

Environmental impacts & risks of deep-sea mining: recommendations for exploitation regulations

Edited by

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Environmental impacts & risks of deep-sea mining: recommendations for exploitation regulations

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Role of polymetallic-nodule dependent fauna on carbon cycling in the eastern Clarion-Clipperton Fracture Zone (Pacific)

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The abyssal seafloor in the Clarion-Clipperton Fracture Zone (CCZ) in the central Pacific is covered with large densities of polymetallic nodules, i.e., metal concretions containing iron, manganese, nickel, cobalt, and copper. Nodules are of economic importance for these metals, but they also host a variety of deep-sea fauna. In a recent study it was estimated that the removal of these nodules would lead to a loss of up to 18% of all taxa in the CCZ. Here, I assess the impact of removing these nodule-dependent taxa on carbon cycling at two sites (B4S03, B6S02) of the Belgian exploration license area in the eastern CCZ. For this purpose, I developed two highly resolved carbon-based food web models with 71 (B6S02) to 75 (B4S03) food-web compartments consisting of different detritus pools, bacteria, metazoan meiobenthos, macrobenthic isopods, polychaetes and other macrobenthos, megabenthic cnidarians, crustaceans, poriferans, holothurians and other invertebrate megabenthos, and fish. These compartments were connected with 303 (B6S02) to 336 (B4S03) links which were reduced by 5–9% when nodule-dependent faunal compartments were removed. The models estimated the “total system throughput” $T_{..}$ i.e., the sum of all carbon flows in the food webs, in intact food webs as $1.18 \text{ mmol C m}^{-2} \text{ d}^{-1}$ and $1.20 \text{ mmol C m}^{-2} \text{ d}^{-1}$ at B4S03 and B6S02, respectively, whereby 69.8% (B6S02) to 71.2% (B4S03) of $T_{..}$ flowed through the microbial loop. A removal of the nodule-dependent fauna did not affect this microbial loop but reduced the scavenger loop by 56.5% (B6S02) to 71.6% (B4S03). Overall, nodule-dependent fauna is responsible for only a small fraction of total carbon cycling at the eastern CCZ. Therefore, when the effect of prospective deep-seabed mining on carbon cycling is investigated, its impact on benthic prokaryotes and the microbial loop should be addressed specifically.

KEYWORDS

abyssal plains, linear inverse model, ferromanganese nodules, network indices, food-web model

1 Introduction

The abyss between 3,000 and 6,000 m water depth is the largest ecosystem on our planet (Gage and Tyler, 1991) and covers 85% of the seafloor (Harris et al., 2014). It consists of abyssal hills, abyssal mountains, and abyssal plains. The latter can have a difference in elevation between hilltops and valleys of 0 to 300 m and are often very flat with slopes of less than 0.05° (Cormier and Sloan, 2018). Abyssal plains are subdivided by mid-ocean ridges, like the East-Pacific Rise, deep-sea trenches, and island arcs, and interspersed with hills and mountains (Smith et al., 2008).

Abyssal plains are covered with polymetallic nodules in areas with well oxygenated bottom waters, sedimentation rates of less than 20 mm kyr⁻¹, and high numbers of shark teeth, shells of plankton, or small rocks at the sediment surface that can act as nuclei for these nodules (Hein et al., 2013; Hein and Koschinsky, 2014; Petersen et al., 2016). The nodules are concretions of iron oxide-hydroxide and manganese oxide (Hein and Koschinsky, 2014) and contain nickel, copper, cobalt, titanium, molybdenum, lithium, rare earth elements, and yttrium (Petersen et al., 2016). They form with growth rates of 1 to 10 mm million yr⁻¹ (Petersen et al., 2016). Due to their high content of metals that are currently in demand for the transition from a fossil fuel-based economy to a renewable-energy based economy, polymetallic nodules are considered a potential future source of cobalt, copper, nickel, and rare earth elements (Hein et al., 2013).

Areas with polymetallic nodule densities of economic interest are the Central Indian Ocean Basin, the seabed around the Cook Islands, and the Clarion-Clipperton Fracture Zone (CCZ) (Kuhn et al., 2017). The CCZ is located south of Hawaii and west of Mexico in the central Pacific Ocean between 0°N, 160°W and 23.5°N, 115°W (International Seabed Authority, 2011). It has very variable nodule densities between on average ~1.5–3 kg m⁻² in the south to on average ~7.5 kg m⁻² in the east (Washburn et al., 2021) and experiences a north-west (1.3 mg C_{org} m⁻² d⁻¹) to south-east (1.8 mg C_{org} m⁻² d⁻¹) gradient in particulate organic carbon flux (Vanreusel et al., 2016). These two environmental variables together with seafloor topography drive the different habitat classes in the CCZ (McQuaid et al., 2020).

Polymetallic nodules are not only interesting from an economic point of view, but also provide essential hard substrate for deep-sea benthos: Stalked sponges, such as *Hyalonema* (*Cyliconemaoida*) *ovuliferum* (Kersken et al., 2018), the encrusting sponge *Plenaster craigi* (Lim et al., 2017), and large xenophyophores (Gooday et al., 2017) grow on them. In fact, Veillette et al. (2007) identified 73 protozoan and 17 metazoan taxa, mainly of macrofaunal size, attached to polymetallic nodules from the CCZ. This nodule epibenthos includes a variety of taxa, e.g., polychaetes, bryozoans, crustaceans, molluscs, and foraminiferans (Mullineaux, 1987; Gooday et al., 2015), of which more than half are suspension or deposit feeders (Mullineaux, 1989). Furthermore, Vanreusel et al. (2016) showed that even mobile benthos might benefit from the presence of nodules as their densities were higher in areas with nodules compared to nodule-free areas. To quantify the role of polymetallic nodules for trophic and non-trophic interactions in the CCZ, Stratmann et al. (2021) recently presented a highly resolved interaction-web model. When the authors removed the nodules

from the interaction web, they detected knock-down effects causing the loss of 17.9% of all taxa and 30.6% of all network links. Their detailed model estimated that 4% of all meiobenthos (i.e., benthos >32 µm), 50% of the macrobenthos (i.e., benthos >250 µm/500 µm), 45% of the invertebrate megabenthos (benthos >1 cm), and 0.5% of the fish were missing and the most impacted phyla were Bryozoa, Cnidaria, Platyhelminthes, and Porifera. Several of the taxa affected by nodule removal in the CCZ as modelled by Stratmann et al., (2021) occur in the exploration license area of the Belgian company *Global Sea Mineral Resources* (GSR) in the eastern CCZ (Supplementary Table 1). Therefore, by evaluating which of the taxa that disappeared due to the modelled knock-down effects are part of the present food-web model (short summary is presented in Supplementary Table 1), and by subsequently excluding these from the model, one should be able to assess the impacts of polymetallic nodule removal on carbon cycling.

Short-term carbon cycling in the abyss is dominated by prokaryotes (Stratmann et al., 2018b; Sweetman et al., 2019), with the contribution of macrobenthos to phytodetritus cycling increasing after several days (Witte et al., 2003). In fact, carbon-based food-web models for deep-sea stations in the NE Atlantic, the NE Pacific, and the SE Pacific estimated that prokaryotes contribute >70% to total benthic respiration at these specific sites (van Oevelen et al., 2012; Dunlop et al., 2016; Durden et al., 2017; de Jonge et al., 2020). These carbon-based food web models are based on the principle of mass conservation and combine physiological parameters (e.g., assimilation and growth efficiency, secondary production, mortality, respiration), with site-specific flux constraints on carbon influx and loss, and biomasses of individual food-web compartments to calculate the carbon flows between compartments in the pre-defined topological food-web (Soetaert and van Oevelen, 2009; van Oevelen et al., 2010). They have been used previously to assess the potential recovery from a small-scale sediment disturbance in the SE Pacific (Stratmann et al., 2018a; de Jonge et al., 2020) and here, they will be applied to estimate changes in carbon flows depending on the presence and absence of nodule-dependent fauna at two sites of the GSR exploration license area in the CCZ.

I will assess (1) the potential small-scale variability in carbon cycling between two sites in the north-eastern CCZ that lay approximately 280 km apart, and (2) the potential reduction in total carbon cycling that the removal of nodule-dependent fauna from the abyssal food web might cause.

2 Materials and methods

2.1 Study site

In the north-eastern CCZ, the GSR exploration license area stretches from 16°N, -128°E to 13°N, -122°E and includes three non-adjacent areas, the so-called B2, B4, and B6 areas (Figure 1). The most western area B2 is located at ~15°43' N, -126°42' E, the central area B4 is located at ~14°6' N, -125°52' E, and the most eastern area B6 is located at ~13°51' N, -123°17' E. Inside the B4 and B6 areas, two 10×20 km sampling sites, the B4S03 (14.112°N,

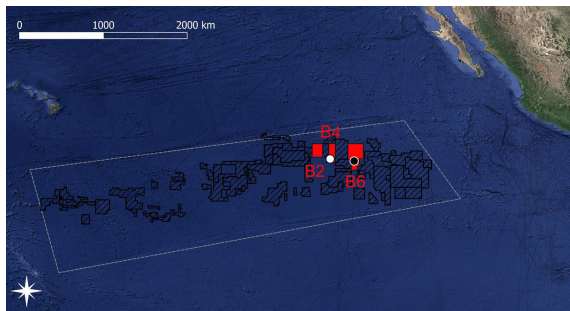


FIGURE 1

Map of the exploration license areas (black dashed areas) within the Clarion-Clipperton Fracture Zone (white dashed polygon based on the working definition of the CCZ by [Glover et al. \(2015\)](#)). The Global Sea Mineral Resources (GSR) exploration license areas are shown in red and the sites B4S03 and B6S02 are indicated by white and black dots, respectively. Copyright for shapefile for the exploration license areas within the CCZ area: © International Seabed Authority 2007–2020.

-125.871°E) and B6S02 (13.894°N, -123.297°E) sites, were identified by [de Smet et al., \(2017\)](#) based on differences in polymetallic nodule properties.

Water depth at the two sites ranges between 4,470 and 4,560 m and the sediment is mainly muddy (mud content: 88.7–91.5%, sand content: 11.3–8.5%) with a median grain size of 16.5–18.5 μm ([de Smet et al., 2017](#)). Nodule density is $22.2 \pm 2.41 \text{ kg m}^{-2}$ ($\pm$ standard error) at B4S03 and $27.3 \pm 0.62 \text{ kg m}^{-2}$ at B6S02 with a nodule surface coverage of $36.2 \pm 2.95\%$ and $31.1 \pm 8.43\%$, respectively, and a nodule volume of $92.6 \pm 6.95 \text{ cm}^3$ and $69.7 \pm 13.7 \text{ cm}^3$ ([de Smet et al., 2017](#)).

2.2 Food-web structure

Food webs in the GSR exploration license area consisted of the abiotic compartments detritus (phytodetritus, semi-labile detritus, refractory detritus), dissolved organic carbon (DOC), and carrion, and the biotic compartments bacteria, metazoan meiobenthos (4 compartments), macrobenthos (39 compartments), invertebrate megabenthos (25 compartments), and fish (3 compartments). Metazoan meiobenthos and macrobenthic isopods were divided in feeding types based on peer-reviewed literature ([Supplementary Tables 2, 3](#)). The food-web compartments of all other macrobenthos, invertebrate megabenthos, and fish compartments resembled the highest taxonomic resolution possible, i.e., family level for macrobenthic polychaetes, (mainly) class level for arthropods, and phyla and class level for all other fauna.

Metazoan meiobenthos ($>32 \mu\text{m}$) included copepods and crustacean nauplii ([Pape et al., 2017](#)) that both deposit-feed on bacteria, phytodetritus, and semi-labile detritus ([Supplementary Table 2](#)). Nematodes were classified as epistrate feeders, selective, and non-selective deposit feeders following [Wieser \(1953\)](#) based on the nematode genera present in the GSR exploration license area ([Pape et al., 2017](#)). They feed on bacteria, phytodetritus, and semi-labile detritus ([Supplementary Table 2](#)).

Macrobenthos ($>300 \mu\text{m}$) contained annelids, arthropods, molluscs, brachiopods, nematodes, nemerteans, echinoderms, and sipunculans ([de Smet et al., 2017](#)), that were identified to be carnivorous, (non-selective or subsurface) deposit feeders, filter- and suspension feeders, scavengers, and omnivores using peer-reviewed literature ([Supplementary Table 3](#)). The polychaete feeding guild catalogue of [Jumars et al. \(2015\)](#) was used to classify the feeding types of polychaetes on family level. Isopods were grouped in the feeding types (selective, non-selective) deposit feeders based on the genera present in the GSR exploration license area ([de Smet et al., 2017](#)) and using information on feeding type and food sources presented in [Menzies \(1962\)](#).

Invertebrate megabenthos ($>5 \text{ cm}$) comprised decapods, anthozoans and hydrozoans, poriferans, and echinoderms, i.e., crinoids, echinoids, holothurians, and ophiuroids ([de Smet et al., 2021](#)). These taxa were classified as deposit feeders, carnivorous suspension feeders, filter and suspension feeders, omnivores, and carnivores based on peer-reviewed literature ([Supplementary Table 4](#)). The fish observed in the GSR exploration area were *Coryphaenoides* spp., *Ipynops* sp., and Osteichthyes morphotypes ([de Smet et al., 2021](#)) feeding on fish, crustaceans, polychaetes, and carrion ([Supplementary Table 5](#)). A detailed list with taxon-specific feeding types and prey as well as food sources, respectively, is presented in [Supplementary Tables 2–5](#).

2.3 Food-web links

Links of carbon transfer between the food-web compartments were implemented as presented in [Figure 2](#).

The sedimentary phytodetritus pool receives carbon input *via* degrading phytoplankton in marine snow, the semi-labile and refractory detritus pools are maintained by marine snow particles. Furthermore, fecal pellets, that are not taken up by specialized coprophagus feeders (i.e., organisms feeding on feces), contribute to the semi-labile and refractory detritus pool. Also dying metazoan meiobenthos becomes part of the semi-labile detritus pool. Dead macrobenthos, invertebrate megabenthos, and fish, in contrast, add to the carrion pool. All three detritus pools hydrolyze to DOC that is taken up by bacteria. Bacteria convert this DOC to dissolved inorganic carbon (DIC) *via* respiration and they contribute to the DOC pool when they burst due to virus-induced bacterial lysis. Carbon is lost from the modeled system *via* burial of refractory detritus, respiration of all fauna and bacteria, and by external predators that predate upon invertebrate megabenthos.

Deposit feeders graze upon bacteria, semi-labile detritus, and phytodetritus. Suspension and filter feeders filter, depending on the taxon, marine snow, crustaceans of macrobenthic size and fish out of the water column. Porifera, i.e., *Chonelasma* sp., *Docosaccus* sp., *Euplectella* sp., and other unidentified poriferans, furthermore, take up marine snow and DOC. Carnivores predate upon their specialized prey reported in [Supplementary Tables 3, 4](#), except for the polychaetes of the family Sigalionidae, for which no prey types were published. Hence, this compartment was defined to feed on prey of the same size class. The diet composition of the individual fish taxa was constrained based on the contribution of each prey

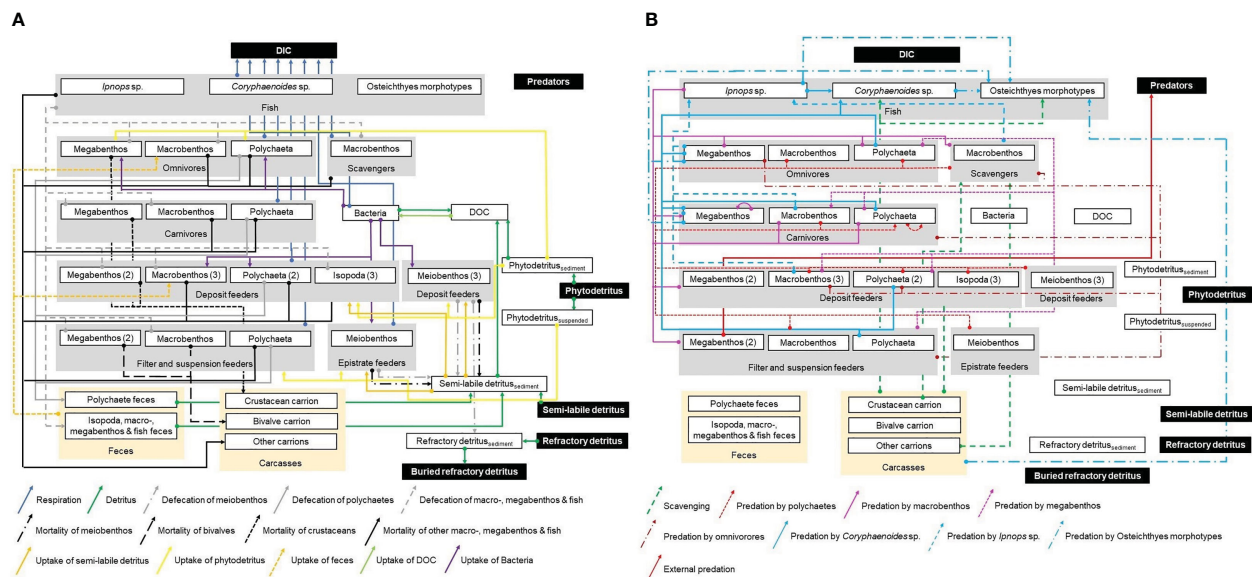


FIGURE 2

Presentation of the topological food web implemented in the linear inverse models for the GSR exploration license area. White boxes represent all compartments of the food web inside the model, in contrast to black boxes that show compartments outside the model that were not specifically modelled. Gray boxes display the feeding types epistrate feeders, filter- and suspension feeders, deposit feeders, carnivores, omnivores, scavengers, and fish. Yellow boxes represent feces and carcasses. White boxes enclosed by gray boxes show the size classes meiobenthos, macrobenthic isopods and polychaetes, other macrobenthos, invertebrate megabenthos, and the three fish taxa *Ipnotops* sp., *Coryphaenoides* spp., and *Osteichthyes* morphotypes. Numbers in brackets indicate how many kinds of a specific feeding type exist for a particular size class. Arrows show the flow of carbon from the carbon source (arrow ending with a big dot) to the carbon sink (head of arrow). Figure panel (A) shows respiration in the form of dissolved inorganic carbon (DIC), fluxes of detritus, DOC, and feces, mortality of fauna, and uptake of Bacteria, detritus, and DOC. Figure panel (B) presents scavenging and predation.

taxon to the total prey carbon stock (Supplementary Table 5); the contribution of carrion to the fish diet, however, was not further constrained.

