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# Uncovering potential mangrove microbial bioindicators to assess urban and agricultural pressures on Martinique island in the eastern Caribbean Sea.

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## HIGHLIGHTS

- Martinique's mangroves face human pressures, which cause pollutant accumulation in sediments.
- Regulatory thresholds for DDT, dieldrin, Cu, Ni, and As were exceeded in all samples.
- Microbial biomass and bacterial/archaeal abundance were higher in less-impacted stations.
- A total of 65 potential microbial bioindicators of human pressures were identified.
- 9 taxa abundant in high-impacted stations (e.g., *Spirochaeta*, *Pseudolabrys*, *Truepera*).

## ABSTRACT

Martinique's mangroves, which cover 1.85 hectares of the island (<0.1% of the total area), are considerably vulnerable to local urban, agricultural, and industrial pollutants. Unlike for temperate ecosystems, there are limited indicators that can be used to assess the anthropogenic pressures on mangroves. This study investigated four stations on Martinique Island, with each being subject to varying anthropogenic pressures. An analysis of mangrove sediment cores approximately 18 cm in depth revealed two primary types of pressures on Martinique mangroves: (i) an enrichment in organic matter in the two stations within the highly urbanized bay of Fort-de-France and (ii) agricultural pressure observed in the four studied mangrove stations. This pressure was characterized by contamination, exceeding the regulatory thresholds, with dieldrin, total DDT, and metals (As, Cu and Ni) found in phytosanitary products. The mangroves of Martinique are subjected to varying degrees of anthropogenic pressure, but all are subjected to contamination by organochlorine pesticides. Mangroves within the bay of Fort-de-France experience notably higher pressures compared to those in the island's northern and southern regions. In these contexts, the microbial communities exhibited distinct responses. The microbial biomass and the abundance of bacteria and archaea were higher in the two less-impacted stations, while in the mangrove of Fort-de-France, various phyla typically associated with polluted environments were more prevalent. These differences in the microbiota composition led to the identification of 65 taxa, including Acanthopleuribacteraceae, Spirochaetaceae, and Pirellulaceae, that could potentially serve as indicators of an anthropogenic influence on the mangrove sediments of Martinique Island.

## 1. INTRODUCTION

Mangrove forests are salt-tolerant tree assemblages in tropical and subtropical zones (Bunting et al., 2018), that cover 137,000 km<sup>2</sup> of the worldwide coastal areas and provide numerous ecological services. The one hundred and twenty million people who live within or

around mangrove areas (UNEP, 2014) closely depend on mangrove resources in terms of provisioning services such as firewood, charcoal, and construction materials (Baba et al., 2013), nutritive feeds for farm animals (Kathiresan, 2012), fisheries (Nagelkerken et al., 2008), and medicines (Bunyapraphatsara et al., 2003). Mangroves contribute to reducing the “greenhouse effect” by removing CO<sub>2</sub> from the atmosphere (Kathiresan, 2012), protect the coast against cyclones and tsunamis (Kathiresan and Rajendran, 2005), trap suspended sediments, recycle nutrients (Furukawa et al., 1997), and slow down coastal erosion (Harada et al., 2002). Mangroves also serve as feeding, breeding, and nursery areas for fishes, and the leaves are a food resource for insects and crabs (Nagelkerken and Van Der Velde, 2004). These forests benefit the adjacent ecosystems (*e.g.*, seagrass, coral reef) since they provide these ecosystems with nutrients and organic matter (OM; Kathiresan, 2012).

Despite their importance, mangrove areas have experienced degradation and losses in recent decades due to human activities (Hamilton and Casey, 2016; Cuny et al., 2020). Models and indicators have been developed over several years to evaluate and follow the health of mangrove ecosystems around the world, integrating several mangrove components such as trees and mollusks (Faridah-Hanum et al., 2019; Wang et al., 2021). Similarly, benthic organisms (such as crabs, meiofauna, bivalves, mollusks, and microorganisms) could be proposed as bioindicators in relation to the types and levels of anthropogenic pressure (inorganic or organic contamination or pollution) exerted on mangroves (Zuloaga et al., 2009; Debenay et al., 2015; Torres et al., 2019; Hong et al., 2020; Lavezzo et al., 2020; Michelet et al., 2021; Fiard et al., 2022). Among them, bacteria and archaea (referred to herein as microorganisms) are of particular interest for bioindication, as they are highly sensitive to environmental changes, which can result in the disappearance, appearance, or change in abundance of specific taxa (Tiralerdpanich et al., 2021). Microbial bioindicators have already proven to be valuable tools in the evaluation of the impacts of heavy metal contamination (De La Rosa-Acosta et al., 2015;

Fernandez-Cadena et al., 2020), aquaculture effluents (Erazo and Bowman, 2021), land use changes (Dinesh and Ghoshal Chaudhuri, 2013; Gong et al., 2019), and enrichment with OM, organochlorines, or polycyclic aromatic hydrocarbons (Fiard et al., 2022).

Coastal ecosystems in the Caribbean islands are highly vulnerable to agricultural effects, i.e., the cultivation of sugarcane, bananas, and other tropical fruits. Historically, these islands have been reliant on monocrop agricultural economies that heavily rely on the use of fertilizers and pesticides. Martinique, a lesser French Indies Island located in the eastern Caribbean Sea, is no exception to this trend. The island's primary economic activities include the production of bananas and sugarcane, which has led to significant soil and water pollution (Clostre et al., 2014). To control banana crop predation, several organochlorine insecticides, including the persistent chlordecone (commercialized as Curlone and Kepone), have been widely used for over twenty years (1972 – 1993). Besides agriculture, tourism is another significant source of income for Martinique, with several hotel complexes situated along the island's west coast. These various sources of pressure have adversely affected the Martinique mangroves. Given their proximity to contamination sources on the island and their ability to concentrate pollutants (Tam and Wong, 1995), mangroves are an ideal area for identifying microbial bioindicators of urban and agricultural pressures.

This study aimed to evaluate the response of the mangrove sediment microbiota to the environmental context of Martinique mangroves in order to identify potential microbial bioindicators of specific pressures. A focus was placed on the level of organic (e.g., pesticides, polychlorobiphenyl and HAP) and inorganic (e.g., trace metals and metalloids) contamination in mangrove sediments.

## **2. MATERIALS AND METHODS**

### **2.1. Study area and sampling stations**

Martinique is a volcanic island located in the French West Indies, situated between the Atlantic Ocean and the Caribbean Sea (Figure 1). The island has a tropical climate with a dry season from December to June and a rainy season from July to November that favors cyclones. The mangroves in Martinique are dominated by red mangrove (*Rhizophora mangle*), black mangrove (*Avicennia germinans*), and white mangrove (*Laguncularia racemosa*).

In this study, four sampling stations (S4, S5, S6, and S7 hereinafter) were selected from four watersheds with varying anthropization pressures (Figure 1). Watershed delineation and land identification were performed using advanced GIS techniques, leveraging an array of data sources. Precise bathymetric and topographic data, obtained through a continuous land-sea digital elevation model known as Litto3D, were used. This model provided detailed information about both underwater and terrestrial terrain features. Additionally, we incorporated orthophotos (DB Ortho 2017) to map various environmental settings, including agricultural areas, open natural spaces, forested regions, and specific aquatic habitats. The spatial identification of surface aquatic habitats across Martinique was achieved by the CarTHAgE<sup>®</sup> database. The exact extent of each study site's watersheds was ascertained by meticulously tracing the contour lines of altimetry data derived from the MNT. Delimiting agricultural zones involved the utilization of BD\_Culture DAAF 2008, which was subsequently refined with the latest orthophotography data. To ensure the precision of these delineations, on-site observations were conducted for thorough verification.

Station 4 (S4; Baie du Trésor; 60.88°E; 14.81°N) was in the less-anthropized zone in the north-east of the island. This colluvial mangrove is situated in the Caravelle nature reserve and is a natural zone of ecological interest for fauna and flora. The watershed is small (42.1 ha) and is mostly composed of natural zones (Table 1). As no direct pressure was detected for the mangrove in S4, it served as the reference station for this study.

Station 5 (S5; Pointe Marin; 60.87°E; 14.44°N) was in Marin Bay in the south of the island (Figure 1). The watershed (67.9 ha; Table 1) is minimally impacted from an industrial or agricultural standpoint, but the colluvial mangrove forest is under direct pressure from the larger marina of the Lesser Antilles and is located near the outflow of a wastewater treatment plant (Belfond).

The alluvial mangrove forest located at Station 6 (S6; Pointe Merle; -61.01°E; 14.56°N) in the Fort-de-France Bay (Figure 1) is fed by multiple watersheds and situated in an agricultural area (245 ha; Table 1) and artificial area (271 ha; Table 1). Fishing is prohibited due to the presence of a high concentration of chlordecone in the food webs (prefectorial decision, decree n°2012335-0003).

