Bacterial contributions to the formation of polymetallic nodules in the Pacific Ocean

Xinyi He [1,2,3,4], Qian Liu [5], Xiaohu Li [1,2,3*], Zhenggang Li [1,2], Hao Wang [1,2], Zhimin Zhu [1,2], Yanhui Dong [1,2], Jie Li [1,2] and Huaiming Li [1,2]

[1]State Key Laboratory of Submarine Geoscience, Hangzhou, China, [2]Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou, China, [3]School of Oceanography, Shanghai Jiao Tong University, Shanghai, China, [4]Bureau of Marine Development, Qingdao West Coast New Area, Qingdao, China, [5]Key Laboratory of Marine Ecosystem Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou, China

Polymetallic nodules, widely distributed in the deep seafloor of the Pacific Ocean, are characterized by their abundance of diverse metal elements and considerable economic value. Previous studies have suggested a partial biogenic origin of these nodules. This study investigated the role of microorganisms in nodule formation by examining biological-like structures and bacterial communities within nodules and sediments. Scanning electron microscopy revealed bacteria-like microspheres, skeleton-like structures and extracellular polymeric substances-like structures in the nodules. Energy dispersive spectroscopy showed that these biological-like structures facilitated metal enrichment, enabling subsequent mineral precipitation. *Shewanella*, *Colwellia*, *Leptospirillum*, *Sulfitobacter*, and other bacteria may possess mineralization potential due to their Mn or Fe oxidation capabilities. Differences in internal structures and bacterial community composition between nodules from the western and eastern Pacific Ocean could potentially suggest that growth environment factors may contribute to nodule formation variation. These findings highlight the involvement of microorganisms in nodule formation and contribute to a better understanding of the biogenic mineralization process.

KEYWORDS

polymetallic nodules, sediment, bacterial composition, biomineralization, scanning electron microscope, Pacific Ocean

1 Introduction

Polymetallic nodules, also known as ferromanganese nodules, are widespread mineral resources in the deep seafloor. They are rich in Fe, Mn, Ni, Co, Cu, rare earth elements (REEs), and other metal elements, such as Ti, Mo, Zr, Li, and Y (Hein et al., 2020). These elements occupy crucial positions in various economic sectors, including the automotive, aerospace, and renewable energy industries (Hein et al., 2013). The substantial reserves of

nodules are found primarily in the Clarion-Clipperton Fracture Zone (CCZ) in the eastern Pacific Ocean (Hein et al., 2013).

Initially, it was widely believed that nodule formation was primarily driven by physicochemical interactions (Takematsu et al., 1989; Roy, 1992). This process was thought to be dominated by water-rock interactions and precipitation dynamics, with iron and manganese oxides precipitating under redox gradient conditions as the main mineralization driver (Goldberg and G.O.S, 1958; Crerar and Barnes, 1974).

Nodules that form through the continuous precipitation of Fe and Mn minerals from oxygen-rich near-bottom waters are known as "hydrogenetic nodules." These nodules typically exhibit a Fe/Mn ratio of approximately 1 in bulk analyses and are enriched in elements such as Fe and Co (Hein et al., 2013). In contrast, nodules that precipitate from oxygenated or suboxic sediment pore waters are referred to as "diagenetic nodules," which typically have an Mn/Fe ratio > 5 and are enriched in elements such as Mn and Cu (Halbach et al., 1988). Additionally, nodules that exhibit a mixture of diagenetic and hydrogenetic origin are called "mixed-type nodules" (Halbach et al., 1981).

Since Graham and Cooper (1959) suggested that manganese-rich deposits on the seafloor may result from biological processes, research into the role of microorganisms in nodule mineralization has steadily gained attention. Subsequent studies employing techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have further highlighted the independent relationships between microorganisms and the metals and minerals within the nodules (Burnett and Nealson, 1983; Hu et al., 2000; Wang and Müller, 2009; Nayak et al., 2013). The involvement of microorganisms in nodule formation has become an increasingly acknowledged and explored topic within the field.

Nodules exhibit distinct distribution patterns in various marine regions and are significantly influenced by the underlying environments (Glasby, 1976; Cochonat et al., 1992; Molari et al., 2020). The western Pacific contains one of the oldest oceanic crusts (Glasby et al., 1982) and the seafloor is coated with red clay (Glasby et al., 1987). The CCZ mainly develops on the smooth relief of abyssal hills (Halbach et al., 1981), and the seafloor is covered by rather thick, extensive layers of siliceous oozes (Margolis and Burns, 1976). Furthermore, the western Pacific mainly produces hydrogenetic nodules (Jiang et al., 2020b), while nodules from the CCZ are mostly of diagenetic or mixed-type origin (Wegorzewski and Kuhn, 2014; Hollingsworth et al., 2021).

In recent years, microorganisms within nodules have been acknowledged to significantly contribute to the process of nodule formation (Jiang et al., 2020a). Microorganisms significantly contribute to the cycling and mineralization of metals, utilizing their metabolic activities to influence nucleation and crystallization processes (Banfield and Nealson, 2018). By altering microenvironments such as pH and redox conditions, they facilitate mineral precipitation and shape mineral compositions (Konhauser, 1997). The deposition of minerals is crucial for the formation and growth of nodules, and microorganisms may exhibit a promoting effect on nodule formation due to their ability to mediate mineral formation and deposition (Hoffmann et al., 2021).

Moreover, microbial mineralization activities are also associated with the rough surface structure and the growth of internal laminae in the nodules (Akai et al., 2013; Jiang et al., 2017).

However, the current understanding of microbial communities and their biomineralization functions in nodules in different regions of the Pacific Ocean remains limited. Comprehensive assessments of the variations in environmental conditions, nodule types, and abundance, as well as benthic organism activities across different regions, are challenging. Further research is needed to provide a more in-depth and comprehensive understanding.

In this study, we analyzed elemental composition of nodules and applied scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) to analyze biological structures and surface elemental composition in nodules from the western and eastern Pacific. Furthermore, full-length 16S rRNA gene analysis was conducted to examine bacterial community composition within the nodules and underlying sediments, aiming to uncover potential bacterial mineralization processes and their role in the formation of mineralized structures in nodules.

2 Materials and methods

2.1 Sample collection

Samples of nodules and underlying sediments were collected using a multicorer at depths of 5302-5562 m from zone M2 (MC02, BC38A, and BC78) in the western Pacific during August and September 2022 aboard the research vessel *Dayangyihao* on voyage 75 (Figure 1, Table 1). The M2 area in the northern Magellan Seamounts of the western Pacific is a geologically stable region with limited volcanic and tectonic activity (Hein et al., 1997). The surface sediments are mainly composed of brownish-yellow clay. The study area represents a typical tropical oligotrophic environment, with low surface productivity (Jiang et al., 2020a), moderately high bottom water oxygen concentrations (175–200 mmol/m³) (Dutkiewicz et al., 2020; Ren et al., 2022), and a bottom seawater temperature of approximately 1.5°C and salinity of around 34.6. The nodules in this area are primarily formed through hydrogenic processes, characterized by Co enrichment and a spheroidal morphology.

