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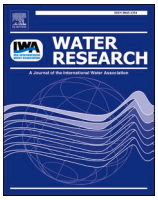

Anaerobic mercury methylators inhabit sinking particles of oxic water columns

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ABSTRACT

Increased concentration of mercury, particularly methylmercury, in the environment is a worldwide concern because of its toxicity in severely exposed humans. Although the formation of methylmercury in oxic water columns has been previously suggested, there is no evidence of the presence of microorganisms able to perform this process, using the *hgcAB* gene pair (*hgc*$^+$ microorganisms), in such environments. Here we show the prevalence of *hgc*$^+$ microorganisms in sinking particles of the oxic water column of Lake Geneva (Switzerland and France) and its anoxic bottom sediments. Compared to anoxic sediments, sinking particles found in oxic waters exhibited relatively high proportion of *hgc*$^+$genes taxonomically assigned to Firmicutes. In contrast *hgc*$^+$members from Nitrospirae, Chloroflexota and PVC superphylum were prevalent in anoxic sediment while *hgc*$^+$ Desulfobacterota were found in both environments. Altogether, the description of the diversity of putative mercury methylators in the oxic water column expand our understanding on MeHg formation in aquatic environments and at a global scale.

1. Introduction

In the environment, inorganic divalent mercury (Hg^{II}) can be transformed into methylmercury (MeHg), a neurotoxic compound that bioaccumulates and biomagnifies in aquatic food webs ultimately leading to severe environmental and human health risks (Doney et al., 2010; Mason et al., 2012; Driscoll et al., 2013). This process, also called Hg methylation, is mediated by microorganisms carrying hgcA and hgcB genes (hgc+ microorganisms) coding respectively for a corrinoid protein and a ferredoxin (Parks et al., 2013; Smith et al., 2015). The discovery of *hgc* genes opens avenues to unravel the diversity and distribution of putative Hg methylators in the environment in microbial groups recognized well to include Hg methylators (Desulfobacterota, Firmicutes, Methanomicrobia) (Gilmour et al., 2013; Podar et al., 2015) and in newly discovered groups such as Chloroflexota and PVC superphylum (McDaniel et al., 2020; Gionfriddo et al., 2020; Capo et al., 2022a). Additionally, the study of the abundance of HgcAB-encoding genes, transcripts and *HgcAB* proteins may, in theory, provide information about the occurrence and importance of Hg methylation in the environment. First biodiversity studies using the *hgc* gene pair were based on the polymerase chain reaction (PCR) amplification with primers targeting *hgcA* followed by cloning (Bae et al., 2014; Liu et al., 2014; Schaefer et al., 2014), while recent studies used metabarcoding (Bravo et al., 2018a,b; Xu et al., 2019, 2021) and metagenomic approaches to detect full-length *hgc* genes (Jones et al., 2019; Peterson et al., 2020; Capo et al., 2020). Hg methylators are known to thrive in oxygen-deficient environments (e.g., rice paddies, wetlands, sediments, anoxic waters) in which redox conditions play an important role regulating both the activity of Hg^{II} methylating microorganisms and the availability of Hg^{II} for methylation (Bravo and Cosio, 2020). Recent works further highlighted the huge diversity of Hg methylators in aquatic water columns – mostly oxygen-deficient - and underlying

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sediments (Lin et al., 2021; Azaroff et al., 2020; Capo et al., 2022b).

Although Hg methylation to form MeHg is a process known to occur in the absence of oxygen, the *in-situ* production of MeHg was often reported in oxic marine waters (Monperrus et al., 2007; Heimbürger et al., 2010; Lehnherr et al., 2011; Blum et al., 2013) and rarely in oxic lake water columns (Gascón Díez et al., 2016; Gallorini and Loizeau, 2021) which raises questions about the compartments where this process might occur. The understanding and quantification of the potential MeHg formation in oxic environments are crucial since it may significantly contribute to the overall budget of MeHg production in the environment (Gallorini and Loizeau, 2021), especially considering that oxygenated water bodies represent the vast majority of the total volume of water on Earth (Gnanadesikan et al., 2012). Such a question is of importance because of the need to understand the source and fate of Hg in the environment to predict how current environmental change and subsequent consequences, i.e., deoxygenation (Jane et al., 2021) will impact the formation of MeHg in our near-future ecosystems.

For Lake Geneva, the largest freshwater reservoir of western Europe, Gascón Díez et al. (2016) reported relatively high Hg methylation rates constant (k_m) in sinking particles ($1.6–6.5{\cdot}10^{-2}$ day^{-1}) compared to nearby sediments ($0.1–0.7\ 10^{-2}$ day^{-1}). Moreover, k_m measured in sinking particles of the oxic water column presented similar ranges as those reported from anoxic water bodies such as Canadian lakes ($0.56–14.8{\cdot}10^{-2}$ day^{-1}) (Eckley et al., 2005) and the Baltic Sea ($1.6–4.8{\cdot}10^{-3}$ day^{-1}) (Soerensen et al., 2018). Sinking particles – also called settling particles or lacustrine/marine snow – provide microhabitats with anaerobic niches for microorganisms (Bianchi et al., 2018) and are thus suspected to be suitable habitats for Hg methylators (Gallorini and Loizeau, 2021; 2022) because this process happens in absence of oxygen (Bravo and Cosio, 2020). For instance, an experiment by Ortiz et al. (2015) showed the potential of sinking particles to be adequate for Hg methylation by detecting significant k_m in controlled microcosms. Another study focusing on polar marine waters highlights the role of sinking particulate matter in Hg^{II} methylation (Lehnherr et al., 2011) although most studies focused on marine and not freshwater sinking particles. Recently, metagenomes obtained from sinking particles of the Baltic Sea (DNA captured on 3 μm pore size filters) were found to exhibit higher abundance of *hgc* genes in comparison to metagenomes from anoxygenic water columns (DNA captured on subsequent 0.2–3 μm fractions) (Capo et al., 2020). Villar et al. (2020) found *hgcA* genes in the oxic water of the open ocean suggesting the presence of Hg methylators in this environment. In freshwater systems, while two previous study showed that sinking particles were an important environment prone to MeHg formation (Gascón Díez et al., 2016; Gallorini and Loizeau, 2022), no evidence on the presence of the *hgcA* gene was reported, hampering the understanding of anaerobic biological processes involved in MeHg formation in sinking particles from oxic water columns. Describing the anaerobic microorganisms involved in MeHg formation in sinking particles improves current knowledge and understanding on the niches that Hg methylators can successfully colonize. Furthermore, determining the concentration of MeHg in sinking particles is of major importance to quantify the contribution of this pelagic source of MeHg to food web biomagnification rates.