Lastly, Station 7 (S7; Cohé du Lamentin; -61.02°E; 14.60°N) was the largest and most impacted watershed of this study (Table 1). This station was in an agricultural and artificialized area near the bigger city of the island Fort-de-France (Figure 1; Table 1). The alluvial mangrove forest is surrounded by several industrial activities (*e.g.*, food industry, chemical industry), a refinery, and a domestic discharge and is near the main airport of the island.

## 2.2. Core sampling

During the dry season at low tide in June 2018, three sediment cores were collected from each station using plexiglass tubes measuring 10.4 cm in diameter and 20.0 cm in height. Each core was sliced into 0.5 cm thick layers for the first two centimeters and 1 cm thick layers thereafter, until the end of the core. Random subsamples were taken from these layers to create three composite samples representing different sediment depth horizons: 0 to 2 cm, 2 to 10 cm, and 10 cm to the end of the core. In each layer of each core, we measured the pH and redox potential (Eh) with a multi-parameter probe (WTW Multi 3500i) and the pore water salinity with a hand refractometer (ATAGO S-28). The instrument precision was 0.01 Eh and 0.001



pH, and the latter was calibrated with NBS buffers. Subsamples of the slices were taken and allocated for laboratory analyses to determine the grain size, total organic carbon (TOC), total nitrogen (TN), organic contaminants, and trace metals and metalloids (TMMs), and to conduct microbial analyses. The samples were frozen and stored at  $-80^{\circ}\text{C}$  until the analysis.

### **2.3. Laboratory analyses**

#### **2.3.1. Grain size, TOC, and TN measures**

The sediments were freeze-dried for 24 h, crushed, and gently homogenized. The grain size (0.011 to 3000 microns) was analyzed using a laser beam diffraction analyzer (Partica LA-950V2; Horiba Instruments, Inc.) at the EcoLab-LEFE Platform for Physical and Chemical Analyses (PAPC, Univ Toulouse UPS, INPT, CNRS). The TOC and TN were analyzed through combustion at  $930^{\circ}\text{C}$  using a CHN carbon analysis (Flash-2000; ThermoFisher Scientific Inc., Milan, Italy). The TOC and TN were used as proxies of the organic matter, and the molar TOC:TN ratio (mol:mol) was calculated to infer a proxy of the refractory versus labile nature of the organic matter.

#### **2.3.2. Analysis of trace metals and metalloids and organic contaminants**

Analyses of Al, Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn were performed with an Agilent 5800VDV ICP-OES and an Agilent 8900 ICP-MS. To this end, 0.25 g of each sample were digested using a 3:1 (v/v) 67–70%  $\text{HNO}_3$ /34–37% HCl mixture (Fisher, trace metal quality). Acidic digestion of the samples was carried out overnight at room temperature and then in a Milestone microwave oven (30 min with constantly increasing temperature up to  $120^{\circ}\text{C}$ , then 15 min at this maximal temperature). Each sample was made up to 50 mL with ultrapure quality water (Milli-Q). Four certified reference materials (CRMs) were treated and analyzed in the same way as the samples. CRMs, from the National Research Council Canada

(NRCC), were DOLT-5 (dogfish liver), TORT-3 (lobster hepatopancreas), PACS-1 (marine sediment) and PACS-2 (marine sediment). For Total Hg, samples ranging from 5 to 10 mg of dried material were directly analysed in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). Hg determination involved evaporation of the metal by progressive heating until 800 °C was reached and then held under oxygen atmosphere for 3 min, and subsequent amalgamation on a gold-net. Afterwards, the net was heated to liberate the collected Hg, which was then measured by atomic absorption spectrophotometry. Accuracy was checked using a certified reference material DOLT-5 (dogfish liver), from the National Research Council Canada (NRCC).

Six groups of organic contaminants were analyzed: 16 polycyclic aromatic hydrocarbons (PAHs; acenaphthene, acenaphthylene, anthracene, benzo[ghi]perylene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[b+k]fluoranthene, benzothiophene, biphenyl, chrysene, dibenzothiophene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene), 12 organochlorines (2,4-DDT, 2,4-DDD, 2,4-DDE, 4,4-DDD, 4,4-DDE, 4,4-DDT, aldrin, chlordecone, dieldrin, endrin, hexachlorobenzene, and isodrin), 6 polychlorinated biphenyls (PCBs; PCB-7, PCB 28, PCB-52, PCB-105, PCB-156, and PCB-169), 2 alkylphenols (4-nonylphenol and 4 tert-octylphenol), 7 polybrominated diphenyl ethers (PBDEs; BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183), and 6 phthalates (DMP, DEP, DBP, BBP, DEHA, and DEHP). The extraction, clean-up, and analysis of the sediment were performed by following Munschy (2005) method by Alzaga et al. (2004). Two reference materials were used for quality control: the WEPAL SETOC 767 reference material (LGC Standard, Germany) and SRM 1941b (National Institute of Standards and Technology, US). To increase the sensitivity, a GC-MS/MS was used as the analytical equipment. The analytical method used described by Lacroix et al. (2014).

All values for the certified materials used for trace elements and organic compounds are provided in Supplementary Materials 2 and 3.

### 2.3.3. Total DNA extraction and quantification

The samples were freeze-dried (48 h) and crushed into a powder with a sterilized mortar and pestle. The total DNA was extracted with a DNeasy PowerSoil Kit (Qiagen) according to the supplier's recommendations for 0.25-0.30 g of dried sediment. The DNA was quantified with a Quantifluor dsDNA system kit (Promega) using a fluorometric dosage according to the manufacturer's protocol with the Qubit 4 Fluorometer (Invitrogen). The measured DNA quantity (molecular microbial biomass) was used as a proxy of the microbial biomass in the sediment (Dequiedt et al., 2011).

### 2.3.4. Quantitative Polymerase Chain Reaction (qPCR)

The bacterial and archaeal abundances in the sediment were determined by quantifying the 16S rRNA genes (*rrs*) using qPCR with GoTaq qPCR Master Mix (Promega). Specific bacterial (300F: 5'-GCCTACGGGAGGCAGCAG and univ516R: 5'-GTDTTACCGCGGCKGCTGRCA-3') and archaeal (931F: 5'-AGGAATTGGCGGGGAGCA-3' and m1100R: 5'-BTGGGTCTCGCTCGTTRCC-3') primers sets were used (Klindworth et al., 2013). The thermal cycle for bacteria was composed of a step of initial denaturing that lasted 2 min at 98°C, followed by 30 cycles of (1) initial denaturation (5 s at 98°C), (2) primer hybridization (10 s at 55°C) and (3) elongation (12 s at 72°C). For archaea, the cycles consisted of initial denaturing (3 min at 98°C), followed by 35 cycles composed of (1) denaturation (10 s at 98°C), (2) primer hybridization (10 s at 62°C) and (3) elongation (20 s at 72°C). The final elongation was performed at 95°C for 10 s and the melt-

curve was built between 65 °C and 95 °C (0.5 °C per 5 s). The DNA standards used came from *Pseudomonas stutzeri* for bacteria and a euryarchaeal species for the archaea.

### 2.3.5. Amplification and sequencing of 16S rRNA genes

The variable regions V4-V5 of the 16S rRNA genes were amplified using PCR with the following primer set (Wang and Qian, 2009; Parada et al., 2016): 515f (5'-GTG YCA GCM GCC GCG GTA A-3') and 928r (5'-CCG YCA ATT CMT TTR AGT-3'). The reaction mixes were composed of 1-10 ng of DNA, 10 µM of 512f forward primer, 10 µM of 928r reverse primer, 10 mM of dNTPs, 0.4 µL of Pfu DNA polymerase (Promega), and 2.5 µL of Pfu buffer (Promega). The PCR amplifications were performed using a T100 Thermal Cycler (Biorad) and consisted of denaturing (2 min at 94°C) followed by 35 cycles of (1) denaturation (20 s at 94°C) (2) primer hybridization (20 s at 50°C) and (3) elongation (25 s at 72°C). The amplicons were checked using agarose gel electrophoresis (1.5 % w/v), purified with the Agencourt AMPure XP system, and quantified with QuantIT PicoGreen. The 16S rRNA genes were sequenced on an Illumina MiSeq platform (Genotoul, Toulouse, France). The raw sequence data set was deposited in the National Center for Biotechnology Information database under the PRJNA874590 BioProject and the BioSample accession Nos. from SRX17300545 to SRX17300510.

### 2.3.6. Sequence analysis

The reads and the sequences analysis were performed with the Rstudio interface (v3.2.3) and the dada2 package (v.3.9) using the workflow proposed by Callahan et al. (2016). The amplicon sequence variants (ASV) obtained were affiliated with the Silva database, v.132 (Quast et al., 2013). The ASVs affiliated with chloroplastic and mitochondrial sequences were excluded from the dataset. The phyloseq package (v3.9; McMurdie and Holmes, 2013) was

used to rarefy the dataset at an even number of sequences (8,638 reads) and calculate the diversity indices.

