrast, MET3 shows values that are approximately
539 overlapping between the pockmark and reference sediment cores. At MET4, both cores show a
540 pronounced downcore decrease in LOI, particularly in the reference core, where values decline to ca.
541 4% in the deepest part of the profile.

542

543 **3.3 iGDGTs in pockmark and non-pockmark marine surface sediments**

544 The abundance and distribution of iGDGTs (GDGT-0 to GDGT-3, crenarchaeol, and its isomer)
545 were analysed in pockmark core sediments and in surrounding non-pockmark reference core
546 sediments (Fig. 6). All targeted iGDGTs were detected in both settings.

547 Unless stated otherwise, concentration values are reported as ranges, followed by the median
548 and the mean \pm standard deviation (SD). Summed iGDGT concentrations (Σ iGDGTs) span
549 0.02–58.85 $\mu\text{g g}^{-1}$ sediment across all samples, with values generally higher and more variable
550 in pockmark sediment cores (0.92–58.85 $\mu\text{g g}^{-1}$ sediment; 5.10 $\mu\text{g g}^{-1}$; 10.58 \pm 13.08 $\mu\text{g g}^{-1}$)
551 than in reference sediment cores (0.02–16.81 $\mu\text{g g}^{-1}$ sediment; 2.59 $\mu\text{g g}^{-1}$; 3.73 \pm 3.41 $\mu\text{g g}^{-1}$),
552 that is, approximately twice the median and three times the mean. Spatially, the highest
553 Σ iGDGT concentrations occur at sites in the Gdańsk Deep (MET3, MET4), whereas lower
554 concentrations are generally observed in the MET1 area (Fig. 6). With depth, Σ iGDGTs
555 commonly show shallow to mid-depth maxima, particularly between approximately 15 and 45
556 cm bsf, followed by downcore depletion. Excluding the anomalously low values in the S/MET4
557 reference core, Σ iGDGTs concentrations typically decline by a factor of \sim 2.6 to \sim 64, although
558 the fold decrease is much larger where the deepest reference intervals approach near-zero values
559 in S/MET4.

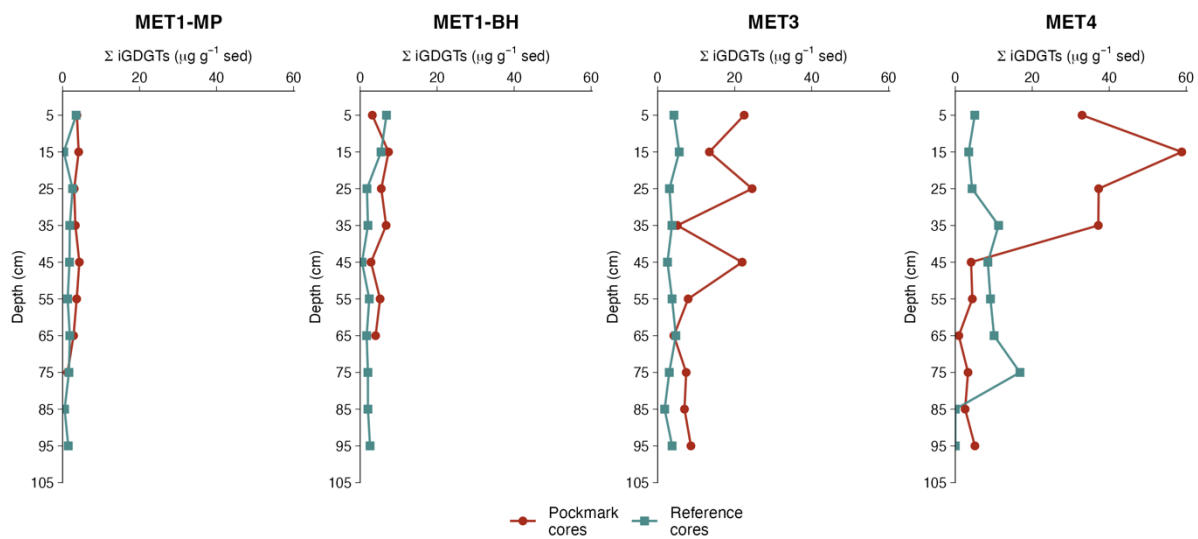
560 Crenarchaeol (cren) is the dominant iGDGT in both pockmark cores (mean fractional
561 abundance of 0.53 \pm 0.03 SD) and reference cores (0.55 \pm 0.02 SD) (Fig. 7). GDGT-0 is the
562 second most abundant in pockmark and reference cores (0.43 \pm 0.03 SD; 0.41 \pm 0.02 SD,
563 respectively) (Fig. 7). Together, crenarchaeol and GDGT-0 account for \sim 96 % of the total
564 iGDGT pool in both pockmark and reference cores, indicating broadly similar iGDGT
565 distributions between pockmarks and references. Consistently, GDGT-0 covaries very strongly
566 with crenarchaeol ($r = 0.996$, $p < 0.001$).

567 The remaining iGDGTs, GDGT-1 to GDGT-3, occur only in minor proportions and at much
568 lower concentrations (Fig. 7). Across the analysed depth intervals from 0-5 to 90-95 cm bsf,
569 GDGT-1, GDGT-2, and GDGT-3 show a highly consistent pattern of relative abundance. In 74
570 of 75 samples, the order is GDGT-1 > GDGT-2 > GDGT-3, and GDGT-2 is not the dominant
571 compound in any sample. The only exception is sample P/MET4/3, where the order is GDGT-
572 1 > GDGT-3 > GDGT-2.

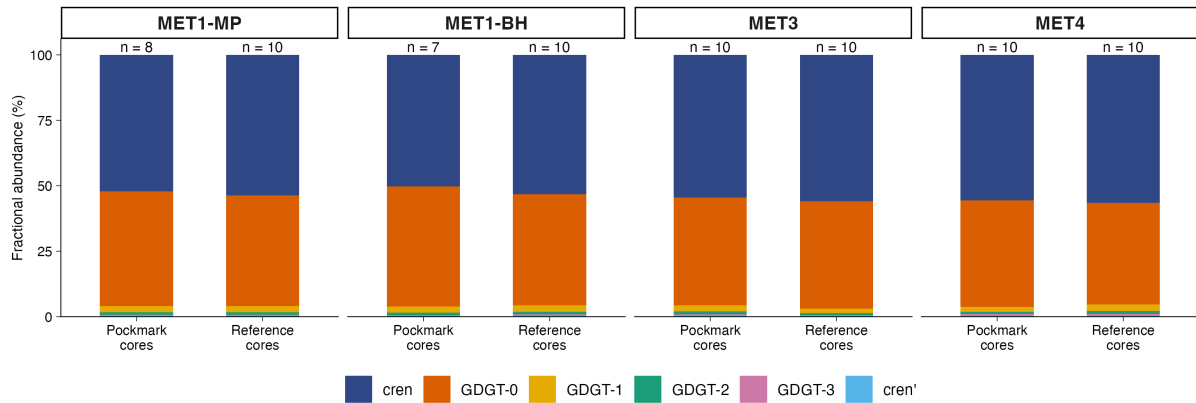
573 Concentration profiles of the individual iGDGTs (Supplementary material Fig. S2) generally
574 follow similar downcore patterns, except for isolated concentration maxima of GDGT-1 in

575 P/MET1-BH/6 and GDGT-3 in P/MET4/3, minor changes in the crenarchaeol isomer (cren') at
 576 MET1-MP and MET1-BH, and even smaller differences at MET3 and MET4. GDGT-1,
 577 GDGT-2, and GDGT-3 also show strong covariation with crenarchaeol ($r = 0.988, 0.954, 0.951,$
 578 $p < 0.001$). Across all iGDGT pairs, correlations are consistently strong ($r = 0.847\text{--}0.996, p <$
 579 0.001), indicating that the individual iGDGTs vary largely in concert rather than exhibiting
 580 compound-specific enrichments.

581 The mean \pm SD values for the indices are reported in Table 1. The GDGT-0/cren ratio is
 582 moderate and broadly comparable between pockmark and reference cores, ranging from 0.65
 583 to 0.99 in pockmark cores and from 0.63 to 0.91 in reference cores. No consistent downcore
 584 trend is observed across all cores; where changes occur, they are site-specific and modest
 585 relative to the overlap between pockmark and reference cores. The GDGT-2/cren ratio is
 586 consistently low (0.01–0.04), and MI values remain low, ranging from 0.04 to 0.09 in
 587 pockmarks and from 0.03 to 0.07 in references. The BIT index values are generally low, ranging
 588 from 0.008 to 0.143 in pockmarks and from 0.034 to 0.468 in references, with the slightly higher
 589 values (negligible) confined mainly to the deepest intervals of the MET4 reference core.



590
 591 Fig. 6. Downcore profiles of summed isoprenoidal glycerol dialkyl glycerol tetraethers (Σ iGDGTs; μg
 592 g^{-1} dry sediment) for sediment cores from four sites in the south-eastern Baltic Sea: MET1-MP and
 593 MET1-BH (Gulf of Gdańsk), and MET3 and MET4 (Gdańsk Deep). Concentrations are plotted as a
 594 function of depth below the sea floor (cm bsf), showing a general downcore decrease. Σ iGDGTs exhibit
 595 elevated concentrations in pockmark sediment cores relative to adjacent reference sediment cores, with
 596 the most pronounced enrichment observed at the Gdańsk Deep sites (MET3, MET4), where elevated
 597 near-surface values are followed by a downcore decrease. Error bars are not shown because each point
 598 represents a single sediment horizon analysed once (see section 2.5).



