# **Deep-ocean Manganese Nodules:** Formation Processes, Geomicrobiology, and Economic Prospects

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# **1. Introduction & History**

Manganese nodules are comprised of Mn–Fe oxide precipitates and are found in a limited number of marine environments in the abyssal plains of the global ocean. Perhaps the most studied is the Clarion and Clipperton Zone (CCZ) in the Pacific Ocean due to its economically significant deposits. This area stretches from the west coast of Mexico to Hawaii, approximating six million square km (Van Nijen et al., 2018). The composition of polymetallic nodules in the CCZ includes Mn, Fe, Cu, Ni, and Co, alongside enrichments in Mo, Zr, Li, Y (Wegorzewski & Kuhn, 2014). The CCZ seafloor, characterized by oxic conditions down to approximately 2–2.5 m in sediment depth, holds sediments primarily consisting of clay and siliceous ooze at water depths ranging from 4000–6000 m (Cronan, 2017).

Manganese nodules were first discovered on the Challenger Deep-Sea Expedition which took place from 1872 to 1876. Dredging equipment brought up metal-rich concretions from the deep ocean floor, subsequently sparking interest and curiosity among scientists about the nature and origin of them. The following years saw increased research and exploration focused on understanding their composition, distribution, and formation processes (Barnes & Dymond, 1967). Additional expeditions of the late 19<sup>th</sup> to mid 20<sup>th</sup> century such as the German Gazelle Expedition (1874-1876) and the American Albatross Expedition (1888-1904), contributed to the collection of nodules from the ocean. Post-World War II brought about many technological advancements including the development of remotely operated vehicles (ROVs) which were subsequently used in the study of nodules (Martin Ludvigsen et al., 2014). This period was one of renewed interest in marine resources including manganese nodules as a potential source of critical metals.

The initial decades of research on manganese nodule formation led to a general and temporary consensus among the scientific community consisting of the following: The occurrence of processes involving the primary oxidation of manganese to MnO2, followed by the agglomeration of MnO2 into nodules and a potential role played by bacterial oxidation, transforming manganese in seawater into Mn(IV) and incorporating it into the nodule matrix (Ehrlich et al., 1973). Alongside this consensus from the scientific community, came the industry consensus on how economically significant manganese nodules could be for the future. Together, science and industry slowly popularized nodules and their status as potential resource reservoirs

for diverse elements, recorders of regional and global oceanic and climatic conditions, and controllers of element concentration and metal redox states in the global ocean (Sparenberg, 2019).

In 1982 the United Nations Convention on the Law of the Sea (UNCLOS) was established as a legal foundation for exploitation of resources in international waters. In association, the International Seabed Authority was created to actively monitor and regulate seabed mining activities. After several countries attempted experimental deep-sea mining projects in the late 80's and 90's, environmental concerns and economic challenges led to a pullback in commercial mining interest. For the better part of the 30 years, the outlook on utilizing nodules as a source for critical materials has remained stagnant. However, the increasing technological advancements of the modern day and hopes that they will one day provide the means necessary for mining nodules efficiently has kept research related to them relevant.

# 2. Environmental Implications

#### 2.1 Background

To many, the most familiar part of the ocean is that which is filled with sunlight known as the epipelagic zone (0-200m). This is largely because most of the marine life that inhabits this zone, including the ever-important photosynthetic microorganisms, are commonly discussed. However, the epipelagic is just a sliver of the ocean biome which descends to depths of around 11,000m at its deepest point in the Mariana Trench. To better conceptualize just how vast the ocean is, one should consider that 79% of the entire volume of Earth's biosphere consists of waters with depths that exceed 1,000m. It's true that many scientists claim we in fact understand more about the surface of the Moon and Mars than we do about our own ocean. Thus, deep sea research is vitally important and continuously reshapes the way we think about aqueous geochemistry, biogeochemical cycling, geomicrobiology, and habitability.

The ocean has been divided into two major realms known as *pelagic* and *benthic*. Pelagic, derived from the ancient Greek word pélagos ('open sea'), is a zone that is home to free swimming and floating organisms. It can be understood as a water column that is further subdivided by depth due to the environmental changes that are associated with consecutively

deeper waters such as, pressure increase, temperature and light decrease, oxygen decrease and micronutrient change. These subdivisions are known as zones and are formally divided into the following: Epipelagic (<200m), mesopelagic (200-1,000m), bathypelagic (1,000 - 4,000m), abyssopelagic (4,000 - 6,000m) and the hadopelagic (6,000 - 11,000m). Everything beyond the mesopelagic is void of sunlight entirely.

Benthic, derived from the ancient Greek word béthos ('the depths'), describes the environment defined as the bottom sedimentary surface of a body of water. The organisms that thrive there are commonly known to live in close relationship with the bottom substrate. Many of them are attached to the bottom permanently, also known as *sessile*, while others use it to burrow. In the ocean, benthic environments are mostly divided by depth in correspondence to the pelagic zones. The intertidal zone is an exception to this and has no pelagic equivalent as it describes the area where sea meets land. The subtidal (continental shelves, to about 200m), the bathyal (continental slopes, to about 4,000m), the abyssal (most of the deep seafloor, 4,000-6,000m) and the hadal (the trenches, 6,000-11,000m).



Fig 1. Pelagic zones shown in an arbitrary virtual cross section of the Mariana Trench. Source: www.epicdiving.com

#### 2.2 Impact

The removal of manganese nodules will undoubtedly disturb and damage benthic organisms living on and around the nodules. Organisms known as megabenthic, which includes species such as anemones, barnacles, corals, and other sessile life have a close association with the environment that the nodules are in. Although it may be difficult to determine exactly how these disturbances will affect specific species, we know from decades of climatology and marine sciences that these are often complex systems, and any preconceived perturbation may turn out to be a forcing (Jackson, 2010; Tennant et al., 2016).

The nematode assemblage in the CCZ extends to an estimated 400 species and stand as just one example of a community that would be directly affected. Their community has raised concern with biologists due to the phylogenetic clustering among them, exhibiting a high risk of local extinctions even before mining activities are accounted for (Macheriotou et al., 2020). Sediment plumes produced by mining technology are expected to impact pelagic phytoplankton communities which serve as one of the oceans greatest primary producers. Disruption of the base of a food chain often has far-reaching consequences, many of which are impossible to predict. It's also unclear how the resuspension of heavy metals and other contaminants produced during mining could introduce toxins into the food chain. In addition, noise and vibrations created by mining activity are of great concern as they can disturb marine mammals. Studies conducted on orca whales and dolphins have demonstrated that tampering with local sound frequency can disrupt feeding, communication, and even migration (Erbe et al., 2019; Solé et al., 2023).

