A Genome Catalogue of Mercury-Methylating Bacteria and Archaea from Sediments of a Boreal River Faced by Human Disturbances

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Abstract

A Genome Catalogue of Mercury-Methylating Bacteria and Archaea from Sediments of a Boreal River Faced by Human Disturbances

Charlène Lawruk-Desjardins

Methylmercury (MeHg), the most bioavailable form of mercury (Hg), is a neurotoxin produced by anaerobic microbes. MeHg generated in aquatic sediments can be transferred to aquatic organisms and biomagnified along food webs, ultimately reaching fish consumers. This is a particular concern for rivers, as they are connective bodies for aquatic ecosystems and play crucial roles in the transport of nutrients. Moreover, rivers exhibit heightened susceptibility to environmental disturbances within their watershed, which have been linked to increased Hg-methylation. Rivers impacted by run-of-river dams hold specific significance, given the growing preference for these dam types over reservoir dams. Early studies have identified sulfate reducers, methanogens, and iron reducers as the main contributors to Hg-methylation. More recently, proteins encoded by the *hgcAB* genes have been found to confer the ability to methylate Hg. Recent metagenomic studies have expanded our knowledge of *hgcAB*-carrying lineages in the environment. Nevertheless, genome-based exploration of Hg-methylators remains limited, particularly in the context of river systems. To fill this knowledge gap, we created a genome catalogue of Hg-methylating microorganisms from the sediments of a river impacted by two run-of-river dams, logging, and a forest fire. We assessed the taxonomic and metabolic diversity of these putative Hg-methylators. Additionally, we assessed their abundance and diversity across sites along the river that were subject to different disturbances to gain insight into the ecological impact on Hg-methylators. For a deeper understanding of the environmental factors shaping Hg-methylator diversity, we juxtaposed the genome catalogue with the wider microbial community to which these methylators belong. We uncovered a unique and diverse assemblage of Hg-methylators dominated by members of metabolically versatile and fermentative *Bacteroidota*. This assemblage was particularly enriched in butyrate fermentative, carbon fixing and nitrite reducing microbes. We found that sites affected by press-like disturbances such as logging were particularly favorable to the establishment of a Hg-methylating niche. Lastly, we argue that the effects of watershed disturbances are likely not specific to Hg-methylators, but rather shared across the greater microbial community.

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I am very grateful for the incredible support of my supervisor, Dr David Walsh. His advice and input not only have made this work possible, but also helped me greatly to enrich my scientific intuition. His passion for all things microbial ecology fueled mine and made for very interesting, sometimes far-fetched brainstorming. I am also immensely thankful for my co-supervisor Dr Marc Amyot. His expertise in ecotoxicology was indispensable for this project, and despite his busy schedule, was always available to provide guidance. I would also like to thank my committee members, Dr Selvadurai Dayanandan and Dr Laurent Potvin-Trottier for their counsel.

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Special thanks to Veronicka Storck, my first mentor who welcomed me into Dr Walsh's lab and Dr Amyot's lab. Her incredible patience in teaching me the metagenomic approach to study Hg-methylating microorganisms paved the way for this project and allowed me to be independent in my research. Her constant encouragements empowered me to confidently approach this research subject. I am also thankful for Dominic Ponton and his input on biogeochemistry in regards to Hg-methylation. His readiness to answer any questions I had was truly appreciated.

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Contribution of Authors

Lise Millera Ferriz and Dominic Ponton sampled the sediments in 2018.

Veronicka Storck extracted the DNA from the sediment cores, prepared it and sent it for sequencing to Genome Quebec.

Lise Millera Ferriz and Dominic Bélanger carried out the mercury and biogeochemical testing on the sediment cores.

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Abbreviations

CA: Chutes Allard

C/N ratio: Carbon to Nitrogen ratio

Hg: Mercury

MeHg: Methylmercury

MAG: Metagenome-assembled Genome

OM: Organic Matter

PCoA: Principal Coordinates Analysis

RDC: Rapide-des-Coeurs

rOTU: ribosomal Operational Taxonomic Unit

TAD80: Truncated Average Depth

Introduction

The fate of mercury (Hg) in the environment is the subject of widespread research due to its capacity for biomagnification in food webs in its organic form (methylmercury, MeHg), coupled with its severe impact on both animal and human health. Instances of acute or chronic exposure to methylmercury have been associated with neurotoxic and harmful reproductive effects, documented across various animal species, including humans (Depew et al., 2012; Hong et al., 2012). The Hg cycle is complex due to its chemistry, and Hg pollution mitigation requires robust understanding of this cycle specific to the ecosystem affected (Ullrich et al., 2001). The main source of exposure to MeHg for humans is through consumption of contaminated fish (Ferreira-Rodríguez et al., 2021), thereby emphasizing the need to focus research on Hg contamination in aquatic ecosystems. Potential sources of external Hg in aquatic environments include atmospheric deposition, precipitation, and runoff (Ullrich et al., 2001). In freshwater ecosystems, sediments represent reservoirs of Hg and are the main sites of Hg transformation to MeHg through microbial activity (Hong et al., 2012; Regnell & Watras, 2019; Ullrich et al., 2001). Lakes and rivers with impacted watersheds are more susceptible to Hg contamination, because the release of organic matter (OM) into the water body increases microbial activity and allochthonous Hg input (De Bonville et al., 2020). Indeed, inorganic Hg exhibits a remarkably high affinity for organic matter particles (Bravo et al., 2017), leading to sedimentation of allochthonous Hg. Additionally, microbial activity in freshwater sediments is known to be influenced by the source and type of organic matter input (Pusch et al., 1998).

Rivers play a vital role in providing a diverse range of ecosystem services, including the transportation of nutrients and organic matter along the land-water continuum. The complex microbial communities within river sediments are highly sensitive to human impact and play a crucial role in various biogeochemical cycles (Gibbons et al., 2014). Consequently, anthropogenic contamination of rivers can have far-reaching consequences on other ecosystems and the overall ecological health of a region (Sofi et al., 2020). Hydroelectric dams have long been studied in relation to their impact on the Hg cycle (Potter et al., 1975) because of the increased Hg mobilization created by flooding of large areas. Run-of-river dams have become a popular alternative to large reservoir dams due to their ability to harness a river's natural flow to generate energy without the need for extensive flooding of surrounding areas (Anderson et al., 2015). Nonetheless, the potential environmental and social repercussions of run-of-river dams may surpass those of reservoir dams, owing to the compounded effects arising from their escalating proliferation and widespread distribution (Lange et al., 2019). Despite their significance, sediment

microbial communities of such impacted rivers remain understudied, and research on mercury methylators in these environments is even scarcer.

A wide diversity of microorganisms is involved in Hg-methylation. Traditionally, our understanding of mercury methylating groups was confined to experimental testing of MeHg production by microbial isolates in laboratory settings, which identified methanogens, sulfate-reducing bacteria, and iron-reducing bacteria as the primary contributors to Hg-methylation (Choi et al., 1994; Compeau & Bartha, 1985; Kerin et al., 2006; Yu et al., 2013). However, the discovery of *hgcAB* as the genetic basis for Hg-methylation (Parks et al., 2013) provided a more comprehensive view of the taxonomic breadth of Hg methylators in the environment. For instance, *hgcAB* has been found in various taxonomic groups, including members of the *Planctomycetota-Verrucomicrobiota-Chlamydiota* (PVC) superphylum, *Bacteroidota*, *Firmicutes*, and *Chloroflexota*, albeit sporadically (Capo, Peterson, et al., 2023; Cooper et al., 2020). Despite this significant advancement, many aspects regarding the factors that regulate Hg-methylating community composition and the abundance of Hg-methylators in the environment remain unknown. Therefore, deepening our knowledge of the taxonomic and functional diversity of Hg-methylators within their ecological context is essential for better predicting and understanding the role of Hg-methylators in the Hg cycle.

Shotgun metagenomic approaches have revolutionized our ability to investigate microbial communities inhabiting diverse environments, overcoming the limitations imposed by the culturing of microorganisms (Quince et al., 2017). In particular, analysis of metagenome-assembled genomes (MAGs) has been instrumental in unveiling microbial diversity (Baker et al., 2021) and inferring the metabolic capacities of uncultured microorganisms (Vavourakis et al., 2018). Given the current uncertainties surrounding the environmental factors regulating Hg-methylating communities, metagenomic studies have become even more relevant to study the diversity of Hg-methylating microorganisms. To date, thirty-two studies have employed metagenomics to investigate Hg methylator diversity (**Table S1**), with two of them specifically focusing on Hg-methylating communities in rivers (Leclerc et al., 2021; Millera Ferriz et al., 2021). These studies utilized approaches based on *hgcA* gene diversity and abundance in metagenomes. In contrast, twelve studies have employed MAGs to study Hg-methylating communities. Collectively, these studies recovered *hgcA* containing MAGs from metagenomes obtained from diverse environments, including marine, soil, brackish water, lakes, permafrost, mangroves, aquifers, sludges, hot springs, contaminated sites, wetlands, built environments, endosymbionts, and reservoirs (Capo, Feng, et al., 2022; Jones et al., 2019; Langwig et al., 2022; Lin et al., 2021;

Jibao Liu et al., 2022; McDaniel Elizabeth et al., 2020; Peterson, Krabbenhoft, et al., 2023; Peterson et al., 2020; Peterson, Poulin, et al., 2023; Vigneron et al., 2021; Zhang et al., 2023; Zheng et al., 2022). However, among the thousand *hgcA* containing MAGs collectively obtained, only three were identified in freshwater river systems, specifically limited to urban waterways or city moat rivers (McDaniel Elizabeth et al., 2020). Nevertheless, through genome-resolved metagenomics, these studies expanded our understanding of the ecological niches, phylogenetic breadth, and metabolic diversity of putative Hg-methylators. Notably, a few studies provided evidence of potential horizontal gene transfer (HGT) of Hg-methylating genes (Jibao Liu et al., 2022; McDaniel Elizabeth et al., 2020; Zhang et al., 2023; Zheng et al., 2022).

In this thesis, I focused on mercury-methylating microbial communities along a length of the St. Maurice River in Québec, Canada, where two run-of-river dams were recently constructed, and the Atikamekw community of Wemotaci is located. This length of the river has experienced several environmental disturbances, including wildfires and logging, which have the potential to impact the Hg cycle by causing the release of OM and mercury into the river (Millera Ferriz et al., 2021). Additionally, artificial wetlands were constructed to restore lost wetland habitat following dam construction. However, such conservation efforts have been associated with increased MeHg production in certain cases (Hsu-Kim et al., 2018; Obrist et al., 2018). A previous study on the St. Maurice River concluded that the interactions between these disturbances have the potential to favour MeHg production by microorganisms (Millera Ferriz et al., 2021). Therefore, the St. Maurice River represents a relevant study system for broadening our knowledge on the global diversity of Hg-methylators. The first objective of the study was to create a genome catalogue of mercury methylators from human-impacted river sediments, with the purpose of expanding our general understanding of the taxonomic diversity of mercury methylators. Secondly, we investigated the functional diversity of Hg-methylators and assess their potential enrichment in metabolic capacities. Lastly, we aimed to compare the ecological drivers for the structure of Hg-methylating assemblages with those shaping the broader microbial community structure.

