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Microbial diversity and abundance of Hg related genes from water, sediment and soil the Colombian amazon ecosystems impacted by artisanal and small-scale gold mining

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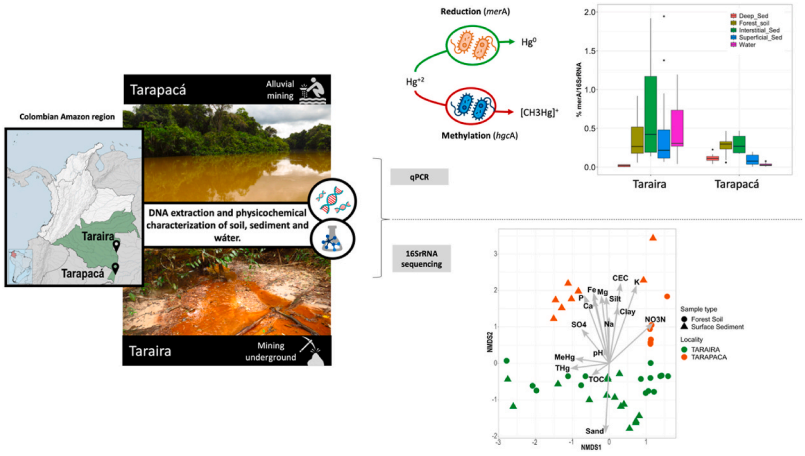
HIGHLIGHTS

- In long-polluted sites bacterial communities are more affected by environmental variables than by Hg.
- *MerA* was more abundant in Taraira sediments in line with higher mercury levels.
- The Hg-immobile form was dominant, probably limiting microorganism access.
- *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes,* and *Chloroflexi* dominated in high Hg sites.

GRAPHICAL ABSTRACT



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ABSTRACT

The Amazon region abounds in precious mineral resources including gold, copper, iron, and coltan. Artisanal and small-scale gold mining (ASGM) poses a severe risk in this area due to considerable mercury release into the surrounding ecosystems. Nonetheless, the impact of mercury on both the overall microbiota and the microbial populations involved in mercury transformation is not well understood. In this study we evaluated microbial diversity in samples of soil, sediment and water potentially associated with mercury contamination in two localities (Taraira and Tarapacá) in the Colombian Amazon Forest. To this end, we characterized the bacterial

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merA gene
hgcA gene

community structure and mercury-related functions in samples from sites with a chronic history of mercury contamination which today have different levels of total mercury content. We also determined mercury bioavailability and mobility in the samples with the highest THg and MeHg levels (up to 43.34 and 0.049 mg kg^{-1}, respectively, in Taraira). Our analysis of mercury speciation showed that the immobile form of mercury predominated in soils and sediments, probably rendering it unavailable to microorganisms. Despite its long-term presence, mercury did not appear to alter the microbial community structure or composition, which was primarily shaped by environmental and physicochemical factors. However, an increase in the relative abundance of *mer*A genes was detected in polluted sediments from Taraira. Several Hg-responsive taxa in soil and sediments were detected in sites with high levels of THg, including members of the *Proteobacteria, Acidobacteria, Actinobacteria*, *Firmicutes* and *Chloroflexi* phyla. The results suggest that mercury contamination at the two locations sampled may select mercury-adapted bacteria carrying the *merA* gene that could be used in bioremediation processes for the region.

1. Introduction

The Amazon rainforest is one of the Earth's most important reservoirs of biodiversity; it contains a significant portion of the world's freshwater, providing crucial ecosystem services like water and nutrient recycling, climate regulation, and carbon storage (Venturini et al., 2022). In recent decades, this unique ecosystem has come under increasing threat from anthropogenic activities, such as artisanal and small-scale gold mining (ASGM). The main methods used by artisanal miners to extract gold involve mining underground, or open pit or wet alluvial ores followed by amalgamation or the concentrates or the whole ore (Veiga et al., 2014) (). The first method involves extraction followed by grinding through cyanidation or amalgamation, while the second method extracts gold from riverbeds using suction pumps or panning (Cardona et al., 2022). ASGM is a significant problem due to the misuse of mercury (Hg) in the amalgamation process to extract gold from ores or concentrates. The process involves forming a gold-mercury amalgam, which when heated releases mercury vapors, leaving behind a mixture of gold and other metals in smaller proportions. Alluvial ores, with low gold grades, are concentrated in sluice boxes and the concentrates are

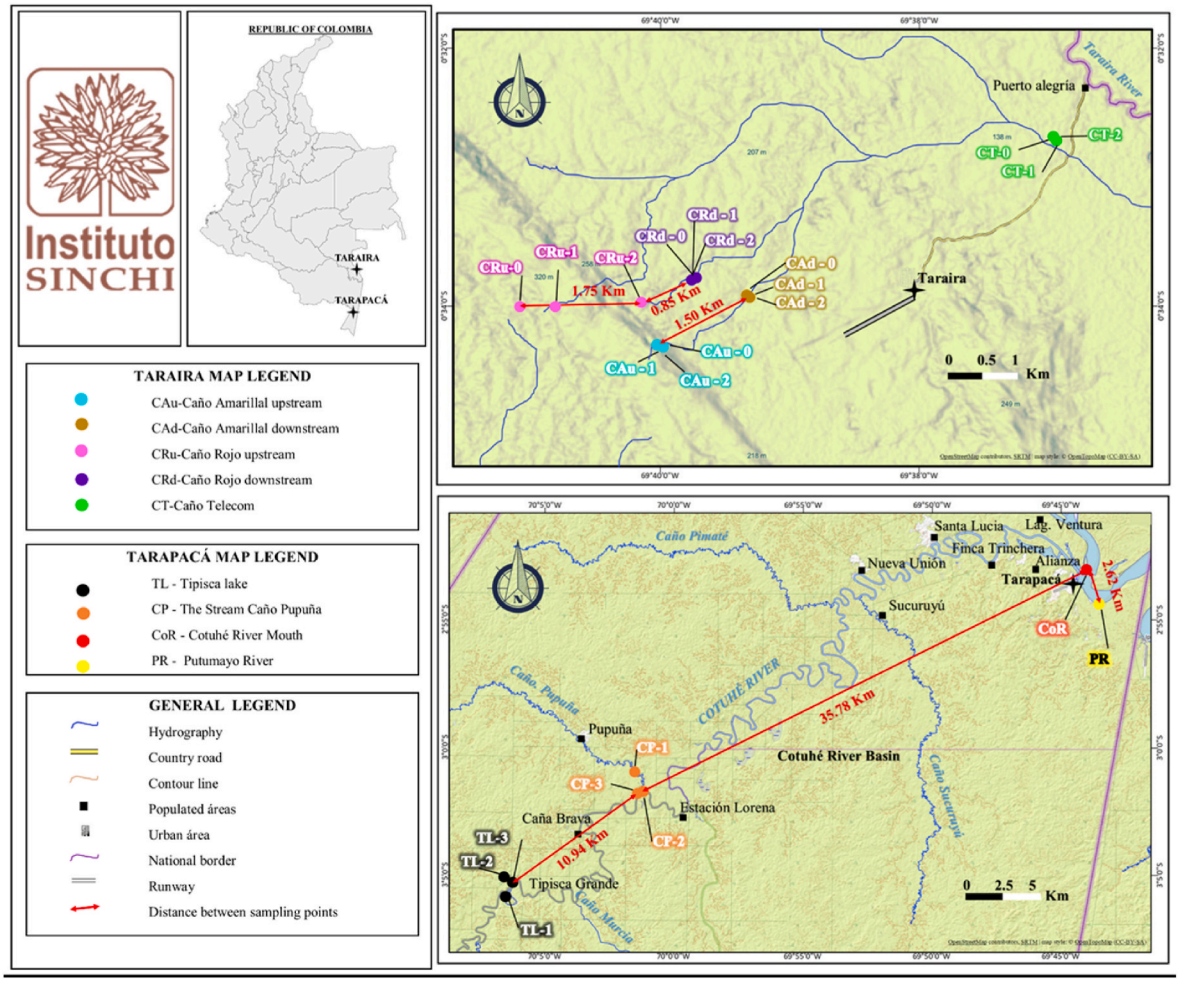

Fig. 1. Map showing the location and distribution of sites across the sampled regions (Tarapacá and Taraira). (a). Map of location of Tarapacá sites: Tipisca Lake (TL), Caño Pupuña, Cotuhé river (CoR) on the Cotuhe River Basin and Putumayo rivers (PR) on the Putumayo River Basin. (b). Map of Taraira sites where the highest mercury pollution samples were collected: Caño Amarillal (CAu), Caño Amarillal downstream strech (CAd), Caño Rojo (CRu), Caño Rojo downstream stretch (CRd) and Caño Telecom (CT). The names correspond to the sampling points where the water and sediment samples were collected. The forest soil samples were collected in the area of influence of the water bodies (not indicated in the maps). The numbers indicate the three sites collected in each site.

amalgamated. Using rudimentary techniques to amalgamate the free particles of gold, about 30–50% of the mercury introduced in the amalgamation process of concentrates is lost (García et al., 2015; Cordy et al., 2011; Veiga and Gunson, 2020).