In order to fill this gap of knowledge, the present study aims to unveil the diversity (and potentially the abundance) of hgc^{+} microorganisms inhabiting sinking particles of the oxic water column of Lake Geneva (Switzerland, France). By combining the analyses of different chemical forms of Hg and the characterization of organic matter (OM) composition with molecular tools (metagenomics and qPCR analyses), our study provides scientific evidence of the presence of Hg methylators in sinking particles from oxic lake water columns and points out the important role of these environments as a source of MeHg for the aquatic food webs.

2. Material and methods

2.1. Sampling and conditioning

Sinking particles and bottom sediments were collected in Lake Geneva (Fig. 1) on September 21, October 17 and November 16, 2017 at two sites: NG2 (6° 35′ 0″ E, 46° 30′ 6″ N; Swiss coordinates (m): 534,350/ 150,400) at 137 m water depth, and NG3 (6° 34′ 46″ E, 46° 29′ 40″ N; Swiss coordinates: 534,050 /149,600) at 192 m water depth. No sinking particle samples were obtained in November at site NG2 due to a trap system recovery problem. Surface sediments were collected by scrapping the first oxic millimeters (up to 1 cm) obtained from a Van Veen grab sampler. Sinking particles were collected using a trap system described by Gascón Díez et al. (2016, 2018). Briefly, it was composed of a weight, an acoustic release, and a particle trap frame composed of six 80 cm-long Plexiglas tubes of 11 cm internal diameter placed 5 m above the lake bottom (actual sampling depth: 132 at NG2 and 187 m at NG3) and maintained vertically by two buoys during 26–29 days. Sinking particles from the six Plexiglas tubes were pooled. Bottom sediment and sinking particles samples were transported to the laboratory in a cool box prior to processing. Sediments were freeze-dried in a CHRIST BETA 1–8 K freeze-drying unit (−54 °C, 6 Pa) for at least 48 h. Dry samples were ground and homogenized with an agate mortar.

2.2. Sediment grain size distribution and organic matter characterization

A laser diffraction analyzer Coulter LS-100 (Beckman Coulter, USA) was used to determine particle grain size distributions in the size range 0.4 900 μm, following the procedure described by Loizeau et al. (1994) on wet samples.

Rock-Eval® analyses were performed at the Institute of Earth Sciences of the University of Lausanne (Switzerland) using a Rock-Eval® 6 (Technologies Vinci, Rueil-Malmaison, France). During Rock-Eval pyrolysis ~ 160–170 mg of ground and homogenized sample is subject to a pyrolysis step followed by the complete oxidation of the residual sample. A flame ionization detector measures the hydrocarbon released during pyrolysis, while CO_2 and CO are detected by infrared absorbance during both steps. The applied standard cycle consists of pyrolysis starting isothermally at 300 °C, then the sample is heated to 650 °C. The oxidation step starts isothermally at 400 °C and then heats up to 850 °C. Organic carbon decomposition results in 4 main peaks: the S1 peak (hydrocarbons released during the isothermal phase), the S2 peak (hydrocarbons produced between 300 and 650 °C), the S3 peak (CO_2 from pyrolysis of OM up to 400 °C), and the S4 peak (CO_2 released from residual OM below ~ 550 °C during the oxidation step). Mineral carbon decomposition is recorded by the S3' peak (pyrolysis-CO_2 released above 400 °C), and the S5 peak (oxidation-CO_2 released above ~ 550 °C). These peaks are used to determine the amount of total organic carbon (TOC) and the amount of mineral carbon (MINC). In addition, the calculated hydrogen index (HI = S2/TOC) and oxygen index (OI = S3/TOC) are proportional to the H/C and O/C ratios of the organic matter, respectively. They are used for OM classification in a Van-Krevelen-like diagram (Espitalié et al. 1985/1986). The HI and OI signatures allow a rough distinction between algal/bacterial (types I and II kerogen) and terrestrial plant (types III and IV kerogen) OM, as well as the degree of degradation and oxidation (Behar et al., 2001). The calibration standard used was the IFP-160,000, a Toarcian marine sediment collected in the Paris Basin.

Analytical precision was better than 0.05 wt.% (1σ) for TOC, 1.5 °C (1σ) for Tmax, 10 mg HC/g TOC (1σ) for HI, and 10 mg CO2/g TOC (1σ) for OI.

2.3. Measurements of total mercury and methylmercury concentrations

Total mercury (THg) was analyzed using a Direct Mercury Analyzer (DMA-80 III, MWS GmbH, Switzerland) that consists of thermal

