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Abstract

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

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

Introduction

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

The biogeochemical cycling of Hg remains understudied in the open ocean, particularly on continental margins and associated slopes (Mason et al., 2012). Submarine 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

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

Chemical analysis

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

DNA extraction and bacterial community composition: 16S rDNA gene

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

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

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

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

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

Statistical analysis

Pearson correlations were calculated between chemical parameters using the R software environment version (http://www.r-project.org/). To remove redundancy of the chemical parameters data in order to avoid collinearity, we used the variance inflation factor (VIF) with the mctest package (Imdadullah et al., n.d.) in R using the Farrar-Glauber test. Multivariate analysis (Principal Component Analysis (PCA) were performed with FactomineR and Vegan package in R (Lê et al., 2008; Oksanen, 2007). PCA for 16S rDNA diversity analysis was performed at the phylum level because of PCA constrains, with relative abundance above > 0.05%. We used the factoextra package (Kassambara and Mundt, 2017) for extracting and 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 (<63µm), 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 ± 3.6% of the total relative abundance, followed by

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

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

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

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

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

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

Discussion

Geochemical conditions along Submarine canyons offer a diversity of ecological niches

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

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

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

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

Conclusion

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

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

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Compliance with ethical standards

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

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Fig. 1

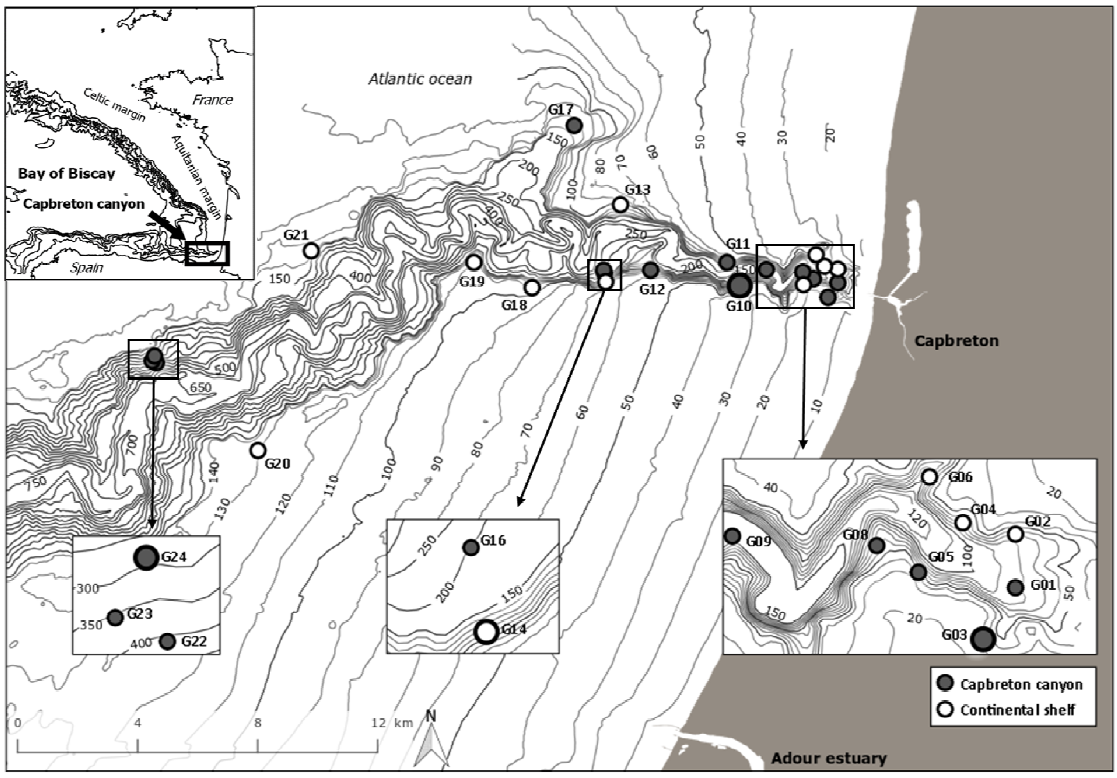

Atlantic ocean
France
Celtic margin
Armorican margin
Bay of Biscay
Capbreton canyon
Spain
Capbreton
Adour estuary
G17
G13
G21
G19
G18
G12
G11
G10
G20
G24
G23
G22
G16
G14
G06
G04
G02
G09
G08
G05
G01
G03
Capbreton canyon
Continental shelf
0
4
8
12 km
N