#### 2.3 Policy

Environmental impact investigations began in 1972 with a comprehensive study conducted at Columbia University's Lamont-Doherty Earth Observatory (Ozturgut et al. 1997). This laid the foundation for the large-scale environmental investigations that followed the Deep Ocean Mining Environmental Study (DOMES: 1975–1980) conducted by the USA (Ozturgut et al. 1978) and the listing of potential environmental impacts within the Programmatic Environmental Impact Study (PEIS: 1981). Research on environmental impacts continued with the Marine Environmental Research Plan 1981–1985, which emphasized significant concerns regarding two crucial environmental impact factors. This study highlighted the destruction of benthic organisms

in and near the collection path. It also emphasized the risks associated with blanketing of the benthos and the dilution of their food supply away from the mining site (NOAA 1982; Ozturgut et al. 1997).

Verification of the Marine Environmental Research Plan was conducted by the USA, Japan, the Interoceanmetal Joint Organization (IOM), and India. These collective examinations verified significant environmental impacts associated with deep-sea mining activities (Kaneko et al. 1997; Sharma et al. 2003). Additionally, long-term monitoring investigations were carried out 17 years, 5 years, and 44 months after the environmental impact by Japan, IOM, and India, respectively (Ingole et al. 2005; Stoyanova 2014; Deep Ocean Resources Development Co Ltd 2015). This emphasis on environmental implications was marked by the G7 Summit's call for the International Seabed Authority to develop a transparent and effective framework for sustainable deep-sea mining that incorporates the considerations of all stakeholders, especially the interests of developing nations.

Additionally, the increasing attention towards deep-sea mining has coincided with the execution of projects like Blue Mining, MIDAS, INDEEP, JPI Oceans, and DOSI, which are directed towards environmental impact assessment and conservation actions linked to deep-sea mining . Certain non-governmental organizations (NGOs) have advocated for a stop to deep-sea mining activities under the rationale that the environmental impacts are ambiguous and that the extraction of deep-sea minerals is fundamentally unsustainable. In view of this, the ISA undertook a comprehensive survey in 2015 to engage and understand the perspectives of stakeholders on deep-sea resource development, categorizing the inquiry into economy, environmental conservation, safety, and a general overview (G7 Germany, Schloss Elmau 2015).

To achieve consonance with environmental impact assessments, and to parallel the examinations of many other leading bodies, the ISA devised nine regulated marine areas, known as Areas of Particular Environmental Interest (APEIs). These APEIs are strategically positioned around and within the CCZ and are designed to ensure that the totality of the CCZ's biodiversity is preserved, despite mining activities in portions of it. Concurrently, within Japan's jurisdiction, the Japan Oil, Gas and Metals National Corporation has investigated environmental conservation strategies at the genetic level for seabed hydrothermal deposits, selecting various locations with ongoing gene flow for excavation and distinguishing areas where development should be

avoided . Considering the combined global endeavors, including those by the UN, there is an understanding that deep-sea resource development must advance in harmony with international environmental conservation actions.

# **3. Economic Prospects**

## 3.1 Background

After the initial studies on manganese nodules (Ehrlich, 1963; Ehrlich et al., 1973), exploratory programs began which led to several organizations and governmental bodies laying claim to massive sections of seafloor. These sections held potential resources in international waters. Exclusive rights from the ISA was seen as extremely valuable. From 1994 to 2010 eight Pioneer Investors, otherwise known as contractors, (Japan, China, Russia France, Korea, Germany and InterOceanMetal) were given rights to mine polymetallic nodules (Table 2). By 2015, the number of contractors had increased to 25 and the mining parameters were broadened to nodules, crusts, and sulfides (Table 1). Continuous evaluation of information on the mining techniques and resource distribution has driven the interest in nodule deposits throughout the last few decades (Pearson 1975; Glasby 1977; Cronan 1980, 2000; UNOET 1982, 1987; Dick 1985; Rona 2003).

Research on ferromanganese deposits from the Christmas Island region and seamounts of the Indian Ocean (Exon et al. 2002; Banakar et al. 2007) as well as the Marshall Island area of the Pacific Ocean (Usui et al. 2003) and the granting of permits to private companies for exploration of seafloor sulphides near Papua New Guinea and New Zealand (Gleason 2008) support the case for persistent interest from researchers and mining companies. This ultimately indicates that the possibility of developing new technology for mining and exploiting ferromanganese nodules is quite real and is moving forward as a developing field. Environmental awareness giving rise to the implementation of mass recycling and the fluctuation in metal prices have in many ways, slowed the commercial exploitation of ferromanganese nodules, however this does not delineate their importance as a substantial mining resource that would aid in meeting the increasing demand for metals such as Fe, Ni, Mn, Co, Mo, Cu and Rare Earth Elements (Kotlinski 2001). Several estimates have been conducted to show that the average

metal content in ferromanganese nodules are higher than those found in terrestrial deposits by factors of 1.1 for Ni and >5 for Co as of 2021.

Nodule/ metal	Mean concentr-ation <sup>a</sup>	Resource potential t (Mt) <sup>b</sup>	Metal production per year t (Mt) @ 1.5 Mt/year @ 3 Mt/year		Price of metal (\$/Kg) <sup>c</sup>	Gross in-place value of metal \$/year @ 1.5 Mt/year @ 3 Mt/year		Gross in-place value of metal \$/20 years @ 1.5 Mt/year @ 3 Mt/year	
Wet nodules	-	375,000,000 (375)	-	-	-	-	-	-	-
Dry nodules	25% of wet nodulesd	281,250,000 (281.25)	-	-	-	-	-	-	-
Manganese	22/24% of dry nodules	61,800,000 (61.8)	330,000 (0.33)	660,000 (0.66)	1.32	435,600,000 (435.6 million)	871,200,000 (871.2 million)	8.712 billion	17.424 billion
Nickel	1.0/1.1% of dry nodules	2,810,750 (2.81)	15,000 (0.015)	30,000 (0.030)	23.00	345,000,000 (345.0 million)	690,000,000 (690 million)	6.90 billion	13.80 billion
Copper	0.78/1.04% of dry nodules	2,190,000 (2.19)	11,700 (0.0117)	23,400 (0.0234)	8.30	97,110,000 (97.11 million)	194,220,000 (194.22 million)	1.9422 billion	3.8844 billion
Cobalt	0.23/0.1% of dry nodules	281,250 (0.281)	1500 (0.0015)	3000 (0.003)	39.20	58,800,000 (58.8 million)	117,600,000 (117.6 million)	1.176 billion	2.352 billion
Total (metals)	24.01/26.24%	67,081,000 (67.081)	358,200 (0.3582)	716,400 (0.7164)	-	936,510,000 (936.51 million)	187,302,000 (1873.02 million)	18.7302 billion	37.460 billion