### **2.3.7. Statistical analysis**

Statistical analyses were performed with the RStudio interface (v3.2.3). A multiple factor analysis (MFA), an extension of principal component analyses (PCA), was performed to compare the mangrove stations (Escofier and Pagès, 1994) by following the custom function `bc.mfa` (<https://github.com/Hubas-prog/BC-MFA>). An MFA enables the analysis of complex data tables with distinct groups of variables. Rather than using all the variables simultaneously, an MFA focuses on examining the relationships between these variable groups. This methodology is particularly well suited for situations where tables contain numerous explanatory variables while sharing the same statistical individuals. An MFA assigns weights to these variables, ensuring that imbalanced groups do not disproportionately influence the analysis. Variables within each group receive identical weights, although these weights can vary from one group to another. Importantly, these weights are chosen to standardize the maximum axial inertia of each group to 1. Four groups of variables were constituted in this study (physicochemical parameters, OM, organic contaminants, and TMMs) to determine the characteristics of each station and depth (statistical individuals). The FactoMineR package (Lê et al., 2008) was used to run the MFA. Only variables that exhibited significant differences in their concentrations between stations (Kruskal – Wallis test;  $p < 0.05$ ), as well as those that exceeded the regulatory thresholds (ERLs), were plotted using the factoextra package (Kassambara and Mundt, 2020).

To compare the difference in the microbiota compositions and the environmental parameters, non-parametric tests (SRH – test and Wilcoxon pairwise test) were performed using the R companion packages (Mangiafico, 2021).

A heat tree was constructed with the Metacoder package (Foster et al., 2017) for the top 30 phyla of the dataset. The abundances of these phyla were determined and compared using a Wilcoxon test. The heat tree highlights the significant differences in archaeal and bacterial abundances with a log<sub>2</sub> ratio of median proportions. Finally, Spearman correlations were determined between the taxa abundance and environmental parameters.

### 3. RESULTS

#### 3.1. Physicochemical characteristics of sampling stations

Overall, the sediments from the four study stations had rather similar physicochemical characteristics. The pH was slightly acidic, with the vertical average values ranging from  $5.4 \pm 0.1$  (S6) to  $5.8 \pm 0.3$  (S5). The redox potentials varied between -200 mV and  $-318.3 \pm 55.7$  mV indicating reduced conditions that would be suitable for anaerobes. The granulometry of the sediments was dominated by silt, which accounted for 77.2 – 37.3 % of the grain size, while a higher clay content ( $1.1 \pm 0.4$  %,  $p < 0.05$ ) was found at S5. Finally, the pore water salinities were similar ( $32.5 \pm 3.3$  ‰) across all stations, except at S5 ( $24.1 \pm 6.9$  ‰; Supplementary Data 1). The MFA allowed for the identification of the environmental parameters that discriminated between the stations. For example, the first axis of the MFA (44.1 % explained inertia) showed discrimination between the samples from S4 – S5 and S6 – S7 (Figure 2). Indeed, S4 – S5 had higher levels of TOC ( $15.6 \pm 5.7$  %) and TN ( $0.7 \pm 0.2$  %) compared to the other stations (TOC =  $6.0 \pm 1.8$  %; TN =  $0.2 \pm 0.7$  %; Supplementary Data 1). The results showed that stations S4 – S5 had sediment concentrations  $7.5 \pm 2.0$   $\mu\text{g. g}^{-1}$  for Co,  $187.8 \pm 127.5$   $\mu\text{g. g}^{-1}$  for Mn,  $43.2 \pm 9.5$   $\mu\text{g. g}^{-1}$  for Zn,  $0.07 \pm 0.01$   $\mu\text{g. g}^{-1}$  for Ag and  $0.1 \pm 0.02$   $\mu\text{g. g}^{-1}$  for Cd which were 1.3-fold to 3.5-fold higher compared to S6 – S7 (Supplementary data 1). Compared to S4 – S5, the sediments in S6 and S7 were characterized by the presence of higher concentrations of organic contaminants (Figure 2), including pyrolytic PAHs such as phenanthrene and anthracene. The

concentration of phenanthrene, which was not detected in S4 – S5, was found to be  $0.4 \pm 1.1 \text{ ng. g}^{-1}$  in S6 and  $0.7 \pm 1.3 \text{ ng. g}^{-1}$  in S7 (Supplementary Data 1). Anthracene was detected in S6 (a maximum value of  $57.06 \text{ ng. g}^{-1}$  in surface sediments; a mean concentration of  $6.3 \pm 8.8 \text{ ng. g}^{-1}$ ), in S7 (a maximum value of  $19.23 \text{ ng. g}^{-1}$  in the deeper sediments of  $>10 \text{ cm}$ ; a mean concentration of  $7.5 \pm 8.9 \text{ ng. g}^{-1}$ ), and only once in S5 ( $14.17 \text{ ng. g}^{-1}$  in subsurface sediments of  $2 - 10 \text{ cm}$ ). Furthermore, S6 and S7 were found to have high mean levels of several organochlorine pesticides, including aldrin ( $4.5 \pm 0.01 \text{ ng. g}^{-1}$ ), HCB ( $1.8 \pm 1.8 \text{ ng. g}^{-1}$ ), chlordecone ( $6.3 \pm 5.0 \text{ ng. g}^{-1}$ ), endrin ( $8.8 \pm 4.9 \text{ ng. g}^{-1}$ ), total DDT ( $14.0 \pm 4.4 \text{ ng. g}^{-1}$ ), and dieldrin ( $9.2 \pm 4.5 \text{ ng. g}^{-1}$ ; Supplementary Data 1). These compounds were found in the other stations at concentrations 2 to 10 times lower. Notably, the concentrations of total DDT (min of  $5.4 \pm 3.2 \text{ ng. g}^{-1}$ , S4; max of  $14.3 \pm 4.8 \text{ ng. g}^{-1}$ , S6) and dieldrin (min of  $5.0 \pm 1.6 \text{ ng. g}^{-1}$ , S4; max of  $10.4 \pm 3.2 \text{ ng. g}^{-1}$ , S6) exceeded the ERL values (total DDT =  $1.58 \text{ ng. g}^{-1}$ ; dieldrin =  $0.02 \text{ ng. g}^{-1}$ ) in all samples (Buchman, 2008). The sediment samples from S4, S5, S6, and S7 contained mean chlordecone concentrations of  $4.6 \pm 1.5 \text{ ng. g}^{-1}$ ,  $4.9 \pm 2.5 \text{ ng. g}^{-1}$ ,  $9.5 \pm 3.2 \text{ ng. g}^{-1}$ , and  $3.4 \pm 4.6 \text{ ng. g}^{-1}$ , respectively.

The second axis of the MFA (14.1 % explained inertia) discriminated stations based on the biphenyl, Al, Cu, Pb, Ni, and, As concentrations (Figure 2). Biphenyl was only detected in S4 ( $3.6 \pm 2.4 \text{ ng. g}^{-1}$ ). The aluminum concentrations were higher in S7 and reached  $15.57 \pm 3.86 \text{ } \mu\text{g. g}^{-1}$ . The arsenic concentrations were similar in S4 ( $12.5 \pm 2.9 \text{ } \mu\text{g. g}^{-1}$ ), S6 ( $10.2 \pm 3.2 \text{ } \mu\text{g. g}^{-1}$ ) and S7 ( $9.3 \pm 2.2 \text{ } \mu\text{g. g}^{-1}$ ) but were lower in S5 ( $2.2 \pm 0.6 \text{ ng. g}^{-1}$ ). The copper quantities were higher in S6 – S7 ( $31.3 \pm 8.4 \text{ } \mu\text{g. g}^{-1}$ ) than S4 – S5 ( $28.2 \pm 5.0 \text{ } \mu\text{g. g}^{-1}$ ), and the Ni content was higher in S4 ( $20.1 \pm 6.8 \text{ } \mu\text{g. g}^{-1}$ ) and S7 ( $22.2 \pm 10.8 \text{ } \mu\text{g. g}^{-1}$ ; Supplementary Data 1).

### 3.2. Microbial community characteristics

The microbial molecular biomass (total DNA extracted from the sediment) was six times higher for S4 – S5 ( $p < 0.05$ ) than for S6 and S7, with mean values of  $10.8 \pm 7.6 \text{ } \mu\text{g. g}^{-1}$  and  $1.8$

$\pm 1.9 \mu\text{g. g}^{-1}$ , respectively (Table 2). Similarly, the mean bacterial population size was found to be significantly higher at stations S4 and S5 ( $1.63 \times 10^9 \pm 2.0 \times 10^9$  *rrs* gene copies.  $\text{g}^{-1}$ ) compared to S6 and S7 ( $1.7 \times 10^8 \pm 2.0 \times 10^8$  *rrs* gene copies.  $\text{g}^{-1}$ ;  $p < 0.05$ ; Table 2). The archaea abundance followed a similar trend, with a mean number of *rrs* gene copies.  $\text{g}^{-1}$  in the sediment of  $8.6 \times 10^7 \pm 5.0 \times 10^7$  for S4 and S5 compared to  $2.7 \times 10^7 \pm 3.5 \times 10^7$  for S6 and S7, respectively (Table 2).

