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► To cite this version:

Alyssa Azaroff, Marisol Goñi, Claire Gassie, Mathilde Monperrus, Remy Guyoneaud. Marine mercury-methylating microbial communities from coastal to Capbreton Canyon sediments (North Atlantic Ocean). *Environmental Pollution*, 2020, 262, pp.114333. <10.1016/j.envpol.2020.114333>. <hal-02543039>

HAL Id: hal-02543039

<https://hal.science/hal-02543039v1>

Submitted on 22 Aug 2022

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1 **Submitted to Environmental Pollution**

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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⁻¹ ampicillin, 64 µg ml⁻¹ 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

425 **Acknowledgements**

426 This work was supported by the European Union and the Adour Garonne Water Agency.
427 Europe commits the New Aquitaine with the European Regional Development Fund. We
428 thank the crew of INSU-CNRS research vessel (Côte de la Manche). We gratefully thank
429 Carl Lamborg (University of California, Santa Cruz) for his proofreading and anonymous
430 reviewers for helpful criticize of the paper

431

432 **Compliance with ethical standards**

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

434

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Fig. 1

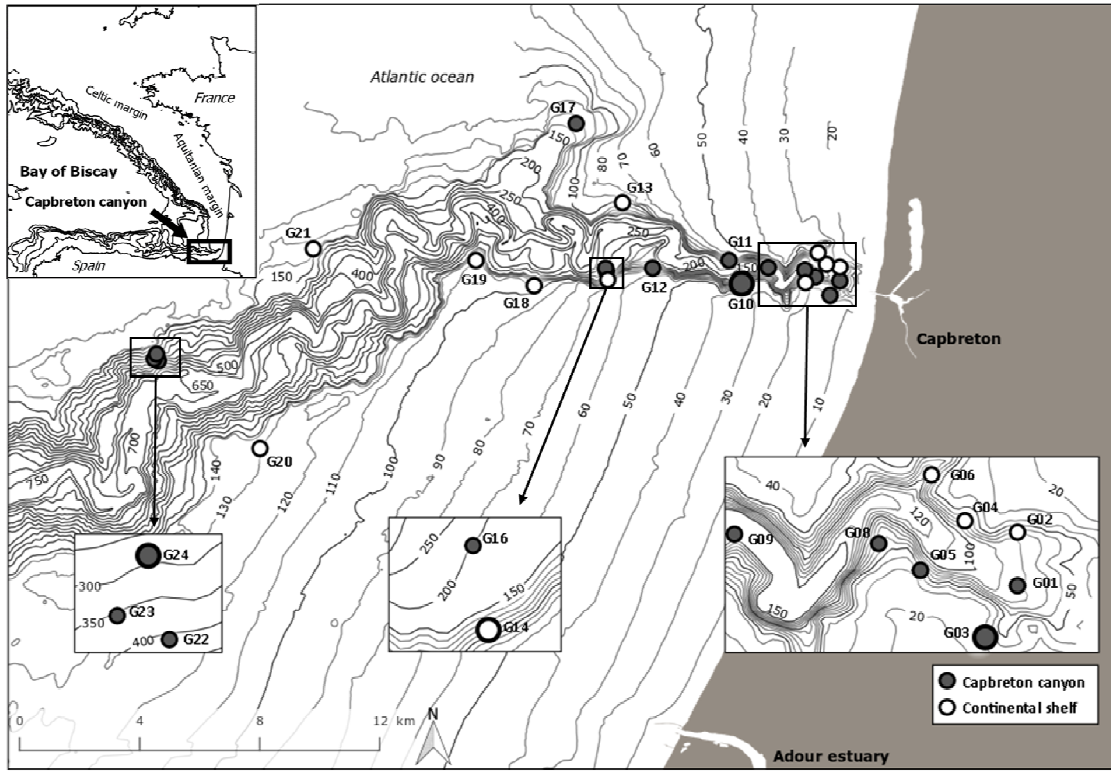


Fig. 2

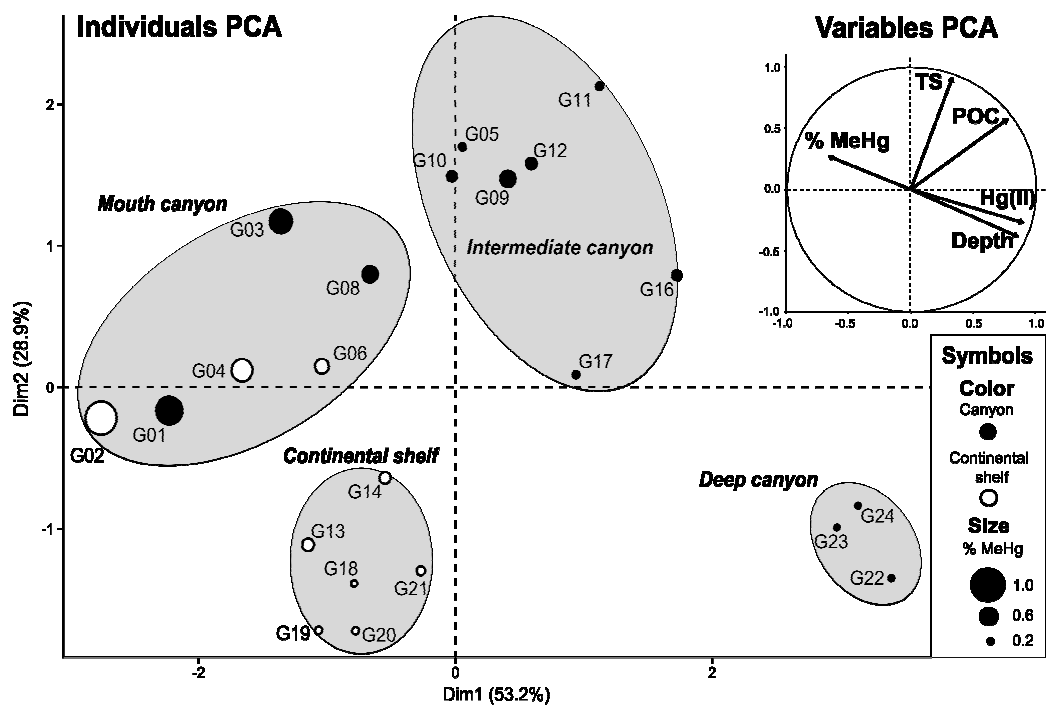


