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3 **Marine mercury-methylating microbial communities from coastal to Capbreton Canyon**  
4 **sediments (North Atlantic Ocean)**

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25 **Abstract**

26 Microbial mercury (Hg) methylation transforms inorganic mercury to neurotoxic  
27 methylmercury (MeHg) mainly in aquatic anoxic environments. Sampling challenges in  
28 marine ecosystems, particularly in submarine canyons, leads to a lack of knowledge about  
29 the Hg methylating microbes in marine sediments. A previous study showed an enrichment of  
30 mercury species in sediments from the Capbreton Canyon where both geochemical  
31 parameters and microbial activities constrained the net MeHg production. In order to  
32 characterize Hg-methylating microbial communities from coastal to deeper sediments, we  
33 analysed the diversity of microorganisms' (16S rDNA-based sequencing) and Hg methylators  
34 (*hgcA* based cloning and sequencing). Both, 16S rDNA and *hgcA* gene analysis  
35 demonstrated that the putative Hg-methylating prokaryotes were likely within the  
36 *Deltaproteobacteria*, dominated by sulfur-compounds based reducing bacteria (mainly sulfate  
37 reducers). Additionally, others clades were also identified as carrying HgcA gene, such as,  
38 *Chloroflexi*, *Spirochaetes*, *Elusimicrobia*, PVC superphylum (*Plantomycetes*,  
39 *Verrucomicrobia* and *Chlamydiae*) and *Euryarchaea*. Nevertheless, 61% of the *hgcA*  
40 sequences were not assigned to specific clade, indicating that further studies are needed to  
41 understand the implication of new microorganisms carrying *hgcA* in the Hg methylation in  
42 marine environments. These first results suggest that sulfur cycle drives the Hg-methylation  
43 in marine ecosystem.

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48 **Keywords:** Mercury-methylation, Hg-methylating prokaryotes, 16S rDNA diversity, HgcA  
49 diversity, marine sediments

## 50 **Introduction**

51 Methylmercury (MeHg) is a neurotoxin that is biomagnified in aquatic food webs, with fish  
52 consumption as a primary route for human MeHg exposure (Mason et al., 2012; Sunderland,  
53 2007). In aquatic ecosystems, methylation of mercury (Hg) to MeHg is carried out by some  
54 anaerobic microorganisms. Studies have identified the sulfate-reducing bacteria (SRB) as  
55 the main contributors to Hg methylation (Compeau and Bartha, 1985). MeHg production has  
56 also been associated with the activity of sulfur-reducing bacteria, iron-reducing bacteria  
57 (FeRB) and methanogenic archaea (Fleming et al., 2006; Hamelin et al., 2011; Ranchou-  
58 Peyruse et al., 2009). The recent identification of some genes required for Hg methylation,  
59 *hgcA* and *hgcB* (Parks et al., 2013), expanded our knowledge on the diversity of potential Hg  
60 methylators (Gilmour et al., 2013; Podar et al., 2015). *hgcAB* genes are present in the  
61 genome of diverse sulfate, sulfur and iron reducing *Deltaproteobacteria*, in methanogens, but  
62 also in *Clostridia* (firmicutes), fermentative and acetogenic microorganisms (Gilmour et al.,  
63 2018, 2013; Jones et al., 2019; Parks et al., 2013; Podar et al., 2015). Metagenomic data  
64 suggested the presence of *hgcAB* in members of others microbial phyla including the  
65 *Chloroflexi*, *Chrysiogenetes*, *Nitrospina*, the PVC superphylum (*Planctomycetes*,  
66 *Verrucomicrobia*, *Chlamydia*) and *Spirochaetes* (Gionfriddo et al., 2016; Jones et al., 2019;  
67 McDaniel et al., 2020; Podar et al., 2015). Microbial Hg-methylating communities that have  
68 recently been described in wetlands, paddy soils, lakes, dam reservoir and sewage treatment  
69 plant effluent included methanogens, SRB, FeRB, syntrophs and also unaffiliated  
70 microorganisms (Bravo et al., 2018; Du et al., 2017; Liu et al., 2014; Podar et al., 2015;  
71 Schaefer et al., 2014; Xu et al., 2019). Hg-methylating microbial communities in marine  
72 environment remain largely un-investigated with regards to their importance for methylation  
73 and the specific types of microorganisms involved.

74 The biogeochemical cycling of Hg remains understudied in the open ocean,  
75 particularly on continental margins and associated slopes (Mason et al., 2012). Submarine  
76 canyons are large incisions into the continental margin which contribute to the enrichment of

77 the sediments with organic matter (OM) and various trace metals (Azaroff et al., 2019;  
78 Oliveira et al., 2011; Palanques et al., 2008). Indeed, a previous study on the Capbreton  
79 Canyon (North Atlantic Ocean) has shown that the MeHg formation is driven by the OM and  
80 the sulfur content (Azaroff et al., 2019). Comparatively to offshore canyon sediment, coastal  
81 sediment had the highest biological Hg methylation rates whereas ambient mercury  
82 compounds concentrations were lower (Azaroff et al., 2019). Currently, no data exist about  
83 the microbial populations carrying *hgcA* in submarine canyons or even in marine sediments.  
84 Knowledge of the relationship between community composition and MeHg production may  
85 help to elucidate the origins of the large variability in MeHg/Hg ratios in oceanic ecosystem.

86 Building on these earlier discoveries and recognizing the implication of microbial  
87 communities on Hg methylation in Capbreton submarine canyon sediments, this study aimed  
88 to provide a mechanistic understanding of MeHg formation in sediments along the canyon  
89 transect. For this purpose, we linked sediment geochemistry and Hg speciation with parallel  
90 analysis of the global prokaryotic diversity (16S rDNA) and microorganisms carrying *hgcA*.  
91 This is the first study in marine sediments, from coastal to canyon samples exploring the  
92 diversity of microorganisms involved in Hg methylation.

## 93 **Materials and methods**

### 94 **Study area and sampling strategy**

95 The present study was undertaken in one of the longest canyons in the world, located in the  
96 North Atlantic Ocean: the Capbreton submarine canyon. It deeply incises the Aquitaine  
97 continental slope and shelf, and runs along the Spanish coast for 300 km and sinks into the  
98 abyssal plain at 3500 meters depth. Its head is at only 200 meters from the coast, where the  
99 population and human activities, are constantly increasing. Like many coastal ecosystems, it  
100 is a highly productive and ecologically rich zone due to large and continuous continental  
101 inputs of nutrients and organic matter coming from a rich network of effluents and the Adour  
102 estuary. Surface sediments were collected with a grab sampler and a core sampler along the

103 canyon transect starting at 1.2 km from the coast to 23.5 km. Samples were located in slopes  
104 and terraces into the canyon (n=13) and in the adjacent continental shelf (n=9; Fig. 1). G03 is  
105 considered a canyon location because it is located in a gully connected to the mouth of this  
106 canyon (Mazières et al., 2014). For each sediment sample, Hg compounds analysis (Hg(II)  
107 and MeHg) geochemical parameters as well as 16S rDNA diversity were analysed. *hgcA*  
108 diversity has been determined along the canyon transect at locations G03, G10, G14 and  
109 G24. On board, samples for DNA were collected in triplicate with sterile cryotubes then  
110 immediately frozen at -80 °C until analysis.

### 111 **Chemical analysis**

112 Sample processing, geochemical parameters (organic carbon (POC), total sulfur (TS)) and  
113 speciation of mercury (Hg(II) and MeHg) as well as the Hg transformation potential are  
114 detailed in a previous study (Azaroff et al., 2019).

### 115 **DNA extraction and bacterial community composition: 16S rDNA gene**

116 DNA was extracted from frozen sediments with the Power Soil DNA extraction kit (Mo-Bio  
117 Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Diversity  
118 of the 16S rDNA were determined by sequencing the V4-V5 hypervariable regions of the 16S  
119 rDNA with universal primers V4-515F (5' GTGYCAGCMGCCGCGGTA 3') and V5-928R (5'-  
120 ACTYAAAKGAATTGRCGGGG 3') (Parada et al., 2016; Walters et al., 2015; Wang and  
121 Qian, 2009). PCR was performed using *ampliTaq Gold® 360* master mix (Applied  
122 Biosystems, CA, USA), 0.5 µM of each primer and 3 ng of extracted DNA. PCR cycling was  
123 as following: after 10 min of initial denaturation at 95 °C, 30 cycles of 30 s denaturation at 95  
124 °C, 30 s annealing at 60 °C and 40 s elongation at 72 °C with 7 min final elongation at 72 °C.  
125 Amplicons were sequenced by the *Get-PlaGe* sequencing service (INRA, Toulouse, France)  
126 using *MiSeq 250 pb* paired-end technology and reagent kit V3. Data were preprocessed  
127 using the *Galaxy FROGS* pipeline (Escudié et al., 2018). Chimeric and PhiX reads were  
128 removed, Operational Taxonomic Units (OTUs) clustering, after a de-noising step allows

129 building fine clusters with minimal differences, with an aggregation distance equal or above  
130 3. Data were normalized to the minimum number of reads. Taxonomic assignments were  
131 performed using the Silva database v.128 (Pruesse et al., 2007). Sequences data have been  
132 deposited in GenBank under the accession number PRJNA608532.