Samples were also collected using a multicorer at depths of 5177-5267 m from zone KW1 (MC04 and BC05) and zone A5 (BC12) in the eastern Pacific during voyage 73 of the research vessel *Dayanghao* within the same timeframe (Figure 1, Table 1). KW1 and A5 are adjacent blocks, both located in the western part of the CCZ in the eastern Pacific. The feature surface sediments with a brown hue, is primarily consisting of siliceous oozes. The dissolved oxygen concentration in the bottom seawater is approximately 150 mmol/m³ (Dutkiewicz et al., 2020), with a temperature of 1.5°C and salinity around 34.7 (Washburn et al., 2021). The nodules are predominantly poly-nodules and have a hydrogenetic or mixed-type origin (Reyss et al., 1985).

The collected nodules and sediments were stored at −80°C and transported on dry ice to the laboratory.

Locations of nodules and sediments sampling sites.
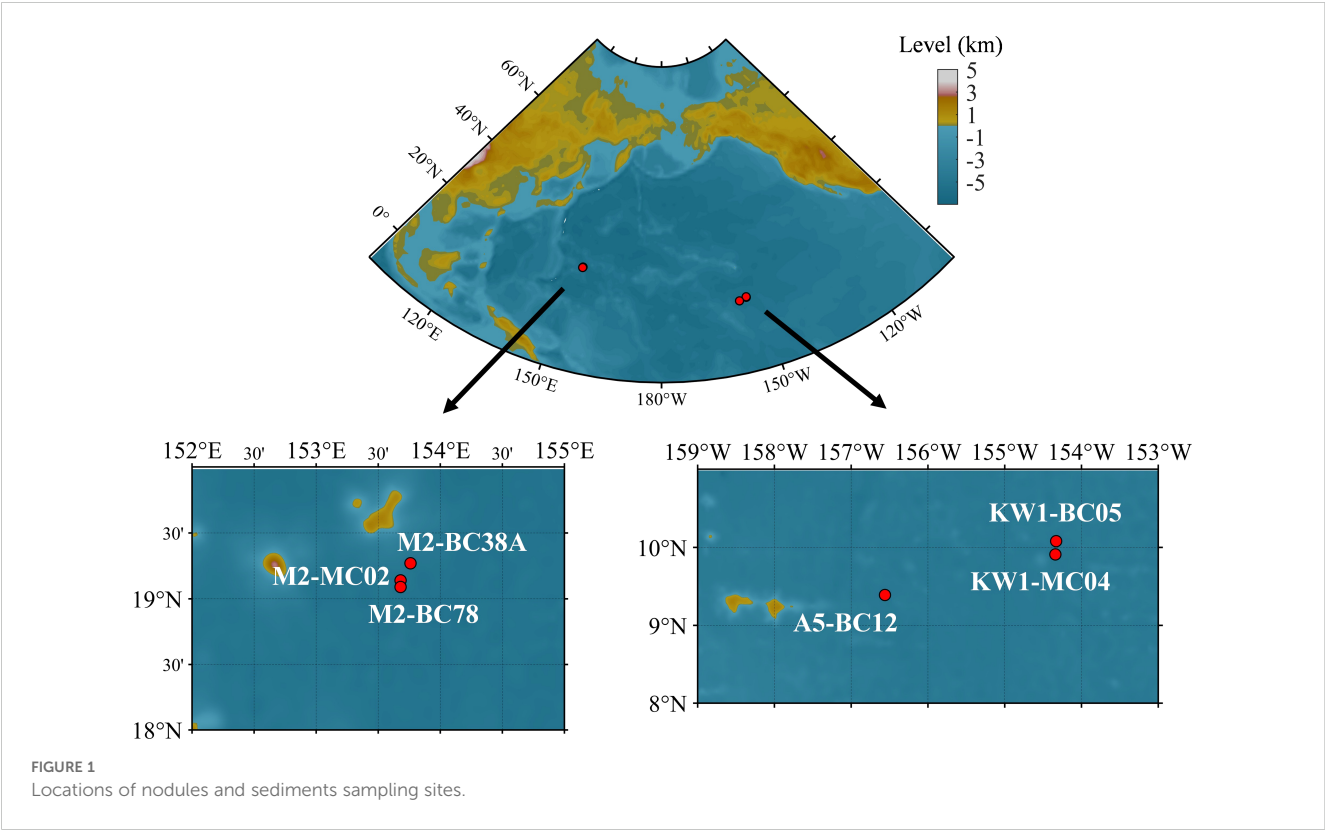
2.2 Elemental composition analysis

The polymetallic nodules were dried, crushed, weighed (5 g) and then ground into a homogeneous powder of 200 mesh size using an agate pestle and mortar. Elemental analyses were conducted at the Key Laboratory of Submarine Geosciences, Second Institute of Oceanography, Ministry of Natural Resources.

The major elements in the nodules and sediments were determined using X-ray fluorescence spectroscopy (XRF, AxiosMAX, PANalytical, Netherlands). The trace elements in the nodules and sediments were determined using inductively coupled plasma-mass spectrometry(ICP-MS, Elan DRC-e, Perkin Elmer). All measured concentrations exceeded detection limits, with a relative standard deviation of laboratory precision less than 10%.

TABLE 1 Description of the polymetallic nodules and surrounding sediments sampled at six sites in the Pacific Ocean.

Site	Western Pacific			Eastern Pacific		
	M2-MC02	M2-BC38A	M2-BC78	KW1-MC04	KW1-BC05	A5-BC12
Longitude	153.68˚E	153.76˚E	153.68˚E	154.34˚W	154.33˚W	156.56˚W
Latitude	19.14˚N	19.27˚N	19.09˚N	9.91˚N	10.08˚N	9.39˚N
Depth	5519 m	5302 m	5562 m	5219 m	5177 m	5267 m
Sampling Method	Multicorer	Multicorer	Multicorer	Multicorer	Multicorer	Multicorer
Sample Type (Symbol)	Nodule (WNA) Sediment (WSA)	Nodule (SA)	Nodule (SB)	Nodule (ENB) Sediment (ESB)	Nodule (SC)	Nodule (SD)
Nodule Morphology	Spheroidal	Spheroidal	Spheroidal	Poly nuclei	Poly nuclei	Poly nuclei
Type of Sediment	Siliceous clay	Siliceous clay	Siliceous clay	Siliceous oozes	Siliceous oozes	Siliceous oozes
Technology	16S rRNA sequencing	SEM-EDS	SEM-EDS	16S rRNA sequencing	SEM-EDS	SEM-EDS

2.3 Scanning electron microscopy and energy dispersive X-ray spectroscopy

The nodules were mounted to epoxy and then cut in along the maximum axis to make thin sections. SEM images were performed using a TESCAN MIRA3 field-emission scanning electron microprobe (FE-SEM) at the Testing Center, Tuoyan Analytical Technology Co. Ltd. (Guangzhou, China). After the samples were carbon-coated, SEM images were acquired under an acceleration voltage of 20 kV, a beam current of 15 nA and a magnification of 300–500×. The surface elemental composition of carbon coated sample was characterized by energy dispersive X-ray analysis (SEM-EDAX) EDS detector (EDAX Element EDS detector) device attached to an SEM operating at 20 kV. Observing SEM images provides information about the internal structures within nodules, and further analysis can be conducted by combining the corresponding EDS results.