600

601 Fig. 7. Mean fractional abundances of iGDGTs in pockmark and reference cores at sites MET1-MP,
 602 MET1-BH, MET3, and MET4 in the south-eastern Baltic Sea. Stacked bars show mean fractional
 603 abundances of iGDGTs (GDGT-0–3, crenarchaeol, and cren'), averaged by site and core type; n
 604 indicates the number of horizons (samples). The upper panel shows the full scale (0–100%).
 605 Crenarchaeol and GDGT-0 dominate across all sites, whereas GDGT-1–3 and cren' constitute minor
 606 fractions.

607

608 Table 1. Mean GDGT-based indices for pockmark (P) and reference (S) sediment cores at MET1 (MP,
 609 BH), MET3, and MET4. Values are reported as mean \pm sample standard deviation, rounded to a
 610 maximum of two significant figures. Metrics include OH-GDGT%, hydroxylated GDGT ring indices
 611 (RI-OH, RI-OH'), the Branched and Isoprenoid tetraether index (BIT), the Methane Index (MI), and
 612 diagnostic ratios (GDGT-0/cren, GDGT-2/cren). RI-OH shows minimal variation across sites and
 613 between pockmark and reference cores (\sim 1.1–1.2), indicating comparable OH-GDGT cyclisation
 614 patterns. OH-GDGT% is moderately elevated at MET1 relative to MET3–MET4, with minimal within-
 615 site variation. MI values remain consistently low (0.05–0.07). GDGT-0/cren ratios are consistently
 616 slightly elevated in pockmark cores relative to paired references, whereas GDGT-2/cren remains
 617 uniformly low (0.01–0.02), indicating that pockmark influence primarily affects GDGT-0 abundance
 618 rather than higher-cyclised GDGTs.

619

Sediment core	OH-GDGT%	RI-OH	RI-OH'	BIT	MI	GDGT-0/cren	GDGT-2/cren
P/MET1-MP	9.0 \pm 0.4	1.2 \pm 0.03	0.19 \pm 0.02	0.12 \pm 0.01	0.065 \pm 0.003	0.84 \pm 0.02	0.019 \pm 0.001
P/MET1-BH	9.3 \pm 0.4	1.2 \pm 0.04	0.19 \pm 0.02	0.09 \pm 0.01	0.066 \pm 0.003	0.92 \pm 0.04	0.019 \pm 0.002
P/MET3	7.9 \pm 0.9	1.2 \pm 0.02	0.21 \pm 0.03	0.05 \pm 0.02	0.064 \pm 0.008	0.76 \pm 0.11	0.021 \pm 0.004
P/MET4	7.8 \pm 1.3	1.1 \pm 0.02	0.19 \pm 0.02	0.03 \pm 0.02	0.054 \pm 0.016	0.74 \pm 0.08	0.014 \pm 0.003
S/MET1-MP	8.1 \pm 0.8	1.2 \pm 0.04	0.25 \pm 0.04	0.14 \pm 0.03	0.066 \pm 0.002	0.79 \pm 0.05	0.019 \pm 0.001
S/MET1-BH	8.1 \pm 0.9	1.2 \pm 0.02	0.23 \pm 0.03	0.12 \pm 0.02	0.066 \pm 0.003	0.80 \pm 0.06	0.019 \pm 0.001

S/MET3	7.0±0.6	1.2±0.03	0.19±0.02	0.07±0.01	0.047±0.003	0.73±0.07	0.013±0.001
S/MET4	7.2±0.9	1.2±0.06	0.21±0.07	0.13±0.16	0.065±0.036	0.69±0.03	0.019±0.013

620

621 **3.4 OH-GDGTs in pockmark and non-pockmark sediments**

622 The abundance and distribution of hydroxylated GDGTs (OH-GDGT-0 to -2) in pockmark and
623 reference cores broadly mirror those of iGDGTs, with strong positive cross-correlations ($r =$
624 $0.840\text{--}0.992$, $p < 0.001$) and the tightest coupling with GDGT-0 ($\Sigma\text{OH-GDGTs}$: $r = 0.992$, $p <$
625 0.001) and crenarchaeol ($r = 0.983$, $p < 0.01$). All targeted OH-GDGTs (OH-GDGT-0 to -2)
626 were detected in both settings.

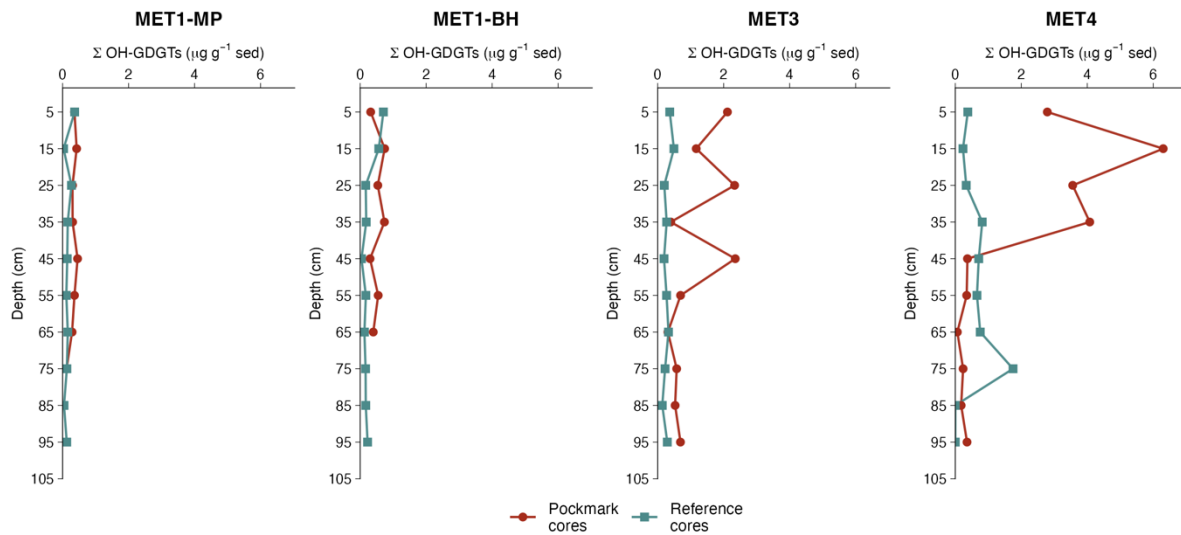
627 Summed OH-GDGT concentrations ($\Sigma\text{OH-GDGTs}$) ranged from 0.00 to 6.30 $\mu\text{g g}^{-1}$ sediment
628 across all samples, with values generally higher in pockmark sediment cores (0.06–6.30 $\mu\text{g g}^{-1}$
629 sediment; 0.43 $\mu\text{g g}^{-1}$ sediment; $1.02 \pm 1.36 \mu\text{g g}^{-1}$ sediment) than in reference sediment cores
630 (0.00–1.75 $\mu\text{g g}^{-1}$ sediment; 0.21 $\mu\text{g g}^{-1}$ sediment; $0.31 \pm 0.32 \mu\text{g g}^{-1}$ sediment) (Fig. 8). $\Sigma\text{OH-}$
631 GDGTs are therefore, on average, approximately three times higher and, at the median, about
632 twice as high in pockmarks as in references. Spatially, the highest $\Sigma\text{OH-GDGTs}$ concentrations
633 occur at MET4, intermediate values at MET3, and lower values in the MET1 area, broadly
634 mirroring the spatial pattern observed for ΣiGDGTs . With depth, $\Sigma\text{OH-GDGTs}$ commonly
635 show shallow to mid-depth maxima, typically between 15 and 45 cm bsf, followed by downcore
636 depletion (Fig. 8). This decrease is particularly pronounced at MET4, especially in P/MET4,
637 where ΣiGDGTs declined from 6.30 to 0.06 $\mu\text{g g}^{-1}$ sediment.

638 Across all cores, OH-GDGTs are dominated by OH-GDGT-0 in both pockmark and reference
639 cores (Fig. 9, average fractional abundances of 0.83 ± 0.02 SD and 0.82 ± 0.03 SD, respectively).
640 OH-GDGT-1 and OH-GDGT-2 are consistently minor components (Fig. 9). Overall, OH-
641 GDGT distributions are broadly similar between pockmark and reference cores (Supplementary
642 material Fig. S4), and most variability is evident in downcore concentration profiles, which
643 typically decline by a factor of ~ 1.2 to ~ 7.8 , with most cores showing an average decrease by a
644 factor of ~ 3 (except for S/MET4, which declines to zero at the base). Across all OH-GDGT
645 pairs (OH-0, OH-1, OH-2), correlations are consistently very strong ($r = 0.975\text{--}0.992$, $p < 0.01$).

646 OH-GDGT% and RI-OH show only minor differences between pockmark and reference cores
647 (Table 1). OH-GDGT% values are slightly higher in pockmarks (6.4–9.9) than in references
648 (6.2–9.4). RI-OH is identical between settings (1.1–1.3 in pockmarks; 1.1–1.3 in references),

649 whereas RI-OH' is modestly higher in references (0.16–0.36) than in pockmarks (0.16–0.25).
 650 Overall, variability in OH-GDGTs is mainly reflected in absolute abundances, while OH-
 651 GDGT distribution and indices are broadly comparable between pockmark and reference cores.