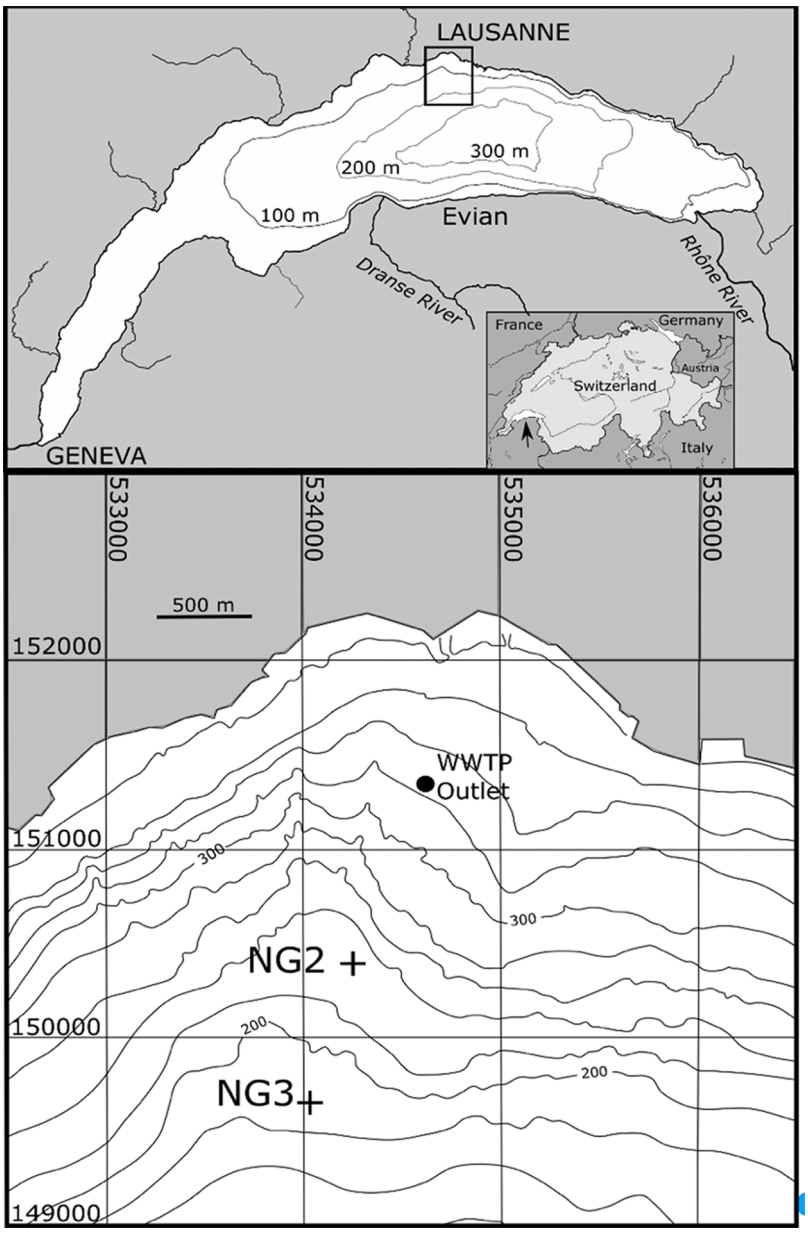

Fig. 1. Location map of the two sampling sites NG2 and NG3 in Lake Geneva. Contours indicate the altitude of the lake botom above sea level. The lake surface is at an altitude of 372 m. Inset maps: small map – Lake Geneva (arrow) at the border between Switzerland and France, large map – Lake Geneva bathymetry, the crosses indicate the position of the sampling sites.

decomposition, amalgamation and atomic absorption spectroscopy. All analyses were run in triplicate using 0.1–0.2 g of dried and homogenized samples. The detection limit and working range were 0.01 mg/kg and 0.03–50 mg/kg, respectively. Certified reference material (marine sediment SRM 2702, 0.4474 ± 0.0069 mg/kg) was measured at the beginning and end of each measurement series. Deviation of analyses was lower than 5% of the certified value.

MeHg in the solid dried phases (sediments and sinking particles) was extracted using the HNO_3 leaching/CH_2Cl_2 extraction method described by Liu et al. (2012) and subsequently analyzed following the US EPA Method 1630. The extract is purged in a vessel allowing the N_2 flow to volatilize ethylated Hg species to a Tenax trap. The trapped Hg is thermally released and carried into a GC column, with Ar as a carrier gas. Hg species are separated on a GC column and then thermally reduced to elemental mercury. Finally, Hg is detected using a Brooks Rand Model III cold vapor atomic fluorescence spectrophotometer (CV-AFS). Manufacturer certified accuracy is 109% and precision 3%. Samples were run in triplicates and the recovery of extractions and analyses of the certified reference material (ERM-CC580) were on average 90%.

2.4. Molecular analysis

DNA was extracted in triplicate from freeze-dried samples with the Power Soil RNeasy Extraction Total RNA Kit and RNeasy PowerSoil Elution kit (Qiagen) under sterile conditions according to instructions from the manufacturer. DNA quantity and quality was addressed on 1% agarose gel by electrophoresis in 0.5% Tris EDTA buffer and by spectrophotometry. DNA was used for quantitative PCR quantification of genes 16S rRNA, *dsrA* and *merA* as described in Bravo et al. (2016), as well as *hgcA* for Desulfobacterota (previously Deltaproteobacteria with NCBI classification) and Firmicutes using primers described in Christensen et al. (2016). Appropriate standard curves and no-template controls were included in triplicate for each reaction and the target copy number per sample was calculated. Metagenome sequencing was

carried out at the facility of the institute for clinical microbiology (IKMB) hosted by Kiel University (Germany), using Nextera DNAflex and NovaSeq SP 2 × 150 bp sequencing kit in pair-end 150 bp read length mode Ion Illumina NovaSeq 6000.

2.5. Bioinformatics

For the 11 obtained metagenomes, between 31.6 and 56.8 million reads were obtained. The raw data are accessible at NCBI (SRA accession: PRJNA831615). Trimmomatic (Bolger et al., 2014) 0.36 was used to quality trim the data with the following parameters: ILLUMINACLIP: NexteraPE-PE.fa:2:30:10, LEADING:5, TRAILING:5, MINLEN:36. The metagenome assembly of the DNA samples, generated using the assembler MEGAHIT 1.1.2 (Li et al., 2016) with default settings, yielded 10,535,672 contigs. This was followed using the software Prodigal 2.6.2 (Hyatt et al., 2010) for prokaryotic gene prediction that detected 15, 658,347 protein-coding genes in the co-assembly. The DNA reads from the 11 metagenomes were mapped against the contigs with bowtie2, and the resulting sam files were converted to bam files using samtools 1.9 (Li et al., 2009). On average 43,763,487 (± 5159,940) DNA sequences were mapped onto the assembly. The .bam files and the prodigal output .gff file were used to estimate reads counts by using the featureCounts software (Liao et al., 2014). The overall community composition was evaluated using the software kraken2 2.0.8 (Wood et al., 2019) and bracken 2.5 (Lu et al., 2017) with default settings.

2.6. Detection and taxonomic identification of hgc genes

To detect hgc genes in the metagenomes, we firstly looked for *hgcAB* homologs with the function *hmmsearch* from HMMER software 3.2.1 (Finn et al., 2011) and using the HMM profiles from the recent database Hg-MATE version 01 (Gionfriddo et al., 2021) as a reference database of protein sequences. We considered 5174 genes with E-values $\leq 10^{-3}$ as significant hits. We used knowledge from the seminal paper of Parks et al. (2013) that described unique motif from *hgcA* (NVWCA(A/G/S)GK) and *hgcB* genes (C(M/I)EC(G/S)(A/G)C). We kept information about *hgcA* genes found alone in their contigs or side-by-side to *hgcB* genes. For distribution plots, only read counts from *hgcA* were used.