ASGM presents a significant environmental risk to the region because of the release of large quantities of Hg into the water and the atmosphere during the extraction process (Moreno-Brush et al., 2020; Fritz et al., 2023). In 2022 alone, 327 kg of gold were extracted in 61 sites. This involved the removal of 12 million tons of river sediments and the discharge of 152 kg of mercury into the rivers (Gasparinetti et al., 2023). Mercury is a toxic element that affects the nervous system, as well as organs like the brain, heart, kidneys, lungs, and the immune system (Basu et al., 2023).

The abundance of mineral resources in the Colombian Amazon region has attracted informal ASGM activities for years, seriously affecting localities like Tarapacá and Taraira (Fig. 1). In the 1980s in Taraira, approximately between 10,000 and 120,000 underground miners were extracting between 100 and 200 g of gold per day. In this process, the waste that did not evaporate was discarded on the ground and in the pipes without any precautions. Currently, there are around 2000 miners, and amalgamation practices continue (Rubiano, 2014). In Tarapacá, the first four rafts were observed in the 1980s. Unlike in Taraira, the number has continued to grow, and currently between 40 and 50 rafts can be observed on the Cotuhé and Putumayo rivers. It is estimated that each raft extracts approximately 20–70 g of gold per day, depending on factors such as the number of miners, the size of the raft, and prevailing weather conditions (Valencia, 2015). ASGM has had a significant impact in terms of the increasing deforestation of the primary tropical rainforest. The quality of the water bodies has been drastically impaired by the removal of sediment during gold mining (Rubiano, 2014).

The mercury found in water, air, soil and sediment is of both natural and anthropogenic origin (Moreno-Brush et al., 2020; Reis et al., 2015), and the contribution of each type to Hg in fish is uncertain. The use of Hg isotope ratio analysis in river sediments from French Guiana showed an important direct contribution of ASGM to THg found in mining areas (Goix et al., 2019). However, the actual contribution of ASGM-related Hg (0) to fish tissue Hg was unclear (Laffont et al., 2021). Furthermore, recent studies related the origin of Hg in fish with the particular feeding habits of the species, with important differences between fish species (Eckley et al., 2023). Also, other studies have shown that deforestation and soil erosion, which in many cases also indirectly originate from ASGM, were the main cause of Hg pollution in the Amazon (Crespo-Lopez et al., 2021). Hg availability depends on its particular chemical form (Singh et al., 2023), and therefore its toxicity in soil is highly dependent on its chemical speciation (organic/inorganic Hg species), and its speciation depends on the physicochemical properties of the soil (Campos et al., 2018). The most mobile form of Hg in the environment is the mercury bound to inorganic ions (Araújo et al., 2019; Gutiérrez-Mosquera et al., 2020) but mercury bound to soil organic matter (humic substances) is also potentially mobile (Matsumoto and Liu, 2020). Hg(II) can be reduced to Hg (0) by mercury-reducing bacteria, which are generally associated with aerobic respiration and possess the *merA* gene encoding mercury reductase along with a variable set of genetic functions related to mercury transport (*merT*, *merP*, *merC*), demethylation (*merB*), and pathway regulation (*merR, merD*) (Priyadarshanee et al., 2022). Depending on the oxygen content and depth, especially in aquatic ecosystems and sediments, anaerobic bacteria, and in particular sulfate-reducing bacteria (SRB), can bring about the methylation of inorganic mercury when in the Hg(II) form. This process can also be undertaken by additional groups of bacteria with *hgcAB* genes, the genetic complement for mercury methylation (Gilmour et al., 2013; McDaniel et al., 2020; Yu and Barkay, 2022). Bacteria bearing the *merB* gene can carry out mercury demethylation and abiotic demethylation also occurs (Bishop et al., 2020). Of all these forms of mercury, methylmercury is the most potent bioaccumulating neurotoxin (Aaseth et al., 2020).

Extensive research has been conducted to study the effects of mercury on microbial communities in both natural settings and controlled microcosm experiments. Short-term exposure produces a rapid response from microbial communities, with higher mercury inputs leading to more significant changes in the abundance and diversity of soil bacteria and fungi (Frossard et al., 2017; Zheng et al., 2022; Du et al., 2022, 2023; Liu et al., 2023; Wang et al., 2020). Some studies observed an increase in the copy number of the *merA* gene in response to high levels of mercury contamination in soils (Liu et al., 2023; Pu et al., 2022; Zheng et al., 2022; Frossard et al., 2017). However, the impact of long-term mercury exposure in historically contaminated areas is less clear due to the influence of physicochemical factors and climate conditions (Frossard et al., 2018; Liu et al., 2014b, ; Liu et al., 2018a). Studies analyzing the *merA* gene in long-term exposure sites have yielded contradictory results (Ruuskanen et al., 2020; Frossard et al., 2018). For example, a recent global analysis of this question suggested increases in the diversity of *merA* rather than in its abundance in long-term exposed areas in the Northern Hemisphere (Ruuskanen et al., 2020).

Few studies have so far explored the impact of mercury on microbial communities in tropical forests, particularly in the Amazon. Most of these have analyzed mercury-resistant strains using culture-dependent techniques or study the effect of Hg on microbial communities in microcosmos with known Hg concentrations. Previous research in the Amazon region emphasized that the chemical forms of mercury must be taken into account when analyzing the impact of Hg on microbial communities, as must the environmental conditions and the physicochemical properties of the ecosystem (Mariano et al., 2020). As there is still limited knowledge about the microorganisms involved in mercury transformation in the Colombian Amazon region, this research is crucial for a better understanding of the impact of mercury in natural Amazonian ecosystems, the role of microbial community in Hg-speciation and the design of *ad hoc* bioremediation strategies based on the bioaugmentation of bacteria carrying the *mer* operon, with the ability to reduce Hg(II) to Hg (0); this reduction not only changes the chemical state of Hg to a volatile and less toxic form, but also decreases the concentrations of Hg(II) which is the substrate for Hg methylation. In the present study, our goal was to determine the extent to which mercury and/or physicochemical variables helped shape the bacteria community structure in two sites affected by a history of ASGM, and identify possible bioindicator taxa of Hg contamination.

2. Methodology

2.1. Study area and sampling

Samples were collected in June (Tarapacá) and October (Taraira) 2016 (Fig. 1) from these two localities in the Colombian Amazon region, where mercury has been used to amalgamate gold and lost to the environment by the artisanal miners (Table S1; Cardona et al., 2022). Goldminers in Tarapacá typically apply the alluvial gold extraction method, whereas underground gold mining is the standard method in Taraira. The sampling protocol and details were published in earlier research (Cardona et al., 2022).

2.2. Physicochemical parameters of water, sediment, and soil samples

The physicochemical properties of water, soil, and superficial sediment samples (Tables S2a, S2b and S2c) were determined using the methods and abbreviations summarized in Table S3. The total mercury and MeHg concentrations were determined previously (Cardona et al., 2022).

2.3. Sequential mercury extraction protocol (SEP) from selected Tarapacá and Taraira samples

The eight samples with the highest THg concentration were selected

for sequential mercury extraction described by (Bloom et al., 2003), which distinguished 5 fractions: F1: water-soluble mercury (Hg-w), F2: human stomach acid-soluble fraction (Hg-a), F3: organo-chelated fraction (Hg-o), F4: elemental Hg (Hg-e), and F5: residual mercury in sulfides and silicates (Hg-s). Subsequently, the fractions were combined as follows: The mobile Hg fraction (Hg-bio) was the sum of the Hg-w and Hg-a fractions; the potentially mobile fraction (Hg-o) was associated with the organic fraction; and the so-called immobile fraction was the sum of Hg-e and Hg-s fractions (Matsumoto and Liu, 2020). These eight samples consisted of three samples of surface sediment (SS) and two of forest soil (FS) from an of Caño Amarillal in Taraira, one sample of SS from Cotuhé River Mouth, and one FS sample of Caño Rojo and from Caño Pupuña. Protocol details are summarized in Table S4.