652

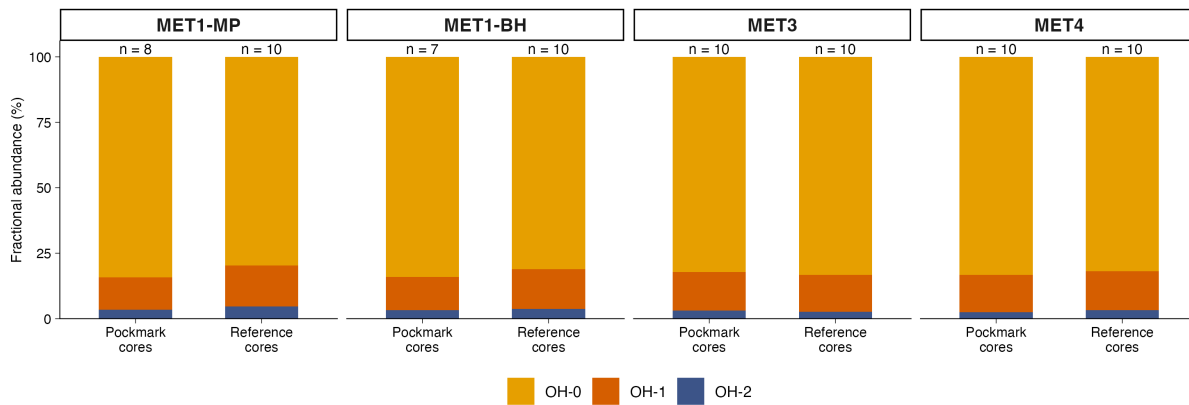


653

654 Fig. 8. Downcore profiles of summed hydroxylated glycerol dialkyl glycerol tetraethers (Σ OH-GDGTs;
 655 $\mu\text{g g}^{-1}$ dry sediment) for sediment cores from four sites in the south-eastern Baltic Sea: MET1-MP and
 656 MET1-BH (Gulf of Gdańsk) and MET3 and MET4 (Gdańsk Deep). Concentrations are plotted against
 657 depth (downcore increase; cm). Σ OH-GDGT concentrations follow the pattern of iGDGTs, showing
 658 strong positive cross-correlations and the highest coupling with GDGT-0 and crenarchaeol (see Section
 659 3.2). In the Gulf of Gdańsk (MET1-MP, MET1-BH), Σ OH-GDGTs show lower overall abundances than
 660 iGDGTs but remain elevated in pockmark intervals relative to reference sediments.

661

662



663

664 Fig. 9. Mean OH-GDGT fractional abundances in pockmark versus reference cores at sites MET1-MP,
 665 MET1-BH, MET3, and MET4 in the south-eastern Baltic Sea. Stacked bars show mean fractional

666 abundances of OH-0, OH-1 and OH-2, averaged by site and core type (pockmarks and references); n
667 denotes the number of horizons (samples). OH-0 dominates across all sites, with OH-1 contributing a
668 smaller yet consistent fraction and OH-2 occurring at low abundance.

669 **3.5 Abundance and composition of archaea**

671
672 Archaeal community composition differs between pockmark and reference sediments (Fig. 10).
673 However, at the class level, most samples are dominated by Nanoarchaeia (Fig. 10a). Additional
674 major contributions, at varying proportions, include Thermoplasmata, Methanosarcinia,
675 Thermococci, Bathyarchaeia, Lokiarchaeia, and Methanomicrobia (Fig. 10a). Pockmark
676 samples generally contain higher proportions of methane-cycling archaeal groups, particularly
677 Methanosarcinia and Methanomicrobia, whereas reference cores more commonly show
678 elevated contributions of Thermoplasmata, Bathyarchaeia, and Lokiarchaeia (Fig. 10a). The
679 general depth trend shows Nanoarchaeia dominating shallower horizons, uniformly across
680 reference and pockmark cores. Nanoarchaeia dominate the archaeal community in pockmarks,
681 even in deeper sediment horizons, particularly at P/MET1-BH and P/MET1-MP. Vertical
682 stratification is less consistent and more site-specific in pockmark cores than in reference cores,
683 but the relative abundance of Methanosarcinia, Thermococci, and Bathyarchaeia increases
684 downcore. Methanomicrobia also show a pockmark-associated pattern, with higher mean
685 relative abundance in pockmarks than in references ($2.6 \pm 1.8\%$ vs. $0.6 \pm 1.0\%$, respectively),
686 indicating additional methanogen-affiliated archaeal populations beyond Methanosarcinia (Fig.
687 10a). In reference cores, in deeper horizons, the community composition shifts towards a higher
688 relative abundance of Thermoplasmata, Bathyarchaeia, Lokiarchaeia, and Deep Sea
689 Euryarchaeotic Group (DSEG, currently within Thermoplasmatota; Rinke et al., 2021),
690 suggesting a greater contribution from uncultivated anaerobic sedimentary archaeal lineages
691 with depth. Across all samples, these classes were more abundant in reference than in pockmark
692 cores, with mean relative abundances of $20.5 \pm 13.6\%$ vs. $9.4 \pm 5.2\%$ for Thermoplasmata, 14.8
693 $\pm 19.5\%$ vs. $5.2 \pm 5.9\%$ for Bathyarchaeia, $5.6 \pm 2.4\%$ vs. $3.5 \pm 2.6\%$ for Lokiarchaeia, and 3.0
694 $\pm 1.7\%$ vs. $2.0 \pm 1.8\%$ for DSEG.

695
696 Archaeoglobi were more abundant in reference sediments, averaging $1.7 \pm 1.9\%$ and $0.4 \pm$
697 0.6% , respectively, particularly in mid-depth horizons, where they may indicate a sulphur-
698 cycling anaerobic archaeal community (Fig. 10a). Other low-abundance classes, including
699 Micrarchaeia, Iainarchaeia, Altiarchaeia and Heimdallarchaeia, formed part of the archaeal rare

700 biosphere and exhibited localised enrichment in selected horizons. ANME-1 was detected only
701 at trace relative abundance.

702

703 Nitrososphaeria were detected in both pockmark and reference cores, with taxonomic profiling
704 revealing that this class is dominated almost exclusively by Nitrosopumilaceae (Fig. 10e,
705 supplementary material Fig. S5) and, at the genus level, primarily by *Candidatus*
706 Nitrosopumilus (Supplementary material Fig. S5a, b). Nitrosopumilaceae showed higher
707 relative abundance in reference cores ($14.0 \pm 8.8\%$) than in pockmark cores ($8.8 \pm 3.7\%$), with
708 maxima in deeper reference sediment strata. *Ca.* Nitrosopumilus was more abundant in
709 reference cores, particularly in S/MET3/10, S/MET1-MP/10, and S/MET1-BH/10
710 (Supplementary material Fig. S5a, b). These distributional trends indicate that
711 Nitrosopumilaceae-affiliated AOA are a more significant component of the biogeochemical
712 nitrogen-cycling community in non-pockmark and deeper sediment horizons, whereas selected
713 pockmark layers are comparatively enriched in archaeal taxa implicated in CH₄ cycling. This
714 indicates that the distinction between pockmark and reference sediments is largely reflected in
715 shifts in the relative abundance of CH₄-cycling, heterotrophic/fermentative, and ammonia-
716 oxidising archaeal groups.

717

718 Hierarchical analysis at the class level showed four distinct clusters (Fig. 10b), with partial
719 clustering of pockmark and reference samples and overlap between the pockmark and reference
720 cores. Cluster 1 comprises the pockmark samples; Cluster 2 combines reference and pockmark
721 samples; Cluster 3 groups mainly reference samples; and Cluster 4 comprises two S/MET1-BH
722 reference samples and the P/MET4/10 sample (outliers). This pattern is consistent with the
723 MDS ordination. The PERMANOVA test (Fig. 10c) indicates that the difference between
724 pockmark and reference archaeal communities is statistically significant ($p = 0.003$).
725 Multivariate analysis using MDS clearly shows clustering of samples into two categories –
726 pockmarks vs references (Fig. 10c). However, some differences are noticeable: pockmark
727 samples are more tightly clustered, whereas reference samples are slightly more dispersed.
728 Based on the above analyses, it can be assumed that the pockmark cores differ significantly in
729 their archaeal composition from the adjacent reference cores. Additionally, PCA analysis
730 indicates the groups that may have the greatest impact on sample differentiation (Fig. 10d). For
731 pockmarks, the greatest impact on sample variability is as follows: Hadarchaeia >
732 Methanobacteria > Methanocellia > Halobacteria. In the reference samples, the order is
733 Methanocellia > ANME-1 > Methanobacteria > Hadarchaeia. In both pockmark and reference

734 cores, the same groups contribute most to sample variability, although the proportions of these
735 contributions differ; therefore, the compositions must differ.

736

737 Because several archaeal groups discussed in relation to methane cycling and ammonia
738 oxidation are not resolved at the class level, the family-level heatmap is presented to clarify the
739 distribution of key taxa (Fig. 10e). Additional figures in the supplementary material show
740 extended family-level relative abundance profiles (Supplementary material Fig. S4), the genus-
741 level heatmap with extended genus-level relative abundance profiles (Supplementary material
742 Fig. S5a, b), and profiles revealing the relative abundances of methanogenic (Supplementary
743 material Fig. S6) and methanotrophic (Supplementary material Fig. S7) archaeal groups.

744

745 At the family level, a few groups dominate both pockmark and reference cores, albeit in
746 different proportions: SCGC_AAA011-D5, uncultured lineages, GW2011, and
747 Nitrosopumilaceae (Fig. 10e). However, there are some site- and horizon-specific distinctions.
748 Pockmark cores are characterised by a strong, localised enrichment of methane-cycling archaea,
749 particularly ANME-2a/2b (mean relative abundance of $5.5 \pm 9.3\%$ vs $1.8 \pm 3.1\%$),
750 Methanosarcinaceae ($5.0 \pm 6.0\%$ vs $3.4 \pm 1.9\%$), Methanomicrobiaceae ($2.2 \pm 1.8\%$ vs $0.3 \pm$
751 0.4%), and Methanosaetaceae ($1.8 \pm 1.5\%$ vs $0.8 \pm 1.6\%$). These taxa show vertical
752 heterogeneity, with relative abundances peaking at the middle and bottom of the profiles, i.e.,
753 ANME-2a/2b in the deeper parts of the pockmark cores (P/MET1-MP/7, P/MET4/10; Fig. 10e;
754 Supplementary material Fig. S4), as well as Methanosarcinaceae (P/MET4/5; Fig. 10e;
755 Supplementary material Fig. S4) and Methanomicrobiaceae (P/MET4/5; Fig. 10e;
756 Supplementary material Fig. S4). Reference cores, by contrast, show a stronger contribution
757 from Nitrosopumilaceae ($14.0 \pm 8.8\%$ vs $8.8 \pm 3.7\%$), Archaeoglobaceae ($2.0 \pm 2.3\%$ vs $0.5 \pm$
758 0.7%), Hadarchaeales ($1.3 \pm 2.0\%$ vs $0.5 \pm 0.7\%$), and uncultured lineages ($25.3 \pm 21.1\%$ vs.
759 $10.1 \pm 7.4\%$) (Fig. 10e; supplementary material Fig. S4), which are characterised by variable
760 depth-related trends.