2.4 Full-length 16S rRNA gene sequence analysis

Under sterile conditions, the surface deposits adhering to the nodules were scraped away. The nodules were then fragmented using a chisel and then ground with a mortar. The nodules and surrounding sediments were individually weighed to 0.5 g. DNA was extracted using an Advanced Soil DNA Kit (MOBIO, Solana Beach, USA) according to the instructions.

The extracted DNA was used as a template for amplifying the V1-V9 regions with the primer set 27 F (5'-AGRGTTY GATYMTGGCTCAG-3') and 1492 R (5'-RGYTACCTTGTT ACGACTT-3'). Polymerase chain reaction (PCR) was conducted using the BioRad (S1000, Bio-Rad Laboratories, USA). Each sample was run in triplicate, and the PCR products from the same sample were pooled. Library preparation followed the 16S Amplification SMRTbell[®] Library Preparation protocol, and sequencing of the amplicon library was performed by Guangzhou Meige Biotechnology Co., Ltd., utilizing the PacBio Sequel II platform (PacBio, USA).

Fastp (v0.14.1) removed sequences over 2000 bp, and Cutadapt (v1.14) eliminated primers, resulting in valid fragments. Uparse defined operational taxonomic units (OTUs) at a 97% similarity threshold, with a confidence level of 0.8. Usearch-sintax (v10.0.240) aligned OTU representative sequences against the Silva v132 database.

Alpha diversity indices, including abundance-based coverage estimator (ACE) and Shannon, were calculated using usearch-alpha div (v10) based on the relative abundances of OTUs in each sample. Rarefaction curves were drawn by usearch-alpha div rare (v10) based on a richness index to estimate the sampling efforts. Community compositions were performed using R software.

3 Results

3.1 Morphologies and structures of nodules

Disparities were observed in the external morphological characteristics and internal structures of polymetallic nodules from the western and eastern Pacific (Figure 2). The nodules from the western Pacific were near-spherical (Figure 2A), with a homogeneous texture and no distinct core (Figure 2B). In contrast, nodules from the eastern Pacific were primarily poly-nodules containing more than three nuclei, exhibiting rough surfaces (Figure 2C) and distinct cores, which were encased by surrounding conduits (Figure 2D).

3.2 Elemental compositions

Nodules exhibited a significant enrichment of metal elements such as Mn, Fe, Ti, Co, Cu and Ni compared to the sediments (Table 2). Mn, Cu and Ni were generally more enriched in nodules from the eastern Pacific, while Fe, Ti and Co were more enriched in nodules from the western Pacific. Based on the Mn/Fe ratio, nodules from the western Pacific were indicative of a pronounced hydrogenetic origin (Mn/Fe ≈ 1) (Verlaan et al., 2004; Hein and Koschinsky, 2014), whereas nodules from eastern Pacific generally show a mixture of diagenetic and hydrogenetic origin, with a predominantly diagenetic input (2.09-2.77) (Wegorzewski and Kuhn, 2014; Hein et al., 2020).

3.3 Internal structures in nodules

Both western and eastern Pacific nodules exhibit regularly arranged laminated structures (Figure 3). The nodules from the western Pacific are characterized by laminated and concentric rims (He et al., in press), where compact layers (brighter) and loose layers (darker) alternate in a ring-like pattern, forming a rhythmic structure (Figures 3A–C). In contrast, nodules from the eastern Pacific predominantly display stromatolite-like rims with a loose texture and abundant pores (darker areas) distributed irregularly (Figures 3D–F). The compact structures in both regions show clear Mn enrichment on their surfaces, identified from the Mn elemental mapping, where lighter purple areas indicate higher Mn concentrations (Figures 3C, F). This is based on qualitative EDS analysis. The Mn distribution follows a rhythmic pattern, corresponding to concentric or stromatolite-like laminations.

Furthermore, many biological-like structures were observed from the fissures and cavities in the thin sections. For example, some bacteria-like microspheres (Figures 4A) exhibited a ring-like arrangement, with morphologies similar to those considered bacteria by Jiang et al. (2019) and Reykhard and Shulga (2019). Additionally, another type of bacteria-like microsphere, characterized by a flattened, centrally concave shape, was densely distributed within the channels (Figure 4B). Larger biological-like structures (>20 μm), including porous spheroidal (Figure 4C) and annular (Figures 4D, E) structures were also observed. Furthermore, a transparent and highly reflective extracellular polymeric substance (EPS)-like structure (Figure 4F) (Ren and Jones, 2021).