### 3.3. Microbial diversity and composition

The filtering and trimming process allowed the identification of 11,463 ASVs ( $751 \pm 167$  ASVs per sample), and the Shannon index varied between 4.7 and 7 ( $p$ -value  $> 0.05$ ). Within the 11,463 ASVs, 67 phyla were identified in the mangrove sediments of the four stations. The dominant phyla observed in this study were Proteobacteria (20.5 – 28.8%), Chloroflexi (17.9 – 25.5%), Bacteroidetes (11.3 – 19.4%), Planctomycetes (3.4 – 10.5%), Calditrichaeota (3.3 – 5.7%), Acidobacteria (2.9 – 5.6%), Actinobacteria (0.7 – 5.3%), Crenarchaeota (1.1 – 5.8%), Euryarchaeota (1.6 – 4.1%), and Spirochaetes (1.3 – 3.1%).

To identify the taxa that exhibited differential responses to the contamination levels, the stations were divided into two groups (S4 – S5 and S6 – S7) based on the level of pressure in each station, as identified previously. A heat-tree clustering analysis was performed specifically on these groups (Figure 3). Several taxa were found to be more abundant in the stations with the lowest levels of anthropization (S4 – S5) or were totally absent in these sediments. This was notably the case for Crenarchaeota (the group formerly called the Miscellaneous Crenarchaeotal Group is now known as Bathyarchaeota), the taxa of which were more abundant in the S4 – S5 group, and the very diversified Acidobacteria phylum (subgroup 18), the taxa of which were four times more abundant in the stations with the lowest levels of anthropization. In contrast, nine taxa had a higher abundance (*Deinococcus-Thermus*, *Mariprofundus*, *Latescibacteria*,



Calditrichaeota, Planctomycetes, *Dragonibacterium*, *Spirochaeta*, Acanthopleuribacteraceae and *Pseudolabrys*) in the most contaminated stations (S6 – S7). Another particularly interesting taxon was the Thermodesulfovibrionaceae family (Nitrospirae), which was totally absent from S6 – S7. Similarly, a lower abundance of the Desulfobacteraceae (7.2 – 2.6%), Desulfobulbaceae (1.0 – 0.4%), and Desulfarculaceae (4.4 – 3.1%) families were observed in stations S6 and S7.

### 3.4. Correlations between taxa and environmental variables

To assess the relationships between all the identified taxa and the environmental variables of concern, including the total organic carbon, organochlorine compounds, and total metal concentrations exceeding the effects range low (ERL) values, Spearman's rank-order correlations were performed. The correlations were conducted at the family level due to the taxonomic assignment of 60% of the sequences at this level, whereas only 30% of the sequences could be identified at the genus level. Furthermore, by working at the family level, we were able to analyze groups of organisms that exhibited a greater similarity in terms of their functioning and physiology, in contrast to higher taxonomic levels such as order, class, and phylum. The analysis revealed that 65 families showed positive or negative correlations with these six environmental variables (Figure 4). First, thirty-seven families showed a positive correlation with the TOC in the analysis (Figure 4), including Acetobacteraceae, Acidobacteraceae and Gemmataceae ( $p$ -value < 0.05); Balneolaceae, Hydrogenedensaceae, and Mariprofundaceae ( $p$ -value < 0.01); Acanthopleuribacteraceae, Devosiaceae, Micropepsaceae ( $p$ -value < 0.001). Regarding pesticides, thirty-one families were positively correlated with dieldrin and total DDT (Figure 4): Chromatiaceae, Magnetospiaceae, and Pianococaceae ( $p$ -value < 0.05); Beggiatoaceae, Mycobacteraceae, and Thermoanaerobaculaceae ( $p$ -value < 0.01); and Devociaceae, Nocardiodaceae, and Rhodobacteraceae ( $p$ -value < 0.001). Finally,

thirteen families were positively or negatively correlated with the three TMMs (As, Cu, and Ni; Figure 4), whereas only four families were positively correlated with these metals (Moduliflexaceae, Rhodomicrobiaceae, Pirellulaceae, and Kineosporiaceae).

## 4. DISCUSSION

### 4.1. Characteristics and anthropization levels of sampling stations

As seen previously, the sediment characteristics were globally similar except for the pore water salinity, which was lower in S5. This difference may be attributed to the discharge of freshwater effluents from the upstream wastewater treatment plant of Belfond, located near the station. One other dissimilarity was the higher OM content in S6 and S7. Indeed, these two stations had significant leaf litter deposits (field observation), possibly due to the natural characteristics of the alluvial mangroves in these areas, which receive regular nutrients and OM inputs from the agricultural and artificialized watershed basins of the Lézarde and the Longvilliers rivers, respectively. Although S5 was near a wastewater treatment plant, the sampling period corresponded to a less touristic period, and the plant had the capacity to treat all water inputs, making it less likely for the wastewater treatment plant to be the source of the enriched OM in this mangrove.

Another difference between the stations was the higher concentration of pyrolytic PAHs (phenanthrene and anthracene) in S6 – S7. These contaminants are released into the environment particularly during accidental oil release or during combustion activities, such as power production, residential heating, incineration, refineries, and traffic (Pereira et al., 2019; Pichler et al., 2021). More specifically, the high concentrations of PAHs detected in S6 and S7 could have been due to the urban context of these stations (industries and vehicular and air traffic). Biphenyl was only detected in S4, but its concentration was lower than the ERL value (73 ng. g<sup>-1</sup>). Usually, this PAH is found in crude oils and refined products (diesel, lubricants,

and heavy oils). The origin of this benzenoid aromatic compound in this station is unclear. Although the production of PCBs is prohibited, they remain a toxic legacy as they were widely used from the 1930s to the 1980s in various applications, such as in PCB-filled transformers, paints, capacitor fluids, fire retardants, and extenders in pesticides (De Boer, 2005). The illegal use of pesticides or the dumping of waste containing PCBs could be possible explanations.

Despite higher quantities of organochlorine pesticides in S6 – S7, the omnipresence of pesticides in all stations underscores the widespread prevalence of agricultural activity in Martinique. The presence of dieldrin and total DDT highlights the past legacy of agricultural practices on the island. DDT was widely used as an insecticide between the 1940s and the 1950s (Kim et al., 2016) to fight banana weevils, even after its harmful effects were known. Despite its banishment in the 1970s, it can still be detected in the environment, along with its biodegradation products. Similarly, dieldrin is a persistent organic pollutant (POP) listed in the Stockholm convention (2001) and was used as an alternative to DDT for many years before being banned in France in 1972. An interesting fact is that the chlordecone content, a pesticide used during many years in the French Lesser Antilles, was found below the regulatory threshold (19.9 ng. g<sup>-1</sup>) in this study (INERIS, 2021). These findings are consistent with those of a previous study (Bocquené and Franco, 2005) that reported the leaching of this pesticide from Martinique soil and the consequent contamination of nearby waters (Dromard et al., 2022).

The concentrations of Co, Zn, Ag, and Mn were higher in S4 – S5; these differences may have been due to variations in the natural inputs between watersheds, since the TMM concentrations were below the regulatory thresholds (Buchman, 2008). In the same way, the sediment of S7 had a higher Al content. This station was in Le Lamentin Bay, which is the second most populated town in Martinique and the island's economic and industrial center. Le Lamentin has various industries, an oil refinery, landfills, and waste incinerators, which could be responsible for the inputs of Al in S7. In contrast, the concentrations of As, Cu, and Ni were

higher than the corresponding ERL values (Buchman, 2008). These TMMs are commonly found in the composition of fertilizers, herbicides, and pesticides (Defarge et al., 2018). Their presence, along with that of organochlorine compounds, most likely indicates intense agricultural activity on the island. Some of the zinc may have also been derived from the widespread use of metal sheet roofs on the island; however, this aspect requires further investigation (SDAGE, 2022).

The Martinique mangroves studied in this work can be categorized into two levels of anthropization. Although all four stations were polluted with dieldrin, total DDT, As, Cu, and Ni, S6 and S7 showed higher levels of anthropization than the other stations. The sediments of S6 and S7 were exposed to PAH contamination and had higher eutrophication levels with a higher TOC and TN than that in S4 and S5. These differences can be attributed to the watershed basins of S6 – S7, which are more anthropogenic than those of the other two stations.