761

762 At the genus level, both pockmark and reference cores are dominated by AR15, *Ca.*
763 *Nitrosopumilus*, *Methanosarcina*, and AR20 (Supplementary material Fig. S5). However, the
764 archaeal community in pockmark cores shows a higher relative contribution of methanotrophic
765 taxa than in reference cores, i.e., ANME-3 (minor component; $0.7 \pm 1.0\%$ vs $0.1 \pm 0.4\%$),
766 particularly in cores P/MET1-BH and P/MET4, and ANME-2b ($7.5 \pm 12.3\%$ vs $1.9 \pm 4.1\%$),
767 particularly in the upper and lower parts of the profiles P/MET1-MP and P/MET4; as well as

768 enrichment of methanogens, particularly at mid-depth in P/MET4: Methanosarcina (29.3%),
769 Methanosaeta (8.2%), and Methanogenium (5.9%). In contrast, reference cores show a stronger
770 representation of *Ca. Nitrosopumilus* ($34.1 \pm 17.7\%$ vs. $22.3 \pm 7.4\%$), particularly at mid-depth
771 in S/MET3, as well as AR20 ($7.5 \pm 6.1\%$ vs. $2.4 \pm 1.8\%$) and Methanoregula ($3.3 \pm 6.1\%$ vs.
772 $2.8 \pm 3.5\%$), particularly at the bottom of the S/MET1-BH profile.

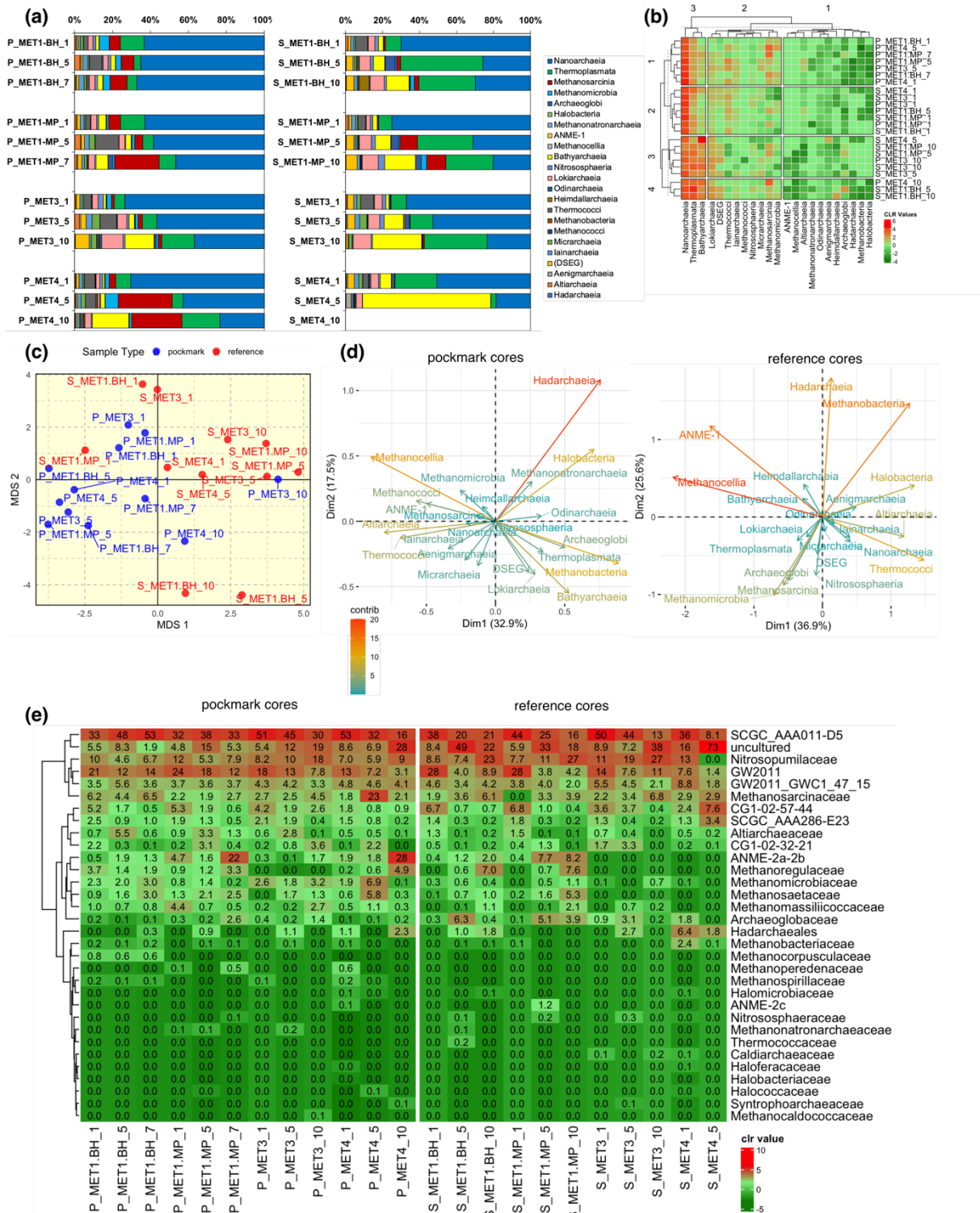
773

774 The methanogenic and methanotrophic groups are not restricted to pockmarks; in the reference
775 cores, their relative abundances are lower, yet they remain common. The summed relative
776 abundance of methanotrophic archaeal groups is approximately fourfold higher in pockmark
777 cores than in adjacent reference cores, averaging $8.3 \pm 12.2\%$ and $2.0 \pm 4.1\%$, respectively.
778 AOM-associated archaea, primarily ANME-2b and, to a lesser extent, ANME-3, are, however,
779 enriched in selected pockmark horizons. Methanogenic archaea are abundant in both pockmark
780 and reference cores, with a moderately higher average summed relative abundance in
781 pockmarks ($18 \pm 11.4\%$) than in references ($14.0 \pm 11.4\%$). However, in pockmark cores, the
782 diversity of methanogenic taxa, and to a lesser extent methanotrophic taxa, is higher
783 (Supplementary material Figs. S6 and S7).

784

785 Archaeal read abundances were generally higher in pockmark cores than in reference cores,
786 particularly at MET4, where abundances peak in the shallow horizon (Supplementary material
787 Fig. S8). In pockmark cores, archaeal abundances typically peak in the upper to middle parts of
788 the sampled profiles, except in P/MET1-BH, where the trend is reversed (Supplementary
789 material Fig. S8). Similarly, in the reference cores, archaeal abundances peak in the upper to
790 middle horizons, except in the S/MET1-BH core, where abundance increases with depth
791 (Supplementary material Fig. S8).

792



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Fig. 10. Archaeal community composition and multivariate structure in pockmark and reference sediment cores from the south-eastern Baltic Sea. (a) Class-level relative abundance profiles (stacked bars) of archaeal communities in pockmark (P) and reference (S) sediment samples from MET1-BH, MET1-MP, MET3, and MET4. Bars show the proportional contribution of archaeal classes in each sample; sample suffixes indicate depth horizons (as explained in section 2.2). (b) Class-level heatmap of centred log-ratio (CLR)-transformed relative abundances, with hierarchical clustering (dendrograms)

800 of samples and archaeal classes, showing covariation among classes and samples, where red indicates
801 higher relative enrichment and green indicates lower relative enrichment. (c) Multidimensional scaling
802 (MDS) ordination of archaeal community composition shows partial separation between pockmark and
803 reference samples (PERMANOVA, $p = 0.003$). NMDS shows significant separation (PERMANOVA,
804 $p = 0.003$), with reference samples more dispersed and pockmark samples more tightly grouped. Taxa
805 driving within-group variability differ between habitats: Hadarchaeia and Halobacteria dominate the
806 pockmark ordination, whereas Methanobacteria, ANME and Hadarchaeia drive the reference ordination.
807 (d) Ordination biplot showing the archaeal classes that contribute most strongly to community variation
808 in pockmark and reference samples, respectively. Nanoarchaeia dominate across all sites. Pockmark
809 horizons show elevated Methanosarcinia (MET1-MP, MET4), whereas reference horizons show
810 stronger contributions from Thermoplasmata and/or Bathyarchaeia. Hierarchical clustering reveals
811 partial intermixing of pockmark and reference samples, indicating that community structure reflects
812 both habitat type and site-specific variability. (e) Family-level heatmap of archaeal relative abundance
813 (CLR-transformed community structure), showing taxonomic groups not visible at the class level,
814 including ANME-2a/2b.