### 133 **Hg methylation community composition: *hgcA* gene cloning and sequencing**

134 *hgcA* gene diversity was studied in four surface sediments along the canyon gradient from  
135 the coast to the offshore (G03, G10, G14 and G24). Primers targeting *hgcAB* sequences  
136 were adopted from Christensen et al, 2016 (Christensen et al., 2016) and *hgcAB* genes were  
137 amplified with the primer set ORNL-HgcAB-uni 268F (5'-AAYGTCTGGTGYGCNGVCGG-3')  
138 and ORNL-HgcAB-uni 167R (5'-CABGCNCCRCAYTCCATRCA-3'). PCR was performed  
139 using ampliTaq Gold® 360 master mix (Applied Biosystems, CA, USA) and 1 µM of each  
140 primer, with 2 min initial denaturation at 95 °C, 5 cycles of 30 s denaturation at 96 °C, 30 s  
141 annealing at 68 °C and 30 s elongation at 72 °C, then 30 cycles of 30 s denaturation at 96  
142 °C, 30 s annealing at 63 °C and 1 min elongation at 72 °C with 7 min final elongation at 72  
143 °C. PCR products were purified using GE Healthcare illustra™ GFX™ PCR DNA and Gel  
144 Band Purification Kit (GE Healthcare, Chicago, Illinois, US) according to the manufacturer's  
145 instructions. Purified products were ligated into a pCR™ 2.1-TOPO®, with TOPO™ TA  
146 Cloning™ Kit (Invitrogen, Carlsbad, CA, US), which was used to transform One Shot™  
147 Mach1™ T1 Phage-Resistant Chemically Competent *E. coli*. Transformed *E. coli* were grown  
148 on lysogeny broth (LB) agar plates with 100 µg mL<sup>-1</sup> ampicillin, 64 µg ml<sup>-1</sup> X-gal and 160 mM  
149 IPTG. Successful transformants picked and screened by colony PCR with M13 primers  
150 (M13F; 5'GTAAAACGACGGCCAG-3' and M13R; 5'-CAGGAAACAGCTATGAC-3'). PCR  
151 was performed using ampliTaq Gold® 360 master mix (Applied Biosystems, CA, USA) and  
152 0.2 µM of each primer, with 10 min initial denaturation at 95 °C, 30 cycles of 30 s  
153 denaturation at 95 °C, 30 s annealing at 54 °C and 40 s elongation at 72 °C with 10 min final  
154 elongation at 72 °C. Clones of the correct sized inserts were sequenced by SANGER  
155 sequencing at Eurofins Genomics (Ebersberg, Germany). Forward sequences were only

156 used and converted to protein with ExPaSy tool (Swiss Institute of Bioinformatics, Lausanne,  
157 Switzerland) then, trimmed with Chromas Pro (Technelysium software). The obtained protein  
158 sequences were aligned with MUSCLE (Edgar, 2004). The alignment was trimmed to the  
159 amplicon, and a tree was generated using MEGAX (Kumar et al., 2018), with the Maximum  
160 Likelihood method based on the Poisson correction model (Zuckerandl and Pauling, 1965)  
161 (with n replication bootstraps = 500). Phylogenetic analyses were processed using HgcA  
162 proteins homemade database including HgcA sequences from isolated strains' genomes and  
163 from metagenomes obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>) and Integrated  
164 Microbial Genomes and Microbiomes platform (IMG/M, developed by the Joint Genome  
165 Institute, CA, USA, <http://img.jgi.doe.gov/>) (Markowitz et al., 2012) and previous studies  
166 (Supporting information, Table S3). HgcA sequences were also collected from Pfam  
167 database (Finn et al., 2016) using custom Pfam models (corrino-iron sulfur proteins  
168 (CFesP) classified under Pfam3599) according to Podar et al. (Podar et al., 2015). From  
169 these references, we selected only sequences with the conservative cysteine in HgcA (Smith  
170 et al., 2015) within the CWA motif. All reference sequences are compiled in Supporting  
171 information, Table S3. Sequences are archived in GenBank under submission number  
172 .2316824

### 173 **Statistical analysis**

174 Pearson correlations were calculated between chemical parameters using the R software  
175 environment version (<http://www.r-project.org/>). To remove redundancy of the chemical  
176 parameters data in order to avoid collinearity, we used the variance inflation factor (VIF) with  
177 the mctest package (Imdadullah et al., n.d.) in R using the Farrar-Glauber test. Multivariate  
178 analysis (Principal Component Analysis (PCA) were performed with FactomineR and Vegan  
179 package in R (Lê et al., 2008; Oksanen, 2007). PCA for 16S rDNA diversity analysis was  
180 performed at the phylum level because of PCA constrains, with relative abundance above >  
181 0.05%. We used the factoextra package (Kassambara and Mundt, 2017) for extracting and  
182 visualizing the results. Hierarchical Clustering on Principal Components (HCPC) were



183 performed using FactomineR package in R. Distances were calculated with the Canberra  
184 method and classification was applied to draw the trends of the dataset.

## 185 **Results**

### 186 **Characterization of habitats and microbial community assemblies**

187 In this study, we used previous chemical parameters in order to discriminate different  
188 habitats in the Capbreton canyon (Azaroff et al., 2019). The variance in the relationship  
189 between depth, TS content, POC content, Hg(II) concentrations and MeHg proportion  
190 (selected among 10 parameters with the variance inflation factor, VIF, Supporting  
191 information, Table S1) was explained by two principal components contributing to 82.1% of  
192 the total variance contribution (Fig. 2). Surface sediments were separated into four distinct  
193 clusters corresponding to different areas in the Capbreton Canyon, with sediments from the  
194 mouth of the canyon, intermediate canyon, deep canyon and continental shelf (Fig. 2).  
195 Overall, the geochemical composition of sediments was dependent on the location (i.e.  
196 Canyon vs Continental Shelf), with an enrichment of TS, POC and Hg(II) levels in  
197 intermediate and offshore canyon locations (Fig. 2). Continental shelf sediments were  
198 characterized by a lower fine fraction content ( $<63\mu\text{m}$ ), highlighting that Capbreton canyon  
199 enhances the transport and the accumulation of particles in the sediments (Azaroff et al.,  
200 2019). In contrast, higher MeHg proportions of total Hg were found in coastal locations  
201 suggesting higher methylation activity close to the coast.

202 The composition of sediment microbial communities was investigated based on 16S rDNA  
203 gene sequences, normalized at 5024 reads per sample allowing assignment of 2600 OTUs  
204 for the whole set of data. Overall, the global diversity was quiet close across samples (Chao1  
205 ranging from 1208 to 1685) and replicates (low standard deviation) and no particular trends  
206 in alpha diversity could be observed (Supporting information, Table S2). *Proteobacteria* was  
207 the dominant phylum in terms of richness and relative abundance; they represented 921  
208 OTUs and an average of  $49.6 \pm 3.6\%$  of the total relative abundance, followed by

209 *Bacteroidetes* and *Planctomycetes* representing  $12.6 \pm 5.7\%$  and  $8.6 \pm 1.9\%$ , respectively. A  
210 PCA analysis was performed on the relative abundance of phyla, in order to discriminate  
211 assemblies of microbial communities. The variance of the relationship between the relative  
212 abundance of phyla, was explained by the two first principal components contributing with  
213 62.5% of the total variance (Fig. 3). Although the global pattern composition was uniform  
214 accross all locations, relative abundance variations led to three separate microbial  
215 community clusters along the canyon axis, distinguishing communities from mouth,  
216 intermediate and offshore locations (Fig. 3). Here, the distance to the coast seems to drive  
217 the microbial composition. Those multivariate analyses discriminated four different habitats  
218 colonized by three distinct microbial communities, themselves allocated along the canyon  
219 axis gradient.

#### 220 **Microorganisms involved in the Hg methylation: 16S rDNA versus *hgcA* gene**

221 A previous study showed that the methylmercury proportion of total Hg decreased along the  
222 canyon axis, associated with a decrease of Hg methylation likely of biotic origin (Azaroff et  
223 al., 2019). *hgcA* diversity was investigated in one sediment of each of previously defined  
224 habitat (three sediments into the canyon along the canyon axis (G03, G10 and G24) and one  
225 from the continental shelf (G14)) in order to observe the diversity of Hg-methylating microbial  
226 communities. In addition, known groups containing Hg-methylators were assessed using the  
227 16S rDNA marker.

228 The alpha diversity in the different Capbreton canyon sediments was similar (Supporting  
229 information, table S2). *Deltaproteobacteria*, which includes most of the known Hg-  
230 methylating bacteria, were the most abundant and accounted for 50% of the *Proteobacteria*  
231 and 25% of the total reads (Fig. 4). Among phyla known to contain Hg-methylators members,  
232 *Planctomycetes* were observed at  $8.6 \pm 1.9\%$  of total reads and *Chloroflexi* at  $3.0 \pm 1.9\%$  of  
233 total reads. The relative abundance of the operational taxonomic units (OTUs) from 16S  
234 rDNA within the *Deltaproteobacteria* group, showed the dominance of *Desulfobacterales*  
235 over *Desulfuromonadales*, NB1-J, Sva0485, *Desulfarculales* and *Syntrophobacterales* (Fig.

236 5), with maximum total relative abundance of 20.8%, 5.4%, 4.9%, 1.8%, 2.2% and 3.3%,  
237 respectively. Genera within the *Deltaproteobacteria* known to methylate Hg  
238 (*Desulfobacteraceae*, *Desulfobulbaceae* and *Desulfuromonodaceae*) were dominated by  
239 *Desulfobulbus* and other uncultivated or unaffiliated genera. The relative abundance of  
240 *Desulfuromonadales* decreased along the canyon transect like the unknown genus of  
241 *Desulfobacterales*. SEEP, SRB1, *Desulfobulbus* follow the trend of the *Desulfobacterales*  
242 order trend and SVA0081 increased along this transect. Although *Geobacter sp.* were found  
243 as a crucial Hg-methylator in continental aquatic ecosystems (Bravo et al., 2018; Jones et  
244 al., 2019; Xu et al., 2019), here, abundance of *Geobacteraceae* members peaked to only  
245  $0.17 \pm 0.10\%$  in the G03 location. Those results based on 16S rDNA may indicate that the  
246 bacterial sulfur-reduction, i.e sulfate and sulfur reduction, is the dominant metabolic guild in  
247 these marine sediments.

248 A total of 84 HgcA clones were obtained from locations G03, G10, G14 and G24 (n= 19, 22,  
249 21, 22, respectively). They were mainly related to the *Deltaproteobacteria* (21% of the total  
250 HgcA clones), dominated by the SRB (10 HgcA clones) followed by ferrireducers  
251 (*Desulfuromodales*) and syntrophs (*Syntrophobacteriales*) (Fig. 6 and Fig.7). HgcA clones  
252 were also related to recent new clades like *Chloroflexi*, *Atribacteria*, *Archae*, PVC  
253 superphylum, *Spirochaetes*, *Elusimicrobia* and *Bacteriodetes* (Fig. 6). Nevertheless, 61 % of  
254 the HgcA clones were unaffiliated (unaffiliated 1 to unaffiliated 7 groups). While 18 clones of  
255 these unaffiliated HgcA clones were unknown, 22 and 11 HgcA clones were likely related to  
256 *Deltaproteobacteria* (BSR like) and PVC superphylum / *Aminicenantes*, respectively (Fig. 6  
257 and Fig. 7) All together, almost half of HgcA clones (43 out of 84) obtained from the  
258 Capbreton submarine canyon sediments are likely related to *Deltaproteobacteria* with  
259 dominance of sulfate reducers. While HgcA clones cannot be representative of the Hg-  
260 methylators diversity due to the low number of clones per location and the selective  
261 approach used (cloning sequencing), those results follow the trend observed with the 16S  
262 rDNA approach, suggesting that microbial methylation is dominated by sulfate reduction.