Fig. 2

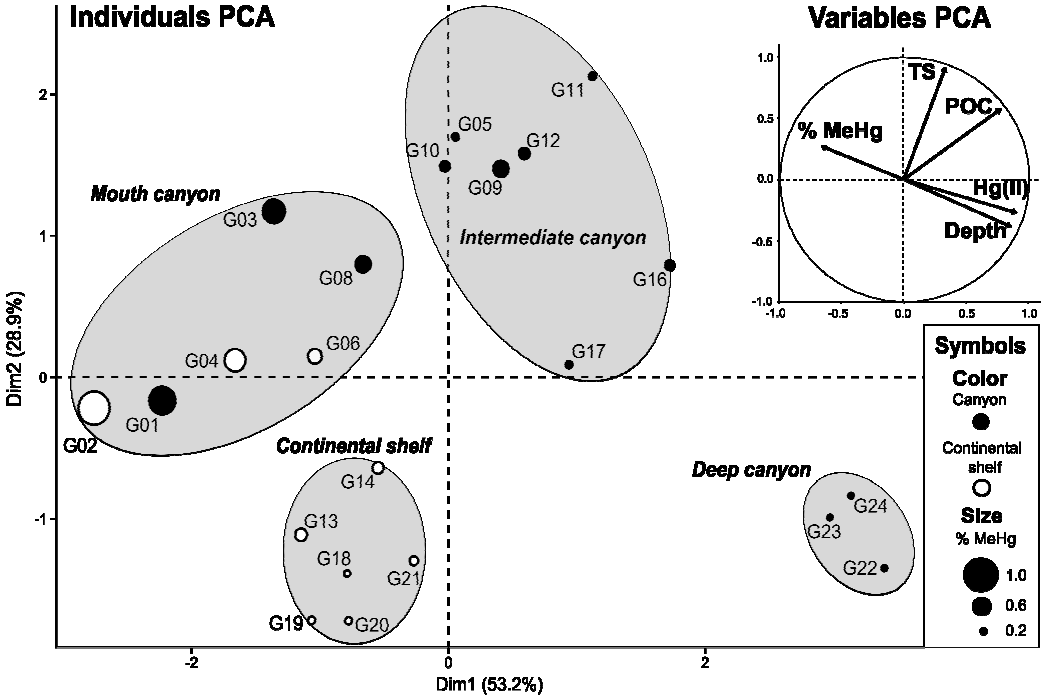

Individuals PCA
Variables PCA
Mouth canyon
Intermediate canyon
Continental shelf
Deep canyon
G01
G02
G03
G04
G05
G06
G08
G09
G10
G11
G12
G13
G14
G16
G17
G18
G19
G20
G21
G22
G23
G24
TS
POC
% MeHg
Hg(II)
Depth
Symbols
Color
Canyon
Continental shelf
Size
% MeHg
1.0
0.6
0.2
Dim2 (28.9%)
Dim1 (53.2%)