815

816 **3.6 Correlation network**

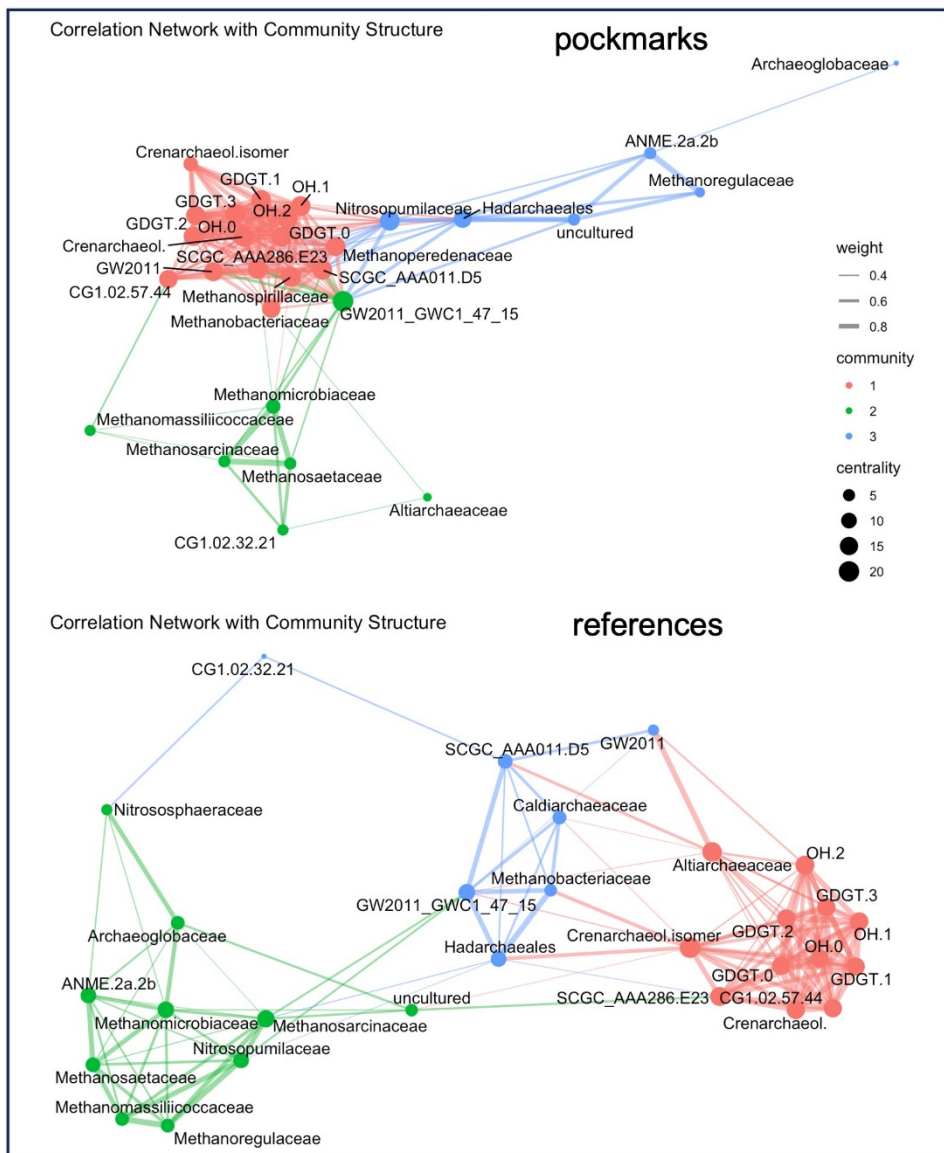
817 To link GDGT patterns to probable biological sources, we combined iGDGT and OH-GDGT
818 abundances with archaeal 16S rRNA relative abundances at the family level using correlation
819 network analysis. This approach identified co-varying lipid–taxon modules that may indicate
820 common sources, ecological niches, or interconnected processes rather than direct biosynthesis
821 alone.

822 In pockmark sediments, the correlation network resolves three main archaeal communities that
823 co-vary (Fig. 11). The first community (red) comprises the full suite of measured GDGTs and
824 is most strongly connected to several Nanoarchaeia lineages (GW2011, SCGC_AAA286_E23,
825 CG1.02.57.44, SCGC_AAA011.D5), with additional links to Methanoperedenaceae. The
826 second community is dominated by ammonia-oxidising Nitrosopumilaceae and clusters with
827 Hadarchaeales. Within this module, Nitrosopumilaceae show the strongest positive associations
828 with GDGT-0, the remaining iGDGTs, and OH-GDGTs. The same module contains ANME-
829 2a/2b, Methanoregulaceae, and uncultured lineages that are strongly interconnected with one
830 another but show comparatively weaker links to GDGTs. Archaeoglobaceae forms a peripheral
831 node and does not meet the edge-retention criteria; no GDGTs were detected in pockmarks.
832 The third community comprises methanogenic lineages, including *Methanospirillaceae*,

833 Methanobacteriaceae, Methanosarcinaceae, Methanosaetaceae, Methanomicrobiaceae,
834 Methanomassiliicoccaceae and CG1.02.32.21 (associated with the order Micrarchaeales).
835 These are less directly linked to GDGTs but strongly interlinked with one another. This cluster
836 also includes another representative of Nanoarchaeia (GW2011_GWC1_47_15).

837 In reference sediments, GDGT associations are more broadly distributed across archaeal
838 groups, and the network exhibits higher modularity, with clearer separation among modules. A
839 lipid cluster (crenarchaeol, cren', GDGT-0-3, and OH-GDGTs) correlates with both ammonia
840 oxidisers (Nitrosopumilaceae, Nitrososphaeraceae) and multiple methanogenic families
841 (Methanosaetaceae, Methanosarcinaceae, Methanoregulaceae, Methanomicrobiaceae,
842 Methanomassiliicoccaceae). Compared with pockmarks, methanogens show fewer direct links
843 to the GDGT cluster and more to ANME-2a/2b. Archaeoglobaceae remains peripheral, with
844 weak links to Nitrososphaeraceae and methanogens.

845 Overall, the pockmark network exhibits low modularity and relatively dense connectivity,
846 indicating a closely coupled archaeal community. At the same time, the strongest GDGT links
847 centre on Nitrosopumilaceae, implying that AOA dominate GDGT co-variation in these
848 methane-rich sediments, whereas methanogens/ANME form more distinct sub-clusters with
849 weaker GDGT coupling.



850

851 Fig. 11. Correlation networks linking archaeal lipid biomarkers and community members in pockmark
 852 and reference (non-pockmark) sediments. Nodes represent lipid variables (GDGTs, OH-GDGTs) and
 853 archaeal taxa (family level). Edge thickness reflects correlation strength; node colour denotes
 854 modules/communities (1–3); node size reflects centrality (larger nodes indicate more central or highly
 855 connected features). Lipid variables form a tightly connected subnetwork (module 1; red), while
 856 additional modules comprise methane-cycling and other archaeal lineages. Module connectivity differs
 857 between pockmark and non-pockmark sites, indicating habitat-dependent coupling between biomarkers
 858 and archaeal taxa. Pockmark networks show tighter coupling between lipid biomarkers and methane-
 859 cycling lineages, whereas reference sediments display clearer module separation, with lipids and the
 860 archaeal community varying more independently.

861

862

863

864 **4 Discussion**

865 **4.1 Archaeal community structure in methane-influenced pockmark sediment cores**

866

867 The archaeal community structure and porewater profiles of CH₄ and SO₄²⁻ indicate that the
868 investigated pockmarks are CH₄-influenced yet spatially heterogeneous sedimentary systems.
869 At the microbial community level, pockmark cores are not characterised by a complete
870 replacement of the reference archaeal groups, but rather by localised enrichment of CH₄-cycling
871 lineages. This is most clearly evident at lower taxonomic levels, where ANME-2a-2b/ANME-
872 2b, Methanosarcinaceae, Methanosaetaceae, Methanomicrobiaceae, and other CH₄-associated
873 taxa are enriched in selected pockmark horizons (Fig. 10e; supplementary material Figs. S4 and
874 S5). Pockmark cores exhibit substantially higher methanogen diversity (11 genera detected)
875 and a higher abundance of CH₄-cycling archaea (approximately threefold) than reference cores
876 (6 genera) (Supplementary materials, Fig. S5a, b). Correspondingly, total iGDGT and OH-
877 GDGT concentrations are approximately three times higher in pockmark cores than in reference
878 cores (Fig. 6, 8). In the correlation network (Fig. 11), pockmarks represent a tightly coupled
879 metabolic system in which various archaeal groups act in concert — likely CH₄-impacted —
880 whereas at the reference sites these groups form less integrated sub-communities, with stronger
881 representation of ammonia-oxidising *Ca. Nitrosopumilus* (Supplementary material Fig. S5a, b),
882 heterotrophic Thermoplasmata associated with acidic, sulphur-rich environments (González-
883 Toril et al., 2006), and organic matter-degrading Bathyarchaeia (Zhou et al., 2018). This
884 suggests that in reference cores, microorganisms have more independent ecological roles,
885 which could be explained by higher sulphate concentration and lower methane concentration
886 (Fig. 2). On the other hand, the summed relative abundances of methanotrophic archaea are
887 approximately fourfold higher in pockmark cores than in reference cores, whereas methanogens
888 are moderately more abundant in pockmark cores than in reference cores. This suggests that
889 AOM-associated lineages are more closely linked to pockmark niches, whereas methanogenic
890 taxa occupy broader anoxic sedimentary niches. However, the Baltic Sea exhibits considerable
891 microbial diversity due to eutrophication and pollution, steep horizontal and vertical gradients
892 in salinity and nutrient levels, and the presence of freshwater and marine phyla, as well as
893 autochthonous brackish populations (Ininbergs et al., 2015).

