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The Origin of the Matter Matters: The Influence of Terrestrial Inputs on Coastal Benthic Microeukaryote Communities Revealed by eDNA

Mathisse Meyneng[1] ⬤ | Raffaele Siano[1] | Naïna Mouras[2] | Dominique Ansquer[2] | Christine Laporte-Magoni[3] | Florence Antypas[2] | Thomas Haize[2] | Hugues Lemonnier[2]

[1]IFREMER, DYNECO, Plouzané, France | [2]French Institute for Research in the Science of the Sea (IFREMER), Research Institute for Development (IRD), University of New Caledonia, University of Reunion, CNRS, UMR 9220 ENTROPIE, Nouméa, New Caledonia | [3]Institute of Exact and Applied Sciences, University of New Caledonia, Nouméa, New Caledonia

Correspondence: Mathisse Meyneng (mathisse.meyneng@ifremer.fr)

ABSTRACT

Estuaries are a key component of the land-sea continuum, and their microbial diversity depends on the connection with terrestrial ecosystems. This work aimed to demonstrate that the terrestrial matter carried by rivers influences the structuration of microeukaryote communities of superficial (0–3 cm) sediments collected at the interface between the land and marine coastal areas. To demonstrate this hypothesis, we have chosen the main island of New Caledonia as a study site, a French overseas territory located in the South West Pacific. Using amplicon sequencing of the 18S-V4 rDNA extracted from sediments, we analyzed microeukaryote community composition in relation to numerous environmental parameters. Samples were collected in five bays influenced by riverine inputs and corresponding to distinct geological features of the watersheds, as revealed by high variations in metal concentrations released from specific minerals in the sediment. Particularly, the influence of ultramafic soils was highlighted by higher nickel concentration (correlated to Co, Cr, Mn, and Fe). Diatoms were the dominant taxonomic group, especially the classes of Bacillariophyceae and Mediophyceae. Then Apicomplexa, Ciliophora, Dinoflagellata, and Cercozoa followed. The metallic composition of the sediment explained 18.46% of the community spatial variability. The selection of ASVs based on their contribution to beta diversity and their correlation with metallic concentrations enabled us to identify spatial patterns. This information could lead to identifying microeukaryote bioindicators of terrestrial influences, particularly of ultramafic origin. We hypothesized that the association between microeukaryotes and metallic compositions is linked to selection processes, given the resistance of some microeukaryotes to some high metallic concentrations. In vitro experiments are needed to confirm this hypothesis. Our results emphasized the role of terrestrial inputs in shaping estuarine diversity and the need to consider the entire land–sea continuum for studying these ecosystems.

1 | Introduction

Estuaries are interface environments connecting terrestrial, riverine, coastal, and oceanic ecosystems. The numerous physical, biological, and chemical processes resulting from these connections create highly dynamic environments (Elliott and Whitfield 2011). In particular, estuaries are influenced by the properties of the upstream watersheds, which,

in turn, vary according to the terrestrial ecosystems that the rivers cross (Xenopoulos et al. 2017). Soil characteristics, vegetation, or human activities can modify the composition and the quantity of the matter transported by rivers, triggered by erosion and run-off processes (McLaughlin et al. 2003; Montgomery et al. 2000).

All along the land–sea continuum, microbial communities change in dependence on the transported matter from the land to the marine system through the estuary (Châtillon et al. 2023; Fagervold et al. 2014). Within the estuarine microbiome, benthic communities living in marine surface substrates are influenced by sediment properties and show variabilities of their structure in both space and time (Crump and Bowen 2024). Although benthic bacteria have received more attention in environmental studies to understand ecosystem functioning and variabilities, microeukaryotes offer several advantages. Their sensitivity to environmental changes, small size, large abundance, rapid response time, and long-time preservation (cyst-forming species) are useful characteristics to study environmental variabilities, supporting the use of these organisms for biomonitoring (Al-Enezi et al. 2022; Pawlowski et al. 2016; Payne 2013; Stoeck et al. 2018; Underwood et al. 2022; Xu and Xu 2017). Studying the diversity changes of benthic microeukaryotes in relation to the organic and inorganic matter transported from land and sedimented at the estuary interface could help understand how the terrestrial ecosystem influences the functioning of coastal ecosystems.

The distribution and composition of benthic microeukaryote communities, along with the drivers shaping their structure, still hold uncertainties. The advances in molecular methods, with the high-throughput sequencing of environmental DNA (eDNA), have provided microbial ecologists with more tools to better assess the diversity of this biological compartment (reviewed in Burki, Sandin, and Jamy 2021). Using this technology, benthic microeukaryotes have been less studied than their pelagic congeners, but it is acknowledged that they show distinct community composition and more species richness (Chen et al. 2017; Forster et al. 2016; Kong et al. 2019; Massana et al. 2015). Microeukaryote distribution can be explained by both stochastic and deterministic processes (Ramond et al. 2023). In the present study, we have focused on deterministic processes, which refer to the abiotic and biotic factors shaping a community by selection and favoring different species′ growth (Stegen et al. 2012; Vellend 2010). The influence of environmental parameters such as depth, salinity, temperature, nutrients, organic matter, sediment grain size, pollutants, oxygen concentration, or metallic compounds on benthic community composition has been highlighted (Chariton et al. 2015; Gong et al. 2015; Pan et al. 2020; Pawlowski et al. 2014; Suzzi et al. 2023; Yang et al. 2023; Zhang et al. 2018; Zhu et al. 2018). Yet, studies on how the matter originated from land can influence microbial benthic communities in coastal ecosystems are quite scarce. Some focusing on bacteria showed that the modification of organic matter sources has an impact on assemblage composition (Fagervold et al. 2014; Zhao et al. 2023). This lack of information is even more important in tropical latitudes, even though the impact of terrigenous components on the ecological functioning of estuaries has been well acknowledged (Bahadori

et al. 2023; Xenopoulos et al. 2017). A strategy to disentangle how the terrestrial matter inputs can influence the diversity of benthic microeukaryote community is to compare sites with different terrestrial landscapes, river catchments, and matter transports while limiting other co-influencing factors such as climate, grain size, or depth.

New Caledonia archipelago is a French overseas territory in the South-West Pacific Ocean that offers this possibility because of its peculiar geological history (detailed in Pelletier 2007). This territory harbors several kinds of soils, including ultramafic soil (1/3 of the surface), showing one of the highest nickel, cobalt, and chrome concentrations worldwide (Kierczak, Pietranik, and Pędziwiatr 2021). Therefore, beyond the typical riverine run-off influences, New Caledonia allows studying the impact of matter rich in numerous metals. The fact that these resources are exploited by important mining activities amplifies the natural erosion and transport of matter toward the marine system and the potential impact of this specific matter on the estuarine ecosystem (Merrot et al. 2022; Sellier et al. 2021). In fact, metallic compounds are transported all along the land–sea continuum and create an in-offshore gradient of concentration and bioavailability across New Caledonian marine ecosystems, including the coral reef lagoon (Ambatsian et al. 1997; Juillot 2019). This lagoon sustains a very wide range of life (Bouchet et al. 2002; Myers et al. 2000; Payri, Richer de Forges, and Colin 2007) and has been inscribed on the UNESCO Heritage List since 2008. The role of those particular inputs is not fully known and needs to be assessed to protect and preserve this biodiversity hot spot.

Taking advantage of the variety of terrestrial landscapes and coastal ecosystems offered by the New Caledonia archipelago, we aimed to better describe if and how variable terrestrial inputs can shape the distribution and composition of benthic microeukaryote communities. One of our hypotheses was that the diversity of those coastal communities reflects the different kinds of organic and inorganic terrigenous matter run-off. To demonstrate this, we have adopted a holistic approach by combining the characteristics of the sediment and its origin with the composition of the benthic communities assessed using microeukaryote DNA. We aimed at disentangling the importance of the organic and inorganic matter, particularly of metallic compounds, in shaping microeukaryote benthic diversity, making the hypothesis that the latter has the most significant influence on the spatial compartmentalization of communities.