Fig. 3

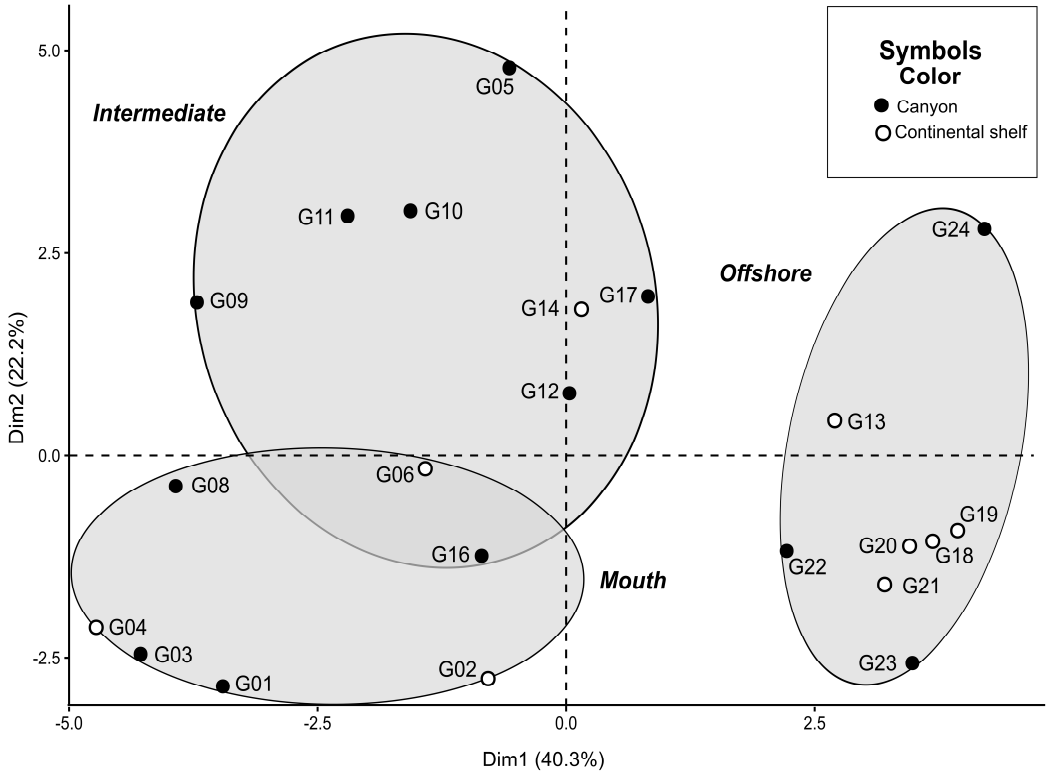

Symbols
Color
Canyon
Continental shelf
Intermediate
Offshore
Mouth
G01
G02
G03
G04
G05
G06
G08
G09
G10
G11
G12
G13
G14
G16
G17
G18
G19
G20
G21
G22
G23
G24
5.0
2.5
0.0
-2.5
-5.0
-2.5
0.0
2.5
Dim2 (22.2%)
Dim1 (40.3%)

Fig. 4

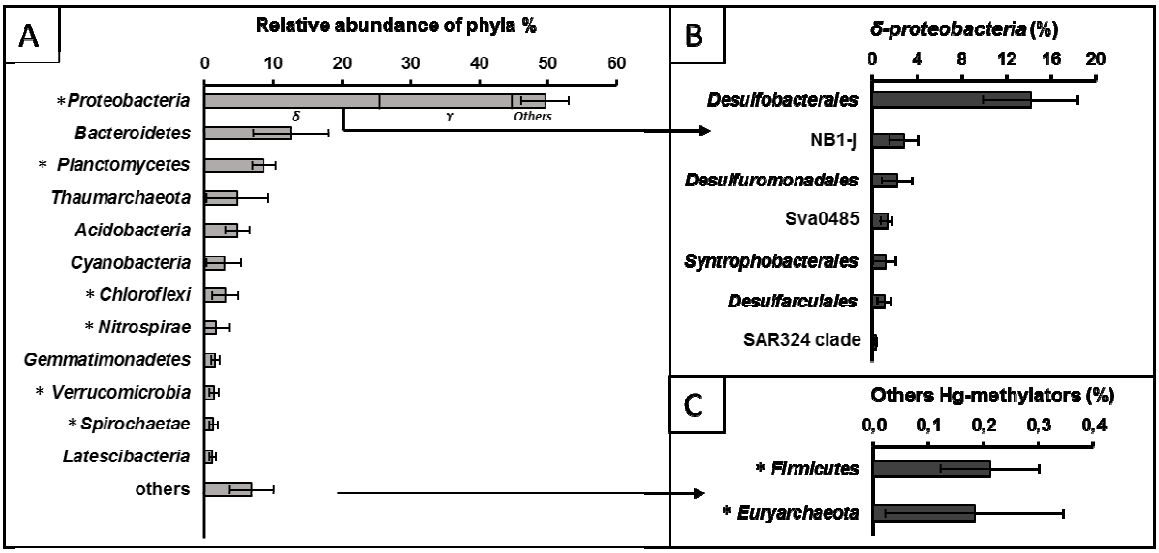

A
Relative abundance of phyla %
0
10
20
30
40
50
60
*Proteobacteria
δ
γ
Others
Bacteroidetes
* Planctomycetes
Thaumarchaeota
Acidobacteria
Cyanobacteria
* Chloroflexi
* Nitrospirae
Gemmatimonadetes
* Verrucomicrobia
* Spirochaetae
Latescibacteria
others
B
δ-proteobacteria (%)
0
4
8
12
16
20
Desulfobacterales
NB1-J
Desulfuromonadales
Sva0485
Syntrophobacterales
Desulfarculales
SAR324 clade
C
Others Hg-methylators (%)
0,0
0,1
0,2
0,3
0,4
* Firmicutes
* Euryarchaeota