2.4 Data sources

2.4.1 Carbon stocks of food-web compartments

Data on carbon stocks of the various food-web compartments were extracted from the literature and standardized to mmol C m⁻² as described below.

The phytodetritus carbon stock in the upper 5 cm of sediment was calculated by converting the chlorophyll content [3.80×10^{-4} µg dry mass sediment⁻¹, (Pasotti et al., 2021)] measured by high performance liquid chromatography to carbon units using a carbon:chlorophyll a-ratio of 40 (de Jonge, 1980) and a porosity of 0.858 (B4S03) and of 0.874 (B6S02) (de Smet et al., 2017) assuming a dry sediment density of 2.55 g cm⁻³. Unfortunately, no chlorophyll a concentration data was available for B6S02, so that the concentration from B4S03 was also used for B6S02. No data on semi-labile detritus, here defined as the so-called biopolymeric carbon (Fabiano et al., 1995), i.e., the sum of proteins, carbohydrates, and lipids, existed for the GSR exploration license area or any other license area in the CCZ. Therefore, the sum of the semi-labile and refractory detritus carbon stock was calculated by subtracting the phytodetritus pool from the total organic carbon

(TOC) stock based on TOC data from de Smet et al. (2017). Subsequently, the relation of semi-labile to refractory detritus of 1:17.3 from the Peru Basin (SE Pacific) (de Jonge et al., 2020) was used to calculate both carbon stocks.

Bacterial carbon stock in the upper 5 cm of sediment was measured by Pape et al. (2017) as polar lipid concentrations following the Bligh and Dyer method (Bligh and Dyer, 1959; Boschker et al., 1999) and subsequently converted to bacterial biomass by the authors using conversion factors from Middelburg et al. (2000) and Brinch-Iversen and King (1990).

Metazoan meiobenthos carbon stock in the upper 5 cm of sediment was calculated by multiplying meiobenthos densities determined in multicorer samples (inner core diameter: 10 cm; n = 3) by Pape et al. (2017) with taxon-specific individual biomass data from Supplementary Table 2. Macrobenthos carbon stock in the upper 10 cm of sediment was estimated by multiplying macrobenthos density data taken from box corer deployments (surface: 0.25 m², sediment depth: 60 cm; n = 4 at B4S03, n = 3 at B6S02) by de Smet et al. (2017) with taxon-specific individual biomasses from Supplementary Table 3. Invertebrate megabenthos and fish density data were taken from an autonomous underwater vehicle megafauna survey of the seafloor in 6 m altitude (de Smet et al., 2021) and multiplied with taxon-specific individual biomasses from Supplementary Table 4 to estimate invertebrate megabenthos and fish carbon stocks. A complete list with carbon stocks of all food-web compartments is presented in Table 1.

TABLE 1 Carbon stocks (mmol C m⁻²) of all food-web compartments at sites B4S03 and B6S02 of the GSR exploration license area.

Compartment	B4S03	B6S02
Detritus		
Phytodetritus (PhytoDet)	2.29×10^{-2}	2.03×10^{-2}
Semi-labile detritus (slDet)	522	445
Refractory detritus (rDet)	8,412	7,170
Bacteria		
Bacteria (Bac)	34.7	19.8
Metazoan meiobenthos		
Deposit-feeding meiobenthos (MeiDF)	0.84	0.78
Epistrate-feeding Nematoda (NEMeDF; Phylum: Nematoda)	3.39×10^{-2}	8.18×10^{-2}
Non-selective deposit feeding Nematoda (NEMnsDF; Phylum: Nematoda)	0.13	0.26
Selective deposit feeding Nematoda (NEMsDF; Phylum: Nematoda)	2.88×10^{-2}	6.90×10^{-2}
Macrobenthos		
Oligochaeta (Mac_Oli; Phylum: Annelida, Class: Clitellata, Subclass: Oligochaeta)	1.81×10^{-3}	0.00
Copepoda (Mac_Cope; Phylum: Arthropoda, Class: Copepoda)	6.11×10^{-2}	5.28×10^{-2}
Amphipoda (Mac_Amp; Phylum: Arthropoda, Class: Amphipoda)*	3.43×10^{-2}	4.42×10^{-2}
Cumacea (Mac_Cum; Phylum: Arthropoda, Class: Malacostraca, Order: Cumacea)	0.00	3.09×10^{-3}
Mysida (Mac_Mys; Phylum: Arthropoda, Class: Malacostraca, Order: Mysida)	3.83×10^{-3}	5.74×10^{-3}
Tanaidacea (Mac_Tan; Phylum: Arthropoda, Class: Malacostraca, Order: Tanaidacea)	0.24	0.36
Ostracoda (Mac_Ost; Phylum: Arthropoda, Class: Ostracoda)	6.05×10^{-3}	2.72×10^{-2}
Pycnogonida (Mac_Pyc; Phylum: Arthropoda, Class: Pycnogonida)	3.83×10^{-3}	0.00
Other Crustacea (Mac_otherCru; Phylum: Arthropoda, Subphylum: Crustacea)	3.71×10^{-3}	8.34×10^{-3}
Bivalvia (Mac_Biv; Phylum: Mollusca, Class: Bivalvia)	1.50×10^{-2}	8.46×10^{-3}
Brachiopoda (Mac_Bra; Phylum: Brachiopoda)*	1.05×10^{-2}	7.87×10^{-3}
Nematoda (Mac_Nema; Phylum: Nematoda)	0.12	0.13
Nemertea (Mac_Neme; Phylum: Nemertea)	5.25×10^{-2}	7.87×10^{-3}
Ophiuroidea (Mac_Oph; Phylum: Echinodermata, Class: Ophiuroidea)	9.13×10^{-3}	2.74×10^{-3}
Scaphopoda (Mac_Sca; Phylum: Mollusca, Class: Scaphopoda)	2.59×10^{-2}	3.88×10^{-2}
Gastropoda (Mac_Gas; Phylum: Mollusca, Class: Mollusca)	0.46	0.09
Sipuncula (Mac_Sip; Phylum: Annelida, Order: Sipuncula)	1.05×10^{-2}	7.87×10^{-3}
Isopoda (Phylum: Arthropoda, Class: Malacostraca, Order: Isopoda)		
Deposit-feeding Isopoda (IsoDF)	0.80	1.11
Non-selective deposit feeding Isopoda (IsonsDF)	0.11	8.56×10^{-2}
Selective deposit-feeding Isopoda (IseDF)	0.34	0.26
Polychaetes (Phylum: Annelida, Class: Polychaeta)		
Acroirridae (Pol_Acro)	1.77×10^{-2}	1.33×10^{-2}
Capitellidae (Pol_Cap)	1.77×10^{-2}	2.66×10^{-2}
Cirratulidae (Pol_Cir)	3.15×10^{-3}	6.70×10^{-3}
Euphrosinidae (Pol_Eup)	1.77×10^{-2}	1.33×10^{-2}
Glyceridae (Pol_Gly)	1.77×10^{-2}	0.00

(Continued)

TABLE 1 Continued

Compartment	B4S03	B6S02
Goniadidae (Pol_Goni)	2.22×10^{-2}	3.33×10^{-2}
Lumbrineridae (Pol_Lumb)	6.67×10^{-3}	5.0010^{-4}
Maldanidae (Pol_Mal)	0.15	0.00
Nephtyidae (Pol_Nep)	1.77×10^{-2}	0.00
Nereididae (Pol_Ner)	2.92×10^{-2}	7.30×10^{-3}
Opheliidae (Pol_Ophe)	2.66×10^{-2}	6.65×10^{-3}
Oweniidae (Pol_Owen)	1.53×10^{-3}	1.15×10^{-3}
Paraonidae (Pol_Para)	4.48×10^{-3}	5.04×10^{-3}
Phyllodocidae (Pol_Phy)	2.66×10^{-2}	6.65×10^{-3}
Sabellidae (Pol_Sab)	0.00	1.33×10^{-2}
Sigalionidae (Pol_Siga)	1.77×10^{-2}	0.00
Spionidae (Pol_Spio)	6.93×10^{-2}	0.12
Syllidae (Pol_Syl)	1.77×10^{-2}	0.00
Trichobranchidae (Pol_Tri)	1.06×10^{-2}	0.00
Invertebrate megabenthos		
Polychaeta (Meg_Pol; Phylum: Annelida, Order: Polychaeta)	8.07×10^{-3}	9.94×10^{-3}
Brisingida (Meg_Bri; Phylum: Echinodermata, Class: Asteroidea, Order: Brisingida)*	0.00	1.24
Other Asteroidea (Meg_othAs; Phylum: Echinodermata, Class: Asteroidea)	0.26	0.53
Crinoidea (Meg_Cri; Phylum: Echinodermata, Class: Crinoidea)*	7.14×10^{-3}	5.12×10^{-3}
Aspidodiadematidae (Meg_Asp; Phylum: Echinodermata, Class: Echinoidea, Order: Aspidodiadematoida, Family: Aspidodiadematidae)	0.16	5.50×10^{-2}
Ophiuroidea (Meg_Oph; Phylum: Echinodermata, Class: Ophiuroidea)	0.70	1.02
Bivalvia (Meg_Biv; Phylum: Mollusca, Class: Bivalvia)	1.25×10^{-2}	0.00
Other Mollusca (Meg_othMol; Phylum: Mollusca)	5.82×10^{-3}	0.00
Megabenthic crustaceans (Phylum: Arthropoda, Subphylum: Crustacea)		
Aristeidae (Meg_Ari)	0.23	3.73×10^{-2}
Galatheidae (Meg_Gal)	0.81	0.59
Other Decapoda (Meg_othDec)	5.62×10^{-3}	1.00×10^{-2}
Megabenthic cnidarians (Phylum: Cnidaria)		
Actiniaria (Meg_Act)*	6.34×10^{-3}	8.22×10^{-3}
Antipatharia (Meg_Ant)	0.28	0.42
Alcyonacea (Meg_Alc)	1.37×10^{-2}	9.41×10^{-2}
Ceriantharia (Meg_Cer)	0.00	0.92
Other Anthozoa (Meg_othAn)	6.80×10^{-2}	0.18
Hydrozoa (Meg_Hyd)	0.00	1.75×10^{-3}
Other Cnidaria (Meg_othCni)	6.24×10^{-5}	0.00
Holothurians (Phylum: Echinodermata, Class: Holothuroidea)		
Elpidiidae gen. sp. 2 (“double velum” morphotype in ccfczatl.com) (Meg_Elp)	0.00	1.06×10^{-2}
Other Elpidiidae (Meg_othEl)	0.00	5.28×10^{-3}
Unknown Holothuroidea (Meg_unHo)	3.17×10^{-2}	1.80×10^{-2}

(Continued)

TABLE 1 Continued

Compartment	B4S03	B6S02
Poriferans (Phylum: Porifera)		
<i>Chonelasma</i> sp. (Meg_Cho)	1.01×10^{-2}	1.86×10^{-2}
<i>Docosaccus</i> sp. (Meg_Doc)	1.09×10^{-2}	1.63×10^{-2}
<i>Euplectella</i> sp. (Meg_Eup)	1.43×10^{-2}	3.24×10^{-3}
Other Porifera (Meg_othPor)	2.90×10^{-2}	3.24×10^{-2}
Fish		
<i>Coryphaenoides</i> spp. (Fish_Cor; Phylum: Chordata, Class: Teleostei, Order: Gadiformes, Family: Macrouridae, Genus: Coryphaenoides)	1.49	1.38
<i>Ipnotis</i> sp. (Fish_Ipn; Phylum: Chordata, Class: Teleostei, Order: Aulopiformes, Family: Ipnotidae, Genus: Ipnotis)	0.19	6.15×10^{-2}
Osteichthyes morphotypes (Fish_Ost; Phylum: Chordata, Parvphylum: Osteichthyes)	0.37	0.35

*Taxa excluded in the models developed for section 3.5.

2.4.2 Site-specific flux constraints

Site-specific flux constraints were estimated by Volz et al. (2018) in a numerical diagenetic model for the GSR exploration license area and were implemented in the food-web models as presented in Table 2.

Phytodetritus deposition corresponds to labile detritus deposition in Volz et al. (2018) and semi-labile and refractory detritus deposition rates are the influx of semi-labile and refractory marine snow particles. Phytodetritus and semi-labile detritus degradation processes describe the reduction of these detritus pools by dissolution of the detritus to DOC and their uptake by fauna. Refractory detritus is degraded via dissolution of this pool to DOC, and it is buried in the sediment via the burial flux. Total C mineralization relates to aerobic respiration that is responsible for 90% of the organic matter consumption at the seafloor in the CCZ (Volz et al., 2018).

2.4.3 Physiological constraints

Constraints of physiological processes that were included in the model are presented in Table 3.

Bacterial growth efficiency *BGE* is defined as

$$BGE = \frac{BCP}{(BCP + BR)} \quad (\text{Equation 1}),$$

with *BCP* being bacterial carbon production ($\text{mmol C m}^{-2} \text{ d}^{-1}$) and *BR* being bacterial respiration ($\text{mmol C m}^{-2} \text{ d}^{-1}$).

Assimilation efficiency *AE* is defined as

$$AE = \frac{(I - F)}{I} \quad (\text{Equation 2}),$$

where *I* is food ingested and *F* relates to the feces produced (Crisp, 1971).

Net growth efficiency *NGE* is defined as $NGE = \frac{G}{(G+R)}$ (Equation 3), where *G* is the growth and *R* is the respiration (Clausen and Riisgård, 1996).

The secondary production *SP* ($\text{mmol C m}^{-2} \text{ d}^{-1}$) is calculated as

$$SP = \frac{P}{B} \text{ratio} \times C \text{ stock} \quad (\text{Equation 4}),$$

with $\frac{P}{B} \text{ratio}$ being the production/biomass ratio (d^{-1}).

The mortality *M* ($\text{mmol C m}^{-2} \text{ d}^{-1}$) always ranges from 0 to the maximum secondary production. Respiration *R* is calculated similar to secondary production as:

$$R = r \times C \text{ stock} \quad (\text{Equation 5}),$$

where *r* corresponds to the biomass-specific faunal respiration (d^{-1}).

TABLE 2 Carbon flux data ($\text{mmol C m}^{-2} \text{ d}^{-1}$) from Volz et al. (2018) were implemented in the model as equalities (single value) or inequalities [min, max].

Carbon flux	Value
Phytodetritus deposition	[0, 0.118]
Semi-labile detritus deposition	[0, 7.945×10^{-3}]
Refractory detritus deposition	[4.658×10^{-4} , ∞]*
Phytodetritus degradation	lDet stock $\times$ [0, 5.479×10^{-5}]
Semi-labile detritus degradation	slDet stock $\times$ [1.918×10^{-8} , ∞]*
Refractory detritus degradation	rDet stock $\times$ [5.479×10^{-10} , ∞]*
Burial flux of refractory detritus	[0.013, ∞]*
Total C mineralization	[7.75×10^{-2} , 0.115]

*Constraints adjusted in the fitting step.

TABLE 3 Constraints of the physiological processes bacterial growth efficiency *BGE* (-), virus-induced bacterial mortality *VIBM* (-), bacteria carbon production *BCP* (mmol C m⁻² d⁻¹), assimilation efficiency *AE* (-), net growth efficiency *NGE* (-), secondary production *SP* (mmol C m⁻² d⁻¹), mortality *M* (mmol C m⁻² d⁻¹), respiration *R* (mmol C m⁻² d⁻¹), and feeding preferences *FP* (-) implemented in the food-web models as equalities (single values) and inequalities ([minimum, maximum] values).