263 **Discussion**

264 **Geochemical conditions along Submarine canyons offer a diversity of ecological**  
265 **niches**

266 We identified four distinct habitats along the canyon transect (Fig. 2) where Hg species, OM  
267 and TS contents were enriched. OM composition and TS play important roles in Hg  
268 biogeochemistry like MeHg formation and accumulation, as seen elsewhere (Azaroff et al.,  
269 2019; Bravo et al., 2017; Choi and Bartha, 1994; Cossa et al., 2014). Higher biotic  
270 methylation in coastal samples as compared to the offshore sediments as determined in a  
271 previous work, may be explained by a higher OM lability (Wakeham and Lee, 2019) and a  
272 differential sequestration of particulate matter along the canyon (i.e. gravitational  
273 sedimentation, hemipelagic sedimentation, remobilization). Otherwise, *Cyanobacteria* that  
274 might have come from the water released by the nearby Adour estuary (Goñi-Urriza et al.,  
275 2007), decrease along the canyon axis. As a primary producer, *Cyanobacteria* may  
276 contribute to the local OM, with higher production in coastal sediments than offshore  
277 sediments, and thus might control the MeHg production (Lázaro et al., 2019; Müller et al.,  
278 2018). Moreover, Capbreton Canyon is still active, meaning it is still filled with particulate  
279 matter (Gaudin et al., 2006), where fine particulate matter is transported along the canyon  
280 transect (gravitational sedimentation), confirmed by the higher fine fraction measured in  
281 offshore sediment relative to the coastal sediments (Azaroff et al., 2019). According to  
282 Mestre et al. (Mestre et al., 2017) microbial community composition varied with particle size  
283 where, Bacteria were more diverse in larger size-fractions, whereas Archaea were more  
284 diverse in smaller particles determining diverse ecotypes with distinct size-fraction  
285 preferences. Considering colonization of the four habitats by three distinct bacterial  
286 communities (Fig. 2 and Fig. 3), different abundances of known groups containing Hg-  
287 methylators (Fig. 4) were correlated with the diverse metabolisms of Hg methylators  
288 identified along this canyon (Fig. 5), our results highlighted that geochemical conditions

289 controlled both the global microbial communities and thus the Hg methylating microbial  
290 communities.

## 291 **Hg-methylating communities in submarine canyon sediments: global and functional** 292 **approaches**

293 For the first time, in submarine canyon sediments, we identified Hg methylating communities  
294 using both 16S rDNA and *hgcA* gene. The organic carbon remineralization in marine  
295 sediments is dominated by bacterial sulfate reduction (Jørgensen, 1990, 1977) performed by  
296 *Deltaproteobacteria* who are known to be the main group of Hg-methylating microorganisms  
297 (Bravo et al., 2018; Christensen et al., 2019; Compeau and Bartha, 1985; Parks et al., 2013;  
298 Podar et al., 2015). Nevertheless, although 16S rDNA analysis showed the  
299 *Desulfobacterales* as dominant, *hgcA* analysis showed SRB were also related to  
300 *Desulfovibrionales*. That is in accordance with the results of SRB identified in the water  
301 column in this canyon (Colin et al., 2017). *hgcA* clones within the “unaffiliated 4”, likely  
302 affiliated with the *Deltaproteobacteria* (Jones et al., 2019), might be related to the uncultured  
303 or unknown bacteria from the 16S rDNA analysis (Fig.5 and Fig.6). We suggest putative Hg  
304 methylating bacteria of unknown genera within the *Desulfobacteraceae*, *Desulfobulbaceae*  
305 and Sva1033 within the *Desulfuromonadaceae*. The relative abundance of those genera  
306 followed the trend of Hg methylation potential along the canyon axis, suggesting their  
307 involvement in this process. Moreover, Sva1033 was related to *Desulfuromonas palmitatis*  
308 (Ravenschlag et al., 1999) and Kerin et al. 2006 (Kerin et al., 2006) demonstrated that the  
309 latter, in pure culture under iron reducing conditions, was able to methylate Hg. Although  
310 sulfate reducing bacteria are widely studied and accepted as the major bacteria involved in  
311 mercury methylation in aquatic environments, these sulfate but also sulfur reducing bacteria  
312 remain largely unknown in marine environments.

313 Like in recent studies, *hgcA* analysis showed that the Hg methylators are also represented  
314 by fermentative organisms, or ferrireducers (Fig. 6) (Bae et al., 2014; Christensen et al.,  
315 2019; Jones et al., 2019; Podar et al., 2015). This highlighted the complexity between

316 geochemical parameters and microorganisms involved into the MeHg formation. As recently  
317 discovered, HgcA clones were related to new phyla containing *hgcA* (Jones et al., 2019),  
318 such as *Aminicenantes*, *Bacteriodetes*, *Kiritimatiellaeota*, *Elusimicrobia* and *Spirochaetes*  
319 (Fig. 6).

320 Hg methylation is a strain-specific trait (Ranchou-Peyruse et al., 2009; Podar et al., 2013;  
321 Gilmour et al., 2013; Yu et al., 2013; Gilmour et al., 2018) that cannot be predicted purely by  
322 16S rDNA taxonomic classification. Although another recent study demonstrated that 16s  
323 rRNA gene pyrosequencing did not have sufficient resolution to identify *hgcAB* harboring  
324 species in soil samples (Christensen et al., 2019), this approach in marine sediments could  
325 provide indications about the *hgcA*-carrying *Prokaryotes*. The unknown *hgcA*-carrying  
326 populations are from uncultivated organisms, and consequently their identification, cultivation  
327 and specific contribution for MeHg remain to be explored.

328 MeHg proportion has previously been used as a proxy for methylation efficiency (Drott et al.,  
329 2008; Skjellberg et al., 2007) and high MeHg proportion has also in a few cases been shown  
330 to correlate positively with the abundance of Hg(II) methylators (Xu et al., 2019). Relative  
331 abundance of some phyla showed a strong correlation with MeHg proportion such as  
332 *Cyanobacteria* or *Gemmatimonadetes* (Table S5) suggesting that they could be involved,  
333 indirectly or directly, in MeHg formation. This may suggest that not only the Hg methylators  
334 themselves, i.e. *Deltaproteobacteria*, but also the supporting and interacting bacterial  
335 communities residing in the sediment may influence the MeHg formation across the studied  
336 submarine canyon sediments (Xu et al., 2019).

337 As previously demonstrated in these sediments, methylmercury production is under control  
338 of both methylation and demethylation processes (Azaroff et al., 2019). Although the  
339 methylation seems to be controlled by microbial activities (for example *hgcA* gene is used as  
340 a proxy of microbial methylation), demethylation can be both abiotic (e.g. photoreduction)  
341 and biotic (e.g. reductive (performed by microorganisms carrying *operon mer*), oxidative  
342 (performed by methylotrophs)) processes (Lu et al., 2017; Mark Marvin-DiPasquale et al.,

2000; Oremland et al., 1991). Such biotic demethylation should be investigated in further studies to better understand microbial involvement in the Hg cycle in marine environment.

### **The S cycling and OM in marine sediments: drivers of Hg biomethylation ?**

The mercury bioavailability for methylating microorganisms in marine sediments and indirectly the MeHg production, is controlled OM mineralization, thus depending on geochemical parameters (Bravo et al., 2017; Goñi-Urriza et al., 2015) and the degree of OM lability. Only a small portion of the total inorganic mercury is likely available for cellular uptake (Hsu-Kim et al., 2013). Indeed, the relative partitioning of Hg(II) between various dissolved and particulate forms will govern the overall mobility of Hg in aquatic systems and the bioavailability of Hg to methylating microorganisms in anaerobic settings. OM could be more labile in coastal sediment (Wakeham and Lee, 2019), which may improve the microbial activities. This suggests that terrestrial inputs, from the high proximity of the canyon mouth to the coast, can influence Hg-methylating microbial composition and thus could explain the higher estimated methylation potential in coastal locations compared to the offshore sediments.

Putative Hg methylators were found among different anaerobic metabolic guilds, including sulfate and sulfur-reducing bacteria, dehalorespiring bacteria, syntrophs, and in some cases FeRB (Fig. 6) but also some newly identified micro-organisms (*Chloroflexi*, *Spirochaetes*, *Elusimicrobia*, PVC superphylum). This suggests that Hg methylation potential is shared by organisms using a wide variety of carbon sources and electrons acceptors. This is in accordance with recent studies which demonstrated that the putative Hg methylators are phylogenetically more diverse than previously, suggesting several independent horizontal gene transfer events (Jones et al., 2019; McDaniel et al., 2020).

Carbon cycling in marine sediments is dominated by sulfate-reduction that could mineralize 50% of the OM (Jørgensen, 1977). This is in accordance with the predominance of sulfur reducing bacteria (observed within 16S rDNA and HgcA) observed in these sediments.

369 Fermenters were also observed in all studied sediments (Fig. 7). This suggests a  
370 fermentative degradation of OM from complex carbon molecules to small carbon products  
371 (i.e. propionate, pyruvate, acetate) and dihydrogen production. They could be used as  
372 electron donors for the SRB and methanogens (Gilmour et al., 2018), improving the Hg  
373 biomethylation. Otherwise, OM degradation depends on the microbial composition and then  
374 both the methylating and non-methylating bacteria may control the MeHg formation (Bravo  
375 et al., 2018; Xu et al., 2019). Specific populations of non-Hg(II) methylating bacteria actively  
376 decomposing OM seem also to create a niche that promotes Hg(II) methylation. Also, it has  
377 been demonstrated that bioturbating fauna disturbed mechanically the electrogenic sulfur  
378 oxidation through long filamentous bacteria (*Desulfobulbaceae*), which likely mediated the  
379 electron transport between the deep and surface marine sediments (at the centimetre scale;  
380 50), could have profound implication for the sulfur cycle. This highlights the complexity of  
381 interspecific relationship at different scales for the S and the Hg cycling in marine sediments  
382 (Liu et al., 2018).