Table 1. Resource potential and metal production estimates courtesy of www.metalprices.com

# 3.2 Outlook & Technical Considerations

Each of the UN contractors have been allotted several thousand square kilometers. If we analyze the resource availability of ferro-manganese nodules in a 75,000km<sup>2</sup> area, the total available resource could be upwards of 281Mt dry and a total metal of ~67Mt with concentration values of Mn = 22%, Ni = 1.0%, Cu = 0.78%, Co = 0.1% (Table 1). The total annual production of metals could range from 0.358 Mt/year to 0.716Mt/year. Considering average metal prices for a given period the value of total metals produced annually will be \$936.5 million, with a total yield of about \$18.73 billion in 20 years from a single mine-site at 1.5 Mt/year mining rate. Despite the undeniable potential that deep sea mining holds, they still cannot be economically recovered under the current economic conditions, however market conditions and technological advancements seem to be moving towards a point in which the profit margins are acceptable.

The size of a given mine site is becoming important for both economic and environmental reasons. A single mining operation carried out under specific geologic and technical conditions is what constitutes a 'mine site'. The following is the criteria proposed for mining ferro-manganese nodules by the UNCLOS:

- Cut off grade = 1.8% Cu + Ni
- Cut off abundance = 5 kg/m2
- Topography = acceptable

- Duration (D) = 20 years
- Annual recovery (Ar) = 3 million dry tones, which has been subsequently pro- posed as 1.5 million tones by ISA (2008a).

Using this information, the total mineable area (M) can be estimated as follows:

$$M = A_t - \left(A_u + A_g + A_a\right)$$

Where,

 $A_t$  = total area  $A_u$  = un-mineable area due to topography  $A_g$  = area below cutoff grade

 $A_a$  = area below cutoff balance

In addition, the size of the mine site  $A_s$ , can be determined via:

$$A_s = \frac{(A_r)(D)}{(A_n)(E)(M)}$$

Where,

 $A_s$  = size of mine-site (km<sup>2</sup>)

 $A_r$  = annual nodule recovery rate (dry tonnes/year)

D = duration of mining operation (years)

 $A_n$  = average nodule abundance in the mineable area

E = overall efficiency in the mining device (%)

M = proportion of mineable area

		General location of the					
Contractor	Sponsoring state	exploration area under contract					
Contractors for exploration for polymetallic nodules							
InterOceanMetal Joint Organization	Bulgaria, Cuba, Czech, Poland, Russia, Slovakia	Clarion-Clipperton Fracture Zone (CCFZ), Pacific Ocean					
Yuzhmorgeologiya	Russia	CCFZ, Pacific Ocean					
Government of the Republic of Korea	Korea	CCFZ, Pacific Ocean					
China Ocean Mineral Resources Research and Development Association	China	CCFZ, Pacific Ocean					
Deep Ocean Resources Development Co.	Japan	CCFZ, Pacific Ocean					
Institut français de recherche pour l'exploitation de lamer	France	CCFZ, Pacific Ocean					
Bundesanstalt fur Geowissen Schaften und Rohstoffe	Germany	CCFZ, Pacific Ocean					
Nauru Ocean Resources Inc.	Nauru	CCFZ, Pacific Ocean					
Tonga Offshore Mining Limited	Tonga	CCFZ, Pacific Ocean					
UK Seabed Resources Ltd.—I	UK	CCFZ, Pacific Ocean					
G-TEC Mineral Resources NV	Belgium	CCFZ, Pacific Ocean					
Marawa Research and Exploration Ltd.	Kiribati	CCFZ, Pacific Ocean					
Ocean Mineral Singapore Pte Ltd	Singapore	CCFZ, Pacific Ocean					
Cook Islands Investment Corporation	Cook Islands	CCFZ, Pacific Ocean					
UK Seabed Resources Ltd.—II	UK	CCFZ, Pacific Ocean					
Government of India	India	Indian Ocean					
Contractors for exploration for ferromanganese crusts							
Government of the Russia	Russia	Pacific Ocean					
China Ocean Mineral Resources Research and Development Association	China	Pacific Ocean					
Japan oil, Gas and Metals National Corporation	Japan	Pacific Ocean					
Contractors for exploration for hydrothermal sulphides							
Institut français de recherche pour l'exploitation de la mer	France	Mid-Atlantic Ridge					
Government of the Russia	Russia	Mid-Atlantic Ridge					
Government of the Republic of Korea	Korea	Central Indian Ridge					
China Ocean Mineral Resources Research and Development Association	China	Southwest Indian Ridge					
Govt of India	India	Southwest Indian Ridge					
Bundesanstalt fur Geowissen Schaften und Rohstoffe	Germany	Southeast and Central Indian Ridge					

**Table 2.** Contractors for exploration for polymetallic nodules, ferromanganese crusts, and hydrothermal sulphides.Source: (Macheriotou et al., 2020)

# 4. Formation & Classification

# 4.1 Initial Formation

Manganese nodules are found in deposits that are within the upper 10-15cm of sea floor due to a combination of current mechanics and upward ionic diffusion through the sediment (Hein and Koschinsky 2013). In many cases, nodule formation is divided up among different types and then individual formation types are studied in detail. It's important to keep in mind that current studies suggest most nodules are the product of multiple formation types, although the exact number is debated. (Duliu et al., 2008; Koschinsky & Hein, 2003; Wang et al., 2008; Wegorzewski & Kuhn, 2014). This should be a core perspective when conducting research on nodules.

The same cool bottom current mechanism is simultaneously responsible for halting the burial of nodules beneath sediment and sorting coarser objects which in turn become nuclei for new nodules (Sanderson, 1985). In essence, the cool bottom currents act as a crude filter for deposited material, sweeping away fine sediments while leaving larger material behind. This can range from shark teeth or the shells of small marine organisms, to pumice or dense sediment



Figure 2. Graphic representation of manganese nodule constituents and associated ratios with respect to nodule type. Source: Blöthe et al., 2015

fragments. These currents are also responsible for transporting oxygen-rich water from the surface to the seafloor which is necessary for hydroxide compounds to precipitate at any significant rate. Minerals are drawn to the nuclei due to local mineral saturation and availability of nucleation sites for mineral growth, leading to the aggradation of initial oxides (Lyu et al., 2021). The type of growth that more often initiates the aggradation process has been difficult to determine, and there are several plausible arguments for both sides (Popoola et al., 2021; Wang et al., 2008; Wegorzewski & Kuhn, 2014).