Physiological process	Size class	Value	Ref.
<i>BGE</i>	Bacteria	[0.27, 0.90]*	1,2
<i>VIBM</i>	Bacteria	[0.87, 0.91]	3
<i>BCP</i>	Bacteria	[0.34, 0.68]	1,3
<i>AE</i>	Metazoan meiobenthos	[0.18, 0.27]	2
	Macrobenthos	[0.68, 0.89]	2
	Invertebrate megabenthos	[0.40, 0.75]	2
	Fish	[0.84, 0.87]	2
<i>NGE</i>	Metazoan meiobenthos	[0.10, 0.96]	2
	Macrobenthos	[0.57, 0.68]	2
	Invertebrate megabenthos	[0.23, 0.61]	2
	Fish	[0.37, 0.71]	2
<i>SP</i>	Metazoan meiobenthos	[2.00 × 10 ⁻² , 0.12] × C stock	2
	Macrobenthos	[2.57 × 10 ⁻³ , 1.67 × 10 ⁻²] × C stock	2
	Invertebrate megabenthos	[3.18 × 10 ⁻⁴ , 1.47 × 10 ⁻³] × C stock	2
	Fish	[0, 6.30 × 10 ⁻⁴] × C stock	2
<i>M</i>	Metazoan meiobenthos	[0, 0.12] × C stock	2
	Macrobenthos	[0, 1.67 × 10 ⁻²] × C stock	2
	Invertebrate megabenthos	[0, 1.47 × 10 ⁻³] × C stock	2
	Fish	[0, 6.30 × 10 ⁻⁴] × C stock	2
<i>R</i>	Metazoan meiobenthos	[7.00 × 10 ⁻³ , 0.15] × C stock	2
	Macrobenthos	[3.57 × 10 ⁻⁵ , 4.21 × 10 ⁻²] × C stock	2
	Invertebrate megabenthos	[9.32 × 10 ⁻⁸ , 1.26 × 10 ⁻³] × C stock	2
	<i>Coryphaenoides</i> spp., Osteichthyes morphotypes	[3.12 × 10 ⁻⁴ , 4.84 × 10 ⁻⁴] × C stock	4-7
	<i>Ipnots</i> sp.	[1.79 × 10 ⁻⁴ , 8.54 × 10 ⁻⁴] × C stock	2
<i>FP</i>		[0.75, 1.00]	2

*Constraint adjusted in the fitting step.

In several cases, individual faunal constraints had to be adjusted in the fitting step. For these cases, the adjusted constraints are presented in [Supplementary Table 6](#).

References (Ref.): ¹(Vonnahme et al., 2020), ²(de Jonge et al., 2020), ³(Danovaro et al., 2008), ⁴(Smith, 1978), ⁵(Drazen and Seibel, 2007), ⁶(Smith and Hessler, 1974), ⁷(Drazen and Yeh, 2012).

Feeding preferences *FP* of mixed deposit feeders and carnivores and omnivores indicate the contribution of predation to their respective diets.

$$E \times x = f \quad (\text{Equation 6}),$$

$$G \times x \geq h \quad (\text{Equation 7}),$$

2.5 Linear inverse model development and network indices

Carbon-based linear inverse food-web models were developed for steady state conditions based on the topological food web presented in [Figure 2](#). These food-web models consist of linear functions in the form of equality (equation 6) and inequality matrix equations (equation 7) ([van Oevelen et al., 2010](#)):

with vector x including the unknown fluxes. The vectors f and h include empirical equality and inequality data, and the coefficients in the matrices E and G describe combinations of unknown fluxes that have to fulfill the requirements defined in the vectors f and h .

Models for the two sites consisted of 654 (B6S02)–829 (B4S03) flows, 74 (B6S02)–79 (B4S03) mass balances (i.e., food-web compartments), 0 data equalities, and 340 (B6S02)–371 (B4S03) data inequalities. This meant that the models were mathematically under-determined (74–79 equalities vs. 654–829 unknown flows). In

this case, either a single ‘best’ solution can be calculated based on the principle of parsimony or simplicity, or the uncertainty of each flow can be quantified using the likelihood approach (van Oevelen et al., 2010). Generally, the likelihood approach is recommended (van Oevelen et al., 2010), but this can be extremely computational demanding when the food-web models are exceptionally complex (see e.g. de Smet et al. (2016)). In fact, a test run on the bioinformatics server of the NIOZ Royal Netherlands Institute for Sea Research (The Netherlands) with 2,000 solutions calculated in one session required more than 1.5 months of calculation. As a result, the author decided to calculate the parsimonious solution of each food-web model and estimate ranges with the R package *LIM* v.1.4.6 (van Oevelen et al., 2010) in R v.1.3.1073 (R-Core Team, 2017). These ranges are reported for each flux in the supplementary material.

The network indices “number of links” L_{total} , “link density” LD , “connectance” C , “total system throughput” T , which is the sum of all carbon flows in the food web, Finn’s Cycling Index FCI , and the trophic level of each compartment were calculated using the R package *NetIndices* v.1.4.4 (Kones et al., 2009).

The trophic levels of the carrion pools were calculated as described in de Jonge et al. (2020) as:

$$TL_{carrion} = \sum_{j=1}^n \left(\frac{T_{j, carrion}^*}{T_{carrion}} \times TL_j \right), \quad (\text{Equation 8}),$$

where n corresponds to the number of internal food-web compartments, j is all food-web compartments, T^* is the flow matrix without external flows, and $T_{carrion}$ is the total carbon inflow to the carrion pool without external sources.

3 Results

3.1 Benthic biomass

Total carbon stocks were 8,977 mmol C m⁻² at site B4S03 and 7,646 mmol C m⁻² at B6S02 (Table 1). Most of these carbon stocks consisted of semi-labile (B4S03: 5.81%, B6S02: 5.82%) and refractory detritus (B4S03: 93.7%, B6S02: 93.8%). Phytodetritus, bacteria, and metazoan fauna accounted for, respectively, 2.55 × 10⁻⁴% (B4S03)–2.66 × 10⁻⁴% (B6S02), 0.26% (B6S02)–0.39% (B4S03), and 0.10% (B4S03)–0.14% (B6S02) of the total carbon stock.

Nematoda accounted for 0.19 mmol C m⁻² and 0.41 mmol C m⁻² of the carbon stocks at B4S03 and B6S02, respectively, while other meiobenthos (i.e., copepods and their nauplii) accounted for 0.84 mmol C m⁻² and 0.78 mmol C m⁻² (Figure 3). The carbon stock of macrobenthic Isopoda was 1.25 mmol C m⁻² and 1.46 mmol C m⁻² at the two different sites and macrobenthic polychaetes had a carbon stock of 0.47 mmol C m⁻² and 0.25 mmol C m⁻² at B4S03 and B6S02, respectively (Figure 3). The other macrobenthos carbon stock included mainly macrobenthic Nematoda, Tanaidacea, and Gastropoda, that contributed between 23.2% (B6S02) and 29.5% (B4S03) to the total carbon stock of this size class. The megabenthic carbon stock accounted for 2.71 mmol C m⁻² at the B4S03 site and 5.23 mmol C m⁻² at the B6S02 site (Figure 3). Fish had a carbon stock of 2.05 mmol C m⁻² and 1.79 mmol C m⁻² (Figure 3).

3.2 Food-web structure and trophic levels

The food webs consisted of 75 and 71 compartments at sites B4S03 and B6S02 that were connected with 336 and 303 links, respectively (Figure 4; Table 4), resulting in a link density of 4.48 at B4S03 and of 4.27 at B6S02. The connectance was 4.77 × 10⁻² at B4S03 and 4.75 × 10⁻² at B6S02.

Maximum trophic level was estimated as 4.22 (macrobenthic Ostracoda) at site B4S03 and as 4.61 (fish of the Osteichthyes morphotypes) at B6S02. Mean modelled trophic level of metazoan fauna ranged from 2.73 ± 0.08 (± standard error; B4S03) to 2.87 ± 0.10 (B6S02). Mean modelled trophic levels of exclusively carnivores were estimated to be 3.36 ± 0.08 and 3.55 ± 0.13 at site B4S03 and B6S02, respectively, and the modelled trophic levels of exclusively deposit feeders were estimated as 2.51 ± 7.75 × 10⁻² and 2.50 ± 8.56 × 10⁻².

3.3 Carbon flows

Modeled total carbon input, i.e., the deposition of detritus and filter- and suspension feeding, had an estimated value of 9.21 × 10⁻² mmol C m⁻² d⁻¹ at site B4S03 and of 9.15 × 10⁻² mmol C m⁻² d⁻¹ at B6S02 (Table 5). This input was dominated by phytodetritus deposition with a contribution of 58.3% (B6S02) to 61.8% (B4S03) to total deposition, followed by refractory detritus deposition (B4S03: 29.6%, B6S02: 33.0%). Additionally, an external pool of swimming copepods as prey for several predators (i.e., fish, antipatharians, hydrozoans, and other anthozoans) contributed 4.78 × 10⁻⁵ mmol C m⁻² d⁻¹ (B6S02) to 5.99 × 10⁻³ mmol C m⁻² d⁻¹ (B4S03) to the modelled benthic ecosystem.

Most carbon was estimated to be lost from the two sampling sites *via* respiration (85.8–86.7% of total carbon loss), whereas carbon burial accounted for 1.30 × 10⁻² mmol C m⁻² d⁻¹.

Estimated respiration ranged from 7.85 × 10⁻² mmol C m⁻² d⁻¹ (B6S02) to 8.51 × 10⁻² mmol C m⁻² d⁻¹ (B4S03). It was dominated by bacterial respiration that contributed between 44.1% (B4S03) and 48.1% (B6S02) to total respiration. Faunal respiration was estimated to be lower at B6S02 (4.08 × 10⁻² mmol C m⁻² d⁻¹) than at B4S03 (4.73 × 10⁻² mmol C m⁻² d⁻¹) and was dominated by metazoan meiobenthos at B6S02 and by metazoan meiobenthos and macrobenthos at B4S03 (Table 5).

Modeled carbon ingestion of fauna is summarized in Supplementary Figures 1–4. Estimated uptake of carbon by metazoan meiobenthos (Supplementary Figure 1) was largest and accounted for 8.63 × 10⁻² mmol C m⁻² d⁻¹ (B4S03) and 8.96 × 10⁻² mmol C m⁻² d⁻¹ (B6S02). Modeled carbon uptake by macrobenthos (Supplementary Figure 2; B4S03: 5.63 × 10⁻² mmol C m⁻² d⁻¹, B6S02: 3.84 × 10⁻² mmol C m⁻² d⁻¹) and megabenthos (Supplementary Figure 3; B4S03: 1.07 × 10⁻² mmol C m⁻² d⁻¹, B6S02: 3.51 × 10⁻² mmol C m⁻² d⁻¹) were of similar magnitude. The estimated carbon uptake of fish (Supplementary Figure 4) was, however, one order of magnitude lower (B4S03: 2.54 × 10⁻³ mmol C m⁻² d⁻¹, B6S02: 1.54 × 10⁻³ mmol C m⁻² d⁻¹).

Macrobenthic polychaetes were responsible for 6.37–11.8%, macrobenthic isopods for 7.81–8.56%, and other macrobenthos

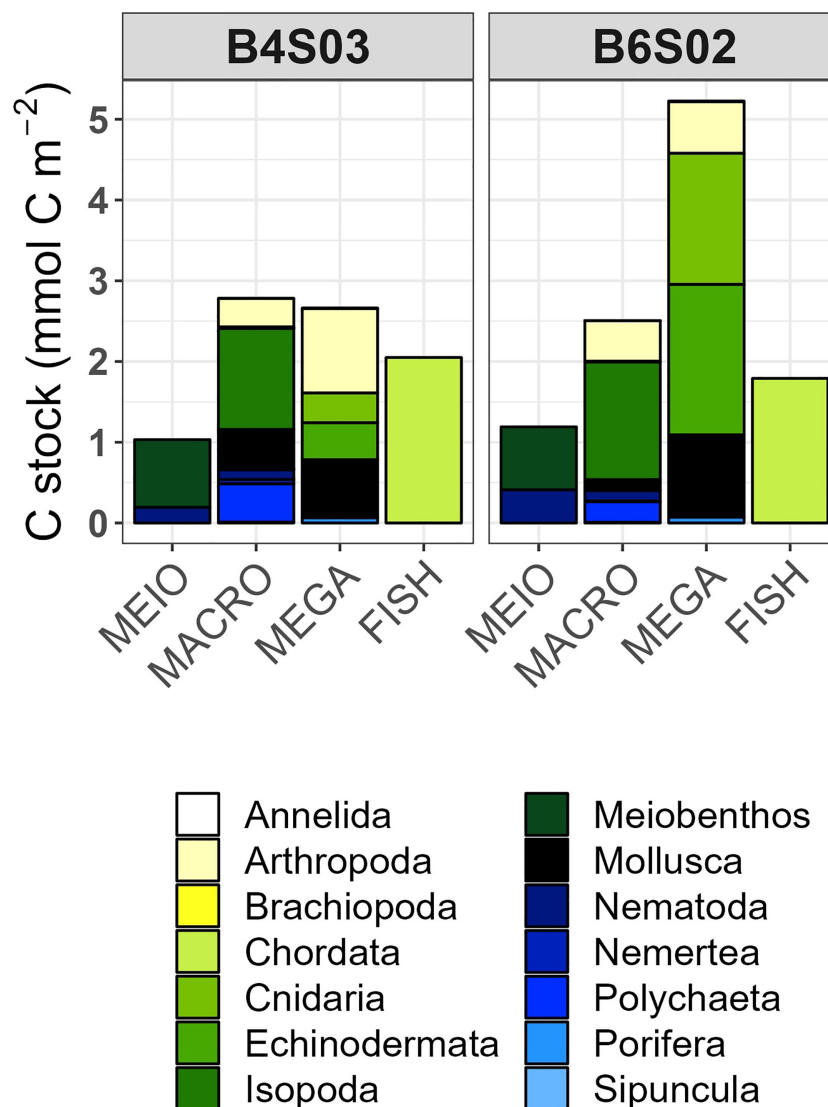


FIGURE 3

Mean carbon stocks (mmol C m^{-2}) of the faunal food-web compartments at sites B4S03 (left columns) and B6S02 (right columns) in the GSR exploration license area.

for 9.13–15.7% of carbon uptake. Megabenthic crustaceans were responsible for 1.40–2.02%, megabenthic cnidarians for 3.29–4.71%, holothurians for 7.40×10^{-2} –8.62%, poriferans for 0.28–1.17%, and all other invertebrate megabenthos for 1.21–5.45% of total faunal ingestion.

Uptake of DOC by bacteria as part of the microbial loop (see section 3.4) was $0.38 \text{ mmol C m}^{-2} \text{ d}^{-1}$.

3.4 Carbon cycling and specific C pathways

T_{f} was estimated to be $1.18 \text{ mmol C m}^{-2} \text{ d}^{-1}$ at site B4S03 and $1.20 \text{ mmol C m}^{-2} \text{ d}^{-1}$ at B6S02 and FCI was modeled to range from $0.53 \text{ mmol C m}^{-2} \text{ d}^{-1}$ (B4S03) to $0.53 \text{ mmol C m}^{-2} \text{ d}^{-1}$ (B6S02).

The microbial loop, i.e., dissolution of detritus, uptake of DOC by bacteria, virus-induced bacterial mortality, bacterial respiration,

and faunal grazing upon bacteria, had an estimated carbon flow of $0.84 \text{ mmol C m}^{-2} \text{ d}^{-1}$, which was 71.2% of modelled T_{f} at B4S03 and 69.8% of modelled T_{f} at B6S02.

An estimated carbon flow of $2.55 \times 10^{-3} \text{ mmol C m}^{-2} \text{ d}^{-1}$ was channeled through the scavenging loop (i.e. scavenging of scavengers on carrion) at B4S03, which was 0.22% of modelled T_{f} . At B6S02, the carbon flow through the scavenging loop was modeled to be $7.25 \times 10^{-4} \text{ mmol C m}^{-2} \text{ d}^{-1}$ (6.19×10^{-2} % of modelled T_{f}).