We calculated *hgcA* corrected read counts by dividing read counts by gene base pair (read/bp). In order to compare the *hgcA* counts obtained from different samples, we extracted the counts of the housekeeping gene *rpoB* from the metagenomes, following the strategy recommended in the consensus protocol for the study of *hgc* genes from metagenomes (Capo et al., 2022c). The *rpoB* genes found in the metagenomes were detected using the function *hmmsearch* from HMMER software 3.2.1 (Finn et al., 2011). We used *rpoB* HMM profiles provided by JCVI (http://tigrfams.jcvi.org/cgi-bin/index.cgi): TIGR02013.hmm for bacterial *rpoB* genes. Verified hits of *rpoB* genes were detected using the trusted cut-off. We calculated *rpoB* coverage values by dividing the number of read mapped to each *rpoB* genes to their number of base pairs (reads/bp). We normalized each *hgcA* gene coverage values by *rpoB* coverage values and obtained the final dataset (Table S1).

To putatively taxonomically annotate the *hgc* genes detected in our metagenomes, we used the recent database Hg-MATE version 01 (Gionfriddo et al., 2021) that compiled a total of 1020 *hgc* sequences from publicly available isolate genomes ($n = 204$), single-cell genomes ($n = 29$) and metagenome-assembled genomes ($n = 787$). To taxonomically identify each Hg methylation gene, we constructed two phylogenetic trees: one for concatenated *hgc* gene pairs and one for *hgcA* genes (Fig. S1). For each phylogenetic analysis, the amino acid sequences were aligned using MUSCLE in the software MEGAX (Kumar et al., 2018). RAxML 8.2.10 (Stamatakis et al., 2014) was used to generate maximum likelihood (ML) tree under the GAMMA distribution with the LG model with the following parameters: raxmlHPC -f a -p 283976 -m PROTGAMMAAUTO -N autoMRE -x 2381 -T 10. Branch support was generated by rapid bootstrapping.

2.7. Statistics

Homoscedasticity and normality of values, and/or residuals, were verified by Bartlett's tests and Kolmogorov-Smirnov's tests in RStudio software 4.0.3. Because these criteria were not met, Wilcoxon-Mann-Whitney tests were applied ($p = 0.05$). A principal coordinate analysis (PCoA) was performed applying the function *wcmdscale* to a Bray-Curtis dissimilarity matrix built with the function *vegdist* from (i) the *hgcA* gene abundance table (ii) the species abundance table provided by the bracken analysis. A PROTEST permutation procedure analysis (1000 permutations) was performed using the function *Procrustes* to evaluate the level of concordance between the distribution of hgc^+ microbial groups and the composition of the prokaryotic community. The functions *wcmdscale, vegdist, procrustes* belong to the R package vegan (Oksanen et al., 2015) and the functions *rcorr* and *corrplot* belong to the R packages Hmisc (Harrell and Harrell, 2013) and corrplot (Wei et al., 2021), respectively.

3. Results and discussion

3.1. Higher MeHg production in lake sinking particles than sediments

Mean values of THg and MeHg concentrations were similar between sites but different between anoxic sediments and sinking particles from oxic water columns. While THg concentrations were significantly higher in sediments than in sinking particles, the opposite trend was observed for MeHg concentrations, showing higher concentrations in sinking particles than in sediments (Fig. 2; Wilcoxon rank test p-value < 0.05). No clear trends were observed between September, October and November samplings therefore data are summarized with range and mean concentrations (Table S2).

The results of these field campaigns (2017) agree with observations made at the same sites in 2010, 2011 and 2014 by Gascón Díez et al. (2016; 2018). Similarly, at both sites, the ratio between MeHg and THg (%MeHg/THg) was significantly higher (Wilcoxon rank test p-value $<$ 0.05) in sinking particles (12% to 38%) than in sediments (1% to 4%) (Fig. 2) and are in good agreement with previously reported Hg methylation rate constants (Gascón Díez et al. 2016). Samples were collected in the northern part of Lake Geneva (Fig. 1), close to the city of Lausanne, not far from the Bay of Vidy, where the Lausanne's wastewater treatment plant releases its effluent of treated and untreated waters. However, Gascón Díez et al. (2018) showed that the effluent has no, or a negligible, influence on sinking particle Hg contamination at the NG2 and NG3 sites.

Particle size distribution was very homogenous in all samples, with an overall mean grain size of 22.1 μm and a range of the mean sizes of 18.4–26.8 μm (Table S3). Most of the particles are in the silt size range (81.2%), with a small contribution of sand size particles (13.5%) and clays (5.4%). No significant differences were observed between bottom sediment and sinking particles (Wilcoxon rank test, p-value > 0.05).

3.2. Contrasted microbial communities in sinking particles and anoxic sediments from Lake Geneva

The overall prokaryotic community from anoxic sediments and sinking particles was dominated by Alpha-, Beta-, Gammaproteobacteria and Actinobacteria being the most abundant taxa (Fig. 4). We used principal Coordinates Analysis (PCoA) to explore and to visualize similarities or dissimilarities of the overall prokaryotic community (Fig. 5A) and the structure of hgc^+(Fig. 5B) across sites but also between niches (sediments versus sinking particles). While no differences in terms of community composition were observed across sites (NG2 and NG3), the community structure of sinking particles and sediments differed (Fig. 5A). Betaproteobacteria, Deltaproteobacteria (Desulfobacterota in GTDB classification), Actinobacteria and Euryarchaeota were more abundant in bottom sediments compared to sinking particle's