2.4. DNA extraction, 16S rRNA amplification and data analysis

Total DNA for metagenomics analysis was extracted using either the PowerSoil® DNA Isolation Kit (MO BIO) or NucleoSpin® Soil for soil and sediments, while the PowerWater ®DNA Isolation kit (MOBIO) was used for the water samples, following the manufacturer's instructions. Water samples (~800 ml) were first filtered *in situ* with 0.22 μm polycarbonate membranes to collect the microbial cells, and the filters were then used to extract the DNA. The quality, integrity, and concentration of the extracted DNA were verified by agarose gel electrophoresis and then quantified using Qubit 2.0 fluorometer and NanoDrop.

Amplicons of V3–V4 hypervariable regions of the 16S rRNA gene were sequenced on an Illumina MiSeq platform in Macrogen Inc. (Seoul, South Korea) using a paired-end method. Sequence reads were assembled with FLASH (Magoč and Salzberg, 2011), selecting minimum overlap -m100 and maximum overlap -M 320. Reads were filtered with a Phred >25, and primers and low-quality reads were removed with QIIME 2 (Callahan et al., 2016). High-quality sequences were grouped into Operational Taxonomic Units/Amplicon Sequence Variants (OTUs/ASVs, >97% similarity) using SWARM. UCHIME was used to eliminate chimeric sequences; the only OTUs retained were those found in >10 samples, and underrepresented sequences (i.e. singletons and doubletons) were eliminated. Finally, representative sequences were aligned against the Ribosomal Database Project (RDP) and SILVA 132 database using the Sklearn classifier (Naive Bayes Classifier). All the raw sequences have been deposited in the NCBI Sequence Read Archive with project number PRJNA995435 (Accession number SRR25305389 -SRR25305476).

2.5. Quantification of functional genes associated with mercury microbial reduction and methylation

qPCR was used to quantify the *merA* (mercury reduction), *hgcA* (mercury methylation), *dsrA* (sulfate reduction), and 16S rRNA (indicator of total bacterial abundance) genes. Quantification was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad Technology; Hercules, CA), using SsoAdvanced SYBR® Green Supermix and primers described in Table S5.

For gene copy number quantitation, calibration curves were established using plasmidic DNA containing the target gene. To this end, each gene was amplified using DNA extracted from the corresponding species as described in Table S5. The resulting PCR products were cloned in pCR4-TOPO (vector pCR™-4-TOPO™TA) and transformed into *E. coli* TOP10 (Invitrogen). To calculate the abundance of each gene in each sample, we first determined the number of copies/ng of DNA using the calibration curves established for each gene (efficiency ≥90%, R2 ≥ 0,98). These values were then corrected with the abundance of the 16S rRNA gene and expressed as a percentage of abundance. The abundances of Deltaproteobacteria and Firmicutes *hgcA* were added together to find total *hgcA* abundance.

2.6. Statistical analyses

Alpha diversity was determined using the richness, evenness, Chao I, Simpson and Shannon indices. These indices were calculated using R-CRAN's implementation of the Vegan package, based on a table of OTUs rarified to 26,759 OTUs. Beta diversity was evaluated using the Non-metric MultiDimensional Scaling method (NMDS) and the permutational multivariate analysis of variance (PERMANOVA) tests with Bray-Curtis index and $p < 0.05$ as a significant value, using the adonis2 function from the "vegan" R package. The association between environmental variables and bacterial community dissimilarities was determined using the envfit test with 999 permutations (vegan package). The abundance of bacterial communities was transformed with the Hellinger transformation. To search for taxa indicative of high levels of mercury (indicator taxa), we categorized the samples into Low (LHg) and High (HHg) mercury concentration based on the accepted concentration limit for soil-sediment (0.094 mg kg^{-1}) according to the Canadian Council of Ministers of the Environment-Threshold Effect Level (ECMDEPQ, 2007). IndVal calculations were performed using the "labdsv" R package (Dufrêne and Legendre, 1997). Indicator taxa were selected with $p \leq 0.05$ and IndVal >0.6. All the analyses were conducted using the RStudio package (version 4.1.3). The univariate and multivariate statistical analysis used to analyze functional genes considered 3 factors: sampling area, sampling site, and sample type. Normality was verified on original or transformed data using the Shapiro-Wilk test in SPSS Statistics 25 (IBM). PERMANOVA and PCoA tests with the Bray-Curtis index (permutations = 999) were carried out in the Past4 program. Past4 software (Scientific Engineering and Manufacture) was also used for the simultaneous ordination of the soil properties and Hg speciation, as correlation with the selected bacterial communities, using the Bray-Curtis correlation index. Pearson's correlation analysis was carried out to establish correlations between soil properties and Hg speciation using Minitab 19.

3. Results and discussion

The samples collected in Tarapacá and Taraira consisted of water, sediments (surface, interstitial and deep) and soils from the surrounding forest with varying degrees of mercury concentrations (Fig. S1, Tables S2a, S2b and S2c) (Cardona et al., 2022). Taraira surface and deep sediments and soils showed the highest THg content. By contrast, the highest THg concentrations in Tarapacá were close to or below this limit. Overall, THg and MeHg concentrations in sediments and forest soils were significantly higher in Taraira than in Tarapacá ($p = 0.0001$ and $p = 0.017$), as evidenced in Fig. S1. All water samples were found to be below the threshold value for raw water (0.001 mg L^{-1}, according to Colombian wastewater guidelines () (Ministerio de Ambiente y Desarrollo Sostenible de Colombia, 2015) (Fig. S1).

3.1. Microbial community structure and diversity in Tarapacá and Taraira localities

Sequencing of the 16S rRNA gene V3–V4 region of all the samples yielded a dataset of 12,618,594 high-quality filtering sequences. After removing the chimeric sequences, singletons, and doubletons, 4,576,900 operational taxonomic units (OTUs) were defined. The resulting dataset contained 61,397 OTUs present in at least 10 samples. Diversity indices were calculated based on OTUs rarefied to 26,759 reads (Table S6). The variation of OTUs and Chao1 index values was significant across almost all the samples, with a coverage index of between 92 and 98% (Table S6), indicating adequate sampling depth for assessing microbial diversity. Sediment samples showed higher Shannon diversity index scores in Tarapacá (average 7.03 ± 0.7) than in Taraira (average 5.27 ± 0.8), as illustrated in Figs. S2a and S2b. Furthermore, sediment and forest soil samples showed the highest diversity levels, probably because they provide a nutrient-rich matrix for microbial

growth (Wang et al., 2012; Du et al., 2020). The samples from aquatic ecosystems exhibited the lowest diversity (Chao I and Shannon indexes, Table S6) in both localities (Figs. S2a and S2b), as recurrently observed in water bodies (Li et al., 2023).

The bacterial community composition of all the samples is depicted in Fig. S3. Overall, soil samples from Tarapacá were primarily dominated by *Alphaproteobacteria* (18%), *Ktedonobacteria* (12%), *Actinobacteria* (9%) and *Planctomycetia* (7%). In sediments (Fig. S3a), *Alphaproteobacteria* were dominant (13%), together with *Deltaproteobacteria* (6%), *Ktedonobacteria* (4%) and *Acidobacteriia* (4%). Water samples had high amounts of *Betaproteobacteria* (26%), *Actinobacteria* (14%), *Alphaproteobacteria* (11%), *Gammaproteobacteria* (7%), and [*Saprospirae*] (7%). As for Taraira, soil and sediment showed similar results, with *Alphaproteobacteria* being one of the most abundant groups in most samples (23% and 16%, respectively). *Betaproteobacteria* (16% and 18%, respectively), *Gammaprotoebacteria* (8% and 9%, respectively) and *Sphingobacteriia* (7% and 7%, respectively) were also prevalent, except for the CRd deep sediment, which was dominated by *Clostridia* (24%–40%). Meanwhile, the predominant phyla in Taraira water samples (Fig. S3b) were *Betaproteobacteria* (35%), *Gammaprotoebacteria* (20%), *Alphaproteobacteria* (16%), *Actinobacteria* (3%) and [*Saprospirae*] (3%), resembling the community composition in a lacustric region in Brazil, dominated by *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Chlorobi*, *Chloroflexi* and *Acidobacteria* (Ávila et al., 2017). Previous reports of bacterial communities from soils with a long history of mercury pollution described the recurrent presence of *Proteobacteria, Chloroflexi,* and *Acidobacteria, Plantomycetes* and *Bacteriodetes* (Ji et al., 2018; Liu et al., 2021; Li et al., 2022), whilst long-term mercury polluted river sediments in China were dominated by *Proteobacteria*, *Bacteriodetes* and *Acidobacteria*.