3.5 Consequences of polymetallic nodule removal

Excluding fauna from the food webs that have been predicted to be lost when polymetallic nodules are removed (Stratmann et al., 2021), led to a decrease in number of food-web compartments of 5.33% (B4S03) to 7.04% (B6S02) and to a loss of 5.28% (B6S02) to

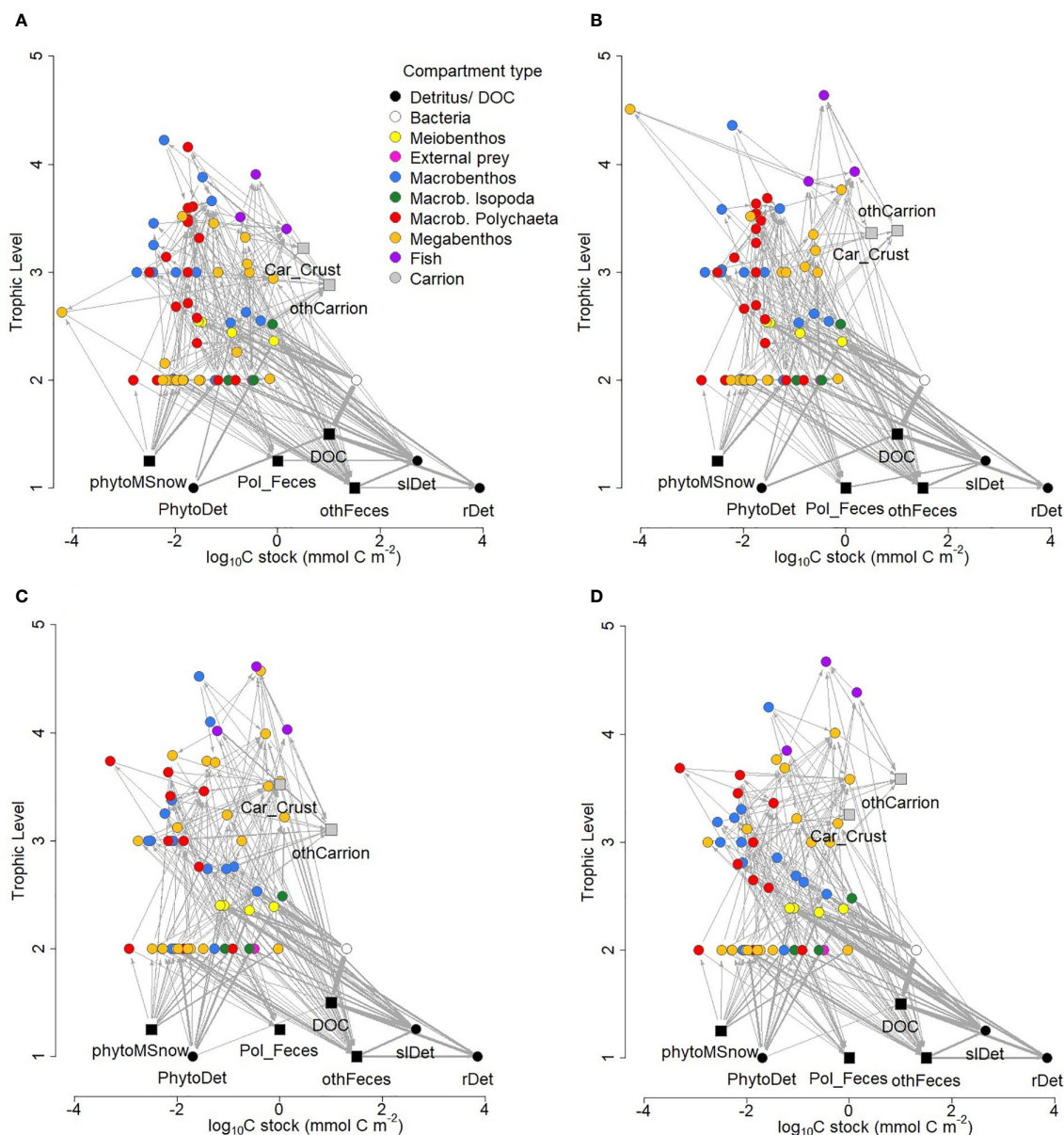


FIGURE 4

Structure of food-web models developed for the B4S03 site (A) in the presence and (B) absence of polymetallic nodules and for the B6S02 site (C) in the presence and (D) absence of nodules in the GSR exploration license area. Round nodes show compartments whose biomass (mmol C) is known, whereas square nodes indicate compartments to which the author assigned values for plotting purposes. Trophic levels 1.25 and 1.5 were assigned to square nodes for plotting purposes only; their true trophic levels are 1. The thickness of the arrows corresponds to the magnitude of carbon flow ($\text{mmol C m}^{-2} \text{ d}^{-1}$) between two food-web compartments after double square root-transformation. Note, that neither import nor export fluxes were plotted and that the x-axis is a $\log_{10}$ scale. Car_Crust = crustacean carrion, DOC = dissolved organic carbon, othCarrion = other carrion, othFeces = isopod, macro-, invertebrate megabenthic and fish feces, PhytoDet = phytodetritus, phytoMSnow = phytodetritus-based marine snow, Pol_Feces = polychaete feces, rDet = refractory detritus, siDet = semi-labile detritus.

9.23% (B4S03) of all links. Link density was reduced by 4.11% at B4S03 and increased by 1.90% at B6S02, and the connectance increased by 0.29% (B4S03) to 10.5% (B6S02). $T.$ decreased by 0.27% at B4S03 and increased by $2.52 \times 10^{-2}\%$ at B6S02, and FCI increased by 0.29% (B6S02) to 0.34% (B4S03). Network indices calculated for both sites in the presence and absence of nodule-related fauna are reported in Table 4.

A summary of the most important changes in modelled carbon flows (ΔC_{flow}) due to the exclusion of nodule-dependent fauna is

visualized in Figure 5. At site B4S03, the largest changes in carbon flows were related to increased respiration of macrobenthic polychaetes ($+3.64 \times 10^{-3} \text{ mmol C m}^{-2} \text{ d}^{-1}$) and decreased predation of fish upon macrobenthic polychaetes ($-3.63 \times 10^{-3} \text{ mmol C m}^{-2} \text{ d}^{-1}$). In comparison, at B6S02, the largest change in carbon flows was related to the increased sedimentary detritus uptake by metazoan meiobenthos ($+6.13 \times 10^{-3} \text{ mmol C m}^{-2} \text{ d}^{-1}$) and increased predation of megabenthic cnidarians on macrobenthic isopods ($+3.90 \times 10^{-3} \text{ mmol C m}^{-2} \text{ d}^{-1}$).

TABLE 4 Network indices calculated for the sites B4S03 and B6S02 when all fauna are present (+nodule) and when fauna predicted to be lost when polymetallic nodules are removed are excluded (-nodule). Relative changes (%) in network indices due to faunal loss are reported for each site.

Network indices	B4S03 site			B6S02 site		
	(+nodule)	(-nodule)	% relative change	(+nodule)	(-nodule)	% relative change
<i>n</i>	75	71	-5.33	71	66	-7.04
<i>L_{total}</i>	336	305	-9.23	303	287	-5.28
<i>LD</i>	4.48	4.30	-4.11	4.27	4.35	+1.90
<i>C</i>	4.77×10^{-2}	4.79×10^{-2}	+0.29	4.75×10^{-2}	5.24×10^{-2}	+10.5
<i>T_{..}</i>	1.18	1.17	-0.27	1.20	1.20	+2.52 $\times 10^{-2}$
<i>FCI</i>	0.53	0.54	+0.34	0.53	0.53	+0.29

n = number of food-web compartments, *L_{total}* = number of links, *LD* = link density, *C* = connectance, *T_{..}* = total system throughput, *FCI* = Finn's Cycling Index.

Removing the nodule-dependent fauna did not affect the strength of the microbial loop. In contrast, the scavenging loop at B4S03 (-nodule) (7.25×10^{-4} mmol C m⁻² d⁻¹) was only 28.4% of the original scavenging loop and at B6S02 (-nodule), the scavenging loop accounted for 43.5% ($= 1.51 \times 10^{-3}$ mmol C m⁻² d⁻¹) of the initial scavenging loop.

4 Discussion

The role played by nodule-dependent fauna in benthic carbon cycling in the eastern CCZ was assessed by investigating differences in modelled carbon flows of intact food webs and of food webs where

nodule-dependent fauna was excluded in the models. The food webs for two sites in the GSR exploration license area contained between 9 mmol C m⁻² and 11 mmol C m⁻² faunal carbon and between 20 mmol C m⁻² and 35 mmol C m⁻² bacterial carbon in 61 to 65 biotic compartments (excluding detritus, DOC, and carrion) (Figures 3, 4; Table 1). The food webs were estimated to be fueled to 58% to 62% by phytodetritus deposition and to 30% to 33% by refractory detritus deposition, whereas most of the carbon was lost from the food webs as respiration. This respiration was dominated by bacterial respiration, followed by metazoan meiobenthos, and other macrobenthos respiration. Removing nodule-dependent faunal compartments had almost no effect on total system throughput *T_{..}*, but reduced the total number of links by 5% to 9% (Table 4).

TABLE 5 Deposition of detritus (phytodetritus, semi-labile detritus, refractory detritus) and respiration of bacteria, metazoan meiobenthos, fish, and different macro- and megabenthos faunal groups at sites B4S03 and B6S02. .

	B4S03	%	B6S02	%
<i>Total deposition</i>	9.21×10^{-2}	100	9.15×10^{-2}	100
Phytodetritus	5.69×10^{-2}	61.8	5.33×10^{-2}	58.3
Semi-labile detritus	7.95×10^{-3}	8.63	7.94×10^{-3}	8.68
Refractory detritus	2.73×10^{-2}	29.6	3.02×10^{-2}	33.0
<i>Total respiration</i>	8.51×10^{-2}	100	7.85×10^{-2}	100
Bacteria	3.78×10^{-2}	44.4	3.78×10^{-2}	48.1
Metazoan meiobenthos	1.11×10^{-2}	13.0	2.00×10^{-2}	25.5
Macrobenthic polychaetes	1.09×10^{-2}	12.8	3.06×10^{-3}	3.90
Macrobenthic isopods	8.66×10^{-3}	10.2	4.92×10^{-3}	6.26
Macrobenthos (except polychaetes, isopods)	1.19×10^{-2}	14.0	5.25×10^{-3}	6.68
Megabenthic crustaceans	1.38×10^{-3}	1.62	8.03×10^{-4}	1.02
Megabenthic cnidarians	1.51×10^{-3}	1.78	2.05×10^{-3}	2.61
Holothurians	3.99×10^{-5}	4.69×10^{-2}	4.27×10^{-5}	5.44×10^{-2}
Poriferans	8.10×10^{-5}	9.52×10^{-2}	8.89×10^{-5}	0.11
Invertebrate megabenthos (except crustaceans, cnidarians, holothurians, poriferans)	9.51×10^{-4}	1.12	3.60×10^{-3}	4.59
Fish	8.58×10^{-4}	1.01	9.18×10^{-4}	1.17

Data are presented as flux values (mmol C m⁻² d⁻¹) and as % contribution to total deposition and total benthic respiration.

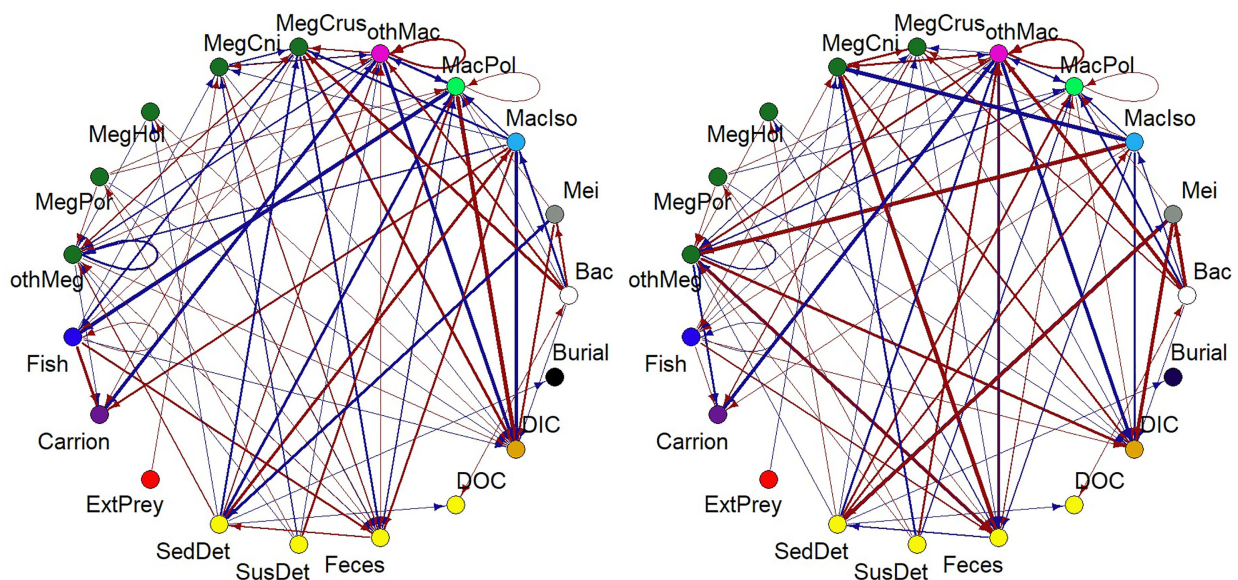


FIGURE 5

Figure summarizing changes in carbon flows between compartments because of the exclusion of polymetallic nodule-dependent fauna at sites (A) B4S03 and (B) B6S02. The width of the arrow corresponds to the double-square root of ΔC_{flow} (in $\text{mmol C m}^{-2} \text{ d}^{-1}$), where $\Delta C_{\text{flow}} = C_{\text{flow+node}} - C_{\text{flow-node}}$; and the color indicates whether an exclusion of the fauna led to an increase in a specific carbon flow (red) or a decrease (blue). Bac, bacteria; ExtPrey, external prey; MacIso, macrobenthic isopods; MacPol, macrobenthic polychaetes; MegCni, megabenthic cnidarians; MegCrus, megabenthic crustaceans; MegHol, holothurians; MegPor, poriferans; Mei, metazoan meiobenthos; othMac, other macrobenthos; othMeg, invertebrate megabenthos; SedDet, sedimentary detritus; SusDet, suspended detritus.

4.1 Model limitations

Like all scientific models, these highly resolved food-web models have limitations.

Biomasses of metazoan meiobenthos, macrobenthos, invertebrate megabenthos, and fish were estimated by converting densities reported by the authors of the original peer-reviewed studies (see section 2.4.1) to carbon stocks using conversion factors (Supplementary Tables 2–4). de Smet et al. (2021) assessed invertebrate megabenthos community composition and densities using autonomous underwater vehicle imagery obtained from ~6 m above the seafloor and only reported specimens >5 cm. In a method paper, Schoening et al. (2020) compared abyssal faunal densities on seafloor images taken at 1.6 to 1.7 m above the seafloor with images taken 4.5 and 7.5 m above the seafloor and found three to 28 times higher densities in the low altitude images compared to the high-altitude images. Consequently, in this study invertebrate megabenthos biomasses and subsequently their role in carbon cycling are underestimated.

Polymetallic nodules in abyssal plains have microbial communities inside their crevices (Blöthe et al., 2015; Cho et al., 2018) and on their surfaces (Wang et al., 2009) that are different from the microbial community in adjacent sediments (Cho et al., 2018; Molari et al., 2020). Hence, for a proper assessment of the role of polymetallic nodules on abyssal carbon cycling, not only the impact of nodule-dependent fauna exclusion, but also the effect of nodule-dependent microorganism exclusion on carbon cycling should be assessed. However, so far, no study has quantified the role of these microorganisms in carbon cycling and data on

bacterial biomass inside and outside the nodules are lacking for the GSR exploration license area or any other license area in the CCZ. Therefore, I could not confidently include this process in the food web models for the sites B4S03 and B6S02, and used one (sedimentary) bacterial carbon pool per site instead.

Sweetman et al. (2019) measured $0.10 \pm 0.03 \text{ mmol C m}^{-2} \text{ d}^{-1}$ DIC uptake by benthic bacteria in the UK Seabed Resources Ltd (UK1) exploration license area and the Ocean Minerals of Singapore exploration area in the eastern CCZ. This value was 1.2 to 2.0 times higher than the uptake of phytodetritus carbon by bacteria at the sites (Sweetman et al., 2019) and therefore likely a very substantial contribution to abyssal carbon cycling. However, as the researchers conducting the baseline studies for GSR at B4S03 and B6S02 did not investigate bacterial dissolved inorganic carbon uptake, I am hesitant to include this process in the model (see also discussion in de Jonge et al. (2020)). Further studies in dark carbon fixation at different sites of the CCZ would therefore provide valuable information about the underlying mechanisms and which microorganisms are involved in them and allow to include this process in future abyssal carbon-based food-web models.

Foraminiferans are one of the dominant compartments of meiobenthos (Gooday and Goineau, 2019), macrobenthos (Goineau and Gooday, 2017; Stachowska-Kamińska et al., 2022; Gooday and Wawrzyniak-Wydrowska, 2023), invertebrate megabenthos (Gooday et al., 2017; Simon-Lledó et al., 2019a; Simon-Lledó et al., 2019b), and nodule-encrusting communities (Veillette et al., 2007; Gooday et al., 2015) in the CCZ (Gooday et al., 2021). Nevertheless, their role in carbon cycling as well as the potential importance of small naked protists (i.e., ciliates, flagellates,

amoebae) for the microbial communities are almost always overlooked, except by a few specialists (Gooday et al., 2020; Gooday et al., 2021). Foraminiferans are particularly difficult to include in carbon-based food-web models as it is often difficult to determine the proportion of living vs. dead specimens. This applies to the large megabenthic xenophyophores visible in seafloor images (Hughes and Gooday, 2004) as well as to the smaller species. We also miss information about assimilation and net growth efficiencies, growth rates/secondary production, and mortality rates required to model them. Having said that, de Jonge et al. (2020) tried to estimate the contribution of foraminiferans to carbon cycling in the Peru Basin. These authors estimated that Xenophyophores take up $0.63 \text{ mmol C m}^{-2} \text{ d}^{-1}$ assuming that all Xenophyophores observed on still images were alive. Even though $T.$ in the Peru Basin is substantially larger than in the CCZ ($6.42 \text{ mmol C m}^{-2} \text{ d}^{-1}$ when dark carbon fixation and Xenophyophores were included, (de Jonge et al., 2020)), I expect a large underestimation of $T.$ for the sites B4S03 and B6S02 of up to 10% due to the neglect of foraminiferans.