Materials and Methods

Sampling and geochemical profiling

Sediment cores were sampled in August 2018 at six sites along a 40 km stretch of the St. Maurice River, between Wemotaci and the Reservoir Blanc, in Mauricie, Quebec, Canada. This section includes two run-of-river dams, Chutes Allard (CA) and Rapide-des-Coeurs (RDC). Furthermore, the watershed of this segment encompasses a significant logging site, a region spanning 180 km^2

that was impacted by a wildfire in 2010, and artificial wetlands established as a response to the loss of natural wetlands resulting from the flooding necessitated by run-of-river dam construction. Six sampling sites were selected according to their location related to different disturbances of the watershed (**Fig. 1A**).

Sediment cores were retrieved using a 3.5L Ekman Grab, cut longitudinally and then cut in 1 cm long sections. For each cm, one half was used for geochemical analysis and the other half was used for metagenomic analysis. As such, 10 samples were taken at each 1 cm interval in each core, to a depth of 9 cm. For geochemical profiles, MeHg was measured by cold vapor atomic fluorescence spectrophotometry (CVAFS; series 2700, Tekran™, Canada) according to the EPA method 1630 and total Hg (THg) was measured by Direct Mercury Analysis system (DMA-80). For MeHg, analytical quality was checked using certified reference material IAEA-158 with a certified value of 1.4 ± 0.4 ng g^{-1}. For THg, analytical quality was verified using the certified reference material TORT-2 and SO-2 with certified values for Hg of respectively 270 ± 60 ng g−1 and 82 ± 9 ng g^{-1}. Organic matter proportion (% OM) of the sediment sample was obtained by using loss on ignition (LOI) method by heating for 2h at 550 ºC (Dean, 1974). Carbon on nitrogen ratio (C/N ratio) was obtain by dividing the concentration of atomic carbon on atomic nitrogen in the sample, which were measured by CHNS-O Element Analyzer (EA-1108 model, Fisons©) calibrated with acetanilide.

DNA extraction, sequencing, and metagenome assembly

DNA was extracted according to the PowerSoil®DNA Isolation Kit manufacturer's manual. DNA was quantified with QubitTM and sent to Genome Québec for shotgun sequencing using the IlluminaNovaSeq 6000 S4 PE150 technology. Raw reads were trimmed with Trimmomatic v0.38 using a minimum length threshold of 36 bp and a base quality threshold of 15 (Bolger et al., 2014). For primary diversity assessment, trimmed reads were classified in ribosomal gene operational taxonomic units (rOTUs) and taxonomically identified by profiling *rplB* for ribosomal protein L2 in metagenomes using SingleM (Woodcroft Ben J). The rOTUs were clustered at 97% nucleotide sequence identity. Trimmed reads were co-assembled with Megahit v.1.2.7 using a k-mer list of 27,37,47,57,67,77,87 (Li et al., 2016). Reads were mapped to the co-assembly using Burrows-Wheeler Aligner (bwa-mem algorithm) (Li, 2013). Co-assembly scaffolds coverage in each metagenomic sample was computed using SAMtools (Danecek et al., 2021) and the jgi_summarize_bam_contig_dept script provided by MetaBAT (Kang et al., 2019).

HgcA *detection in metagenomes*

Co-assembly scaffolds were annotated by MetaGeneMark for gene prediction (Besemer & Borodovsky, 1999). *HgcA* sequences were detected in annotated scaffolds using a hidden Markov-Model provided the HG-MATE database v.1 (Capo, Peterson, et al., 2023) with HMMER v.3.2.1 (Finn et al., 2015). We selected *hgcA* hits with a bitscore of > 300 and e value < 1e-50 for the full sequence (McDaniel Elizabeth et al., 2020). We then dereplicated these sequences to avoid redundancy with CD-HIT using 95% sequence identity (Fu et al., 2012), and kept the best representative for abundance and taxonomy analysis. Abundance of *hgcA* sequences in samples were calculated by summing the coverage of each *hgcA* containing scaffold and dividing by total scaffold coverage in a sample. Taxonomy was assigned by protein alignment to Hg-MATE database (Capo, Peterson, et al., 2023) using DIAMOND blastp command (Buchfink et al., 2015) with the option sensitive, 40% identity cutoff, query cover of ≥ 70% and an e value cut-off of 1e-20.

Genome catalogue generation and functional annotation

Co-assemblies were binned using MetaBAT v.2.14 (Kang et al., 2019), and the quality of produced bins was checked using the lineage_wf pipeline of CheckM v.1.1.3 (Parks et al., 2015). We selected medium to high quality MAGs for further analyses, according to the standard set by Bowers et al. (2017) (contamination < 10% and completeness > 50%). We dereplicated the selected 1,023 MAGs using average nucleotide identity of > 95% with dRep v.3.4 (Olm et al., 2017) and selected the best representative MAG for each species, resulting in 850 MAGs. MAGs were mapped back to metagenomes with BBmap, using a minimum alignment score of 0.98. MAG abundances in each metagenome were quantified by computing the truncated average sequencing depth (TAD_{80}) (Rodriguez et al., 2020). We normalized these values by average genome size of each co-assemblies with MicrobeCensus (Nayfach & Pollard, 2015). When assessing the abundance of taxonomic groups or metabolic pathways of the catalogue in co-assemblies, we used a presence/absence metric, so that if a MAG with said taxonomy or metabolic pathway was present in a co-assembly, its abundance was set at 1. Taxonomy of MAGs was determined using GTDB-tk v.2.1 software (Chaumeil et al., 2022), using the commands identify, align, infer, and classify.

MAGs were searched for genes using Prokka v.1.14.5 (Seemann, 2014), and their function were annotated based on KEGG Orthology using KofamScan (Aramaki et al., 2019) to estimate metabolic capabilities of our putative methylators. The presence of different fermentation pathways was determined by using the genes requirement used by Kirchman et al. (2014).

The *hgcA* genome catalogue was created by selecting dereplicated MAGs that had binned *hgcA* containing scaffolds, resulting in a catalogue of 145 *hgcA* containing MAGs. To confirm that these were putative Hg-methylators, we looked for the presence of a ferredoxin [4Fe-4Fe] annotated by KofamScan downstream of *hgcA* in each MAG (*i.e*. putative *hgcB* gene), within two open reading frames (ORFs). If none was found, we looked if the binned *hgcA* sequence fell within a known clade of Hg-methylator in phylogenetic analysis. A phylogenetic tree was constructed with the alignment of our binned dereplicated *hgcA* sequences with the multiple sequence alignment of *hgcA* provided by McDaniel Elizabeth et al. (2020).

Statistical analyses

To determine if the mean %OM, C/N ratio and %MeHg/THg varied by sampling sites, we first conducted Levene's test on our data to assess variance homoscedasticity between sites. If the null hypothesis was not rejected ($p > 0.05$), an ANOVA was conducted to test if the effect of different sampling site had a significant effect on the measure analyzed. If the null hypothesis was rejected ($p \leq 0.05$), we used Tukey's test to assess which groups differed from each other. If the null hypothesis was rejected in Levene's test, we conducted a Kruskal Wallis test instead of an ANOVA and a Dunn's test instead of the Tukey's test for the same purpose as stated above.

The same statistical analysis was used to determine if the number of *hgcA* gene copies and the number of *hgcA* containing MAGs significantly varied by sampling site. To visualise the differences in microbial community structure between sites, we conducted a principal coordinates analysis (PCoA) on Jaccard distance between samples. Finally, to assess if the two PCoA conducted for each genome catalogue were correlated, we computed the RV coefficient.

Results

Environmental conditions in the sediments along the St. Maurice River

Sediment cores were collected at six locations along a section of the St. Maurice River (**Fig. 1A**). Sampling sites were selected according to their position in relation to the different disturbances that affect the river. One site was located 7 km upstream of the first run-of-river dam, and since this location was not affected by disturbances it was selected as a reference site (REF-2). Downstream of the reference site was the site located in the constructed channels (WEM-4). Two were in the flooded area of the Chutes Allard dam and were near to the location of a wildfire (CA-10 and CA-6). CA-10 was located in the middle of a flooded bay with abundant periphyton and macrophytes, whereas CA-6 was located in a deeper section of the river where sediments

accumulate. The final two sites were located in the flooded area of the Rapide-des-Coeurs dam and near to a logging area (RDC-10 and RDC-14).

To understand the ecological conditions of sediments in relation to the disturbances of the watershed, we compared the biogeochemical profiles of the six sediment cores by using %OM, C/N ratio and percentage of total Hg that is methylated (%MeHg/THg). C/N ratio is an indication of the provenance of the organic matter: a higher C/N ratio in sediments suggest that it contains a higher proportion of allochthonous organic matter from the watershed (Millera Ferriz et al., 2021). Two of the four sites that were flooded by a run-of-river dam (CA-6 and RDC-14) had significantly higher %MeHg/THg than the other sites (**Fig.1B**). These two sites also had the highest %OM along with another flooded site (CA-10). Finally, CA-6 had the highest C/N ratio. These three sites have the highest potential for Hg-methylation, especially CA-6 because of a high C/N ratio, %OM and %MeHg/THg. The reference site (REF-2), the site located in the constructed channels (WEM-4) and one of the sites disturbed by dam construction and logging (RDC-10) had low %OM, %MeHg/THg and C/N ratio compared to the 3 other disturbed sites mentioned.