894

895 **4.2 Methanogenic archaea and depth-related community shifts**

896 *Methanosarcina* predominates across all sediment horizons in both pockmark and reference
897 cores (Supplementary material Fig. S6), likely owing to its metabolic versatility (Sowers et al.,
898 1993; Galagan et al., 2002; Maeder et al., 2006), which confers competitive advantages under
899 fluctuating environmental conditions (e.g., hydrostatic pressure governing freshened porewater
900 seepage, seasonal thermocline dynamics, nutrient availability, and North Sea saline water
901 inflow). The vertical distribution of methanogenic archaea varies with sediment depth
902 (Supplementary material, Fig. S6), whereas *Methanosarcina* and *Methanosaeta* predominantly
903 populate the examined sediment horizons, except in the reference cores S/MET3 and S/MET4.
904 Both taxa drive acetoclastic methanogenesis (Conklin et al., 2006; Welte et al., 2014) and are
905 favoured in sediments rich in labile organic matter (Li et al., 2022). Both *Methanosarcina* and
906 *Methanosaeta* are major contributors to the GDGT-0 pool in estuarine sediments (De Rosa et
907 al., 1977; Schouten et al., 2013; Bauersachs et al., 2015). GDGT-0 is the second most abundant
908 GDGT in the analysed samples (Fig. 7). The remaining major component of the methanogenic
909 community that appears to alternate – sometimes with *Methanosaeta*, sometimes with SDB –
910 is *Methanoregula* and *Methanogenium*, exhibiting a downcore shift.

911 Interestingly, *Methanosaeta* completely disappears in reference cores S/MET3 and S/MET4,
912 being replaced by *Methanosarcinia*. In pockmark cores, *Methanosaeta* abundance declines
913 remarkably in P/MET3 (especially in the uppermost sediment horizon) and in P/MET4
914 (especially in the lowermost horizon) and is replaced by diverse hydrogenotrophic genera,
915 including *Methanospirillum*, *Methanogenium*, *Methanomicrobium*, *Methanoculleus*,
916 *Methanocorpusculum*, *Methanobrevibacter*, and *Methanolinea*. Pockmark core P/MET4,
917 which is characterised by the highest total GDGT concentrations and the most pronounced
918 individual GDGT peaks (Fig. 6; supplementary material Fig. S2), alongside *Methanosarcina*,
919 harbours exceptionally abundant hydrogenotrophic methanogens (particularly *Methanoregula*);
920 pockmark core P/MET3 shows the next highest abundance. Microbial activity hotspots form at
921 interfaces and in mixing zones (Stegen et al., 2016). This methanogenic community shift
922 coincides with elevated CH₄ concentrations in the pockmarks P/MET3 and P/MET4 (Fig. 2).
923 The hydrogenotrophs listed above belong to the class Methanomicrobia and are known to
924 produce acyclic GDGT (Bale et al., 2019; Zeng et al., 2022). A genus such as
925 *Methanobrevibacter* has been identified as a producer of GDGT-0 (Bauersachs et al., 2015;
926 Elling et al., 2017). Similar hydrogenotrophic communities have been documented in an
927 inactive pockmark within the Hanco Basin, northern Baltic Sea (Purkamo et al., 2022). In this
928 dataset, pockmark P/MET3 was inactive during the study period and did not demonstrate

929 porewater freshening (Supplementary material Table S1), and pockmark P/MET4 is
930 characterised by weak activity.

931 **4.3 Porewater geochemistry, SMTZ development**

932 Porewater profiles of CH₄ and SO₄²⁻ show stronger CH₄ accumulation and sharper SO₄²⁻
933 depletion, particularly at MET1-MP, MET1-BH, and MET4, indicating active or recent
934 methane supply and shallow-to-subsurface transition zones (from 5 to ~25 cm bsf) where CH₄
935 and SO₄²⁻ gradients overlap. In MET4, the strongest porewater methane enrichment coincides
936 with high relative abundance of Methanosarcinaceae/Methanosarcina at 40-45 cm bsf and with
937 high relative abundance of ANME-2a/2b/ANME-2b at 90-95 cm bsf (Fig. 10e; supplementary
938 material Figs. S4, S5a, b). MET1-MP also shows pronounced enrichment of ANME-
939 2a/2b/ANME-2b at 60-65 cm bsf (Fig. 10e; supplementary material Figs. S4, S5a, b), consistent
940 with the presence of methane below the shallow SMTZ. However, the microbial maxima do
941 not always coincide with the visually estimated SMTZ intervals, as expected, because the
942 porewater profiles represent a ‘snapshot’ of geochemical gradients at that time, whereas DNA-
943 based community structure may integrate temporal variability.

944 The interpretation is consistent with previous observations of Baltic pockmark sediments
945 reported by Iasakov et al. (2022), in which ANME lineages showed vertical niche separation:
946 ANME-2a/2b were predominant in upper horizons within the SMTZ, which in the Gdańsk Deep
947 region typically occurs between 20 and 40 cm bsf, whereas ANME-1a/1b were found in deeper
948 SO₄²⁻-depleted sediments below the SMTZ, up to 280 cm bsf. In the samples studied, ANME-
949 1 occurs only at trace levels, suggesting that the sampled intervals primarily capture shallow-
950 to intermediate-depth CH₄-affected horizons. Therefore, the combined biomarker, porewater,
951 and microbiological data indicate a mosaic of CH₄-cycling niches, in which S-AOM,
952 methanogenesis, and porewater freshening govern complex redox zonation and mixed archaeal
953 lipid sources.

954 **4.4 Controls on archaeal lipid accumulation: methane cycling, LOI, and pockmark** 955 **activity**

956 The iGDGT distribution profiles offer an additional lipid-based perspective on the spatial
957 heterogeneity of the investigated pockmark systems. Pockmark cores, particularly P/MET3 and
958 P/MET4, show elevated concentrations of archaeal lipids \sum iGDGT and \sum OH-GDGT (Figs. 6,

959 8) relative to adjacent reference cores, indicating enhanced archaeal lipid accumulation in
960 selected pockmark cores. In the pockmark core P/MET4, high iGDGT concentrations coincide
961 with strong porewater CH₄ enrichment, SO₄²⁻ depletion (Fig. 2), lower Cl⁻ concentrations
962 relative to the reference core (Fig. 3), and local enrichment of methane-cycling archaea (Fig.
963 10e), including ANME-2b, ANME-3, and methanogen-affiliated genera (Supplementary
964 material Figs. S5a, b, S6, S7). Taken together, these suggest that CH₄ cycling and possibly weak
965 porewater freshening contributed to the archaeal lipid signal. The elevated GDGT-3
966 concentration in pockmark P/MET4 is also consistent with a locally increased contribution from
967 ANME-3 (at P/MET4/5; supplementary material Fig. S5a), although GDGT-3 is not source-
968 specific.

969 To assess whether elevated Σ iGDGT concentrations might simply reflect higher bulk organic
970 matter content, GDGT profiles were compared with LOI-derived trends in organic matter (full-
971 core elemental TOC data were not available for all sites) (Fig. 4). LOI was used as a screening-
972 level proxy, supported by comparisons of measured TOC and LOI profiles in representative
973 cores, which show comparable vertical trends (Fig. 4). This comparison indicates that Σ iGDGT
974 and Σ OH-GDGT concentrations are not tightly coupled to bulk organic matter (Figs. 4-6, 8).
975 In several cores, GDGT profiles do not align with LOI trends: for example, pockmark P/MET1-
976 BH shows relatively high LOI and clear CH₄ enrichment with SO₄²⁻ depletion, yet does not
977 exhibit the strongest GDGT accumulation, whereas pockmark P/MET3 contains elevated
978 Σ iGDGT and Σ OH-GDGT concentrations despite weaker porewater CH₄ enrichment and a
979 smaller contribution from methane-cycling archaea than P/MET4. Similarly, the strong GDGT
980 enrichment cannot be explained by LOI alone, because the lipid maxima align more closely
981 with steep CH₄/SO₄²⁻ gradients and with pockmark archaeal community abundance than with a
982 simple increase in bulk organic matter. Additionally, the generally low BIT values indicate that
983 terrestrial-derived brGDGT input is minor across the investigated sites. This suggests that the
984 iGDGT pool is dominated by marine archaeal lipids. However, the deepest intervals of the
985 S/MET4 reference core show slightly elevated BIT values, indicating a localised increase in the
986 relative contribution of brGDGTs. BIT can also be affected by the decline in crenarchaeol.
987 However, brGDGTs may also be produced in marine sediments (although their molecular
988 composition differs) (Peterse et al., 2009; Sinninghe Damsté, 2016). Thus, elevated Σ GDGT
989 concentrations cannot be attributed solely to increased TOC or bulk organic matter
990 accumulation. Instead, they appear to reflect multiple, partially decoupled controls, including
991 archaeal community composition and abundance, and the probable contributions of water-

992 column and sedimentary GDGT sources. Lipid preservation could also be a side effect of the
993 less ebullitive nature of methane seepage in pockmarks P/MET3 and P/MET4: the kinetics and
994 episodicity of CH₄ supply and freshened porewater discharge, which influence redox zonation.
995 As reported by Treude et al. (2005) in Eckernförde Bay, the spatial and temporal heterogeneity
996 caused by gas ebullition allows methanogens and sulphate reducers to coexist, feeding the
997 shallow SMTZ, and also enables methane to escape into the overlying water column, bypassing
998 the microbial barrier. This is particularly important for the more hydrologically complex
999 pockmarks MET1-BH and MET1-MP with porewater freshening, where present-day dissolved
1000 CH₄ and SO₄²⁻ profiles may not fully capture the timing or intensity of CH₄ supply.

1001 The partial mismatch among porewater chemistry, archaeal community composition, and
1002 GDGT concentrations therefore suggests that CH₄ cycling in these sediments cannot be
1003 described by a simple sulphate-controlled SMTZ model alone. P/MET1-BH, for instance,
1004 shows strong CH₄ enrichment and SO₄²⁻ depletion, indicating an active or recently active
1005 methane-influenced redox zone (Fig. 2). However, its GDGT concentrations are much lower
1006 than those in pockmarks P/MET3 and P/MET4. Conversely, P/MET3 shows substantial GDGT
1007 accumulation despite weaker porewater methane enrichment and a less pronounced archaeal
1008 presence involved in CH₄ cycling. This decoupling suggests that the bulk GDGT pool integrates
1009 *in situ* archaeal production, pelagic input of Nitrossopaheria-derived crenarchaeol,
1010 contributions from sedimentary lineages such as Thermoplasmata and other uncultivated
1011 archaea, and preservation effects.