3.2. Key factors driving the structure of microbial communities in both Hg-polluted and unpolluted water, sediment and soil samples

To explain the variations among the different samples, a non-metric multidimensional scaling analysis (NMDS) of the 88 samples was carried

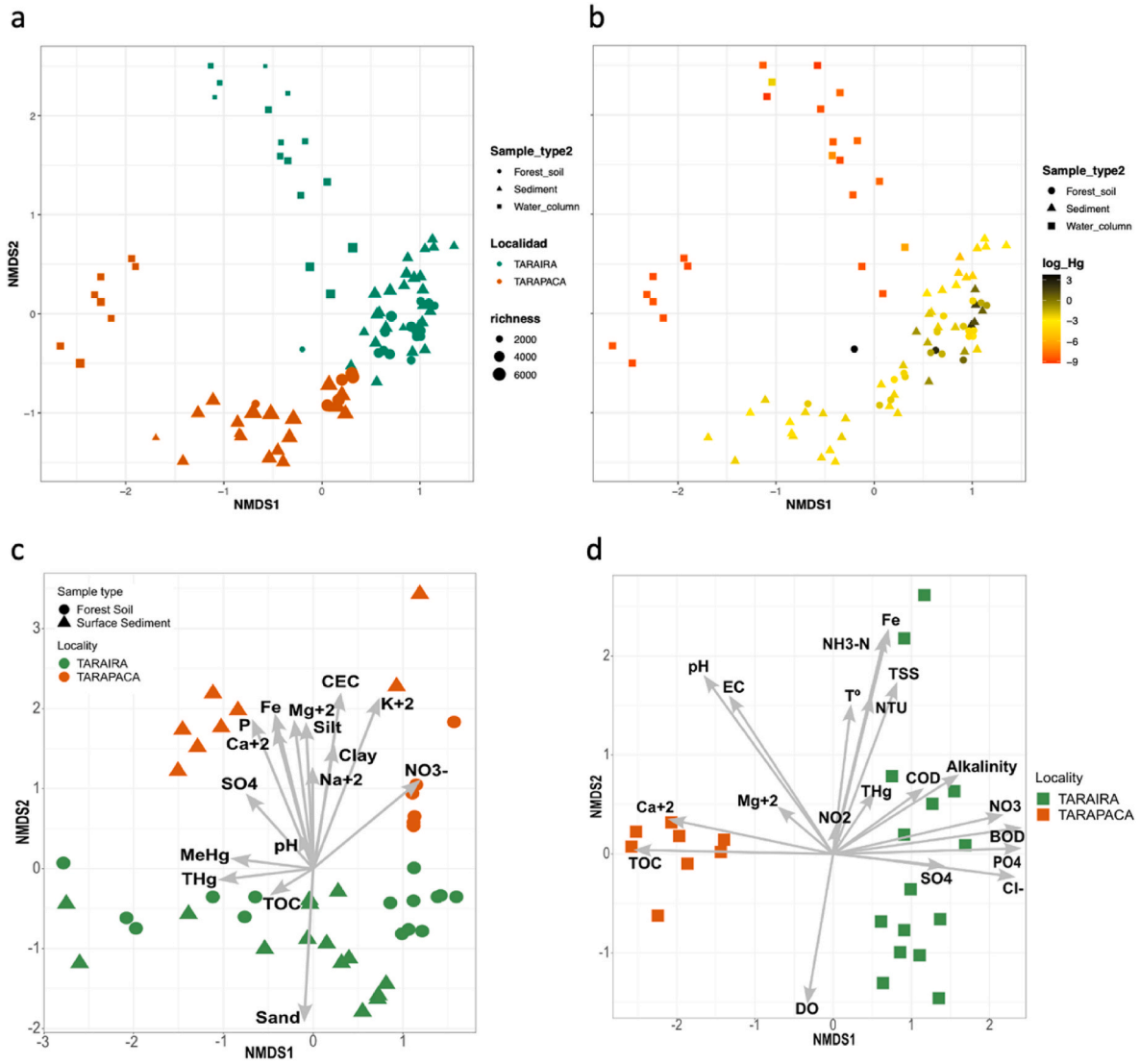

Fig. 2. a and b) NMDS calculated based on the Bray-Curtis dissimilarity of community composition (stress = 0.091). (a) The color code represents the two localities (green, Taraira; orange, Tarapacá), and the size of the symbols is related to the OTUs abundance. The symbol shape indicates the sample type (squares, water samples; triangles, surface sediment samples; circles, soil samples). (b) The color code represents mercury concentration. c and d) NMDS ordination of community composition based on the Bray-Curtis dissimilarity with environmental variable. (c) Surface sediments and soil samples from Taraira and Tarapacá localities (stress = 0.099). (d) Water samples from Tarapacá and Taraira localities (stress = 0.10). The color indicates locality (orange circles, Tarapacá; green circles, Taraira. The symbol shape indicates sample type (circle, forest soil; triangles, surface sediments; squares, water). The size of the arrow is proportional to the strength of the correlation of each variable. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

out. As shown in Fig. 2a, there was a differential pattern in bacterial community composition, based primarily on the sample type and locality (PERMANOVA of Locality, $R = 0.074$ and $P < 0.001$; and Sample Type, $R = 0.134$, $P < 0.001$; Table S7). The bacterial communities in soils and sediments of both localities were more closely clustered, suggesting certain similarity. In contrast, the bacterial communities in aquatic system samples showed a strong dissimilarity. In fact, the waters of the two localities are of different origin and show very different properties (see below). Also, Taraira water samples had the lowest OTU richness values, which increased towards soils and sediments; this increase in richness towards sediments and soils was especially evident in Tarapacá samples (Fig. 2a). Fig. 2b shows that concentrations of total mercury increased towards the soils and sediments especially of Taraira locality and decreased towards the water samples from both localities.

The most relevant environmental variables of water, forest soil and superficial sediment samples were determined and included in the NMDS analysis to assess their influence on bacterial community composition. Forest soil communities in Tarapacá samples showed quite similar results, whilst greater dissimilarity was observed in Taraira (Fig. 2c). The NMDS analysis indicated a correlation between NO3, CEC and K and forest soil from Tarapacá (NMDS2, Fig. 2c), whilst variables such as Ca, P, Fe, Mg were related with surface sediment samples (5 cm upper layer of river beds). By contrast, sand, TOC, THg and MeHg were related to samples from Taraira (NMDS1). THg appeared as a less significant variable in NMDS analysis (Table S8a). In water samples, TOC, pH, Fe, BOD and PO4 were the variables most closely associated with the dissimilarity between the two localities. TOC and Ca were the variables that most influenced Tarapacá water samples, while the Taraira samples were most differentiated by BOD, PO4, NO3, Fe and alkalinity (Fig. 2d–Table S8b). This analysis confirmed that the bacterial community structures showed more dispersion between localities in aquatic systems, and were more clustered in soils and sediments with the highest mercury concentrations. The results in Fig. 2d reflect the differences between the water of the two localities: Tarapacá water originates from white water rivers and contains a high nutrient load, whilst Taraira water originates from the Guiana Shield and shows low mineralization and nutrient load (Moreno-Brush et al., 2020). Texture (sand, silt, and clay %) was also strongly correlated with bacterial community structure (Fig. 2c). Taraira surface sediments and soils had a sandy texture, whilst Tarapacá sediments were clayish. Sandy textures do not allow soil particles to aggregate properly, making them prone to erosion. The sands in the Taraira region have high quartz content, with little or no nutrients and low levels of exchangeable cations (Rubiano, 2014). This may explain the low bacterial diversity in Taraira.

The fact that physicochemical variables are more important than THg levels in structuring the bacterial communities in long-term mercury-polluted soils and sediments has previously been observed in different types of ecosystems. In water-submerged sediments, $NH4^{+}$ nitrogen, followed by CEC, TOC, sulfate, and to a lesser extent MeHg, were the most significant variables affecting bacterial community distribution (Du et al., 2020). In sites with long-term mercury pollution (Li et al., 2022), variables such as land use pattern, nutrients and certain physicochemical parameters were found to have a more significant influence on bacterial community distribution than the presence of mercury, which explained just 5% of the variability. Studies conducted on polluted wetland soils also confirmed that physicochemical properties, such as TOC, moisture, pH, and soil type, were the primary factors driving microbial community structure rather than their heavy metal content (Zhang et al., 2016; Du et al., 2020). It is possible that bacterial communities in Tarapacá and Taraira have adapted to the presence of Hg, either because it already existed (possible natural origin due to soil mineralogy) or due to its prolonged use in gold amalgamation. Fadini and Jardim. (2001) suggested that Amazon soils are naturally abundant in mercury, which could gradually be discharged into aquatic systems or retained on oxy-hydroxides in mineral horizons. In addition, several Hg tolerance mechanisms could have helped local bacterial communities to adapt to chronic exposure to mercury (Das et al., 2016; Mathivanan et al., 2021).