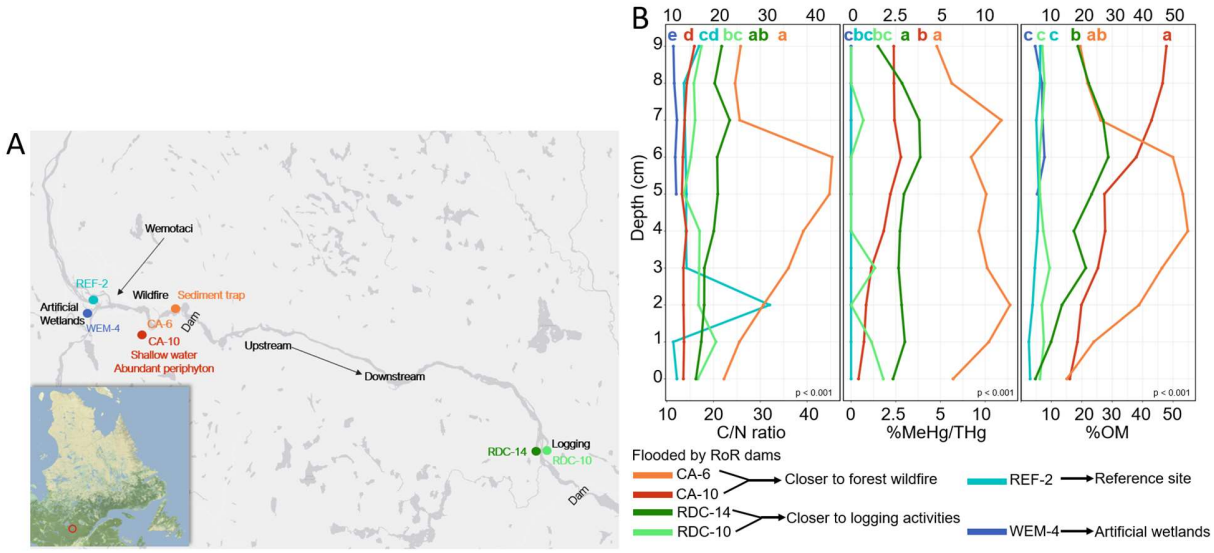

Figure 1. **(A)** Map of the section of the St. Maurice River with the 6 sampling sites where sediment cores were collected along with the location of watershed disturbances. **(B)** OM and Hg related variable profiles in sediment cores along a depth gradient. Different letters indicate statistically different groups according to Dunn's test, colour-coded according to sampling site.

Microbial diversity within river sediment metagenomes

To evaluate whether metagenome assembly and binning resulted in significant loss of diversity, we first assessed microbial community diversity in our metagenomes by profiling the rOTUs in the

unassembled metagenome reads prior to these steps. Analysis of the rOTUs revealed a rich diversity of prokaryotic microorganisms in the sediment samples with the majority of rOTUs assigned to Bacteria (87%) and smaller fraction assigned to Archaea (11.2%) (**Fig. S1A**). In total, we identified 64 phyla in the metagenomes, with rOTUs assigned to *Proteobacteria* (15.5%), *Acidobacteriota* (9.9%), and *Chloroflexota* (6.3%) being the most abundant (**Fig. 2C**). A significant portion of the rOTUs (12.1%) could not be identified at the phylum level, suggesting the presence of novel lineages in our metagenomes.

HgcA *diversity in river sediment metagenome co-assemblies*

To identify *hgcA* diversity in sediments, we first generated metagenome assemblies. To enhance assembly sequencing depth and improve the contig binning outcome for higher quality metagenome-assembled genomes (MAGs), we opted for co-assembly over single assembly. We generated 6 co-assemblies, representing the 6 sediment cores. This approach increases the likelihood of obtaining *hgcA* containing MAGs of sufficiently high quality (Albertsen et al., 2013).

We detected *hgcA* on 1,909 metagenomic scaffolds, yielding 1,560 dereplicated *hgcA* sequences. We taxonomically identified the *hgcA* sequences found in the 66 metagenomes to get a general overview of the diversity of mercury methylators. The majority (52.2%) could not be taxonomically assigned (**Fig. 3**). The most abundant phyla were *Desulfobacterota* (11.5%), *Chloroflexota* (11.0%), *Bacteroidota* (7.9%) and *Halobacteriota* (5.4%). The high abundance of unassigned *hgcA* sequences suggests the presence of Hg-methylating lineages that were previously not identified as putative Hg-methylators in our assemblies.

Construction of a river sediment genome catalogue

To investigate the microbial metabolic diversity in freshwater sediments, we reconstructed composite genomes from microbial populations in the sediments. This was achieved by binning our 6 co-assemblies, which resulted in 1,023 medium to high-quality MAGs. To remove redundancy, we dereplicated these MAGs and obtained 850 MAGs, which constituted our river sediment genome catalogue. Similar to our first diversity assessment, our catalogue was dominated by *Proteobacteria* (7.3% *Alphaproteobacteria* and 5.6% *Gammaproteobacteria*) and *Acidobacteriota* (11.8%), but the abundance of MAGs from *Bacteroidota* (10.2%) surpassed those from *Chloroflexota* (9.6%) (**Fig. 2C, Fig. S2A**). Overall, the phylum-level diversity in rOTUs and in MAGs was similar, with the most abundant phyla found in both (**Fig. 2C**). Some phyla were more represented in the MAGs than in the rOTUs (*Patescibacteria* and *Desulfobacterota*), and rOTUs had captured some eukaryotic diversity whereas MAGs did not (**Fig. 2A, Fig. S1B**). Thus, we

considered that our genome catalogue had the potential to capture most of the diversity of Hg-methylating bacteria and archaea.

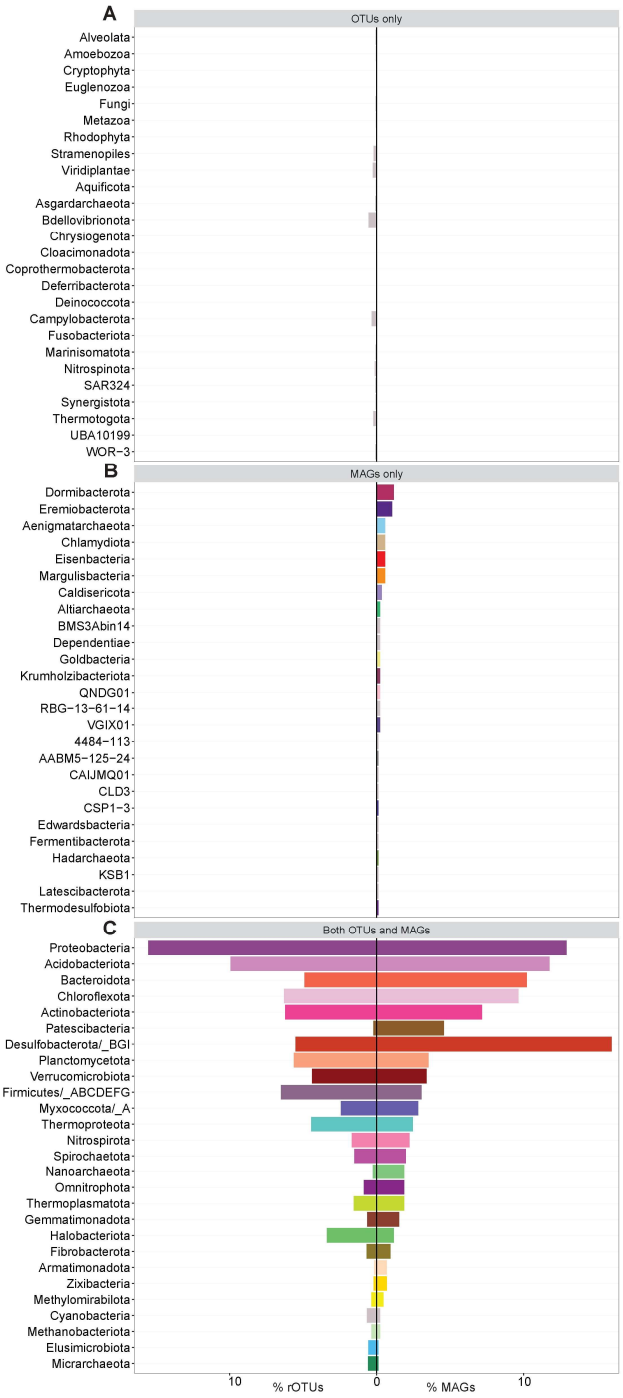

Figure 2. **(A)** Abundance of phyla only represented in all rOTUs extracted from metagenomes prior to assembly and binning. **(B)** Abundance of phyla only represented in MAGs generated from all co-assemblies. **(C)** Comparison of relative abundance of phyla found in both rOTUs and MAGs.

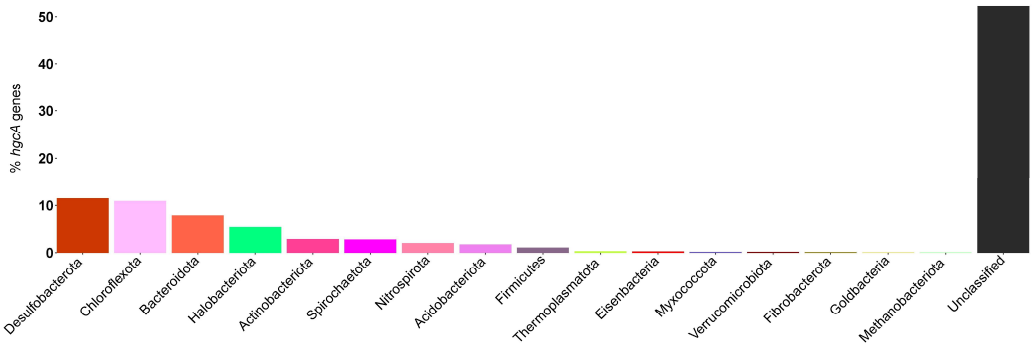

Figure 3. Proportion of hgcA hits with bitscore > 300 found in all co-assemblies belonging to different phyla, with archaeal phyla colored in green shades.

HgcA *diversity in the river sediment genome catalogue*

Since our river sediment genome catalogue accurately represented microbial diversity in the sediments, we proceeded to search for binned *hgcA* containing scaffolds to construct our putative Hg-methylating genome catalogue. We found 145 *hgcA* containing MAGs, representing 17% of the river sediment genome catalogue. The 145 *hgcA* carrying MAGs contained 154 *hgcA* containing scaffolds in total, of which 56 were previously taxonomically unclassified. The presence of a ferredoxin gene downstream of the *hgcA* gene in a MAG or the phylogenetic position of the binned *hgcA* falling within clades of known Hg-methylators confirmed that all the 145 *hgcA* MAGs were putative Hg-methylators (**Fig. S3**). We also uncovered a large amount of evolutionary novelty for putative Hg-methylators, as all of our *hgcA* containing MAGs constituted novel candidate species, and close to 40% (56 *hgcA* MAGs) represented novel candidate genera (**Fig.4A**).