Fig. 5

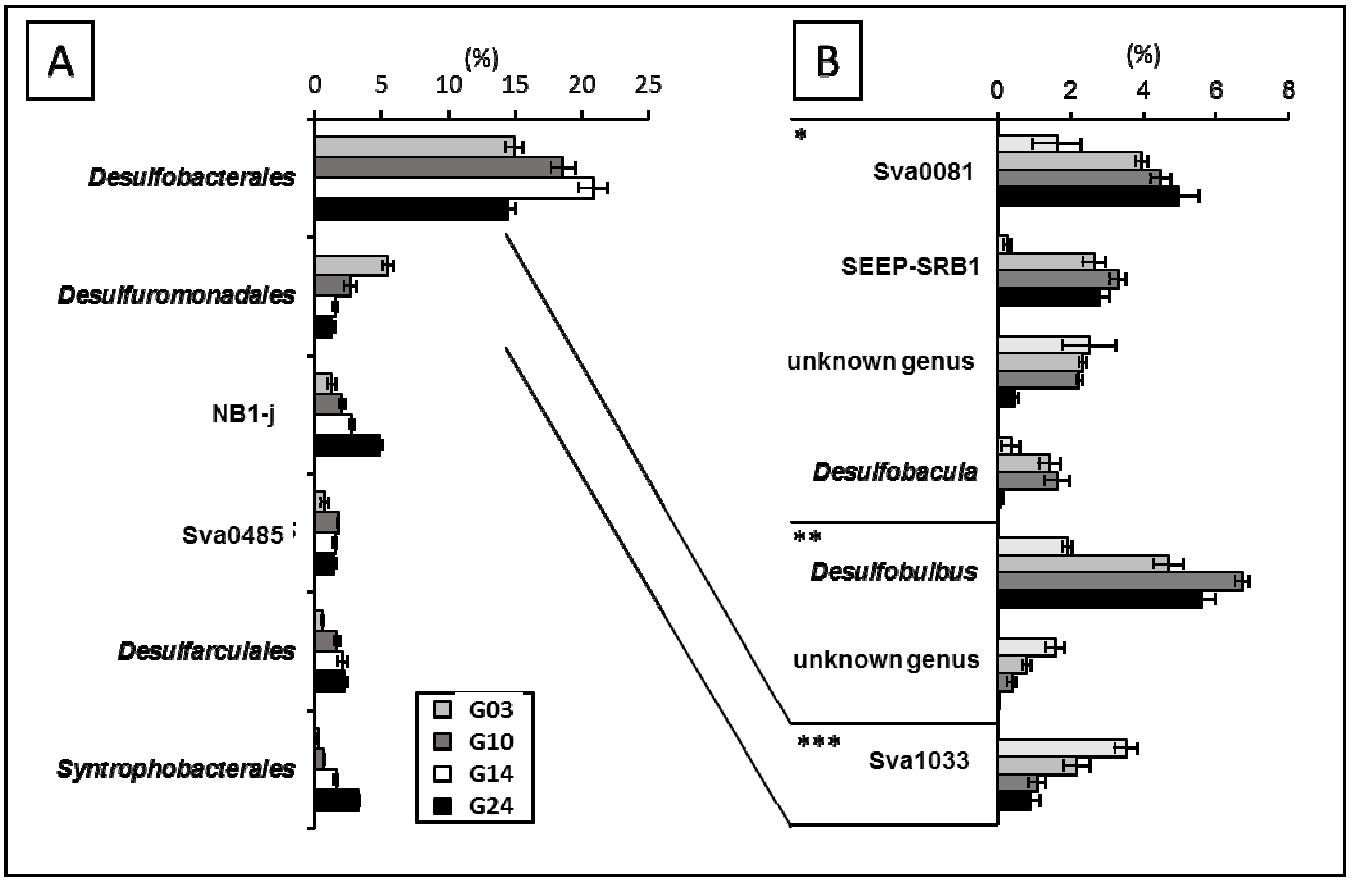

A
(%)
0
5
10
15
20
25
Desulfobacterales
Desulfuromonadales
NB1-j
Sva0485
Desulfarculales
Syntrophobacterales
G03
G10
G14
G24
B
(%)
0
2
4
6
8
*
Sva0081
SEEP-SRB1
unknown genus
Desulfobacula
**
Desulfobulbus
unknown genus
***
Sva1033