4.2 Regional differences in carbon cycling at abyssal plains

The presented food webs estimated a difference in total system throughput $T.$ of $2.49 \times 10^{-2} \text{ mmol C m}^{-2} \text{ d}^{-1}$ between sites B4S03 and B6S02 (Table 4). This difference in $T.$ was partly the result of higher carbon cycling by deposit-feeding nematodes at B4S03 compared to B6S02 (Table 1) and might be related to small-scale variability in environmental parameters between the two sites. Metazoan meiobenthos densities in surface sediments (0–5 cm sediment depth) of the CCZ are influenced by water depth, silt content, and polymetallic nodule density (Hauquier et al., 2019). In a comparison of metazoan meiobenthos across various locations in the eastern CCZ, Hauquier et al. (2019) measured the highest meiobenthos density at a location with low nodule density, high pigment concentrations as a proxy for (fresh) phytodetritus availability, and low clay content compared to the other sampling locations. Further sediment characteristics that influence meiobenthos distribution and densities in the eastern CCZ were identified in a modeling exercise by Uhlenkott et al. (2021). They include total organic and inorganic carbon content of the sediment, dry bulk sediment density, and shear strength. In this study, site B4S03, where higher metazoan biomass was detected, was indeed characterized by decreased nodule abundance compared to the B6S02 site, though mud content was higher and total organic carbon content was lower at B4S03 than at B6S02 (de Smet et al., 2017).

Another reason for the differences in $T.$ was the higher carbon uptake by invertebrate megabenthos (i.e., cnidarians, holothurians, poriferans, other invertebrate megabenthos except crustaceans) at B6S02 compared to B4S03. Differences in invertebrate megabenthos and fish densities at various spatial scales have been described for the mid-eastern CCZ by (Simon-Lledó et al., 2020). The authors found that geomorphology and polymetallic nodule cover control faunal abundance and community composition, areas with the lowest nodule cover having consistently decreased faunal densities compared to areas

with higher nodule cover. Simon-Lledó et al. (2020) further speculated that the shape and volume of nodules might be important for community structure. The two study sites in the GSR exploration license area investigated in this study are also characterized by different nodule densities, volumes, and coverages, whereby nodule density is higher at B6S02, but nodule volume and coverage are lower. Hence, I conclude that environmental conditions and particularly polymetallic nodule densities exert an important influence on carbon cycling at a regional scale (280 km) in the eastern CCZ.

4.3 Assessment of polymetallic nodule removal on C cycling and outlook to prospective deep-seabed mining effects

Excluding nodule-dependent fauna from the food webs developed for sites B4S03 and B6S02 impacted the faunal contribution to $T.$, but had no effect on the microbial loop. The taxa, that were removed, belonged to filter and suspension feeders taking up marine snow (Brachiopoda, Crinoidea), but also to scavengers (Amphipoda observed always in pairs at sponge stalks; (Stratmann et al., 2021; personal observations)), and carnivorous suspension feeders (Brisingida, Actinaria) predated upon small crustaceans (Table 1, Supplementary Tables 3, 4).

Scavenging amphipods were responsible for 89% of scavenging occurring at B4S03, so that their absence as a result of nodule removal strongly reduced the scavenging loop. Scavengers in the CCZ include demersal fish, such as *Coryphaenoides* spp., *Bassozetus* sp., and *Pachycara nazca*, and invertebrate megabenthos, like the amphipod *Eurythenes gryllus*, squat lobster *Munidopsis* sp., and the shrimp *Cerataspis monstrosus* (Harbour et al., 2020). Their taxon density and diversity vary regionally and Drazen et al. (2021) observed that the scavenger assemblage in the eastern CCZ was very different from the assemblage in the western APEIs due to higher abundances of the shrimp *Hymenopenaeus nereus* and lower abundances of the fishes *Barathrites iris* and *Coryphaenoides* spp. The latter fish was also observed in the GSR exploration license area (de Smet et al., 2021) and the carbon-based food-web model for B4S03 estimated that *Coryphaenoides* spp. partly compensated the loss of scavenging amphipods by increasing its contribution to the scavenging loop from 0% to 44%.

Abyssal scavengers react relatively fast to the deposition of carrion at the seafloor: *Coryphaenoides* spp. were attracted to fish bait (i.e., albacore tuna) at the seafloor of the eastern CCZ within 1 h (Harbour et al., 2020) and synbranchid eels reached the fish bait (i.e., Pacific mackerel) in the western CCZ after ~7 h (Leitner et al., 2020; Leitner et al., 2021). Also, during a natural mass deposition event of gelatinous pyrosome carcasses in the Peru Basin in 2015, actinarians, ophiuroids, asteroids, and isopods were observed to (putatively) scavenge on the pyrosomes carcasses within a couple of days (personal observation, Hoving et al. (in review)). Hence, an industrial deep-seabed mining operation will likely attract scavenging fish and invertebrate megabenthos within hours. For the first months to years, this will overcompensate the loss of nodule-dependent scavengers and the importance of the scavenging loop for benthic carbon cycling will strongly increase, unless scavengers are chased off

by the sediment plume or by the toxic metals that might be released during the mining operation (Koschinsky et al., 2001; Hauton et al., 2017; Müller et al., 2018).

At B4S03, filter and suspension feeding Bivalvia benefitted from the loss of nodule-dependent filter and suspension feeding Brachiopoda and Crinoidea by ingesting 8% more labile marine snow. More labile marine snow particles, that nodule-dependent filter and suspension feeders normally would have filtered out of the water column, however, settled on the sediment at B4S03 and at B6S02 where they contributed to the sedimentary phytodetritus pool. As a result, epistrate feeding, selective and non-selective deposit-feeding nematodes were estimated to consume more labile phytodetritus at B6S02, while deposit-feeding isopods, polychaetes of the family Maldanidae and non-selective deposit-feeding nematodes were predicted to feed on more labile phytodetritus at B4S03.

The top 1 m of seafloor sediment is assumed to contain 10^8 prokaryotic cells cm^{-3} (Jørgensen and Boetius, 2007) and Nomaki et al. (2021) measured prokaryotic cell abundances of 3.6×10^7 cells g^{-1} in the 0–1 cm depth layer to 1.4×10^7 cells g^{-1} in the 5–10 cm depth layer in the abyssal west Pacific. In the eastern CCZ, bacterial biomasses range from 238 mg C m^{-2} to 530 mg C m^{-2} (Pape et al., 2017; Sweetman et al., 2019) in the upper 5 cm of sediment which will be removed during industrial deep-seabed mining operations. As the models presented here indicate, the microbial loop in the upper 5 cm of sediment contributes between 70% and 71% to T_r at the GSR exploration license area. Hence, we can expect that an impairment of the microbial loop during deep-seabed mining operations will have the largest impact on benthic carbon cycling. Vonnahme et al. (2020) measured an up to fourfold lower prokaryotic activity in a 5-week-old epibenthic sledge track in the more eutrophic (compared to the CCZ) abyssal Peru Basin. Assuming that sediment removal by mining will have a similar, if not stronger effect on the microbial loop in the CCZ, benthic carbon cycling at the GSR exploration license area will be further reduced by 0.63 $\text{mmol C m}^{-2} \text{d}^{-1}$. Hence, this study indicates that deep-seabed mining in the GSR license area will likely reduce ecosystem function in the form of carbon cycling by minimum 57 to 61% on a millennial timescale (Volz et al., 2020). Further expected loss of nodule-independent metazoan meiobenthos, macrobenthos, and other invertebrate megabenthos has not been taken into account.

5 Conclusion

Based on very highly resolved abyssal food-web models for two sites in the GSR exploration license area, I show that benthic carbon cycling in the eastern CCZ varies regionally (280 km) on the same order of magnitude as benthic carbon cycling 26 years after a small-scale sediment disturbance experiment between reference sites and areas likely affected by re-sedimentation (i.e., sites called ‘outside the plough tracks’ in de Jonge et al. (2020)). Hence, the International Seabed Authority should request a spatially (and temporally) very homogenous and fine-scale (environmental baseline) sample coverage for each sub-exploration license area in the CCZ in order to assess serious harm to the marine environment (Levin et al., 2016) and to determine potential recovery from subsequent

deep-seabed mining later on. The models furthermore indicate that polymetallic nodule-dependent fauna has a very minor contribution to benthic carbon cycling, though it is important for marine biodiversity (Niner et al., 2018; Stratmann et al., 2021) and therefore the author recommends concentrating on the microbial loop when investigating whether this specific ecosystem function is affected by deep-seabed mining as well as on biodiversity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of interest

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

The handling editor MH declared a past collaboration with the author TS.

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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.2023.1151442/full#supplementary-material>

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Major fine-scale spatial heterogeneity in accumulation of gelatinous carbon fluxes on the deep seabed

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Abyssal plain communities rely on the overlying water column for a settling flux of organic matter. The origin and rate of this flux as well as the controls on its fine-scale spatial distribution following seafloor settlement are largely unquantified. This is particularly true across regions where anthropogenically-induced seafloor disturbance has occurred. Here, we observed, quantified and mapped a mass deposition event of gelatinous zooplankton carcasses (pyrosomes) in July–September 2015 across one such physically disturbed region in the Peru Basin polymetallic nodule province (4150 m). Seafloor in this area was disturbed with a plough harrow in 1989 (as part of the DISCOL experiment) causing troughs in the sediment. Other parts were disturbed with an epibenthic sled (EBS) during a cruise in 2015 resulting in steep-walled, U-shaped troughs. We investigated two hypotheses: a) gelatinous food falls contribute significantly to the abyssal plain carbon pump and b) physical seafloor disturbance influences abyssal distribution of organic matter. We combined optical and bathymetric seafloor observations, to analyze pyrosome distribution on seabeds with different levels of disturbance. 2954 pyrosome colonies and associated taxa were detected in > 14,000 seafloor images. The mean regional carbon (C) deposition associated with pyrosome carcasses was significant compared to the flux of particulate organic C (182 to 1543%), and the total respired benthic C flux in the DISCOL Experimental Area (39 to 184%). EBS-disturbed seafloor tracks contained 72 times more pyrosome-associated C than an undisturbed reference site, and up to 4 times more than an area disturbed in 1989. Deposited pyrosomes collected had a higher proportion of labile fatty acids compared to the sediment. We document the temporal and spatial extent of an abyssal food fall event with unprecedented detail and show that physical seafloor disturbance results in the accumulation of detrital material. Such accumulation may reduce oxygen availability and alter benthic community

structure. Understanding both the relevance of large food falls and the fine scale topography of the seafloor, is necessary for impact assessment of technologies altering seafloor integrity (e.g. as a result of bottom-trawling or deep seabed mining) and may improve their management on a global scale.

KEYWORDS

abyssal plain, DISCOL, food fall, *Pyrosoma*, seafloor disturbance, benthopelagic coupling

Introduction

The abyssal seafloor (areas of 3000–6000 m depth) covers an estimated 75% of the global deep ocean floor surface, but *in situ* observations are still very few, with less than 1 percent explored visually by cameras and submersibles (Ramirez-Llodra et al., 2010). Abyssal plains are typically characterized by a relatively flat seafloor surface topography, predominantly composed of fine sediments (Harris et al., 2014). In oligotrophic to mesotrophic areas these plains can host high abundances of polymetallic nodules (i.e., oblate to spherical authigenic mineral concretions of typically less than 20 cm in diameter) containing a range of economically valuable metals (Hein and Koschinsky, 2014). Nodules provide hard seafloor habitat niches (Vanreusel et al., 2016; Boetius and Haeckel, 2018; Stratmann et al., 2021), increase rugosity of benthic topography on a fine scale (Simon-Lledó et al., 2019b) and alter benthic community composition and faunal density (Vanreusel et al., 2016; Simon-Lledó et al., 2020). Typically, abyssal benthic communities are highly biodiverse but with relatively low faunal densities, limited by low rates of organic matter deposition from the overlying water column (Smith et al., 2008). The majority of this organic matter originates from primary production in the photic zone. The deposition of carcasses of larger pelagic life (e.g. kelp, whales, sharks, fish, crustaceans, squid and jellyfish), also known as food falls, leads to temporarily available and highly localized patches of organic matter. Such rich depositions are set against an oligotrophic background with carcasses therefore rapidly attracting benthic and demersal megafauna scavengers (Stockton and DeLaca, 1982; Jones et al., 1998; Smith et al., 2008; Treude et al., 2009; Bernardino et al., 2010; Higgs et al., 2014). Observations of food falls on abyssal plains are rare, and their contribution to local carbon fluxes remains unquantified for many regions.

Understanding abyssal carbon fluxes is essential for evaluating the ocean's role in marine C sequestration, nutrient remineralization and biodiversity (Thurber et al., 2014), i.e. all major ecosystem services of the deep sea. Regionally, C budgets may be imbalanced where the flux of particulate organic matter measured in sediment traps is not sufficient to explain biomass and respiration rates of seafloor communities (Burd et al., 2010). This suggests that there are additional sources of C reaching the seafloor. Episodic deposition of carcasses may help to explain and partly counterbalance these C budget deficits (Smith et al., 2018), given that this flux is wholly

missed by sediment-trap sampling. Gelatinous zooplankton are a prominent group in pelagic communities, fulfilling pivotal roles as both predator and prey (Robison, 2004; Lucas et al., 2014; Choy et al., 2017; Luo et al., 2020), and are therefore vectors for the transport of carbon (C) and nitrogen (N) through marine food webs. The C and N contained in the carcasses of dead gelatinous zooplankton falling to the deep seafloor is made available to benthic communities, establishing a connection between benthic and pelagic food webs (Sweetman et al., 2014; Luo et al., 2020). The available quantifications suggest significant local contributions to the C and N flux by jellyfish and pelagic tunicates (Robison et al., 2005; Lebrato and Jones, 2009; Smith Jr. et al., 2014; Sweetman and Chapman, 2015). Recent modelling and meta-analysis efforts show a global relevance of jelly falls in carbon flux (Lebrato et al., 2019) in particular in the context of warming oceans and finfish overexploitation. Carcass deposition events may also provide 'essential' fatty acids to the deep-seabed environment. Fatty acids are both principle components of lipids and an energy source and a building component of cell membranes (Burdge and Calder, 2014) as well as playing a role in signal transduction and gene expression (Burdge and Calder, 2014). Since some essential fatty acids can only be synthesized by algae or plants (with some exceptions (Pond et al., 1997; Pond et al., 2002)), they are trophic markers that are transferred conservatively from lower trophic levels (i.e., primary producers, primary consumers) to higher trophic levels and may provide indications of diets (Dalsgaard et al., 2003). Such information about diets is often unknown for abyssal fauna. Owing to a lack of observational and biochemical data, coupled with limited geographical coverage, the significance, biogeochemical contribution and fine scale distribution of gelatinous carcass deposition events in the abyssal deep sea remains uncertain. Here we quantified the deposition of pyrosome carcasses on the seafloor and analyzed their biochemical composition to test the hypothesis that jelly falls contribute significantly to local carbon budgets on the abyssal plain.

Pyrosomes are increasingly recognized as pivotal components of oceanic food webs. The pyrosome species we observed here is likely *Pyrosoma atlantica*, a species that is very common in the Atlantic, Pacific and Indian Ocean (van Soest, 1981), and recently expanded its range into the North Pacific from its typical tropical and subtropical habitats (Kuo et al., 2015; Sutherland et al., 2018; Cornwall, 2019; Décima et al., 2019; Henschke et al., 2019). Its populations may locally reach exceptionally high numbers (200,000

kg WM km⁻³ (Brodeur et al., 2019)), clogging fishing nets and waste water treatment plants. Pronounced ecological impacts of pyrosome blooms include reductions in crustacean zooplankton (Sakuma et al., 2016), and high grazing impact. In one grazing event 22% of the phytoplankton stock in the Northern California Current offshore Oregon (USA) were consumed by pyrosomes (O'Loughlin et al., 2020). Pyrosomes occur in high densities in midwater, forming a biological substrate for pelagic crustaceans. They migrate on a daily basis from mesopelagic depths at night to the surface, to benefit from the increased productivity of sunlit waters. Pyrosome food falls may contribute significantly to local carbon fluxes in the Atlantic and Pacific and attract a diversity of megafaunal scavengers. Quantifications of the biogeochemical contribution of pyrosome food falls on the abyssal plain are absent at the time of writing.