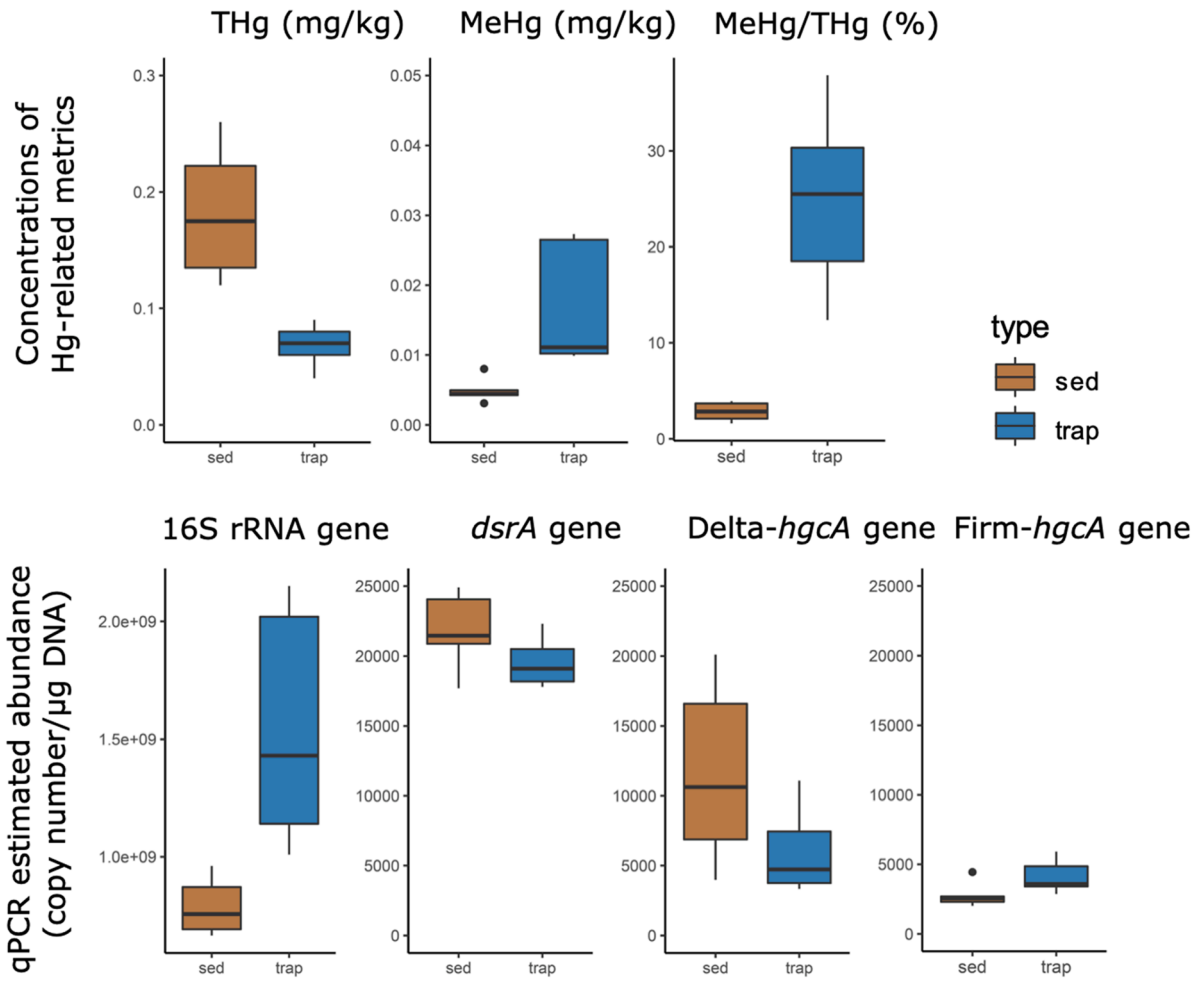

Fig. 2. Upper panels: THg concentrations, MeHg concentrations, MeHg/THg ratio (%). Lower panels qPCR estimates abundance (in copy number/µg DNA) of 16S rRNA genes, *dsrA* genes, *hgcA* genes specific to Desulfobacterota (Delta-*hgcA*) and *hgcA* genes specific to Firmicutes (Firm-*hgcA*) in sediment (sed, color brown) and sinking particles (trap, color blue).

Mean TOC content in sinking particles (4.57% ± 0.90%) was significantly higher (p-value < 0.05) than in sediments (2.10% ± 0.22%). The hydrogen index (HI) values in sinking particles are significantly higher ($p = 0.004$) than in sediment, whereas oxygen index (OI) values are similar ($p = 1$) (Fig. 3A). HI values around 500 mg HC/g TOC and OI values below 200 mg CO_2/g TOC measured in sinking particles correspond to fresh phytoplankton (Carrie et al., 2012). They plot close to the type II kerogen, usually associated with OM of marine algal origin (Fig. 3A), which cannot be the case in the present situation. This suggests that sinking OM in these sampling locations was a mixture of in-situ OM production (type I) and terrestrial OM inputs (type III). The decrease of HI values alone in sediments suggests that sediment has undergone early diagenesis that preferentially reduces lipids, enriched in hydrocarbon (e.g., cell walls) (Langford et al., 1990) as reflected by the reduction of S2 peak (Fig. 3B) with a concomitant decrease in TOC content. Therefore, there is no evidence to suggest a different origin of OM in the two sample types but rather a more processed OM in sediments compared to the OM characterized in sinking particles. Data here confirms the role of sinking particles as an environment enriched in fresh organic matter and thus prone to MeHg formation in oxic water columns in line with previous studies (Gascón Díez et al., 2016; Bravo et al., 2017).

metagenomes (Fig. 4). In contrast, higher proportions of Gammaproteobacteria and Cyanobacteria were observed in sinking particles compared to bottom sediments. Alphaproteobacteria, Firmicutes, Bacteroidetes and Deinococcus-Thermus were found with similar proportions in bottom sediment and sinking particlés metagenomes (Fig. 4). Previous studies also reported higher seasonal variability in the composition of bacterial communities from pelagic ecosystems (Allgaier et al., 2006; Lima et al., 2016) than sediments (Tšertova et al., 2011; 2013). These differences were explained by the occurrence of episodic disturbances having a larger impact in open water column than in sediments, such as rainfall (Andersson et al., 2014), environmental impacts (Rösel et al., 2012), storms and strong winds (Jones et al., 2008), solar radiation (Pérez et al., 2007) and anthropogenic impacts (Zhang et al., 2020).