Fig. 6

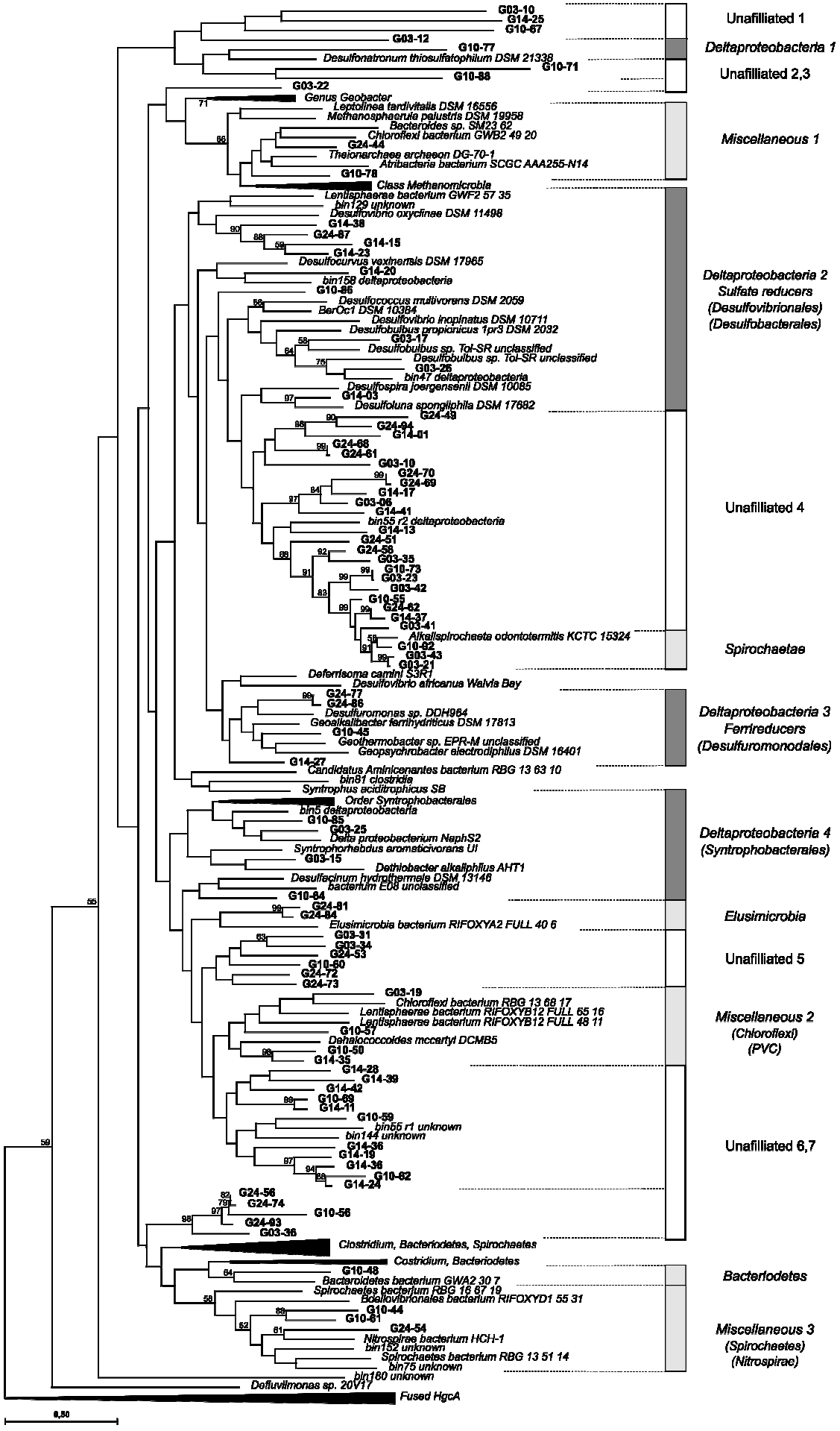