We were able to resolve unknown Hg-methylator diversity in our co-assemblies by assigning the taxonomy of the MAGs that contained these unassigned *hgcA* sequences. MAGs from putative Hg-methylators belonged to 23 phyla, with the majority belonging to phyla associated with fermentative and sulfate reducing metabolisms. Phyla containing organisms that were previously experimentally shown to methylate mercury (Gilmour et al., 2018; Gilmour et al., 2013; Waite et al., 2020) are also represented in our *hgcA* containing MAGs: 17 MAGs belong to *Desulfobacterota,* 6 to *Halobacteriota,* 6 to *Thermoplasmatota* and 3 to *Firmicutes* (**Fig. 4B**).

Furthermore, the most represented phyla in our putative Hg-methylating catalogue have also been identified as putative methylators by other metagenomics studies (Capo, Peterson, et al., 2023), such as *Bacteroidota* (34), *Chloroflexota* (18), *Spirochaetota* (12) and *Nitrospirota* (5). A good proportion of MAGs were found to belong to the phyla *Actinobacteriota* and *Acidobacteriota* (12

and 11, respectively), which have only been recently shown to include putative methylators (McDaniel Elizabeth et al., 2020).

We identified 2 *hgcA* MAGs belonging to newly characterized bacterial *Candidatus* phyla that were not previously shown to carry *hgcA*. One was assigned to the *QNDG01* phylum and the other to the *VGIX01* phylum (**Fig. 4B**). We also identified 2 *hgcA* MAGs belonging to the phylum *Zixibacteria*, a group newly identified as containing putative Hg-methylators by Zhang et al. (2023).

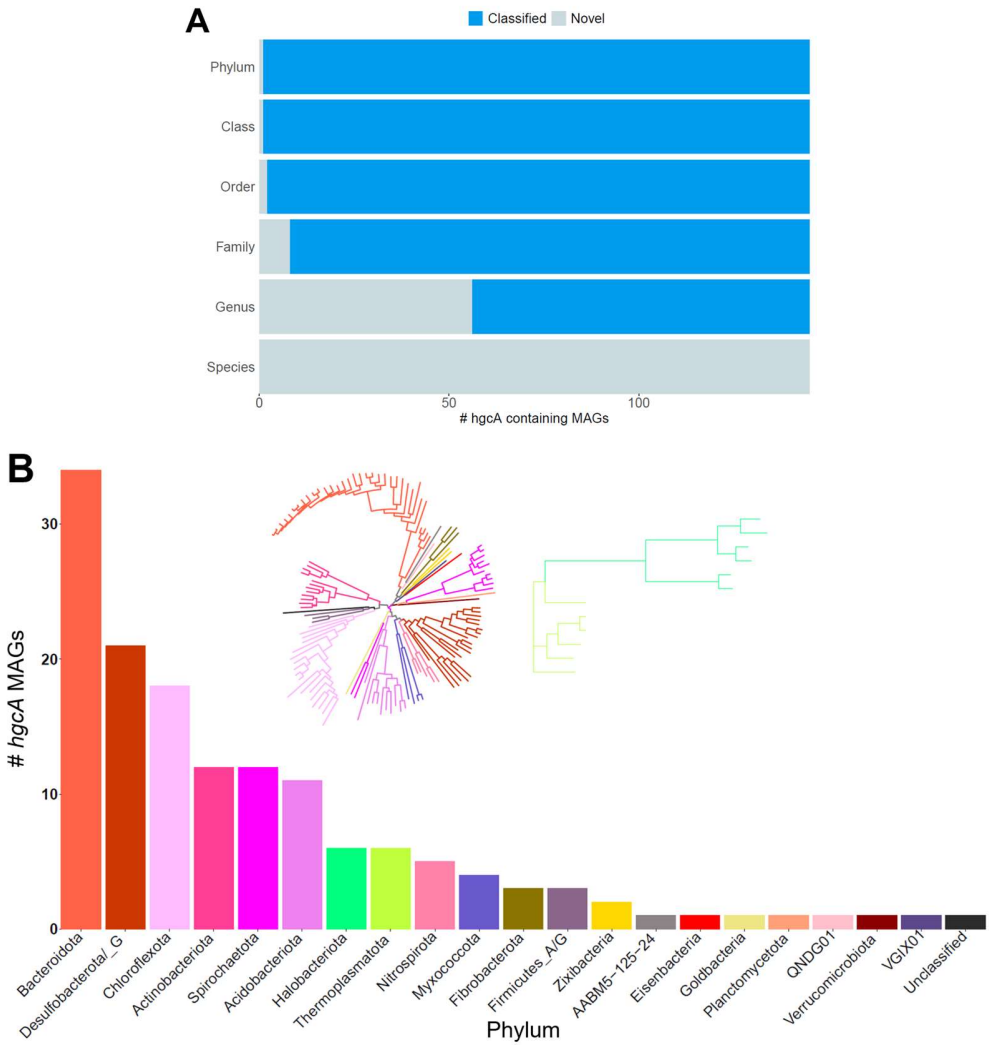

Figure 4. (A) Number of *hgcA* containing MAGs recovered across all co-assemblies combined representing taxonomic novelty in each taxonomic levels. **(B)** Percentage of *hgcA* containing MAGs recovered across all co-assemblies combined belonging to different phyla, along with phylogenetic trees of bacterial and archaeal MAGs, coloured by phyla.

Compared to the full river sediment genome catalogue, the *hgcA* containing MAGs were enriched in *Bacteroidota*, *Desulfobacterota*, *Halobacteriota*, *Spirochaetia*, and *Thermoplasmatota* (**Fig. 5**). No MAG from the most abundant phylum in the whole community (*Proteobacteria*) was found to harbour *hgcA*, despite that *Alphaproteobacteria* and *Gammaproteobacteria* were previously shown to include putative Hg methylators (**Fig. 5, Fig. S2**) (Capo, Peterson, et al., 2023). Members of *Patescibacteria* were the sixth most abundant in the whole community, but absent in the putative Hg-methylating assemblage, however they were not previously shown to harbour *hgcA* in the literature (**Fig. 5)**. Moreover, some of the most abundant classes in the complete MAG dataset belonging to phyla found to be represented in *hgcA* containing MAGs, such as *Acidobacteriae* (*Acidobacteriota*), *Bacilli* (*Firmicutes*), *Limnocylindria* and *Dehalococcoidia* (*Chloroflexota*) were not represented in our Hg-methylating genome catalogue (**Fig. S2**).

Metabolic diversity within putative Hg-methylating MAGs

To identify enriched metabolisms in *hgcA* carrying microorganisms compared to the complete MAG catalogue, we analyzed specific functional marker gene content in our MAGs. We first looked for the presence of metabolisms usually linked to Hg-methylation, such as sulfate reduction and methanogenesis. Only 15.9% of our *hgcA* containing MAGs possessed at least one marker gene for sulfate reduction, and 2.8% possessed the marker gene for methanogenesis (*mcrA*) (**Fig. 6**). Furthermore, only 10 of the 21 *hgcA* containing MAGs in *Desulfobacterota,* a phylum associated with sulfate reduction, possessed at least one marker gene for this pathway, and only a third of *hgcA* containing archaeal MAGs had the marker gene for methanogenesis (**Fig. 7**). When comparing with the whole river sediment genome catalogue, marker genes for sulfate reduction were only slightly less abundant, with 9.8% of MAGs possessing these genes. It was also the case with methanogenesis, with 0.7% of MAGs being putative methanogens (**Fig. 6**).

As fermentation is an important pathway in anaerobic microbial communities, we looked for marker genes specific to seven fermentation pathways that differed in their end products. As such, 76% of *hgcA* containing MAGs possessed fermentative pathway genes, with those producing acetate and lactate as the most abundant end-products (57% and 26%, respectively) (**Fig. 6**). They were also more abundant in phyla that are considered sulfate reducers, such as *Desulfobacterota*. In *hgcA* containing MAGs from this group, 76% possessed gene markers for fermentation pathways (**Fig. 7**). Fermentative marker genes were only absent in two identified phyla. Compared to the whole river sediment genome catalogue, *hgcA* carrying MAGs were particularly enriched in fermenters producing butyrate (16% vs 5%) and lactate (25.5% vs 17.5%) (**Fig. 6**). Most *hgcA* containing MAGs that showed evidence of butyrate fermentation were in the class *Bacteroidia*

(**Fig. 7**). One *hgcA* containing MAG from the family lineage vadinHA17 (genus LD21) of *Bacteroidota* was particularly versatile for fermentation, containing genes for fermentation yielding acetate, butyrate, hydrogen, and lactate. Furthermore, the two novel groups of Hg-methylators that were identified in our catalogue were fermenters. MAGs assigned to *QNDG01* possessed the genes for acetate fermentation and MAGs assigned to *VGXI01* possessed the gene for butyrate fermentation.