A pressing question in deep-sea ecology is how seafloor disturbance may alter food distribution. Abyssal food availability is spatially variable. The spatial heterogeneity of food availability appears to be controlled by local hydrodynamic regimes, often affected by topography across fine (centimeters to meters) (Snelgrove et al., 1994) and broad landscape scales (Durden et al., 2015; Simon-Lledó et al., 2018). Temporal patterns include clear seasonal and inter-annual variation in C flux (Smith and Kaufmann, 1999; Durden et al., 2020). Long-term variations with associated impacts on the benthos also result from climatic variations (Smith et al., 2013; Soltwedel et al., 2016; Sweetman et al., 2017). This heterogeneity can structure deep-sea communities at various spatial and temporal scales and hence is relevant to local biodiversity (Grassle and Morse-Porteous, 1987), community structure, and affect functional processes, such as bioturbation and C distribution, cycling and remineralisation (Thurber et al., 2014). The combined interaction of abyssal benthic fauna and microbes results in a higher proportion of C stored in the seafloor sediments, when compared to nutrients such as N and P, of which a greater relative proportion is re-mineralized and released back into the ocean (Van Cappellen, 2003). Disturbing the biologically active layer of the deep-sea sediment (e.g. via anthropogenic activities) is therefore expected to alter the processing of C within sediments (Vonnahme et al., 2020), but may cause other impacts on seabed ecosystems as well. For example, the increased roughness which can result from direct physical disturbance of the seafloor can also influence the deposition of organic matter by altering the hydrodynamical and mechanical processes governing the spatial distribution of organic matter deposition, as shown for coastal systems in laboratory experiments (Yager et al., 1993) and in the field (Cacchione et al., 1978; Lebrato and Jones, 2009). Today, little information is available on the interaction between seafloor physical disturbance and organic matter deposition in the deep sea (König et al., 2001), even though large areas of seafloor have already been disturbed by deep-sea fisheries (Gage et al., 2005; Sala et al., 2021). It is likely that physical impacts on seafloor integrity influence not only the release of inorganic C (Sala et al., 2021), but also the spatial distribution of organic matter deposition in the deep sea. This hypothesis has not been tested, owing to the stochastic, thus hard to predict, nature of zooplankton blooms and other large-scale organic matter deposition events in the deep-sea.

One of the most comprehensive experiments aimed at assessing the impacts of deep-sea sediment disturbance to date, the so-called 'Disturbance and Recolonization Experiment' (DISCOL), commenced in 1989 in an area of abundant polymetallic nodules (>4000 m depth) in the Peru Basin, southeast Pacific (Thiel, 1991; Thiel et al., 2001). At that time, the seafloor was physically disturbed by dragging a plough-harrow almost 78 times across a nodule-covered area of 10.8 km², ploughing most of the nodules under the overturned sediment surface (Thiel and Schrieffer, 1989; Thiel et al., 2001). The effects of seafloor disturbance on the deposition of organic matter were studied here using the results from a research cruise that returned to the DISCOL site 26 years after the initial disturbance as part of the European project MiningImpact (Greinert, 2015). The plough tracks from 1989 were still clearly visible in 2015 (Gausepohl et al., 2020), when additional trawl tracks were created with an epibenthic sled (EBS) to collect epifauna. Using an array of observational tools, we quantified zooplankton food falls to determine if gelatinous carcasses contribute significantly to the local carbon pump of the abyssal plain of the Peru Basin. We also investigated the effect of physical impacts on seafloor integrity, as induced by ploughing and trawling disturbance, on the local C deposition of sinking food fall material within the deep sea.

Methods

Study site

The DISCOL area (07° 04.40 ' S/088° 27.60 ' W) lies in the Peru Basin (Southeast Pacific) between 3,850 m and 4,250 m water depth (Figure 1). Towards the south and east extent of the area, the morphology of the area is gentler, consisting of large basins descending from 4,150 m water depth at the DISCOL experimental area (DEA) down to 4,250 m water depth. The DEA itself covers an area of 3.7 km diameter, the centre of which is situated in a 25 m deep N-S striking valley of 1.4 km width (Figure 2).

Sediment disturbance creation

During the DISCOL experiment in 1989, an 8-m wide plough-harrow was ploughed 78 times across the circular DISCOL experimental area (DEA; diameter: 3.7 km) creating plough tracks (Figure 2). These tracks altered seafloor integrity by creating troughs (approximately 20 cm in 2015) (Paul et al., 2019), depressions, piles and ridges in the sediments (Vonnahme et al., 2020) (Figure S1). By disturbing the biologically active layer, microbial and benthic faunal communities were altered and with that the biogeochemical properties of the seafloor (Haeckel et al., 2001; Vonnahme et al., 2020).

During RV *Sonne* cruise SO242 leg 1 (28 July 2015 – 25 August 2015), a fresh seafloor disturbance were created with an epibenthic sled (Greinert, 2015) (EBS) towed across previously unploughed, nodule-abundant seafloor, starting with the first EBS track on 5 August 2015 (#081-EBS-3) and the second EBS track on 13-14

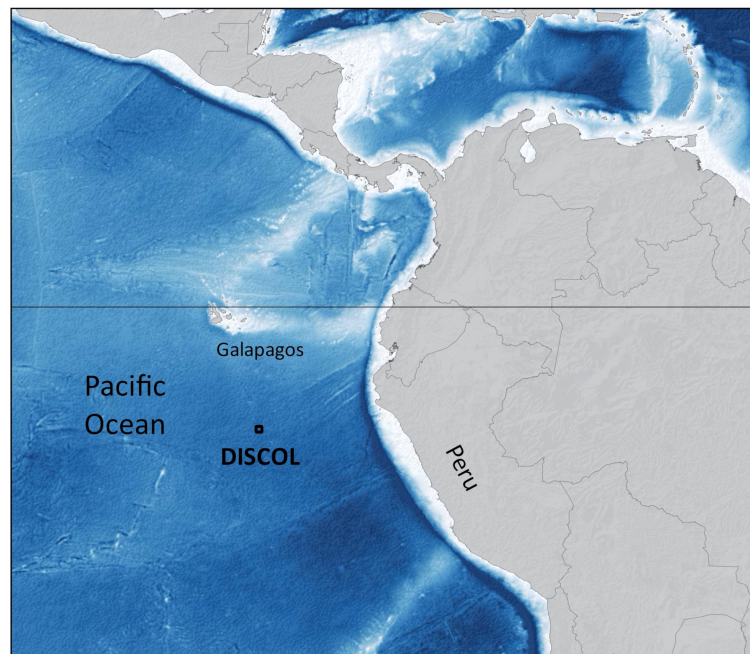


FIGURE 1
DISCOL Experimental area in the Peru Basin. Geographic location of the DISCOL area.

August 2015 (#085-EBS-4) (Figure 2). The purpose of conducting these new EBS deployments was to create a disturbance with freshly exposed deeper suboxic and more compacted sediment strata for infauna and biogeochemical process impact analyses. The EBS disturbance differed somewhat in nature from the initial plough-harrow disturbance methodology employed in 1989. The EBS pushes material to the side, resulting in the formation of a steep, a few 10s of cm deep, U-shaped through approximately 2 m wide, in

which polymetallic nodules are completely removed. Compared to the 1989 plough tracks, the EBS disturbance exposed more compacted sediment from below the bioturbated layer, which have a more heterogeneous character. The recent EBS trawling tracks also resulted in piles of overturned sediment blocks smothering seafloor surface sediments, with deeper, potentially less oxygenated and nutrient-abundant sediments displaced onto the adjacent seafloor sediments. The disturbance of the EBS resulted

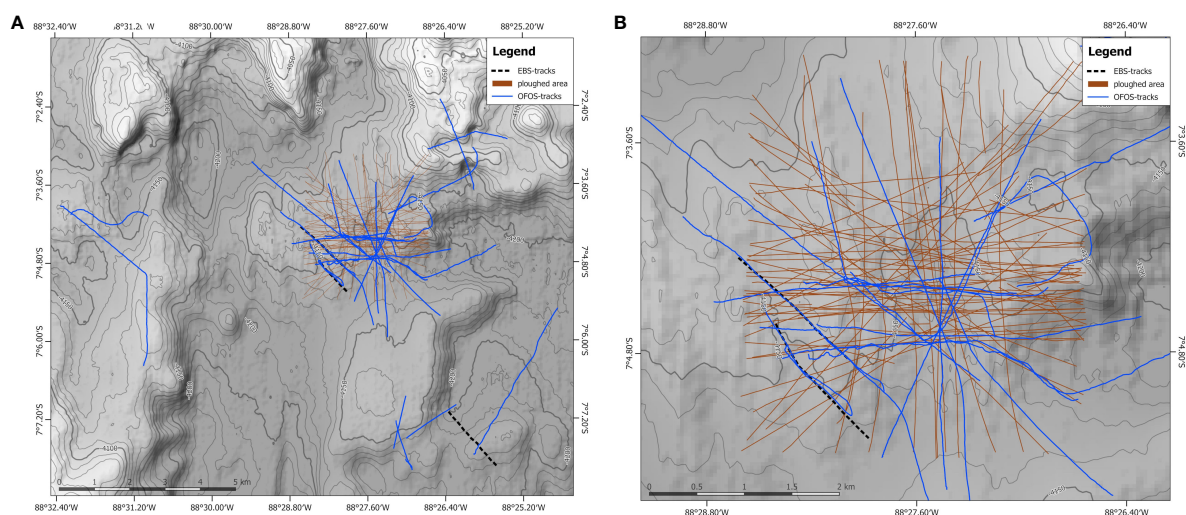


FIGURE 2
Bathymetric maps of the survey area. (A) The complete seafloor area that was surveyed during SO242. (B) the DISCOL Experimental Area. The plough tracks from 1989 are indicated in brown lines, and the EBS tracks from 2015 are in a dashed black line, the Ocean Floor Observations System (OFOS) tracks are in blue. Bathymetric data were acquired during cruise SO242 and published by Gausepohl et al. (2019; <https://doi.org/10.1594/PANGAEA.905579>).

in 15 – 20 cm deep track marks with steep sides (Vonnahme et al., 2020), contrasting with the older plough harrow tracks, that were similar in depth but had smoother and more gently sloping sides (Figure S1).

Seafloor imagery

During SO242 leg 1, 6 video transects (covering 3.6 km) were conducted between 3 August and 23 August 2015, with a custom-built Ocean Floor Observation System (OFOS) (Greinert, 2015). During RV *Sonne* cruise SO242 leg 2, OFOS collected more than 14,000 seafloor images during 16 transects from 31 August to 25 September 2015 each of which covered between 462 and 7,365 m² of seafloor area and collected between 88 and 1,403 images per transect (Table S1). OFOS was towed at an altitude of 1.7 m above the seafloor and used a 23 megapixel downward-facing still camera (iSiTEC, Canon EOS 5D Mark III) to take images every 15 s, each of which also captured the laser points projected by a tri-laser (50 cm spacing) sizing device (iSiTEC, custom built). Ship speed was maintained at 0.1–0.2 m s⁻¹ (Boetius, 2015). After imagery collection, the seafloor within each image was characterized as representing one of four contrasting categories of seafloor type, following earlier classification (Bluhm et al., 1995), and a corrected version (Bluhm, 2001). These categories were: 1) Disturbed Areas (inside plough mark in DEA), 2) Undisturbed Areas (outside plough mark in DEA, but showing various levels of re-sedimentation of sediment plumes created by the ploughing), 3) Reference Areas (outside DEA, not affected by ploughing) with polymetallic nodules, and 4) Reference Areas without polymetallic nodules. A fifth category of seafloor was added to represent images of the seafloor disturbed by the EBS trawl; 5) EBS disturbed seafloor. However, sidescan sonar imaging conducted during the cruise revealed some inconsistencies in Bluhm's category 4, as nodule patches were detected in the seabed area representing this category (Reference East); the only relatively large nodule-free areas (i.e. low backscatter) across the study area were found within the DEA⁴³. For the current study, the focus was on the accumulation of pyrosomes across physically undisturbed and disturbed areas of seafloor. With this in mind, we defined three seafloor categories: "Undisturbed" includes the categories with no physical disturbance 2 to 4 (see above), "plough-disturbed" corresponds to category 1 above, and "EBS disturbed" is category 5 above.

All seafloor images were inspected, the number of pyrosome carcasses quantified and the seafloor category noted. In cases where more than one habitat category was present in an image, the image was identified as belonging to the most spatially dominant category. Pyrosome densities were calculated by dividing the number of pyrosomes by the area surveyed. Image scaling was conducted by measuring the spacing of the laser points in a subset of 3,663 images using the PAPARA(ZZ)I software application (Marcon and Purser, 2017). The image area of all remaining images was calculated from the camera altitude (distance to seafloor) using a second-order polynomial regression of the laser-based measurements. The average seafloor image area was 5 m² (interquartile range 4.45 – 6.25 m²). To determine the average dimensions of the pyrosome

carcasses observed on the seafloor, 20 pyrosome colonies were measured on OFOS images collected on each of three different days (onset of pyrosome carcass deposition: 10 August 2015; maximum carcass deposition: 16 September 2015; decline of carcass deposition: 25 September 2015) using the PAPARA(ZZ)I software application (Marcon and Purser, 2017). A one-way ANOVA was conducted using the SPSS 17.0 software application to determine whether pyrosome lengths differed significantly by date of observation.

Additionally, pyrosome colonies were opportunistically imaged when observed on the seafloor during seafloor activities conducted by the remotely operated vehicle ROV KIEL 6000 (GEOMAR, Kiel, Germany). Associations between the carcasses and other organisms, such as scavengers, were also recorded. High-resolution photographs (24 Megapixel) of such pyrosome colony associations were taken by the SubC-Imaging 1 Alpha Cam, which was mounted on the lower pan & tilt unit of the ROV KIEL 6000.

Water column imagery

ROV KIEL 6000 was deployed 22 times during SO242/2 to a maximum depth of 4,160 m. The ROV was equipped with two colour zoom cameras (Kongsberg OE14-366, standard definition (SD), PAL, resolution 470 TV lines, recorded with 720x756 pixel, Mpeg encoded), which recorded footage continuously throughout the entire ROV dives including the water column during downcast and upcast. The cameras were mounted on a lower and an upper pan & tilt unit, respectively. During downcast and upcast, both SD cameras were usually pointed forward and zoomed out.

After an initial inspection of the SD video footage, the depth intervals for analysis per dive were defined as 300 to 450 m on downcast and 450 m to the surface on upcast (depth sensor data are shown in the image overlay). Videos of both SD cameras were inspected for all stations except one when the depth could not be determined due to malfunction of sensor. Numbers of pyrosome colonies present in the water column were categorized as 1) possible, 2) occasional (1 – 5 ind.), 3) numerous (6 – 22 ind.) and 4) many (50+ ind.). For graphical illustration, the higher density category of both downcast and upcast was chosen for each dive.

Pyrosome carcass collections and processing

Pyrosome carcasses were collected (n = 3) from the seafloor using the manipulator arm of the ROV KIEL 6000, stored in its "biobox", and recovered to the vessel where they were measured, weighed and stored frozen at -20°C. Pyrosome biomass (g org. C pyrosome⁻¹) was determined by total length (TL)-wet mass (WM) relationships and subsequent conversion to dry mass (DM), organic carbon content (orgCC), and total nitrogen content (TNC) using conversion factors.

WM (g WM pyrosome⁻¹) of pyrosomes was calculated following the equation (Miller et al., 2019):

$$WM_{pyrosome} = 3.6 \times 10^{-4} \times TL_{pyrosome}^{2.32}$$

with the mean pyrosome length (from photographs) from 2015 (133 ± 5 mm). This resulted in a WM of $30.5 \text{ g WM pyrosome}^{-1}$.

DM (% WM) was determined from three collected pyrosomes. At the shore-based laboratory facilities of NIOZ-Yerseke (the Netherlands), the frozen pyrosomes were weighted on a precision balance, freeze-dried, and weighted again to determine DM. Subsequently, the individual freeze-dried pyrosomes were ground to fine powder with mortar and pestle and organic C and N content as well as their stable isotope signature $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ~ 10 mg pyrosome tissue were measured with a Thermo Flash EA 1112 elemental analyzer (EA; Thermo Fisher Scientific, USA). Results of the stable isotope analysis were presented in standard δ notation (‰) as:

$$\delta X = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000$$

where X corresponds to ^{13}C and ^{15}N , respectively, and R is the ratio of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, respectively.

Pyrosome biomass (g C pyrosome^{-1} , $\text{g TN pyrosome}^{-1}$) was calculated as:

$$\text{orgCC}_{pyrosome} = WM_{pyrosome} \times DM_{pyrosome\text{conversion}} \times C_{pyrosome\text{conversion}}$$

with $DM_{pyrosome\text{conversion}} = 0.047$ and $C_{pyrosome\text{conversion}} = 0.085$, because DM of the three pyrosomes was $4.70 \pm 0.08\%$ WM and orgCC of the pyrosomes was $8.51 \pm 1.45\%$ DM.

$$TNC_{pyrosome} = WM_{pyrosome} \times DM_{pyrosome\text{conversion}} \times TN_{pyrosome\text{conversion}}$$

with $TN_{pyrosome\text{conversion}} = 0.0199$, because the TNC of the freeze-dried pyrosomes was $1.99 \pm 0.38\%$ DM.