2 | Material and Methods

2.1 | Study Area

New Caledonia soils are naturally diverse and present different compositions depending on their geological history (Figure 1). Anthropogenic pressures concern mainly the exploitation of valuable metals (e.g., Nickel) in ultramafic rocks, which supports a significant part of the archipelago's economic activity. About urbanization, two-thirds of the population is concentrated around the city of Nouméa. The rest of the main island has a low population density of 5 people.km^{-2} (Payri
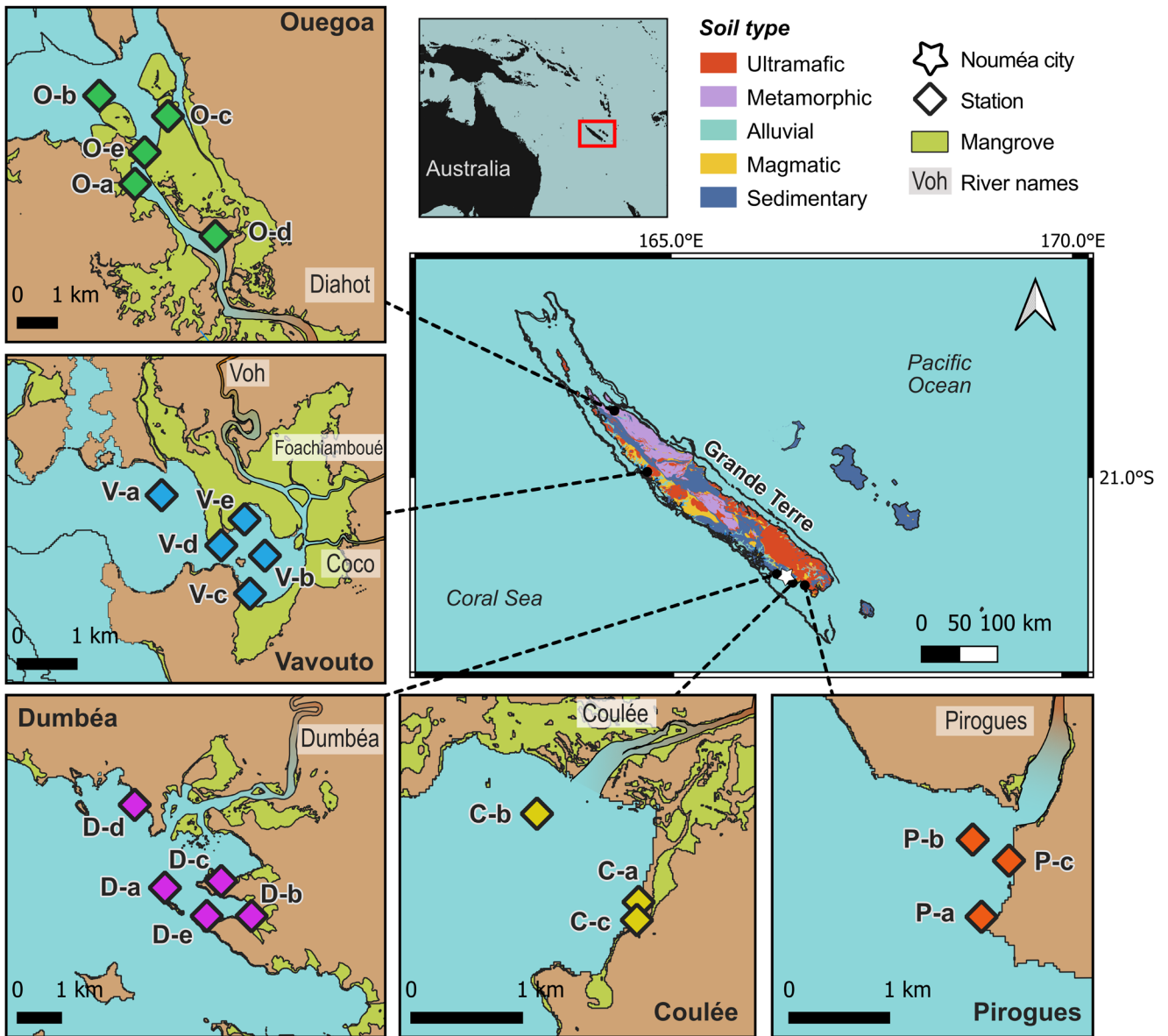
FIGURE 1 | Map of the sampled stations at the five sites associated with different types of soils of the Grande Terre Island of New Caledonia (based on the available shape file "Gouvernement de la Nouvelle-Calédonie/Carte de la géologie de la Nouvelle-Calédonie au 1000000ème, DIMENC/SGNC-BRGM, 2009").

et al. 2019). Agriculture activities are limited, essentially livestock farming on 13% of the land (Bouard, Sourisseau, and Zenou 2020).

On the main island, the Grande Terre (Figure 1), numerous rivers are present, and watersheds are typical of high islands, with small surfaces, steep slopes, and low annual flow rates like in Hawaii, Fiji, or Tahiti (Desclaux et al. 2018). The climate is considered subtropical, with two main seasons: a dry season (June–November), where temperature and precipitation are the lowest, and a rainy season (December–May), where they are the highest. During the latter, tropical depressions and cyclones can induce massive rains, and river inputs rich in suspended matter affect large parts of the lagoon (Terry, Kostaschuk, and Wotling 2008) and the coastal microbial communities (Meyneng et al. 2024).

2.2 | Sampling Strategy

In order to link the origin of terrestrial matter to the diversity of the benthic microeukaryote community, five estuarine ecosystems were sampled all around the Grande Terre. Each site was chosen for its watershed peculiarities (Table 1). Stations within each site were chosen based on similar grain size (Debenay and Dugas 1978; Dugas et al. 1982) characteristics to maintain comparable sedimentary habitats.

In each ecosystem, sampling stations were chosen close to the river mouth, in subtidal and low-depth areas (between 0.2 and 1.5 m) with usual low hydrodynamic. Samples were collected in different field campaigns that took place between March and April 2022. Sediment samples were taken manually at low tide using a 40 cm long and 10 cm wide PVC core rinsed and cleaned

TABLE 1 | Characteristics of each sampling site. The percentage of ultramafic soil was derived from the shape file used in Figure 1 using QGIS 3.32.

Site	River	Watershed surface (km^2)	Ultramafic soil (% of surface watershed(s))	Human pressure
Ouegoa	Diahot	460	0.1	Old mining sites (Cu, Pb, Zn, Ag) that stopped before 1930 (Bird, Dubois, and Iltis 1984).
Vavouto	Voh	164	37	Active mining activity in Koniambo massif since 1998. This site has been studied to assess the high concentration and availability of Fe, Mn, Cr, Ni, and Co (Boula et al. 2022; Merrot et al. 2019).
	Coco	86		
	Foachiamboue	8		
Dumbéa	Dumbéa	219	76	Close to Nouméa City (urbanization). Presence of agriculture activities with poultry, cattle farming, and market gardening
Coulée	Coulée	84	99	Close to Nouméa City. Old mining activities between 1904 and 1981 (Ni) (Bird, Dubois, and Iltis 1984; Fernandez et al. 2006). Presence of poultry farm and market gardening.
Pirogues	Pirogues	148	99	High concentration of Ni, Co, Mn, and Cr (Bird, Dubois, and Iltis 1984). Mining activity since 2016.

with bleach between sampling dates. Three stations were sampled in Coulée and Pirogues, and five in Dumbéa, Vavouto, and Ouegoa (Table S1). Three replicates were done for all stations, for a total of 63 sediment cores.

2.3 | Biogeochemical Analyses

The core's temperature, pH, Redox, and oxygen concentration were measured in situ at 1.5 cm depth using sensors (Fibox 4 DBL ST20-YOP, WTW 3320 pH sentix 81, WTW pH315i sentix ORP, and DP-PSt3-L5-St20-YOP1 Oxygen Dipping Probe, respectively). About 10 mL of pore water was directly extracted from the core at 1.5 cm depth using soil moisture samplers (Rhizon) filtered on 0.2 μm. The pore water salinity was measured with an ATC refractometer (Brix). On the same core, sediments were sampled and conditioned at a suitable temperature depending on the preconized analyses.

Sediment particle size (granulometry) was characterized based on their scattering pattern using laser technology with a Mastersizer hydro 2000S (Malvern Panalytical). Water content, expressed as a percentage of wet weight, was determined based on the difference in the weight between the dry and wet sediment samples. Total metal concentrations were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES) after alkaline fusion, following the normalized EN ISO 14869–2 procedure. Metal concentrations are expressed per gram of dry sediment. Total organic carbon (TOC) was quantified using the Walkey & Black method, using wet oxidation (Ulmer et al. 1992; Walkley and Black 1934). Dissolved organic carbon (DOC) and nitrogen

(DON) concentrations were determined with a TOC-VSCH analyzer (Shimadzu) (Louis et al. 2009). Nutrients were measured only in one sample per station. Nitrite (NO_2^-) and nitrate (NO_3^-), summed as NO_x, were measured by colorimetry after the reduction of NO_3^- and NO_2^- (Wood, Armstrong, and Richards 1967) with an Autoanalyzer SEAL (Oudot and Montel 1988; Raimbault et al. 1990). Silicates ($Si(OH)_4$) and soluble reactive phosphate (SRP) were determined following Hansen and Koroleff (1999). Dissolved sulfide (H_2S) was determined by the methylene blue method (Fonselius, Dyrssen, and Yhlen 2007). Chlorophyll a (Chl a) and pheophytin (Pheo) were measured before and after acidification using a TD-700 fluorimeter and are expressed per gram of wet sediment (Holm-Hansen et al. 1965).