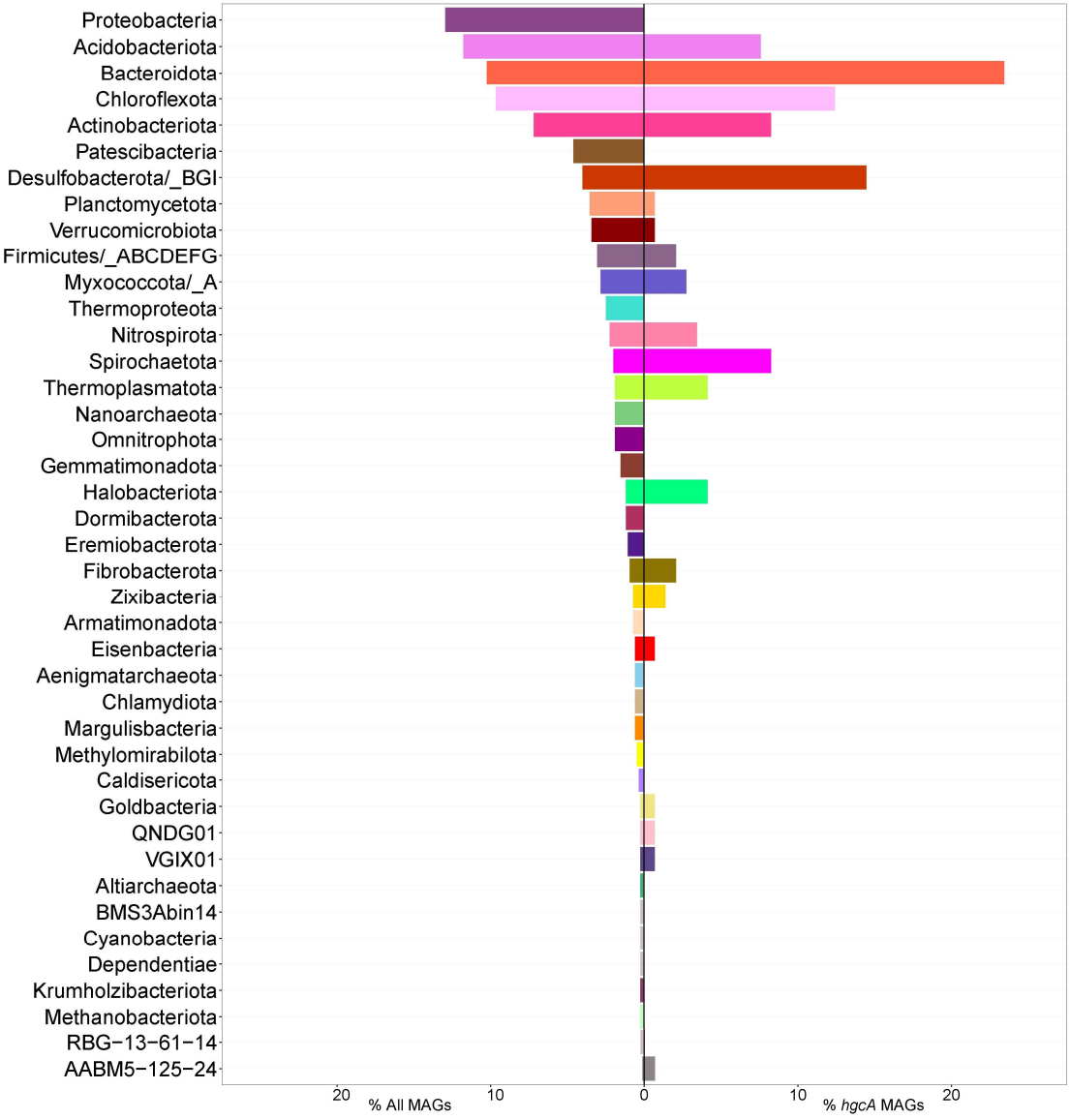

Figure 5. Relative abundance of phyla in all river sediment MAGs versus *hgcA* containing MAGs, recovered across all co-assemblies.

We also looked for marker genes indicating the presence of three anaerobic carbon fixation pathways in MAGs harbouring *hgcA,* namely the 3-hydroxypropionate-4-hydroxybutyrate (3HP-4HB) cycle, the Wood–Ljungdahl (WL) pathway, and the reductive TCA cycle. At least one marker gene for either of these pathways was present in 48% of our *hgcA* MAGs. More specifically, 26% had the WL pathway gene marker, and 21% had the 3HP/4HB cycle gene marker (**Fig. 6**). Only 2 *hgcA* containing MAGs had the marker gene for the rTCA cycle, both belonging to the class *Polyangia* of Myxococcota (**Fig.7**). Otherwise, carbon fixation pathways were widely distributed amongst different groups of *hgcA* containing MAGs, with the majority belonging to *Desulfobacterota* and *Actinobacteriota*. The newly identified putative Hg-methylator from the VGIX01 phylum also contained genes for carbon fixation for both the 3HP/4HB and WL pathway. Carbon fixation pathways were also enriched in *hgcA* containing MAGs, because either one of the marker genes for the three pathways was present in 20% (vs 48% for *hgcA* MAGs) of all river sediment MAGs (**Fig.6**).

Next, we investigated the presence of marker genes involved in the nitrogen cycle in *hgcA* containing MAGs. Fifteen percents of *hgcA* MAGs possessed gene markers for nitrogen fixation (**Fig.6**), and they belonged to eight different phyla (**Fig.7**). Nitrate reduction marker genes were found in almost 60% of our *hgcA* MAGs, and spanned many different phyla (**Fig. 6-7**). Five percents of MAGs had inferred dissimilatory nitrate reduction potential, 50% had inferred dissimilatory nitrite reduction potential, and 22% had inferred nitric oxide reduction potential. We found that a higher proportion of *hgcA* containing MAGs showed putative nitrogen fixation (15% vs 5%) and dissimilatory nitrite reduction potential (50% vs 36%) than in the whole river sediment genome catalogue.

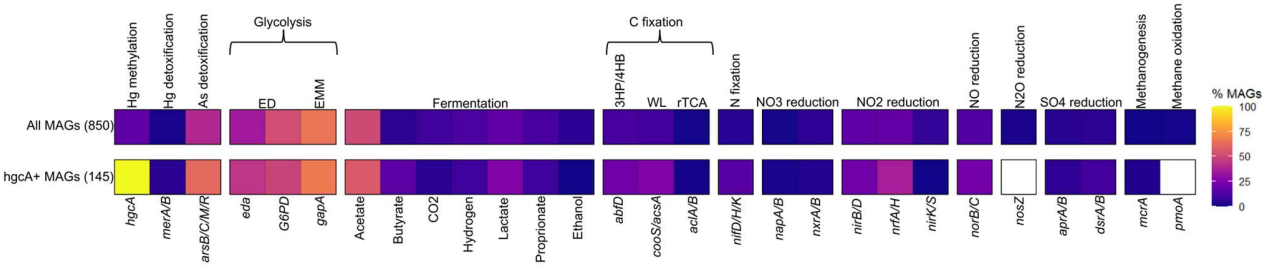

Figure 6. Abundance of MAGs containing marker genes for selected anaerobic pathways in all river sediment genome catalogue versus Hg-methylating genome catalogue recovered from the six co-assemblies combined.

Lastly, we looked for arsenic (As) detoxification and Hg detoxification genes. The majority of *hgcA* containing MAGs, 88%, possessed at least one of the As detoxification gene (**Fig.6**). We also

found that As detoxification genes were often within one ORF of the *hgcAB* genes in our *hgcA* containing MAGs. These genes were also abundant in the full MAG community (83%). Genes for Hg detoxification were depleted in our putative methylators (5%) compared to all MAGs (12%).

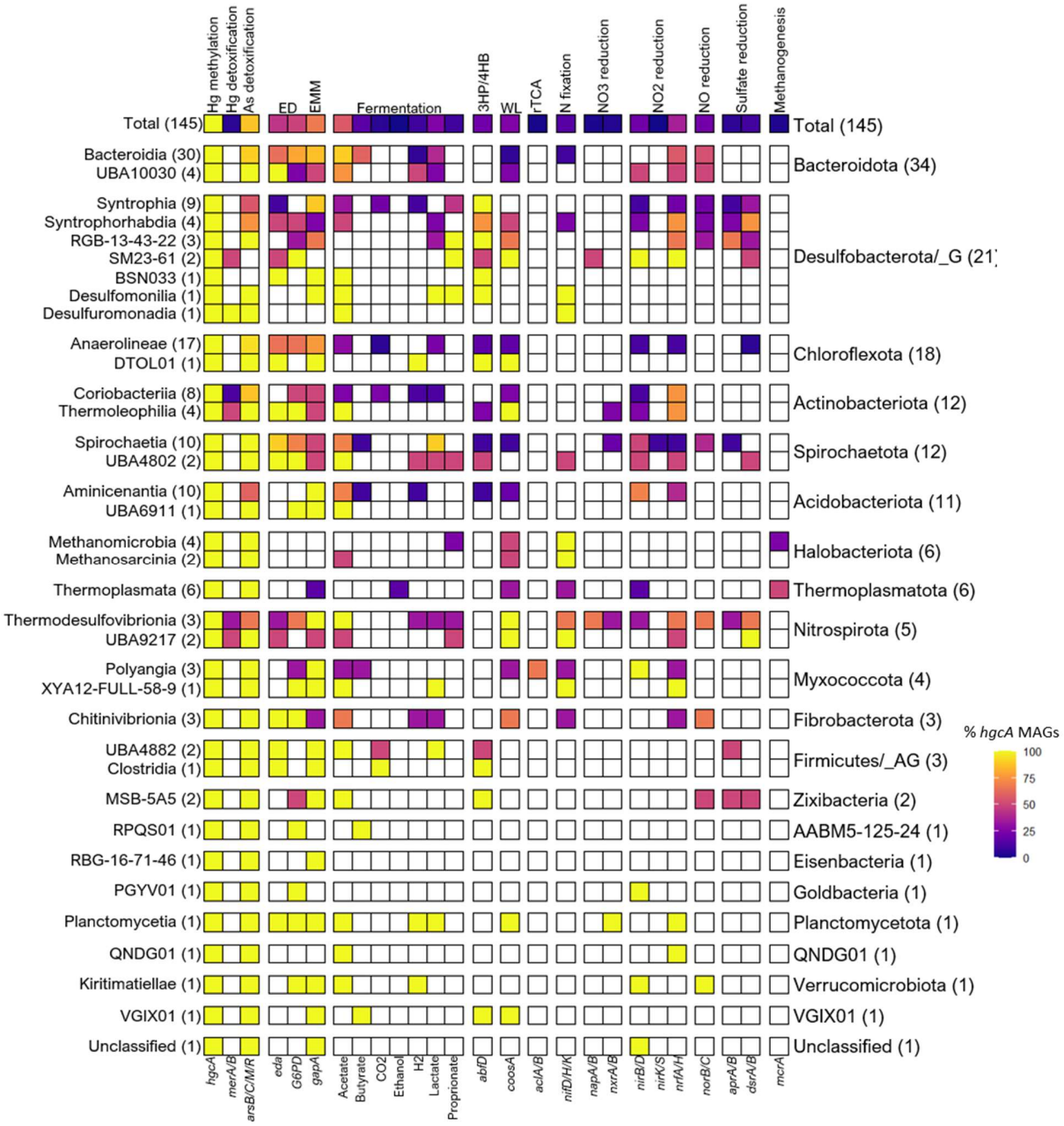

Figure 7. Relative abundance of *hgcA* containing MAGs recovered from all co-assemblies combined belonging to different classes that possess gene markers for selected anaerobic pathways.

Influence of rivershed disturbances on Hg-methylating microbial community

To explore the link between ecological conditions and the effects of watershed disturbances on the sediment Hg-methylating community, we first compared the abundance of Hg methylators between sites and depth in the sediment core. Two measures were used for this aim, namely the abundance of the *hgcA* gene and the abundance of *hgcA* containing MAGs in our metagenomes. Variation between sampling sites was more important than variation by depth in the sediments, so we focused on differences between sampling sites for further discussion (**Table S2**)**.** One site closer to the logging area (RDC-10) contained significantly more *hgcA* copies than the sites closer to the wildfire (CA sites) and the artificial wetland site (WEM-4). However, RDC-10 did not contain significantly more *hgcA* copies than the reference site (REF-2) (**Fig. 8A**). The abundance of *hgcA* containing MAG was significantly higher in RDC-10 than the other sites. The *hgcA* containing MAGs were similarly abundant in both sites closer to logging, but one of these sites (RDC-14) also had similar abundance to the reference site and the other sites (**Fig. 8B**). With both measures, the sediments closer to logging and upstream of a run-of-river dam contained the most Hg-methylators.