383 In oceans and seas, methylation of mercury occurs both in column water and sediment  
384 compartment (Monperrus et al., 2007; Rosati et al., 2018; Smith et al., 2018). At a global  
385 scale, Boyd et al. 2019 (Boyd et al., 2019) showed that carbon sequestration in the open  
386 ocean is probably more influenced by solubility than biological activity. These insights into  
387 carbon sequestration can be taken into consideration to better understand the Hg  
388 biogeochemical cycle of mercury in ocean sediments. Although the OM is very reactive in the  
389 euphotic layer of the ocean, the carbon can directly sink to the bottom surface sediment  
390 (Wakeham and Lee, 2019) and could be an important carbon pump reacting into the Hg  
391 cycle, i.e. increase Hg methylation. Marine environment are the largest habitat on the earth  
392 accounting for more than 90% of total biosphere volume. The microorganisms colonizing  
393 marine habitats are responsible for more than 50% of the global primary production and  
394 nutrient cycling (Lauro et al., 2009).



395 Carbon sedimentation at the bottom ocean of the ocean represents a significant fraction of  
396 global oceanic carbon sequestration (Boyd et al., 2019) and could influence the bacterial  
397 composition, and consequently, can be a determinant for the formation of methylmercury  
398 (Bravo et al., 2018; King et al., 2001, 2000, 1999). The high anthropogenic Hg levels found in  
399 the North Atlantic ocean (Lamborg et al., 2016, 2014) associated with the high affinity of Hg  
400 for OM, could sink and trap Hg in those marine sediments. It is crucial to improve our  
401 knowledge of the Hg-methylating bacteria in marine sediments, in order to estimate their  
402 impact on MeHg production in a-the global Hg cycle.

403

#### 404 **Conclusion**

405 In this study, an assessment of marine Hg-methylating microbial communities was conducted  
406 in submarine canyon sediments by combining pluridisciplinary approaches including  
407 geochemical parameters, mercury speciation, global microbial diversity and *hgcA*-carrying  
408 bacteria diversity. Results highlighted that the distribution of microbial communities in  
409 submarine canyon sediments as well as methylation of mercury, were driven by the  
410 environmental parameters. Although Hg(II) and MeHg concentrations increased along the  
411 canyon axis from the coast to the offshore, the fraction of MeHg was higher in coastal  
412 locations due to the higher biomethylation potential estimated in coastal sediments (Azaroff  
413 et al., 2019). These results may indicate that methylation of Hg in marine sediments is driven  
414 by OM composition and the S cycle. Indeed, 16S rDNA and *hgcA* analysis suggested that  
415 *Deltaproteobacteria* dominated by sulfur-compounds-reducing bacteria are the main  
416 contributors for the methylation of Hg in marine sediments. Nevertheless, further studies are  
417 needed to identify and characterize/isolate the unaffiliated and unknown *hgcA* carrying  
418 microorganisms that could be novel Hg-methylators. Their methylation and demethylation  
419 capacities must be also determined in order to estimate their impacts on MeHg production in  
420 marine environments at the global scale. To fully understand and quantify the relationship

421 between Hg, sulfur, OM and microorganisms, further studies are also needed to decipher the  
422 biogeochemical cycle of Hg in marine sediments.

423

424

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431

#### 432 **Compliance with ethical standards**

433 **Conflict of interest** The authors declare that they have no conflict of interest.

434

435 **References**

- 436 Azaroff, A., Tessier, E., Deborde, J., Guyoneaud, R., Monperrus, M., 2019. Mercury and  
437 methylmercury concentrations, sources and distribution in submarine canyon  
438 sediments (Capbreton, SW France): Implications for the net methylmercury  
439 production. *Sci. Total Environ.* 673, 511–521.  
440 <https://doi.org/10.1016/j.scitotenv.2019.04.111>
- 441 Bae, H.-S., Dierberg, F.E., Ogram, A., 2014. Syntrophs Dominate Sequences Associated  
442 with the Mercury Methylation-Related Gene *hgcA* in the Water Conservation Areas of  
443 the Florida Everglades. *Appl. Environ. Microbiol.* 80, 6517–6526.  
444 <https://doi.org/10.1128/AEM.01666-14>
- 445 Boyd, P.W., Claustre, H., Levy, M., Siegel, D.A., Weber, T., 2019. Multi-faceted particle  
446 pumps drive carbon sequestration in the ocean. *Nature* 568, 327.  
447 <https://doi.org/10.1038/s41586-019-1098-2>
- 448 Bravo, A.G., Bouchet, S., Tolu, J., Björn, E., Mateos-Rivera, A., Bertilsson, S., 2017.  
449 Molecular composition of organic matter controls methylmercury formation in boreal  
450 lakes. *Nat. Commun.* 8, 14255. <https://doi.org/10.1038/ncomms14255>
- 451 Bravo, A.G., Zopfi, J., Buck, M., Xu, J., Bertilsson, S., Schaefer, J.K., Poté, J., Cosio, C.,  
452 2018. Geobacteraceae are important members of mercury-methylating microbial  
453 communities of sediments impacted by waste water releases. *ISME J.* 12, 802.  
454 <https://doi.org/10.1038/s41396-017-0007-7>
- 455 Choi, S.-C., Bartha, R., 1994. Environmental factors affecting mercury methylation in  
456 estuarine sediments. *Bull. Environ. Contam. Toxicol.* 53, 805–812.  
457 <https://doi.org/10.1007/BF00196208>
- 458 Christensen, G.A., Gionfriddo, C.M., King, A.J., Moberly, J.G., Miller, C.L., Somenahally,  
459 A.C., Callister, S.J., Brewer, H., Podar, M., Brown, S.D., Palumbo, A.V., Brandt, C.C.,  
460 Wymore, A.M., Brooks, S.C., Hwang, C., Fields, M.W., Wall, J.D., Gilmour, C.C.,  
461 Elias, D.A., 2019. Determining the Reliability of Measuring Mercury Cycling Gene

462 Abundance with Correlations with Mercury and Methylmercury Concentrations.  
463 Environ. Sci. Technol. 53, 8649–8663. <https://doi.org/10.1021/acs.est.8b06389>

464 Christensen, G.A., Wymore, A.M., King, A.J., Podar, M., Hurt, R.A., Santillan, E.U., Soren,  
465 A., Brandt, C.C., Brown, S.D., Palumbo, A.V., Wall, J.D., Gilmour, C.C., Elias, D.A.,  
466 2016. Development and Validation of Broad-Range Qualitative and Clade-Specific  
467 Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment.  
468 Appl Env. Microbiol AEM.01271-16. <https://doi.org/10.1128/AEM.01271-16>

469 Colin, Y., Goñi-Urriza, M., Gassie, C., Carlier, E., Monperrus, M., Guyoneaud, R., 2017.  
470 Distribution of Sulfate-Reducing Communities from Estuarine to Marine Bay Waters.  
471 Microb. Ecol. 73, 39–49. <https://doi.org/10.1007/s00248-016-0842-5>

472 Compeau, G.C., Bartha, R., 1985. Sulfate-Reducing Bacteria: Principal Methylators of  
473 Mercury in Anoxic Estuarine Sediment. Appl. Environ. Microbiol. 50, 498–502.

474 Cossa, D., Buscail, R., Puig, P., Chiffoleau, J.-F., Radakovitch, O., Jeanty, G., Heussner, S.,  
475 2014. Origin and accumulation of trace elements in sediments of the northwestern  
476 Mediterranean margin. Chem. Geol. 380, 61–73.  
477 <https://doi.org/10.1016/j.chemgeo.2014.04.015>

478 Drott, A., Lambertsson, L., Björn, E., Skyllberg, U., 2008. Do Potential Methylation Rates  
479 Reflect Accumulated Methyl Mercury in Contaminated Sediments? Environ. Sci.  
480 Technol. 42, 153–158. <https://doi.org/10.1021/es0715851>

481 Du, H., Ma, M., Sun, T., Dai, X., Yang, C., Luo, F., Wang, D., Igarashi, Y., 2017. Mercury-  
482 methylating genes *dsrB* and *hgcA* in soils/sediments of the Three Gorges Reservoir.  
483 Environ. Sci. Pollut. Res. 24, 5001–5011. <https://doi.org/10.1007/s11356-016-8213-9>

484 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high  
485 throughput. Nucleic Acids Res. 32, 1792–1797. <https://doi.org/10.1093/nar/gkh340>

486 Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S.,  
487 Hernandez-Raquet, G., Combes, S., Pascal, G., 2018. FROGS: Find, Rapidly, OTUs  
488 with Galaxy Solution. Bioinforma. Oxf. Engl. 34, 1287–1294.  
489 <https://doi.org/10.1093/bioinformatics/btx791>

490 Finn, R.D., Coghill, P., Eberhardt, R.Y., Eddy, S.R., Mistry, J., Mitchell, A.L., Potter, S.C.,  
491 Punta, M., Qureshi, M., Sangrador-Vegas, A., Salazar, G.A., Tate, J., Bateman, A.,  
492 2016. The Pfam protein families database: towards a more sustainable future.  
493 *Nucleic Acids Res.* 44, D279–D285. <https://doi.org/10.1093/nar/gkv1344>

494 Fleming, E.J., Mack, E.E., Green, P.G., Nelson, D.C., 2006. Mercury Methylation from  
495 Unexpected Sources: Molybdate-Inhibited Freshwater Sediments and an Iron-  
496 Reducing Bacterium. *Appl. Environ. Microbiol.* 72, 457–464.  
497 <https://doi.org/10.1128/AEM.72.1.457-464.2006>

498 Gaudin, M., Mulder, T., Cirac, P., Berné, S., Imbert, P., 2006. Past and present sedimentary  
499 activity in the Capbreton Canyon, southern Bay of Biscay. *Geo-Mar. Lett.* 26, 331.  
500 <https://doi.org/10.1007/s00367-006-0043-1>

