Exploring the diversity and structural response of sediment-associated microbiota communities to environmental pollution at the siangshan wetland in Taiwan using environmental DNA metagenomic approach

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One of the most crucial struggles for the aquatic ecosystem and modern society is environmental pollution. New approaches, such as the environmental DNA Metagenomic approach, have become a powerful tool to investigate the abundance and diversity of sediment-associated microbiota communities. Nevertheless, information on the response of microbial populations to heavy metals (HMs) in the coastal sediments of Taiwan is scarce. 44 sediment samples were collected from the Siangshan wetland in Taiwan (Taiwan Strait) at two different depths (surface and sub-surface) to measure various environmental variables viz., heavy metals, total organic matters, and sediment composition. Moreover, the abundance and structure of sediment-associated microbiota were examined using high-throughput 16S rRNA gene (V3-V4) next-generation sequencing, to explore the response of the microbiota community to environmental variables. According to the computed pollution indices viz., sediment quality guidelines (SQGs), enrichment factor (EF), geo-accumulation index (I_{geo}), pollution index (PI), and pollution load index (PLI), the studied sites were classified as unpolluted, moderately polluted, and extremely polluted. Our results revealed that the majority of high-quality reads were assigned to bacteria (~ 95.05%), Archaea (~ 4.83%), and 0.13% were unclassified. Study sites were dominated largely by *Proteobacteria* with a total of 38.02% across all sediment samples, followed by *Bacteroidota* (7.18%), *Desulfobacterota* (6.97%), *Actinobacterota* (6.68%), *Cyanobacteria* (5.84%), *Chloroflexota* (4.18%), *Planctomycetota* (4.16%), and *Firmicutes* (3.69%). Beta diversity (Non-metric dimensional scaling analysis, nMDS) explained that

surface and sub-surface groups had significantly different microbial community compositions (p = 0.01). Meanwhile, polluted sites exhibited more richness and diversity than unpolluted sites. Redundancy analysis (RDA) illustrated that Mn, Cu, Al, Co, Ni, Sand, and Cr had a sizable effect on the structure of microbiota communities (at the class level). This work highlights the potential responsibility of environmental variables in shaping the sedimentary microbiota of the Siangshan wetland *via* integrating various ecological variables with alteration of the microbiota composition.

1 Introduction

Nowadays, environmental contamination is one of the most serious problems confronting modern human society. Most near-shore marine environments around the world are under growing threat (David and Obura, 2000). Heavy metal (HM) contamination in aquatic environments is a global environmental issue that has garnered increasing attention over the last few decades because of its negative impacts (Iksandar and Keeney, 1974; Allen-Gil et al., 1997; Kim et al., 2003a; Ma et al., 2013; Wang et al., 2013). Heavy metals in the aquatic ecosystems originate from natural activities, i.e., geogenic (erosion and weathering) and anthropogenic sources (Bai et al., 2011; Wang et al., 2015b; Lee et al., 2017; Yazidi et al., 2017; Barut et al., 2018). The major sources of HMs are anthropogenic activities, such as smelting, mining, agriculture, aquaculture, and domestic or industrial discharge. (Vareda et al., 2019). Once HMs enter the aquatic ecosystem, they can be bioaccumulated by marine species and biomagnified through the food web, posing a serious menace to humans and animal hygiene (Tian et al., 2020) or adsorbed by suspended particles, then heavily accumulated in sediments (Gümgüm et al., 1994; Yi et al., 2011) while only ions persist soluble in the water column. As a result, sediments appear to be the main repository and sink for heavy metals in comparison to water, and may also serve as sources of contamination. Hence, sediments are commonly employed as geo-markers for the management and detection of likely sources of contamination in aquatic habitats (Hakanson, 1980; Ahmad et al., 2010; Bastami et al., 2012; Wang et al., 2012; Alves et al., 2014; Tavakoly Sany et al., 2014). Due to their biotoxicity, widespread origins, persistence, non-degradability, and subsequent bio-accumulation, heavy metal pollution, particularly in sediments, is one of the greatest risks to the aquatic environment (Forstner and Wittmann, 1983; Barlas et al., 2005; Gargouri et al., 2011; Fu et al., 2014).

Additionally, coastal sediments are important habitats for a wide range of microbiota such as bacteria and archaea that mediate the uptake, stashing, release, and transport of heavy metals and play a pivotal role in the biogeochemical transformations of nutrients or pollutants. (Ul-Hasan et al., 2019; Liu and Yang, 2020; Custodio et al., 2021). Since, the pollution by heavy metals not only jeopardizes the health of the native people and animals, but is also noxious to almost all the microbiomes by interfering with the growth, shape, and metabolism and inhabiting fundamental cellular activities including the synthesis of proteins and the cell membrane integrity (Kandeler et al., 2000; Walker et al., 2000) leading to shifts in structure, abundance, and distribution pattern of sediment microbiota community (Gillan et al., 2005; Deng et al., 2020; Di Cesare et al., 2020). Due to the extreme sensitivity of these communities to environmental disturbances in their natural habitats, the structure and diversity of sediment-associated microbiota communities are increasingly explored.

High-throughput sequencing (HTS) technology is improving in rate, efficiency, and cost (Di Bella et al., 2013). Consequently, it is becoming increasingly prevalent to investigate the entire population of prokaryotes in many habitats. Metagenomic approaches are culture-independent molecular tools used to explore ambient microbiota, including activity, function, diversity, and direct DNA sequencing extracted from the environment (Thies, 2015; Liao et al., 2020; Liu et al., 2020). Currently, using HTS technology to analyze microbiota in heavy metal-contaminated sediments has received more attention (Wang et al., 2010; Li et al., 2020a; Li et al., 2020b). Although the environmental DNA metagenomic approach is used to analyze microbial communities in heavy metal-contaminated sediments, this approach is rarely used in Asia, especially in Taiwan. Therefore, in our study, the microbiota communities in metal-contaminated sediments were analyzed using the eDNA technique for the first time at the Siangshan wetland in Taiwan.

In the current study, we collected the surface and sub-surface sediments from the Siangshan wetland in Hsinchu city of Taiwan. Environmental variables comprising sediment grain size, total organic matter, and heavy metals content such as Fe, Al, Mn, Zn, Cu, Ni, Co, Cr, Ga, In, Cd, and Pb were analyzed. In parallel, the composition and diversity of the microbiota community as well as how the microbiota community structure responds to environmental variables were investigated using high-throughput 16S rRNA gene (V3-V4) next-generation sequencing. The main aims of this work were to: (1) ascertain the sediment composition, total organic matter (TOM) content, and the levels of Fe, Al, Mn, Zn, Cu, Ni, Co, Cr, Ga, In, Cd, and Pb, (2) evaluate the pollution degree of HMs using stander pollution indices such as sediment quality guidelines (SQGs), geo-accumulation index (I_{geo}), enrichment factor (EF), pollution index (PI), and pollution load index (PLI) (Ali et al., 2015; Xu et al., 2017; Salam et al., 2019), (3) profile the diversity and structure of the sediment-associated microbiota in Siangshan wetland, and (4) explore the most important environmental variables that influence the sediment microbiota community structure in Siangshan wetland. This research can help us understand the role of heavy metals, as well as the responses and potential adaptability of microbiota communities to different metal-contaminated habitats. This will upgrade the existing understanding of the ecological risks posed by HMs deposition in the Siangshan wetland and provide a useful benchmark for future metal-microbial interaction investigations.

2 Materials and methods

2.1 Study area and sampling process

The Siangshan wetland stretches west of Hsinchu, Taiwan, from the Ke-Ya stream to the Haishan Fishing Port. The coastline is roughly 8 km and the overall study area covers about 1,600 ha and is a major muddy wetland with a variety of species and biodiversity. Siangshan Wetland was given the official name Hsinchu City Coastal Wildlife Sanctuary in 2001. The muddy intertidal region is a breeding habitat for numerous crabs, shrimp, shellfish, and benthic invertebrates, as well as a variety of threatened bird species (Young et al., 2005; Young, 2009; Young, 2019; Lee, 2020).The wetland's southwest region has vast-scale oyster beds and mangrove forests. Historical pollution events caused by traditional electroplating factories happened from 1970 to 1990. After that, copper contamination in sediment became a major concern because of heavy metal pollution that affected oyster culture and the safety of seafood collected from the west coast of Taiwan (Han and Hung, 1990). After the 1980s, a new era of industrial development happened in Hsinchu city, Hsinchu Science Industrial Park became the top semiconductor manufacturing community in the world contemporary. Except for semiconductor production, this park also included many advanced manufacturers of optoelectronic

plates, biomedical supplies, liquid crystal displayers, light-emitting diodes, etc. A new era of industrial development also brought new kinds of pollution. Based on the manufacturing process of high-tech products, many different kinds of materials are used in large quantities. Even under strict control, there is still a trace amount of contamination present in the final effluent water after the wastewater treatment plant. The consumption of water is more than 200 thousand CMD in this park, and more than 100 thousand CMD of final effluent water from the Hsinchu Science Industrial Park wastewater treatment plant is discharged into the middle course of the Ke-Ya stream. In the Siangshan wetland, the Ke-Ya stream was the major terrigenous freshwater source, more than 40% of freshwater output was effluent water from the treatment plant, about 40% was untreated domestic sewage, and less than 20% was natural water collected in the catchment area. All the freshwater from the Ke-Ya stream estuary flows into the wetland, then all pollutants from industrial use will either settle in the wetland or be carried away by the tides to the Taiwan Strait.

Total forty-four sediment samples were collected from nine main areas at Siangshan wetland, Hsinchu City, Taiwan; Ke-Ya (KY), Ke-Ya Water resource center (KW), Da-Juang (DJ), Hui-Min (HM), Fong-Cin (FC), Hai-Shan (HS), Oyster-Bed (OB), Yen-Kan (YK), Mangrove-Area (MA) in April 2021. Each area was divided into different sites that extended from the shore to the inside (perpendicular to the coastline) and were roughly 400 meters apart (Figure 1). From each site, the sediment samples were collected from the surface (0 cm) and sub-surface (~ 40 cm depth) and we collected four sub-samples (representing one sample), using a sterilized plastic spatula (to avoid cross-contamination), and mixed them thoroughly in individual sterile plastic bags, sealed, and rapidly kept in an icebox till returned to the laboratory. At our laboratory, sediment samples were divided into two groups. One group contained 200 g of each sediment sample for environmental variables assessment (grain size analysis, heavy metals, and organic matters), oven-dried at a temperature of 110°C for four hours to eliminate the moisture content, and kept at room temperature. The remainder was kept at -80°C for microbial DNA extraction and later sequencing analysis. To avoid cross-contamination, hygienic gloves were always used and replaced between collections. Each site was GPS-located (Supplementary Table 1), and all sediment samples were gathered during the low tide period.