## **4.2. Microbiota of mangroves in Martinique**

### **4.2.1. Microbial community characteristics**

The total DNA extracted from the mangrove sediment samples was used to estimate the microbial molecular biomass, a parameter that has been employed as a bioindicator of soil quality in metropolitan France for several years (Dequiedt et al., 2011; Terrat et al., 2012; Horrigue et al., 2016). A higher microbial biomass in soils is generally linked to better soil quality (Horrigue et al., 2016). The quality of soil corresponds to the monitoring of its proper functioning, such as the biomass production or the support of habitats and biodiversity (Ritz et al., 2009). In this study, the significantly higher microbial biomass at S4 – S5 may, therefore, suggest a better sediment quality than that at the other two stations. Furthermore, the molecular biomass measured at S6 – S7 was lower than the range that has been reported for other mangrove sediments (3.1 to 19.8  $\mu\text{g. g}^{-1}$ ; Jiang et al., 2011; Fernandes et al., 2014; Fiard et al.,

2022), probably because they are less impacted by anthropogenic pressures. Indeed, Jiang et al. (2011) conducted their research within the confines of the mangrove national nature reserve in China. Similarly, Fiard et al. (2022) delved into the microbial realm of French Guyana's mangroves, recognized for their relatively robust protection against human disturbances.

In the same way, the higher bacterial and archaeal populations observed in S4 and S5 compared to S6 and S7 suggest that the environmental conditions at S4 and S5 are more suitable for the growth and development of these populations. This is consistent with previous studies, which have shown that mangrove sediments enriched in OM can support larger bacterial populations (Fiard et al., 2022). However, in the case of S6 and S7, which were the most anthropized stations, the inhibitory effects of large amounts of PAHs and organochlorines appear to have outweighed the stimulatory effects of OM enrichment on the bacterial and archaeal populations.

#### **4.2.2. Microbial diversity and composition**

The alpha diversity values obtained in this study fell within the lower range of the diversity indices observed in other mangroves worldwide. For example, studies by Gomez-Acata et al. (2023), Gong et al. (2019), and Liu et al. (2023) reported Shannon indices of around 9-10. In contrast, other investigations on sediment samples from mangroves in Kenya (Muwawa et al., 2021), Brazil (De Santana et al., 2021), and Malaysia (Mai et al., 2021) have obtained alpha diversity values more similar to those observed in this study on Martinique mangroves (4.7 to 7). Furthermore, a previous study by Fiard et al. (2022) examined well-preserved mangroves in French Guiana and found Shannon index values ranging from 5.1 to 6.3, which aligns perfectly with the range of values obtained in Martinique using identical bioinformatics methods. The specific richness ranged from 717 to 909, while the Shannon index ranged from 5.8 to 6.4. A systematic review on the microbial diversity of preserved and disturbed mangrove forests

showed that impacted mangroves tend to exhibit a higher microbial diversity compared to that of sediments from preserved forests (Taketani et al., 2009; Fernandes et al., 2014; Fernández-Cadena et al., 2020; Yang et al., 2020). However, in the Martinique mangroves investigated in this study, no significant differences were observed in the richness or diversity index among the different sampling stations ( $p$ -value > 0.05). It is important to note that all the stations under investigation were exposed to pressures, primarily due to the presence of organochlorine pesticides (dieldrin and total DDT). The widespread and persistent occurrence of these pollutants throughout the island could explain the lack of a significant difference in the richness and diversity among the studied mangroves.

Ten dominant phyla were consistently found in high abundance in mangrove sediments, regardless of various factors such as the pressure level, location, season, or forest type. Among these, Proteobacteria taxa were the most dominant, with random reports of Firmicutes and Chloroflexi (see review by Lai et al., 2022). They constitute the core microbiome of mangroves (Andreote et al., 2012; Haldar and Nazareth, 2018; Santana et al., 2019; Fiard et al., 2022). Firmicutes taxa were indeed present in the sediments of Martinique mangroves; however, their abundance was lower (0.1% to 1.1%) than the abundance typically found in mangroves and therefore they were not among the top 10 most abundant phyla.

The heat-tree clustering analysis allowed for the identification of taxa that were more abundant in the two groups of stations (S4 – S5 and S6 – S7). Within these taxa, the Thermodesulfobibrionaceae family was completely absent from the most anthropized stations. However, this family is known to be highly abundant in mangrove sediments (Cao et al., 2017; Santana et al., 2019; Fiard et al., 2022). The contamination level in S6 – S7 seemed to inhibit Thermodesulfobibrionaceae in Martinique mangrove sediments. Additionally, Crenarchaeota and the very diversified Acidobacteria phylum were both less abundant in S6 – S7. The first one is often dominant in anoxic sediments (Frag et al., 2020; Zhou et al., 2018) and its phylum

are known for their ability in dissimilatory nitrite/nitrate reduction to ammonium (DNRA), sulfur reduction, and can degrade a wide range of OM such as plant-derived carbohydrates, detrital proteins, and refractory organic matter (Meng et al., 2014; Lazar et al., 2017; Zhou et al., 2018). In addition, the Acidobacteria phylum, (subgroup 18) is typically found in soil (Naether et al., 2012) and has been shown to be abundant in pasture soils with an acidic pH (Navarrete et al., 2015; De Chaves et al., 2019). Interestingly, the distribution of this taxon was shown to be mainly driven by the sediment grain size and by the Cu and Cr concentrations in Mediterranean coastal sediments highly contaminated with PAHs and heavy metals (Dell'Anno et al., 2021). The environmental conditions at Stations 6 and 7 should, therefore, have allowed for a more significant development of these two taxa. However, a higher level of anthropization appears to have a negative effect on these organisms. Similarly, Desulfobacteraceae, Desulfobulbaceae, and Desulfarculaceae were found in sediments with high OM content but also low levels of organic contaminants. This suggests that these taxa may be sensitive to organic contaminants detected in these stations.

On the contrary, some taxa showed higher abundances in the two most impacted stations. Within these taxa, the Deinococcus-Thermus phylum was represented by the *Truepera* genus, which is known to contain chemoorganotrophic bacteria commonly found in polluted mangroves (Albuquerque et al., 2018). *Truepera* has been implicated in the degradation of several environmental contaminants such as PAHs, bisphenol, and microplastics in sediments, soils, and water (Guo et al., 2020; Shabbir et al., 2020; Chen et al., 2021; Tong et al., 2021). The lower abundance of Deinococcus – Thermus in S4 and S5 suggests that this taxon may prefer the environmental conditions of the two contaminated stations. Another notable taxon was the chemolithoautotroph *Mariprofundus* genus, which belongs to the Zetaproteobacteria class, and which was totally absent in the S4 – S5 sediments. It can participate in the degradation of PAHs (Guo et al., 2016), and it has been found in polluted sediments (Obi et al., 2017; Torres

et al., 2019). Similarly, Acanthopleuribacteraceae, a family in the Acidobacteria phylum, is known to degrade organic compounds and is used for bioremediation purposes in the environment (Falkiewicz-Dulik et al., 2015). Due to its affinity for PCBs, it is also a potential bioindicator of PCB contamination in mangrove sediments (Fiard et al., 2022). The Spirochaetes phylum, particularly the *Spirochaeta* genus, was two time more abundant, is known to utilize detrital biomass and recycle necromass in coastal anoxic polluted environments (Dong et al., 2018), and is a frequent member of contaminated-sediment microbial communities in coastal areas (Quero et al., 2015). Within the Alphaproteobacteria class, some taxa are known to degrade organic pollutants, such as bisphenol A as well as other organic compounds (Falkiewicz-Dulik et al., 2015). One such taxon is the *Pseudolabrys* genus, which is a nitrogen-fixing and nitrifying bacterium (Yu et al., 2022). It is commonly found in hydrocarbon-rich soils and is considered a hydrocarbon degrader (Medina et al., 2018; Manucharova et al., 2021).

#### **4.3. Mangrove microbial bioindicators of anthropic pressures**

To identify indicator organisms that reflect the primary urban and agricultural pressures affecting the mangroves of Martinique, we performed Spearman correlations between microorganism families and the various variables linked to these pressures. A positive correlation may indicate that the taxon can degrade the compound or tolerate the recorded concentration of OM or the pollutant. Conversely, a negative correlation may suggest that the taxon is sensitive to the pollutant or the measured concentration of OM. Out of the 65 identified families, 31 are particularly interesting, as a link has been established among the total organic carbon, organochlorines, and total metal concentrations, as documented in the literature (refer to Tables 3, 4, and 5). Therefore, these families have therefore the potential to serve as microbial bioindicators of the environmental pressures present in the mangroves of Martinique.



#### 4.3.1. Potential bioindicators of OM enrichment (urban pressures)

The quantified TOC and TN values at Stations 6 and 7 indicated an enrichment of organic matter sourced from the urbanized watershed of Lamentin Bay. Out of all the families correlated with the TOC, only 13 of them have been described in the literature for their marked response to OM enrichment (Table 3). These heterotrophic taxa, which can be either aerobic or anaerobic, are found in various environments, such as marine sediments, soils, plant rhizospheres, seawater, and mangrove sediments (Huang et al., 2014; Lino, 2014; Shivani et al., 2016; Fiard et al., 2022; Krohn et al., 2022; see Table 3). In contrast, 54 other families also showed significant correlations with OM enrichment (higher TOC content; Figure 4). However, there is limited knowledge about these organisms, and further research is needed to understand their behavior towards OM. Nonetheless, these families warrant special attention as potential indicators of OM enrichment.