501 Gilmour, C.C., Bullock, A.L., McBurney, A., Podar, M., Elias, D.A., 2018. Robust Mercury  
502 Methylation across Diverse Methanogenic Archaea. *mBio* 9.  
503 <https://doi.org/10.1128/mBio.02403-17>

504 Gilmour, C.C., Podar, M., Bullock, A.L., Graham, A.M., Brown, S.D., Somenahally, A.C.,  
505 Johs, A., Hurt, R.A., Bailey, K.L., Elias, D.A., 2013. Mercury Methylation by Novel  
506 Microorganisms from New Environments. *Environ. Sci. Technol.* 47, 11810–11820.  
507 <https://doi.org/10.1021/es403075t>

508 Gionfriddo, C.M., Tate, M.T., Wick, R.R., Schultz, M.B., Zemla, A., Thelen, M.P., Schofield,  
509 R., Krabbenhoft, D.P., Holt, K.E., Moreau, J.W., 2016. Microbial mercury methylation  
510 in Antarctic sea ice. *Nat. Microbiol.* 1, 16127.  
511 <https://doi.org/10.1038/nmicrobiol.2016.127>

512 Goñi-Urriza, M., Corsellis, Y., Lancelleur, L., Tessier, E., Gury, J., Monperrus, M.,  
513 Guyoneaud, R., 2015. Relationships between bacterial energetic metabolism,  
514 mercury methylation potential, and *hgcA/hgcB* gene expression in *Desulfovibrio*  
515 *dechloroacetivorans* BerOc1. *Environ. Sci. Pollut. Res. Int.* 22, 13764–13771.  
516 <https://doi.org/10.1007/s11356-015-4273-5>

517 Goñi-Urriza, M.S., Point, D., Amouroux, D., Guyoneaud, R., Donard, O.F.X., Caumette, P.,  
518 Duran, R., 2007. Bacterial community structure along the Adour estuary (French  
519 Atlantic coast): influence of salinity gradient versus metal contamination. *Aquat.*  
520 *Microb. Ecol.* 49, 47–56. <https://doi.org/10.3354/ame01128>

521 Hamelin, S., Amyot, M., Barkay, T., Wang, Y., Planas, D., 2011. Methanogens: principal  
522 methylators of mercury in lake periphyton. *Environ. Sci. Technol.* 45, 7693–7700.  
523 <https://doi.org/10.1021/es2010072>

524 Hsu-Kim, H., Kucharzyk, K.H., Zhang, T., Deshusses, M.A., 2013. Mechanisms Regulating  
525 Mercury Bioavailability for Methylating Microorganisms in the Aquatic Environment: A  
526 Critical Review. *Environ. Sci. Technol.* 47, 2441–2456.  
527 <https://doi.org/10.1021/es304370g>

528 Imdadullah, M., Aslam, M., Altaf, S., n.d. Mctest: An R package for detection of collinearity  
529 among regressors. *R J* 8, 499–509.

530 Jones, D.S., Walker, G.M., Johnson, N.W., Mitchell, C.P.J., Wasik, J.K.C., Bailey, J.V., 2019.  
531 Molecular evidence for novel mercury methylating microorganisms in sulfate-  
532 impacted lakes. *ISME J.* 1. <https://doi.org/10.1038/s41396-019-0376-1>

533 Jørgensen, B.B., 1990. A Thiosulfate Shunt in the Sulfur Cycle of Marine Sediments. *Science*  
534 249, 152–154. <https://doi.org/10.1126/science.249.4965.152>

535 Jørgensen, B.B., 1977. The sulfur cycle of a coastal marine sediment (Limfjorden,  
536 Denmark)1. *Limnol. Oceanogr.* 22, 814–832.  
537 <https://doi.org/10.4319/lo.1977.22.5.0814>

538 Kassambara, A., Mundt, F., 2017. Package ‘factoextra’. Extract and visualize the results of  
539 multivariate data analyses 76.

540 Kerin, E.J., Gilmour, C.C., Roden, E., Suzuki, M.T., Coates, J.D., Mason, R.P., 2006.  
541 Mercury Methylation by Dissimilatory Iron-Reducing Bacteria. *Appl Env. Microbiol* 72,  
542 7919–7921. <https://doi.org/10.1128/AEM.01602-06>

543 King, J.K., Kostka, J.E., Frischer, M.E., Saunders, F.M., 2000. Sulfate-Reducing Bacteria  
544 Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments. *Appl*  
545 *Env. Microbiol* 66, 2430–2437. <https://doi.org/10.1128/AEM.66.6.2430-2437.2000>

546 King, J.K., Kostka, J.E., Frischer, M.E., Saunders, F.M., Jahnke, R.A., 2001. A Quantitative  
547 Relationship that Demonstrates Mercury Methylation Rates in Marine Sediments Are  
548 Based on the Community Composition and Activity of Sulfate-Reducing Bacteria.  
549 *Environ. Sci. Technol.* 35, 2491–2496. <https://doi.org/10.1021/es001813q>

550 King, J.K., Saunders, F.M., Lee, R.F., Jahnke, R.A., 1999. Coupling mercury methylation  
551 rates to sulfate reduction rates in marine sediments. *Environ. Toxicol. Chem.* 18,  
552 1362–1369. <https://doi.org/10.1002/etc.5620180704>

553 Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular  
554 Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* 35,  
555 1547–1549. <https://doi.org/10.1093/molbev/msy096>

556 Lamborg, C.H., Hammerschmidt, C.R., Bowman, K.L., 2016. An examination of the role of  
557 particles in oceanic mercury cycling. *Philos. Transact. A Math. Phys. Eng. Sci.* 374.  
558 <https://doi.org/10.1098/rsta.2015.0297>

559 Lamborg, C.H., Hammerschmidt, C.R., Bowman, K.L., Swarr, G.J., Munson, K.M., Ohnemus,  
560 D.C., Lam, P.J., Heimbürger, L.-E., Rijkenberg, M.J.A., Saito, M.A., 2014. A global  
561 ocean inventory of anthropogenic mercury based on water column measurements.  
562 *Nature* 512, 65–68. <https://doi.org/10.1038/nature13563>

563 Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S., DeMaere, M.Z.,  
564 Ting, L., Ertan, H., Johnson, J., Ferriera, S., Lapidus, A., Anderson, I., Kyrpides, N.,  
565 Munk, A.C., Detter, C., Han, C.S., Brown, M.V., Robb, F.T., Kjelleberg, S.,  
566 Cavicchioli, R., 2009. The genomic basis of trophic strategy in marine bacteria. *Proc.*  
567 *Natl. Acad. Sci.* 106, 15527. <https://doi.org/10.1073/pnas.0903507106>

568 Lázaro, W.L., Díez, S., Bravo, A.G., da Silva, C.J., Ignácio, Á.R.A., Guimaraes, J.R.D., 2019.  
569 Cyanobacteria as regulators of methylmercury production in periphyton. *Sci. Total*  
570 *Environ.* 668, 723–729. <https://doi.org/10.1016/j.scitotenv.2019.02.233>

571 Lê, S., Josse, J., Husson, F., 2008. FactoMineR: An R Package for Multivariate Analysis. J.  
572 Stat. Softw. 25, 1–18. <https://doi.org/10.18637/jss.v025.i01>

573 Liu, J., Jiang, T., Wang, F., Zhang, J., Wang, D., Huang, R., Yin, D., Liu, Z., Wang, J., 2018.  
574 Inorganic sulfur and mercury speciation in the water level fluctuation zone of the  
575 Three Gorges Reservoir, China: The role of inorganic reduced sulfur on mercury  
576 methylation. Environ. Pollut. 237, 1112–1123.  
577 <https://doi.org/10.1016/j.envpol.2017.11.045>

578 Liu, Y.-R., Yu, R.-Q., Zheng, Y.-M., He, J.-Z., 2014. Analysis of the Microbial Community  
579 Structure by Monitoring an Hg Methylation Gene (*hgcA*) in Paddy Soils along an Hg  
580 Gradient. Appl Env. Microbiol 80, 2874–2879. <https://doi.org/10.1128/AEM.04225-13>

581 Lu, X., Gu, W., Zhao, L., Haque, M.F.U., DiSpirito, A.A., Semrau, J.D., Gu, B., 2017.  
582 Methylmercury uptake and degradation by methanotrophs. Sci. Adv. 3, e1700041.  
583 <https://doi.org/10.1126/sciadv.1700041>

584 Malkin, S.Y., Rao, A.M., Seitaj, D., Vasquez-Cardenas, D., Zetsche, E.-M., Hidalgo-Martinez,  
585 S., Boschker, H.T., Meysman, F.J., 2014. Natural occurrence of microbial sulphur  
586 oxidation by long-range electron transport in the seafloor. ISME J. 8, 1843–1854.  
587 <https://doi.org/10.1038/ismej.2014.41>

588 Mark Marvin-DiPasquale, \*, Jennifer Agee, †, Chad McGowan, †, Ronald S. Oremland, †,  
589 Martha Thomas, ‡, David Krabbenhoft, § and, Gilmour#, C.C., 2000. Methyl-Mercury  
590 Degradation Pathways: A Comparison among Three Mercury-Impacted Ecosystems  
591 [WWW Document]. <https://doi.org/10.1021/es0013125>

592 Markowitz, V.M., Chen, I.-M.A., Palaniappan, K., Chu, K., Szeto, E., Grechkin, Y., Ratner, A.,  
593 Jacob, B., Huang, J., Williams, P., Huntemann, M., Anderson, I., Mavromatis, K.,  
594 Ivanova, N.N., Kyrpides, N.C., 2012. IMG: the integrated microbial genomes  
595 database and comparative analysis system. Nucleic Acids Res. 40, D115–D122.  
596 <https://doi.org/10.1093/nar/gkr1044>

597 Mason, R.P., Choi, A.L., Fitzgerald, W.F., Hammerschmidt, C.R., Lamborg, C.H., Soerensen,  
598 A.L., Sunderland, E.M., 2012. Mercury biogeochemical cycling in the ocean and



599 policy implications. *Environ. Res., Mercury in Marine Ecosystems: Sources to*  
600 *Seafood Consumers* 119, 101–117. <https://doi.org/10.1016/j.envres.2012.03.013>