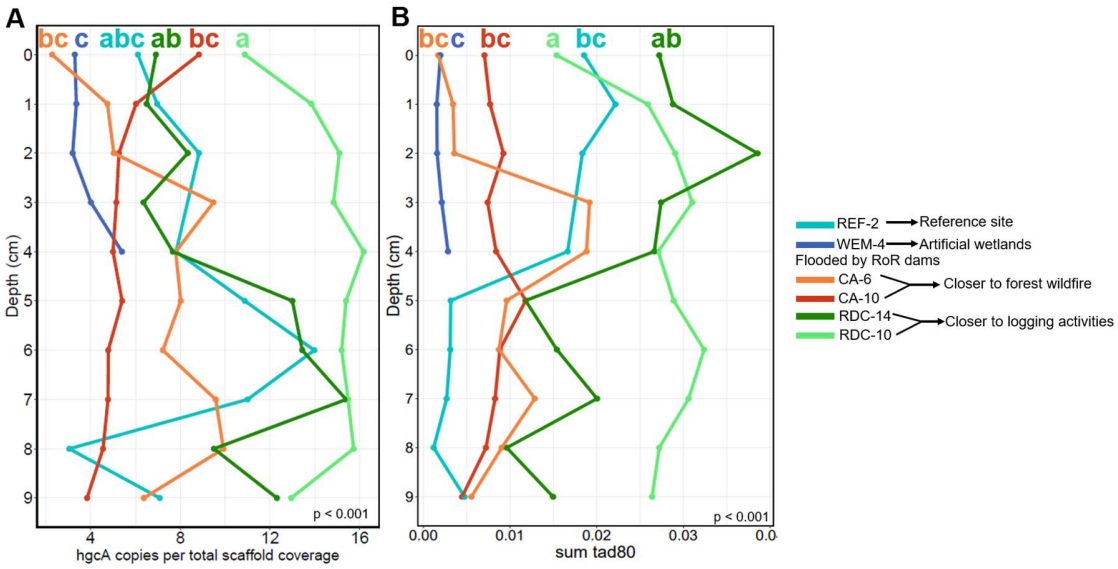

Figure 8. **(A)** Normalized abundance of *hgcA* copies in metagenomes. **(B)** Abundance of *hgcA* containing MAGs in each metagenomes, with TAD80 values of *hgcA* containing MAGs summed in each sample. Different letters indicate statistically different groups according to Dunn's test, colour-coded according to sampling site.

We also investigated if disturbances could have an effect on the composition of the Hg-methylating assemblage in the sediment. Overall, the membership of dominant phyla was relatively similar

between sites, but some differences were still notable. For example, *Bacteroidota hgcA* containing MAGs were more abundant in wildfire and logging disturbed sites (CA and RDC) (**Fig. S4**). Also, the highly versatile VadinHA17 *Bacteroidota* MAG was significantly (p = 0.003) most abundant in metagenomes from RDC-10 and was found in all metagenomes from this site. This MAG was also found in four metagenomes from RDC-14 and five metagenomes from CA-6. Sites affected by logging and dam construction also contained more Hg-methylating taxonomic diversity than the other sites, because they harboured all 35 classes found in our Hg-methylating genome catalogue, except for the class UBA6911 of the *Acidobacteriota* phylum.

Lastly, to investigate if the Hg-methylating community structure was different from the overall sediment microbial community structure, we compared Principal Coordinates Analyses (PCoAs) of microbial community structure between sites for these two genome catalogues. As represented by both PCoAs, the community structure varied by site more than depth, and CA-10 harboured a community structure that is unique from all the other sites (**Fig. 9**). Additionally, microbial community structure in WEM-4 was similar to microbial community structure in REF-2 site. To evaluate the statistical significance of the similarity between these two ordinations, the RV coefficient was used, comparing the first two axes. The RV coefficient was 0.36 with a $p < 0.001$, which mean that the ordinations were not significantly different from each other. The strong similarity between the two ordinations suggests that the drivers for the microbial community structure in the sediments of the St. Maurice River are probably the same drivers for the structure of the Hg-methylating community.

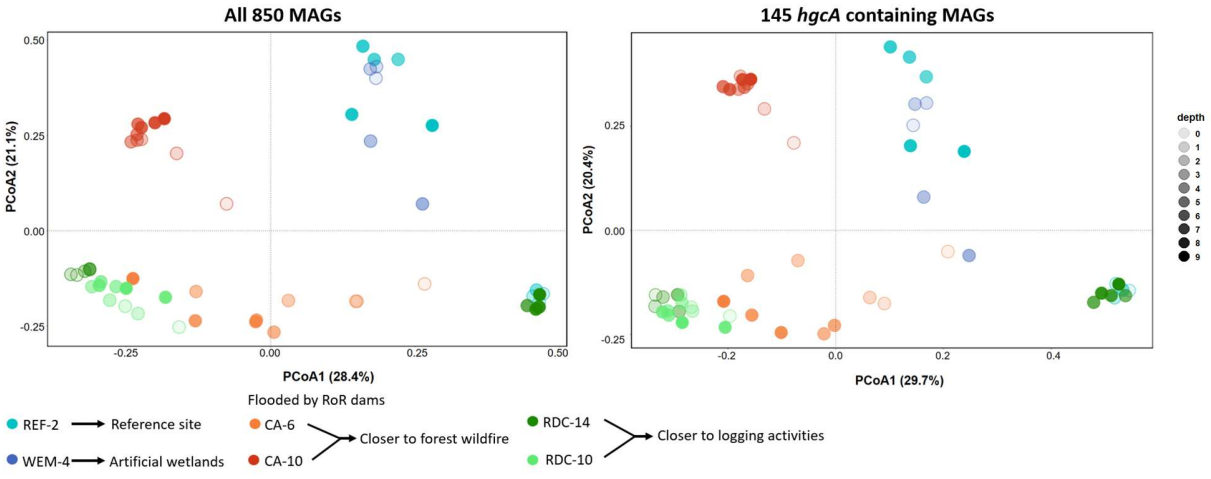

Figure 9. PCoA of Jaccard index of membership of MAGs in each metagenomes for each genome catalogue, colour coded by sampling site and with transparency according to depth. The amount of variance explained by PCoA axes is indicated in parenthesis.

Discussion

In summary, our study has expanded the known diversity of Hg methylators by obtaining a highly diverse, unique, and novel catalogue of Hg-methylating metagenome-assembled genomes (MAGs) from the sediments of an impacted river. Additionally, this research marks the first attempt to compare the Hg-methylating microbial community to the broader microbial community within which they exist. Despite Hg-methylating capacity seemingly restricted to certain taxa and some metabolic capacities being more prevalent in the Hg-methylating assemblage, our findings demonstrate that the Hg-methylating assemblage overlaps both taxonomically and ecologically with the more complex and diverse microbial communities typically found in freshwater sediments.

Unique and novel diversity of Hg-methylators from the St. Maurice River

The sediment microbial community of the St. Maurice River contained a high proportion of putative Hg-methylators. The frequency of *hgcA* containing MAGs varied in other genome-centered studies on Hg-methylation, ranging from less than 1% to almost 30%. The highest incidence was found in a study that reconstructed MAGs from deep-sea hydrothermal sediments (Langwig et al., 2022), and similar proportions of Hg-methylators to ours were found in MAGs recovered from sulfate impacted lake water column and sediments (Jones et al., 2019; Peterson et al., 2020), and from sediments associated with mangroves, permafrost, lakes and ocean (Zhang et al., 2023). Distinct from previous studies, the Hg-methylating community of the St. Maurice River sediments were dominated by members of *Bacteroidota* (Capo, Feng, et al., 2022; Jones et al., 2019; Lin et al., 2021; Jibao Liu et al., 2022; McDaniel Elizabeth et al., 2020; Peterson, Krabbenhoft, et al., 2023; Peterson et al., 2020; Vigneron et al., 2021; Zhang et al., 2023; Zheng et al., 2022), a diverse phylum found in almost every habitat on earth and that are highly versatile organic matter degraders (Thomas et al., 2011). A majority of previous studies found environments dominated by *Deltaproteobacteria* (Capo, Feng, et al., 2022; Jones et al., 2019; McDaniel Elizabeth et al., 2020; Vigneron et al., 2021; Zhang et al., 2023; Zheng et al., 2022), a phylum now separated in *Desulfobacterota* and *Myxococcota* (Waite et al., 2020). Other dominant groups included *Marinimicrobia* (Lin et al., 2021), the PVC superphylum (Peterson et al., 2020), and *Halobacteriota* (Peterson, Krabbenhoft, et al., 2023). We also expanded the diversity of known Hg methylators to include members from *QNDG01,* a candidate phylum previously linked to terpene production (Qiu et al., 2023), and *VGIX01*, another candidate phylum that was shown to be linked to arsenic cycling in a deep sea cold seep (Helfrich et al., 2019). Although our *hgcA* MAGs belonged for the most part to known or putative Hg-methylating groups, they represented a unique, novel, and diverse community of Hg-methylators potentially driven by OM degradation.

Versatile fermentation by Hg-methylators

As previous studies on Hg-methylators have highlighted, metabolic guilds other than sulfate reducers and methanogens are likely to play an important role in Hg-methylation (Jones et al., 2019; McDaniel Elizabeth et al., 2020; Peterson et al., 2020). We found that putative Hg-methylators from the St. Maurice River sediments were distributed across the anaerobic food web, but most importantly in fermentative microorganisms. Fermentation products, such as acetate, CO_2 and H_2 can in turn be used in methanogenesis and sulfate reduction, making these processes closely linked in the sediments. Interestingly, we also found that nitrite reduction genes were enriched in our Hg-methylating assemblage, further supporting the link between denitrification and Hg-methylation that was highlighted in a recent study (Peterson, Poulin, et al., 2023). Given the potential horizontal gene transfer of *hgcAB*, it is not surprising that Hg methylation capacity can be found in fermenters, methanogens and sulfate reducers alike because of their physical proximity.

Surprisingly, the butyrate fermentation pathway was especially enriched in our Hg-methylating genome catalogue. Although the reasons behind this enrichment remain unclear, it is possible that specific environmental conditions in the sediments favour this particular type of fermentation in Hg-methylators over others. Butyrate fermentation can occur from diverse substrates, including sugars, fatty acids, and amino acids. However, this process has received limited attention in natural environments, despite its significance in gut health due to the role of butyrate-fermenting bacteria (Louis & Flint, 2009). Future investigations could focus on examining the full metabolism of the more complete butyrate fermenting MAGs identified in our study to gain valuable insights into the factors driving this enrichment.