2.2 Environmental variables assessment

2.2.1 Grain Size Analysis (GSA) and Total Organic Matter (TOM)

Sediment grain size analysis (GSA) was carried out mechanically utilizing sieve techniques (Folk, 1974). To segregate the particle-size fractions, about 100 g of dry sediments were sieved through a stainless steel mesh. (phi)
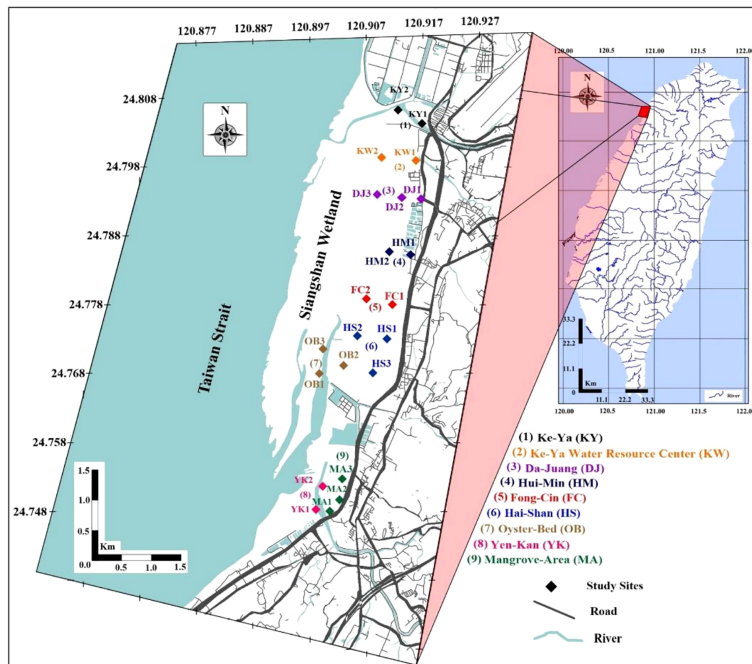
FIGURE 1
Map of the areas of the 22 sampling sites at the Siangshan wetland, Hsinchu, Taiwan.

was utilized to express particle size since the logarithmic scale is more practical than the equimultiple scale. Seven fractions were obtained; gravel ($\Phi_{-1} > 2.00$ mm), very coarse sand ($\Phi_0 > 1$ mm), coarse sand ($\Phi_1 > 0.5$ mm), medium sand ($\Phi_2 > 0.250$ mm), fine sand ($\Phi_3 > 0.125$ mm), very fine sand ($\Phi_4 > 0.063$ mm) and silt or clay ($\Phi_5 < 0.063$ mm). The resulting sediment fractions were divided into three major categories: gravel (Φ_{-1}), sand ($\Phi_0 + \Phi_1 + \Phi_2 + \Phi_3$), and mud ($\Phi_4 + \Phi_5$).

Total organic carbon (TOC) was analyzed *via* potassium dichromate oxidation-colorimetric titration (Peech, 1947; Walkley, 1947; Greweling, 1960). The organic carbon concentrations were then transformed into total organic matter (TOM) by multiplying the results by 1.8. To standardize the potassium dichromate ($Cr_2O_7^{-2}$), a blank titration was performed in the same manner. All reagents used in the analysis were Merck PA.

2.2.2 Determination of heavy metal concentration

Heavy metals (HMs) in 44 sediment samples were measured at National Tsing Hua University following USEPA 3051A method (Element, 2007). Briefly, the samples were pre-sieved (2 mm) to remove shells, roots, and pebbles before being pulverized into a very fine powder (0.063 mm) using a

porcelain mortar and pestle. Then, 0.5 g of the powdered sediment samples were digested with a 12 ml mixture of nitric and hydrochloric acids (3:1) and held inside a closed system, namely a microwave oven (MarsXpress) for 8min 40s on the temperature ramp, the period required to reach 175°C; this temperature was retained for an additional 4min 30s. Following digestion, each extract was diluted into 50 ml of ultrapure water (Millipore Direct-Q System) and then filtered through 40 mm filter paper (ADVANTEC, Japan). Twelve elements; Iron (Fe), Nickel (Ni), Zinc (Zn), Aluminum (Al), Copper (Cu), Manganese (Mn), Cobalt (Co), Chromium (Cr), Gallium (Ga), Indium (In), Cadmium (Cd), and Lead (Pb) were examined utilizing an inductively coupled plasma (ICP-OES). Calibration curves for HMs determination were established using standard 1000 ppm (ICP multi-element standard solution IV, Merck), and samples were analyzed only when the calibration curve's r^2 was greater than 0.999. After primary calibration, it was tested again after analyzing 10 samples; if there was more than 10% variance, the equipment was recalibrated. The recovery percentages for measured heavy metals were between 95.4% and 107.8%. High-purity acids were utilized in the investigation (Merck PA). All equipment was washed and sanitized for 24 hours in a 10% HNO_3 solution before being rinsed with distilled water. Each sample was digested in duplicate, and the findings were represented as mg/kg.

2.3 Sediment quality assessment

2.3.1 Sediment quality guidelines (SQGs)

To more precisely portray the Siangshan wetland's heavy metal pollution, we compared our findings to various sediment quality guidelines. These guidelines were (1) the national standard guidelines of (Taiwan, 2010) and those of (2) the Canadian Council of Ministers of the Environment (CCME, 2002), (3) the National Oceanic and Atmospheric Administration (NOAA) (Long et al., 1995), and (4) the Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand (Anzecc, 2000). Each of the four standard guidelines has lower and upper limits for various heavy metals. When the concentration of metal exceeds the lower limit, adverse effects "rarely or occasionally occur"; whenever the concentration exceeds the upper limit, adverse effects "frequently occur." (Lin et al., 2016).

2.3.2 Potential ecological risk indices

2.3.2.1 Enrichment factor (EF)

The enrichment factor (EF) is a pollution index that is utilized to quantify the level of anthropogenic metal contamination in sediments based on metal enrichments (Sakan et al., 2009) by standardizing the measured metal against a reference metal. The metal values in the samples were primarily standardized using the conservative element. Here, Iron (Fe) was picked as the standardization element (Daskalakis and O'Connor, 1995; Zhang et al., 2007) because it is the fourth most abundant in the earth's crust, has a fine solid surface, it's close to that of many metals and its natural concentration tends to be uniform. According to (Ergin et al., 1991), EF is defined by Equation 1:

$$EF^i = \left(\frac{C_m^i}{Fe_m}\right)_{Sample} / \left(\frac{C_b^i}{Fe_b}\right)_{Shale} \qquad (1)$$

Whereby C_m^i and C_b^i are the ratios between the concentrations of heavy metal i in the sample and its corresponding shale value, respectively, whilst Fe_m and Fe_b refer to the iron concentrations in the sample and its corresponding shale value, respectively. In this study, the background values are the average shale values obtained by Turwkian and Wedepohl (Turekian and Wedepohl, 1961) as follow; Fe: 47200, Al: 80000, Mn: 850, Zn: 95, Cu: 45, Ni: 68, Co: 19, Cr: 90, Ga: 19, In: 0.1, Cd: 0.3, and Pb: 20 mg/kg. Because there is no authorized classification system for pollution degrees based on the enrichment factor approach, a tentative five classes are proposed in Table 1 according to Sutherland (Sutherland, 2000).

2.3.2.2 Geo-accumulation index (I_geo)

The geo-accumulation index is another indicator utilized in the evaluation of HM contamination by excluding the influence of geological contributions (Müller, 1969). I_{geo} can be obtained by Equation 2:

$$Igeo^i = \log_2\left(\frac{C_m^i}{1.5\, C_b^i}\right) \qquad (2)$$

Factor 1.5 is the background matrix correction factor that reduces the geogenic effects (Chen et al., 2007; Zhang et al., 2009). The sediments could be classified into six classes as shown in Table 1.

2.3.2.3 Pollution load index (PLI)

The PI and PLI were used to analyze the studied metals in order to understand heavy metal accumulation in sediments. The pollution index (PI) has been proven to be an effective method for assessing pollution over time, with a lower index value indicating less contamination (Maanan et al., 2015; Tian

TABLE 1 Different pollution indices with their degree.

Class	Enrichment factor (EF)	Pollution degree	Geo-accumulation index (I_geo)	Pollution degree	Pollution index (PI)	Pollution degree	Pollution load index (PLI)	Pollution degree
0	EF < 2	Depletion to minimal enrichment.	$I_{geo} \leq 1$	Unpolluted.	PI < 1	Low contamination.	< 1	Unpolluted.
1	$2 \leq EF < 5$	Moderate enrichment.	$1 < I_{geo} \leq 2$	Very slightly polluted.	$1 < PI \leq 3$	Moderate contamination.	$1 \leq PLI < 2$	Moderately polluted.
2	$5 \leq EF < 20$	Significant enrichment.	$2 < I_{geo} \leq 3$	Slightly polluted.	$3 < PI \leq 6$	Considerable contamination.	$2 \leq PLI < 3$	Strongly polluted.
3	$20 \leq EF < 40$	Very highly enriched.	$3 < I_{geo} \leq 4$	Moderately polluted.	PI > 6	High contamination.	$PLI \geq 3$	Very strongly polluted.
4	EF > 40	Extremely enriched.	$4 < I_{geo} \leq 5$	Heavily polluted.	—	—	—	—
5	—	—	$I_{geo} > 5$	Extremely polluted.	—	—	—	—
	(Sutherland, 2000)		(Förstner et al., 1990)		(Tian et al., 2017)			

et al., 2017). PLI can assess overall pollution in the Siangshan wetland sites under consideration (Tomlinson et al., 1980; Tian et al., 2017). PLI was estimated for 10 elements in the present study after PI_{Cd} and PI_{Pb} were discarded since they were below detection limits (BDL). PI and PLI are calculated by Equations 3 and 4:

$$PI^i = \left. C_m^i \middle/ C_b^i \right.$$ (3)

$$PLI^i = \sqrt[10]{(PI_{Fe} \times PI_{Al} \times PI_{Mn} \times PI_{Zn} \times PI_{Cu} \times PI_{Ni} \times PI_{Co} \times PI_{Cr} \times PI_{Ga} \times PI_{In})}$$ (4)

PI is a single-factor pollution index worth. The PI and PLI are classified into several classes, see Table 1.

2.4 Microbiota analysis

2.4.1 Environmental DNA isolation

We recovered the DNA from all sediment samples within a month of collection in extraction sets of 10 samples at a time. Prior to DNA extraction of each sample, all laboratory instruments and surfaces were disinfected using 70% ethanol, then 10% bleach liquid, and then 70% ethanol again, before a 2 h UV light. Gloves were donned constantly and replaced between each extraction process, and sample handling from individual sites. For all surface and sub-surface samples, DNA extraction, sequence amplification, visualization, and purification were performed at National Tsing Hua University (NTHU, Taiwan). Whilst library preparation and sequencing were performed at Genomics BioSci & Tech. company, Taiwan. Before sample mashing and DNA extractions, sediment samples were defrosted at 4°C for 2 hrs.