2.4 | Mineralogic Data

The mineralogical composition of pre-crushed sediment was done by X-ray diffraction (XRD) using an AERIS (Malvern Panalytical). XRD analyses of all samples identified 35 different minerals (Table S2). These mineral phases were fully consistent with the geology of each studied site. Geological knowledge allowed us to associate these mineral phases with their initial sources. Some minerals originated from the erosion of parent rocks in the watershed (primary minerals), while others were formed from reactions during sediment transport and deposition (secondary minerals from surface alteration). The primary minerals reflected the geological typology of the area, while secondary minerals resulted from chemical reactions due to changes in physico-chemical conditions. Combining geological knowledge of the different watersheds

(Maurizot and Vendé-Leclerc 2009) with the physicochemical conditions at each sampling site, we identified the most discriminating minerals among the 35 identified to classify the different environments and account for observed geochemical differences. For instance, quartz (SiO_2), present in each sample, was not retained, whereas halite (NaCl), a secondary mineral associated with seawater, was retained as it indicates a transition from terrestrial to marine environments.

Integrating XRD mineralogy with geological knowledge allowed us to narrow the mineralogical information to six key phases: goethite, willemseite, albite, laumontite, glaucophane, and halite (in bold in Table S2). These minerals characterized the geological categories of the studied sites: ultramafic, volcano-sedimentary, and metamorphic. Ultramafic watersheds transport goethite and willemseite from altered peridotite outcrops. Volcano-sedimentary watersheds carry albite fragments resistant to transport. Volcanic components in these rocks alter to laumontite, a zeolite mineral. Glaucophane, characteristic of metamorphic formations in Northwest New Caledonia, is sometimes associated with albite. Halite, a marine salt, indicates evaporite rocks. These six selected mineral phases thus represent the sample groups identified through XRD measurements.

2.5 | Genetic Analyses

2.5.1 | eDNA Extraction and Amplification

Surface sediments (0–3 cm) were sampled with a sterilized syringe, secured in cryofalcon tubes, and immediately frozen in liquid nitrogen on the field. DNA was extracted with a DNeasy PowerMax Soil kit (Qiagen) from 10 g of sediment, following the manufacturer's instructions. The concentration of extracted eDNA was quantified using a Quibit 4 (Thermo Fisher Scientific). Despite some limitations in microeukaryote diversity representation (e.g., for foraminifera), the V4 barcode region of the 18S rDNA was chosen as it is considered to be a good descriptor of the diversity of the microeukaryote community, and since it is more widely represented in the reference sequence databases (Hugerth et al. 2014; Pawlowski et al. 2012). The following set of primer and the sequencing adapter (from GeT-Biopuces platform) was used: V4f-PlaGe (5′ CTT TCC CTA CAC GAC GCT CTT CCG ATC TCC AGC ASC YGC GGT AAT TCC 3′) and V4r-PlaGe (5′ GGA GTT CAG ACG TGT GCT CTT CCG ATC TAC TTT CGT TCT TGA TYR A 3′) (Stoeck et al. 2010). PCR mix was composed of 10 µL Buffer 5×, 1 µL dNTP (10 mmol.L⁻¹), 1.5 µL DMSO 3%, 2 µL forward primer (0.4 µmol.L⁻¹), 2 µL reverse primer (0.4 µmol.L⁻¹), 31 µL water, 0.5 µL Taq Phusion 2 U/µL. Then, 2 µL of sample was added for a final volume of 50 µL. The amplification program consisted in an initial amplification at 98°C for 30s, 12 cycles of 98°C for 10s, 53°C for 30s and 72°C for 30s, then 18 cycles of 98°C for 10s, 48°C for 30s, and 72°C for 30s, with a final extension of 72°C for 10 min. Extraction and amplification blanks were realized to detect eventual contaminations. PCR products were sequenced using a standard kit V3 (2×250 bp) on Illumina MiSeq by the GeT-Biopuces platform (INSA, Toulouse, France). Sequencing data are available at https://doi.org/10.12770/62cde5b9-1888-4b2c-b8a5-a490a696b078.

2.5.2 | Bioinformatic Treatment

The raw data were treated using the open-source pipeline SAMBA (V4, https://github.com/ifremer-bioinformatics/samba) developed by the SeBiMer (Ifremer's Bioinformatics Core Facility). The workflow used DADA2 (Callahan et al. 2016), QIIME2 (Bolyen et al. 2019), dbOTU3 (Olesen, Duvallet, and Alm 2017), Swarm (Mahé et al. 2021), and MicronDecon (McKnight et al. 2019) to infer a high-quality Amplicon Sequence Variants (ASVs) database. The taxonomic assignation was done using the PR^2 reference database (V5.0.0) adapted to microeukaryote sequence annotation (Guillou et al. 2013). Before any taxonomic and statistical filters, a total of 15,988 ASVs were found in the 63 samples. Rarefaction curves were produced to assess sequencing depth quality (Figure S1). In order to focus on microeukaryote microorganisms, ASVs assigned to non-Eukaryote and multicellular taxa (36 and 718 ASVs, respectively) were removed (Metazoa, Streptophyta, Florideophyceae, Ulvophyceae, and Agaricomycetes). Singleton and Doubleton were also removed to avoid sequencing artifacts (Zhan and MacIsaac 2015). We ended up with 6424 ASVs in the final database, with 2,532,263 reads in total and a mean of $40{,}194 \pm 10{,}283$ reads/sample.

2.6 | Statistical Analyses

All statistical analyses were performed on R Statistical Software v. 4.3.3 (R Core Team 2024). In the environmental dataset, 1 data (1 value of pH in D-c3) was missing. Instead of discarding this sample, the missing value was extrapolated using 'kNN()' in the 'VIM' package (Kowarik and Templ 2016). Several measures of metal concentrations went below or above the detection threshold. The threshold values were used in these measures to complete our data set. These measures concerned Si (3 samples in Vavouto and 5 in Ouegoa $> 510{,}000\,\mu g.g^{-1}$), Cu (1 sample $< 11.3\,\mu g.g^{-1}$ in Dumbéa), Pb (5 samples in Vavouto, 5 in Ouegoa and 4 in Dumbéa $< 6.0\,\mu g.g^{-1}$), and Cd (1 sample in Vavouto and 3 in Ouegoa $< 0.4\,\mu g.g^{-1}$). The differences in environmental conditions among sites were assessed using the Kruskall–Wallis test. A choice of metal to consider was made based on Spearman correlation and principal component analyses (using 'PCA()' from 'FactoMineR'; Lê, Josse, and Husson 2008) in order to keep only parameters that best explain the environmental variability among our 63 samples. The number of ASVs evaluated alpha-diversity to describe the microeukaryote community richness. PERMANOVA was computed to assess differences at the ASV level at site and station scales. Total community composition specific to each site and shared among sites were analyzed at different taxonomic levels, from subdivision to genus, considering that the amplicon sequencing method used is limited for robust species distinguishing (Plessis et al. 2023; Strube 2021). CSS (Cumulative Sum Scaling) normalization was done to limit sequencing depth bias in beta-diversity analyses (Paulson et al. 2013). Principal coordinates analyses (PCoA), based on Bray Curtis dissimilarity, were performed on dominant subdivisions to evaluate their variability at a spatial scale. A distance-based redundancy analysis (db-RDA, using 'capscale()' in the 'vegan' package; Oksanen et al. 2022) was done with 16 chosen parameters to understand the link

between environmental parameters and the community distribution. The influence of four groups of measured parameters was assessed with a hierarchical partitioning ('rdacca.hp()' in 'rdacca.hp' package) (Lai et al. 2022). The factor "site" was implemented in this analysis to add a spatial dimension and consider the contribution of non-measured parameters. Species contribution to beta-diversity (SCBD) was calculated using 'beta.div()' in 'adespatial' package (Legendre and De Cáceres 2013). In order to highlight ASVs which both show a high contribution to our beta diversity and interesting link with metals, we used two thresholds: SCBD >0.001 (resulting in 320 ASVs) and a significate correlation (Spearman, p-value <0.05) with at least one metal concentration > |0.50| (among Ca, Mg, Ni, Ti, and Al). This resulted in 92 ASVs (shown in Figure S2), with 25 of them selected due to their interesting patterns and relevant literature.

3 | Results

Our data set was composed of 31 quantitative and 35 qualitative environmental parameters. Some of the quantitative parameters were significantly correlated (Figure S3), so only 16 (in bold in Figure S3) were selected as major factors to discriminate environmental differences among our stations. The selection of metals (e.g., Ni over Fe) was guided by a balance between measurement precision and accuracy in identifying matter origin. Fe, while having lower analytical errors, is ubiquitous, making source attribution difficult. Our five chosen metals (Ca, Mg, Ni, Ti, and Al) well resumed the distribution of our 63 samples when considering all 17 measured metals (Figure S4). The same was done on the 35 qualitative parameters concerning minerals, keeping only six minerals that highlight different influences on the sediment composition (see section 2.4).