Duliu et al., made important insights by considering what the formation itself can reveal about the chemistry. Nodules act as elemental scavengers that take advantage of the elements which are only present in trace concentrations. It's because of this action that nodules contain relatively high concentrations of elements such as Li and Co. Since the incorporation of trace metals is largely governed by the mineralogy of the nodule, we can conclude that the variation in trace metal uptake is controlled by changes in formation type.



Figure 3. Graphic representation of manganese nodule mineralogy and chemistry with respect to nodule type. Source: Blöthe et al., 2015

### 4.2 Classification

In *hydrogenetic* formation, the growth rate is 1-2 millimeters per million years. Dissolved Mn2+ and Fe2+ in oxygen rich ocean waters give rise to the subsequent accretion of Mn4+ and Fe3+ around the nucleus via microbial oxidation (Greenslate, 1974). Hydrogenetic precipitates are known to contain a near 1:1 ratio of Fe and Mn, high Th/U and low Y/Ho ratios. High concentrations of Co, Te, Ce and Pt are due to their oxidation at the MnO2 surface (Fig 2). This has been recognized as a hyper-efficient mechanism for enriching trace metals that can overcome the electrostatic repulsion of negatively charged elements in solution by the anions at the surface (Jiang et al., 2020).

X-ray absorption near-edge structure measurements have shown a presence of Co, Ce and Te in a higher oxidation state within the hydrogenetic layers than in the surrounding water which that suggests manganese derived redox processes are a major contributor in the incorporation of trace elements in high concentrations (Wegorzewski & Kuhn, 2014). Studies utilizing X-ray diffraction have revealed amorphous ferrihydrite and cryptocrystalline Fe-vernadite to be predominant resulting phases of this process (Jiang et al., 2019). These phases have positive (ferrihydrite) and negative (Fe-vernadite) surface charges, which will become important in future subchapters. The surface charges of hydrogenetic growth layers result in attraction, surface absorption and enrichment of metals. Negatively charged MnO2 attract Cu2, Ni2 and Co2 cations while positively charged iron oxyhydroxides FeOOH attract the anions of aqueous uranium carbonate species derived from foraminiferal calite UO2(CO3)22-. The low charge density of FeOOH on the surface also allows for covalent bonding to neutral molecules of titanium hydroxide Ti(OH)4 (Duliu et al., 2008; Lyu et al., 2021).

In contrast, the phyllomanganates that constitute *diagenetic* growth such as buserite, balance negative charge disparities by incorporating cations of Ni2, Cu2 and Li2 (Fig 3). At the root cause of these disparities are either prevalent lattice defects or the substitution of Mn2/Mn3 cations in place of Mn4. Diagenetic layers consist of high Mn/Fe ratios typically >10 in bulk analyses, high Y/Ho, high Ni, Cu, and Li, and low Th/U ratios (Duliu et al., 2008; Popoola et al., 2021). In diagenetic growth, the growth rate is placed at tens of millimeters per million years. Metals derived from micronodules in the sediment are remobilized via biomixing and organic material decomposition in underlying sediment layers (Jeong et al., 1994). As organic matter is

oxidized it causes the reduction and dissolution of Mn oxides and the release of associated elements such as Ni, Li, Cu, Co, and more (de Lange et al., 1992). The fluctuation of the concentration gradient caused by sedimentary mineral variation, allows the now reduced minerals to diffuse upwards until they are re-oxidized upon contact with the oxygen rich ocean water giving rise to disordered phyllomanganates. This type of growth requires higher sedimentation rates than hydrogenetic growth which has been shown to reflect climatic variation in biological activity and suboxic conditions in underlying sediments (Duliu et al., 2008).



Fig. 4 A ternary diagram of pore filling hydrogenetic crystal growth versus structural diagenetic growth (Duliu et al., 2008).

## 4.3 Additional Formation

High- resolution computer tomography (μ-CT) analysis of manganese nodules has provided high-quality information about the interplay between different growth types. In many cases, dendritic crystals that are diagnostic of diagenetic growth have been identified in areas that are crucial for the nodule framework, allowing pore systems to give rise to secondary oxic hydrogenetic

growth when the climatic conditions are suitable (Fig 4). Once the nodule is in an oxic environment, the presence of pore systems act as nutrient and molecular oxygen conduits to fuel microbial growth and redox activity (Jiang et al., 2019; Nitahara et al., 2011). Nutrients and molecular oxygen provide the necessary components for the nodule to function as a microbial habitat. Pore spaces later fill with the formation of oxic-hydrogenetic growth unique to the environment. These findings have also been supported by electron microprobe data that shows high Mn/Fe ratios (11-505) and high Ni +Cu contents (1.5-6 wt%) in areas with dendritic growth and low Mn/Fe ratios (0.98-3.27) and low Ni + Cu contents (0.3-1.9 wt%) with increase Co (0.3-1.9 wt%) those without (Nitahara et al., 2011).

Marine microbes, particularly bacteria and archaea are believed to play a significant role in hydrogenetic nodule formation. Filamentous bacilli and coccoid bacteria have been found associated with biofilms that are commonly found on the surface of manganese nodules (Blöthe et al., 2015; Zhang et al., 2019). The precipitation of manganese oxides carried out by microorganisms is the byproduct of metabolic redox mechanisms. This role of manganese oxide precipitation is not unique to the realm of manganese nodule microbes. Metabolic activities that utilize Mn(II) and Mn(III/IV) have been well documented in the precipitation of other ferromanganese oxide crusts on the seabed and rock varnishes in terrestrial regions (Nagy et al., 1991; Palmer et al., 1986).