#### 4.3.2. Potential bioindicators of agricultural practices (pesticides)

As mentioned earlier, agricultural practices in Martinique are causing pressures on mangroves, leading to high levels of organochlorine pesticides (dieldrin and total DDT) and the associated TMMs (As, Cu, and Ni).

Thirty-one families were positively correlated with dieldrin and total DDT (Figure 4), and among them, 13 taxa are already known as bioindicators of agricultural activities in soils or sediments (Table 4). These families have representatives that are resistant to or can degrade organochlorine compounds (*e.g.*, Acanthopleuribacteraceae, Chromatiaceae, Xanthobacteraceae, Gallionellaceae and Trueperaceae), and some are even used in bioremediation processes (Acanthopleuribacteraceae, Nocardioiaceae and Sphingomonadaceae) (Table 4). Therefore, these families are promising candidates for the bioindication of agricultural pressures in mangrove ecosystems, particularly for dieldrin and

total DDT contamination. However, the lack of knowledge about the ecology of the remaining 18 taxa prevents us from understanding the observed correlations, which may be directly or indirectly related to the contamination levels (Figure 4).

#### **4.3.3. Potential bioindicators of agricultural practices (TMM)**

It may sometimes be difficult to define whether a metal is a contaminant or whether its strong concentrations are due to the geochemical background of the study site. The concentrations of As, Cu, and Ni in Martinique mangroves were found to be higher than the corresponding ERL guideline values, providing strong evidence that they are pollutants introduced into the environment through agricultural practices on the island (Defarge et al., 2018). These metals can have harmful effects on the environment and the health of living organisms, including mangroves and their associated microbiota (Bayen et al., 2012). The consequences of these effects may lead to various outcomes, such as changes in the physical features of the microbiota (Kruitwagen et al., 2006; Paixão et al., 2011), alterations in their behavior (Mackey and Hodgkinson, 1996), or even their mortality (Cannicci et al., 2009).

Therefore, understanding the behavior of microbial communities in the presence of these pollutants is important for the development of effective strategies for their remediation and the conservation of mangrove ecosystems. Seventeen taxa were found to be correlated with As, Cu, and Ni (Figure 4), but only five of them have established links with TMMs and could most likely serve as bioindicators of As, Cu, and Ni (Table 5). Among these families, Rhodomicrobiaceae is particularly interesting because it has been found in As-rich sediments and have been shown to be resistant to As (Table 5). Similarly, Caldilineaceae is known to be inhibited by metals, which seems to be the case in mangrove sediments as well (Kou et al., 2018). However, for the other families, further research is needed to determine the reasons for the observed correlations.

## 5. CONCLUSION

Our study highlights the significant influence of agricultural and urban pressures on Martinique's mangroves. It demonstrates organic matter enrichment in the two stations within the Fort-de-France Bay, as well as concentrations of pesticides (dieldrin and total DDT) and trace metal elements (As, Cu, and Ni) that exceed the ERL reference values across all four island stations. Despite the presence of organochlorine compounds across all four stations, the mangroves in the Fort-de-France Bay (S6 and S7) face a greater impact due to their proximity to Martinique's largest urban areas. In this context of contrasting levels of impact, the mangrove microbiota showed significant responses on multiple levels, including the molecular microbial biomass and the bacterial and archaeal population sizes which were lower in the two most impacted stations. The sensitivity and preferences of microbial taxa have led to shifts in the microbial community composition across the various pressure levels experienced by the stations. For instance, organisms commonly found in polluted environments were identified in the mangroves of Fort-de-France. These differences enabled us to identify interesting taxa at the family level that serve as bioindicators, reflecting the significant agricultural and urban pressures on Martinique's mangrove forests. Our identification of bioindicator taxa for the agricultural pressure in Martinique's mangroves constitutes a unique database of microbial taxa in mangroves and presents a promising approach for bioindication in these particular ecosystems, although further investigations are needed to confirm their applicability on a global scale. Overall, our findings emphasize the importance of monitoring and managing agricultural practices to protect the ecological integrity of mangrove ecosystems.

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#### **Credit authorship contribution statement**

**Maud Fiard:** Conceptualization, Software, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Cécile Militon:** Conceptualization, Methodology, Field work, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Léa Sylvi:** Resources, Field work, Investigation. **Jonathan Migeot:** Field work, characterization, and selection of field stations; **Emma Michaud:** Conceptualization, Methodology, Resources, Writing – review & editing, Project administration, Funding acquisition. **Ronan Jézéquel:** Investigation. **Franck Gilbert:** Methodology, Investigation. **Isabelle Bihannic:** Resources, Investigation. **Jeremy Devesac:** Investigation. **Guillaume Dirberg:** Conceptualization, Methodology, Field Work, Resources, Writing – review & editing, Project administration, Funding acquisition. **Philippe Cuny:** Conceptualization, Methodology, Field work, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

**Data availability:** The environmental parameters data were available in the supplementary material of this paper. The raw sequence data set was deposited in the National Center for Biotechnology Information database under the PRJNA874590 BioProject and the BioSample accession nos. from SRX17300545 to SRX17300510.

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**Table 1:** General information about the four watersheds and wastewater treatment plants located near the study stations.

	<b>S4</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>
<b>Total surface (ha)</b>	42.1	67.9	660	1823
<b>Agricultural area (ha)</b>	0	31.2	245	392
<b>Artificial area (ha)</b>	0.1	13.7	271	867
<b>Natural area (ha)</b>	42	23	144	564
<b>Wastewater treatment plant (population equivalent)</b>	NA	16 001 – 35 000	16 001 – 35 000	35 001 – 60 000

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**Table 2:** Values of the microbial biomass (total DNA extracted), and the bacterial and archaeal population sizes in the sediment of the four sampling stations (mean  $\pm$  SD, n=9).

Station	S4	S5	S6	S7
<b>Microbial biomass</b> ( $\mu\text{g. g}^{-1}$ )	9.0 $\pm$ 7.8	12.7 $\pm$ 7.4	1.33 $\pm$ 1.5	2.37 $\pm$ 2.2
<b>Bacterial abundance (number</b> <b>of <i>rrs</i> gene copies. <math>\text{g}^{-1}</math>)</b>	2.2E+09 $\pm$ 2.6E+09	1.1E+09 $\pm$ 1.2E+09	2.7E+08 $\pm$ 2.5E+08	7.7E+07 $\pm$ 7.3E+07
<b>Archaeal abundance (number</b> <b>of <i>rrs</i> gene copies. <math>\text{g}^{-1}</math>)</b>	5.8E+07 $\pm$ 3.0E+07	1.1E+08 $\pm$ 5.5E+07	7.9E+06 $\pm$ 1.0E+07	7.9E+06 $\pm$ 1.0E+07

**Table 3:** Short list of the thirteen potential bioindicator bacterial families of mangrove sediment OM enrichment.

<b>Family</b>	<b>Known Ecology</b>	<b>Reference</b>
<b>Nocardioideae</b>	Chemoorganotrophic bacteria; implicated in OM turnover	Goodfellow, 2014
<b>Sphingomonadaceae</b>	Recalcitrant organic compound degraders (lignocellulose or lignin)	Di Gregorio et al., 2013; Gołębiewski et al., 2014
<b>Xanthobacteraceae</b>	Use of decaying OM as carbon source	Bárta et al., 2017
<b>Prolixibacteriaceae, Ignavibacteraceae, Cyclobacteraceae</b>	Chemo-organoheterotrophic bacteria	Huang et al., 2014; Lino, 2014
<b>Demequinaceae</b>	Cellulose degrader	Ue et al., 2011
<b>Mycobacteriaceae</b>	Use of decaying or detrital OM as carbon source	Bastakoti et al., 2019; Robertson, 2022
<b>Mariprofundaceae, Spirochaetaceae</b>	Involvement in OM decomposition	Shivani et al., 2016
<b>Gallionellaceae</b>	Denitrifying bacteria; carbon fixation	Mendes and Tsai, 2018; Torres et al., 2019
<b>Beggiatoaceae</b>	Denitrifying and DNRA bacteria	Schutte et al., 2018; Huang et al., 2021; Fiard et al., 2022
<b>Pirrelulaceae</b>	Found in sulfur-rich environments; OM mineralization; potentially involved in nitrogen cycle	Muwawa et al., 2021

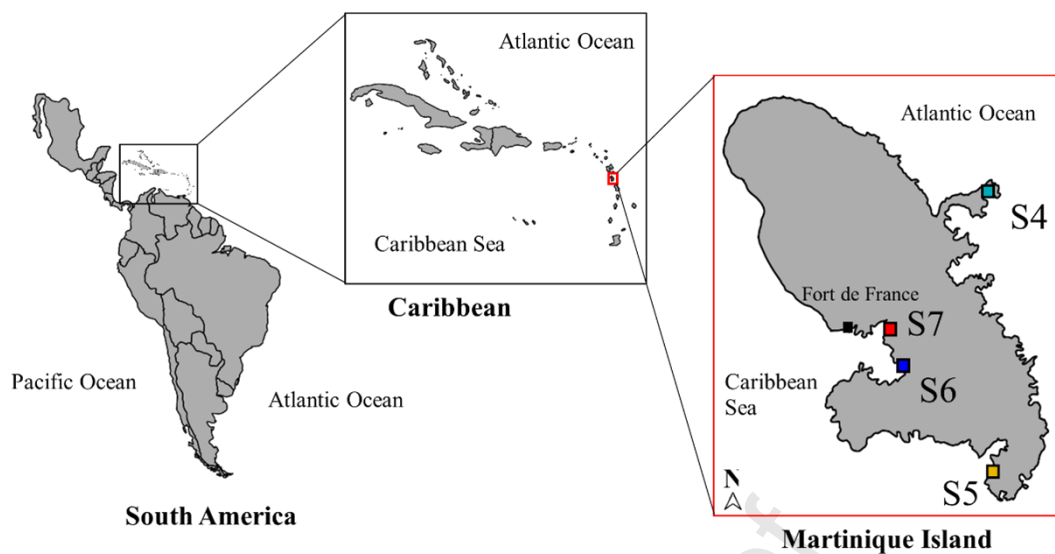
**Table 4:** Short list of the thirteen potential bioindicator bacterial families of mangrove sediments contaminated with organic compounds or subjected to pressures due to agricultural practices.