Differential influence of continuous and acute OM inputs on Hg methylators

The abundance of putative Hg methylators in sites affected by logging and dam construction (RDC sites) suggests that these disturbances have the largest effect on Hg-methylation in the St. Maurice River sediments. This is also supported by the lack of clear depth profiles in Hg-methylators abundance, which indicates that the sampling site location has more effect on their abundance than sediment depth. This might be caused by the nature of these disturbances, such as whether the disturbances are short and acute (pulse), or long term (press). The nature of anthropogenic disturbances has been shown to affect microbial communities differently in the past (Beattie et al., 2020). Indeed, the RDC sites are exposed to continuous logging activities as well as the flooding from the Rapides-des-Coeurs dam. The continual input of OM from logging could have permitted the microbial community to adapt to this press-like type of disturbance and have

higher microbial activity. Thus, this steady inflow of terrigenous OM to the river sediments might have helped to establish an active Hg-methylating niche. For example, members of *Bacteroidota* and the metabolically versatile VadinH17 MAG are more abundant in these sites as well, potentially because these microorganisms may possess a competitive edge and thrive in the sediments of press-impacted sites. The lower abundance of Hg-methylators in sites affected by both a wildfire and dam flooding could be related to the pulse-like nature of the disturbance, and the fact that the wildfire happened 8 years before sampling of the sediments, so the microbial community was given more time to recover. Furthermore, fires release OM-trapped Hg to the atmosphere, which can in turn be deposited in the river. Consequently, it does not lead to direct Hg input in the surrounding water. Therefore, it is important to consider the nature and duration of the disturbance when studying its effect on Hg-methylating communities.

Hg-methylating community dynamics echoes the broader microbial community

We found no significant difference in community structure between Hg-methylating assemblages and the microbial community as a whole. This finding implies that disturbances affecting the watershed likely impact both the microbial community and the subset Hg-methylating members in a similar manner. Therefore, we propose that the increase in MeHg observed in rivers after disturbances such as dam construction is likely a consequence of the enhanced input of organic matter (OM), which in turn stimulates general microbial activity in the sediments. The native function of *hgcAB* remains unknown (Parks et al., 2013), but its widespread presence across various taxonomic and functional groups suggests that this gene pair must have conferred a competitive advantage to microorganisms at some point. The potential co-regulation between Hg-methylation and arsenic detoxification has been recently proposed (Gionfriddo et al., 2023), and co-occurrence of *hgcA* and arsenic detoxification genes in MAGs has also been noted in other studies (Lin et al., 2021; McDaniel Elizabeth et al., 2020; Peterson, Poulin, et al., 2023; Zhang et al., 2023; Zheng et al., 2022), a trend similarly identified in our study. It is plausible that the absence of specific drivers for the structure of Hg-methylating communities is due to the potential loss of the native function of *hgcAB*. This might explain why Hg-methylation can seemingly occur without any specific regulating environmental factors. Consequently, since Hg-methylators are likely to respond to environmental conditions in a manner similar to the overall microbial community, it becomes crucial to interpret the diversity and abundance of Hg-methylators within their specific ecological context.

Conclusion

In conclusion, we found that Hg-methylators were part of a diverse set of taxonomic and metabolic groups that represent the complexity of the microbial diversity within which they live. Once more, fermentation emerged as a crucial pathway for Hg-methylators. Future research endeavors could delve into discerning the specific types of fermentation most closely linked to them and under which ecological conditions. Additionally, we have proposed that the ecological ramifications of disturbances influencing the type and duration of organic matter input from the watershed, as reflected in Hg-methylating assemblages, are likely comparable to the broader ecological effects experienced by the entire microbial community. This underscores the importance of accounting for all disturbances affecting a freshwater ecosystem when assessing the influence of run-of-river dams on the Hg cycle. For a more in-depth investigation into the specific environmental drivers shaping Hg-methylating assemblage structure, an exploration involving the comparison of the respective contributions of distinct environmental factors to the variance in both Hg-methylating assemblage and complete microbial community structures could be pursued. Finally, our study has permitted some insights on mitigations strategies for disturbances of freshwater ecosystems. Our results indicates that the construction of artificial wetlands in response to habitat loss following hydroelectric dam construction did not produce conditions for increased Hg-methylation in the St. Maurice River. In alignment with Peterson, Poulin, et al. (2023), we also infer that the implementation of nitrate addition as a mitigation strategy for Hg pollution in aquatic ecosystems might not universally apply, as evidenced by the proliferation of Hg-methylators exhibiting nitrate reduction capabilities within our system.

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Appendices

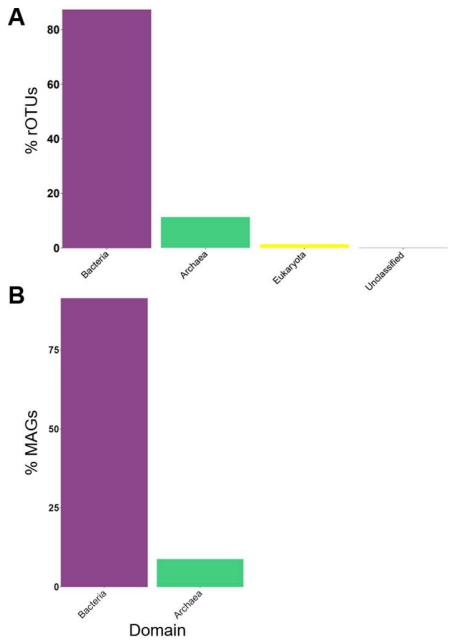

Figure S1. Proportion of rOTUs belonging to different domains (**A**). Proportion of all river sediment MAGs belonging to different domains (**B**).

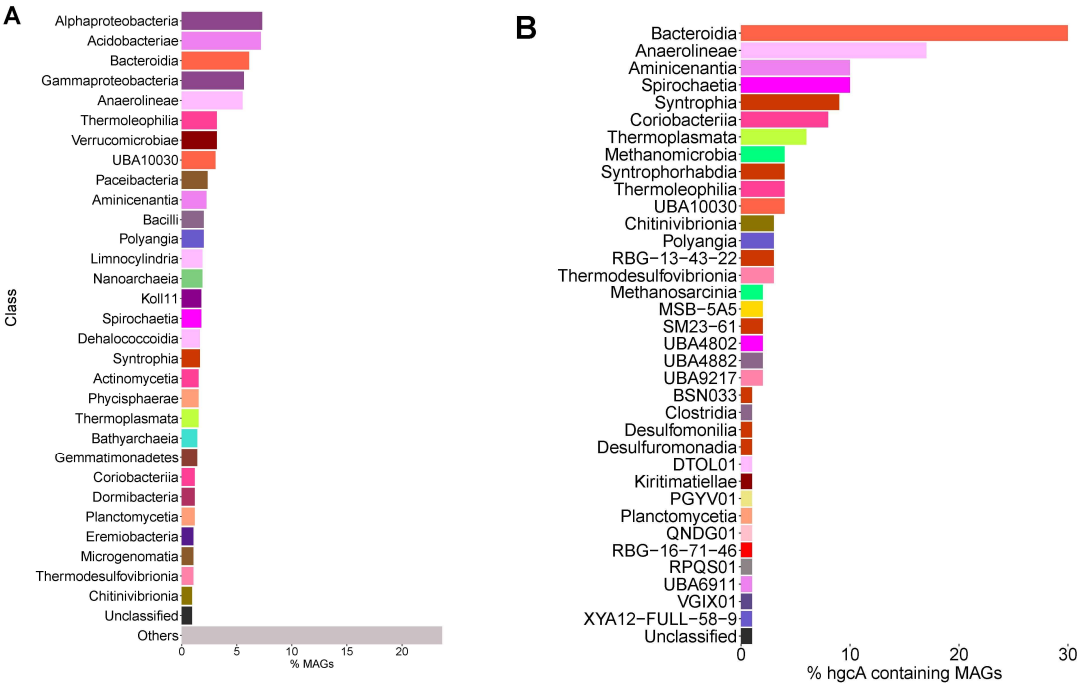

Figure S2. Relative abundance of all river sediment MAGs belonging to the top 30 most abundant classes out of 116 in the river sediment genome catalogue (**A**). Relative abundance of *hgcA* containing MAGs belonging to different classes (**B**). Classes are coloured according to phyla.