Genomic DNA was extracted from the sediment samples utilizing the DNeasy Power Soil Pro-Kit (Qiagen). For each extraction, 250 mg of sediment was treated according to the manufacturer's instructions (https://www.qiagen.com/us/ Resources/ResourceDetail?id=9bb59b74-e493-4aeb-b6c1- f660852e8d97&lang=en). Negative controls were provided with samples to detect the presence of pollutants. Then, the DNA extracts were stored at −20°C until PCR amplification.

2.4.2 PCR amplification, visualization, and purification

The universal prokaryotic primer pair 515F (5′- GTGYCAGCMGCCGCGGTAA-3′) and 805R (5′- GACTACHVGGGTATCTAATCC-3′) were used to amplify the 16S rRNA gene (Caporaso et al., 2011; Herlemann et al., 2011), which identifies 291 bp of the V3-V4 hypervariable region (Fierer et al., 2008; Bates et al., 2011). Previous studies have demonstrated that these primers provide effective phylogenetic information and have minor biases towards any bacterial taxa (Liu et al., 2007; Bates et al., 2011; Bergmann et al., 2011).

Each sample was distinguished by a unique paired barcode or tag (Supplementary Table 2) to enable the multiplexing of PCR products in sequencing libraries (Esling et al., 2015; Steyaert et al., 2020). Each 20 μl PCR reaction contained 1 μl of DNA extract, 1 μl per primer, 10 μl of the 2x O'in 1 DNA Polymerase Premix II w/blue dye, and 7 μl PCR-grade water. Three PCR reactions were run per sample using touchdown temperature (to minimize potential PCR bias) and pooled as described in Supplementary Table 3. PCR negative controls were included in each session to ensure that no contaminations occurred. Following that, amplicons were estimated with the Quant-IT Picogreen dsDNA reagent.

An aliquot (60 μl) of each amplification process was visualized on an agarose gel (1.5%) and ran in a TBE buffer (0.5%) to check the expected size of PCRs yielded amplicons (291pb). Gels were stained with Nucleic Acid Stain (BioKit) and Every gel had a 100 base pair DNA ladder (BioKit).

Thereafter, we cut out the target DNA fragment and purified it with the PCR & Gel Clean-up kit (Enzo Life Sciences Technology Co. USA). Briefly, by melting the DNA fragment in binding DNA buffer (200 μl/100mg) and applying the wash steps by washing buffer (650 μl) and finally eluted in 25 μl of elution buffer. After cleanup, samples were visualized again by electrophoresis and quantified by the PicoGreen kit, and equivalent concentrations were mixed into one single pool for sequencing. A NanoDrop spectrophotometer was utilized to measure the pool's cleanliness and DNA content.

2.4.3 Metagenomic sequencing

Next-generation sequencing generates a tremendous amount of possible identification data, with hundreds of thousands of reads for each sediment sample. The sequencing libraries were produced by applying the Illumina TruSeq® DNA PCR-Free Library Preparation reagent. At the Genomics BioSci & Tech. firm in Taiwan, the purified PCR amplicons were analyzed on the Illumina MiSeq technology utilizing a 2x300 bp paired-end protocol, resulting in paired-end reads that almost entirely overlap. The amplification primers are equipped with MiSeq sequencing adapters and dual-index tags that facilitate PCR product pooling and direct sequencing (multiplexing).

2.5 Bioinformatics analysis

In the present study, we used the open-source program DADA2 version 1.22.0 to quality check, filter, trim, and eliminate chimeras from the Fastaq files (raw demultiplexed sequences) following the online DADA2 workflow Tutorial 1.16 (https://benjjneb.github.io/dada2/tutorial.html) in R platform version 4.1.3 (Callahan et al., 2016). The DADA2 pipeline, which relies on clustering identical reads into distinct sequences known as amplicon sequence variants (ASVs), was

more efficient and produced fewer erroneous sequences than other regularly used pipelines. Using ASVs to examine metabarcoding datasets yields more accurate information about composition and diversity than conventional operation taxonomic unit (OTU) approaches, in which cluster sequencing reads based on a pre-determined threshold of dissimilarity (Callahan et al., 2017; Needham et al., 2017). During our pipeline, the forward and reverse reads produced with next-generation sequencing were trimmed and filtered using the 'filterAndTrim' function. Thereafter, calculate and plot the error rates (machine-learning algorithm problems) for trimmed reads (learnErrors function), and the ASVs were figured out utilizing the dada function (describing DADA2 denoising results) depending on the trimmed reads from all samples. Merge the aligned reads by matching the denoised forward reads with the reverse-complement of the matching denoised reverse reads, to obtain the full denoised reads (mergePairs function), and chimeric sequences were removed using the removeBimeraDenovo function. For the taxonomy assignment, we aligned the resulting list of ASVs from DADA2 with the reference database available at Genomic Taxonomy Database, GTDB (Parks et al., 2022).

2.6 Statistical analysis

ASV abundance was rarified at the lowest sequencing depth (94,861) using 'vegan' package version 2.6.2 (Oksanen et al., 2019) in R version 4.1.3 to reduce the biases resulting from differences in sequencing depth. The rarefaction analysis was plotted employing the 'rarecurve' function in the 'vegan' package, to assess the gain in ASVs richness as we increase the number of reads for each sample. Alpha diversity indices (S_{obs}, Chao1, and Shannon) were computed by the 'estimate richness' function in the 'phyloseq' package (McMurdie and Holmes, 2013) and visualized using the 'ggbetweenstats' function in the 'ggstatsplot' package version 0.9.1 (Patil, 2021). Alpha diversity indices among surface and sub-surface sites were compared using Student or Welch's t-test (Welch, 1947). Moreover, beta diversity was calculated by non-metric dimensional scaling (nMDS) depending on Bray-Curtis distances using the 'plot_ordination' function of the 'vegan' package version 1.38.0 (Oksanen et al., 2019) in R to assess the differences in the microbiota community structure among the surface and sub-surface sediment samples. The analysis of similarity (ANOSIM with 999 iterations) was conducted to test the significance of the dissimilarities in microbial community structure between sediment layers (Clarke, 1993). All alpha and beta diversity indices were calculated from rarified ASVs abundance. The distribution of top taxa (> 1% of all sequences in all sites) was visualized at phylum and class levels in both sediment layers using 'plot_bar' of the 'phyloseq' package in R. Differences were considered significant when P< 0.05. In addition, to visualize the

association between environmental variables and sediment-associated microbiota community structures (most abundant classes > 1%), an interpretive method such as redundancy analysis (RDA) was conducted using CANOCO 4.5 (Lepš and Šmilauer, 2003). For RDA, all variables were log (x + 1) transformed before analysis. Also, the Monte Carlo permutation test (with 999 iterations) was used to identify the factors that had the most influence on microbiota structure. Sediment compositions (GSA), total organic matters (TOM), and pollution indices were visualized using Origin 9.

3 Results

3.1 Granulometric analysis (GSA%) and total organic matters (TOM%)

The collected surface and sub-surface sediment samples at the Siangshan wetland were subjected to GSA, TOM, and heavy metals analyses. According to our results of GSA, sediment compositions were distinguished into seven fractions with different sizes, thereafter we classified them into three main groups (gravel, sand, and mud). Surface and sub-surface sediments of KY1, KW2, HS1, HS2, and YK1 sites were predominated with sand, while HS3, YK2, and MA3 were characterized by muddy sediments at both sediment layers. However, surface sediments of the Oyster beds (OB1, OB2, OB3) were highly abundant with mud (Figure 2, Supplementary Table 4). In contrast, the gravel sediments exhibited limited spatial distribution at all studied sites.

Figure 3 and Supplementary Table 4, illustrate the total organic matter in different layers of Siangshan wetland's sediments. TOM ranged between 10.76% (KY1) and 0.13% (KW2) at the surface sediment, while 4.71% (MA2) and 0.07% (HS2) at the sub-surface layer. Surface sediment of the KY1 site was highly enriched with organic matter (10.76%), and TOM was slightly enriched in surface and sub-surface sediments of the Mangrove areas (MA1, MA2, MA3).

3.2 Distribution of heavy metals in Siangshan wetland sediments

The concentrations of 12 heavy metals were determined in surface and sub-surface sediments and depicted in (Supplementary Tables 5, 6). At surface sediments, the concentrations ranged from 72900 to 12310 for Fe, from 64560 to 7829 for Al, from 917.40 to 184.10 for Mn, from 438.90 to 17.40 for Zn, from 210.70 to 0.10 for Cu, from 74.40 to 3.70 for Ni, from 147.30 to 34.10 for Co, from 123.70 to 24.40 for Cr, from 138 to 41.10 for Ga, from 62.40 to 5.20 for In, and finally, Cd and Pb were below detection limits (BDL). KY1 recorded the highest concentrations for Zn, Cu, and Ni
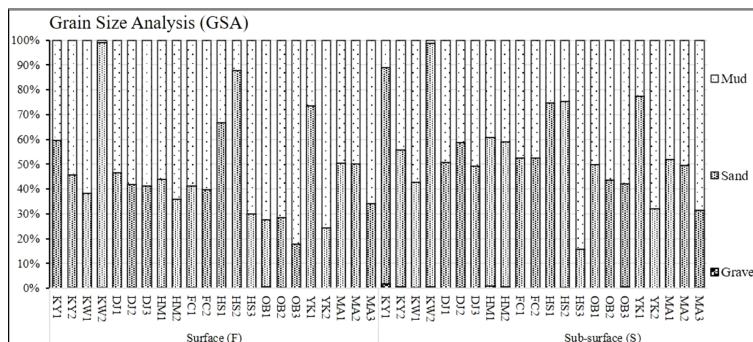
FIGURE 2
Granulometric analysis of surface and sub-surface sediments at Siangshan wetland, Hsinchu, Taiwan.

(438.90, 210.70, and 74.40 mg/kg, respectively), while Fe (72900 mg/kg), Co (147.30 mg/kg), and In (62.40 mg/kg) highly accumulated in MA2. Moreover, DJ1 showed the highest values of Al (64560 mg/kg), Cr (123.70 mg/kg), and Ga (138 mg/kg), and OB3 for Mn (917.40 mg/kg). Inversely, YK1 recorded the lowest concentrations for most metals like Fe, Zn, Cu, Ni, Co, Cr, and In (Supplementary Table 5). Also, HMs concentrations varied between sub-surface sediment sites, they fluctuated between 41040 – 12750 for Fe, 65330 – 12680 for Al, 676.90 – 98.90 for Mn, 196.40 – 19.70 for Zn, 98 – BDL for Cu, 37.30 – 3.80 for Ni, 156 – 38.20 for Co, 122.40 – 27.80 for Cr, 135 – 48.70 for Ga, 57.20 – 2.10 for In, Cd, and Pb were below detection limits (BDL). Zinc (Zn), Copper (Cu), and Nickel (Ni) recorded the maximum concentrations in DJ3, while Iron (Fe), Manganese (Mn), Cobalt (Co), Chromium (Cr), and Indium (In) at DJ1. In addition, Aluminum (Al) and Gallium (Ga) were observed high in MA2. In contrast, YK1 recorded the lowest concentrations for Fe, Zn, Cu, Ni, Co, Cr, and In. Ga observed low in KY1. Cadmium (Cd) and Lead

(Pb) were below the detection limits in all sites (Supplementary Table 6).