1012 **4.5 Porewater freshening and potential alternative methane oxidation pathways**

1013
1014 In addition, porewater freshening and CH₄ ebullition to the water column may modify the redox
1015 zone of the active pockmark sediments at stations P/MET1-BH and P/MET1-MP
1016 (Supplementary material Table S1). Chloride profiles, particularly in the MET1 area and MET4,
1017 show lower chloride concentrations in pockmark cores relative to adjacent reference cores (Fig.
1018 3), indicating a potential influence of freshened porewater in diluting SO₄²⁻ and shifting the
1019 depth, intensity, and archaeal community structure of the methane oxidation zone. Although S-
1020 AOM is expected near the CH₄-SO₄²⁻ overlap, pockmarks P/MET1-BH and P/MET1-MP,
1021 affected by freshened groundwater seepage, may also host deeper CH₄ oxidation zones, where
1022 electron acceptors other than SO₄²⁻ could become more relevant.

1023 Kurowski et al. (2024) concluded that Fe-AOM may occur in MET1-BH sediments.
1024 Additionally, it was suggested that freshened groundwater seepage dilutes the already low
1025 chloride and sulphate concentrations in the pockmarks MET1-BH and MET1-MP (Brodecka-
1026 Goluch et al., 2022; Idczak et al., 2020; Łukawska-Matuszewska and Dwornik, 2025). Water
1027 salinity inside the MET1-BH pockmark crater was measured at ~2 PSU, with Cl⁻ concentrations
1028 93% lower than in typical marine porewater in the region. Fluctuating low-oxygen to anoxic
1029 conditions in the bottom waters of the MET1 area, promoting steep redox gradients (sharply
1030 decreasing SO₄²⁻ concentrations) that favour methanogenesis; these conditions, further
1031 enhanced by anthropogenic contamination, create conditions for intensive organic matter
1032 mineralisation, were also reported (Idczak et al., 2020; Brodecka-Goluch et al., 2022).
1033 Furthermore, Purkamo et al. (2022) showed that SGD-driven advection in northern Baltic Sea
1034 pockmarks can compress redox and reaction zones to the uppermost centimetres, reduce organic
1035 matter accumulation, and suppress SMTZ development. In such active pockmarks, archaeal
1036 communities are dominated by AOA (Purkamo et al., 2022). Conversely, inactive pockmarks
1037 dominated by diffusion accumulate organic-rich, fine-grained sediments that are characterised
1038 by SO₄²⁻ reduction and methanogenesis, with methanogens prominent within the archaeal
1039 community.

1040 **4.6 Evaluation of GDGT-based methane proxies in Gdańsk Basin pockmarks**

1041 **4.6.1 Limitations of GDGT-based AOM proxies in ANME-2/ANME-3-dominated** 1042 **pockmarks**

1043 Peaks in archaeal abundance (Supplementary material Fig. S8) and in GDGT concentrations at
1044 sediment depths of 5–45 cm could mark the SMTZ (Fig. 2). However, iGDGTs indices (Table
1045 1) do not support a strong AOM imprint, as values remain uniformly low. MI values (<0.09)
1046 fall well below the 0.3-0.5 threshold indicative of CH₄-impacted sediments observed by Zhang
1047 et al. (2011). However, for the MI value to reach 0.5, methanotrophic-derived GDGT must
1048 constitute approximately 30-50% of the total GDGT pool (Kim and Zhang, 2023).

1049 A low MI index, calculated as the ratio of GDGT-1-3 to crenarchaeol, typically indicates
1050 minimal methanotrophic contribution relative to Nitrososphaeria-derived sources (Zhang et al.,
1051 2011). The uniformly low MI values observed here likely reflect a strong crenarchaeol signal
1052 from ammonia-oxidising archaea thriving above, in the water column and/or limited GDGT-1-
1053 3 production by the dominant AOM lineages, rather than definitively excluding AOM. GDGT-
1054 1 to -3, which increase substantially in ANME-1-dominated systems (Rossel et al., 2008), are

1055 two orders of magnitude less abundant in the analysed samples than GDGT-0 and crenarchaeol,
1056 consistent with the near-absence of ANME-1 (maximum relative abundance of 0.6% in
1057 S/MET3/5; Fig. 10a).

1058 Although the applicability of MI to ANME-2 and ANME-3 (which dominate the AOM
1059 community here; supplementary material Fig. S7) has been questioned, comprehensive
1060 biomarker investigations generally support its utility for AOM detection (Kim and Zhang,
1061 2023). Nevertheless, ANME-2 and ANME-3 alone are unlikely to contribute substantially to
1062 GDGT production (Niemann and Elvert, 2008; Weijers et al., 2011), making MI non-diagnostic
1063 in this study. The consistently low GDGT-2/cren ratios (maximum 0.04; Table 1) support this
1064 interpretation. This ratio typically indicates CH₄-rich AOM conditions, under which ANME-1-
1065 synthesised GDGT-2 (Rossel et al., 2008) is elevated relative to crenarchaeol. To further
1066 evaluate the GDGT distribution characteristics of an AOM-related SMTZ, the relative
1067 abundances of GDGT-1, GDGT-2, and GDGT-3 were compared across cores. In contrast to the
1068 GDGT-2-dominated pattern reported by Weijers et al. (2011), GDGT-2 was not the dominant
1069 compound in any of our samples. Instead, 74 of 75 samples showed the order GDGT-1 >
1070 GDGT-2 > GDGT-3. This indicates that the investigated cores do not show enrichment of
1071 GDGT-2, consistent with uniformly low GDGT-2/crenarchaeol ratios.

1072 Nevertheless, ANME lineages are more prevalent in pockmark cores (Supplementary material
1073 Fig. S7), suggesting, relatively to reference cores, enhanced AOM activity, particularly at
1074 P/MET3 and P/MET4. Peaks in GDGT-1 to -3 concentrations also occur in reference cores,
1075 following the general trend for all iGDGTs but at lower concentrations (Supplementary material
1076 Fig. S2). In addition, certain core GDGTs (e.g., GDGT-1) may arise from diagenetic and
1077 degradative processes acting on phosphohexose headgroups predominantly produced by *Ca.*
1078 *Nitrosopumilus* in the Baltic Sea (Wittenborn et al., 2023).

1079 Overall, AOM activity appears constrained within the studied pockmarks, consistent with
1080 previous investigations demonstrating weak S-AOM confined to thin, shallow sediment layers
1081 and potentially dependent on alternative electron acceptors in deeper layers (Broclawik et al.,
1082 2020; Idczak et al., 2020; Brodecka-Goluch et al., 2022; Łukawska-Matuszewska et al., 2022;
1083 Ehlert von Ahn et al., 2024). The minor contribution of *Ca.* *Methanoperedens* in the dataset
1084 (MET1-MP; supplementary material Fig. S7) indicates traces of AOM coupled to nitrate and/or
1085 metal oxide reduction. Members of the family *Methanoperedenaceae* (formerly ANME-2D)
1086 typically inhabit sulphate-depleted freshwater systems and perform AOM independently of

1087 syntrophic partnerships (Haroon et al., 2013; Ettwig et al., 2016; Vaksmaa et al., 2017; Leu et
1088 al., 2020).

1089 **4.6.2 Methanogens and GDGT-based proxies**

1090 Within the CH₄-cycling archaeal community, methanogens appear to be the primary
1091 contributors to GDGT biosynthesis (Fig. 7). Although GDGT-0 and crenarchaeol predominate
1092 in marine sediments (Schouten et al., 2002), their elevated concentrations across all investigated
1093 gas systems indicate that they serve as primary iGDGT biomarkers in the sediments of the
1094 Gdańsk Basin, even in CH₄-rich settings harbouring both methanogenic and methanotrophic
1095 communities. However, GDGT-0 lacks source specificity and can be synthesised by multiple
1096 archaeal lineages, including methanogens and methanotrophs (Pancost et al., 2001; Blaga et al.,
1097 2009; Inglis et al., 2015; Słowakiewicz et al., 2016; Petrick et al., 2019). These lineages may
1098 also produce cyclised GDGTs (Koga et al., 1993; Weijers et al., 2006; Schouten et al., 2013;
1099 Bauersachs et al., 2015), although this is not always replicable in culture studies (Bauersachs et
1100 al., 2015).

1101 The network analysis indicates that AOA-associated crenarchaeol and the statistically co-
1102 varying GDGT-0 are the primary drivers of variation in the bulk GDGT pool (Fig. 11), likely
1103 masking the methanogen signal by influencing the GDGT-0/cren ratio through pelagic
1104 Nitrososphaerota contribution. The observed GDGT-0/cren ratios in Gdańsk Basin samples are
1105 <0.99, well below the 2 threshold characteristic of methanogen-dominated systems (Schouten
1106 et al., 2002; Blaga et al., 2009), indicating minimal methanogen contribution and AOA
1107 predominance. In the northern Baltic Sea pockmarks (Hanko Basin), Nitrososphaeria –
1108 including some groundwater-origin populations – constitute a major component of the archaeal
1109 community (Purkamo et al., 2022), consistent with evidence that *Ca. Nitrosopumilus* is
1110 widespread in the Baltic Sea and is an important GDGT-producing lineage (Wittenborn et al.,
1111 2023). However, amplicon-based relative abundance reflects the compositional distribution of
1112 recovered 16S rRNA gene reads after DNA extraction (Gloor et al., 2017) and does not directly
1113 indicate lipid production rates. By contrast, sedimentary iGDGTs integrate archaeal lipid
1114 production and export over longer timescales, and their distribution is further shaped by
1115 preservation conditions (Lengger et al., 2013).