3.1 | Sediment Mineralogy and Chemistry

The sediments showed clear variability among our sites (Figure 2). The six chosen minerals allowed a sharp distinction between three groups: Ultramafic sediments of Pirogues/ Coulée/ Dumbéa sites; Volcano-sedimentary dominant sediments of Vavouto; Metamorphic sediments of Ouegoa (Figure 2B). Pirogues, Coulée, and Dumbéa were the only sites showing the presence of exclusive ultramafic-associated minerals (Willemseite and Goethite). Vavouto was characterized by a complex mix of minerals, with a major influence of volcano-sedimentary clasts (Albite and Laumontite) and less ultramafic ones. Finally, Halite was detected in Dumbéa, Vavouto, and Ouegoa sediments but not at Coulée and Pirogues, where it could be under the detection limit of XRD due to the overabundance of ultramafic-associated minerals.

Linked to mineralogical composition, metal concentrations showed clear variations among our five study sites (Figure 2A). First, Ni and Ti seem to have an opposite distribution. At Coulée and Pirogues, the highest mean concentration per site of Ni (5347 ± 945 and $4961 \pm 634\,\mu g.g^{-1}$, respectively) and the lowest mean concentration of Ti (1455 ± 328 and $1482 \pm 126\,\mu g.g^{-1}$) were measured. Conversely, Ouegoa and

Vavouto had lower Ni concentrations (29 ± 6 and $820 \pm 260\,\mu g.g^{-1}$, respectively) and the highest Ti concentration (4592 ± 160 and $4384 \pm 608\,\mu g.g^{-1}$). Dumbéa showed intermediate concentrations of Ni ($2081 \pm 965\,\mu g.g^{-1}$) and Ti ($2471 \pm 770\,\mu g.g^{-1}$). Ouegoa showed the highest concentration of Al ($66,045 \pm 9375\,\mu g.g^{-1}$) compared to the other sites ($54,845 \pm 1718$, $38,741 \pm 844$, $37,697 \pm 11,254$, and $55,419 \pm 4523\,\mu g.g^{-1}$, respectively, in Pirogues, Coulée, Dumbéa, and Vavouto). For some metals, local variability within a site was highlighted. Ca concentration was quite low in general, but some stations within a site showed higher values compared to others of the same site ($20,542 \pm 3157\,\mu g.g^{-1}$ in C-a, $27,518 \pm 1485$ in C-c, $22,759 \pm 2857$ in P-a, $62,133 \pm 11,434$ in D-d and $86,612 \pm 10,431$ in D-e). Other metals showed similar patterns, like Ni-correlated metals: Co (Spearman correlation: 0.99), Cr (0.90), Fe (0.98), and Mn (0.95). Some measured metals showed low concentrations in each site, such as Cd ($< 2.75\,\mu g.g^{-1}$) (all metal concentrations are available in Table S3).

Variabilities among sites were also notable in terms of the significant differences of TOC, DOC, and DON (Kruskal–Wallis, p-value < 0.05) (Figure 2C). Pirogues showed the lowest concentrations with $16 \pm 5.1\,mg.g^{-1}$ of TOC, $3.1 \pm 0.5\,mg.L^{-1}$ of DOC, and $0.7 \pm 0.3\,mg.L^{-1}$ of DON. Conversely, the Ouegoa site showed the highest mean values ($33 \pm 9\,mg.g^{-1}$ of TOC, $10 \pm 8.7\,mg.L^{-1}$ of DOC, and $2.6 \pm 2.4\,mg.L^{-1}$ of DON), specifically driven by station d, which showed higher values than the other stations within this site ($48 \pm 2\,mg.g^{-1}$ of TOC, $27 \pm 5\,mg.L^{-1}$ of DOC, and $7 \pm 1\,mg.L^{-1}$ of DON).

3.2 | Physio-Chemical Environment Description

Mean temperatures in the water column ranged between 27.4 ± 1.1 (Coulée) and $31.0°C \pm 0.45°C$ (Vavouto). The majority of physicochemical parameters (Table 2) showed comparable values among our five sites with some exceptions (e.g., Redox and SRP). According to our measurement, water content, oxygen concentration, NO_x, SiO_2, and H_2S did not show significant differences (Kruskal–Wallis, p-value > 0.05). The overall sediment grain size was mainly characterized by silt ($57 \pm 13\%$; p-value > 0.01) (Table 2). Among sites, the proportion of Clay, Fine sand, and Sand was more variable, especially at Ouegoa. According to the texture soil classification (United States Department of Agriculture 1987) (Figure S5A), "silt loam," "silty clay loam," and "sandy loam" textures accounted for 57%, 19%, and 14% of the samples, respectively (Figure S5B). The texture loam and clay loam represented each 5% of the samples.

3.3 | Microeukaryote Community Diversity

The sediment microeukaryote community accounted for a total of 6424 ASVs after data filtering (see section 2.5.2). Coulée and Pirogues showed the highest alpha diversity, with 883 ± 157 and 870 ± 158 ASVs/sample, compared to Dumbéa, Ouegoa, and Vavouto, with 618 ± 107, 605 ± 104 and 598 ± 84 ASVs/sample, respectively (Figure 3A). As a proxy of photo-autotrophic microorganism biomass, Chl a values were also clearly higher in Pirogues $2.5 \pm 1.1\,mg.g^{-1}$ (Figure 3B). The other sites showed a similar mean concentration of Chl a around $1.0\,mg.g^{-1}$.
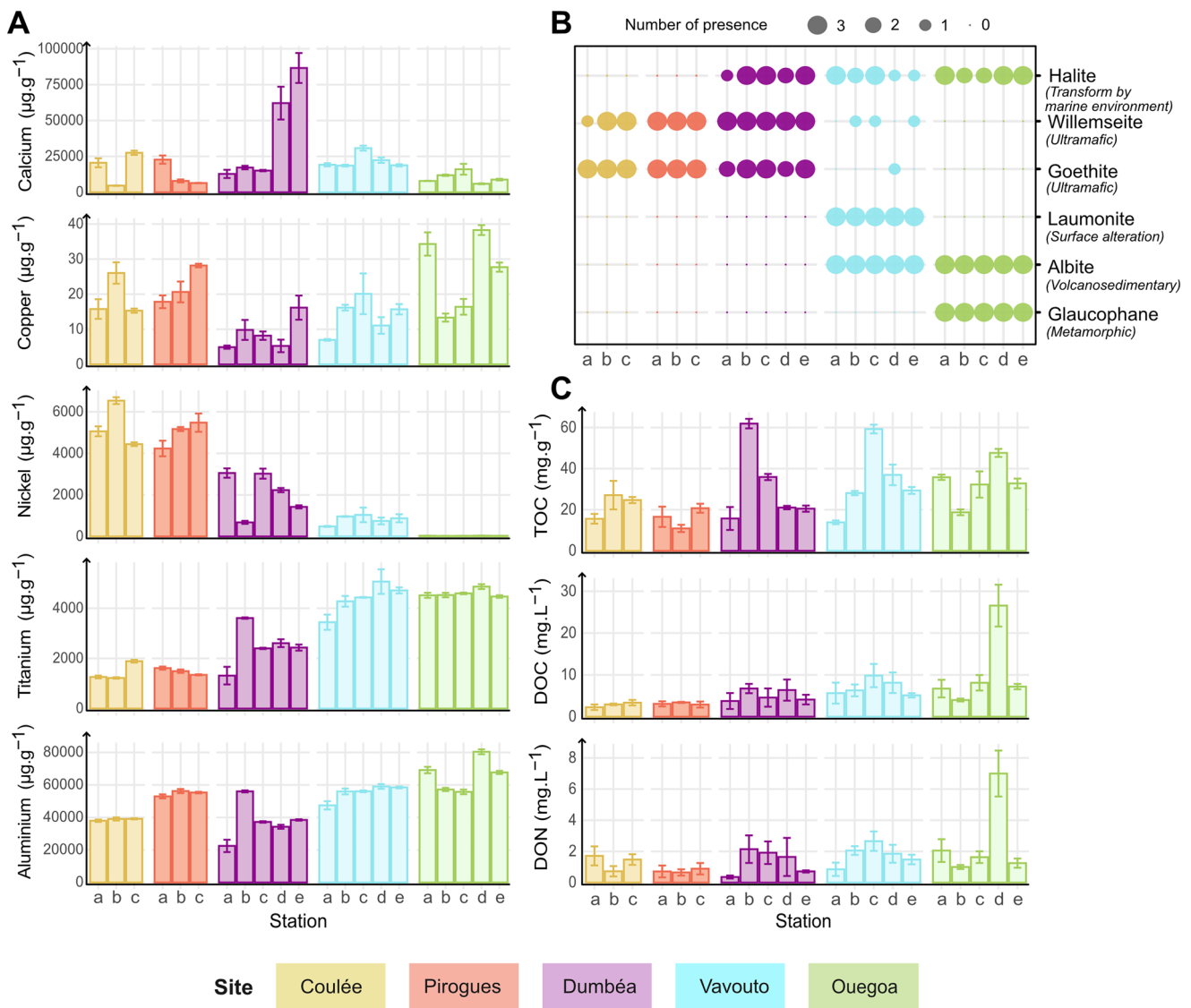
FIGURE 2 | (A) Mean concentrations of five selected metals, (B) presence of 6 selected minerals (the size of the dots represents the number of detections of the mineral per station, between 0 [total absence] and 3 [present in each replicate]) and (C) mean organic carbon and nitrogen concentrations among the 21 stations (DOC, Dissolved Organic Carbon; DON, Dissolved Organic Nitrogen; TOC, Total Organic Carbon).