# 5. Geomicrobiology of Manganese Cycling Communites

#### 5.1 Biofilms

Although there is substantial evidence that microorganisms are responsible for nodule formation and growth, there is still some debate on the exact mechanisms and relationships at play (Shiraishi et al., 2016). The discovery and pioneering research by Ehrlich (1963), examined manganese nodule formation via incubation experiments in the laboratory. The results of these experiments revealed a complex bacterial community structure within manganese nodules, with Proteobacteria being the dominant group. Additionally, other bacterial groups such as Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, and Planctomycetes were also identified in the clone library at the time. The study indicated the presence of ammonia-oxidizing bacteria and archaea, sulfate-reducing bacteria, and Acidobacteria, supporting his suggestion of enzymatic manganese oxidation. In order to gain a comprehensive understanding of the biogenic relationships we will work from the foundation up, starting with biofilms. A *biofilm* is a complex community of microorganisms that adhere to surfaces which exert a primary control on their formation process by factors such as textutre and chemistry (Zhang et al., 2019).

Their distinct structural patterns reflect the intricate and highly organized communities of microorganisms that form them. The structural and spatial organization of microorganisms in biofilms is based on the cellular properties such as growth differentiation, chemotaxis, and cell-to-cell signaling in concert with the external conditions/constraints of surface texture and chemistry (Zhang et al., 2019). Biofilms are in many ways constructed to serve as protection against environmental stresses and are commonly made up of multiple types of organisms. Here we will briefly discuss a few examples and the broad classification scheme. Biofilms are classified into external (surficial) communites known as *exolithic* and internal (subsurface)

communites known as *endolithic* (Wang et al., 2008). Filamentuous bacteria and actinobacteria living on the surface of a nodule from the CCZ shown in fig 5, are a great examples of common exolithic organisms. The bacilli bacterial cells exhibit division of transverse septa while the actinobacteria exhibit a concave helical morphology and are loosely attached to the substrate.

The endolithic communities are known for being far more ordered and compact (Fig 6). The structure seen in D,E, and F consists of cone-like cells characterized by a size of 800 nm in their longitudinal axis and 300 nm in the cross axis. These cones are covered by secondary mineral deposits. They are arranged in a highly ordered manner, with individual cones separated evenly and aligned in perfect orientation with perpendicular linear patterns. Additionally, they exhibit a loose embedding in the matrix, leaving well-defined trace when removed from the support. J, K, and L show a honeycomb



Fig. 5 Exolithic bacilli and coccus bacteria (Wang et al., 2008).

patterned biofilm structure, characterized by individual heavily mineralized cells. They form equant to tabular hexagonal prisms with granular surface texture and exhibit a unilayered palisade when viewed laterally.

#### 5.2 Biomineralization

In many cases it is the biofilms in the nodules that are responsible for the biomineralization that makes up hydrogenetic growth. *Biomineralization* is the formation of inorganic minerals or polymers by a biological organism and is known to contribute greatly to global mineral deposition. Biomineralization is classified into the following categories: (I) biologically induced mineralization, meaning precipitation of minerals on a biological/environmental interphase, (II) biologically controlled mineralization, which allows the biologically guided formation of defined structures, e.g. bone formation or – in general – skeletal formation. The latter category is further divided into (II-A) biologically controlled extracellular mineralization, during which minerals are formed extracellularly at three-dimensional organic matrices, e.g. on collagen and (II-B)

biologically controlled intercellular mineralization, during which mineralization starts between cells (Mann, 2001).

Biofilms present on nodules are largely believed to be associated with biomineralization in the from of Mn(II) oxidation leading to the presence of Mn(III/IV) oxides on the surface of nodules due to enzymatic oxidative metabolism. Manganese oxidation as a whole requires the use of ligands such as Br, F, I or Cl ions. Ligands can significantly change the rate of oxidative reactions by altering the availability of electrons or by modifying the reactivity of the oxidants involved (Morgan et al., 2021). This effect can be observed in various chemical and biological systems, where the presence of specific ligands can either hinder or facilitate the oxidation of certain substances. Microorganisms that utilize enzymatic oxidation take advantage of this and effectively accelerate aqueous Mn2+ oxidation up to five orders of magnitude compared to that of abiotic processes (Saratovsky et al., 2006).



**Fig. 6** Endolithic microbial biofilms. Overview of general area (A, B, C). Cone shape microbes (D, E, F). Hexagonal microiail biofilm (J, K, L)(Wang et al., 2008).

In addition to mineralized biofilms, tangled manganese oxide filaments produced in spherule aggregates can be found inside the pores of nodules contributing to hydrogenetic growth (Fig 7). Some sources suggest these are mineralized methane oxidizing bacteria (MOB) such as the classification scheme of mineralized microbes in nodules by Wang and Müller, 2009. The oxide filaments are characteristic of a high catalytic effect due to abundant enzyme sites, which is likely to improve the efficiency of mineralized microbes in nodules, along with surficial bacilli bacteria. MOB are one of the most common mineralized microbes in nodules, along with surficial bacilli bacteria. MOB are often found to be more abundant in pores that are deep in the nodule and close to the core. The variations in microbe distributions in nodules imply that microbes respond



Fig. 7 Manganese oxide filamentslinked to MOB (Jiang et al., 2020).

to variable environmental conditions. These are II-A biomineral producing biofilms and bacteria characteristic of a polymer network with various chemical compositions based on complex gene clusters (Wolfaardt et al., 1995). It is the micro-consortia of species that provides stability and in turn, the capability to sustain harsh conditions of low temperatures and a nutrient deprived deep-sea environment (Flemming and Wingender, 2010)

#### 5.3 Taxonomy

This subsection considers various methods that are commonly deployed to perform genetic sequencing and then reviews the taxonomic identifications made thus far. 16S rRNA gene analysis performed on 10g of nodule from the CCZ has showed that the number of bacterial operational taxonomic units (OTUs) ranges from 1087 to 1304 (97% sequence identity), which is much higher than that for other crusts and seafloor basalts (Santelli et al., 2008; Blöthe et al., 2015; Jiang et al., 2020)(Fig. 8). Archaeal taxonomic diversity was found



Fig. 8 Operational taxanomic units identified in a subset of CCZ managense nodules (Jiang et al., 2020).

to be low, with archaeal OTUs ranging from 3 to 7. The bacterial community diversity is high in manganese nodules where different positions have similar compositions, with only the percentages of Cyanobacteria and Proteobacteria varying. Phylogenic gene analyses indicate that bacterial clones related to redox functions represent up to 10% of all obtained clones (Jiang et al., 2020). Some OTUs (OTU23654, OTU3455, OTU4495, OTU49706, OTU50415, and OTU54186) belonging to Bacteroidetes, Gemmatimonadetes, Firmicutes, and Actinobacteria are 99% identical to uncultured Fe-reducing microorganisms extracted from anoxic soils and river sediments.