601 Mazières, A., Gillet, H., Castelle, B., Mulder, T., Guyot, C., Garlan, T., Mallet, C., 2014. High-  
602 resolution morphobathymetric analysis and evolution of Capbreton submarine canyon  
603 head (Southeast Bay of Biscay—French Atlantic Coast) over the last decade using  
604 descriptive and numerical modeling. *Mar. Geol.* 351, 1–12.  
605 <https://doi.org/10.1016/j.margeo.2014.03.001>

606 McDaniel, E.A., Peterson, B., Stevens, S.L.R., Tran, P.Q., Anantharaman, K., McMahon,  
607 K.D., 2020. Expanded Phylogenetic Diversity and Metabolic Flexibility of Microbial  
608 Mercury Methylation. *bioRxiv* 2020.01.16.909358.  
609 <https://doi.org/10.1101/2020.01.16.909358>

610 Mestre, M., Ferrera, I., Borrull, E., Ortega-Retuerta, E., Mbedi, S., Grossart, H.-P., Gasol,  
611 J.M., Sala, M.M., 2017. Spatial variability of marine bacterial and archaeal  
612 communities along the particulate matter continuum. *Mol. Ecol.* 26, 6827–6840.  
613 <https://doi.org/10.1111/mec.14421>

614 Monperrus, M., Tessier, E., Point, D., Vidimova, K., Amouroux, D., Guyoneaud, R., Leynaert,  
615 A., Grall, J., Chauvaud, L., Thouzeau, G., Donard, O.F.X., 2007. The biogeochemistry  
616 of mercury at the sediment–water interface in the Thau Lagoon. 2. Evaluation of  
617 mercury methylation potential in both surface sediment and the water column. *Estuar.*  
618 *Coast. Shelf Sci., Biogeochemical and contaminant cycling in sediments from a*  
619 *human-impacted coastal lagoon* 72, 485–496.  
620 <https://doi.org/10.1016/j.ecss.2006.11.014>

621 Müller, A.L., Pelikan, C., de Rezende, J.R., Wasmund, K., Putz, M., Glombitza, C., Kjeldsen,  
622 K.U., Jørgensen, B.B., Loy, A., 2018. Bacterial interactions during sequential  
623 degradation of cyanobacterial necromass in a sulfidic arctic marine sediment.  
624 *Environ. Microbiol.* 20, 2927–2940. <https://doi.org/10.1111/1462-2920.14297>

625 Oksanen, J., 2007. *Multivariate Analysis of Ecological Communities in R: Vegan Tutorial*. UR  
626 [Httpcc Oulu Fi~ Jarioksaopetusmetodivegantutor Pdf 1](http://cc.oulu.fi/~jarioksa/opetus/metodivegantutor/Pdf/1).

627 Oliveira, A., Palma, C., Valença, M., 2011. Heavy metal distribution in surface sediments  
628 from the continental shelf adjacent to Nazaré canyon. *Deep Sea Res. Part II Top.*  
629 *Stud. Oceanogr., The Geology, Geochemistry, and Biology of Submarine Canyons*  
630 *West of Portugal* 58, 2420–2432. <https://doi.org/10.1016/j.dsr2.2011.04.006>

631 Oremland, R.S., Culbertson, C.W., Winfrey, M.R., 1991. Methylmercury Decomposition in  
632 Sediments and Bacterial Cultures: Involvement of Methanogens and Sulfate  
633 Reducers in Oxidative Demethylation. *Appl. Environ. Microbiol.* 57, 130–137.

634 Palanques, A., Masqué, P., Puig, P., Sanchez-Cabeza, J.A., Frignani, M., Alvisi, F., 2008.  
635 Anthropogenic trace metals in the sedimentary record of the Llobregat continental  
636 shelf and adjacent Foix Submarine Canyon (northwestern Mediterranean). *Mar. Geol.*  
637 248, 213–227. <https://doi.org/10.1016/j.margeo.2007.11.001>

638 Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small  
639 subunit rRNA primers for marine microbiomes with mock communities, time series  
640 and global field samples. *Environ. Microbiol.* 18, 1403–1414.  
641 <https://doi.org/10.1111/1462-2920.13023>

642 Parks, J.M., Johs, A., Podar, M., Bridou, R., Hurt, R.A., Smith, S.D., Tomanicek, S.J., Qian,  
643 Y., Brown, S.D., Brandt, C.C., Palumbo, A.V., Smith, J.C., Wall, J.D., Elias, D.A.,  
644 Liang, L., 2013. The genetic basis for bacterial mercury methylation. *Science* 339,  
645 1332–1335. <https://doi.org/10.1126/science.1230667>

646 Podar, M., Gilmour, C.C., Brandt, C.C., Soren, A., Brown, S.D., Crable, B.R., Palumbo, A.V.,  
647 Somenahally, A.C., Elias, D.A., 2015. Global prevalence and distribution of genes  
648 and microorganisms involved in mercury methylation. *Sci. Adv.* 1, e1500675.  
649 <https://doi.org/10.1126/sciadv.1500675>

650 Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O.,  
651 2007. SILVA: a comprehensive online resource for quality checked and aligned  
652 ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35, 7188–  
653 7196. <https://doi.org/10.1093/nar/gkm864>

654 Ranchou-Peyruse, M., Monperrus, M., Bridou, R., Duran, R., Amouroux, D., Salvado, J.C.,  
655 Guyoneaud, R., 2009. Overview of Mercury Methylation Capacities among Anaerobic  
656 Bacteria Including Representatives of the Sulphate-Reducers: Implications for  
657 Environmental Studies. *Geomicrobiol. J.* 26, 1–8.  
658 <https://doi.org/10.1080/01490450802599227>

659 Ravenschlag, K., Sahm, K., Pernthaler, J., Amann, R., 1999. High Bacterial Diversity in  
660 Permanently Cold Marine Sediments. *Appl Env. Microbiol* 65, 3982–3989.

661 Rosati, G., Heimbürger, L.E., Canu, D.M., Lagane, C., Laffont, L., Rijkenberg, M.J.A.,  
662 Gerringa, L.J.A., Solidoro, C., Gencarelli, C.N., Hedgecock, I.M., Baar, H.J.W.D.,  
663 Sonke, J.E., 2018. Mercury in the Black Sea: New Insights From Measurements and  
664 Numerical Modeling. *Glob. Biogeochem. Cycles* 32, 529–550.  
665 <https://doi.org/10.1002/2017GB005700>

666 Schaefer, J.K., Kronberg, R.-M., Morel, F.M.M., Skyllberg, U., 2014. Detection of a key Hg  
667 methylation gene, *hgcA*, in wetland soils. *Environ. Microbiol. Rep.* 6, 441–447.  
668 <https://doi.org/10.1111/1758-2229.12136>

669 Skyllberg, U., Drott, A., Lambertsson, L., Björn, E., Karlsson, T., Johnson, T., Heinemo, S.-  
670 A., Holmström, H., 2007. Net methylmercury production as a basis for improved risk  
671 assessment of mercury-contaminated sediments. *Ambio* 36, 437–442.

672 Smith, K.L., Ruhl, H.A., Huffard, C.L., Messié, M., Kahru, M., 2018. Episodic organic carbon  
673 fluxes from surface ocean to abyssal depths during long-term monitoring in NE  
674 Pacific. *Proc. Natl. Acad. Sci.* 115, 12235–12240.  
675 <https://doi.org/10.1073/pnas.1814559115>

676 Smith, S.D., Bridou, R., Johs, A., Parks, J.M., Elias, D.A., Hurt, R.A., Brown, S.D., Podar, M.,  
677 Wall, J.D., 2015. Site-Directed Mutagenesis of *HgcA* and *HgcB* Reveals Amino Acid  
678 Residues Important for Mercury Methylation. *Appl. Environ. Microbiol.* 81, 3205–3217.  
679 <https://doi.org/10.1128/AEM.00217-15>

680 Sunderland, E.M., 2007. Mercury Exposure from Domestic and Imported Estuarine and  
681 Marine Fish in the U.S. Seafood Market. *Environ. Health Perspect.* 115, 235–242.  
682 <https://doi.org/10.1289/ehp.9377>

683 Wakeham, S.G., Lee, C., 2019. Limits of our knowledge, part 2: Selected frontiers in marine  
684 organic biogeochemistry. *Mar. Chem.* <https://doi.org/10.1016/j.marchem.2019.02.005>

685 Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert,  
686 J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2015.  
687 Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed  
688 Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems* 1.  
689 <https://doi.org/10.1128/mSystems.00009-15>

690 Wang, Y., Qian, P.-Y., 2009. Conservative Fragments in Bacterial 16S rRNA Genes and  
691 Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies. *PLoS*  
692 *ONE* 4. <https://doi.org/10.1371/journal.pone.0007401>

693 Xu, J., Buck, M., Eklöf, K., Ahmed, O.O., Schaefer, J.K., Bishop, K., Skjellberg, U., Björn, E.,  
694 Bertilsson, S., Bravo, A.G., 2019. Mercury methylating microbial communities of  
695 boreal forest soils. *Sci. Rep.* 9, 518. <https://doi.org/10.1038/s41598-018-37383-z>

696 Zuckerkandl, E., Pauling, L., 1965. Evolutionary divergence and convergence in proteins. In :  
697 *Evolving genes and proteins.* Acad. Press 97–166.