3.3 Sediment quality assessment

3.3.1 Sediment quality guidelines (SQGs)

In this study, we compared our data with reference values established in different SQGs as shown in Table 2. The overall concentration of Zn (113.41 mg/kg) at Siangshan wetlands' sediments fell among Taiwan EPA's lower & upper limits and below the other SQGs. Cu (40.18 mg/kg) fell between NOAA ERL & ERM and CCME ISQG & PEL, but less than Taiwan EPA's lower & upper limits, and ANZECC & ARMCANZ's low and high. Ni (24.70 mg/kg) was close to Taiwan EPA's lower limit and fell among NOAA ERL & ERM, and ANZECC & ARMCANZ low and high. Moreover, Cr (80.31 mg/kg) greatly exceeded CCME ISQG and fell among Taiwan EPA's lower and upper limits, but close to ANZECC & ARMCANZ low and lower
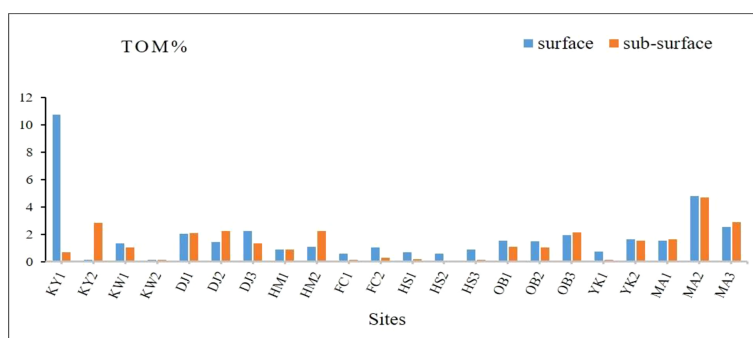

FIGURE 3
Total organic matter percentages of surface and sub-surface sediments at Siangshan wetland, Hsinchu, Taiwan.

TABLE 2   Comparison among the overall average of heavy metal concentrations in the Siangshan wetlands' sediments (current study) and sediment quality guidelines (SQGs) worldwide.

Current study & SQGs	Heavy metal values (mg/kg)												
	Fe	Al	Mn	Zn	Cu	Ni	Co	Cr	Ga	In	Cd	Pb
Current study	30852.95	37036.41	501.18	113.41	40.18	24.70	100.54	80.31	95.65	36.85	BDL	BDL
[a]Taiwan EPA lower limit	–	–	–	14	50	24	–	76	–	–	0.65	48
[a]Taiwan EPA upper limit	–	–	–	384	157	80	–	233	–	–	2.5	161
[b]NOAA ERL	–	–	–	150	34	20.9	–	81	–	–	1.2	46.7
[b]NOAA ERM	–	–	–	410	270	51.6	–	370	–	–	9.6	218
[c]ANZECC & ARMCANZ low	–	–	–	200	65	21	–	80	–	–	1.5	50
[c]ANZECC & ARMCANZ high	–	–	–	410	270	52	–	370	–	–	10	220
[d]CCME ISQG	–	–	–	123	35.7	–	–	37.3	–	–	0.6	35
[d]CCME PEL	–	–	–	315	197	–	–	90	–	–	3.5	91

BDL, below the detection limit.
[a]Taiwan Environmental Protection Agency (Taiwan EPA)'s sediment quality guidelines lower & upper limits (Taiwan, 2010).
[b]National Oceanic and Atmospheric Administration (NOAA)'s effects range-low (ERL) and effects range-median (ERM) (Long et al., 1995).
[c]Australian and New Zealand Environment and Conservation Council (ANZECC) & Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ)'s low and high levels (Anzecc, 2000).
[d]Canadian Council of Ministers of the Environment (CCME)'s interim sediment quality guideline (ISQG) and probable effect level (PEL) (CCME, 2002).
Bold values refer to the overall averages of studied HMs in all sites.

than the others. While Cd and Pb (below detection limit) were observed below all the reference values of different SQGs.

3.3.2 Potential ecological risk indices
3.3.2.1 Enrichment factor (EF)

The calculated heavy metal EFs are shown in Figure 4. Based on EF values, Al, Mn, Ni, Cr, Cd, and Pb in all studied sites showed minimal enrichment (class 0 < 2) (Figures 4A–D), while EF values for the elements viz. Zn, Cu, Co, and Ga ranged between classes 0 and 2 (Figures 4E–H). Interestingly, Indium (In) recorded high EF values > 40 (class 4) at all studied sites (Figure 4I).

3.3.2.2 Geo-accumulation index (I$_{geo}$)

The geo-accumulation index (I$_{geo}$) for the sampling sites is shown in Figure 5. I$_{geo}$ for Fe, Al, Mn, Zn, Cu, Ni, Cr, Cd, and Pb are falling under class 0 (unpolluted) (Figures 5A–G), while Co and Ga are marked as unpolluted to very slightly polluted (class 0, 1) at different sites (Figure 5H–I). Also, all Siangshan sites are extremely contaminated by Indium (class 5 > 5) except the YK2 sub-surface (Figure 5J).

3.3.2.3 Pollution index (PI)

The heavy metal PI is shown in Figure 6. PI values varied most widely among the sediment samples, where PI for Fe, Al, Mn, Ni, Cd, and Pb was less than 1 (class 0), except PI for Fe, Mn, and Ni at the surface sediments of MA2, OB3, and KY1 higher than1 (Figures 6A–C, F). There was low to high contamination of Zn, Cu, Co, Cr, and Ga in the studied sites (Figures 6D, E, G–I). In the same manner, all studied sites were highly polluted with Indium (> 6) (Figure 6J).

3.3.2.4 Pollution load index (PLI)

PLI was computed for the total content of heavy metals at the different sites as shown in Figure 7. High PLI values (class 2, > 2) were found in the surface sediments of KW1, DJ1, DJ2, DJ3, OB2, OB3, YK2, and MA2, but KY1 was subjected to class 3 (very strongly polluted). The remaining surface sediments were recorded as unpolluted or moderately polluted (class 0 and 1). Additionally, most sub-surface sediment sites are falling under class 1 (moderately polluted), except KW2 and YK1 are marked as unpolluted sites (class 0 < 1).

3.4 Overview of high-throughput sequencing (HTS)

The PCR products of the sediment samples were sequenced by the Illumina MiSeq platform and resulted in 6,027,882 paired-end raw reads for 44 samples, out of which 5,496,315 met the quality criteria. The 971 potential chimeras were removed, and 4,075,656 (68% of the total count) non-chimeric high-quality reads were maintained after implementing rigorous quality control procedures (Table 3). The average number of qualified reads per sample was 92,629 (max = 145,870, min = 46,276). In the present study, high-quality reads that were kept after filtering requirements were clustered into 27,872 ASVs for 44 samples with an average of 633 ASVs per sample and were classified into 104 phyla, 227 classes, 573 orders, 1126 families, 2608 genera, and 3314 species. All rarefaction curves slant to reach the saturation plateau, proving that the NGS sequencing depth used in this work was adequate for characterizing microbiota community structure (Supplementary Figure 1).
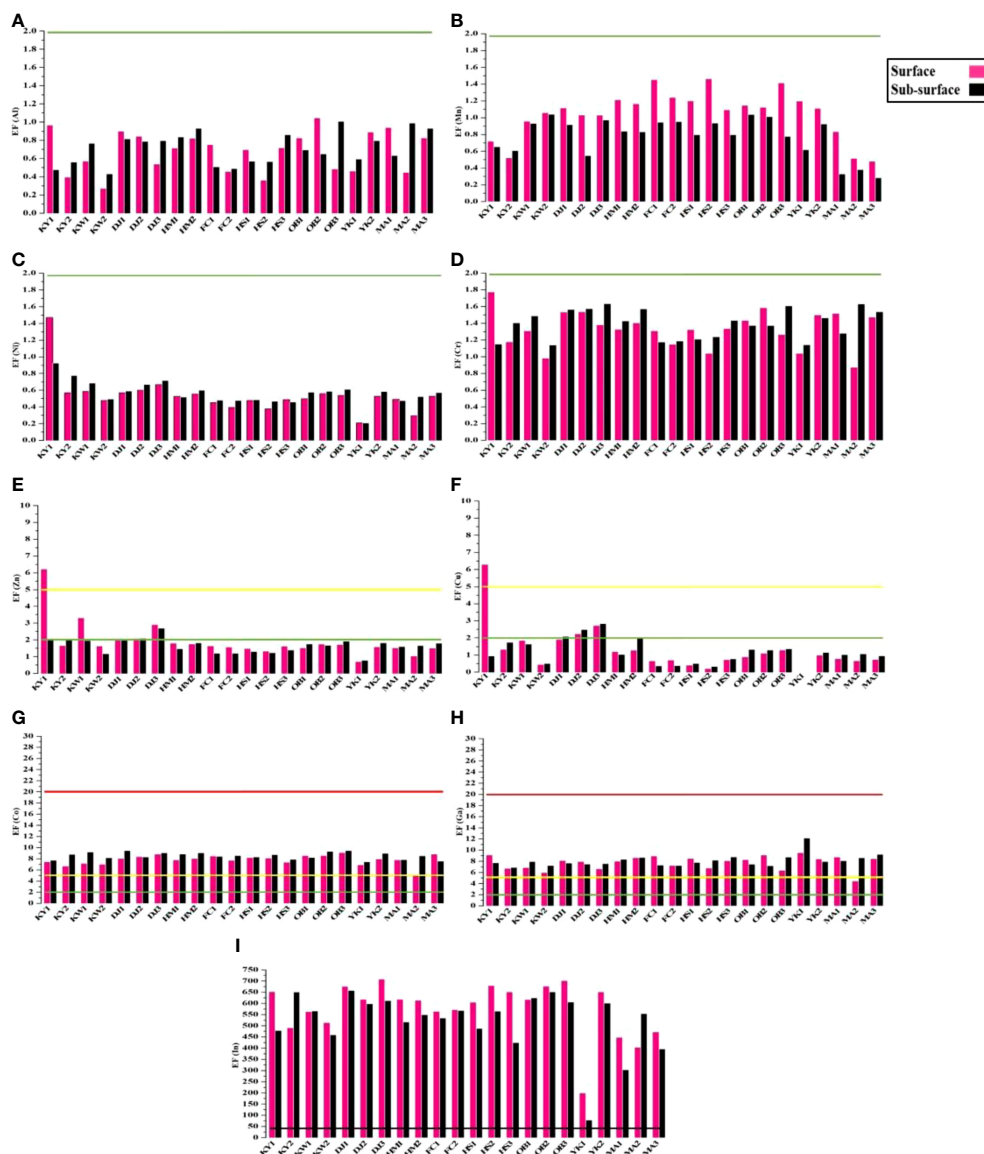
FIGURE 4
The values of Enrichment factor (EF) with pollution degree for studied metals in surface and sub-surface sediments of Siangshan wetland. Green, yellow, red, and black lines refer to classes 0, 1, 2, and 4, respectively (Table 1).