Within the total eukaryotic community observed with our sequencing strategy (V4-18S amplicon sequencing), before taxonomic ASV filtering to target microeukaryotes, metazoans were dominant in some samples (Figure S6) showing a mean relative abundance of 39% ± 13%. Terrestrial plants (e.g., *Montrouziera* sp.), multicellular algae (e.g., Ulvophyceae), and terrestrial Fungi (e.g., *Phallus* sp.) were also recorded before their removal for the final analysis. Among microeukaryotes, the community was highly dominated by Gyrista (58 ± 10%). Generally, diatom sequences constituted on average over 97% of the Gyrista sequences and two diatom classes, Bacillariophyceae (35 ± 15%) and Mediophyceae (21 ± 16%), dominated (Figure 3D). Other ecologically distinct classes of Gyrista were present but at lower relative abundances (e.g., *Developea* or *Pirsoniales*; Figure S7). Therefore, to highlight the ecological perspective of this result, the term "diatoms" will be used throughout the rest of the manuscript instead of Gyrista. The observed variability in diatom community composition,

both at ASV (Figure S8) and genus level (Figure S9), was of significant importance. This variability was evident across the site and among the stations within a site. The other major subdivisions were in order of relative importance: Apicomplexa (9.7 ± 8.3%), Cercozoa (7.6 ± 4.4%), Dinoflagellata (5.5 ± 2.5%), Ciliophora (4.3 ± 2.3%), Chlorophyta (1.9 ± 2.1%), and Fungi (1.3 ± 1.1%). The taxa diversity of these groups, along with the diatoms, showed inter- and intra-site variabilities at ASV and genus levels as detailed in Figures S8–S15 and depended on the subdivision analyzed (Figure S8). The variability of the microeukaryote community at the ASV level was confirmed by a PERMANOVA ($p < 0.001$).

Across all samples, 285 ASVs were found at every site (Figure 3C), showing the existence of a potential core community for benthic microeukaryotes. They accounted for a mean relative abundance of 40% ± 9.5%, with 87% of those ASVs that can be considered as abundant (mean relative abundance > 0.01% among all samples,

TABLE 2 | Mean values of physicochemical parameters in sediment samples of each site.

		Coulée	Pirogues	Dumbéa	Vavouto	Ouegoa
	Salinity (pw)	35 ± 3	30 ± 4	35 ± 1	32 ± 3	29 ± 4
	pH	7.58 ± 0.10	7.58 ± 0.21	7.51 ± 0.14	7.41 ± 0.15	7.45 ± 0.14
	Redox (mV)	-122 ± 114	-22 ± 80	-45 ± 29	-95 ± 54	-73 ± 37
	Oxygen (mg.L^{-1})	0.8 ± 1.2	0.1 ± 0.1	0.2 ± 0.3	1.0 ± 1.2	0.2 ± 0.2
	Chl a (mg.g^{-1})	0.9 ± 0.5	2.5 ± 1.1	1.1 ± 0.5	0.7 ± 0.3	0.9 ± 0.4
	Pheo (mg.g^{-1})	0.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.2	0.5 ± 0.2	0.6 ± 0.3
	Water content (%)	37 ± 4.1	34 ± 5.5	40 ± 15	40 ± 13	42 ± 12
Grain size	Sand 125–2000 μm (%)	32 ± 20	12 ± 11	18 ± 18	26 ± 11	30 ± 18
	Fine Sand 63–125 μm (%)	20 ± 9.9	8.2 ± 4.2	12 ± 7.4	13 ± 5.6	21 ± 7.1
	Silt 3.9–63 μm (%)	44 ± 11	57 ± 7.7	58 ± 14	58 ± 8.3	61 ± 15
	Clay 0–3.9 μm (%)	24 ± 16	31 ± 6.3	24 ± 7.5	17 ± 4.1	8.3 ± 3.4
Nutrients	NO$_x$ (μmol.L^{-1})*	0.20 ± 0.19	0.13 ± 0.14	0.09 ± 0.06	0.28 ± 0.22	0.04 ± 0.06
	SRP (μmol.L^{-1})*	0.06 ± 0.01	0.07 ± 0.03	0.5 ± 0.3	1.3 ± 0.8	7.7 ± 8.5
	SiO$_2$ (μmol.L^{-1})*	109 ± 18	138 ± 31	168 ± 54	221 ± 83	254 ± 123
	H$_2$S (μmol.L^{-1})*	0.3 ± 0.1	0.8 ± 1.0	0.5 ± 0.4	4.0 ± 8.2	1.8 ± 0.4

Note: parameters with * were measured in one replicate per station.
Abbreviation: pw: Pore water.

Logares, Mangot, and Massana 2015). Among those 285 ASVs, diatoms dominated ($30 \pm 9.4\%$) (Figure S16). Yet, each site showed specific ASVs (Figure 2C), with Pirogues and Coulée showing the highest number of specific ASVs (765 and 527 ASVs, respectively) despite fewer stations sampled at these sites. Again, site-specific ASVs were dominated by diatoms but also by Cercozoa, especially in Coulée and Pirogues (Figure S16).

3.4 | Microeukaryote Community Variability in Relation to Environmental Characteristics

Sample distribution based on microeukaryote community composition suggested a spatial ASV diversity differentiation among our different sites (Figure 4A), with some ASVs contributing highly to the beta-diversity observed (Figure 5). The representation of our 63 samples was well explained on our two first axes (29.9%), with a third dimension explaining 8.3% of the variance (Figure S17). In addition to the distinction among sites, an intra-site variability was also visible. Pirogues showed more homogeneity in community composition, whereas Vavouto and Ouegoa showed one distinctive station (V-a and O-b, respectively), with the three sample replicates separated from the others. Community differentiation was variable depending on the taxonomic group. For example, the composition of Ciliophora was more distinct in Pirogues, whereas the one of the Chlorophyta was more distinct in the Vavouto site (Figure S8).

Groups of environmental variables (metals, organic matter, grain size, and physicochemical parameters) showed different degrees of influence on this community distribution (Figure 4B). All our parameters explained 63% of the microeukaryote community composition. Five chosen metal concentrations explained 18.46%, followed by grain size (8.19%), physicochemical (7.33%), and OM parameters (5.4%). As expected, the highest individual effect was the site, with 23.47%.