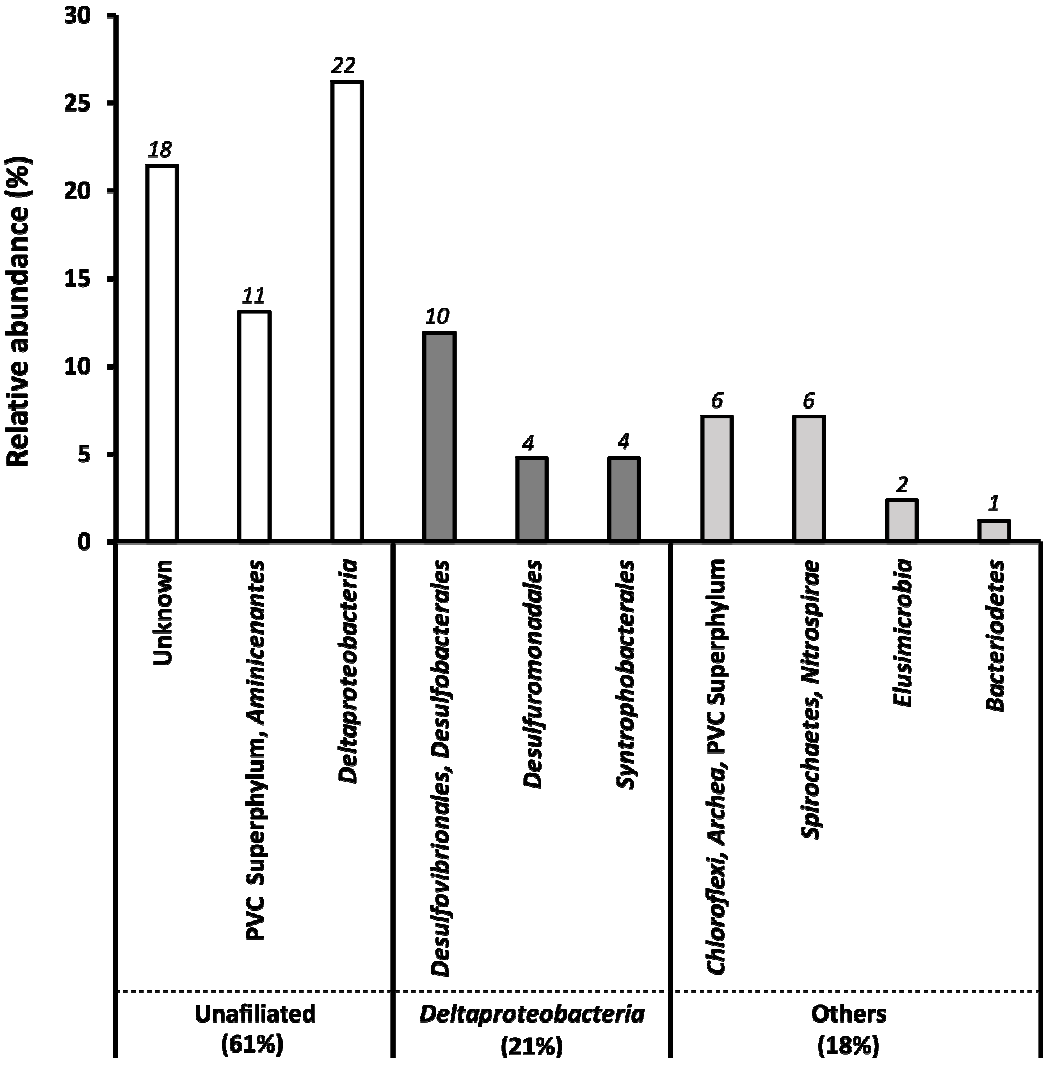