3.5 Microbiota community diversity

To characterize the microbiota community structure in the Siangshan wetland's sediments, we conducted the α-diversity and β-diversity analyses. These analyses were applied for normalized datasets using median sequencing depth (94,861). The observed ASVs (S_{obs}) and Chao1 indices represent microbiota richness, while the Shannon index reflects microbiota diversity. The microbiota communities within the Siangshan sediments at each site recorded relatively similar alpha diversity (Figure 8, Supplementary Table 7). Among the analyzed sediments, the highest richness (S_{obs}) was observed in DJ1.F (2571), followed by MA1.S (2536), DJ2.F (2459), and DJ3.F (2392), while the lowest richness showed in KW1.S (1083), KW2.S (1153), KY2.F (1214) as shown in Figure 8A. Moreover, the Chao1 index has a similar trend as the observed ASVs (Figure 8B). Results of S_{obs} and Chao1 were consistent with tendencies of the rarefaction curve (Supplementary Figure 1). The corresponding Shannon diversity index also displayed a narrow range, from 5.69 to 7.20 (Figure 8C). Overall, this study
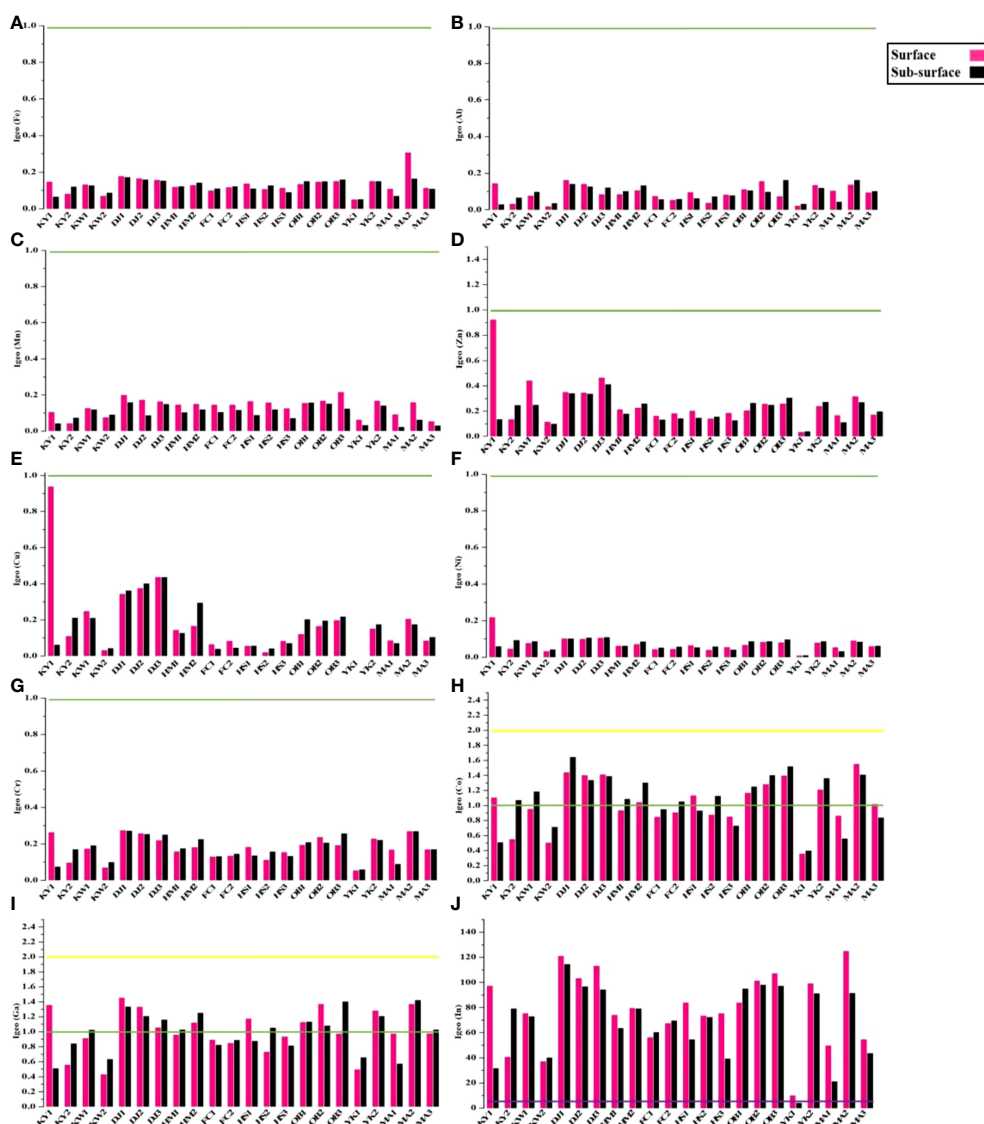
FIGURE 5
Geo-accumulation index (I_{geo}) with pollution degree for studied metals in surface and sub-surface sediments of Siangshan wetland. Green, yellow, and purple lines refer to classes 0, 1, and 5, respectively (Table 1).

observed a minor difference in α-diversity among sites and lacked significance between the surface and sub-surface sediments (Student or Welch's t-test, $p > 0.05$).

In addition, β-diversity was conducted by a non-metric dimensional scale (nMDS) using Bray-Curtis distances to evaluate the dissimilarity between the microbiota communities. The nMDS ordination revealed significant differences in microbiota community composition grouping patterns between surface and sub-surface sites (Figure 9). The microbiota community at surface sites was similar and more clearly separated from the other, except for only 4 samples (KY1.F, KY2.F, MA2.F, and MA3.F). The analysis of similarity

($ANOSIM$) further confirmed that microbial community structure between the surface and sub-surface sediments groups was significantly dissimilar ($ANOSIM$ with 999 iterations, R = 0.2526, p = 0.01).

3.6 Spatial distribution of microbiota community in sediments

In our study, the majority of the high-quality reads (~ 95.05%) were assigned to bacteria while about 4.83% were specified as archaea and 0.13% as unclassified. In total, 104 and 227 microbial
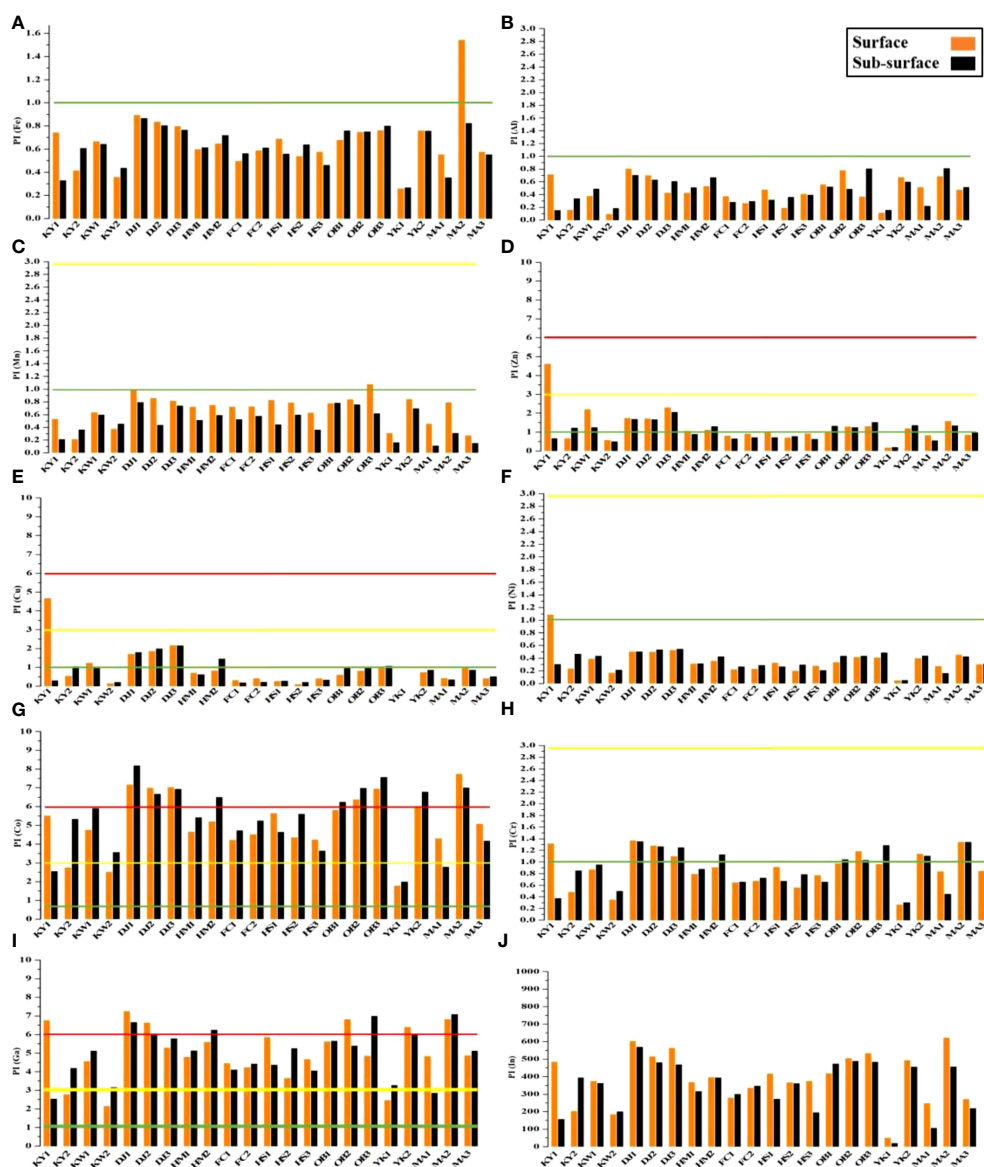
FIGURE 6
Pollution index (PI) with pollution degree for studied metals in surface and sub-surface sediments of Siangshan wetland. Green, yellow, and red lines refer to classes 0, 1, and 2, respectively (Table 1).

phyla and classes, respectively were identified in the sediments. Of these phyla, 13 were considered dominant (> 1% of all sequences in all sites) (Figure 10A; Supplementary Table 8). The most abundant phylum across all sediment sites was *Proteobacteria* (38.02%, 1,460,037 reads). *Bacteroidota* was the second most abundant phylum (7.18%, 275,779 reads), followed by *Desulfobacterota* (6.97%, 267,806 reads), *Actinobacterota* (6.68%, 256,564 reads), *Cyanobacteria* (5.84%, 224,359 reads), *Chloroflexota* (4.18%, 160,703 reads), *Planctomycetota* (4.16%, 159,869 reads), and *Firmicutes* (3.69%, 141,774 reads). Other dominant phyla included *Acidobacteriota*, *Gemmatimonadota*, and *Myxococcota*,

all of which contributed < 5% of overall community composition across all sites. *Asgardarchaeota* and *Halobacteriota*, both archaeal phyla, contributed 4.14% and 1.17%, respectively to microbiota community composition. Among the 18 most abundant classified classes, *Gammaproteobacteria, Alphaproteobacteria, Bacteroidia, Cyanobacteria, Acidimicrobiia, Anaerolineae, Planctomycetes, Lokiarchaeia (Archaea), Desulfobacteria* were the top 10 most abundant classes (exceeding 70% of total sequences) in all sediment sites (Figure 10B; Supplementary Table 8).