Two OTUs (OTU15067 and OTU29034) belong to Proteobacteria and Firmicutes, respectively, OTU15067 is closely (99% sequence identity) related to uncultured Mn-oxidizing bacteria from a soil manganese nodule in China and to bacteria found in deep-sea polymetallic nodules (Wu et al., 2013) and the ocean crust (Santelli et al., 2008). OTU29034 is closely related (99% sequence identity) to an uncultured bacterium from soil manganese nodules (He et al., 2008) and identified Mn-oxidizing bacteria from a contaminated former uranium mine (Akob et al., 2014). In addition, ten OTUs (OTU17884, OTU23947, OTU25201, OTU25567, OTU35571, OTU45804, OTU52876, OTU6259, OTU7227, and OTU9450) detected in nodules share 99% identity with anaerobic ammonium oxidation bacteria extracted from anoxic environments such as lake sediments, wastewater, or agricultural soil (Tal et al., 2005; Yang and Li, 2012; Wan et al., 2014). One identified OTU, OTU46265, belongs to the Proteobacteria family and shares 99% identity with uncultured clones that are related to the nitrogen cycle (Jung et al., 2012; Oshiki et al., 2018). OTU29034 is also closely related (99% sequence identity) to the anaerobic redox cycling of iron (Weber et al., 2006).

Ammonium- and manganese-oxidizing bacterial clones dominate the microbial community related to redox functions in manganese nodules (Wu et al., 2013). The archaea Thaumarchaeota, Bathyarchaeota, and Euryarchaeota have been detected, of which, Thaumarchaeota and Euryarchaeota are the dominant phyla, but Bathyarchaeota was only detected in deeper layers and represents 14% of the total archaeal sequences conducted on nodules (He et al., 2008). Assuming that Bathyarchaeota can undergo mineralization the same as bacterial counterparts, it may be them that are responsible for the spheroid filaments found only deep within the nodule (fig of spheroid balls). Especially given that the filaments have been associated with methane oxidizers and Bathyarchaeota is known to utilize methane as an energy source (Zhou et al.,

2018). The major archaeal OTU (with an average of 311 sequences in each layer) belongs to marine group I Thaumarchaeota and shares  $\geq$ 98% sequence identity to Nitrosopumilus maritimus SCM1, which is an ammonia-oxidizing archaeon (Könneke et al., 2005; Shiraishi et al., 2016). The remaining OTUs in the Euryarchaeota and Thaumarchaeota share high identity with uncultured bacteria from abyssal marine sediments. Different analyzed positions in the nodules have variable OTU compositions at the kingdom, phylum, class, order, family, genus and species classification levels.



*Fig. 9* OTUs, Oxides, and nodule structure with respect to various layers. L2-L3 represents core and L1-L4 represents outer layers (Jiang et al., 2020).

#### 5.4 Distribution

Primary controls on the biodiversity may also take place in the form of total available pore space, leading to the importance of studying genus and species distribution. Younger surface layers have more micro-scale pores than the older central areas of most nodules (Greenslate, 1974; Yang and Li, 2012; Wan et al., 2014). OTU numbers are representative parameters of community diversity that is greatly influenced by the distribution of micron-scale pores; thus, younger surface layers must provide a better micro-environment for microbes. The distribution of organic matter and reduced Mn content is consistent with this control (fig 9) (Jiang et al., 2020). Different organic carbon components have been detected in the profile of XPS results from over 200 Pacific and Atlantic nodules including hydrocarbons, carboxylic acids, esters, carbonyl groups, and peptides which could act as an index for microbial activity in the pore structure within the nodules (Flemming and Wingender, 2010; Blöthe et al., 2015). Metal oxide formation caused by biogeochemical cycling within nodules, i.e., secondary precipitation, could

explain the opposite distributions of Mn and microscale pores (Fig. 9); such metal oxides fill the pores and enable further enrichment of Mn through the utilization of reduced Mn within pore structure. The Fe distribution is consistent with the microscale pores because it is mainly due to external input and could imply secondary precipitation from inner areas to younger growth areas.



Fig. 10 Phyolgenetic clusters of bacterial and archael 16s rRNA gene clones obtained from the nodule outside, inside, and surrounding sediment (Shiraishi et al., 2016)

To better understand community distribution, we must incorporate new methods in addition to genetic sequencing and taxonomic identification. Nucleic acid staining and cryo-sectioning techniques combined with PCR has allowed researchers to achieve precise and accurate distribution results (Shiraishi et al., 2016). These techniques have shown that bacterial composition had small variations between layers, but a large variation between the whole nodule and surrounding sediments (Fig. 10). A prominent difference was the dominance of Deltaproteobacteria in the nodule and Chloroflexi in the surrounding sediment. Some taxa were detected only from the outside of the nodule (Deferribacteres, Actinobacteria, order Rickettsiales



Fig. 11 Nucleic acid stained microorganisms in a cryo thin section (Shiraishi et al., 2016)

of the Alphaproteobacteria, and order Xanthomonadales of the Gammaproteobacteria) and from the surrounding sediment (Bacteriodetes, genus Nitrospira of the Nitrospirae, and family Anaerolineaceae of the Chloroflexi).

In contrast, the diversity of the archaeal community was very low, and almost all clones from both the manganese nodule and surrounding sediment belonged to the Marine Group I (MGI) Thaumarchaeota, which

supports the findings from the previously discussed taxonomic studies. Among them, the percentage of the candidatus 'Nitrosopumilus maritimus' was higher at the outside of the nodule than the nodule inside, and few clones were detected from the surrounding sediment. Relative abundance of archaea among the prokaryotes within the same samples, were estimated at about 23% for nodule exterior (exolithix community), 52% for nodule interior endolithic community, and 29% for the surrounding sediment. Overall cell density on the surface of manganese nodules have been estimated at  $\sim 10^{8}$  cells cm=3, in contrast to the extremely low cell density of the surrounding sediment ( $\sim 10^{5}$  cells cm=3; D'Hondt et al., 2009, 2015). et al., 2015).