<b>Family</b>	<b>Known ecology and/or bioindication potential</b>	<b>Reference</b>
<b>Acanthopleuribacteraceae</b>	Bioremediation of PCB polluted environments; potential bioindicator of agricultural activity	Falkiewicz-Dulik et al., 2015; Fiard et al., 2022
<b>Chromatiaceae</b>	Bioindicators of agricultural activity	Jimenez-Bueno et al., 2016
<b>Cyclobacteriaceae</b>	PAH and complex molecule degrader; involved in the catabolic routes of DDT and its metabolites	Lee et al., 2018; Tiralerdpanich et al., 2021
<b>Rhodobacteraceae</b>	PAHs degraders	Lee et al., 2018; Tiralerdpanich et al., 2021
<b>Intrasporangiaceae</b>	Pyrene degraders; use of dioxin and dibenzofuran as carbon sources	DeBruyn et al., 2012; Yamazoe et al., 2004
<b>Rhizobiaceae</b>	Diazotroph bacteria; hexacyclohexane and lindane tolerance	Yuan et al., 2021
<b>Micropepsaceae</b>	Acclimatization to lindane contamination	Desrosiers et al., 2022
<b>Nocardioiaceae, Sphingomonadaceae</b>	Degradation of recalcitrant chemicals and xenobiotics; used in bioremediation	Acosta-Gonzalez et al., 2015
<b>Trueperaceae</b>	Bioindicator of organochlorine contamination	Dealtry et al., 2016
<b>Mycobacteriaceae</b>	PAHs and organochlorine degraders	Pagnout et al., 2007; De Schrijver and De Mot, 1999
<b>Xanthobacteraceae</b>	Dieldrin degraders; bioindicators of agricultural activity	Krohn et al., 2022
<b>Gallionellaceae</b>	Bioindicators of agricultural activity	Torres et al., 2019

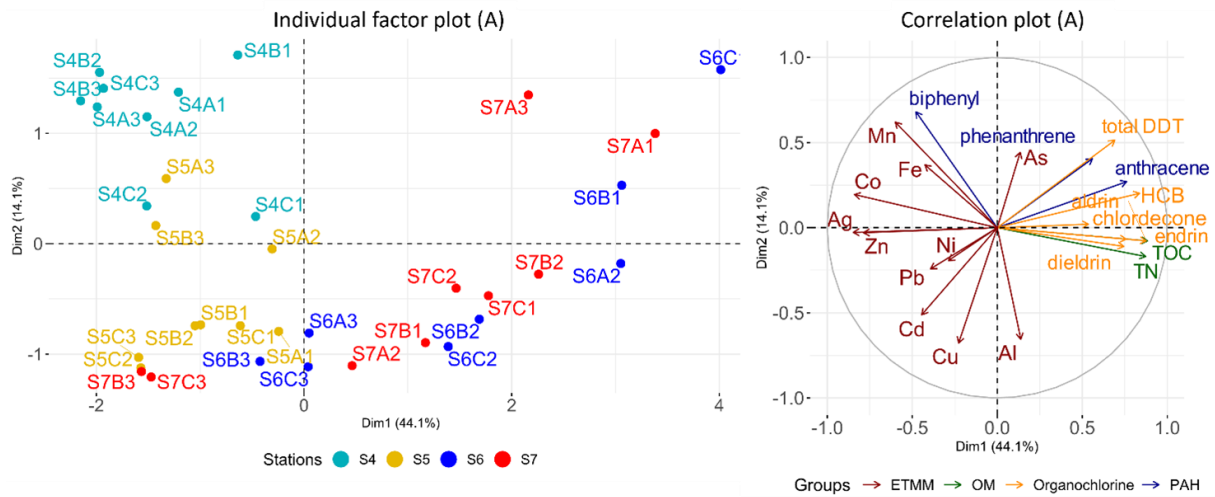


**Table 5:** Short list of the five potential bioindicator bacterial families of metal contamination.

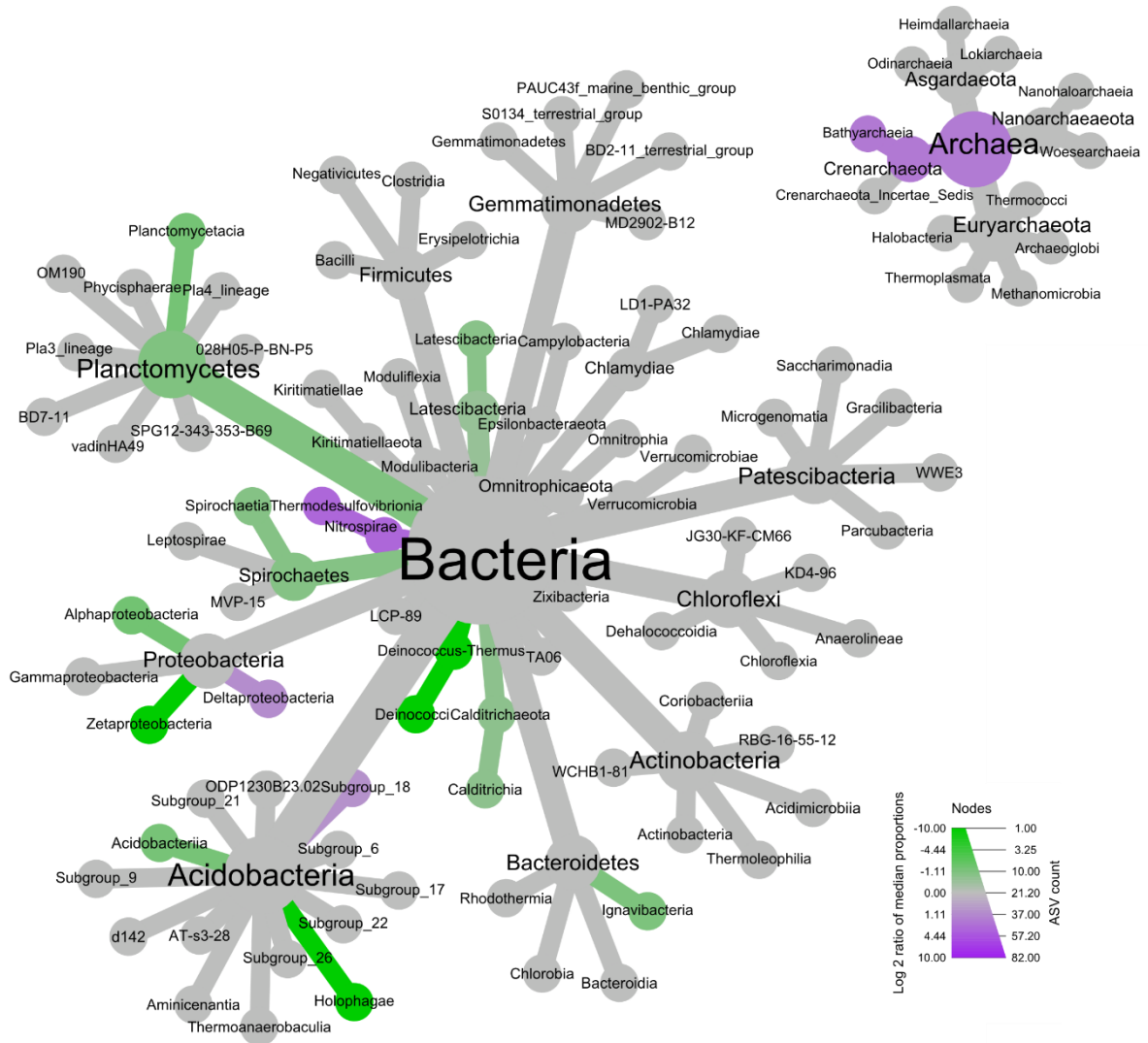
<b>Family</b>	<b>Known ecology and/or bioindication potential</b>	<b>Reference</b>
<b>Pirellulaceae</b>	Potential herbicide degraders; bioremediation of polluted environments	Wiegand et al., 2018
<b>Kineosporiaceae</b>	Bioindicators of agricultural activity	Jiménez-Bueno et al., 2016
<b>Rhodomicrobiaceae (Hyphomicrobiaceae)</b>	Resistant to As	Andres et al., 2013; Laha et al., 2021 ; Zhang et al., 2017
<b>Caldilineaceae</b>	Inhibited by metals	Kou et al., 2018
<b>Rhodothermaceae</b>	Contain genes encoding proteins implicated in Cu and Ag transformation	Goh et al., 2016