To further elucidate the elemental composition of these structures, qualitative EDS analysis was performed. Higher peak intensities indicate greater element enrichment (Figure 4). Mn, Fe, and Ti are the primary metals that exhibit substantial enrichment
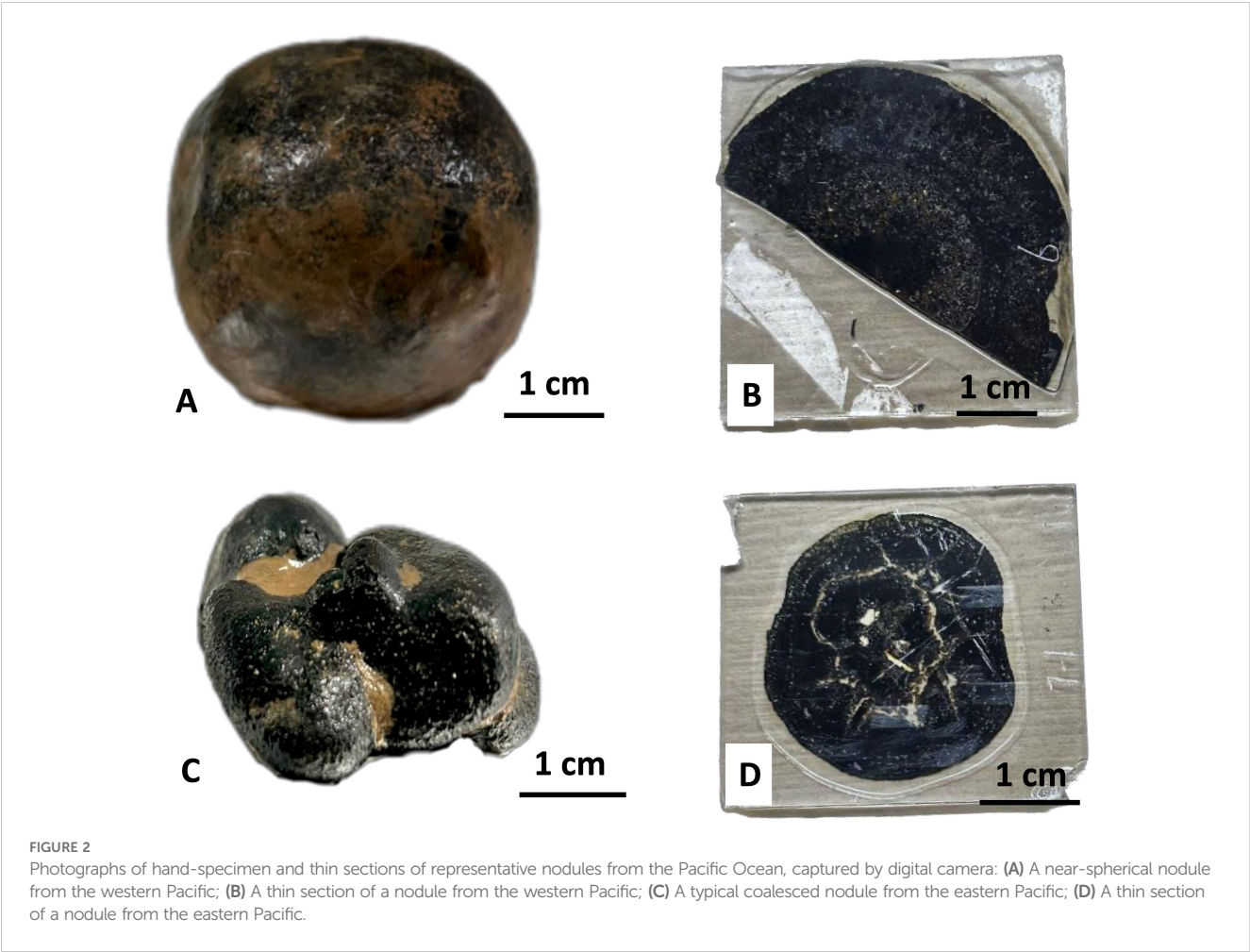
FIGURE 2
Photographs of hand-specimen and thin sections of representative nodules from the Pacific Ocean, captured by digital camera: **(A)** A near-spherical nodule from the western Pacific; **(B)** A thin section of a nodule from the western Pacific; **(C)** A typical coalesced nodule from the eastern Pacific; **(D)** A thin section of a nodule from the eastern Pacific.

TABLE 2 Partial major and trace element compositions of the nodules and sediments. Co, Cu, and Ni in ppm; Mn/Fe dimensionless; others in wt.%.

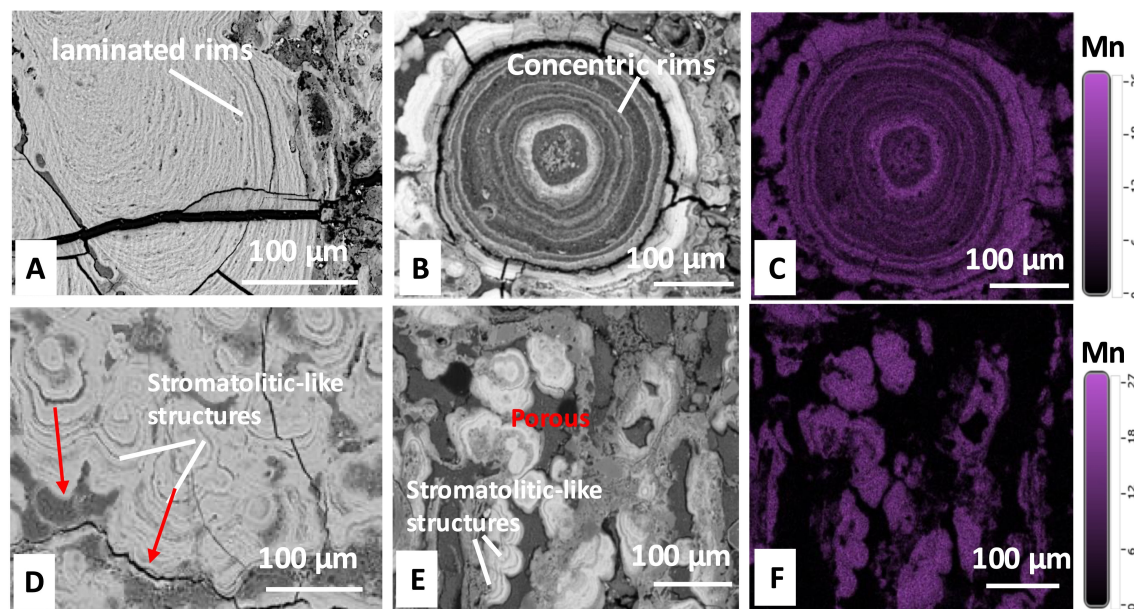|	Western Pacific				Eastern Pacific			
	Nodule (WNA)	Sediment (WSA)	Nodule (SA)	Nodules (SB)	Nodule (ENB)	Sediment (ESB)	Nodule (SC)	Nodule (SD)
Mn	17.47	0.65	17.50	17.27	23.54	0.50	23.81	25.43
Fe	18.57	6.19	17.98	15.86	11.28	4.52	11.20	9.19
Si	7.84	24.08	7.80	8.84	6.67	25.43	6.86	6.83
Al	3.10	9.21	2.90	3.59	2.58	6.66	2.56	2.89
Mg	1.30	2.13	1.22	1.46	1.34	1.93	1.09	1.86
Ca	1.77	1.49	1.82	1.84	1.87	0.93	1.90	1.73
Na	1.44	2.80	1.45	1.51	1.74	4.65	1.71	1.90
K	0.67	2.66	0.67	0.83	0.85	2.22	0.88	0.86
Ti	1.39	0.53	1.37	1.12	0.84	0.44	0.92	0.63
Co	3906	88	4148	3191	3271	95	3105	2549
Cu	2229	217	2168	3349	4974	364	5669	8582
Ni	3702	205	4018	4753	8159	188	9113	11157
Mn/Fe	0.94	0.11	0.97	1.09	2.09	0.11	2.13	2.77