The results of SYBR nucleic acid staining revealed that most microbial cells were in the original small pores near the nodule surface (Fig. 11). A possible explanation for the high cell density found near the surface is active primary production by chemolithoautotrophs. However, in many cases, the sequences of most of the bacterial 16S rRNA genes isolated from the near surface are related to heterotrophs. Several cloned sequences of the family Rhodospirillaceae (Alphaproteobacteria) have been detected, and some genera in this family can grow heterotrophically under aerobic conditions (Baldani et al., 2014). Multiple studies have found chemolithoautotrophs among bacterial sequences and identified them as the notably metabolically versatile SAR324 group of the Deltaproteobacteria which includes a large group of anaerobic oxidizers (Tully and Heidelberg, 2013; Wu et al., 2013). In contrast, archaeal 16S rRNA gene sequences have been identified in relation to the MGI Thaumarchaeota. Many of these archaea conduct their chemolithoautotrophy by oxidizing ammonia in the ocean water and

provide primary carbon to the biogeochemical cycles involved (Ingalls et al., 2006; Nitahara et al., 2011; Walker et al., 2010)

The common presence of MGI Thaumarchaeota in manganese nodules in the studies discussed, suggests that anammox, a globally important component of the nitrogen cycle, is common in the nodule environments (Tully and Heidelberg, 2013; Wu et al., 2013; Shiraishi et al., 2016; Nitahara et al., 2017). A two step oxidation of ammonia to nitrite has been considered (Saratovsky et al., 2006). First and second oxidation are carried out by ammonia monooxygenase and multi-Cu oxidase (MCO protein) during biotic ammonia oxidation (Walker et al., 2010). MCO is an enzyme containing one or several Cu ions as cofactors and is the most common among Mn oxidase (Zhang et al., 2015b). This implies that anammox is most likely accompanied by Mn oxidation leaving much to consider about nodules and the potential weight of their contribution to several biogeochemical cycles.

# 6. Conclusion

The study of manganese nodules has provided many insights into the dynamic ecosystem that harbor them. The bacterial diversity of over 1000 OTUs shows just how remarkable the microbial communities in nodules are. The identification of specific functional groups, such as manganese oxidizers, iron reducers, and anammox bacteria underscores their pivotal role in the biogeochemical cycling of key elements at the sea floor. Contrasting distribution in the community structure between surface, interior and surrounding sediments emphasizes these points further.

As the demand for critical minerals grows, the potential large-scale mining of manganese nodules becomes more of a reality. However, the ecological significance of these deep-sea habitats, and crucial role played by their residents underscores how important it is that we proceed with caution. Continued research in this field, integrating multiple approaches from microbiology, marine biology, geochemistry, and conservation science will be crucial in maintaining responsible management of deep-sea mining.

# References

Barnes, S. S., & Dymond, J. R. (1967). Rates of Accumulation of Ferro-manganese Nodules. *Nature*, *213*(5082), 1218–1219. https://doi.org/10.1038/2131218a0

Blöthe, M., Wegorzewski, A., Müller, C., Simon, F., Kuhn, T., & Schippers, A. (2015).
Manganese-Cycling Microbial Communities Inside Deep-Sea Manganese Nodules. *Environmental Science & Technology*, 49(13), 7692–7700.
https://doi.org/10.1021/es504930v

David Spencer Cronan. (2017). Handbook of Marine Mineral Deposits. Routledge.

- de Lange, G. J., van Os, B., & Poorter, R. (1992). Geochemical composition and inferred accretion rates of sediments and managanese nodules from a submarine hill in the Madeira Abyssal Plain, eastern North Atlantic. *Marine Geology*, *109*(1-2), 171–194. https://doi.org/10.1016/0025-3227(92)90227-9
- Duliu, O. G., Alexe, V., Moutte, J., & Szobotca, S. A. (2008). Major and trace element distributions in manganese nodules and micronodules as well as abyssal clay from the Clarion-Clipperton abyssal plain, Northeast Pacific. *Geo-Marine Letters*, 29(2), 71–83. https://doi.org/10.1007/s00367-008-0123-5
- Ehrlich, H. L. (1963). Bacteriology of Manganese Nodules. *Applied Microbiology*, *11*(1), 15–19. https://doi.org/10.1128/am.11.1.15-19.1963

Ehrlich, H. L., Yang, S. H., & Mainwaring, J. D. (1973). Bacteriology of manganese nodules.
VI. Fate of copper, nickel, cobalt, and iron during bacterial and chemical reduction of the manganese (IV). *Zeitschrift Für Allgemeine Mikrobiologie*, *13*(1), 39–48.
https://doi.org/10.1002/jobm.19730130103

- Erbe, C., Marley, S. A., Schoeman, R. P., Smith, J. N., Trigg, L. E., & Embling, C. B. (2019).
  The Effects of Ship Noise on Marine Mammals—A Review. *Frontiers in Marine Science*, 6(606). https://doi.org/10.3389/fmars.2019.00606
- Greenslate, J. (1974). Microorganisms participate in the construction of manganese nodules. *Nature*, *249*(5453), 181–183. https://doi.org/10.1038/249181a0

Jackson, J. B. C. (2010). The future of the oceans past. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1558), 3765–3778. https://doi.org/10.1098/rstb.2010.0278

- Jeong, K. S., Kang, J. K., & Chough, S. K. (1994). Sedimentary processes and manganese nodule formation in the Korea Deep Ocean Study (KODOS) area, western part of Clarion-Clipperton fracture zones, northeast equatorial Pacific. *Marine Geology*, *122*(1-2), 125–150. https://doi.org/10.1016/0025-3227(94)90208-9
- Jiang, X.-D., Gong, J.-L., Ren, J.-B., Liu, Q.-S., Zhang, J., & Chou, Y.-M. (2020). An interdependent relationship between microbial ecosystems and ferromanganese nodules from the Western Pacific Ocean. *Sedimentary Geology*, 398, 105588. https://doi.org/10.1016/j.sedgeo.2019.105588
- Jiang, X.-D., Sun, X.-M., & Guan, Y. (2019). Biogenic mineralization in the ferromanganese nodules and crusts from the South China Sea. *Journal of Asian Earth Sciences*, 171, 46– 59. https://doi.org/10.1016/j.jseaes.2017.07.050
- Koschinsky, A., & Hein, J. R. (2003). Uptake of elements from seawater by ferromanganese crusts: solid-phase associations and seawater speciation. *Marine Geology*, 198(3), 331– 351. https://doi.org/10.1016/S0025-3227(03)00122-1