3.3. Correlations in mercury-related gene abundance in the polluted sediments and soils from Tarapacá and Taraira

To estimate the functional potential of microbes for mercury metabolism, we quantified the abundance of *merA* and *hgcA* genes in all the samples. We also assessed the abundance of the *dsrA* gene (sulfate reduction) in order to estimate the population of anaerobic SRB, the primary host of mercury methylation activity in nature. The *hgc*A and *merA* genes were significantly more abundant in Taraira than in Tarapacá (Fig. 3a and b), reflecting the higher mercury levels in the Taraira samples, and suggesting that the microbial community within these sediments have the potential to methylate Hg. However, although we know from these data that there are bacteria carrying the *hgc*A gene, and that they could potentially methylate Hg, the extent to which Hg is actually being methylated is unpredictable, since only the Hg(II) form is expected to be methylable by these bacteria, and in these sediments Hg

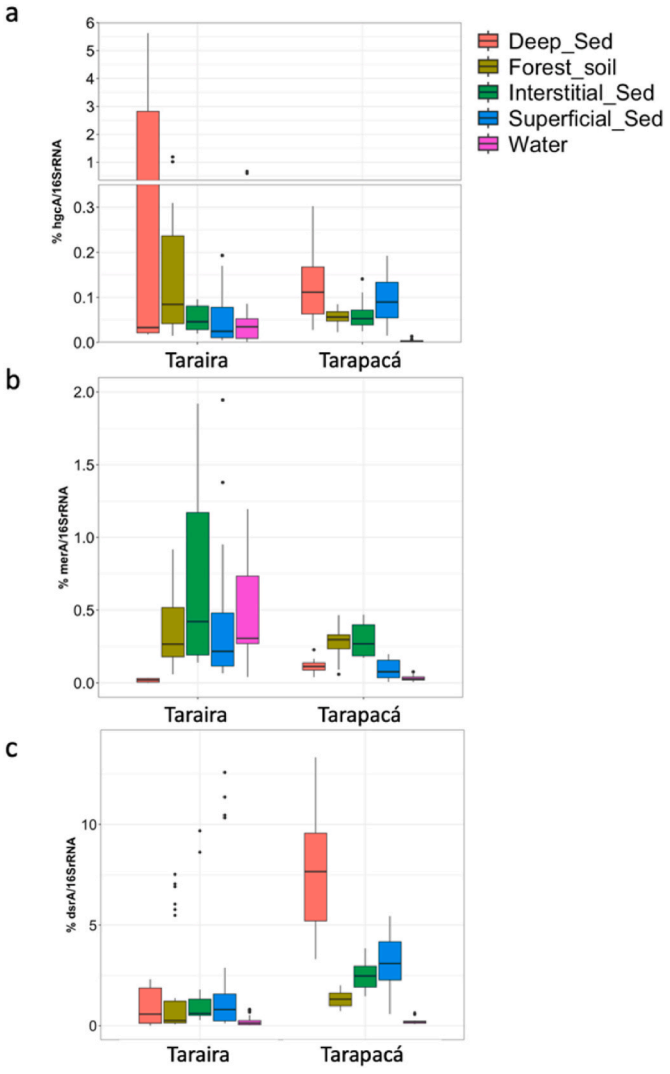

Fig. 3. Percentages of (a) *hgc*A/16SrRNA, (b) *dsr*A/16SrRNA, and (c) *mer*A/16SrRNA functional genes according to the sampling region (Taraira and Tarapacá) and sample type (superficial, interstitial, and deep sediments, forest soils, and waters). Boxplots indicate the percentage of functional genes (*mer*A, *hgc*A and *dsr*A) corrected for the abundance of the general microbial community (16SrRNA gene) in water, sediment and soil forest samples from Tarapacá and Taraira. In a) the ordinate axis was broken to encompass the whole value range.

was mainly found as Hg-s, Hg-e and Hg-o (Fig. 5). However, when all 88 samples were analyzed together, no significant correlation was found between the abundance of mercury-related functional genes and THg concentrations ($p = 0.795$ and $p = 0.901$ for *hgcA*/16SrRNA and *merA*/16SrRNA, respectively (Fig. S4a, Table S10). When the data were divided by sampling location, significant positive correlations were found between the concentrations of total mercury (THg) and methylmercury (MeHg) in river sediment samples of Tarapacá ($n = 32$) and the prevalence of mercury-related functional genes for the two genes: $\rho = 0.593$ ($p = 0.000$) and $\rho = 0.498$ ($p = 0.004$) for *hgcA*/16SrRNA, and *merA*/16SrRNA, respectively (Fig. S4c, Table S10). In contrast, no significant correlation was observed between functional gene abundance and mercury concentration in Taraira (Fig. S4b). Mercury concentration in Taraira soils, sediments and water samples was highly heterogeneous and did not allow the samples to be arranged according to a regular THg value gradient, which probably explains the absence of a significant correlation. By contrast, the *dsrA* gene was more abundant in Tarapacá, where sulfate levels were higher in the surface sediment samples (Fig. 3c–Table S2b). There were significant differences between the sample types in terms of functional gene abundance ($p = 0.0001$) (Table S10). As expected, the *dsrA* and *hgc*A genes were most abundant in deep sediments and least abundant in water. The influence of redox potential on the distribution of mercury methylation was expected, as this process is generally carried out by anaerobic bacteria that inhabit environments with little or no oxygen content, such as deep sediments (Du et al., 2017). By contrast, *merA* genes were scarce in deep sediments, especially in Taraira (Fig. 3b), as expected for this aerobic-dependent pathway which is mainly present in aerobic bacteria living in aerobic and microaerophilic environments such as water, forest soils, and surface and interstitial sediments (Naguib et al., 2018).

The functionality of SRB relies on the presence of oxidized sulfur species (sulfite, sulfate, and thiosulfate), which serve as terminal electron acceptors for the oxidation of organic substances, including methylmercury (Achá et al., 2011). This biological process occurs above all in the anoxic transition zone of sediments situated beneath layers of oxygen-rich water (Bravo et al., 2016). Further analysis established a significant positive association between the concentration of sulfate and the levels of THg (Fig. S5), as well as a linkage with the abundance of *hgcA*/16SrRNA and *dsrA*/16srRNA genes. However, no such relationship was found with the *merA* gen. (Spearman $p > 0.05$; Fig. S4). These observations are in line with those of Bravo et al. (2016) who detected a significant correlation between the *hgcA* and *dsr*A genes; they also found the greatest abundance of *merA* genes in a sediment surface layer with high mercury levels. In previous short-term studies, a huge increase in the *mer*A copy number was observed in response to the highest concentrations of Hg (Frossard et al., 2017; Christensen et al., 2019). Finally, it is important to underscore that while the abundant presence of these genes indicates a potential for microbial mercury transformation (reduction and/or methylation), their active biological function has not been demonstrated. Detailed expression analyses would be required to unveil the real role of these genes and their bacterial hosts in Hg speciation *in situ* in the region.

3.4. Bacterial taxa potentially related to mercury biotransformation

To investigate the relationship between the abundance of *hgcA, merA*, and *dsrA* genes and microbial taxa, as well as THg and MeHg levels, we selected the 15 most prevalent classes in soil and sediment samples with high Hg concentrations through a canonical analysis of the principal coordinates (CAP). In forest soils, a correlation was observed between the high levels of THg and MeHg and the presence of *Betaproteobacteria, Sphingobacteriia* (*Bacteroidetes*) and ZB2 (OD1 candidate phylum), also referred to as *Parcubacteria* (Rinke et al., 2013), whereas the abundance of *mer*A and *hgc*A genes was linked to [*Saprospirae*] (*Bacteroidetes*) ($r = 0.53$; $p = 0.001$) (Fig. 4a). Prior research found OD1 (*Parcubacteria*) to be associated with metal contamination in soils close to mining areas in China (Liu et al., 2021). The presence of *Parcubacteria* was observed in sediments located in an ore-rich region in Minas Gerais (Brazil) (Reis et al., 2016) and also in sulfur-rich and anoxic environments (Barberán and Casamayor, 2011). Yuan et al. (2019) found that *Parcubacteria* was a crucial phylum harboring antibiotic- and mercury-resistance genes in municipal wastewater treatment plants in China with high levels of THg and MeHg Liu et al., 2018b). Although this question is poorly understood, it has been suggested that this group may have limited metabolic capacities (Rinke et al., 2013). An increase of *Parcubacteria* was also observed in naphthalene-treated marine sediments (Acosta-González, unpublished), which might indicate that, for some unknown reason, the group is more resistant to environmental stressors.