FIGURE 3
Images of internal structures in thin sections captured by SEM **(A, B, D, E)** and Mn distribution by EDS **(C, F)**: **(A, B)** Internal structures of nodules from the western Pacific, with **(A)** densely distributed laminated rims and **(B)** Concentric rims growing around a micro-core; **(C)** Mn distribution map of the structure shown in **(B)**; **(D, E)** Stromatolitic-like structures in nodules from the eastern Pacific; **(F)** Mn distribution map of the structure shown in **(E)**. Lighter purple areas indicate higher Mn concentrations.

within the nodules, and due to their considerable economic value, they have been the subject of extensive research (Hein et al., 2020). Furthermore, Mn and Fe play pivotal roles in cellular processes such as development, metabolism, and enzymatic activity (Bruins et al., 2000; Helmann, 2014), and are also known to facilitate the formation of bacterial biofilms (Avidan et al., 2010; Mhatre et al., 2016). Of particular interest, Mn, Fe, and Ti are also found to exhibit markedly high concentrations on the microstructural surfaces, which warrants further attention. The surfaces of microspheres exhibited the ability to enrich metals (Mn, Fe, Ti), particularly Mn (Figures 4A, B). Surfaces of other types of structures have similar elemental enrichments (Fe, Mn, Ti), particularly Fe (Figures 4C–E). EPS-like structure displayed comparable levels of enrichment for both Mn and Fe (Figure 4F).

3.4 Microbial community composition

The sequencing depth was sufficient, capturing most of the community diversity with a rich variety of bacterial taxa (Supplementary Figure S1; Figure 5) (He et al., in press). Proteobacteria (38.2%-46.9%) was predominant across the samples, with the dominance of the class Gammaproteobacteria (18.1% to 32.3%). *Woeseia* exhibited dominance in WNA (12.26%) and WSA (9.56%), while *Arcicella* was the dominant genus in ENB (28.02%) and ESB (22.22%).

The experiment identified some unique genera that appeared to be exclusive to specific samples (Table 3; Supplementary Table S1). For example, *Leptospirillum* and *Lentisphaera* were found only in western Pacific samples (WNA, WSA), while *Psychrobium* and *Sulfitobacter* were detected exclusively in eastern Pacific samples (ENB, ESB). *Halomonas* was enriched in ENB but was not detected

in WNA. Similarly, *wb1-A12*, *Blastocatella*, and *IS-44* were enriched in sediment samples (WSA, ESB) and were not identified in WNA. However, reliance on relative abundances alone was insufficient to definitively confirm the presence or absence of these genera, though it suggests the possibility of such patterns. It is important to note that these results are based on sequencing data from a single sample, highlighting the need for further replicates to robustly assess microbial enrichment preferences across different sample types.

The 16S full-length sequencing technique enabled species identification 29 species were detected from the nodule samples. Excluding the unsigned and uncultured species, 9 species could be identified (Table 4). These species, belonging to the Gamma or Alpha classes of Proteobacteria, were from various genera (Supplementary Table S2), indicating potential variations in their physiological characteristics and functions. It is also noteworthy that the occurrence of these species may be influenced by experimental factors, which may hinder the definitive determination of their exclusivity to a specific sample. Consequently, the zero abundances observed (Table 4) cannot be conclusively interpreted as the absence of these species but instead represent the potential for these species to be unique to certain samples.