- Lyu, J., Yu, X., Jiang, M., Cao, W., Saren, G., & Chang, F. (2021). The Mechanism of Microbial-Ferromanganese Nodule Interaction and the Contribution of Biomineralization to the Formation of Oceanic Ferromanganese Nodules. *Microorganisms*, 9(6), 1247– 1247. https://doi.org/10.3390/microorganisms9061247
- Lyu, J., Yu, X., Jiang, M., Cao, W., Saren, G., & Chang, F. (2021b). The Mechanism of Microbial-Ferromanganese Nodule Interaction and the Contribution of Biomineralization to the Formation of Oceanic Ferromanganese Nodules. *Microorganisms*, 9(6), 1247– 1247. https://doi.org/10.3390/microorganisms9061247
- Macheriotou, L., Rigaux, A., Derycke, S., & Vanreusel, A. (2020). Phylogenetic clustering and rarity imply risk of local species extinction in prospective deep-sea mining areas of the Clarion–Clipperton Fracture Zone. *Proceedings of the Royal Society B: Biological Sciences*, 287(1924), 20192666. https://doi.org/10.1098/rspb.2019.2666
- Martin Ludvigsen, Johnsen, G., Sørensen, A. J., Lågstad, P. A., & Ødegård, Ø. (2014). Scientific Operations Combining ROV and AUV in the Trondheim Fjord. *Marine Technology Society Journal*, 48(2), 59–71. https://doi.org/10.4031/mtsj.48.2.3
- Morgan, J. J., Schlautman, M. A., & Bilinski, H. (2021). Rates of Abiotic Mn<sup>II</sup> Oxidation by O<sub>2</sub>: Influence of Various Multidentate Ligands at High pH. *Environmental Science & Technology*, 55(21), 14426–14435. https://doi.org/10.1021/acs.est.1c01795
- NAGY, B., NAGY, L. A., RIGALI, M. J., JONES, W. D., KRINSLEY, D. H., & SINCLAIR, N.
  A. (1991). Rock varnish in the Sonoran Desert: microbiologically mediated accumulation of manganiferous sediments. *Sedimentology*, *38*(6), 1153–1171.
  https://doi.org/10.1111/j.1365-3091.1991.tb00376.x

- Palmer, F. E., Staley, J. T., Murray, Counsell, T. J., & Adams, J. B. (1986). Identification of manganese-oxidizing bacteria from desert varnish. *Geomicrobiology Journal*, 4(4), 343– 360. https://doi.org/10.1080/01490458609385943
- Popoola, S. O., Adegbie, A. T., Akinnigbagbe, E. A., & Unyimadu, J. P. (2021). Geochemistry of ferromanganese micronodules recovered from sediment-core in the western Nigeria continental margin, Eastern Equatorial Atlantic: Implications on the genesis and depositional history. *Journal of African Earth Sciences*, *184*, 104369. https://doi.org/10.1016/j.jafrearsci.2021.104369
- Rahul Sharma, & Springerlink (Online Service. (2017). *Deep-Sea Mining : Resource Potential, Technical and Environmental Considerations*. Springer International Publishing.
- Sanderson, B. (1985). How bioturbation supports manganese nodules at the sediment-water interface. *Deep Sea Research Part A. Oceanographic Research Papers*, 32(10), 1281– 1285. https://doi.org/10.1016/0198-0149(85)90010-x
- Saratovsky, I., Wightman, P. G., Pastén, P. A., Jean François Gaillard, & Poeppelmeier, K. R. (2006). Manganese Oxides: Parallels between Abiotic and Biotic Structures. 128(34), 11188–11198. https://doi.org/10.1021/ja062097g
- Shiraishi, F., Mitsunobu, S., Suzuki, K., Hoshino, T., Morono, Y., & Inagaki, F. (2016). Dense microbial community on a ferromanganese nodule from the ultra-oligotrophic South Pacific Gyre: Implications for biogeochemical cycles. *Earth and Planetary Science Letters*, 447, 10–20. https://doi.org/10.1016/j.epsl.2016.04.021
- Solé, M., Kaifu, K., Mooney, T. A., Nedelec, S. L., Olivier, F., Radford, A. N., Vazzana, M.,
  Wale, M. A., Semmens, J. M., Simpson, S. D., Buscaino, G., Hawkins, A., Aguilar de
  Soto, N., Akamatsu, T., Chauvaud, L., Day, R. D., Fitzgibbon, Q., McCauley, R. D., &

André, M. (2023). Marine invertebrates and noise. *Frontiers in Marine Science*, *10*. https://doi.org/10.3389/fmars.2023.1129057

- Sparenberg, O. (2019). A historical perspective on deep-sea mining for manganese nodules, 1965–2019. *The Extractive Industries and Society*, 6(3), 842–854. https://doi.org/10.1016/j.exis.2019.04.001
- Tennant, J. P., Mannion, P. D., Upchurch, P., Sutton, M. D., & Price, G. D. (2016). Biotic and environmental dynamics through the Late Jurassic-Early Cretaceous transition: evidence for protracted faunal and ecological turnover. *Biological Reviews*, 92(2), 776–814. https://doi.org/10.1111/brv.12255
- Van Nijen, K., Van Passel, S., & Squires, D. (2018). A stochastic techno-economic assessment of seabed mining of polymetallic nodules in the Clarion Clipperton Fracture Zone. *Marine Policy*, 95, 133–141. https://doi.org/10.1016/j.marpol.2018.02.027
- Wang, X., Schloßmacher, U., Wiens, M., Schröder, H. C., & Müller, W. E. G. (2008). Biogenic
  Origin of Polymetallic Nodules from the Clarion-Clipperton Zone in the Eastern Pacific
  Ocean: Electron Microscopic and EDX Evidence. *Marine Biotechnology*, *11*(1), 99–108.
  https://doi.org/10.1007/s10126-008-9124-7
- Wegorzewski, A. V., & Kuhn, T. (2014). The influence of suboxic diagenesis on the formation of manganese nodules in the Clarion Clipperton nodule belt of the Pacific Ocean. *Marine Geology*, 357, 123–138. https://doi.org/10.1016/j.margeo.2014.07.004
- Zhang, W., Ding, W., Li, Y.-X., Tam, C., Bougouffa, S., Wang, R., Pei, B., Chiang, H., Leung,
  P., Lu, Y., Sun, J., Fu, H., Bajic, V. B., Liu, H., Webster, N. S., & Qian, P.-Y. (2019).
  Marine biofilms constitute a bank of hidden microbial diversity and functional potential. *Nature Communications*, 10(1). https://doi.org/10.1038/s41467-019-08463-z

Zhou, Z., Pan, J., Wang, F., Gu, J.-D., & Li, M. (2018). Bathyarchaeota: globally distributed metabolic generalists in anoxic environments. *FEMS Microbiology Reviews*, 42(5), 639– 655. https://doi.org/10.1093/femsre/fuy023