A separation was visible between Pirogues/Coulée and Ouegoa/Vavouto sites at the ASV level, illustrated by their non-overlapping distribution of samples of these stations along the first axis of Figure 4A. Ni and Ti concentrations contributed the most on this axis, which explained 19.8% of community distribution. This was also visible in the first clustering of Figure 5, where two groups of ASVs were separated based on their higher abundances in relation to high concentrations of Ni, found in Coulée and Pirogues, and Ti, found in Vavouto and Ouegoa. Abundant ASVs associated to Ti were assigned to *Cyclotella* sp. ($5.0 \pm 2.0\%$ and $22\% \pm 11\%$, respectively, in Vavouto and Ouegoa), two Gregarinomorphea ($1.5 \pm 1.6\%$ in Vavouto for the first and $3.7 \pm 3.1\%$ in Ouegoa for the second), *Skeletonema* sp. ($3.0 \pm 1.1\%$ in Ouegoa), *Tetraselmis* sp. ($1.6 \pm 1.1\%$ in Vavouto), *Marinomyxa* sp. ($0.4 \pm 0.8\%$ in Ouegoa), and *Picochlorum* sp. ($1.5 \pm 0.8\%$ in Ouegoa). On the other group, positively correlated to Ni, several genera of diatoms were found abundant in Coulée and Pirogues, with *Pleurosigma* sp. ($0.7 \pm 1.2\%$ in Pirogues), *Nitzschia* sp. ($0.5 \pm 0.8\%$ in Coulée and $1.0 \pm 0.7\%$ in Pirogues), *Navicula* sp. ($1.2 \pm 1.5\%$ in Pirogues), *Seminavis* sp. ($2.4 \pm 2.9\%$ in Coulée and $2.6 \pm 1.9\%$ in Pirogues), *Tryblionella* sp. ($1.5 \pm 0.7\%$ in Pirogues), and *Diploneis* sp. ($1.1 \pm 0.8\%$ in Coulée), but also a member of Ciliophora with *Pseudocyclidium* sp. ($0.5 \pm 0.7\%$ in Pirogues) and an unassigned Fungi ($0.7 \pm 1.0\%$ in Pirogues). The second axis of Figure 4A, driven by concentrations of Cu and Ca, explained 11.1% of the distribution and the
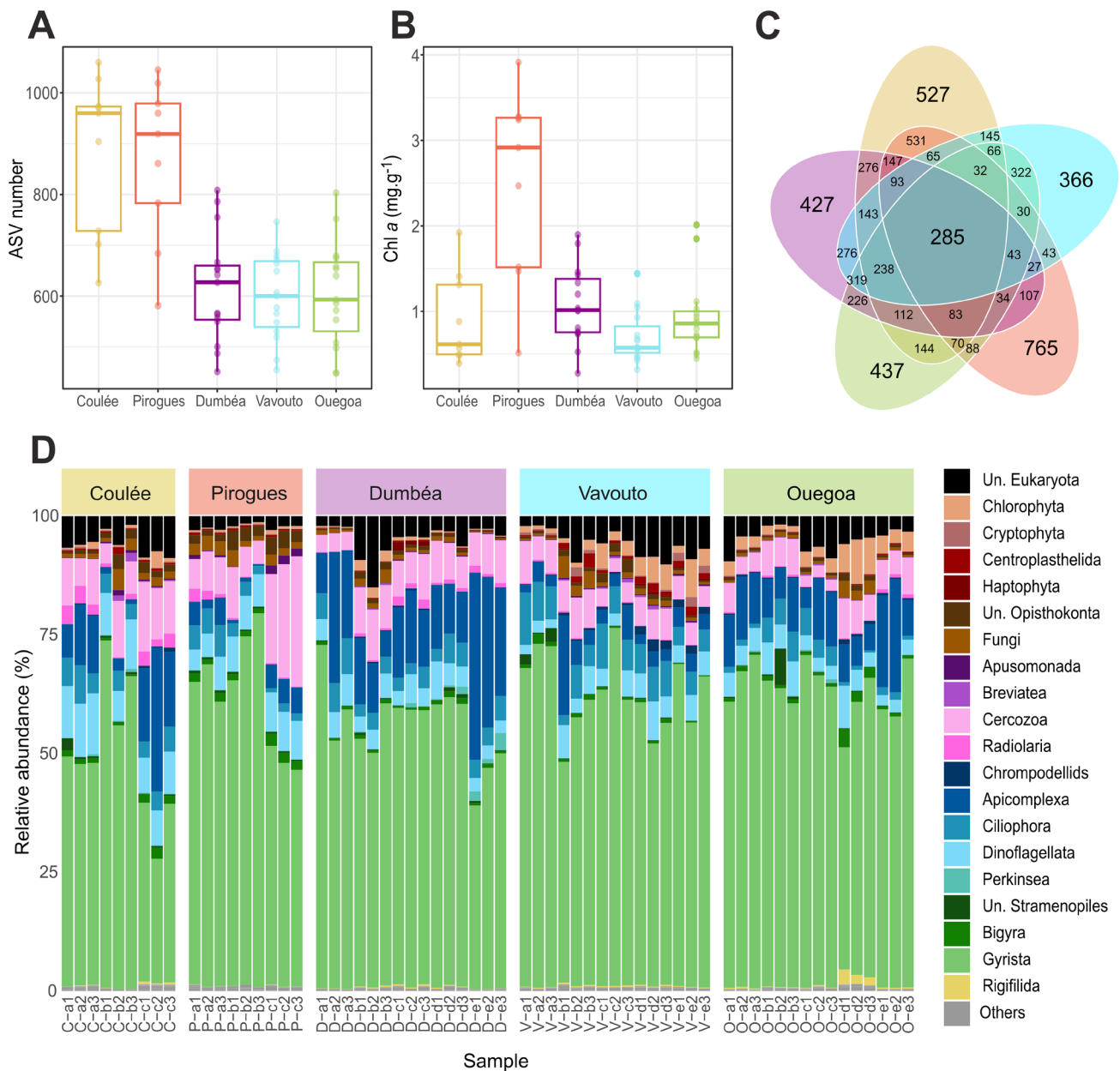
FIGURE 3 | Microeukaryote community description, including (A) the ASV number per site, (B) Chlorophyll *a* concentrations per site, (C) the Venn diagram of specific and common ASVs among sites, and (D) the taxonomic composition at the Subdivision level per sample of all sites ("Others" category gathered subdivision that did not exceed 1% in any sample).

separation of Dumbéa stations. A small group gathering 4 ASVs were abundant in Dumbéa and assigned to: Eugregarinorida (0.5 ± 0.7%), *Navicula* sp. (1.0 ± 0.6%) and two Cercozoa with op14-lineage (0.4 ± 0.4%), and *Thalassomyxa* sp. (0.5 ± 0.4%), showing higher positive correlation with Ca.

4 | Discussion

4.1 | Estuarine Benthic Microeukaryote Community Diversity

This work describes the benthic microeukaryote diversity at a regional scale in New Caledonia using an eDNA amplicon sequencing approach. Based on the ASV number, we found a relatively higher and distinct diversity compared to surface waters of the same territory (Meyneng et al. 2024), a difference also shown at other latitudes in various ecosystems (Forster et al. 2016; Kong et al. 2019; Massana et al. 2015; Tee et al. 2021). The species diversity of our community could be enhanced by the coastal influence, an impact known and referred to as "coastal heterogeneity" (Xu, Yang, and Xu 2017; Zinger et al. 2011), also visible with different taxonomic composition compared to deep-sea sediment (Cordier et al. 2022; Wu and Huang 2019). The sedimentary habitat could harbor a high number of species acting as a reservoir of microorganisms. This reservoir would play a crucial role in the benthic-pelagic coupling, being a species inoculum source for the water column (Forster et al. 2016; Marcus and Boero 1998), especially in coastal shallow water where physical processes easily remobilize sediment (Hochard
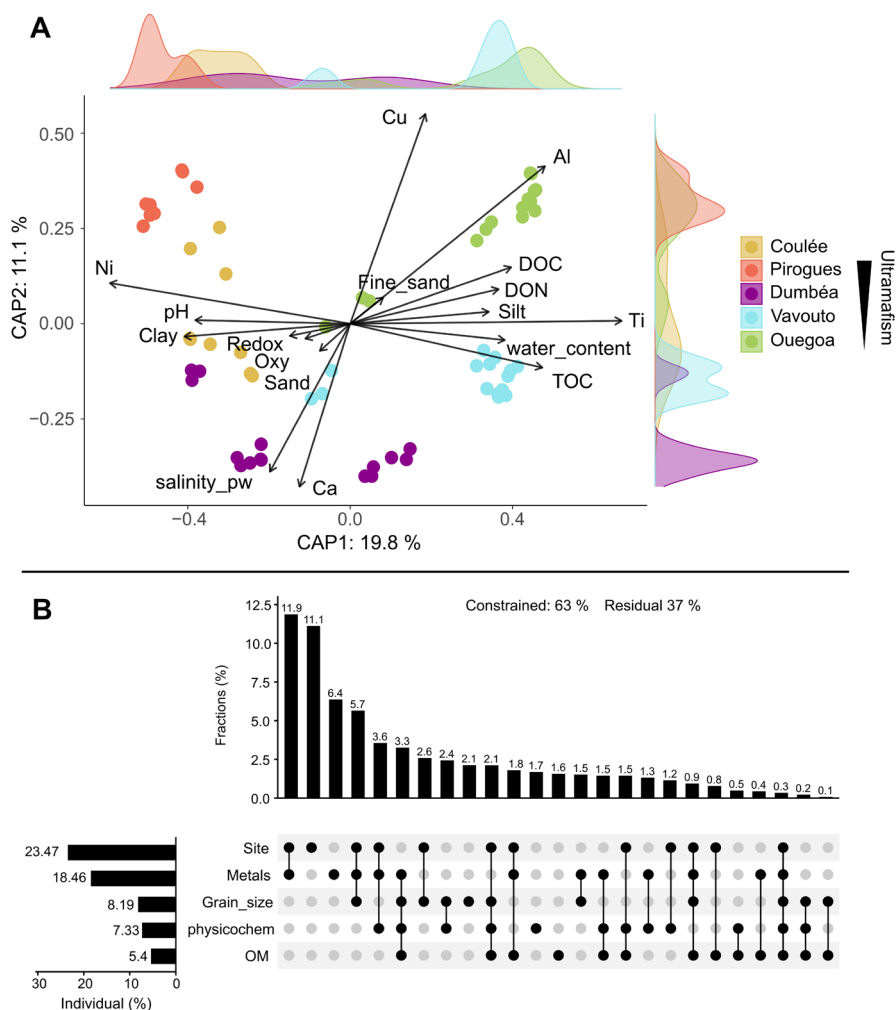
FIGURE 4 | (A) Distance-based redundancy analysis based on microeukaryote distribution calculated with Bray–Curtis dissimilarity constrained with 16 environmental parameters (salinity_pw: Pore water salinity). Density plots represent the overlap of samples among sites on each ax. (B) Hierarchical influences of these different groups of parameters on this distribution (OM, Organic matter; physicochem, Physicochemical parameters).

et al. 2012; Ouillon et al. 2010) and provoke frequent exchanges between the sediment and the water realms.