In sediment samples, a diverse array of bacterial classes was found to be interconnected with mercury levels. Specifically, *Betaproteobacteria*, *Sphingobacteriia*, *Acidimicrobiia*, [*Saprospirae*], and *Acidobacteriia* were associated with total mercury (THg) concentrations, whereas *Alphaproteobacteria* correlated with methylmercury (MeHg) levels. *Deltaproteobacteria*, along with classes like *Nitrospira*, *Ktedonobacteria* from the phylum *Chloroflexi*, *Actinobacteria*, *Planctomycetia, DA052,* and *ZB2*,

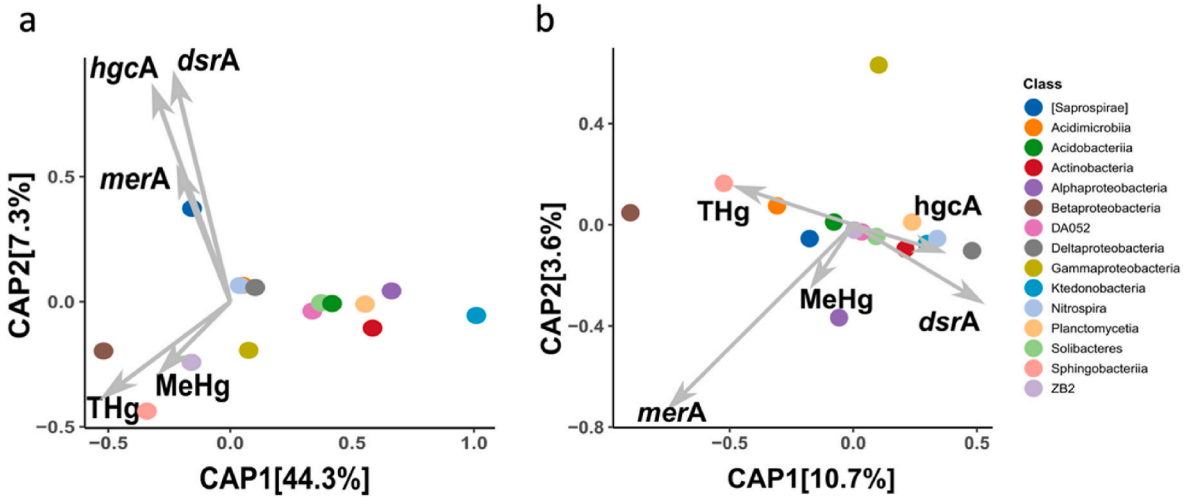

Fig. 4. Principal component analysis (PCA) of the OTUs most abundant at the class level, functional genes and THg and MeHg concentrations in (a) forest soil samples and (b) sediments samples. The different classes are indicated by different colors. The size of the arrows is proportional to the strength of the correlation of each variable. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

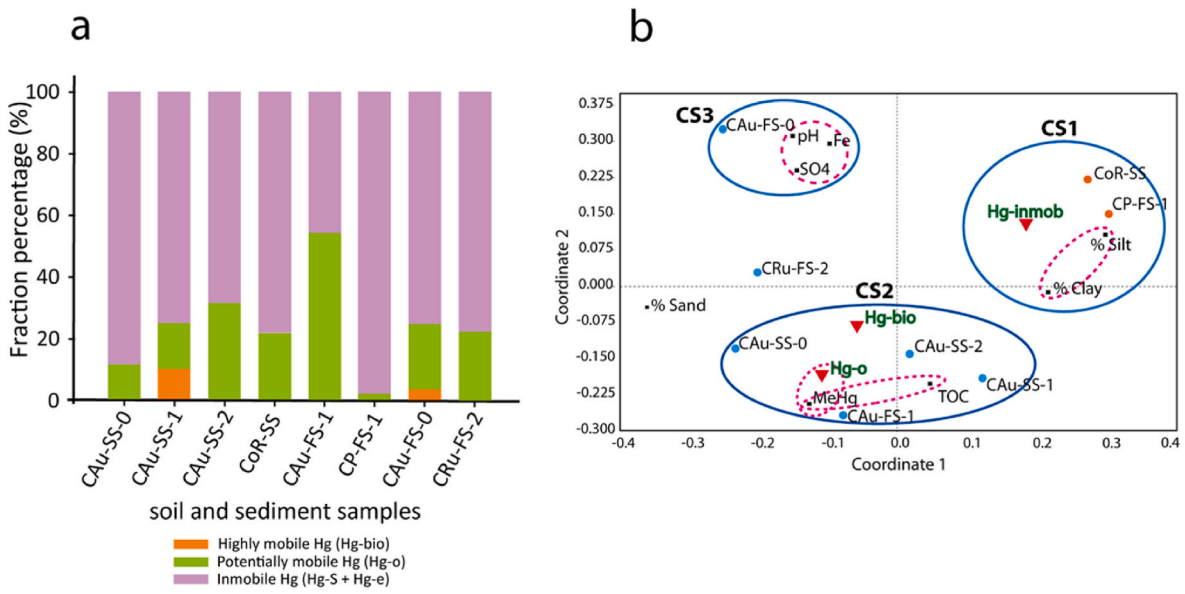

Fig. 5. (a) Percentage of mercury speciation in the evaluated sediments and soil samples. The proportion of the immobile fraction (Hg-s + Hg-e), potentially mobile (Hg-o) and highly mobile (Hg-bio) is presented. **(b)** MDS analysis of the relationships among mercury bioavailability (red triangles), soil sampling sites (blue dots) and physicochemical variables (black squares) according to Bray–Curtis's distance. The blue circles show the clusters (CS) found by MDS, and dotted pink circles show the positive Pearson correlated ($r => 0.600$, $P < 0.005$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

showed a clear association with the *hgcA* gene, a key genetic marker for mercury methylation. *Deltaproteobacteria* were additionally linked to the *dsrA* gene, as indicated by a modest correlation coefficient ($r = 0.22$) with a statistically significant p-value ($p = 0.007$, Fig. 4b). Overall, the study revealed that while there were correlations between class-level taxa abundance, mercury concentrations, and functional gene abundance, these correlations were not particularly robust. This suggests that factors other than mercury might be affecting the diversity of microbial taxa in long-polluted sites (Fig. 4b–Table S8a). However, a consistent pattern emerges across various studies: certain dominant phyla recur in environments with high mercury levels. Research by Frossard et al. (2017) found that the microbial communities in sites with high mercury concentrations were dominated by *Proteobacteria*, *Acidobacteria, Bacteriodetes, Actinobacteria, Chloroflexi, Gemmatimonadetes* and *Planctomycetes*. *Chloroflexi, Cyanobacteria*, *Firmicutes, Planctomycetes, Nitrospira*, *Chlorobi,* and *Verrucomicrobia* were found in sediment samples associated with mining-impacted streams (Reis et al., 2013), while *Proteobacteria, Chloroflexi* and *Acidobacteria* were the most abundant phyla in sites with high mercury levels (Pu et al., 2022). Studies of subjects as diverse as sediments from a Tibetan Lake or large-scale databases have noted the prevalence of *Actinobacteria* and *Betaproteobacteria* and new *Actinobacteria* lineages as hosts of *hgcA* genes (McDaniel et al., 2020). Zheng et al. (2022) highlighted a positive correlation between the relative abundance of *Actinobacteria* and *Acidobacteria* and mercury, and found that *Firmicutes* was more resistant to mercury in acidic soils. Similarly, *Actinobacteria* was identified as the dominant phylum in gold mine tailings (Sibanda et al., 2019). The class *Alphaproteobacteria* (especially the genus *Sphingomonas*) have been described as Hg-resistant bacteria, containing *merA* and *merB* genes, in soils with high levels of mercury pollution (Mahbub et al., 2017; Hu et al., 2022).