Biochemical analysis of pyrosome tissue and deep-sea sediment

Total fatty acids (tFA) were extracted from $\sim 110 - 250$ mg freeze-dried and homogenized pyrosome tissue powder with a modified Bligh and Dyer extraction method (Bligh and Dyer, 1959; Boschker, 2008): The pyrosome powder was mixed with 6 ml MilliQ-water, 15 ml ultra-pure methanol (HPLC grade, 99.8%), and 7.5 ml ultra-pure chloroform (HPLC grade, 99.5%) in pre-cleaned test tubes. Prior to use, these tubes and all other glass ware were washed in the dishwasher and subsequently rinsed with ultrapure *n*-hexane (HPLC grade) and methanol. After shaking for 2 h, 7.5 ml chloroform were added, the tubes were shaken again, and 7.5 ml MilliQ-water were added. The tubes were stored at -21°C over night to let the solvent layers separate. The lower layer contained the chloroform extract that was transferred to pre-weighed test tubes. The weight of this chloroform extract was determined before it was evaporated to complete dryness. About 0.5 ml chloroform was added to the test tube and evaporated again. For the derivatisation of tFAs to fatty acid methyl esters (FAMES), 1 ml methanol-toluene mix (1:1 volume/volume), 20 μl of a first internal standard (1 mg 19:0 FAME mL^{-1}), and 1 ml 0.2 M

metanolic NaOH (0.58 g Na in 100 g methanol) were added to the test tube containing the tFA extract. After 15 min of incubation at 37°C , 2 ml *n*-hexane, 0.3 ml 1 M acetic acid, and 2 ml MilliQ-water were added and the solution was mixed very well. After the layers had separated, the (upper) *n*-hexane layer was transferred to new test tubes, 2 ml *n*-hexane were added to the old test tubes containing the acetic acid-MilliQ-water solution, and the step was repeated. The *n*-hexane layer was transferred to the same new tubes and 20 μl of a second internal standard (1 mg 12:0 FAME mL^{-1}) were added. The *n*-hexane was evaporated to complete dryness and the FAMES were transferred in 200 μl *n*-hexane to measuring vials. All FAMES were separated on a BPX70 column (50 m length, 0.32 mm inner diameter, 0.25 μm film thickness; SGE Analytical Science) with a HP 61530 gas chromatograph (Hewlett Packard/Agilent, USA). Their concentrations ($\mu\text{g C g DM pyrosome}^{-1}$) and $\delta^{13}\text{C}$ values (‰) were measured on a Finnigan Delta Plus IRMS (Thermo Fisher Scientific, USA) that was coupled with the gas chromatograph via a combustion GC-c-III interface (Thermo Fisher Scientific, USA). Peaks of the FAME chromatogram were identified based on the equivalent chain length (ECL) and the peak area was calculated using the two internal standards (12:0 and 19:0) for area correction.

Total NLFAs and PLFAs in pyrosome tissue were extracted from similar amounts of freeze-dried, homogenized pyrosome powder as described above. After the extraction, 0.5 ml chloroform was added to the test tube containing the total lipid extract. This extract was fractionated into different lipid classes over a silicic acid (Merck Kieselgel 60; activated by heating to 120°C for min. 2 h) column by eluting with 7 ml chloroform, 7 ml acetone, and 15 ml methanol. The acetone fraction was discarded, but the chloroform fraction containing the NLFAs and the methanol fraction, in which the PLFAs were dissolved, were collected in separate test tubes and evaporated to dryness. NLFAs and PLFAs were derivatized to FAMES by mild alkaline transmethylation as described for tFA above, separated on the same BPX70 column and measured on the same GC-c-IRMS.

Concentrations of essential PLFAs in pyrosomes deposited at a specific seafloor area ($c(\text{essential PLFA}_{pyrosome, spatial})$, mg C m^{-2}) were calculated as follows:

$$\begin{aligned} c(\text{essential PLFA}_{pyrosome, spatial}) \\ = density_{pyrosome} \times DM_{pyrosome} \times PLFA\ content_{pyrosome} \end{aligned}$$

PLFAs were also measured for deep-sea sediment. Sediment samples were extracted from 0 – 2 cm and 2 – 5 cm sediment layers as stated in (Stratmann et al., 2018a). Briefly, PLFAs from 2.5 g freeze-dried sediment were extracted following the same protocol that was used for pyrosomes (see above). The total lipid extract was fractionated into lipid classes over the silicic acid column and the PLFAs in the chloroform fraction were derivatized to FAMES as mentioned above. Subsequently, the FAMES were separated on a ZB5-5MS column (60 m length, 0.32 mm diameter, 0.25 μm film thickness; Phenomenex, USA) and their concentrations and $\delta^{13}\text{C}$ values were determined by the same GC-c-IRMS that was used for tFAs- PLFA-, and NLFA-FAMES from pyrosome tissue.

Calculation of pyrosome deposition rate

To calculate the pyrosome C and N contribution across the differing disturbed and undisturbed seafloor categories of the study region, we multiplied the above calculated pyrosome density (pyrosome m^{-2}) by the pyrosome C and N content, which was $0.12 \text{ g C pyrosome}^{-1}$ and $0.03 \text{ g N pyrosome}^{-1}$.

Calculating the deposition rate of pyrosome colonies and hence C and fatty acid flux was somewhat hampered by the temporal restrictions of our data. There were no data available beyond the cruise duration and hence we do not know the full duration of this deposition event, i.e. if the observed pyrosome bloom was a temporary event or if deposition happens throughout the year. Additionally, the removal rates were not rates of pyrosome removal, but of *Phacellaphora camtschatica* that is not scavenged by fish (Sweetman, personal observation). Hence, the removal rate in the DISCOL area could be higher than calculated here. On the other hand, experimental food falls of pyrosomes in Monterey Bay were still largely untouched on the seafloor after 24 hours (Hoving, personal observation).

Statistical analysis of seafloor observations

Our main variable of interest was the calculated pyrosome density, labeled Pyrosome *concentration* (Table S1), as measured by the pyrosomes encountered per square meter of the area imaged.

Our unit of analysis was the density per day, $(C_{i,t})$ $t = 1, \dots, 26$, by area classification, $i = \text{U, E, P}$, where U represents undisturbed seafloor, E the EBS disturbed seafloor, and P the plough-disturbed seafloor. Note that although our unit of analysis was at the date/classification level, each cross-sectional unit represented a unique area imaged.

In order to test our hypothesis that seafloor classification had an impact on the observed pyrosome density, we required two modifications of the raw data. The first modification was motivated by theoretical considerations. If no pyrosomes were encountered, reflected by a density level equal to zero in the data, it could be considered possible that either pyrosomes were never present in the first place, or that pyrosomes were at some point present but the seafloor was highly unfit to maintain the pyrosome presence. As both possibilities are *observationally* equivalent, we regarded it as inappropriate to include these observations in testing our main hypothesis. Therefore, we excluded the days when we observed no pyrosomes from statistical analysis. The second modification was a simple transformation of the variable of interest; the distribution in density was highly skewed and of course non-negative, rendering it unlikely that residuals be normally distributed. We therefore assumed that density levels were log-normally distributed, and used the natural logarithm of the pyrosome density in our analysis accordingly.

Figure 3 visualizes the modified data over time, by seafloor classification. Two observations stand out in this figure. First, our panel data set was rather unbalanced, both over time and in cross-

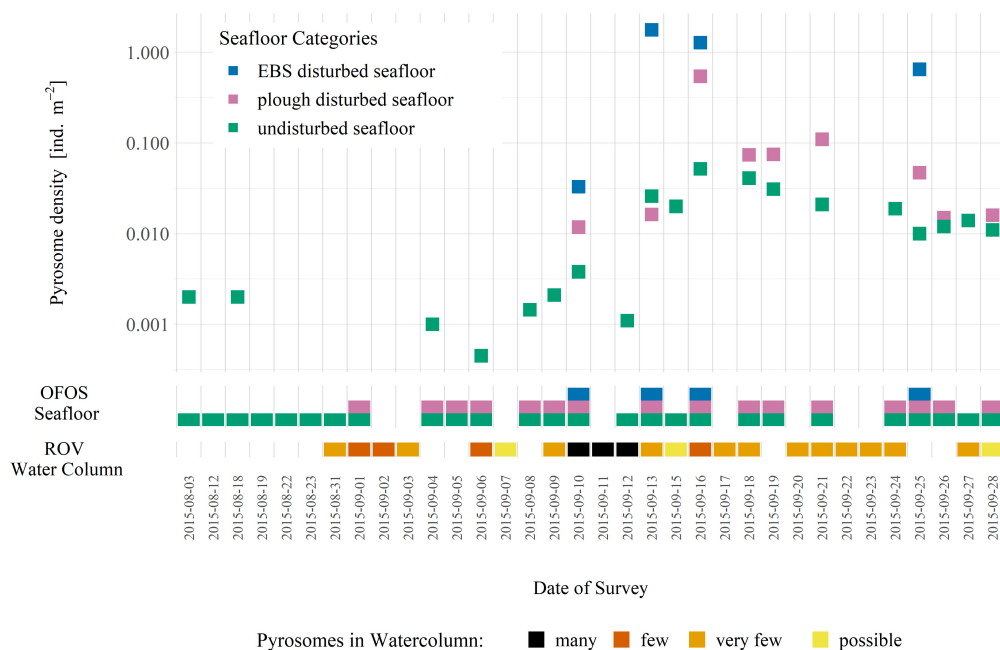


FIGURE 3

Temporal change of observed pyrosomes in the DISCOL Experimental Area in 2015. Seafloor observations with Ocean Floor Observations System (OFOS) allowed quantification of pyrosome densities (y-axis) over time (x-axis). The blue, pink and green symbols on the primary x-axis (OFOS Seafloor) indicate OFOS surveys in EBS, plough-disturbed and undisturbed seafloor, respectively. On all dives with the remotely operated vehicle (ROV), pyrosomes in the water column were estimated and illustrated on the secondary x-axis (ROV Water Column). Degrees of presence include possible (shapes in distance that are not identified as pyrosomes with confidence), occasional (1–5), numerous (6–22) and many (>50) is indicated by colours. Absence of a square on the x-axis under “OFOS Seafloor” and “ROV Water Column” equals no data collection. Absence of a y-value on a day with surveys means no pyrosomes were observed. Note the logarithmic scale on the y-axis.

section, and second, the overall variation over time (*within variation* = 1.88) was large compared to the variation between seafloor classification (*between variation* = 1.14). Moreover, a clear dynamic pattern was visible in the data, directly related to the pyrosome bloom.

To assess the differences in density per seafloor classification we used a *time fixed effects* regression, which accounted for differences in overall average levels of density over time, for all seafloor types. Though there may be more efficient and/or powerful tests to account for dynamic patterns, we approached the bloom effects in a statistically robust manner instead of trying to parametrically model the dynamic pattern. The only implicit assumption we made by doing so was that the temporal pattern affected all seafloor types in a homogenous way. We acknowledge that there may be

particular deviations for each unique imaged area, but these were reflected by the residuals, and we assumed that there was no particular bias in these deviations. To detect statistical differences for the seafloor classification, we ran these regressions with an EBS dummy variable and a Plough-disturbed dummy variable, capturing differences in log density relative to undisturbed seafloors.

To justify our assumption of log-normality and the associated statistical inference, we conducted a test for normality on the residuals and provide a normal-quantile plot (Figure S3). Based on the separate skewness and kurtosis tests, as well as a joint test on both skewness and kurtosis, we cannot reject that the residuals are normally distributed. Moreover, the quantile plot (Figure S3) shows some slight deviation in the tails, yet nothing too severe as to qualitatively affect our tests.

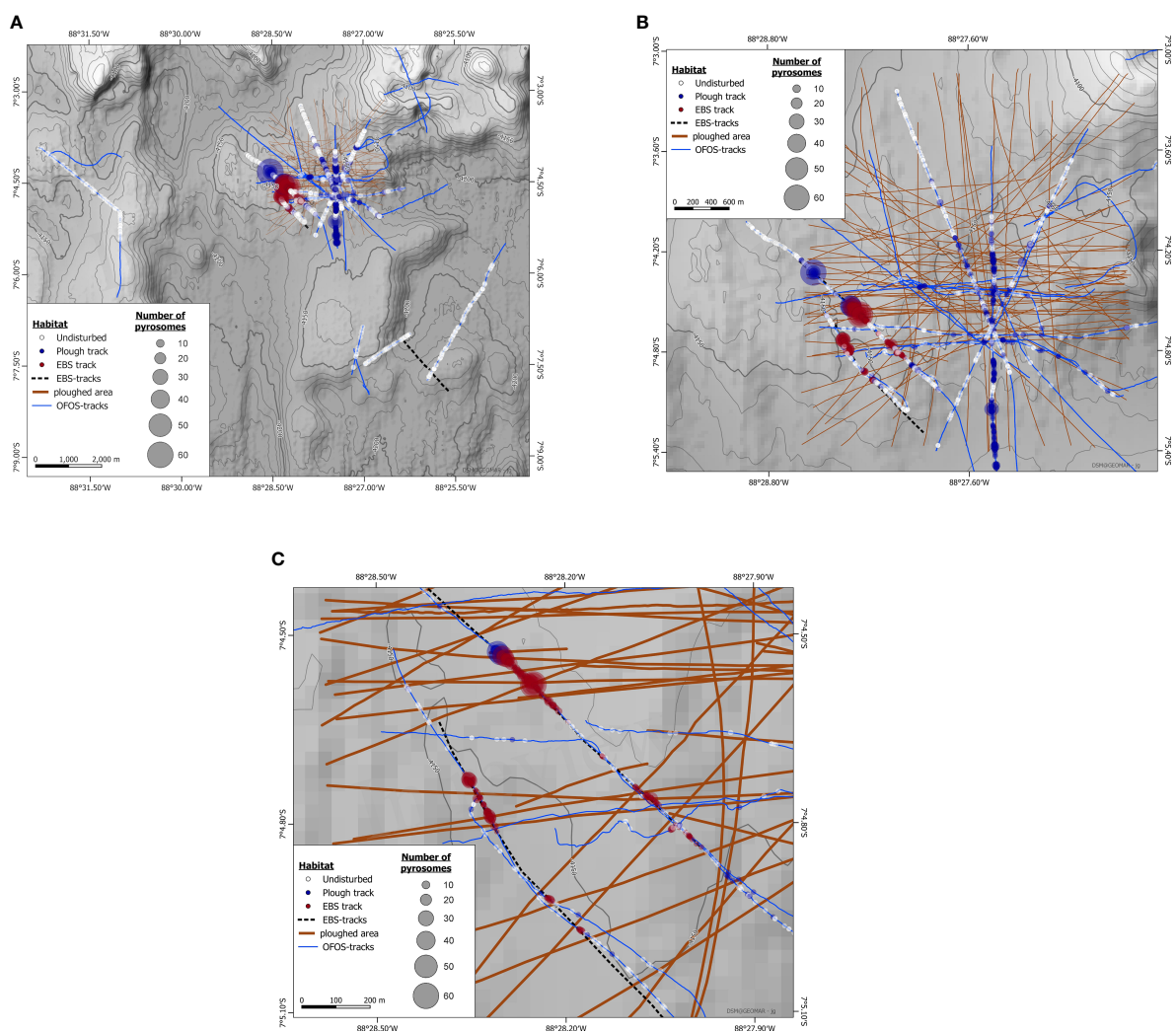


FIGURE 4

Pyrosome deposition in relation to seafloor type. (A) Overview of pyrosome deposition in the complete area surveyed during SO242, (B) Pyrosome deposition in the DISCOL Experimental Area. (C) Detail of (B) showing the southwest DISCOL region that was disturbed with epibenthic sled (EBS) and where most pyrosomes were observed. The circles indicate the presence of pyrosome carcasses observed along a track with the Ocean Floor Observation System (blue), and the colour of the circles indicate the seafloor type (undisturbed, plough track, EBS) where the pyrosomes were observed. The size of the circles depicts the number of pyrosomes in an image, showing that more pyrosomes were observed in the EBS and plough tracks than on the undisturbed seafloor. Images were collected throughout all transects with a spacing of several meters. Where no circles are placed over a survey track, no pyrosomes were observed.