Fig. 3

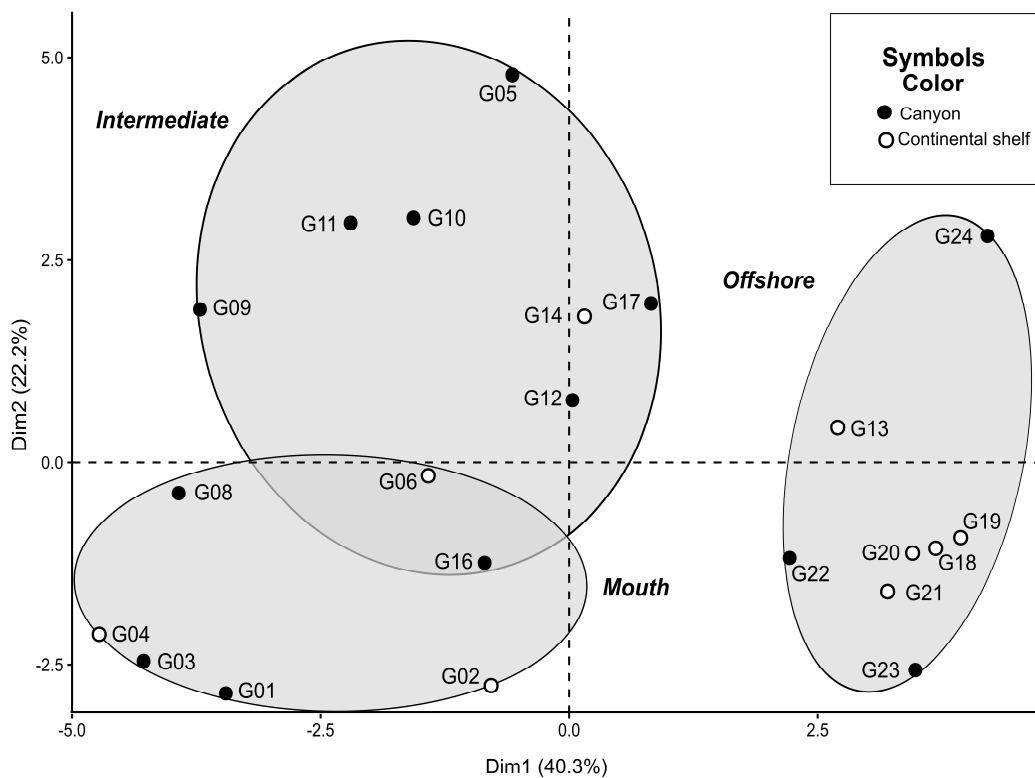


Fig. 4

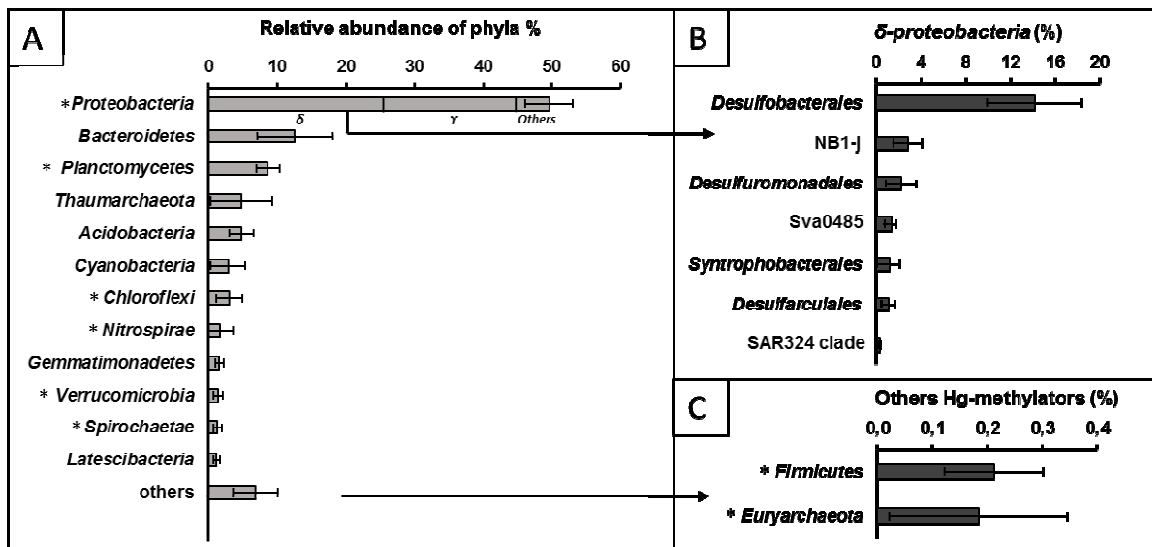


Fig. 5

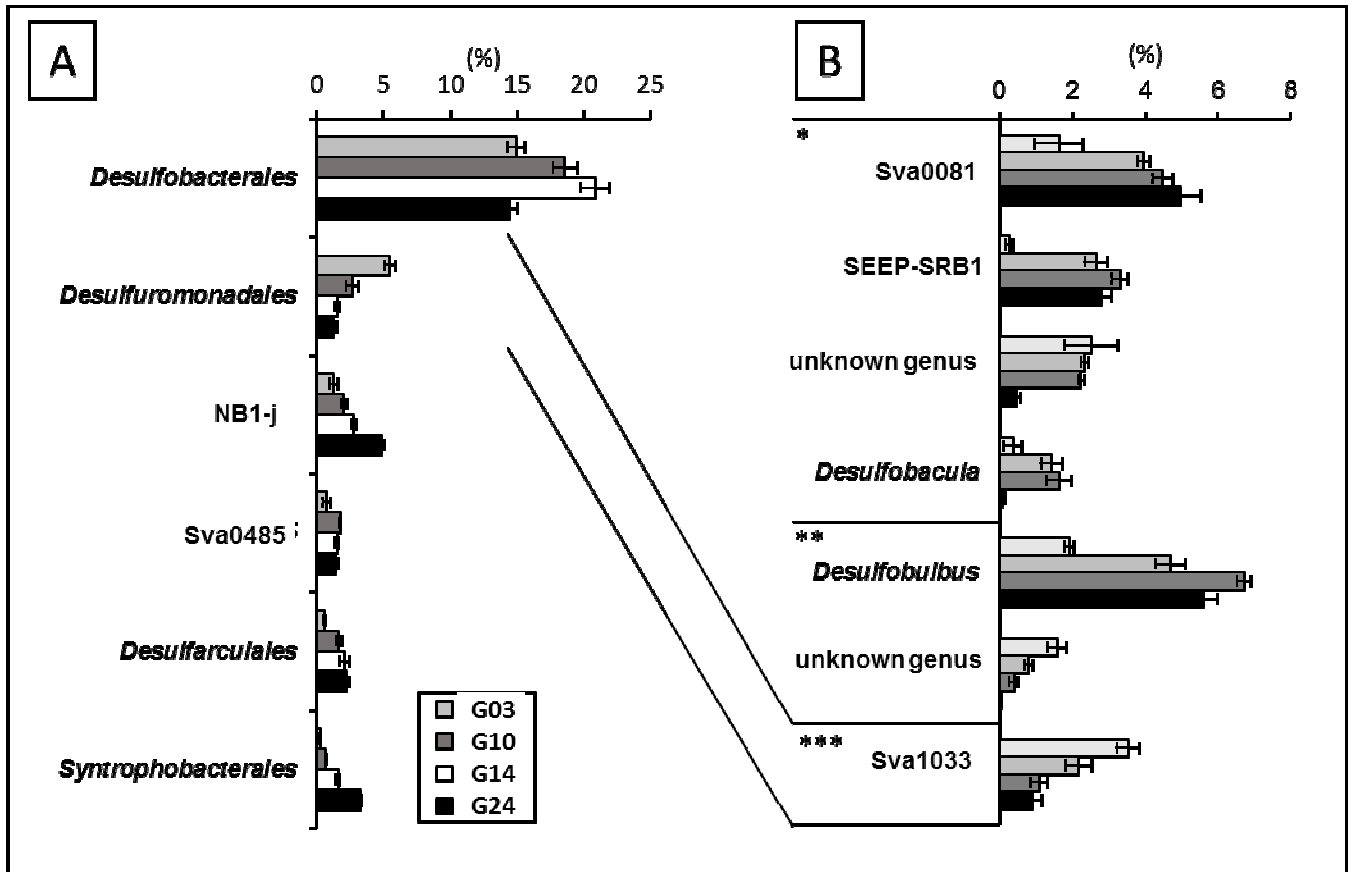


Fig. 6

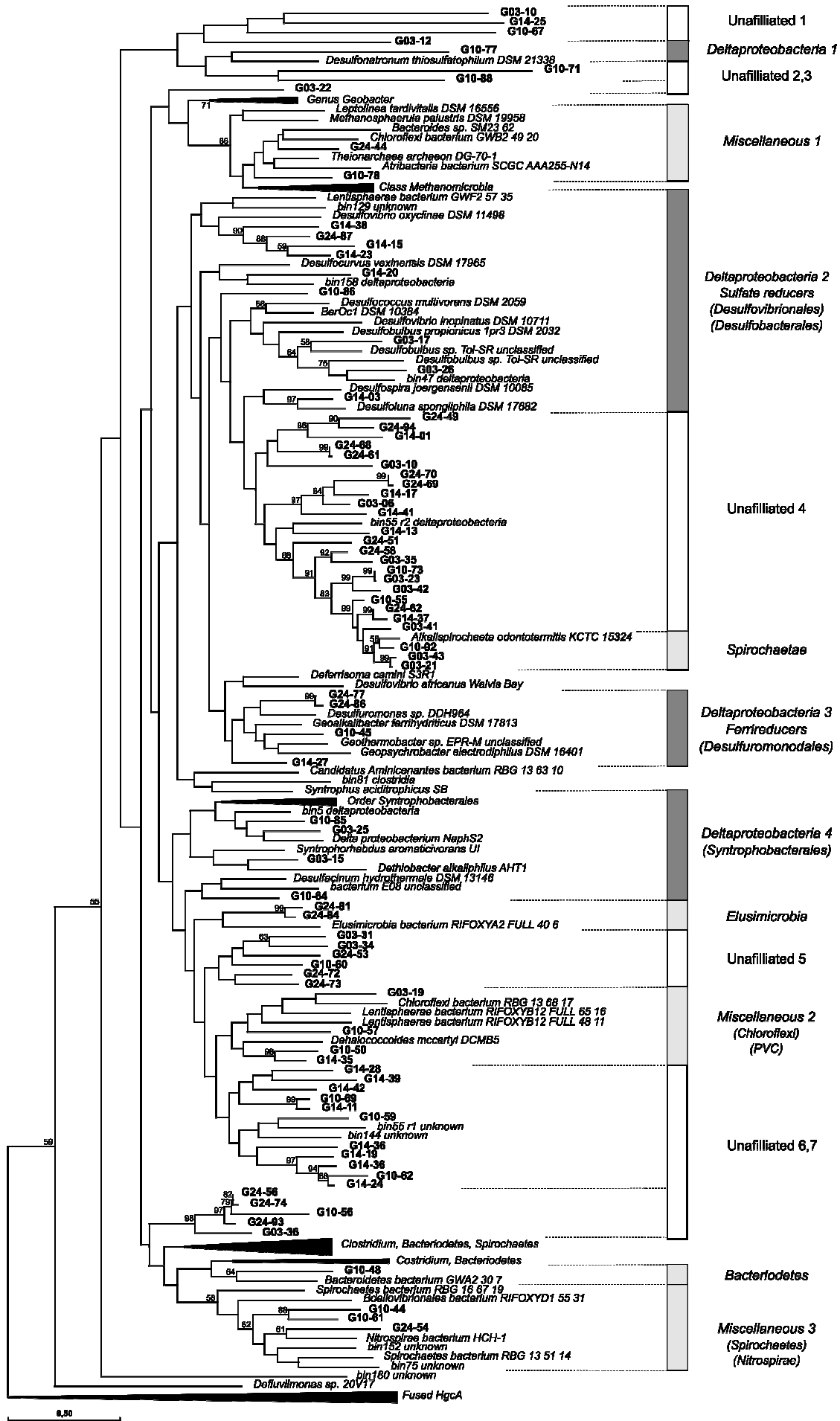
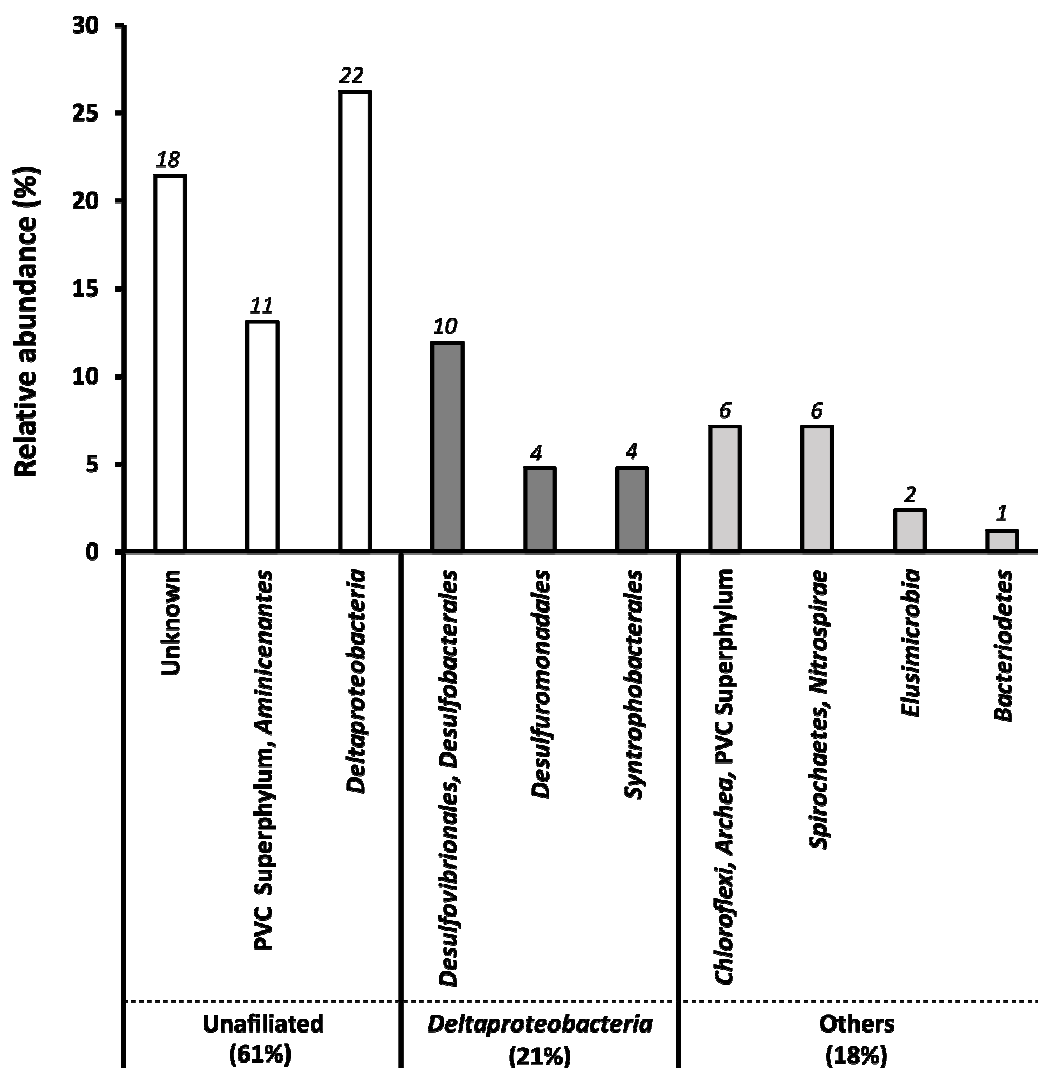


Fig. 7



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

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