Interestingly, we discovered unforeseen connections between the functional gene *hgcA* and members of the following taxa: [*Saprospirae*] (phylum *Bacteriodetes*), *Nitrospira (phylum Nitrospirae)*, *Ktedonobacteria (Phylum Chloroflexi), Actinobacteria,* and *Plantomycetia.* This contradicts the general literature consensus that *hgcA* genes are predominantly found in SRB and iron-reducing bacteria (FeRB) (Schaefer et al., 2014; Parks et al., 2013; Gilmour et al., 2013; Bae et al., 2019; Tang et al., 2020; Gustin et al., 2020; Feng et al., 2022). Recent global studies have analyzed the potential presence and expression of *hgcAB* homologs in ocean waters. The studies found that these homologs occur in oxic subsurface waters and are linked to phylogenetically diverse microorganisms, including those belonging to the *Deltaproteobacteria*, *Firmicutes,* and *Chloroflexi* (Villar et al., 2020). This finding is consistent with previous studies in paddy soils (Liu et al., 2014a). Of particular interest was the high abundance of *hgcA* associated with *Nitrospina* sp., a microaerophilic mercury methylator found in sea ice (Gionfriddo et al., 2016). Furthermore, Feng et al. (2022) showed that the *Gammaproteobacteria Raoultella terrigena* TGRB3 isolated in China could carry out Hg bio-methylation under facultative and even aerobic conditions, supporting the previous finding of seven *Verrucomicrobia* genera positively correlated with increases in the *hgcA* gene copy number, THg, and MeHg concentrations in a site with a history of Hg mmining (ishnivetskaya et al., 2018). Also, Lin et al. (2021) found *hgcA* genes affiliated to the *Verrucomicrobia* in metagenomic sequences from marine waters. Thus, the current view of mercury methylation ecology is changing towards a more widespread capacity in the microbial world and suggests that in both aquatic and terrestrial ecosystems, a large number of aerobic and facultative anaerobic bacteria could also be contributing to net environmental MeHg production from Hg(II) (Xing et al., 2018; Gustin et al., 2020; Feng et al., 2022; Villar et al., 2020; McDaniel et al., 2020). We cannot rule out that something similar is occurring in the sites we sampled, although additional research would be required to identify alternative mercury methylators in our samples.

3.5. *Speciation and bioavailability of mercury in highly contaminated sediment and soil samples*

We narrowed our analysis to the eight sediment and forest soil samples with the highest reported Hg concentrations to determine the fraction of the different Hg chemical species present (including immobile, potentially mobile, and highly mobile mercury). From Taraira, we selected surface sediments (CAu-SS-0, CAu-SS-1, and CAu-SS-2) and forest soils (CAu-FS-0 and CAu-FS-1), while from Tarapacá we selected a surface sediment from Cotuhé River mouth (CoR-SS) and two forest soil samples, one from Caño Rojo (CRu-FS-2) and one from Caño Pupuña (CP-FS-1) (Table S11). The sequential extraction protocol (SEP, see the

methods section, Table S4) identified five essential mercury fractions (Table 1). Fig. 5a shows the SEP results for the eight selected samples. In all the samples except CAu-FS-1, most of the mercury was in immobile form, probably unavailable to microorganisms. This was probably sulfide-bound mercury (Hg-s extracted fraction, cinnabar and meta-cinnabar), but could also include elemental mercury (Hg-e). This immobile fraction was highest in CP–FS–1 (97.5%), followed by CAu-SS-0 (89.6%) and lowest in CAu-FS-1 (45.3%), while the remaining samples averaged 75.7 ± 3.8 %. The mobile fraction (Hg-bio) was only detected in two of the samples and constituted a minor fraction of THg, 10.5% in CAu-SS-1 and 3.92% in CAu-FS-0 samples (Table 1). The potentially mobile fraction (Hg-o), in which mercury is associated with organic ligands such as humic and fulvic acids and amino acids, was highest in CAu-FS-1 (54.5%), followed by CAu-SS-2 (30%), and lowest in CP–FS–1 (2.4%), the remaining samples averaging (17.5 ± 4.9 %). The highly mobile fraction, the bioavailable form of mercury, is composed of the sum of the water-soluble fraction (Hg-w) and the exchangeable, acid-soluble fraction Hg-a, which includes mercury species adsorbed to the matrix through weak electrostatic bonds, from which mercury can be released by ion exchange processes and co-precipitate with carbonates. Changes in cation composition or low pH can release Hg from the Hg-a fraction, although in our samples, the mercury present in the Hg-a fraction was below detection limits. This fraction is considered a semi-mobile and semi-available fraction that is largely mobilizable through microbial activity (Gutiérrez-Mosquera et al., 2020).

The retention and accumulation of Hg in soils and sediments is highly dependent on its chemical form, and on the texture and grain size, redox conditions, pH, organic matter, iron, sulfur and aluminum (Vasques et al., 2020; Gutiérrez-Mosquera et al., 2020). We therefore analyzed these parameters in the samples with the highest mercury content (Table S11). Most of the Taraira samples evaluated by SEP had a sandy to sandy-silt texture, whilst the Tarapacá samples (CoR-SS and CP–FS–1) had a sandy-clay-silt texture.

To quantitatively assess the similarity between the groups while accounting for the potential influences of physicochemical variables on mercury bioavailability at the selected sampling sites, a multidimensional scaling (MDS) analysis was conducted, which revealed two distinct clusters relevant to mercury bioavailability dynamics (Fig. 5b). Cluster CS1 represented the mercury immobile fraction (Hg-inmob); a positive correlation was observed between % silt content and % clay (Pearson correlation coefficient of 0.778, $P < 0.005$). These variables were grouped close to the CoR-SS and CP–FS–1 samples from Tarapacá, which exhibited the lowest MeHg concentrations (Table S11), supporting the negative correlation (Pearson $r = -0.626$, $P < 0.005$) observed between the immobile fraction and MeHg concentration. Cluster CS2 comprised bioavailable mercury encompassing Hg-bio and Hg-o fractions (Table 1). This cluster exhibited a positive correlation with MeHg and TOC, (Pearson $r = 0.852$ and 0.885, $P < 0.005$, respectively), and included four samples: CAu-SS-0, CAu-SS-1, and CAu-SS-2 and CAu-FS-1. The Hg-bio and Hg-o forms typically constitute a small portion of total mercury content (Huang et al., 2020), as observed in the soils and sediments from Taraira (Table 1, Fig. 5a). As described by (Matsumoto and Liu, 2020; Huang et al., 2020), these Hg-o fractions are bound to soil humic or acid-soluble compounds, rendering them bioavailable. Furthermore, these potentially mobile fractions (Hg-bio and Hg-o) facilitate the burial of mercury in sediments or its transportation to adjacent water bodies, where the formation of MeHg species from Hg(II) becomes plausible. The conditions conducive to MeHg production, such as elevated temperatures and reduced dissolved oxygen, are often prevalent in Amazon River sediments (Ramirez et al., 2021), as manifested in the high MeHg levels in the three surface sediment samples and the forest soil sample from Caño Amarillal in Cluster CS2.

Finally, although cluster CS3 exhibited a negative correlation with Hg-bio and Hg-o (Pearson $r = -0.746$ and -0.627, $P < 0.005$, respectively), it showed positive associations with soil properties: a positive correlation between pH and Fe and SO_4^{2+} concentrations (Pearson $r = 0.891$ and 0.751, $P < 0.005$, respectively). These variables were clustered with CAu-FS-0 from Taraira, which displayed the highest Fe and SO_4^{+2} concentrations and the highest pH value (Table S11).

Because Hg-bio is presumably bioavailable to soil microorganisms and could reveal potential Hg-resistant microbial groups, we evaluated the taxonomy at the class level to identify the taxonomic groups that appeared as enriched in samples with higher Hg-bio concentrations. The data were organized in a gradient from higher to lower Hg-bio concentration (Fig. S6). Although the permanova analyses showed no significant differences ($p > 0.05$) for the totality of the data, in the samples with the highest Hg-bio concentrations, CAu-SS-1 and CAu-FS-0 (Fig. 5a–Table 1), the classes with the highest relative abundance were *Sphingobacteriia* (23%) and *Saprospirae* (24%), respectively, as compared to the other samples (1 ± 0,002 % on average for *Sphingobacteriia* in sediments and 1 ± 0,01% for *Saprospirae* in forest soils) (Fig. S6). These two classes belong to *Bacteroidetes*, which is known for having hydrocarbon-degrading representatives (Kwon et al., 2019). *Saprospirae* has been mainly reported as a dominant bacterial class in wastewater treatment reactors (Tian and Wang, 2020), although it was also reported as one of the most abundant classes in soils contaminated with Hg (Frossard et al., 2017). For its part, *Sphingobacteriia* was found to be highly abundant in Hg-contaminated soils (Mahbub et al., 2017) and uranium and heavy metal-contaminated environments (Radeva et al., 2013). This may be because members of this class can produce sphingolipids, which protect the cell surface against different environmental stressors (Rickard et al., 2004).

Table 1
Sequential mercury extraction of the eight samples with the highest total mercury content.