Similarly, to the overall prokaryotic community, the structure of hgc^+ community differed between sediments and sinking particles (Fig. 5B). In other words, although the hgc^+ community was quite similar between sites (NG2 and NG3) and sampling times (September, October and November), a larger variability was observed in terms of structure in sinking particles compared to sediments, suggesting the occurrence of several microenvironments in the former. Accordingly, the procrustean analysis, providing a descriptive summary and graphical comparison of the ordination of the communities (prokaryotic Fig. 5A and the distribution of $hgcA^+$ Fig. 5B) showed a significant level of concordance ($r = 0.96$, pval = 0.001) between the composition of the prokaryotic community and the distribution of $hgcA^+$ microorganisms (Fig. 5C), confirming that both communities are similar in sediments and sinking particles across sites and sampling times but different between sinking particles and sediments. Previous studies have shown that the molecular composition of organic matter had impact in the composition of hgc^+ community (Bravo et al., 2018a) and in the overall prokaryotic community (Pérez and Sommaruga, 2006). Accordingly, the

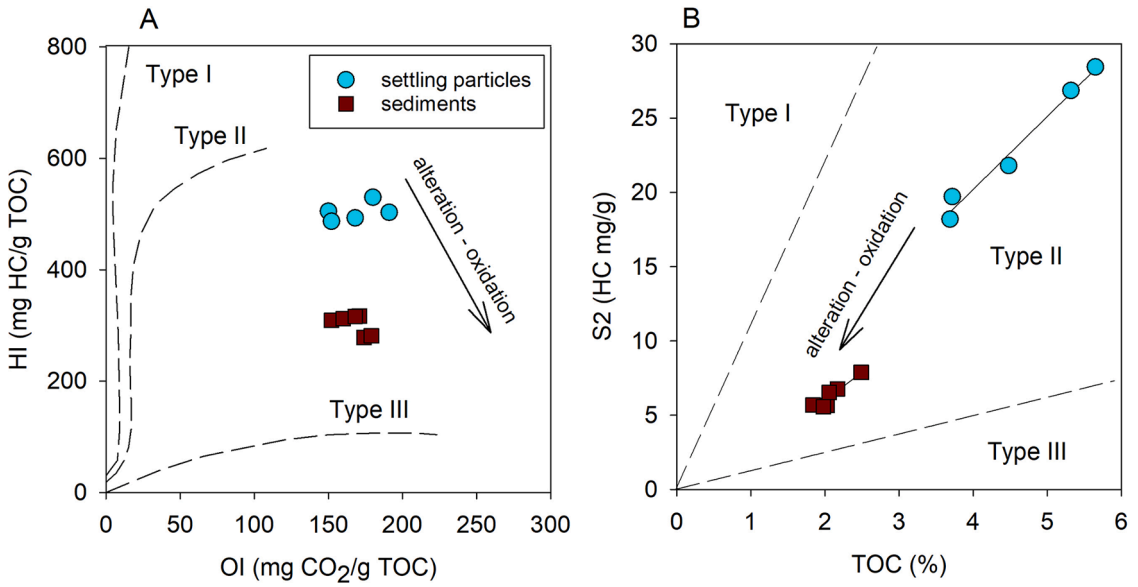

Fig. 3. Characterization of the organic matter with Rock-Eval analyses. (A) HI index vs OI index. OM type I ("lacustrine aquatic"), II ("marine aquatic") and III ("terrestrial") correspond to references (Langford et al., 1990; Steinmann et al., 2003; Ariztegui et al., 2001). (B) S2 peak vs. TOC (Ariztegui et al., 2001).

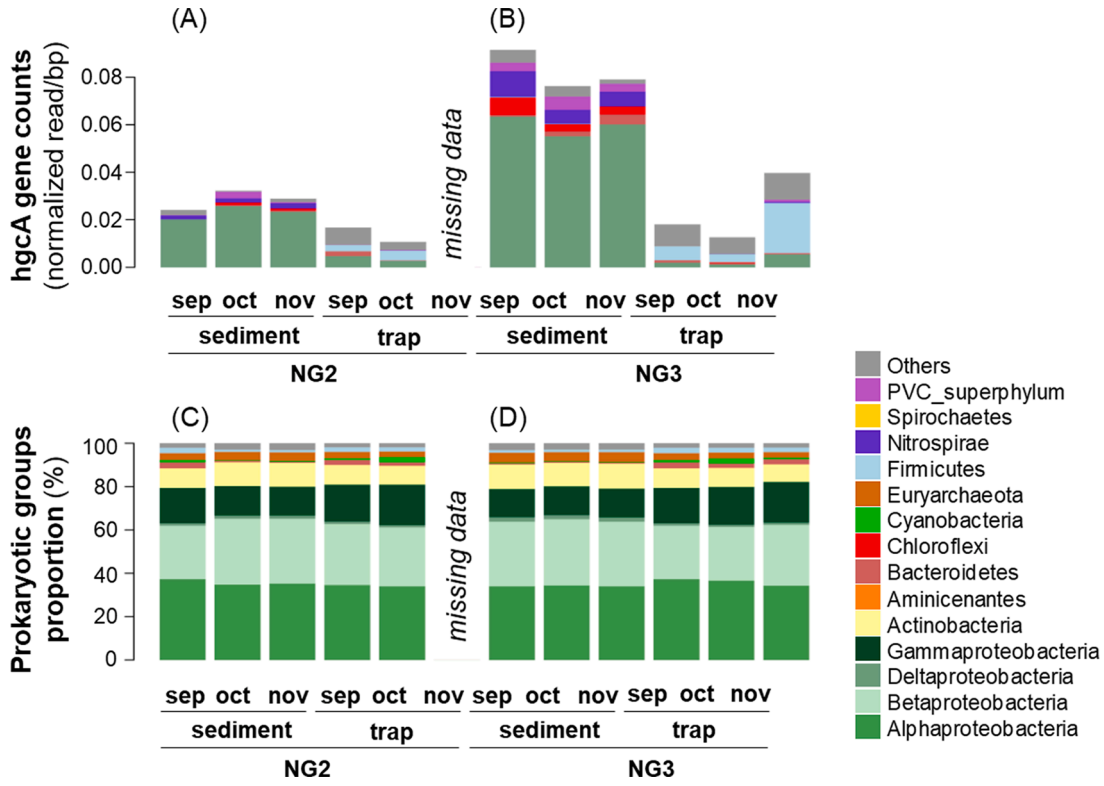

Fig. 4. Distribution of the normalized corrected read counts of *hgcA* genes identified from prokaryotic groups in metagenomes in NG4 (A) and NG3 (B) and proportion of prokaryotic groups DNA sequences found in NG2 (C) and NG3 (D) metagenomes.

differences found in the HI and OI indexes, used as proxies for OM characteristics, could in part explain the differences in community compositions between sinking particles and sediments.