1116 **4.7 AOA and non-methane archaeal lipid sources**

1117 Given that AOA are abundant in the Baltic Sea under low-oxygen conditions and along redox
1118 gradients (Berg et al., 2015b), the consistently low GDGT-0/cren ratios (<1) observed herein
1119 indicate a crenarchaeol-rich iGDGT pool, suggesting a substantial pelagic contribution from
1120 the settling and export of AOA-derived crenarchaeol from the water column. Methane seepage
1121 creates chemically reducing conditions that limit the growth of oxygen-dependent organisms.
1122 However, seep systems also generate sharp chemical gradients and microenvironments (e.g.,
1123 thin oxic/suboxic boundary layers) in which AOA can persist and function (Jakobs et al., 2016).
1124 Martens-Habbena and Qin (2022) showed that *Nitrosopumilus maritimus* can sustain ammonia
1125 oxidation under oxygen-depleted conditions, further indicating that AOA may be flexible and
1126 physiologically adapted to redox-variable conditions. Nevertheless, elevated summed iGDGT
1127 and OH-GDGT concentrations in pockmarks MET3 and MET4 (especially GDGT-0 and
1128 crenarchaeol) may indicate an indirect ecological linkage between the nitrogen and carbon
1129 biogeochemical cycles in the studied system, in which degradation of organic matter,
1130 methanogenesis, sulphate reduction, and other anaerobic processes release NH_4^+ and other
1131 reduced compounds into porewaters. These compounds may subsequently be transported
1132 (diffused) into the bottom waters and the overlying water column, where Nitrososphaeria thrive.
1133 Consequently, crenarchaeol remains the dominant iGDGT in both pockmark and reference
1134 cores (Supplementary material Fig. S2), as Nitrososphaeria is abundant in the overlying water
1135 column and may feed on the products of enhanced processes occurring in the sediments. Despite
1136 this AOA dominance, GDGT-0 concentrations remain relatively elevated across the pockmark
1137 sites, particularly at the inactive pockmark MET3 and the weakly active pockmark MET4,
1138 which experiences weak, occasional porewater seepage, compared with active venting systems
1139 characterised by advective methane flow, such as mud volcanoes on the Canadian Beaufort Sea
1140 slope (Lee et al., 2018) and studied here pockmarks MET1-MP and MET1-BH (Supplementary
1141 material Fig. S2).

1142 Average OH-GDGT% values align with those reported for Baltic Sea surface sediments
1143 (Sinninghe Damsté et al., 2022), and RI-OH and RI-OH values are within the Baltic/Skagerrak
1144 Surface sediment ranges (Sinninghe Damsté et al., 2022). While salinity primarily controls OH-
1145 GDGT behaviour (Sinninghe Damsté et al., 2022), recent studies show strong responses to
1146 nitrate availability and water-column stratification (Harning and Sepúlveda, 2024), indicating
1147 an ecological influence on RI-OH/RI-OH'. The most robust interpretation is that OH-GDGTs
1148 primarily track AOA, particularly Nitrososphaeria, as supported by culture studies (Sinninghe

1149 Damsté et al., 2022). In our data, OH-GDGTs closely covary with crenarchaeol, consistent with
1150 previous findings indicating thaumarchaeal source (Kaiser and Arz, 2016).

1151 Nanoarchaeota, prevalent across the samples, may be involved in ectosymbiosis with
1152 Nitrososphaeria, consistent with their reliance on symbiotic relationships (Waters et al., 2003).
1153 Nanoarchaeota may also possess GDGTs, previously attributed to their biological hosts (Zeng
1154 et al., 2022), which could explain their correlation with GDGTs (Fig. 6). They can also associate
1155 with methanogens (Brick et al., 2025), which may explain their high relative abundance (~40%
1156 in non-pockmark and ~55% in pockmark sediments). Other frequent groups (e.g., AR15, AR20)
1157 are likewise symbiotic or parasitic; the latter, linked to groundwater (Castelle et al., 2015),
1158 underscores the influence of porewater freshening in the Gulf of Gdańsk in pockmarks MET1-
1159 MP and MET1-BH.

1160 Several archaeal groups, including Thermoplasmata, Bathyarchaeia, Lokiarchaeia,
1161 Heimdallarchaeia, Archaeoglobi, and the Deep Sea Euryarchaeotic Group (DSEG; within
1162 Thermoplasmata; Rinke et al., 2021), are associated with the degradation of complex organic
1163 matter, aromatic carbon degradation, protein catabolism, and fermentation (Zinke et al., 2019).
1164 These groups — particularly Bathyarchaeia and Thermoplasmata — are more abundant in
1165 reference cores than in pockmarks and may contribute to GDGT production in anoxic
1166 environments (Besseling et al., 2018, 2020; Baxter et al., 2021). Bathyarchaeia thrive in anoxic
1167 environments, degrading recalcitrant organic matter (Baxter and Zalar, 2019; Blewett et al.,
1168 2022; Zeng et al., 2022), whereas Archaeoglobi mediate both sulphate reduction and
1169 methanogenesis (Lynes et al., 2024). Bathyarchaeia may also occupy a central position in
1170 archaeal carbon-nitrogen networks, co-occurring with methanogens and Nitrososphaeria, and
1171 potentially linking organic carbon degradation with reduced nitrogen availability (Yi et al.,
1172 2024). However, despite the abundance of Bathyarchaeia and Nitrososphaeria in the archaeal
1173 community structure, this mechanism remains inferential, as NH_4^+ and AOA activity were not
1174 directly measured herein. Asgard archaea, including hydrocarbon-degrading Lokiarchaeia and
1175 hydrogen-dependent acetogenic Heimdallarchaeia (Zhang et al., 2025), also show higher
1176 abundance in reference cores and may contribute to iGDGT production, likely GDGT-0 (Zeng
1177 et al., 2022). Although the tetraether synthase (Tes) gene, essential for GDGT biosynthesis, has
1178 been identified in Hadarchaeia and Altiarchaeia, GDGTs have not yet been detected in these
1179 groups (Zeng et al., 2022). Notably, some Hadarchaeia grow syntrophically with methanogens
1180 (Yu et al., 2024).

1181 5. Conclusions

1182 This study presents the first integrated analysis of archaeal 16S rRNA communities,
1183 sedimentary iGDGT/OH-GDGT distributions, and microbial correlation networks in methane
1184 pockmarks of the Gdańsk Basin. Pockmarks host more diverse and abundant archaeal
1185 communities than adjacent reference sites and function as tightly coupled metabolic systems,
1186 whereas reference sites exhibit more niche-partitioned ecological structures. Despite CH₄-rich
1187 conditions, evidence for anaerobic oxidation of methane (AOM) remains limited. Low MI
1188 values and the near absence of ANME-1 reduce the reliability of GDGT-based AOM proxies
1189 in settings with low ANME-1 abundance. Low GDGT-0/cren and GDGT-2/cren ratios indicate
1190 dominance of pelagic AOA, particularly *Ca. Nitrosopumilus*, which may mask methanogenic
1191 GDGT signals. However, the dominance of GDGT-0 and crenarchaeol within specific
1192 pockmarks may reveal intricate linkages between microbial community structure, i.e.,
1193 Bathyarchaeia and Nitrososphaeria, and the underlying biogeochemical processes. The
1194 transport of reduced compounds from anaerobic reactions across the sediment-water interface
1195 may enhance the proliferation of Nitrososphaeria.

1196 Results suggest that porewater freshening and pockmark activity, including gas ebullition,
1197 strongly influence geochemical conditions and archaeal distributions. The mixing of freshened
1198 groundwater with marine porewater, together with gas seepage, may create localised microbial
1199 “hotspots” with higher archaeal diversity. Differences between active (P/MET1) and inactive
1200 (P/MET3) or weakly active (P/MET4) pockmarks are reflected in archaeal community structure
1201 and GDGT concentrations. Methane cycling appears highly localised rather than continuous
1202 downcore. The clearest combined microbiological and biomarker evidence for methane cycling
1203 is observed at P/MET4, where elevated CH₄, SO₄²⁻ depletion, increased iGDGT concentrations,
1204 enrichment of ANME-2b, ANME-3, hydrogenotrophic methanogens, *Methanosarcina*, and
1205 *Methanosaeta* co-occur. However, the iGDGT distribution in the weakly active pockmark
1206 P/MET4 probably indicates a pelagic AOA source. In contrast, active MET1 pockmarks show
1207 iGDGT distributions similar to those of adjacent reference cores. The inactive pockmark
1208 P/MET3 shows increased archaeal lipid accumulation, but, as in pockmark P/MET4, this
1209 accumulation is primarily associated with pelagic AOA rather than with CH₄-cycling
1210 communities.

1211 Future studies should integrate GDGT analyses with methane-specific biomarkers (e.g.,
1212 crocetane, PMI, archaeol, hydroxyarchaeol), NH₄⁺ concentrations, compound-specific carbon

1213 isotopes, and functional gene or transcriptomic approaches (e.g., *mcrA*) to better constrain
1214 active CH₄ cycling and identify the responsible ANME clades, as well as spatial activity of
1215 Nitrososphaeria. Measuring both core and intact polar lipids in sediments and the water column
1216 would further clarify GDGT production, transport, and preservation. Overall, this study
1217 highlights the need for integrated multi-proxy and multi-omic approaches to distinguish active
1218 microbial processes from preserved diagenetic signals in methane seep systems influenced by
1219 submarine groundwater discharge, porewater freshening, and dynamic redox conditions.

1220

1221 *Data availability.* All iGDGT data is available in the repository 10.5281/zenodo.18414700.

1222 *Author contributions.* IDMS and MS designed the research; IDMS evaluated the geochemical
1223 and microbiological data; AB, ABG, and KLM collected the samples; AB prepared the
1224 microbiological dataset; IDMS and AB conducted the statistical analyses; IDMS wrote the
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1234

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