Sample name	Mercury Concentrations (mg Kg^{-1})							% of THg		
	F1 (Hg-w)	**F2** (Hg-a)	**F3** (Hg-o)	**F4** (Hg-e)	**F5** (Hg-s)[a]	**Total**	**Recovery (%)**[b]	**Hg-bio**[c]	**Hg-o**[d]	**Hg-immob**[e]
CAu-SS-0	nd	nd	0,18	0,31	1,24	1,73	107,12	nd	10.40	89.60
CAu-SS-1	1,04	nd	1,48	1,11	6,32	9,95	106,85	10.45	14.87	74.67
CAu-SS-2	nd	nd	1,16	2,72	nd	3,87	97,90	nd	29.97	70.28
CoR-SS	nd	nd	0,12	0,46	nd	0,58	105,12	nd	20.69	79.31
CAu-FS-1	nd	nd	30,49	3,26	22,21	55,97	103,69	nd	54.48	45.51
CAu-FS-0	0,49	nd	2,61	1,99	7,28	12,36	98,53	3.96	21.12	75.00
CP–FS–1	nd	nd	0,59	2,79	21,06	24,43	96,81	nd	2.42	97.63
CRu-FS-2	nd	nd	0,09	0,21	0,12	0,42	99,60	nd	21.43	78.57

nd, not detected (below the detection limit, 0.002 mg/kg).
[a] Hg-w (Water soluble), Hg-a (Acid soluble), Hg-o (Organic), Hg-e (Elemental Hg) and Hg-s (residual mercury in sulfides and silicates.).
[b] Calculated from previously determined total mercury concentrations (Cardona et al., 2022).
[c] Bioavailable mercury, calculated as the sum of F1 and F2 fractions.
[d] Potentially mobile, organic mercury corresponds to the F3 fraction.
[e] Immobile mercury, calculated as the sum of F4 and F5 fractions.

3.6. Differential abundance analysis and selection of possible bioindicators of Hg contamination

The effect of mercury contamination on the overall bacterial communities in the Amazon samples analyzed in this study probably varied according to sample type and location. Thus, we sought to pinpoint the specific taxa that appeared in abundance in the different samples in association with mercury in any of its forms, so as to identify potential mercury-responsive groups that significantly discriminated between samples based on mercury content, using the indicator value analysis (IndVal) proposed by (De Cáceres and Legendre, 2009). Due to their nil Hg content, the water samples were not included in this analysis. The categorical variable "THg" was defined as high (H, >0.094 mg kg^{-1}) or low (L, <0.094 mg kg^{-1}) (ECMDEPQ, 2007) to sort the samples and we compared the taxa abundance between the two categories. Table S12 summarizes the number of OTUs over-represented in each group for the two sample types analyzed ($p < 0.05$ and IndVal >0.6).

Fig. S7 shows that specific OTUs from high mercury soils and sediments showed higher abundance and occurrence in high mercury samples, and lower abundance in low mercury samples (Figs. S7a and S7b). In forest soils, OTU 2943 and 3584, which belong to the DA052 classes of *Acidobacteria* and to the *Actinobacteria*, respectively, were significant indicators for soils with high mercury content (p-values 0.032 and 0.009; IndVal values 0.75 and 0.69, respectively) (Table S13a). This is consistent with previous studies in long-term Hg-contaminated soils (Liu et al., 2014a; Guo et al., 2017; Li et al., 2022). Using a similar differential abundance analysis to estimate specialized indicator taxa, (Li et al., 2022) observed that *Actinobacteria* were predominant in mercury mining areas. Members of the *Acidobacteria* were amongst the dominant groups (16%) in abandoned mining sites with high concentrations of metals and metalloids, including Fe, As, Pb, and Hg (Brito et al., 2023) and in bulk soil from a highly mercury-contamination mining region in Almadén (Spain) (5.3%) (González et al., 2022). Furthermore, in short-term Hg-contaminated soil microcosms, the Hg-tolerant OTUs included *Acidobacteria* and *Actinobacteria* as Hg indicator taxa (Mahbub et al., 2017; Frossard et al., 2017; Zheng et al., 2022).

In sediment samples, OTUs 149 and 396 (*Chloroflexi*), 1148 (*Euryarchaeota*), 1174 (*Firmicutes*), 6837 (*Deltaproteobacteria*) and 58,970 (*Alphaproteobacteria*), with p-value 0.001, 0.001, 0.006, 0.002, 0.001, and 0.001 and IndVal value 0.60, 0.66, 0.66, 0.61, 0.60 and 0.61, respectively, tended to be poorly represented in low mercury samples (Fig. S7b blue horizontal frame) and increased in occurrence and abundance in high mercury samples, and could be considered significant indicator taxa for high mercury sediments (Table S13b). These results are in line with the positive correlation detected between *Proteobacteria* and *Firmicutes*, together with *Cyanobacteria*, *TA06*, *Tenericutes,* and *Bacteriodetes*, with heavy metals in the Dongdagou River (Chen et al., 2018). *Firmicutes* have also been detected in mercury-contaminated rice paddy soils, where THg and methylmercury concentrations were high and *hgcAB* genes were present (Vishnivetskaya et al., 2018).

4. Conclusions

In sites in the Amazon region that are chronically polluted with mercury, the effect of mercury and its derivatives on microbial communities and mercury biotransformation processes are not yet evident. In this study, we evaluated the concentrations of THg and MeHg and their speciation in samples collected from water, soil and sediments from long-polluted sites from the Colombian Amazon region. The comparison of the Shannon diversity index revealed higher diversity in Tarapacá than in Taraira samples, particularly than in sediment samples of this last locality, which showed the highest Hg levels. Our study demonstrated that the chronic presence of mercury in Amazonian ecosystems did not appear to affect the structure of the microbial community to the same degree as certain physicochemical variables, including soil texture components. The apparent lack of effect of long-term mercury pollution on microbial communities may be attributed to multiple factors. A baseline mercury level in Amazon soils and sediments could render community structures insensitive to additional mercury inputs. Alternatively, the low bioavailability of the long-term adsorbed mercury could diminish the community response. The results indicated that the majority of the mercury found in the sampled soils and sediments was in an immobile chemical form probably unavailable to microorganisms, partially explaining its limited effect on the bacterial communities, however, still a fraction of the mercury present seemed available for biotransformations. Overall, we have shown the presence of both a bacterial community with the potential to transform Hg into highly toxic MeHg, and a community with the potential to detoxify Hg, and possibly also MeHg. Furthermore, the highest relative abundance of *merA* was identified in sediments from Taraira, consistent with the highest average THg concentrations found in the soil and sediments from this locality, suggesting a positive selection of this function. In this ecosystem, Hg-resistant bacteria bearing the *mer* pathway could play a significant role in the detoxification of this site by helping reduce Hg(II) to elemental Hg (0), so representing an interesting source for potential mercury detoxification strains for use in remediation strategies (Cardona et al., 2022; MC Escobar, unpublished). The bacterial taxa associated with high mercury concentrations, including members of *Proteobacteria*, *Acidobacteria*, *Actinobacteria, Chloroflexi, Euryarchaeota,* and *Firmicutes*, could be interesting candidates as biomarkers in Hg-polluted Amazon ecosystems, although further screening of Amazonian sites and localities is required to confirm their suitability as biomarkers.

5. Statement of novelty

The present manuscript is part of a more extensive study to generate baseline data on mercury contamination in Amazonian ecosystems in Colombia, a region affected by illegal and small-scale gold mining (ASGM), and its effects on microbial communities in soil, sediment, and water. The final objective will be to create practical monitoring tools and to design bioremediation strategies for future long-term recovery of polluted sites. In the first approach, we assessed mercury pollution and characterized mercury-highly resistant bacteria and yeast strains (Cardona et al., 2022). Our current study takes advantage of available molecular tools to analyze both the microbial diversity and the abundance of specific functional genes related to mercury metabolism in samples associated with long-term mercury contamination. Furthermore, we determined mercury the actual mercury bioavailability and mobility in selected samples with the highest total mercury and methylmercury levels (up to 43.34 mg kg^{-1}). This allowed us to provide for the first time a comprehensive view of the effect of long-term mercury pollution on the microbial communities in its mercury related functions. Overall, our data suggested that the chronic presence of mercury in Amazonian ecosystems did not affect the structure of the microbial community to the same extent as certain physicochemical variables did, in accordance with the low bioavailability of mercury observed.

CRediT authorship contribution statement

Gladys Inés Cardona: Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **María Camila Escobar:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alejandro Acosta-González:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Natalie Díaz-Ruíz:** Writing – review & editing, Software, Formal analysis. **Juan Pablo Niño-García:** Writing – review & editing, Software, Formal analysis, Conceptualization. **Yaneth Vasquez:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation.

José Marrugo-Negrete: Writing – review & editing, Validation, Methodology, Conceptualization. **Silvia Marqués:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141348.

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