3.3. Unveiling the diversity of Hg methylators in sinking particles of oxic water and anoxic sediments

In metagenomes obtained from sinking particles and anoxic sediments of Lake Geneva, a total of 59 *hgcA* genes were found including 9 paired with *hgcB* genes (i.e., side-by-side on the same contig) (Table S1). Among them, 27 were identified as Desulfobacterota particularly, Geobacterales (9 *hgc* genes), Desulfatiglandales (3 *hgc* genes), Desulfobulbales (1 *Desulfobulbus*) and Desulfuromonadales (1 Desulfuromonas). Other microbial lineages identified included Firmicutes (6 genes), Nitrospirae (3 genes), Bacteroidetes (4 genes), Chloroflexota (4 genes) and members of the PVC superphylum (4 genes).

In terms of composition, while sinking particle's metagenomes exhibited higher proportion of hgc^+ Firmicutes (Fig. 4), hgc^+ members of

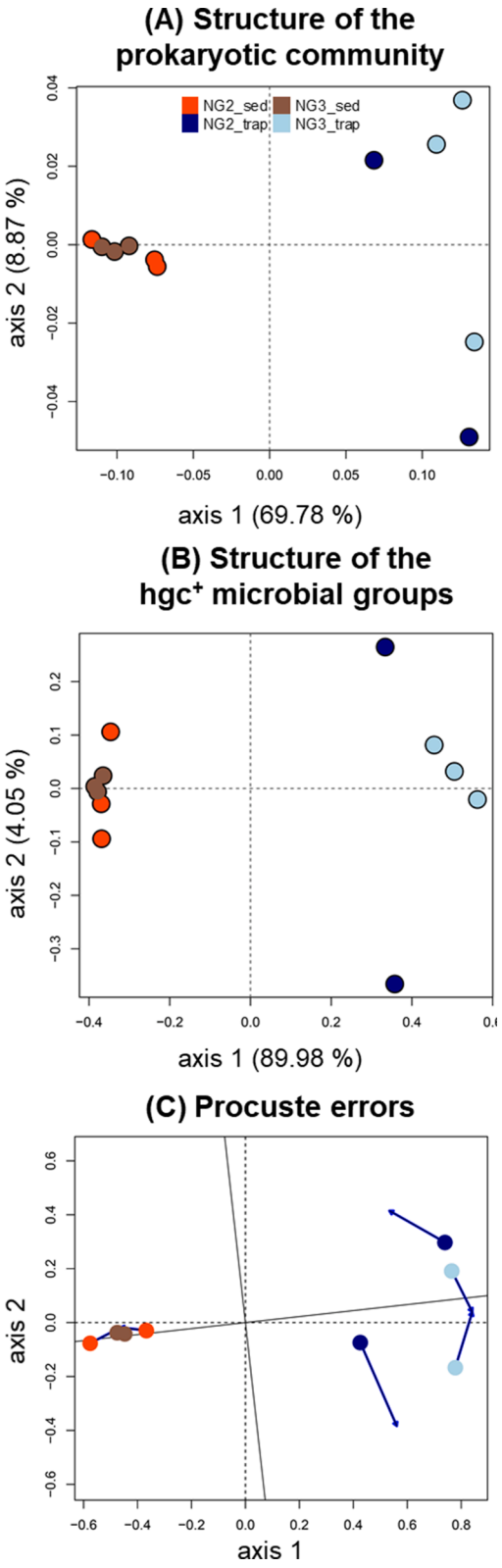

Fig. 5. Differences observed between metagenomes in terms of (A) prokaryotic community composition. (B) Relative abundance of *hgcA* genes. (C) Plot of procrustean rotation analysis illustrated the potential differences in structure of prokaryotic community and relative abundance of *hgcA* genes. The directions of arrows illustrate differences in structure from the prokaryotic community to the relative abundance of *hgcA* genes.

Desulfobacterota, Nitrospirae, Chloroflexota and PVC superphylum were prevalent in sediment metagenomes. Compared to a previous study describing the diversity of putative Hg methylators in the nearby Vidy Bay sediments (Bravo et al., 2018b), the use of the *hgc* gene catalog Hg-MATE (Capo et al., 2022c; Gionfriddo et al., 2021) allowed to achieve a refined identification of Hg methylators of anoxic sediments and sinking particles from Lake Geneva. For instance, this study allowed a better identification of hgc^+ Desulfobacterota, which were up to 60% of the unidentified Hg methylators in Bravo et al. (2018b). We also detected the presence of hgc^+ members of the PVC superphylum, a microbial group for which the microbial ecology is still scarcely revealed and that was recently identified as potential important Hg methylators (Peterson et al., 2020; Capo et al., 2020).

Our results are in good agreement with the three lake metagenomes analyzed by Podar et al. (2015), highlighting the dominance of *hgcA* genes from Desulfobacterota (*Geopsychrobacter, Syntrophobacter, Geobacter*) in anoxic bottom waters and a higher diversity in hgc^+ Desulfobacterota, Firmicutes and methanogenic Archaea in sediments. Also, previous studies have highlighted the importance of methanogenic Archaea and iron-reducing bacteria (from the Geobacteraceae family) in Hg methylation in boreal lakes (Bravo et al., 2018b). Additionally, two recent studies based on the analysis of metagenomes from sulfate-enriched lakes revealed the presence of other hgc^+ microbial lineages including Kiritimatiellaeota (from PVC superphylum), Spirochaetes or Aminicenantes (Jones et al., 2019; Peterson et al., 2020). The contrasted diversity between water column and sediments was also recently observed in a brackish ecosystem, the Baltic Sea, in which hgc^+ Spirochaetes were predominant in sinking particles (Capo et al., 2020) but not in anoxic sediments (Capo et al., 2022a), while certain hgc^+ Desulfobacterota were abundant in both environments.

Previous studies suggested the presence of potential anaerobic Hg methylators in sinking particles of oxic lake water column (Gascón Díez et al., 2016); this study provides evidence of the presence of those microorganisms in such environments and reveal the differences between sediments and sinking particles in terms of Hg methylators composition. Our results not only confirm the role of Desulfobacterota in sediments but also unveil the diversity of Hg methylators in sinking particles, highlighting an important contribution of hgc^+Firmicutes to the Hg methylating microbial community of Lake Geneva.