Comparing the surface and sub-surface layer sediments in the Siangshan wetland, microbial phyla *Proteobacteria*, 42.31%
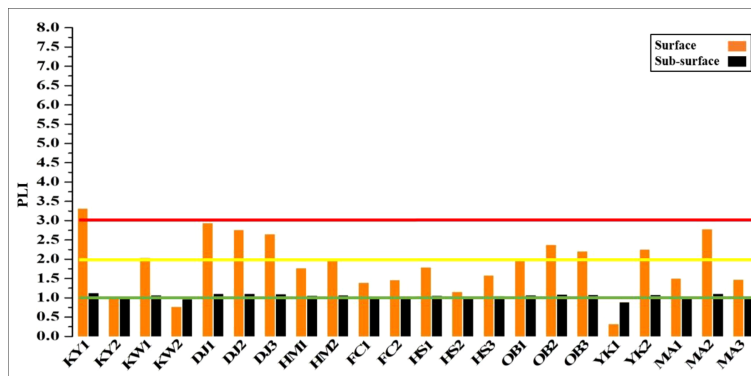
FIGURE 7
Pollution load index (PLI) with pollution degree for different studied sites in surface and sub-surface sediments of Siangshan wetland. Green, yellow, and red lines refer to classes 0, 1, and 2, respectively (Table 1).

(class *Gammaproteobacteria* and class *Alphaproteobacteria*), *Cyanobacteria*, 7.90% (class, *Cyanobacteria*), *Bacteroidota*, 10.12% (class *Bacteroidia*), and *Asgardarchaeota*, 5.52% (class *Lokiarchaeia*) were more abundant in most surface sediments, while *Chloroflexota*, 7.20% (class *Anaerolineae*), *Desulfobacterota*, 9.36% (class *Desulfobacteria* and class *Desulfobulbia*), *Actinobacterota*, 7.12% (class *Acidimicrobiia* and *Actinomycetia*) were more dominant in most sub-surface sediments (Supplementary Figure 2).

3.7 Influences of environmental variables on microbiota community structure

To elucidate the influences of environmental variables including heavy metals, grain particles, and organic matters on sediment-associated microbiota structure, redundancy analysis (RDA) was performed (Figure 11). 14 measured environmental variables and the most dominant microbiota (top 18 classes > 1% of all sequences across all sites) were subjected to RDA. The RDA showed two components of the graph jointly explained 47.9% (axis 1: 36.6% and axis 2: 11.3%) of the total variances of sediment microbiota community structure at the class level and the species-environment correlations were as high as 0.898 for the first axis and 0.801 for the second axis (Supplementary Table 10). Forward selection with the Monte Carlo permutation test revealed that 7 environmental variables including Mn, Cu, Al, Co, Ni, Sand, and Cr significantly influenced the structure of microbiota communities in Siangshan sediment sites ($p < 0.05$). Our results showed that Manganese (Mn) and Copper (Cu) with 14% and 13% contributions to the total explained distribution significantly ($p < 0.05$) were the most important variables that shaped the microbiota community composition. The remaining variables (Al, Co, Ni, Sand, and Cr) had relatively small contributions (Supplementary Table 9).

4 Discussion

In this work, we explored the diversity and structural shifts in sediment-associated prokaryotic communities to elucidate the potential impacts of environmental variables including grain size, organic matter, and HMs in shaping microbiota community structure.

Generally, the Siangshan wetland was characterized by sandy or muddy sediments, but the gravel sediments recorded limited percentages at all studied sites. Based on the results of a grain size measurement, sand granules predominated in KY1, KW2, HS1, HS2, and YK1, whereas mud sediments were most plentiful in KW1, HS3, YK2, and MA3. Furthermore, the OB, DJ, HM, and FC areas' surface sediments contained a high percentage of sand particles > 50%. The flow rate and velocity have a considerable effect on grain size (Kim et al., 2017). In fact, variations in the river channel direction and riverbed slope caused by manmade structures are related to changes in flow velocity and temporary stoppage of flowing streams (Kim et al., 2003b). The content of TOM was different between the sites we looked at in our research, where TOM was observed high percentage in the KY1 site's surface sediments (10.76%). It could be ascribed to the presence of freshwater seepage from the Ke-Ya river, which has a high composition rate of organic elements. Furthermore, MA sites (MA1, MA2, MA3) had relatively significant concentrations at two sediment levels. Mangrove forests are regarded as a very productive ecosystem (Odum and Heald, 1975), with significant rates of organic carbon storage (Huc, 1980).

We then determined the heavy metal concentrations (Fe, Al, Mn, Zn, Cu, Ni, Cr, Co, Ga, In, Cd, and Pb) in Siangshan wetland's sediments, and comprehensively assessed the pollution degree using different indices like; Enrichment factor (EF), Geo-accumulation index (Igeo), Pollution index (PI), and

TABLE 3   Summary of bioinformatics analysis using DADA2 pipeline (raw reads count, filtered read count, merged, high-quality reads, and reads retained percent) at all studied sites.

Sample & ID	Sediment layer	Raw reads	Filtered reads	Merged reads	High-quality reads (Nonchimeric)	reads retained %
KY1.F	Surface	92641	83770	61705	60916	65.8
KY1.S	Sub-surface	89710	80780	55171	55143	61.5
KY2.F	Surface	88855	80305	60764	59599	67.1
KY2.S	Sub-surface	108576	98306	75089	73230	67.4
KW1F	Surface	121454	110787	78847	77781	64
KW1S	Sub-surface	90013	81148	54256	53338	59.3
KW2F	Surface	130216	118778	90402	88007	67.6
KW2S	Sub-surface	108913	98653	71740	68685	63.1
DJ1.F	Surface	189617	173708	134478	131160	69.2
DJ1.S	Sub-surface	172061	154680	114937	113130	65.7
DJ2.F	Surface	184853	169188	133135	130721	70.7
DJ2.S	Sub-surface	163038	146779	110992	107591	66
DJ3.F	Surface	166991	151746	116755	114174	68.4
DJ3.S	Sub-surface	132217	119546	98351	97205	73.5
HM1.F	Surface	133688	122344	96027	93906	70.2
HM1.S	Sub-surface	84507	76190	66200	65740	77.8
HM2.F	Surface	150284	138096	108737	105293	70.1
HM2.S	Sub-surface	154763	138988	101630	99488	64.3
FC1.F	Surface	154097	141470	112264	110239	71.5
FC1.S	Sub-surface	103365	94945	72106	69329	67.1
FC2.F	Surface	148363	136254	105131	102854	69.3
FC2.S	Sub-surface	100569	92110	75990	74744	74.3
HS1.F	Surface	163606	149816	118145	115743	70.7
HS1.S	Sub-surface	116932	107052	82193	79828	68.3
HS2.F	Surface	116806	106790	79705	77935	66.7
HS2.S	Sub-surface	114216	104588	80450	78858	69
HS3.F	Surface	93994	86720	66388	65336	69.5
HS3.S	Sub-surface	74161	67692	46716	46276	62.4
OB1.F	Surface	164709	153385	118719	112657	68.4
OB1.S	Sub-surface	151263	138722	97496	94754	62.6
OB2.F	Surface	169996	157588	120418	115617	68
OB2.S	Sub-surface	154909	141623	96392	94968	61.3
OB3.F	Surface	166351	154034	121126	116623	70.1
OB3.S	Sub-surface	129489	118516	87606	86512	66.8
YK1.F	Surface	158863	146585	110381	106122	66.8
YK1.S	Sub-surface	97673	88870	68519	67301	68.9
YK2.F	Surface	156268	144373	114663	108552	69.5
YK2.S	Sub-surface	92255	84499	63291	59707	64.7
MA1.F	Surface	194660	176239	144650	133873	68.8
MA1.S	Sub-surface	218521	196435	148868	145870	66.8
MA2.F	Surface	152768	138846	105669	103874	68
MA2.S	Sub-surface	195820	176003	130644	128389	65.6
MA3.F	Surface	148640	134400	101995	100594	67.7
MA3.S	Sub-surface	127191	114968	84676	83994	66
Total		**6,027,882**	**5,496,315**	**4,183,417**	**4,075,656**	**68%**

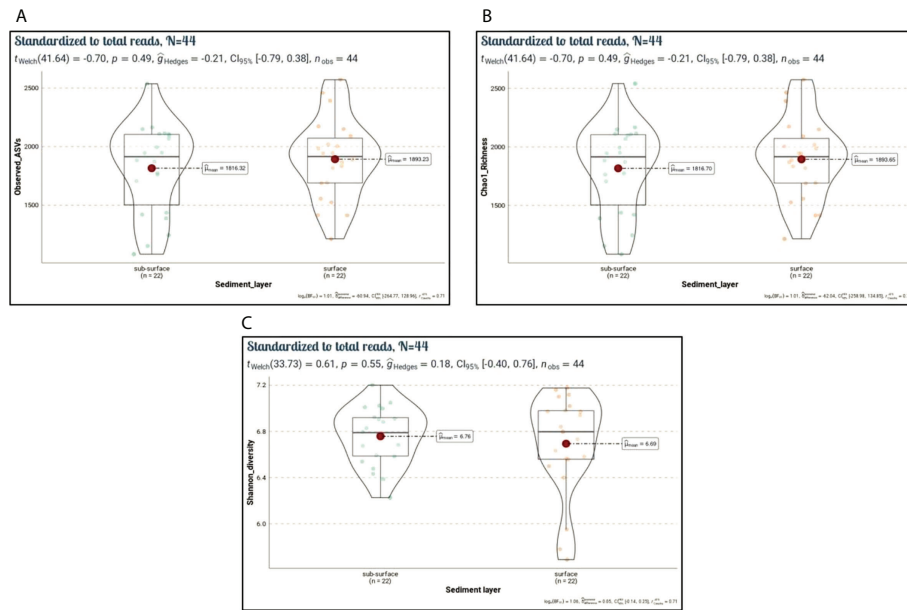F, refer to the surface; S, refer to sub-surface.