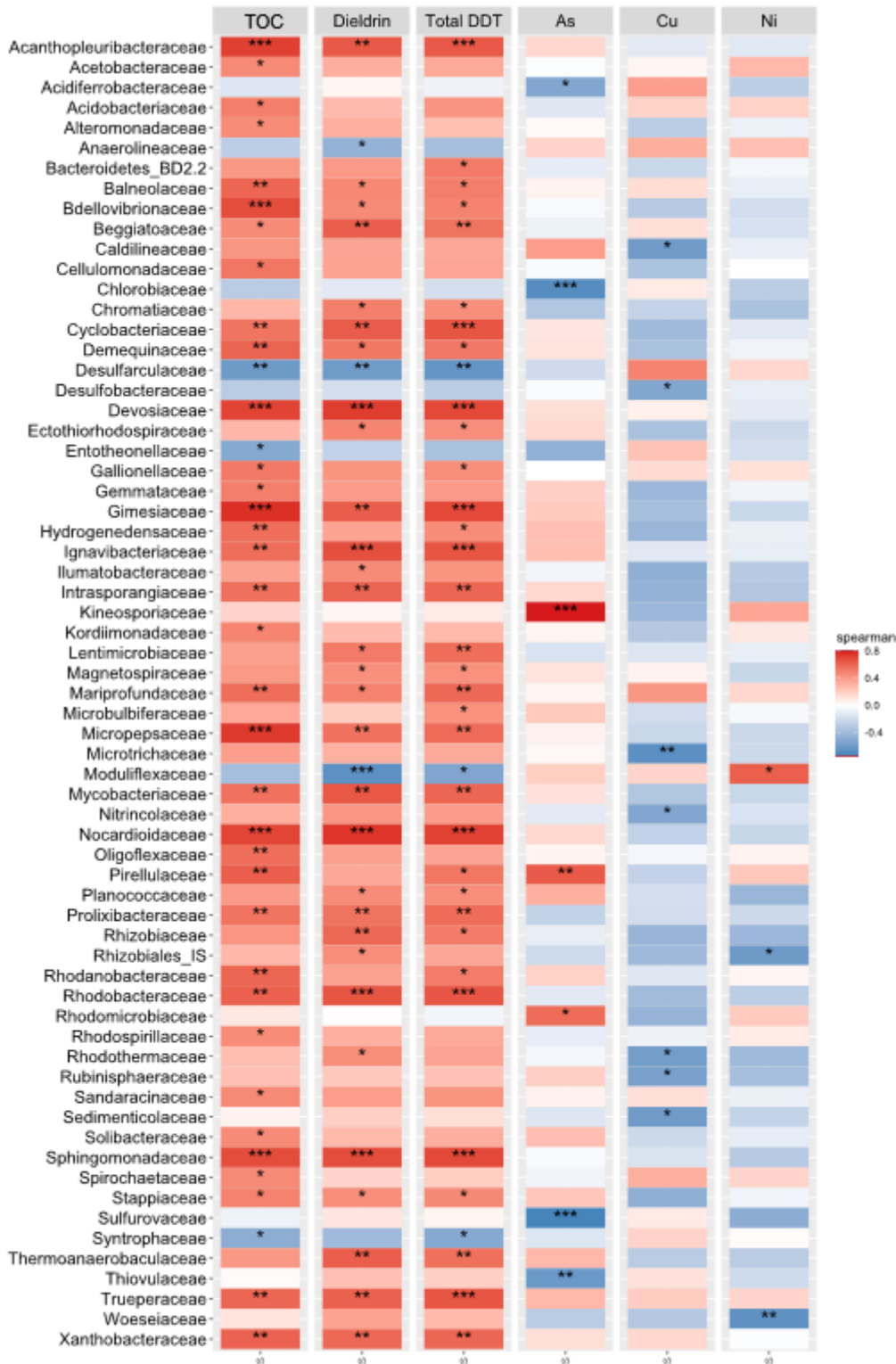
**Figure 1. Location of the sampling stations in Martinique Island.** S4 (blue green) was located at “Trésor” Bay, S5 (yellow) at “Marin” Bay in the south of the island, S6 (blue) at “Pointe Merle”, and S7 (red) at “Lamentin” Bay near the Fort-de-France city.



**Figure 2:** A multiple factor analysis (MFA) of the four groups of environmental variables: OM (pink), PAHs (blue), organochlorine (orange), and TMMs (purple). The individual factor plot (A) and the circle correlation plot (B) are represented. The dots correspond to the samples from the four sampling stations: S4 (green), S5 (yellow), S6 (blue), and S7 (red). The letters indicate replicates (A, B, C) and the last number represents the sediment depth: 0 – 2 cm (1), 2 – 10 cm (2), and >10 cm (3).

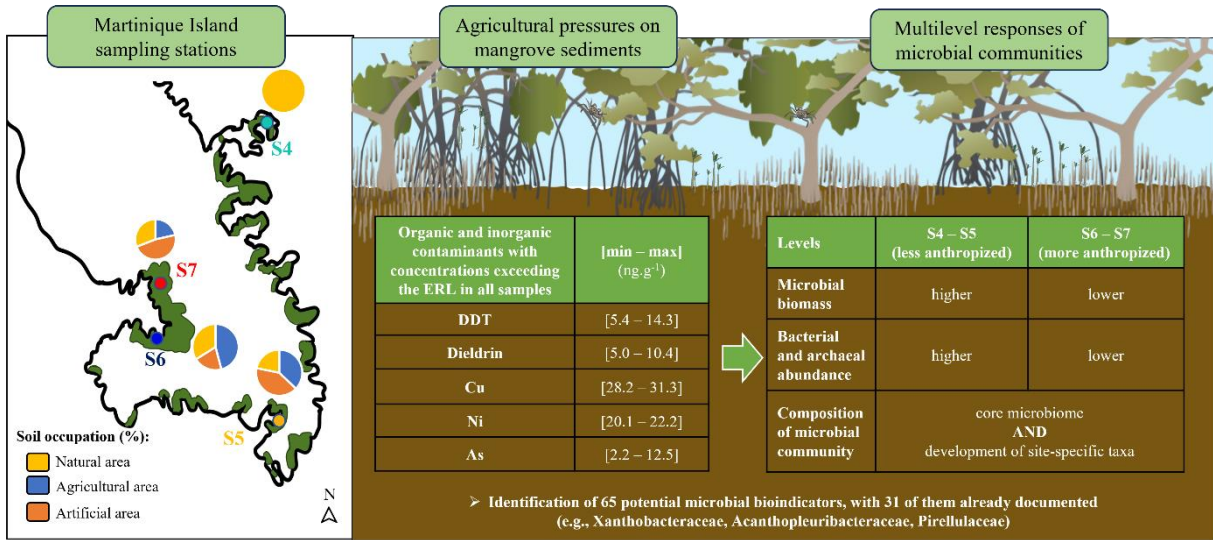


**Figure 3:** Heat tree of the bacteria and archaea community in the two groups of stations (S4 – S5 vs. S6 – S7). The two colors represent the difference in the log<sub>2</sub> ratio of the median proportions of reads between S4 – S5 and S6 – S7. The purple nodes correspond to the taxa significantly more abundant in S4 – S5; the taxa significantly more abundant in S6 – S7 are represented by green nodes and the gray nodes indicate the absence of differences between both station groups.



**Figure 4:** Spearman’s rank correlation heatmap between variables of environmental concern (with the TOC, organochlorine compounds, and TMMs having concentrations higher than the ERL values) and selected families already known for their response to anthropogenic pressures. Significant Spearman correlations are highlighted (\* $p < 0.05$ ; \*\* $p < 0.01$ ; and \*\*\* $p < 0.001$ ).

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**HIGHLIGHTS**

- Martinique's mangroves face human pressures, which cause pollutant accumulation in sediments.
- Regulatory thresholds for DDT, dieldrin, Cu, Ni, and As were exceeded in all samples.
- Microbial biomass and bacterial/archaeal abundance were higher in less-impacted stations.
- A total of 65 potential microbial bioindicators of human pressures were identified.
- 9 taxa abundant in high-impacted stations (e.g., *Spirochaeta*, *Pseudolabrys*, *Truepera*).

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