4 Discussion

4.1 Biological structures potentially involved in nodule formation

Previous studies utilizing SEM-EDS have identified manganese-oxidizing bacteria (MOB) and biological structures involved in
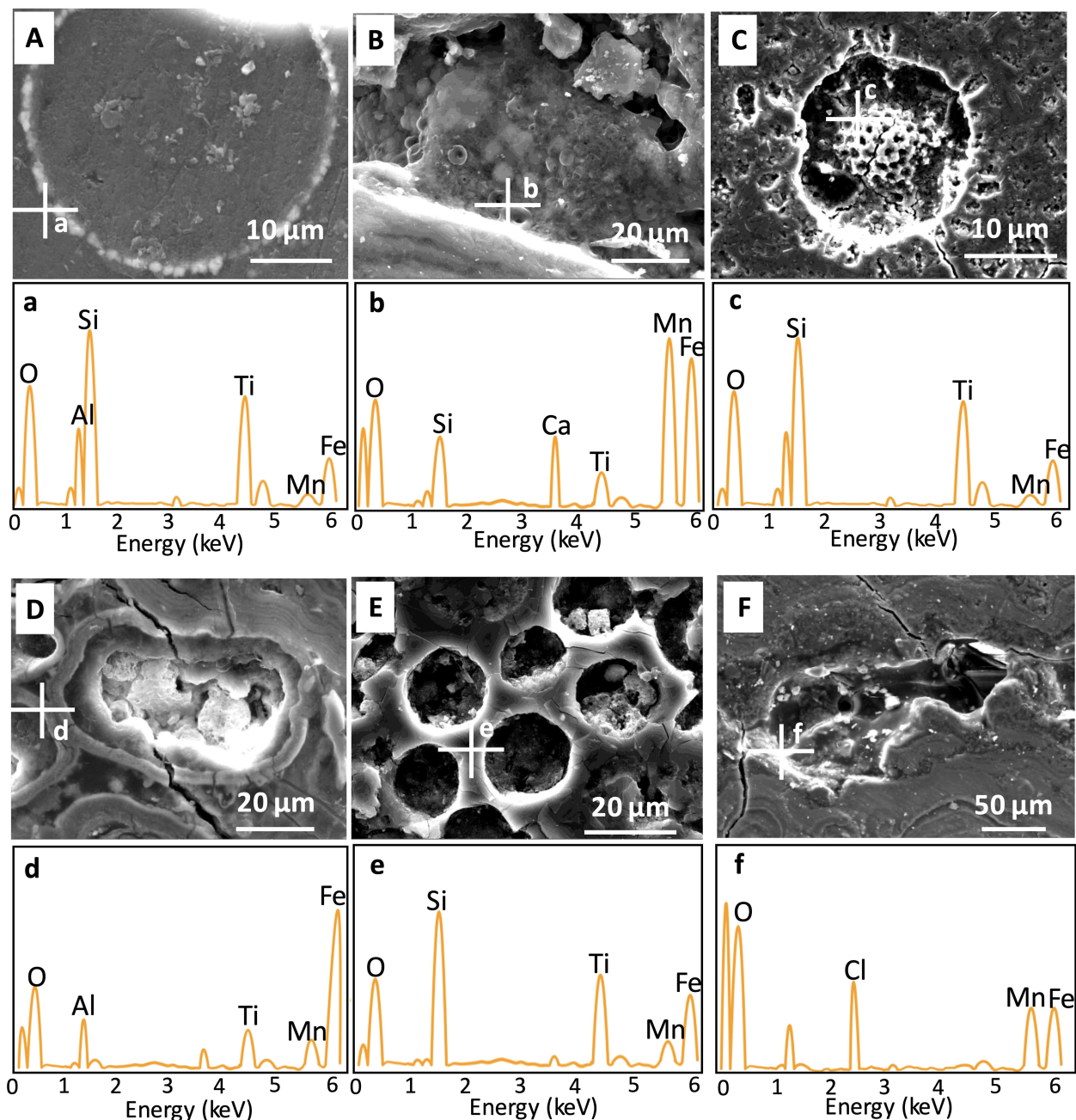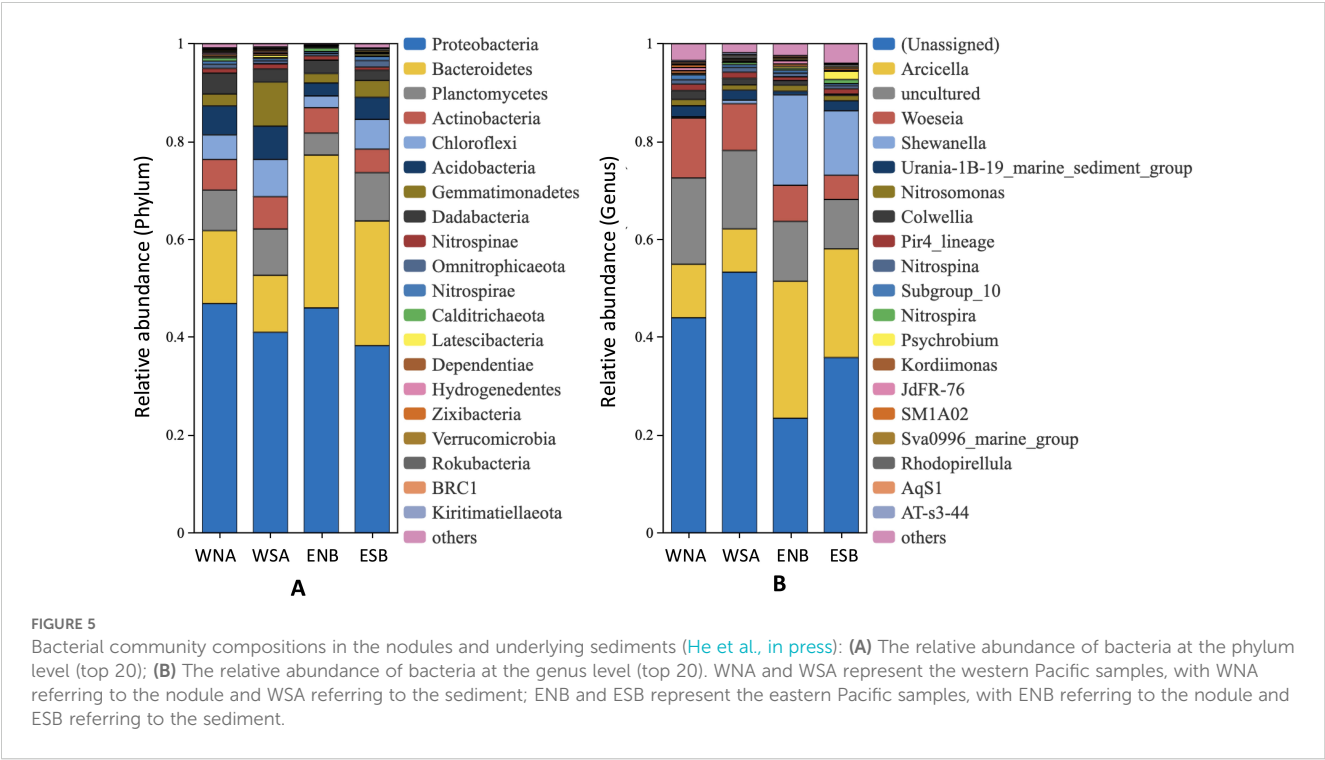
FIGURE 4
Images of biological-like structures in nodules from the western Pacific **(A-C)** and the eastern Pacific **(D-F)** captured using SEM-EDS: **(A)** Microspheres arranged in a ring on a platform; **(B)** Multiple flattened microspheroidal structures with central concavities; **(C)** A porous spheroidal structure; **(D, F)** Annular structures of varying shapes; **(E, F)** EPS-like structures coating tunnel surfaces. White crosses indicate analysis points. a-f indicate the regions in the images **(A–F)** where elemental analysis was performed using EDS.

nodule mineralization (Wang and Müller, 2009; Jiang et al., 2020a; Shulga et al., 2022). In this study, metal-enriched biological-like structures were observed, including bacteria-like structures (Figures 4A, B) and other biomophs (Figures 4C–F), which are also likely involved in the mineralization process. SEM-EDS may have resolution limitations in analyzing biological structures, which can hinder accurate characterization of fine biological features and trace element distributions, making these findings speculative in the absence of direct evidence for their biological origin. However,

careful comparisons with similar structures reported in the literature were made during the SEM-EDS analysis.

In addition to the microspheres potentially representing mineralizing bacteria (Figures 4A, B), other biomorphs that may contribute to the mineralization process include porous structures, likely remnants of radiolarian skeletons, characterized by high Si content and features such as spines or spine-like openings (Figure 4C) (Jiang et al., 2019);annular, skeleton-like structures resembling the solid shells produced by coccolithophores

FIGURE 5
Bacterial community compositions in the nodules and underlying sediments (He et al., in press): **(A)** The relative abundance of bacteria at the phylum level (top 20); **(B)** The relative abundance of bacteria at the genus level (top 20). WNA and WSA represent the western Pacific samples, with WNA referring to the nodule and WSA referring to the sediment; ENB and ESB represent the eastern Pacific samples, with ENB referring to the nodule and ESB referring to the sediment.

(Figures 4D, E), as proposed by Wang et al. (2012); and transparent, highly reflective structures capable of enriching Mn and Fe, similar to extracellular polymeric substances (EPS) (Figure 4F) (Ren and Jones, 2021).The surfaces of these structures seem to serve as sites for the precipitation of metal oxides, resulting in the accumulation of Fe and Mn minerals (Jiang et al., 2019), which fall under the category of biologically induced mineralization (BIM). BIM occurs when organisms secrete metabolites that react with ions or compounds in the environment, leading to the growth and deposition of mineral particles (Frankel and Bazylinski, 2003). Jiang et al. (2019) suggested that these biomorphs can induce mineralization processes through

surface enrichment and proposed a mineralization process of mixed colloids of Mn- and Fe-oxide-hydroxides through biological-induced oxidation of Mn(II) and Fe(II) on the surfaces of biological structures. The significant enrichment of metals observed on the surfaces of biological structures in the present study provides further evidence for this idea (Figures 4).