FIGURE 8
Alpha diversity indices (S$_{obs}$, Chao1, Shannon). Violin plots showing spatial differences in microbiota diversity among surface and sub-surface sediment sites (n = 44). **(A)** Number of observed species (S$_{obs}$, p = 0.49), **(B)** Chao1 richness (Chao1, p = 0.49), and **(C)** Shannon diversity index (p = 0.55). Statistical differences among surface and sub-surface sites were calculated using *Student or Welch's t-test*.

Pollution load index (PLI). The results indicated that the surface sediments of the KY1 site recorded the highest values of Zn, Cu, and Ni, and the maximum concentrations of Fe, Co, and In at MA2. Moreover, DJ1 showed the highest values of Al, Cr, and

Ga, and OB3 for Mn. Inversely, YK1 recorded the lowest concentrations for most metals; Zn, Cu, Ni, Co, Cr, and In. While the DJ3 site's sub-surface sediment recorded the highest concentrations for Zn, Cu, and Ni, as well as Fe, Mn, Co, Cr, and
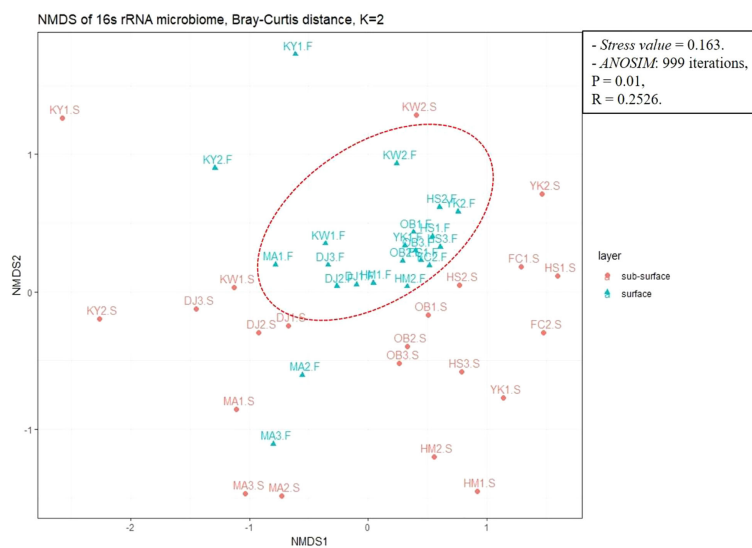

FIGURE 9
Beta diversity analysis showing the dissimilarity or distances between surface and sub-surface sites using non-metric dimensional scaling (nMDS) based on Bray-Curtis distance with stress value < 0.2 (0.163). Analysis of similarity (ANOSIM) was calculated to confirm the significant difference in microbiota community structure between sediment groups (p < 0.05).
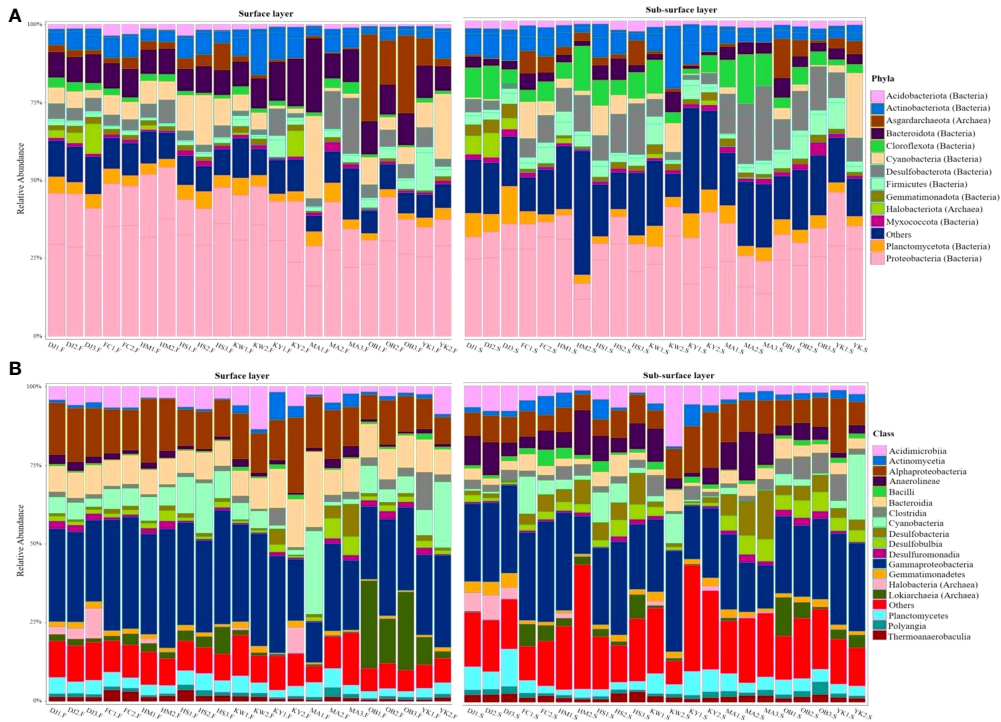
FIGURE 10
Stack bars illustrate the structure or composition of sediment-associated microbiota community with relative abundance > 1% across all sites at phylum **(A)** and class **(B)** levels.
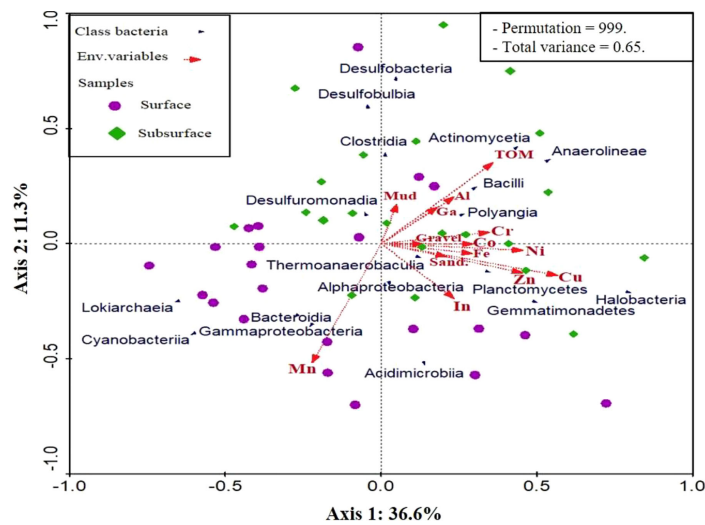

FIGURE 11
Redundancy analysis of the association among environmental variables (red arrows) and microbiota class distribution (blue arrows) across the surface and sub-surface. Statistical significances were determined by the Monte Carlo permutation test (p < 0.05).

In recorded significant values in DJ1; Al and Ga in MA2. On the other hand, the YK1 site recorded the lowest concentrations for Fe, Zn, Cu, Ni, Co, Cr, and In; while Ga was observed at the KY1 site. The accumulation of heavy metals at these sites is due to the presence of a terrigenous freshwater supply, untreated residential sewage, and a large proportion of fine sediments that deposit with high organic matter content, which tend to be associated with heavy metals (Irvine and Birch, 1998; Yu et al., 2012; El-Metwally et al., 2017). Additionally, Siangshan sites are characterized by sandy and muddy sediments, hence the heavy metals were accumulated in high content at these sites due to the vast surface region and the availability of organic materials (Reddy et al., 2004).

In comparison with the previous studies (Supplementary Table 11), the total average concentrations of Fe (30582.95 mg/kg), Al (37036.41 mg/kg), Mn (501.18 mg/kg), and Cr (80.31 mg/kg) in the studied sites were more than those of the previous studies, but they were lower than the average concentration in shale (Turekian and Wedepohl, 1961). Contrary, Cd and Pb (BDL) were less than those in all previous studies, upper limits for natural background concentration in Taiwan (Lai et al., 2018), and average shale value (Turekian and Wedepohl, 1961). The overall mean of Zn (113.41 mg/kg) was close to the concentrations at the Nakdong River in Korea (Kim et al., 2020) and Main River & wetlands in Hsinchu of Taiwan (Ye and Young, 2008), while it lower than the concentrations of (Lai et al., 2018; Ma et al., 2018), but higher than those of other sites and the average shale value (Turekian and Wedepohl, 1961). However, the average concentration of Cu (40.18 mg/kg) was lower than those of Love River (Ma et al., 2018), the upper limit of natural background concentration in Taiwan (Lai et al., 2018), and Shale value, but higher than other previous studies. Additionally, Ni (24.70 mg/kg) was relatively similar to coastal areas of Bohai and Yellow sea, China (Tian et al., 2020), and the results of (Ye and Young, 2008), but lower than Lake Bafa in Turkey (Algül and Beyhan, 2020), Sfax solar saltern in Tunisia (Bahloul et al., 2018), Siangshan wetland, Taiwan (Young, 2019), the upper limit in Taiwan, and Shale value. Furthermore, Co (100.54 mg/kg), Ga (95.65 mg/kg), and In (36.85 mg/kg) were shown more than all the previous studies and average Shale values.

To evaluate sediment quality, we compared the overall average of HMs in sediments to sediment quality guidelines (SQGs). This is the most traditional method (El Nemr et al., 2016) and is particularly successful in assessing HM pollution in sediments (Lin et al., 2016; Duodu et al., 2017). In general, the SQGs have lower and upper limits for the frequency and severity of ecological effects, respectively. The reference values that were implemented by the Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand (Anzecc, 2000) are analogous to the reference values that were established by the National Oceanic and Atmospheric Administration, also

known as NOAA (Long et al., 1995). Those developed by the Canadian Council of Ministers of the Environment (CCME, 2002) and the Taiwan EPA (Taiwan, 2010) are comparable. Generally, the former two guidelines have relatively higher concentrations than the last two, implying that the last two have more rigorous limits for the SQGs strategy. According to our data, the total average of Zn and Ni concentrations surpassed the Taiwan EPA lower limit, NOAA ERL, and ANZECC & ARMCANZ low levels, although Cu exceeded NOAA ERL and CCME ISQG. Furthermore, Cr exceeded the CCME ISQG and Taiwan EPA's low limit. on the other hand, Cd and Pb were below all of the different referenced values. In conclusion, except for Pb and Cd, the sediment averages of heavy metal concentrations in our work surpassed the low reference values in the various SQGs, indicating these metals have infrequent or occasional adverse effects on the aquatic population (Sarkar, 2018).

SQGs have been frequently employed in the evaluation of HMs contamination in sediments, although they do not include the background values. To more precisely assess HMs contamination in sediment, background values (Average Shale Values) of sediments must be considered. Hence, various quantitative indices (EF, I_{geo}, PI, and PLI) have been employed to identify the pollution degree of HMs in sediments. Based on EF values, Al, Mn, Ni, Cr, Cd, and Pb at all studied sites showed minimally enrichment (> 2) suggestive of no or minimal contamination, indicating that they are mostly originating from lithogenic or terrigenous processes. While EF values for Zn, Cu, Co, and Ga have minimal (< 2) to significant pollution (< 20).