3.4. High MeHg concentrations in sinking particles from oxic water columns

One key challenge related to the research about Hg cycling in the environment is the prediction of MeHg formation in the environment. The measurement of Hg methylation rates conducted in the laboratory by spiking isotopic Hg into environmental samples was used for decades to inform about the potential formation of MeHg in environmental conditions (Regnell et al., 2019). Relationships between the abundance of specific genes and corresponding rates are still rarely demonstrated in natural systems (Rocca et al., 2015), particularly in the context of Hg methylation (Bravo and Cosio, 2020). Here, we expanded previously obtained geochemical data obtained by Gascón Díez et al. (2016; 2018) by applying molecular methods.

In the present study, positive and significant correlations were observed between MeHg concentrations and the metagenomics *hgcA* estimates taxonomically assigned to Firmicutes ($r = 0.67$, $p = 0.02$) (Fig. S2), in line with their long-known role in Hg methylation (Bravo and Cosio, 2020). In contrast, negative relationships were found between metagenomics *hgcA* estimates from Desulfobacterota and MeHg concentrations ($r = -0.77$, $p = 0.01$), although reduced Hg methylation rate was observed when SRB are inhibited by molybdate (Gascón Díez et al., 2016). Linking MeHg concentrations with *hgcA* genes abundance is not the aim of this study, however our data suggest that obtaining *hgcA* transcript data, aside Hg chemistry data, could help to identify factors controlling their expression and inform on condition causing

subsequent MeHg formation in the environment, as recently demonstrated in the Baltic Sea (Capo et al., 2022b). It is well recognized that the abundance of *hgc* genes, transcripts or proteins in the environment is not the only factor controlling the MeHg formation. Indeed, the bioavailability of Hg is tightly regulated by environmental factors such as the composition of the organic matter (Chiasson-Gould et al., 2014) and the presence of sulfur compounds, such as thiols (Schaefer and Morel, 2009; Adediran et al., 2019). In our case, Gascón Díez et al. (2016) reported that MeHg concentrations observed in anoxic sediments and sinking particles from oxic water columns of Lake Geneva were mostly explained by Hg methylation (k_m rates) compared to MeHg demethylations (k_d rates).

In this study, we also compared qPCR (copy number/μg DNA) and metagenomic estimates (i.e., normalized length corrected read numbers) for *hgcA* genes from two microbial groups (Desulfobacterota and Firmicutes) (Table 1). This concordance between qPCR and metagenomics estimates was confirmed by significant positive Spearman rankś correlations for both Desulfobacterota ($r = 0.80$, pval<0.01) and Firmicutes ($r = 0.85$, pval<0.01) (Fig. S2), supporting that metagenome efficiently identified those bacterial groups. Both qPCR and metagenomics-based approaches could be useful to estimate the amounts of *hgc* genes present in the environment, although both provided in theory inherently biased estimates due to methods.

Our results suggest the presence of several sulfate-reducers not involved in Hg methylation as the *dsrA* gene (involved in sulfate reduction; Wagner et al., 1998), and *hgcA* counts (qPCR and metagenomics) estimates from Desulfobacterota were not correlated. Although many hgc^+ microorganisms have been described as sulfate reducers (Gilmour et al., 2013; Podar et al., 2015) (Fig. S2), a small proportion of sulfate reducing bacteria isolates are confirmed carrier of hgc^+ and few are experimentally confirmed Hg methylators (Yu and Barkay, 2022). In this context, our results either indicate the presence of sulfate reducers in both sinking particles and sediment lacking the biological Hg-methylation capacity (i.e., not devoid of *hgcA* genes) or might suggest that hgc^+ Desulfobacterota do not have the metabolic capacity for sulfate reduction. Our results support, for the first time, the hypothesis that Firmicutes may be key Hg methylators in the oxic water column of Lake Geneva while being not detected in the oxic water columns of marine systems[20,37] and still understudied in lakes. HI index indicated that OM in sediments was more degraded than in sinking particles. The positive correlation between the abundance of hgc^+ Firmicutes (Fig. S2) and the HI could indicate that those microorganisms might be favored by the freshness of OM.

4. Conclusions

The formation of MeHg in the environment is of high importance because this neurotoxic compound can cause severe threats to the ecosystems and human health. There are still uncertainties about which environmental conditions trigger this microbially-mediated process, as well as the diversity and ecology of microorganisms involved in Hg methylation in aquatic environments. The results of this study highlight the presence of hgc^+ microorganisms in sinking particles of the oxic water column of Lake Geneva and point the Firmicutes as potential key Hg methylators in micro-anaerobic niches from sinking particles. The role of anaerobic microorganisms in oxic ecosystems has been for a long time overlooked. This study shows their presence and potential key role in the largest freshwater reservoir of western Europe with potential effect on global Hg biogeochemical cycle.

CRediT authorship contribution statement

Eric Capo: Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Claudia Cosio:** Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Elena Gascón Díez:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Jean-Luc Loizeau:** Conceptualization, Investigation, Formal analysis, Writing – review & editing, Funding acquisition. **Elsa Mendes:** Investigation, Formal analysis, Writing – review & editing. **Thierry Adatte:** Investigation, Formal analysis, Writing – review & editing. **Sören Franzenburg:** Investigation, Formal analysis, Writing – review & editing. **Andrea G. Bravo:** Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition.

Table 1
Metagenome- and qPCR-estimate of *hgcA* genes from Desulfobacterota and Firmicutes. Units are in *hgcA* coverage values normalized by *rpoB* coverage values for metagenome estimates and in copy number by μg DNA for qPCR estimates.

Sites	Types	Months	Desulfobacterota		Firmicutes	
			metagenome	qPCR	metagenome	qPCR
NG2	sed	sept	1.53E-02	3984	0	2025
NG2	sed	oct	1.54E-02	8058	0	2263
NG2	sed	nov	1.52E-02	6468	0	2429
NG2	trap	sept	2.40E-03	11,078	2.65E-03	2868
NG2	trap	oct	1.49E-03	3342	4.16E-03	3582
NG3	sed	sept	3.79E-02	17,723	2.30E-04	2670
NG3	sed	oct	2.97E-02	13,195	2.30E-04	4444
NG3	sed	nov	3.82E-02	20,103	0	2693
NG3	trap	sept	4.91E-04	4728	5.84E-03	4862
NG3	trap	oct	6.52E-04	3745	3.41E-03	3404
NG3	trap	nov	1.61E-03	7436	2.10E-02	5901

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

The raw data are accessible at NCBI (SRA accession: PRJNA831615, SUB11359380)

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.119368.

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