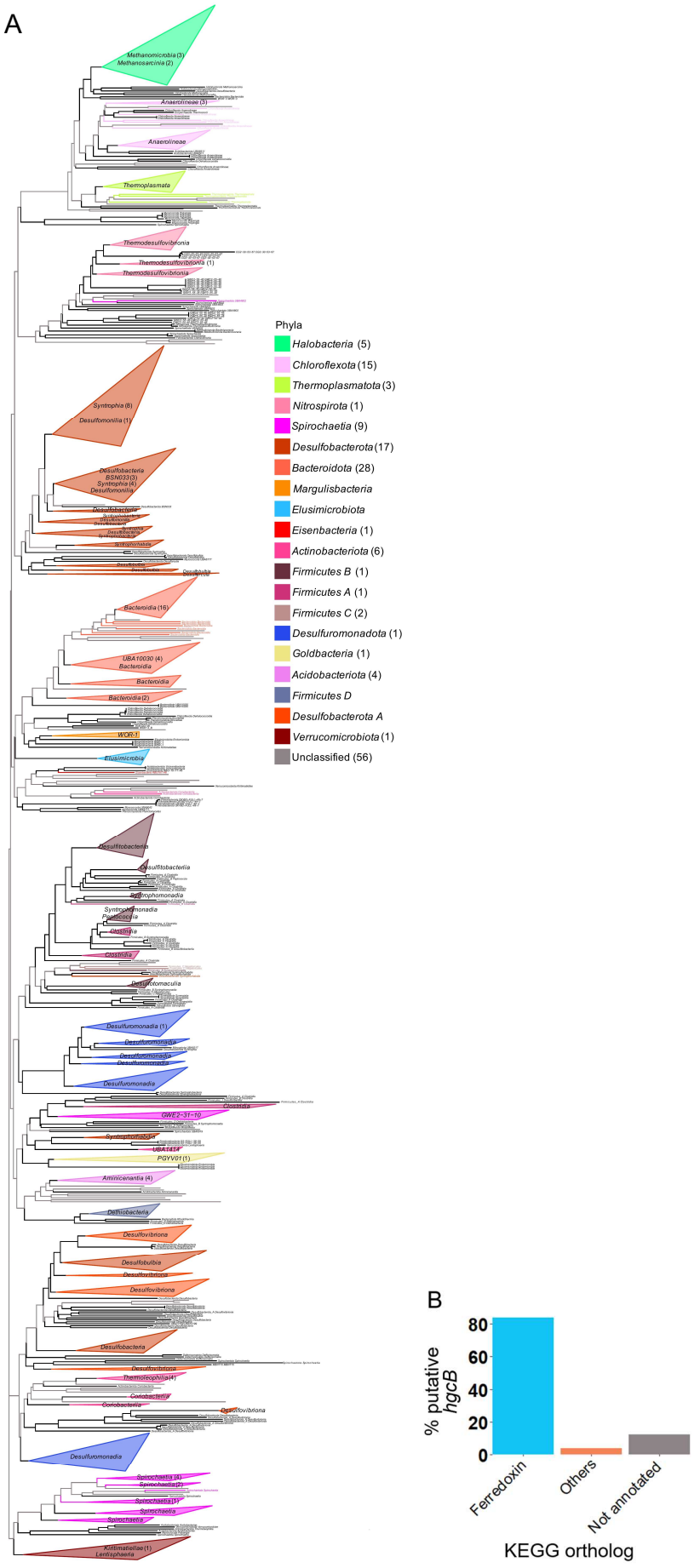
A
Methanomicrobia (3)
Methanosarcinia (2)
Anaerolineae (3)
Anaerolineae
Thermoplasmata
Thermodesulfovibrionia
Thermodesulfovibrionia (1)
Thermodesulfovibrionia
Syntrophia (8)
Desulfomonilia (1)
Desulfobacteria
BSN033 (3)
Syntrophia (4)
Desulfomonilia
Bacteroidia (16)
UBA10030 (4)
Bacteroidia
Bacteroidia
Bacteroidia (2)
WOR-1
Elusimicrobia
Desulfitobacteriia
Desulfitobacteriia
Syntrophomonadia
Dethiobacteria
Desulfovibrionia
Desulfobulbia
Desulfovibrionia
Desulfovibrionia
Desulfobacterota
Desulfovibrionia
Thermovenabulia (4)
Coatabacteria
Coatabacteria
Desulfovibrionia
Desulfuromonadia
Spirochaetia (4)
Spirochaetia (2)
Spirochaetia (1)
Spirochaetia
Spirochaetia
Xiddimarinetales (1)
Lentisphaeria
Phyla
Halobacteria (5)
Chloroflexota (15)
Thermoplasmatota (3)
Nitrospirota (1)
Spirochaetia (9)
Desulfobacterota (17)
Bacteroidota (28)
Margulisbacteria
Elusimicrobiota
Eisenbacteria (1)
Actinobacteriota (6)
Firmicutes B (1)
Firmicutes A (1)
Firmicutes C (2)
Desulfuromonadota (1)
Goldbacteria (1)
Acidobacteriota (4)
Firmicutes D
Desulfobacterota A
Verrucomicrobiota (1)
Unclassified (56)
B
% putative hgcB
80
60
40
20
0
Ferredoxin
Others
Not annotated
KEGG ortholog

Figure S3. Phylogeny of our binned *hgcA* sequences aligned to the multi-sequence alignment provided by McDaniel Elizabeth et al. (2020). Binned hgcA sequences of the current study are colored according to their taxonomy if not in a collapsed node, or number of sequences are in parenthesis in collapsed node class (**A**). KEGG ortholog annotation of putative *hgcB* within 2 ORFs downstream of binned *hgcA* (**B**).

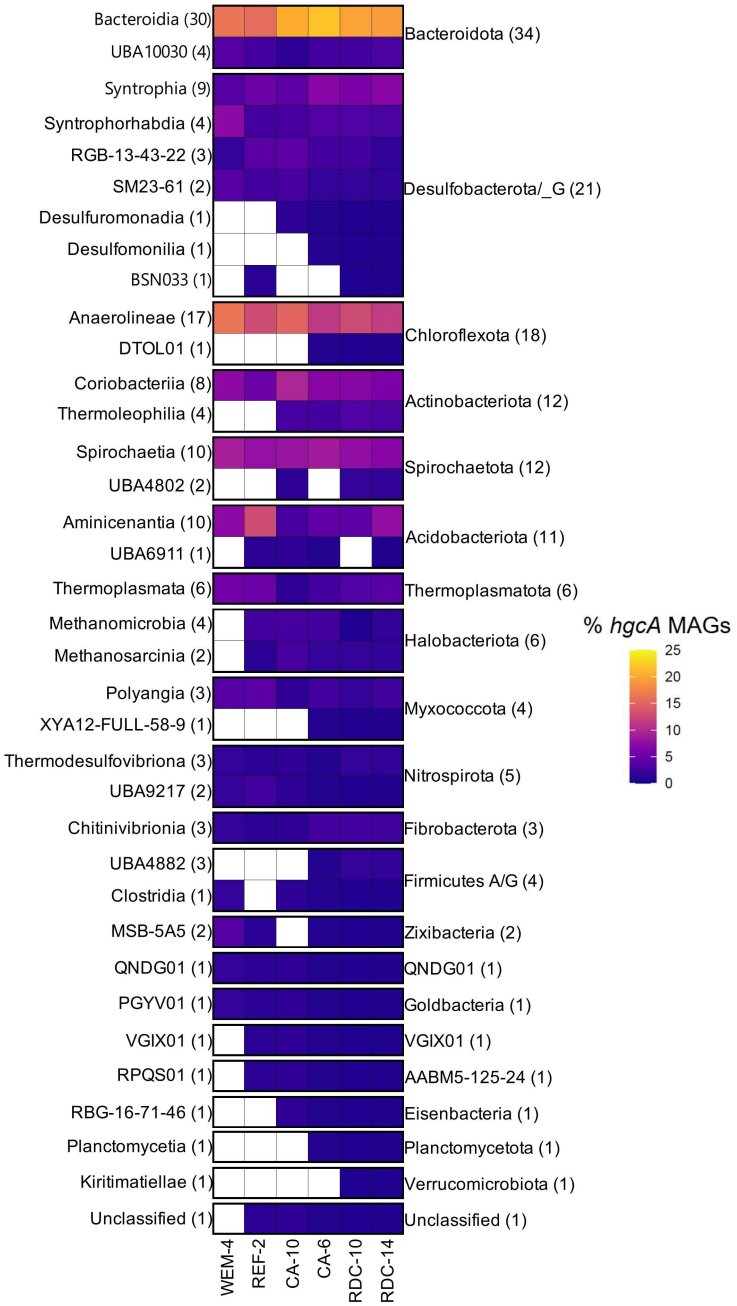

Figure S4. Relative abundance of classes and phyla of *hgcA* containing MAGs in different co-assemblies, MAGs were counted only once if they were present at least in one metagenome of the co-assembly.

Table S1. Published peer-reviewed articles that studied microbial mercury methylation by using metagenomics

Article	MAGs use	Environment	DOI
(Podar et al., 2015)	No	Multiple	https://doi.org/doi:10.1126/sciadv.1500675
(Christensen et al., 2019)	No	Multiple	https://doi.org/10.1021/acs.est.8b06389
(Gionfriddo et al., 2016)	No	Marine	https://doi.org/10.1038/nmicrobiol.2016.127
(Jones et al., 2019)	Yes	Lake	https://doi.org/10.1038/s41396-019-0376-1
(Liu et al., 2018)	No	Wetlands	https://doi.org/10.1021/acs.est.8b03052
(Yuan et al., 2019)	No	Estuary	https://doi.org/10.1016/j.ecoenv.2019.109722
(Bowman et al., 2020)	No	Marine	https://doi.org/10.1002/lno.11310
(Capo et al., 2020)	No	Marine	https://doi.org/10.3389/fmicb.2020.574080
(McDaniel Elizabeth et al., 2020)	Yes	Multiple	https://doi.org/10.1128/mSystems.00299-20
(Peterson et al., 2020)	Yes	Lake	https://doi.org/10.1021/acs.est.0c05435
(Tada et al., 2020)	No	Marine	https://doi.org/10.3389/fmicb.2020.01369
(Villar et al., 2020)	No	Marine	https://doi.org/10.1111/1758-2229.12829
(Zhang et al., 2020)	No	Glacier	https://doi.org/10.1016/j.scitotenv.2019.135226
(Millera Ferriz et al., 2021)	No	River	https://doi.org/10.1016/j.scitotenv.2021.145686
(Leclerc et al., 2021)	No	River	https://doi.org/10.1128/msphere.00021-21
(Lin et al., 2021)	Yes	Marine	https://doi.org/10.1038/s41396-020-00889-4
(Roth et al., 2021)	No	Wetlands	https://doi.org/10.3389/fmicb.2021.741523
(Tada et al., 2021)	No	Marine	https://doi.org/10.1128/Spectrum.00833-21
(Vigneron et al., 2021)	Yes	Ponds	https://doi.org/10.1038/s41522-021-00255-y
(An et al., 2022)	No	Landfill	https://doi.org/10.1016/j.wasman.2022.04.038
(Capo, Feng, et al., 2022)	Yes	Marine	https://doi.org/10.1021/acs.est.2c03784

(Capo, Broman, et al., 2022)	No	Marine	https://doi.org/10.1002/lno.11981
(Frey et al., 2022)	No	Soil	https://doi.org/10.3389/fmicb.2022.1034138
(Langwig et al., 2022)	Yes	Marine	https://doi.org/10.1038/s41396-021-01057-y
(Jibao Liu et al., 2022)	Yes	Compost	https://doi.org/10.1016/j.watres.2022.119204
(Jingli Liu et al., 2022)	No	Mangroves	https://doi.org/10.1016/j.jhazmat.2022.128690
(Zheng et al., 2022)	Yes	Contaminated site	https://doi.org/10.1128/msystems.00736-22
(Capo, Cosio, et al., 2023)	No	Lake	https://doi.org/10.1016/j.watres.2022.119368
(Lin et al., 2023)	No	Multiple	https://doi.org/10.1093/gbe/evad051
(Peterson, Krabbenhoft, et al., 2023)	Yes	Mangroves	https://doi.org/10.1111/1462-2920.16364
(Peterson, Poulin, et al., 2023)	Yes	Reservoir	https://doi.org/10.1038/s41396-023-01482-1
(Tada et al., 2023)	No	Marine	https://doi.org/10.1016/j.marpolbul.2022.114381
(Zhang et al., 2023)	Yes	Mangrove	https://doi.org/10.1038/s41396-023-01360-w

Table S2. Statistical significance of sample depth (cm) and sampling site on the number of *hgcA* copies and number of *hgcA* containing MAGs.

Source of variation	Response variable	Statistical test used	P value
Depth	*hgcA* copies	ANOVA	0.55
	hgcA containing MAGs	ANOVA	0.99
Sampling site	*hgcA* copies	Kruskal-Wallis	1.964e-06
	hgcA containing MAGs	Kruskal-Wallis	1.384e-06