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Fig. 1

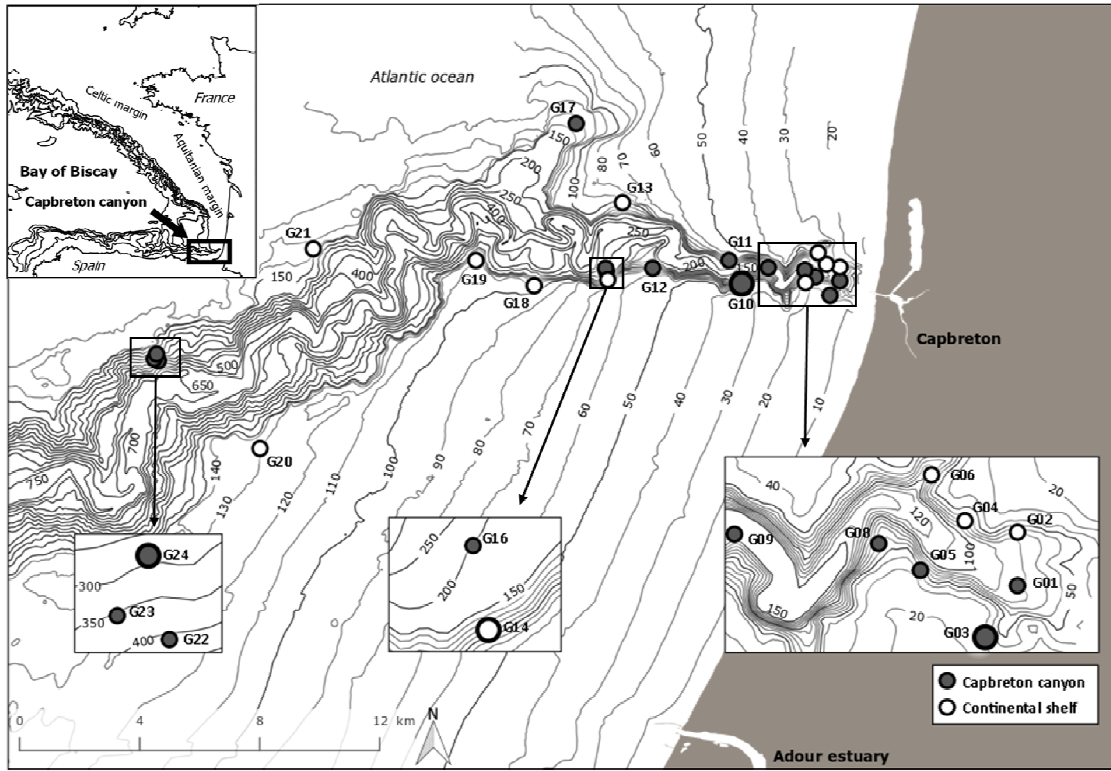


Fig. 2

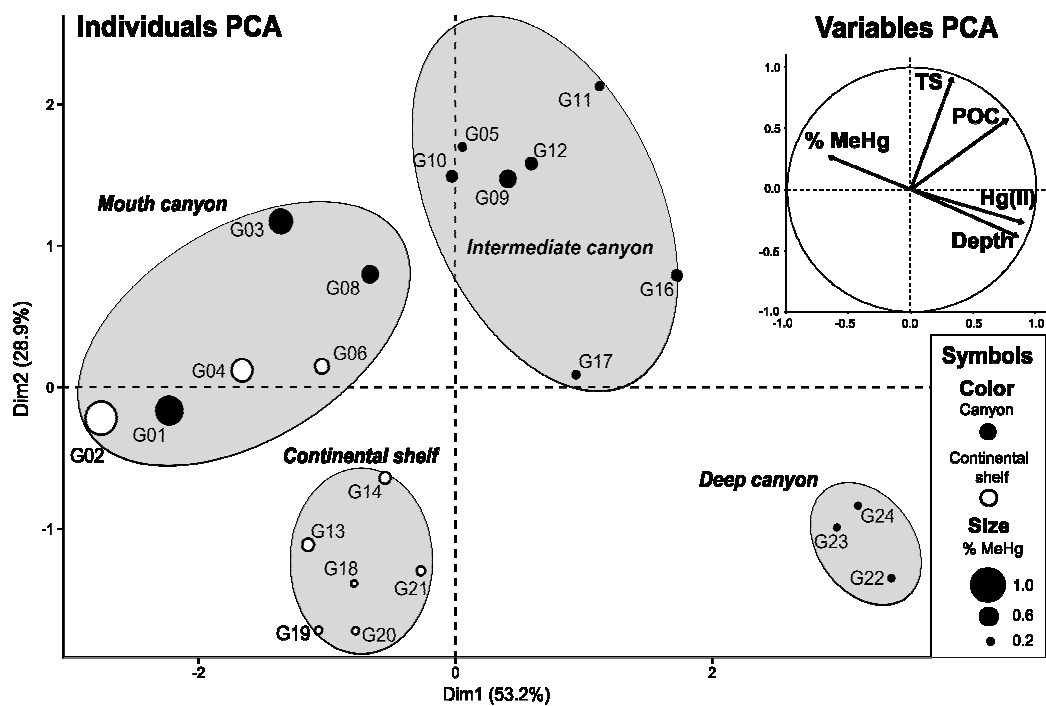


Fig. 3

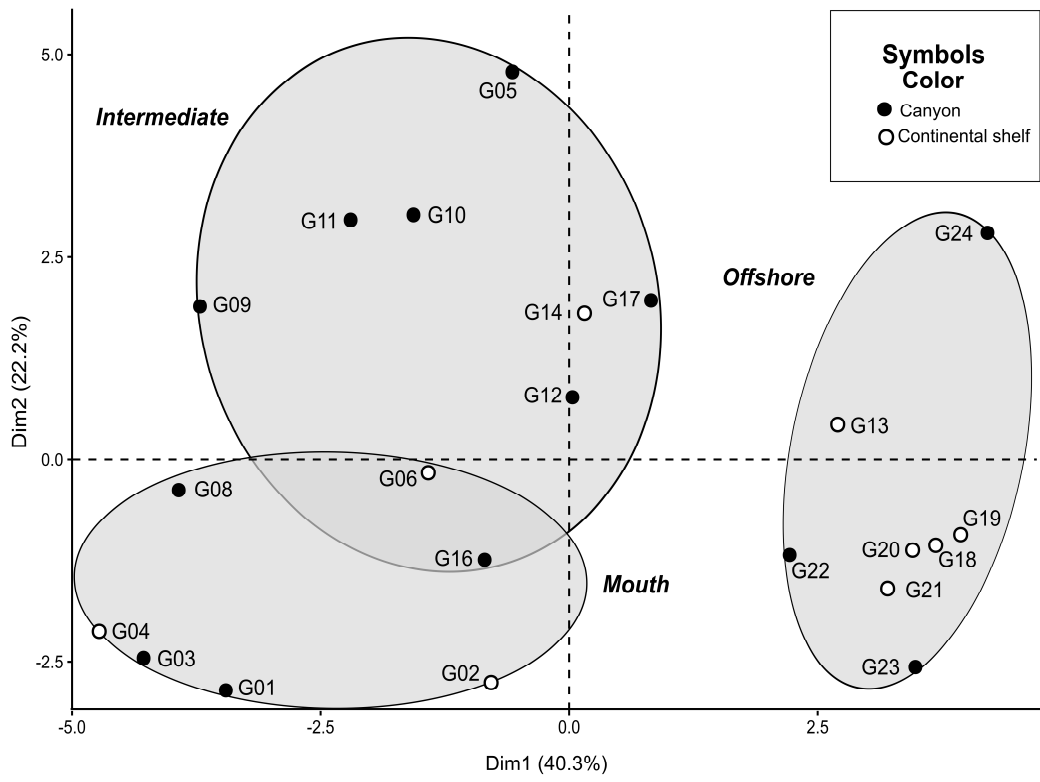


Fig. 4

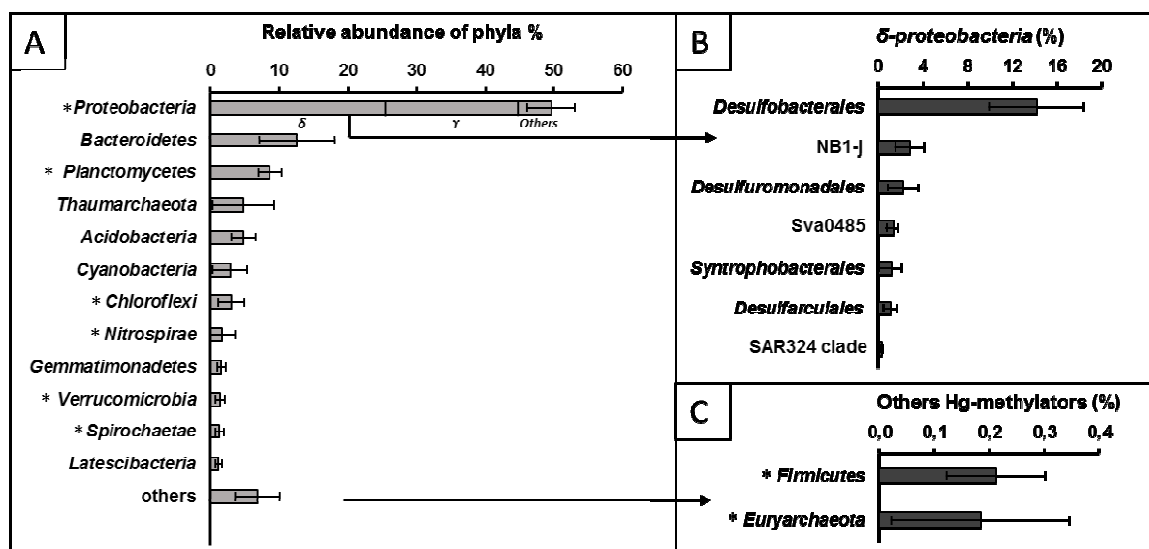




Fig. 5

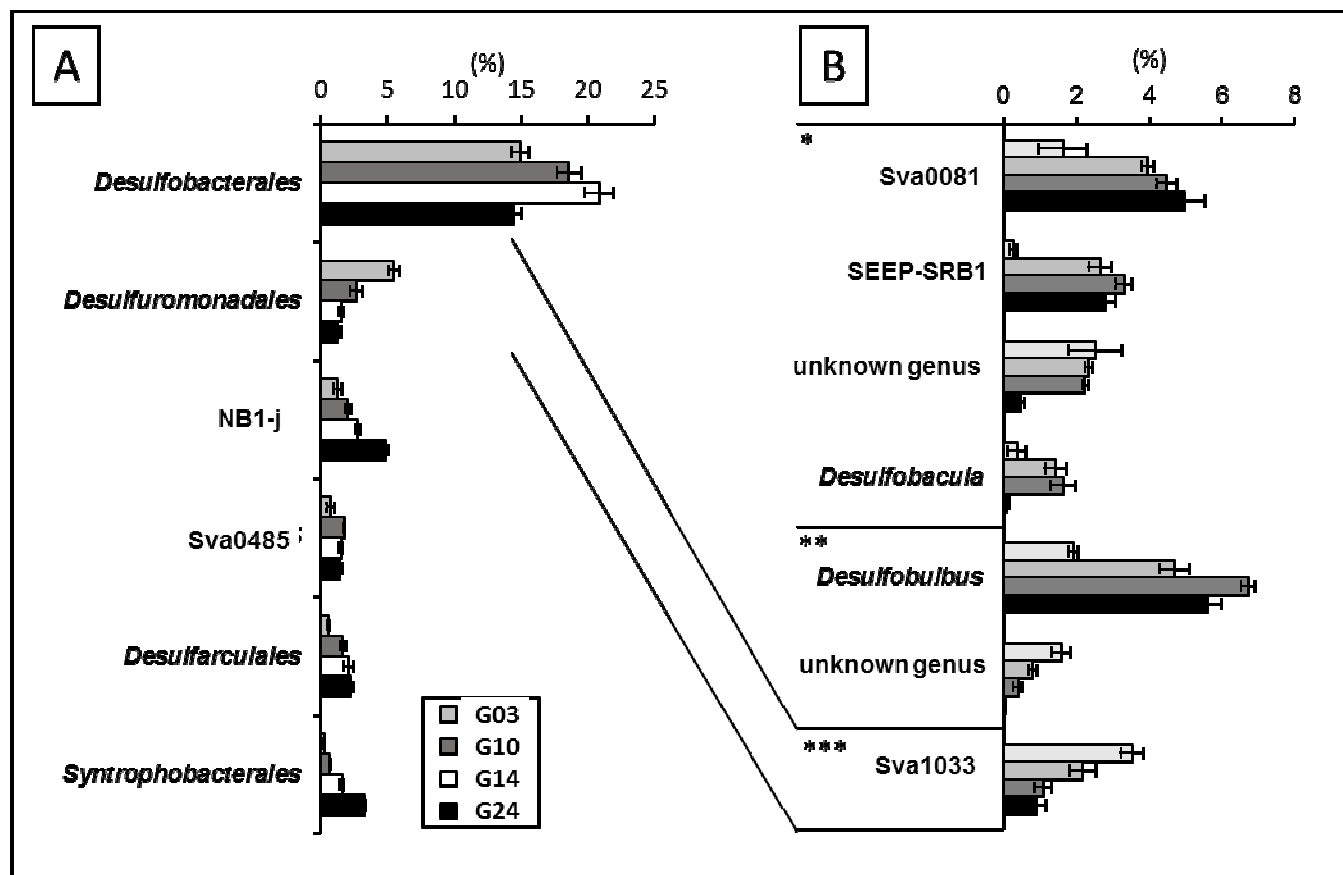