However, it is crucial to acknowledge that the proposed biological structures are primarily qualitative interpretations based on the literature, rather than quantitative determinations. As such, their identification remains speculative, with EDS serving only as a supplementary tool. Consequently, the possibility of non-biological processes contributing to the observed morphological features cannot be excluded. For example, abiotic processes such as mineral precipitation or physical deposition could produce structures that resemble those associated with biological mineralization, complicating the interpretation of the results (Ren and Jones, 2021; Zhang et al., 2024). Moreover, the relationship between biological forms and mineral types in BIM remains unclear. Therefore, further research should aim to establish a more definitive link between specific biological structures and the minerals they may induce, with a focus on acquiring direct evidence of biological processes, while also critically considering the potential role of abiotic mechanisms in shaping these structures.

4.2 Potentials of bacterial community in mineralization

4.2.1 Main bacterial communities with mineralization potential

Previous studies have suggested that mineralizing bacteria, especially manganese-mineralizing bacteria, are present in

TABLE 3 Unique genera that not fully present in all samples (top 10, n.d: not detected).

Unique genera	Relative abundance (%)			
	Nodule (WNA)	Sediment (WSA)	Nodule (ENB)	Sediment (ESB)
Psychrobium	n.d	n.d	0.38	1.69
Sulfitobacter	n.d	n.d	0.15	1.01
Moritella	0.26	n.d	0.07	0.28
Halomonas	n.d	0.01	0.43	0.04
wb1-A12	n.d	0.23	0.02	0.39
Leptospirillum	0.40	n.d	n.d	n.d
Blastocatella	n.d	0.18	0.04	0.13
Ulvibacter	0.16	0.07	n.d	0.04
IS-44	n.d	0.14	0.01	0.13
Lentisphaera	0.11	0.03	n.d	n.d

TABLE 4 Specific species in nodules and the surrounding sediments (removing unsigned and uncultured species).

Species	Relative abundance (%)			
	Nodule (WNA)	Sediment (WSA)	Nodule (ENB)	Sediment (ESB)
Halomonas sp. 148Z-13	0.00	0.01	0.42	0.04
Halomonas sp. CSM-2	0.00	0.00	0.02	0.00
Alcanivorax marinus	0.00	0.00	0.01	0.06
Moritella sp. BSw20747	0.09	0.00	0.03	0.10
Pseudoalteromonas sp. 520P1 No. 423	0.00	0.00	0.03	0.06
Pseudomonas pachastrellae	0.00	0.00	0.02	0.01
Roseovarius sp. TM1035	0.00	0.00	0.01	0.01
Bradyrhizobium japonicum	0.00	0.00	0.02	0.00

nodules and actively contribute to the mineralization process (Blöthe et al., 2015; Reykhard and Shulga, 2019; Jiang et al., 2020b). SEM-EDS analysis has indicated the potential involvement of biological components, including bacteria, in mineralization (He et al., in press). Advances in sequencing technologies could further elucidate the mineralization functions of different bacterial species.

For example, members of the Magnetospiraceae and Hyphomicrobiaceae have been identified as playing significant roles in the iron cycle (Matsunaga et al., 1991; Shulga et al., 2022). The Magnetospiraceae group, characterized by magnetotaxis, also exhibits a unique iron-capturing ability that influences the distribution and migration of iron in the environment (Molari et al., 2020). Meanwhile, the Hyphomicrobiaceae group, as iron-oxidizing bacteria, alters the chemical form and reactivity of iron, thereby affecting its solubility, mobility, and bioavailability. This group may also indirectly impact the cycling of other metal elements (Molari et al., 2020). Additionally, Shewanella and Colwellia are known to participate in manganese reduction processes (Blöthe et al., 2015). Several Shewanella strains are also capable of oxidizing manganese to form manganese oxide nanoparticles (Omoike and Chorover, 2006; Bräuer et al., 2011; Wright et al., 2016). In addition, *Shewanella* may also participate in the Fe cycle, exemplified by *Shewanella oneidensis MR-1*, which is considered a model strain for dissimilatory iron reduction and is capable of mediating metal reduction through the production of EPS (Yan et al., 2021). EPS, composed of polysaccharides, proteins, and lipids, are biopolymers that serve as a prerequisite for biofilm formation (Liu et al., 2020) and act as carriers for biogeochemical processes such as cell adhesion, mineralization, and microbial metal redox cycling (Zheng et al., 2019). It should be noted that 16S rRNA sequencing has limitations in resolution and sensitivity, which may hinder accurate representation of the actual bacterial community composition (Poretsky et al., 2014). Future studies could employ metagenomics for a more comprehensive characterization of microbial community structure and functional traits (Arıkan and Muth, 2023; Satya et al., 2024).

4.2.2 Unique bacteria with mineralization potential

Several genera, which were temporarily detected only in specific samples, also exhibit mineralization potential. For instance, *Leptospirillum*, which was detected exclusively in western Pacific nodule samples, possesses the ability for obligate Fe(II) oxidation and is widely distributed in metal-rich environments, suggesting its potential role in mineralization (Barrie Johnson, 2015). *Lentisphaera*, which was detected exclusively in western Pacific samples, has multiple strains capable of secreting transparent exopolymers, potentially facilitating metal adsorption on the bacterial surface and subsequent precipitation (Choi et al., 2013).

Similarly, several genera, including *Sulfitobacter* (Templeton et al., 2005), *Halomonas* (Templeton et al., 2005), *Marinobacter* (Handley and Lloyd, 2013), *Pseudoalteromonas* (Wu et al., 2013), and *Marinomonas* (Xuezheng et al., 2008), have been temporarily detected exclusively in nodules from the eastern Pacific (Supplementary Table S1) (He et al., in press). Strains of these manganese-oxidizing bacteria have been successfully isolated and cultured under laboratory conditions, demonstrating their ability to oxidize Mn(II) (Templeton et al., 2005; Zhang et al., 2019). Among these, *Pseudoalteromona* has received considerable attention for its production of extracellular organic compounds (Bowman, 2007), with EPS serving as effective carriers for inducing or controlling mineralization processes. For example, the strain *Pseudoalteromonas* TG12 has been reported to produce EPS capable of binding metals in sediments (Gutierrez et al., 2008). In addition, *Marinobacter* and *Alcanivorax*, both exclusively detected in eastern Pacific samples, have shown the ability to oxidize Fe(II) (Handley et al., 2009; Sudek et al., 2009; Smith et al., 2011). Beyond iron and manganese, some bacteria from eastern samples can also facilitate the oxidation of other metals. For instance, *Acinetobacter* has been found to oxidize Cr (Zakaria et al., 2007), while *Marinobacter* can oxidize As (Handley et al., 2009). While this does not diminish the discussion on bacterial roles in mineralization, the limitations of sequencing methods necessitate further evidence to definitively

confirm the presence or absence of bacterial taxa across samples (Mainali et al., 2017).