The I_{geo} values for elements viz. Fe, Al, Mn, Zn, Cu, Ni, and Cr were marked as unpolluted, while Co and Ga range between class 0 and class 1 (unpolluted to very slightly polluted).

PI values for Fe, Al, and Mn were subjected to class 0 means that the studied sites were unpolluted with these metals, while PI values of Zn, Cu, Ni, Co, and Cr range from low to high polluted in different studied sites. Overall, EF, I_{geo}, and PI of Indium (In) recorded high values in all studied sites, indicating that the Siangshan sediments were highly or extremely polluted with this metal, this may be originating from an anthropogenic effluent like industrial seepage from Hsinchu Science Industrial Park. Gallium (Ga) and Indium (In) are critical transition metals utilized in liquid crystal displays (LCD), photovoltaic cells, semiconductors, light-emitting diodes (LED), and light extraction processes that cause deleterious effects on people (Ram et al., 2015; Rocchetti et al., 2015; Wang et al., 2015a; Lee et al., 2016; White et al., 2017). They are discharged into the intertidal zone during the production process such as epitaxy, cleaning activities, and chip preparation in the manufacturing of high-speed semiconductors and light-emitting diodes (Yang and Chen, 2018). Foregs Geochemical Atlas (2006) reported that Gallium content in stream sediments ranged from 1-36 mg/kg (Atlas, 2006). Also, Indium and Gallium have been discovered in

the groundwater under the semiconductor fabrication areas of northern Taiwan (Chen, 2006; Hsu et al., 2011).

The surface sediments of KW1, DJ1, DJ2, DJ3, OB2, OB3, KY2, and MA2 were strongly polluted with studied metals based on PLI (> 2), while KY1 was very strongly polluted (PLI > 3) and the remaining sites were moderately polluted (PLI < 2). Inversely, most sub-surface sediment sites were moderately polluted with metals (PLI < 2) but KW2, and YK1 were unpolluted sites (PLI < 1).

Microbial communities play a vital role in the management of the marine environment, and the abundance or composition of these communities is largely influenced by environmental variables in the ecosystem. We determined 14 environmental variables influencing the microbiota communities in the Siangshan wetland.

High-throughput analysis of the 16S rRNA gene of microbial communities in all surface and sub-surface sites revealed that *Proteobacteria* (including *Gammaproteobacteria* and *Alphaproteobacteria*) are the most diverse and dominant that have a significant function in sediment decomposition and breakdown (Huang et al., 2019). Despite the studied sites being polluted with heavy metals, *Proteobacteria* were dominated. Compared to other phyla, *Proteobacteria* were highly abundant at the metal-contaminated sites may be attributed to the adaptation of its lower taxonomic groups to environmental changes (Bouskill et al., 2010). Moreover, *Proteobacteria* members are widely known for their capacity to tolerate high levels of metals (Guo et al., 2017; Chen et al., 2018). *Proteobacteria* are reported in both HMs contaminated and uncontaminated regions (Ma et al., 2015; Yan et al., 2016). Previous studies documented the response of *Proteobacteria* to HMs contamination in polluted habitats (Hu et al., 2021; Liu et al., 2021b; Serrana and Watanabe, 2022). *Bacteroidota* (class *Bacteroidia*) and *Cyanobacteria* (class *Cyanobacteria*) were more abundant phyla in surface sediments of MA1.F with relative abundances of 23.91% and 26.40%, respectively. Similarly, *Asgardarchaeota* (class *Lokiarchaeia*) were a more enriched phylum in surface sediments of OB1.F (27.84%) than in sub-surface sediments. Inversely, *Desulfobacterota* (represented in 3 classes *Desulfobacteria*, *Desulfobulbia*, *Desulfuromonadia*) and *Chloroflexota* (including class *Anaerolineae*) were more abundant phyla in sub-surface sediments, especially in MA3.S and MA2.S sites, respectively. Other important phyla observed in this study are, *Actinobacterota*, *Planctomycetota*, *Acidobacteriota*, *Gemmatimonadota*, *Myxococcota*, and *Halobacteriota* (Archaea) as the most abundant. All the abundant microbial phyla obtained in this study have been documented previously in other metal-contaminated environments, such as sediments (Lee et al., 2021; Rajeev et al., 2021; Yi et al., 2021; Custodio et al., 2022), iron tailing pond (Li et al., 2021), mining soils (Cao et al., 2017; Hemmat-Jou et al., 2018; Liu et al., 2021a), paddy soils (Tseng et al., 2021), arid loess region (Zeng et al., 2020), and electric waste soils (Jiang et al.,

2019), this suggests that these phyla are associated with metal-polluted sediment.

Generally, the investigation of microbiota community composition revealed that there was variance among these communities, with ecological considerations responsible for a sizable proportion of the variation. This finding is corroborated by 16S rDNA gene sequencing, which observed that these environmental contaminations are also associated with substantial increases in ASVs within the phyla Proteobacteria, Bacteroidota, Actinobacterota, Chloroflexota, Planctomycetota, Acidobacteriota, and Verrucomicrobia (Beattie et al., 2018).

Human activities may cause shifts in microbiota diversity and structure (Wu et al., 2017). Based on the calculated pollution load index, most of the studied sites are polluted with heavy metals. Of these sites, DJ1.F, MA1.S, DJ2.F, and DJ3.F were moderate to strong polluted with heavy metals. Interestingly, these sites had more richness (S_{obs} and Chao1 indices) and diversity (Shannon diversity index) than other sites. The well-developed adaptation capacity of microbial communities and the effectiveness of metal detoxification by exploiting sequestering and precipitation processes may be the causes of higher richness in polluted sites (Rajeev et al., 2021). A pertinent study found that polluted sites have more biodiversity than unpolluted sites (Tao et al., 2017). Prolonged exposure of microorganisms to heavy metal contamination has different impacts on microbiota diversity, and over time, microorganisms progressively build up resistance to metals (Xu and Tang, 2018). The ability of the microbial community to tolerate contaminants through detoxifying mechanisms may enable it to survive in contaminated environments (Bourceret et al., 2016). Nevertheless, other studies have revealed that polluted regions by HMs had markedly lower levels of diversity and the structure or composition of sedimentary microbiota was shifted (Hemme et al., 2010; Frossard et al., 2017; Huaidong et al., 2017; Feng et al., 2018). Here, statistical analysis revealed there were no discernible variations in the microbial diversity and richness of the surface and sub-surface sediments (P > 0.05).

At the same time, the nMDS ordination exhibited short distances and similarities between the surface sites, except KY1.F, KY2.F, MA2.F, and MA3.F. The results illustrated that the sediment-associated microbiota community compositions are significantly dissimilar among surface and sub-surface sediment groups (ANOSIM with 999 iterations, R = 0.2526, p = 0.01).

RDA analysis was performed to further explore the environmental variables that may contribute to microbial community variances between sediment sites. In the present study, Mn and Cu were identified as the most crucial factors (F = 8.07 and p = 0.001; F = 6.04 and p = 0.002, respectively), followed by Al, Co, Ni, Sand, and Cr influencing the microbial community structure. This is consistent with previous studies in the Monkey Head iron tailing pond (Li et al., 2021), contaminated sediments in India (Rajeev et al., 2021), and the arid loess region

(Zeng et al., 2020). Nevertheless, earlier studies contradicted our findings since heavy metals in aquatic sediment have minimal or no association with the composition of the microbial community (Gillan et al., 2005; Gough et al., 2008). Noteworthy it is critical to clarify that the majority of these works relied on traditional molecular methods like direct cell counts or denaturing gradient gel electrophoresis (DGGE) that primarily identify dominant bacterial communities, as result, have the potential to underestimate or misunderstand the structure of the community (Casamayor et al., 2000). Other studies have shown that the effluences of heavy metals on the abundance, structure, and composition of microbiota communities involve several fundamental mechanisms including (i) disrupting replication processes by destroying DNA and protein (Yan et al., 2020; Coclet et al., 2021) (ii) produce harmful reactive oxygen species by acting as redox catalysts, (iii) disrupting enzymatic processes, and (iv) disturbance of the membrane flexibility and ion regulation (Prabhakaran et al., 2016; Igiri et al., 2018). Indeed, the impacts of heavy metals on the communities of sedimentary microbiota are multifaceted. Initially, only bioavailable metals pose a threat to living things (Kunito et al., 1999). Secondly, the level of exposure determines how heavy metals affect microbial populations. Metals either cause a noticeable shift in microbial population and diversity in a short period of time (1 year) (Bouskill et al., 2010) or the diversity of the community remains steady after around a century of pollution, during which susceptible communities dwindle and the impervious population prevails (Reis et al., 2013).

It is worth noting that the current study only analyzed the structural diversity of the sediment-associated microbiota communities; thus, future research should identify the specific functional genes and associated biochemical functions in the communities of microbiota using PICRUSt or Tax4Fun functional predictions and Biolog analyses.

5 Conclusion

This is the first study that utilized eDNA metagenomic analysis to examine the microbial diversity in sediments of Taiwan's Siangshan wetland. The obtained findings concluded that EF, Igeo, and PI of Indium (In) recorded high values in all studied sites, indicating that the Siangshan sediments were highly or extremely polluted with this metal, this may be originating from an anthropogenic effluent like industrial seepage of Hsinchu Science Industrial Park. A comparison of the metal concentrations with the SQGs of Taiwan, USA, Australia & New Zealand, and Canada revealed that pollution surpassed the low reference values indicating these metals have infrequent or occasional adverse effects on the aquatic population. Moreover, we applied the eDNA metagenomic approach to investigate the structure and composition of microbiota community. Study sites were dominated largely by *Proteobacteria*, followed by *Bacteroidota*, *Desulfobacterota*, *Actinobacterota*, *Cyanobacteria*, *Chloroflexota*, *Planctomycetota*, and *Firmicutes*.

NGS sequencing results and alpha diversity indices demonstrated that the diversity of contaminated sites such as DJ1.F, MA1.S, DJ2.F, and DJ3.F was significantly higher than those of uncontaminated sites. The RDA analysis explored that Mn, Cu, Al, Co, Ni, Sand, and Cr had a sizable effect on the structure of microbiota communities (at the class level). Our findings provide a positive glimpse into the potential of eDNA metagenomic to define sediment-associated microbial communities and further the understanding of community structure and composition.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/- PRJNA836729.

Author contributions

AS-T conceptualized the project, gathered samples, performed methodology, bioinformatics & statistical analyses, and manuscript writing. C-SC participated in the fieldwork, laboratory equipment setup, and sample sequencing. M-YL reviewed and revised the manuscript. S-SY conceptualized the study, sample gathering, review and revision of the article, supervision, and funding. All the authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersign.org/articles/10.3389/fmars.2022.990428/full#supplementary-material

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