Fig. 6

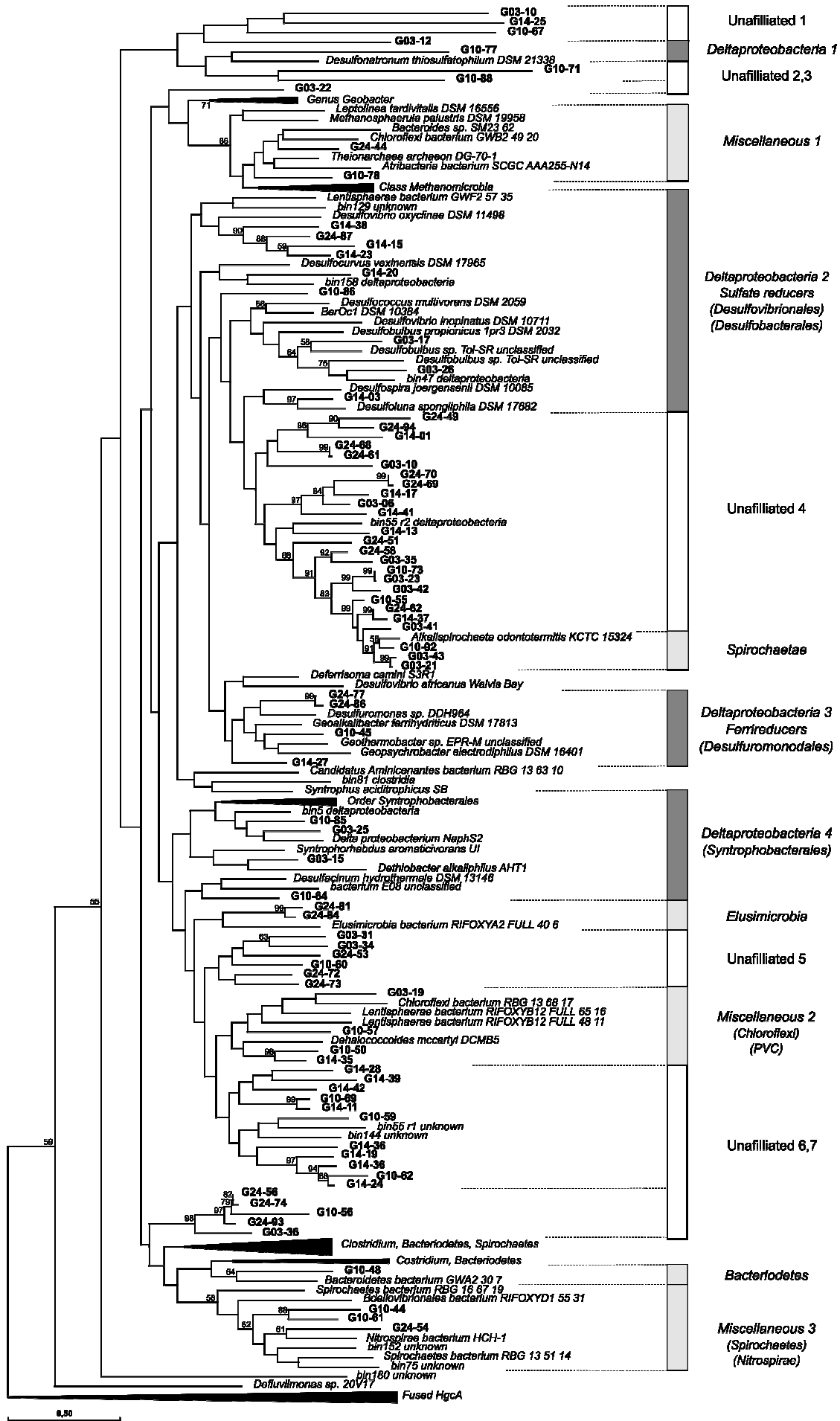
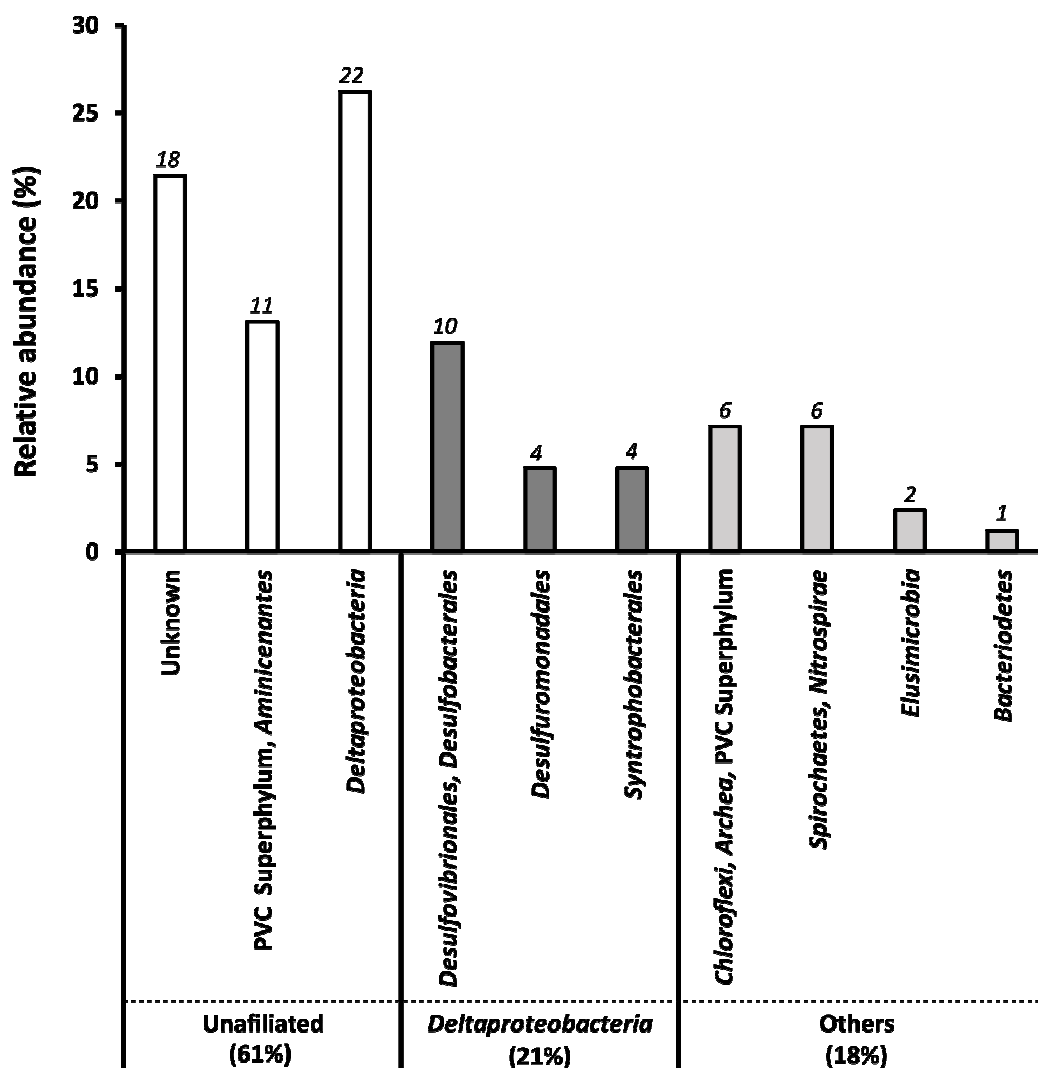


Fig. 7



# Marine mercury-methylating microbial communities from coastal to Capbreton Canyon sediments (North Atlantic Ocean)

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