4.2.3 Mineralization potentials of specific bacterial species

Moreover, by utilizing the BIOCYC database (Caspi et al., 2015, 2016), several species with the potential for Mn(II)-oxidizing have been identified among species detected (Table 4): *Roseovarius* sp. TM1035, *Pseudoalteromonas* sp. 520P1 No. 423, and *Bradyrhizobium japonicum* were all found exclusively in nodules from the eastern Pacific. The enrichment of these species with Mn (II) oxidation capabilities may correlate with the higher Mn content in the nodule from the eastern Pacific (Table 2). However, the specific oxidation pathways and enzymes involved in the Mn oxidation process of these species still require further clarification.

Besides, *Roseovarius* sp. TM1035 can produce biofilm to adapt to changes in the underlying environment (Kent et al., 2018). Biofilm, an EPS containing embedded bacteria, aids in bacterial resistance against adverse environmental stresses and also provides sites on its surface for metal oxidation, mineral aggregation, and precipitation, potentially facilitating the mineralization process (Wang and Müller, 2009; Decho, 2010). Some strains of *Pseudoalteromonas* can also generate biofilms for the removal of Pb(II), Cr(VI), and Cd(II) (Priyadarshanee and Das, 2021), and some strains of *Bradyrhizobium* has been found to employ an EPS-mediated defense mechanism for metal precipitation (Santamaría et al., 2003). However, given our experimental results, more precise functional analysis or prediction of key genes may be required to further enhance our understanding of the mineralization functions of the aforementioned bacteria. It's also important to recognize that the relative abundance data alone may not conclusively indicate the exclusive presence of certain bacterial taxa in specific samples, given potential variations arising from factors like limited sequencing depth and methodological biases.

4.3 Potential discrepancies in the nodule growth environment

The growth environments of polymetallic nodules in the western and eastern Pacific may differ. In the western Pacific, polymetallic nodules are thought to be primarily hydrogenetic (Mn/Fe ~ 1), with metals precipitating from oxygen-rich near-bottom seawater, enriching Fe and Co, while nodules from the eastern Pacific appear to be more significantly influenced by diagenetic processes, with metal precipitation occurring in sediment pore spaces, enriching Mn, Cu, and Ni (Halbach et al., 1981, 1988). The elemental composition of nodules is likely influenced by factors such as surface primary productivity, organic carbon content in the seafloor, and redox conditions. In the western Pacific, low primary productivity and high dissolved oxygen in bottom waters may contribute to the rapid oxidation of Fe and Co to stable +3 oxidation states (Liu and Millero, 2002; Dutkiewicz et al., 2020; Jiang et al., 2020a). In contrast, in the eastern Pacific, higher primary productivity and organic carbon content in sediments may promote the formation of metal-organic

complexes, potentially enriching Mn, Cu, and Ni in the sediments (Verlaan et al., 2004). As organic matter degrades, reducing conditions at the seafloor may release Mn and other elements in their 2+ states into pore waters, which could further contribute to the enrichment of these elements in the nodules (Jorgensen, 2001; Arndt et al., 2013).

The differing genesis of polymetallic nodules may also suggest variations in the hydrodynamic and geological conditions under which the nodules form. Nodules of hydrogenetic origin in the western Pacific typically develop in relatively stable aquatic and geological environments, facilitating the growth of near-spherical nodules characterized by rhythmic, dense, and uniform laminae (Figure 2A, Figures 3A, B) (Li et al., 2020). In contrast, nodules from the eastern Pacific are predominantly influenced by diagenetic processes, displaying stromatolitic-like growth structures with broader fissures and pores (Figure 2D, Figures 3D, E). These features indicate that nodule growth in this region was influenced by ongoing environmental modifications, including seafloor bioturbation and bottom currents (Veillette et al., 2007; Wegorzewski and Kuhn, 2014).

Furthermore, the differences in microbial community composition across nodule fields in different marine regions may also reflect variations in the environmental conditions. For example, notable distinctions were observed when comparing the bacterial communities identified in this study with those from other Mn nodule fields, Such as the Korea Deep Ocean Study (KODOS) (Cho et al., 2018) and the German license area (Blöthe et al., 2015) in the Clarion-Clipperton Fracture Zone (CCFZ), as well as the Kara Sea (Vereshchagin et al., 2019)and the South Pacific Gyre (Shiraishi et al., 2016). For instance, *Marinobacter* and *Idiomarina*, two genera that are mostly strictly aerobic, were abundant in the KODOS area (Ivanova et al., 2000; Green et al., 2006). However, they were not detected in samples from the western Pacific in this study, and their abundances in samples from the eastern Pacific were extremely low (<0.2%), potentially linked to variations in environmental oxygen levels. Moreover, *Shewanella* and *Colwellia*, as potentially important genera in the nodule formation in this study, were not detected in the KODOS area and were only found in nodules from the German license area. However, *Colwellia* exhibited widespread enrichment in sediments from the Kara Sea (2%) and the South Pacific Gyre (>50%). Both of these two regions are characterized by extremely low surface primary productivity (D'Hondt et al., 2009; Demidov et al., 2017), suggesting that *Colwellia* may accumulate in environments with exceptionally low carbon content due to its capability to degrade recalcitrant substances (Tully and Heidelberg, 2013). The acquisition of more detailed environmental data in future studies may provide further empirical support for our hypothesis.

5 Conclusion

This study provides valuable insights into the relationship between microorganisms and polymetallic nodule formation in the Pacific Ocean. Through SEM-EDS analysis, we discovered various biological-like structures capable of enriching metal elements on their surfaces, indicating their potential role in promoting nodule formation and growth. Several bacteria-like microspheres may suggest the

presence of mineralizing bacteria within the nodules. Furthermore, 16S rRNA gene sequencing identified several bacteria with mineralization potential. These bacteria were found to be involved in metal redox cycles through physiological metabolism or by facilitating mineral deposition through the production of biofilms or EPS. Additionally, differences in elemental compositions, internal structures, and bacterial community compositions may indicate variations in the growth environments of nodules across different regions. In the results and discussion, we drew upon literature to clarify the bio-analogous types and the functions of bacteria (especially potential mineralization functions). Further research is needed to explore the specific mechanisms of interaction between microorganisms and minerals, and to investigate the potential applications of these findings in areas such as deep-sea mining and environmental management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

XH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. QL: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – review & editing. XL: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. ZL: Data curation, Methodology, Resources, Writing – review & editing. HW: Methodology, Resources, Writing – review & editing. ZZ: Methodology, Resources, Writing – review & editing. YD: Methodology, Resources, Writing – review & editing. JL: Methodology, Resources, Writing – review & editing. HL: Methodology, Resources, Writing – review & editing.

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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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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1533654/full#supplementary-material

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