G03-10
G14-25
G10-67
G03-12
G10-77
Desulfonatronum thiosulfatophilum DSM 21338
G10-71
G10-88
G03-22
Genus Geobacter
Leptolinea tardivitalis DSM 16556
Methanosphaerula palustris DSM 19958
Bacteroides sp. SM23 62
Chloroflexi bacterium GWB2 49 20
G24-44
Thaumarchaea archaeon DG-70-1
Atribacteria bacterium SCGC AAA255-N14
G10-78
Class Methanomicrobia
Lentisphaerae bacterium GWF2 57 35
bin129 unknown
Desulfovibrio oxyclinae DSM 11498
G14-38
G24-67
G14-15
G14-23
Desulfocurvus vexinensis DSM 17965
G14-20
bin158 deltaproteobacteria
G10-86
Desulfococcus multivorans DSM 2059
BerOc1 DSM 10084
Desulfovibrio inopinatus DSM 10711
Desulfobulbus propionicus 1pr3 DSM 2032
G03-17
Desulfobulbus sp. Tol-SR unclassified
Desulfobulbus sp. Tol-SR unclassified
G03-26
bin47 deltaproteobacteria
Desulfospira joergensenii DSM 10085
G14-03
Desulfoluna spongiphila DSM 17682
G24-49
G24-94
G14-01
G24-66
G24-61
G03-10
G24-70
G24-69
G14-17
G03-06
G14-41
bin55 r2 deltaproteobacteria
G14-13
G24-51
G24-58
G03-35
G10-73
G03-23
G03-42
G10-55
G24-62
G14-37
G03-41
Alkalispirochaete odontotermitis KCTC 15324
G10-92
G03-43
G03-21
Deferrisoma camini S3R1
Desulfovibrio africanus Walvis Bay
G24-77
G24-86
Desulfuromonas sp. DDH964
Geoalkalibacter ferrihydriticus DSM 17813
G10-45
Geothermobacter sp. EPR-M unclassified
Geopsychrobacter electrodiphilus DSM 16401
G14-27
Candidatus Aminicenantes bacterium RBG 13 63 10
bin81 clostridia
Syntrophus aciditrophicus SB
Order Syntrophobacterales
bin5 deltaproteobacteria
G10-85
G03-25
Delta proteobacterium NaphS2
Syntrophorhabdus aromaticivorans UI
G03-15
Dethiobacter alkaliphilus AHT1
Desulfacinum hydrothermale DSM 13146
bacterium E08 unclassified
G10-64
G24-91
G24-84
Elusimicrobia bacterium RIFOXYA2 FULL 40 6
G03-31
G03-34
G24-53
G10-60
G24-72
G24-73
G03-19
Chloroflexi bacterium RBG 13 68 17
Lentisphaerae bacterium RIFOXYB12 FULL 55 16
Lentisphaerae bacterium RIFOXYB12 FULL 48 11
G10-57
Dehalococcoides mccartyi DCMB5
G10-50
G14-35
G14-28
G14-39
G14-42
G10-69
G14-11
G10-59
bin55 r1 unknown
bin144 unknown
G14-36
G14-19
G14-36
G10-62
G14-24
G24-56
G24-74
G10-56
G24-93
G03-36
Clostridium, Bacteriodetes, Spirochaetes
Costridium, Bacteriodetes
G10-48
Bacteroidetes bacterium GWA2 30 7
Spirochaetes bacterium RBG 16 57 19
Bdellovibrionales bacterium RIFOXYD1 55 31
G10-44
G10-61
G24-54
Nitrospirae bacterium HCH-1
bin152 unknown
Spirochaetes bacterium RBG 13 51 14
bin75 unknown
bin180 unknown
Defluviimonas sp. 20V17
Fused HgcA
0,50
Unafilliated 1
Deltaproteobacteria 1
Unafilliated 2,3
Miscellaneous 1
Deltaproteobacteria 2
Sulfate reducers
(Desulfovibrionales)
(Desulfobacterales)
Unafilliated 4
Spirochaetae
Deltaproteobacteria 3
Ferrireducers
(Desulfuromonodales)
Deltaproteobacteria 4
(Syntrophobacterales)
Elusimicrobia
Unafilliated 5
Miscellaneous 2
(Chloroflexi)
(PVC)
Unafilliated 6,7
Bacteriodetes
Miscellaneous 3
(Spirochaetes)
(Nitrospirae)

Fig. 7


Relative abundance (%)
30
25
20
15
10
5
0
18
11
22
10
4
4
6
6
2
1
Unknown
PVC Superphylum, Aminicenantes
Deltaproteobacteria
Desulfovibrionales, Desulfobacterales
Desulfuromonadales
Syntrophobacterales
Chloroflexi, Archea, PVC Superphylum
Spirochaetes, Nitrospirae
Elusimicrobia
Bacteriodetes
Unafiliated (61%)
Deltaproteobacteria (21%)
Others (18%)

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

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[(2)]CNRS/ UNIV PAU & PAYS ADOUR/ E2S UPPA, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux – MIRA, UMR 5254, 64000, Pau, FRANCE

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