A core microeukaryote community composed of 285 ASVs was identified based on their presence at each studied site (Figure 3C). This result suggests a potential core diversity similar to that Pringault et al. (2008) proposed for bacteria in sediments. Yet, this result should be validated considering a larger spatial scale sampling and the temporal variability of microeukaryote, as some taxa could be occasionally abundant/present or have seasonal patterns (Lynch and Neufeld 2015; Neu, Allen, and Roy 2021; Salonen et al. 2019).

The high concentrations of Chl *a* in our sediment samples suggest a high proportion of photo-autotrophic taxa biomass (Bourgeois, Hochard, and Pringault 2010; Hochard et al. 2012). Amplicon sequencing data showed an overall dominance of diatoms, apicomplexans, ciliates, dinoflagellates, and cercozoans similar to other coastal ecosystems (Kalu, Reyes-Prieto, and Barbeau 2023; Marinchel et al. 2024; Sachs, Dünn, and Arndt 2023). In the present work, diatoms largely dominated

with a mean relative abundance of 58% ± 10%. This result was expected since we sampled the top 3 cm of the sediment, including potential biofilm and the microphytobenthos compartment, often dominated by diatoms (Magnusson 2009). Other studies focusing on the water–sediment interface using eDNA also measured a large dominance (> 90%) of diatoms (Kong et al. 2019). Their high proportion could be overestimated by amplicon sequencing because of the bias of the copy number of 18S rDNA per cell within this lineage. This issue is even more important for other groups like Dinoflagellata (Godhe et al. 2008; Zhu et al. 2005), which, however, were not dominant in the sequences from sediments (this study at Dumbea, Coulée, and Pirogues, Figure 3D), but only in the surface water of the same area (Meyneng et al. 2024). Despite this potential bias, we consider that diatoms are the dominant group in sequences from sediment samples of New Caledonia. Within the microphytobenthos, this group could be the major source of oxygen production in such dynamic shallow coastal areas (Karsten et al. 2021; Prelle et al. 2019). In various marine ecosystems, the growth of photosynthetic organisms is often limited by the availability of certain metals, such as iron (Hutchins and Bruland 1998) or
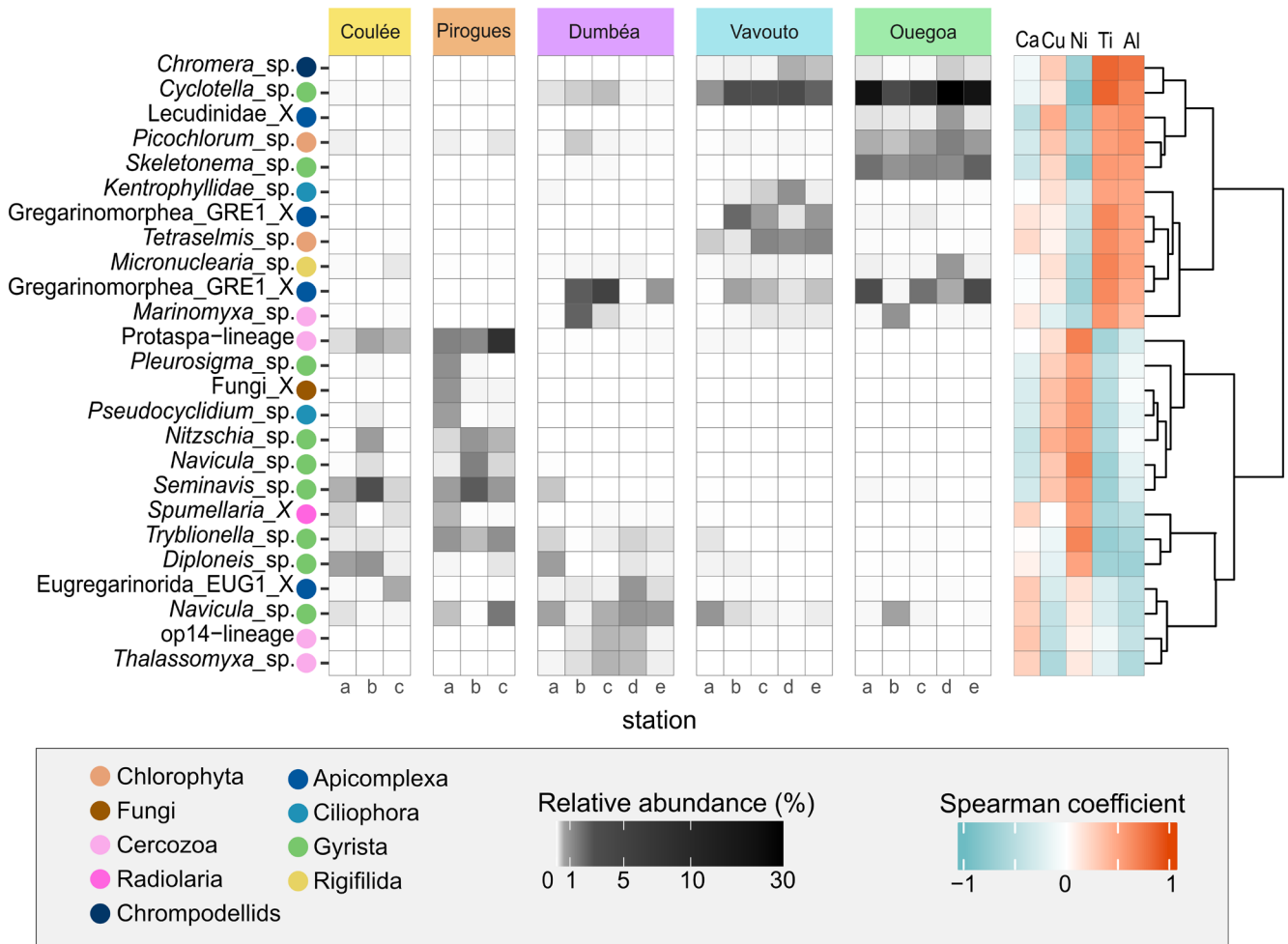
FIGURE 5 | Mean relative abundance per station of the 25 selected ASVs ordered according to a clustering based on their correlation with metal concentrations.

manganese (Browning et al. 2021), or nutrients like nitrogen (Moore et al. 2013). In contrast, in our coastal systems, which are naturally rich in diverse metal concentrations and receive nutrient inputs from rivers, the growth of these taxa might be enhanced (Annett et al. 2008), thereby supporting primary production through oxygenic photosynthesis. However, this question is out of the scope of the present work, and further research on the specific stoichiometry of this ecosystem would be necessary to assess this hypothesis (Redfield 1958; Saito, Goepfert, and Ritt 2008).

4.2 | The Influence of Terrestrial Matter Origin in Shaping Shallow Sediment Diversity

Given the importance of some parameters in the variability of microeukaryote communities, such as depth, salinity, pH, or grain size (Gong et al. 2015; Pan et al. 2020; Tee et al. 2021), and focusing on the main topic of this work which was to test the hypothesis of the influence of terrestrial matter in shaping diversity, we tried to limit the influence of different environmental confounding parameters. For this, we have voluntarily collected samples from similar sediment habitat types. Our samples were all collected in sites close to river mouths, and each station showed silty sediments sampled at a depth of 0.2 to 1.5 m at low

tide. Measurements showed low concentration of NO_x (between $0.04 \pm 0.06\,\mu mol.L^{-1}$ in Ouegoa and $0.28 \pm 0.22\,\mu mol.L^{-1}$ in Vavouto), the limiting element in the area (Jacquet et al. 2006), and generally stable pH values (between 7.41 ± 0.15 in Vavouto and 7.58 ± 0.21 in Pirogues) (Table 2). Still, some parameters showed significant differences. SRP was higher in Ouegoa ($7.7 \pm 8.5\,\mu mol.L^{-1}$), likely explained by a different input from the Diahot River, the largest river of New Caledonia, and the potentially following *in situ* biogeochemical processes linked to phosphorus and organic matter cycles (Li et al. 2021; Meng, Yao, and Yu 2014). The naturally high heterogeneity of sediment (Zhao and Xu 2017), though not the primary factor driving microeukaryote community variations, did contribute to some variations in grain size and physicochemical parameters. These factors accounted for 8.19% and 7.33% of the variations in microeukaryote communities, respectively.

The main environmental difference among the coastal ecosystems we studied was in sediments' metallic and mineralogical composition, a characteristic linked to the watershed geology of the terrestrial landscapes. We showed that this metal variability was associated with different benthic microeukaryote communities. The concentrations of five metals (Ni, Ti, Cu, Al, and Ca) explained 18.46% of the microeukaryote community variability, being the major factor of ASV diversity influence among our

measured environmental parameters. Most of the studies focusing on the link between metals and benthic microbial communities *in situ* have either focused on gradients of concentrations (Bernardino et al. 2019; Gillmore et al. 2021; Kavehei et al. 2021) or on the impact of human activities that disturbed the environment (Gillan et al. 2005; Zhuang et al. 2019). In New Caledonia, this difference in metallic composition is natural, even if past and actual phenomena, like mining activities (Fernandez et al. 2006) or wildfires (Thery et al. 2024, 2023), could have amplified the quantity and modified the form of those metals. Metals of anthropogenic origin, such as Cd (Dalto et al. 2006), were found at low concentrations in our work. Therefore, the soil type of the watershed is suggested to be the principal factor shaping benthic microeukaryote community diversity.

The different vegetation coverings linked to the soil type could also add more variability to our sites. New Caledonia has some of the world's highest nickel and cobalt soil concentrations (Kierczak, Pietranik, and Pędziwiatr 2021), and several studies have tried to understand the ecological implications of this kind of soil on terrestrial biodiversity. They found that the vegetation and the associated terrestrial microbial communities are adapted to this environment's specific constraints, partially explaining the high rate of New Caledonia endemism (Isnard et al. 2016). Those variable landscapes can have a twofold effect. First, the contribution of different organic matter derived from the vegetation (Fagervold et al. 2014), and second, the direct transport of matter and associated organisms of terrestrial but also freshwater origin by coalescence can influence the functioning of coastal communities (Châtillon et al. 2023; Rillig et al. 2015; Snell Taylor et al. 2018). Therefore, terrestrial biodiversity can act as a cofactor in addition to the soil in the watershed in increasing and differentiating shallow sediment microeukaryote diversity.

The transformation of the organic matter during its transport from land to the sea can also affect the biodiversity of the coastal zone. Mangroves, for instance, present in most of our study sites, play a role in filtering and transforming organic matter (Alongi 2014; Kristensen et al. 2008). Studies have proved the influence of mangroves on benthic communities, such as the "rhizosphere effect" (Zhu et al. 2018) or the potential of those plants to host distinct microeukaryote populations like a higher proportion of environmental pathogens (Liu et al. 2024). The presence of mangroves at some of our stations impacted the composition and the quantity of organic matter (Mouras et al. 2024) and might have modified the speciation of metals (Marchand et al. 2012). This transformation might have contributed to the microbiome variations in our ecosystems. Yet, parameters concerning organic matter only explain 5.4% of community composition distribution, but based on the ecological importance of this factor, we suggest that more advanced studies on this aspect should be carried out to better elucidate this aspect.

4.3 | Specific Associations Between Metal Composition and Microeukaryote Diversity

The associations between higher relative abundances of selected ASVs (Figure 5) and metal concentrations allowed us to highlight some potential ecological diversity patterns. Some taxa that highly contributed to our beta-diversity were found in other specific ecosystems, like *Pleurosigma* sp. and Apicomplexa in mangrove sediment (Hendrarto and Nitisuparjo 2011; Liu et al. 2024), *Chromera* sp. in corals (Moore et al. 2008), *Skeletonema* sp. in estuaryies (Cui et al. 2022), or *Cyclotella* sp. in brackish waters (Varona-Cordero, Gutiérrez-Mendieta, and Meave del Castillo 2010). The gradient of ultramafic terrestrial matter that we showed among our sites (Coulée-Pirogues > Dumbéa > Vavouto>Ouegoa) seems to reflect in the presence and relative abundance of some ASVs. Several diatoms showed a significant positive correlation with Ni concentrations, such as *Pleurosigma* sp., *Nitzschia* sp., *Navicula* sp., *Seminavis* sp., *Tryblionella* sp., and *Diploneis* sp. (Figure 5). Some genera and species of diatoms have been showed to have high resistance level to metal stress, as *Navicula* sp. or *Nitzchia* sp. (Araújo, Blasco, and Moreno-Garrido 2010; Potapova, Desianti, and Enache 2016). This could be one potential explanation for their dominance in our work in high Ni-concentrated sediments (also correlated to Cr, Co, Mn, and Fe). The presence of Fungi ($1.3 \pm 1.1\%$) and the high correlation of some of them with metals (Figure 5) was not surprising as they have also been considered metal-resistant (Binsadiq 2015; Hassan et al. 2020). Specific parasitic and predatory taxa also showed spatial patterns that might be explained by the presence of some metals. Members of Gregarine (Apicomplexa), a parasite of benthic invertebrates (Boisard and Florent 2020), *Marinomyxa* sp., a parasite of seagrass (Kolátková et al. 2022), as well as predators of *Thalassomyxa* lineage (Berney et al. 2013), showed different spatial repartition according to metal concentrations. Finally, some species that we associated with metal concentration (Ca, Cu, Ni, Ti, and Al) have little or no environmental description, like *Pseudocyclidium* sp. Our results provide new interesting information to understand the mysterious ecology of these taxa.

The biogeochemical processes that could explain the association between some metals and microeukaryote taxa or groups are not easy to identify. Given the important concentrations of several metals in our ecosystems, the associations found could be based on selection mechanisms (Stegen et al. 2012). Numerous metal components are vital for the proper functioning of microorganisms (Morel and Price 2003), but depending on the metal, its speciation and concentration can become a source of stress for some taxa (Beveridge et al. 1996; Simpson and Batley 2007). The addition of certain metals can lead to the growth of some microorganisms and the disappearance of others, explaining the heterogeneous response at the community level (Gardham et al. 2014). Metal needs could also partially explain the higher abundances of phototrophic taxa that we found in Pirogues estuary, which was characterized by higher concentrations of Mn and Fe, correlated with Ni, two elements that are needed in the photosynthesis process (Beer, Björk, and Beardall 2014; Hansel 2017). Our study was based on total metal concentrations, but it is especially the bioavailable forms that highly impact living organisms (Beveridge et al. 1996). Cu^{2+}, Cd^{2+}, and Cr(VI) are among the most toxic forms, and microeukaryotes can respond with several kinds of metal resistance mechanisms (Rodriguez-Zavala et al. 2007; Slaveykova, Sonntag, and Gutiérrez 2016).

The physiological processes behind the ecological associations that we found remain to be studied, and new environmental data confirming our microeukaryote–metal associations in other

regions are needed. Although we showed that the terrestrial matter origin has a major influence on benthic microeukaryote diversity, there is still a part of the community variability not explained clearly, as suggested by our high individual effect of the "site" factor (Figure 4B). In addition, we identified intra-site community variability that we did not fully explain in this study. We propose that this variability is influenced by other biological and physical factors affecting the distribution of benthic microeukaryotes. This variability could result from stochastic processes (Ramond et al. 2023; Stegen et al. 2012; Zhou and Ning 2017) not considered in our study. Plus, as mentioned before, some taxa may originate from freshwater or terrestrial sources. Likely, upstream sampling in addition to RNA-based or population genetic approaches might help in the future to differentiate between allochthonous and autochthonous species in the marine ecosystems. Finally, unmeasured deterministic parameters such as light (Arias, Saiz, and Calbet 2020), biotic interactions (Nemergut et al. 2013), or pollutants induced by human activities could also explain more spatial variabilities. For instance, the potential presence of organic pollutants in Dumbéa or Coulée, where agricultural activities exert cumulative pressures, could partially explain the specificity of these sites (Bao et al. 2023). Incorporating this information could refine our study and improve our understanding of the variables influencing the taxonomic groups studied (Figure S8).

5 | Conclusions

This work underscores that the origin of matter plays a major role in shaping the diversity of benthic microeukaryote communities. Our findings highlight that, due to the connectivity within the land–sea continuum, the source of materials can significantly impact coastal ecosystems. This demonstrates the importance of considering the entire land–sea continuum to better interpret the biological diversity of coastal communities. To illustrate this, our study was conducted in New Caledonia, a territory with distinct terrestrial landscape differences, particularly with ultramafic soil. The metal influence we found is mainly natural, induced by soil watershed variability, but mining activities can exacerbate it and cannot be distinguished in our work. The study of the impact of such additional human pressures could be relevant to better understanding their consequences on the functioning of the land–sea continuum. We have shown a diversity pattern in shallow sediments, but the association between *in situ* and *in vitro* studies will elucidate if the associations between metals and species are local environmental specificity or based on the physiological resistance of some microeukaryote species to metals. This could ultimately lead to the development of marine environmental bioindicators of ultramafic zones worldwide.

Finally, at the local scale of New Caledonia, further investigation is still needed to fully comprehend the spatial and temporal dimensions of the influence of metals over coastal biodiversity, particularly in such sub-tropical ecosystems characterized by contrasting seasonal variations in river inputs. By considering a larger spatial scale (110 estuaries in New Caledonia), the whole land–sea continuum (using soil, riverine, and offshore samples collected along a land–sea transect), differently oxygenated sediment layers (samples collected along an oxygen concentration gradient), and different periods (the dry and rainy seasons), we could fully assess the spatio-temporal dynamic of the terrigenous influences on benthic communities highlighted in this work. This information is not only crucial for preserving the region's unique, rare, and endemic biodiversity but also for mitigating the impacts of human activities and climate change, which consequently affects ecosystem functionality.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequences are available at https://doi.org/10.12770/62cde5b9-1888-4b2c-b8a5-a490a696b078.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.