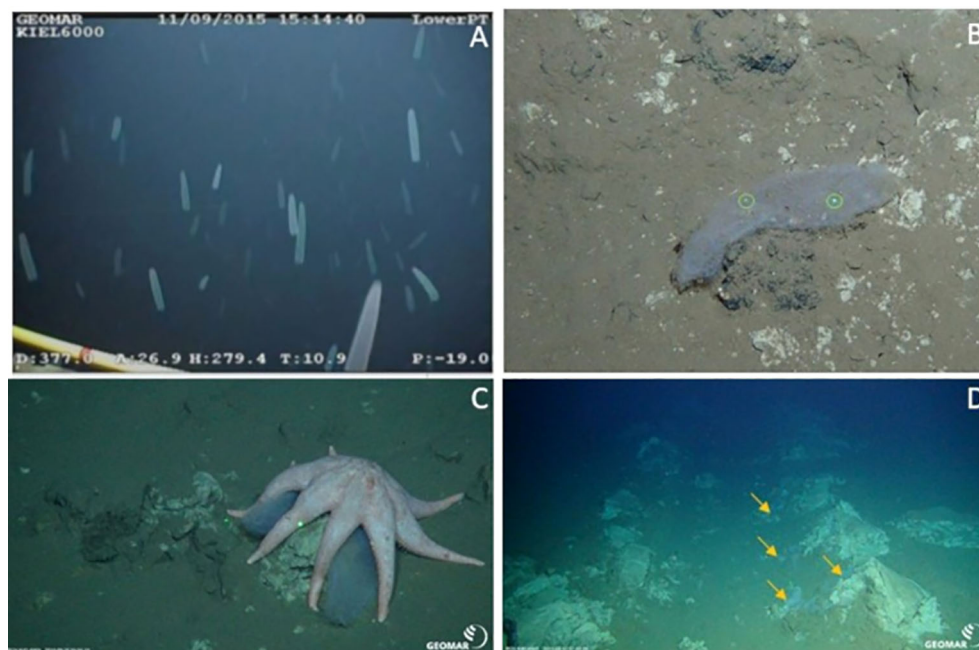


FIGURE 5

In situ observations of pyrosomes by ROV KIEL 6000 in the water column and on the seafloor. (A) Pyrosomes in the water column during ROV descent in the morning of 11.09.2015. (B) A pyrosome on the seafloor next to a polymetallic nodule at 4,150 m in the DISCOL area illustrating that seafloor rugosity can stop pyrosomes from travelling with currents. (C) A seastar presumably feeding on pyrosomes in the EBS track. (D) Pyrosomes in the EBS track, indicated by yellow arrows, showing accumulation of pyrosomes on one side of the EBS track (Images: ROV Team GEOMAR). (Laser points on B, C are 6.3 cm apart, and indicated by green circles).

Results

Temporal change in abundance of pyrosomes

Pyrosomes were observed and quantified on the seafloor by the OFOS (Figures 3, 4) and documented in both the water column and seafloor by the ROV (Figures 3, 5). The first water column observations were made on ascent on 31 August 2015, followed by a peak in water column pyrosome counts in observations between 10 and 12 September 2015 when many (> 50) pyrosomes were observed in the water column during ROV descents and ascents (Figure 3). Subsequently, numerous (6 – 22) or occasional (1 – 5) pyrosomes were seen within the water column between 17 September 2015 and the end of the cruise station work on 28 September 2015. The ROV was only on board the ship on cruise leg 2.

Few dead pyrosomes were observed during SO242/1 on the seafloor. AUV surveys documented ~5 dead pyrosomes in 100,000 m² of seabed surveyed across the DISCOL site (Simon-Lledó personal observation) and OFOS observed 2 pyrosomes during 6 transects across the undisturbed area (Table S2). During SO242/2, pyrosomes were observed on the seafloor on 3 August 2015 during an OFOS survey across the undisturbed areas (Figures 3, 4; Table S1). During the following 12 OFOS surveys in undisturbed areas, pyrosomes were occasionally visible (max. density 0.002 pyrosomes m⁻² on 9 September 2015) and only during five surveys. At these times, none were observed during five OFOS surveys in the DEA.

Pyrosomes were, however, increasingly present on OFOS images taken on transects on and after 10 September 2015, during which pyrosomes were observed in all three areas (plough tracks, EBS tracks and undisturbed) (Figure 3).

On 13 September 2015 the overall highest pyrosome seafloor density was recorded (in the tracks of the EBS; 1.77 pyrosomes m⁻²) (Figures 3, 4; Table S1). Densities of pyrosomes across both the undisturbed and plough-disturbed areas reached maxima (0.052 and 0.54 pyrosomes m⁻², respectively) on 16 September 2015, but were lower than in the freshly trawled EBS tracks on the same day (1.28 pyrosomes m⁻²) (Figure 3; Table S1). The peak abundance observed on 16 September 2015 was followed by a gradual decrease in pyrosome densities on the seafloor across all seafloor categories, with the declining trend in abundances sustained until the end of the survey period. On 25 September, the last day with surveys conducted in all habitat areas, pyrosomes showed low densities on the undisturbed seafloor (0.01 pyrosomes m⁻²), higher densities in the plough-disturbed area (0.047 pyrosomes m⁻²) and highest densities in the EBS track (0.65 pyrosomes m⁻²) (Table S1). Repeated observations in the same region confirmed a similar pattern in pyrosome densities.

Pyrosome accumulation in disturbed seafloor areas

Average densities in the undisturbed areas were 0.01 ± 0.01 pyrosomes m⁻² (with and without including zero values), and in the

plough-disturbed areas this was 0.06 ± 0.13 (including zero values) and 0.1 ± 0.17 (excluding zero values) in the plough-disturbed areas. In the EBS areas the average density was 1.01 ± 0.64 (no zero values). Fixed time effects regression confirmed that there were significant differences in pyrosome density between seafloor classifications (Table S2). Overall, the pyrosome densities in both disturbed areas were significantly higher than in the undisturbed seafloor classification areas (point estimate for EBS dummy variable = 3.88, significance = 1%, t -stat = 8.65; point estimate for plough-disturbed dummy variable = 0.97, significance = 5%, t -stat = 3.00) (Table S2).

Pyrosomes appeared to accumulate against vertical or sloped edges of the flanks of track marks from the plough and the EBS (Figure 5; Figure S1). Individual pyrosomes were additionally observed in association with polymetallic nodules (Figure 5), or the stalks of sponges or crinoids.

Biochemical composition of pyrosomes

During the onset of the pyrosome carcass bloom on 10 September 2015, pyrosomes had a mean total length (TL) of 13.3 ± 4.1 cm ($n = 20$) and on 16 September 2015, the day the maximum density of carcasses was observed on the seafloor, TL was 13.6 ± 3.7 cm ($n = 20$). During the decline of pyrosome carcass density on the seafloor on 25 September 2015, the mean TL was 13.0 ± 7.1 cm length ($n = 20$). The overall mean pyrosome TL was 13.3 ± 0.5 cm ($n = 60$) and did not differ significantly between the different dates of observations (one-way ANOVA test, $F(2,57) = 0.056$, $p > 0.05$).

Carbon content of pyrosomes was $8.51 \pm 1.77\%$ C dry mass (DM) pyrosome⁻¹ ($n = 3$) and their $\delta^{13}\text{C}$ value was $-22.0 \pm 0.08\text{‰}$ ($n = 3$). Pyrosomes contained $1.99 \pm 0.38\%$ nitrogen (N) DM pyrosome⁻¹ ($n = 3$), had a $\delta^{15}\text{N}$ value of $2.59 \pm 0.37\text{‰}$ ($n = 3$) and a C/N-ratio of 4.86 ± 0.34 ($n = 3$). Furthermore, they included 689 ± 109 μg total fatty acids (tFA)-C g DM pyrosome⁻¹, of which $86.1 \pm 25.2\%$ consisted of PLFAs and $11.9 \pm 4.57\%$ of NLFAs (Table 1). Highly unsaturated fatty acids (HUFAs; i.e., fatty acids with ≥ 4 double bonds) contributed with 47% most to tFAs, followed by saturated fatty acids (SFAs) with 28%, and PUFAs (i.e., fatty acids with ≥ 2 double bonds) (15%) (Table 1). Pyrosome PLFAs were similarly dominated by HUFAs and SFAs, whereas NLFAs contained 28% SUFAs, 22% HUFAs, and 21% PUFAs. About 64%, 92%, and 75% of these PUFAs of tFAs, PLFAs, and NLFAs, respectively, were 'essential fatty acids' (Dalsgaard et al., 2003) and ALA (co-eluted with oleic acid, 18:1 ω 9) (Table 1). Other abundant fatty acids in pyrosomes were adrenic acid (22:4 ω 6) (tFA: 20%, PLFA: 21%) and palmitic acid (16:0) (NLFA: 8%) (Tables 1, 2).

In addition to the dominant PLFAs, pyrosomes contained PLFAs that have been identified as trophic markers for specific food sources (Table 2): Pyrosomes had low concentrations of gondoic acid (20:1 ω 9; co-eluted with 20:3 ω 3) and 22:1 ω 11 (together 0.3% of total PLFAs, Table 2), which are markers for calanoid copepods [Table 2, (Dalsgaard et al., 2003)]. They also contained low concentrations of stearidonic acid (18:4 ω 3), 20:4 ω 3, and eicosapentaenoic acid (EPA, 20:5 ω 3) (together 12% of total PLFAs; Table 2) that are markers for brown macroalgae (Phaeophyta), and low concentrations of the markers for Prymnesiophyceae, i.e., oleic acid, moroctic acid (18:4 ω 3), and

TABLE 1 Biochemical composition of pyrosomes ($n = 3$) and sediment collected in the DISCOL area in 2015.

	Total fatty acid in pyrosomes	PLFA in pyrosomes	NLFA in pyrosomes	PLFA in sediment	
				0–2 cm	2–5 cm
Total concentration (μg C g DM pyrosome ⁻¹ , μg C g DM sediment ⁻¹)	689 ± 109	497 ± 146	68.9 ± 26.3	2.01 ± 0.85	2.15 ± 0.43
Contribution of SFA (%)	27.8 ± 2.22	28.5 ± 3.71	27.6 ± 1.46	36.1 ± 2.82	52.1 ± 15.1
Contribution of MUFA (%)	8.54 ± 2.42	8.47 ± 0.16	19.7 ± 3.54	41.9 ± 2.08	31.3 ± 8.52
Contribution of PUFA (%)	14.9 ± 1.35	15.4 ± 1.15	20.5 ± 4.68	11.0 ± 0.52	8.25 ± 2.94
Contribution of HUFA (%)	46.6 ± 2.67	46.3 ± 3.94	22.2 ± 3.36	1.98 ± 1.45	0.60 ± 0.84
Contribution of LCFA (%)	0.95 ± 1.04	1.03 ± 1.46	8.45 ± 7.74	0.30 ± 0.43	0.92 ± 1.31
Contribution of cyclopropyl fatty acids (%)	0.07 ± 0.08	0.01 ± 0.02	0.56 ± 0.79	2.02 ± 0.40	1.19 ± 0.90
Contribution of 10-methyl fatty acids (%)	1.10 ± 1.26	0.15 ± 0.11	1.01 ± 1.43	6.78 ± 0.84	5.66 ± 2.33
Contribution of hydroxy fatty acids (%)	0.06 ± 0.04	0.02 ± 0.03	0.00 ± 0.00	not detected	not detected
Total concentration of essential fatty acids 18:2 ω 6 (linoleic acid) and 18:3 ω 3 (α -linolenic acid; co-eluted with 18:1 ω 9, oleic acid) in pyrosome carcass ($n = 3$) and sediment samples ($n = 3$)	63.6 ± 33.3 μg C g DM pyrosome ⁻¹	68.4 ± 12.0 μg C g DM pyrosome ⁻¹ (equivalent to: 0.10 ± 0.06 mg C m ⁻² in EBS track; 0.006 ± 0.013 mg C m ⁻² in plough track; 0.001 ± 0.001 mg C m ⁻² at undisturbed seafloor)	9.28 ± 1.54 μg C g DM pyrosome ⁻¹	0.12 ± 0.04 μg C/g DM sediment (equivalent to: 0.48 ± 0.05 mg C m ⁻²)	$0.11 \pm 4.23 \times 10^{-3}$ μg C/g DM sediment (equivalent to: 0.43 ± 0.11 mg C m ⁻²)

DM, dry mass; HUFA, highly unsaturated fatty acids (fatty acids with ≥ 4 double bonds); LCFA, long-chain fatty acids (fatty acids with ≥ 24 C atoms); MUFA, monounsaturated fatty acids (fatty acids with one double bond); NLFA, neutral lipid-derived fatty acid; PLFA, phospholipid-derived fatty acid; PUFA, polyunsaturated fatty acids (fatty acids with ≥ 2 double bonds); SFA, saturated fatty acids.

TABLE 2 Sources of fatty acids in the pyrosomes from the DISCOL area (Peru Basin).

Fatty acid	Origin	Ref.
20:1 ω 9, 22:1 ω 11	Calanoid copepods	(Falk-Petersen et al., 1987; Dalsgaard et al., 2003)
16:3 ω 3, 16:3 ω 4, 16:4 ω 3, 18:1 ω 7, 18:2 ω 6, 18:3 ω 3, 18:3 ω 6, 18:4 ω 3, 20:4 ω 3	Chlorophyta (green macroalgae)	(Léveillé et al., 1997; Graeve et al., 2002; Kelly and Scheibling, 2012; Gaillard et al., 2017)
14:0, 18:1 ω 9, 18:2 ω 3, 18:2 ω 6, 18:4 ω 3, 20:4 ω 6, 20:5 ω 3	Phaeophyta (brown macroalgae)	(Graeve et al., 2002; Kelly and Scheibling, 2012; Gaillard et al., 2017)
18:1 ω 9, 18:4 ω 3, 22:5 ω 6	Prymnesiophyceae	
16:1 ω 7, 16:2 ω 4, 16:2 ω 7, 16:3 ω 4, 16:3 ω 6, 16:4 ω 1, 20:5 ω 3; 16:1 ω 7/16:0-ratio >1; 20:5 ω 3/22:6 ω 3-ratio >1	Diatoms	(Jeffries, 1970; Mayzaud et al., 1989; Skerratt et al., 1995; Léveillé et al., 1997; Perissinotto et al., 2007; Kelly and Scheibling, 2012)
16:0, 16:3 ω 4, 18:0, 18:2 ω 6, 18:3 ω 3, 18:4 ω 3, 18:5 ω 3, 20:5 ω 3, 22:6 ω 3; 16:1 ω 7/16:0-ratio <1	Dinoflagellates	(Jeffries, 1970; Mayzaud et al., 1989; Skerratt et al., 1995; Léveillé et al., 1997; Kelly and Scheibling, 2012)(Perissinotto et al., 2007)
12:0, 14:0, <i>i</i> 15:0, <i>ai</i> 15:0, 16:0, 16:1 ω 7, <i>cy</i> -17:0, 18:1 ω 7, 18:0, <i>cy</i> -19:0	Bacteria	(Parkes and Taylor, 1983)
18:1 ω 9/18:1 ω 7-ratio >1	Carnivore	(Graeve et al., 1997)
18:1 ω 9/18:1 ω 7-ratio <1	Suspension feeder	(Graeve et al., 1997)

22:5 ω 6 [Table 2, (Graeve et al., 2002; Perissinotto et al., 2007)]. Furthermore, 7–35% of total PLFAs in pyrosomes contained the dinoflagellate markers C18-PUFAs and C22-PUFAs. The 16:1 ω 7/16:0-ratio- and 20:5 ω 3/22:6 ω 3-ratio-values below 1 also suggest a dinoflagellate-dominated diet [Table 2, (Dalsgaard et al., 2003)]. Unfortunately, the PLFAs 18:1 ω 9 co-eluted with 18:1 ω 7, so that it could not be specified whether the pyrosomes from the DISCOL site were mainly carnivores or suspension feeders. $\delta^{13}C_{FA}$ of the ten most abundant fatty acids in pyrosomes ranged from $-44.4 \pm 22.6\text{‰}$ (EPA) to $-17.2 \pm 12.2\text{‰}$ (adrenic acid) in tFAs, from $-28.1 \pm 1.17\text{‰}$ (LA) to $-9.28 \pm 13.1\text{‰}$ (arachidonic acid, ARA: 20:4 ω 6) in PLFAs, and from $-29.8 \pm 5.86\text{‰}$ (stearic acid: 18:0) to $-15.6 \pm 11.1\text{‰}$ (lignoceric acid: 24:0) in NLFAs (Table 1).

Biogeochemical composition of deep-sea surface sediments

Deep-sea surface sediments (0–2 cm sediment layer, $n=29$) in the surveyed region had a C content of $0.67 \pm 0.08\%$ C DM sediment $^{-1}$ and a N content of $0.15 \pm 0.02\%$ N DM sediment $^{-1}$. The 2–5 cm sediment layer ($n=28$) had a C content of $0.62 \pm 0.06\%$ C DM sediment $^{-1}$, a N content of $0.14 \pm 0.001\%$ N DM sediment $^{-1}$. The resulting C/N-ratios were for both depth layers ~ 5.1 .

The most abundant PLFAs in the deep-sea sediment were palmitoleic acid (16:1 ω 7 cis) (0–2 cm sediment layer: $16.8 \pm 0.64\%$) and behenic acid (22:0) (2–5 cm sediment layer: $14.1 \pm 11.8\%$) (Table 1). $\delta^{13}C_{FA}$ of PLFAs varied from $-19.8 \pm 2.26\text{‰}$ (oleic acid 18:1 ω 9 cis , co-eluted with LA 18:2 ω 6 $trans$ and ALA) to $-11.5 \pm 3.05\text{‰}$ (palmitic acid) in surface sediment (0–2 cm layer) and from $-24.8 \pm 0.69\text{‰}$ (behenic acid) to $-10.4 \pm 1.28\text{‰}$ (palmitoleic acid: 16:1 ω 7 cis) in subsurface sediment (2–5 cm layer).

Carbon and nitrogen contribution of pyrosomes

Mean regional C and N contributions of pyrosomes in the entire surveyed region was $14 \pm 41\text{ mg C m}^{-2}$ and $3.2 \pm 10\text{ mg N m}^{-2}$ (range: 0–216 mg C m $^{-2}$ and 0–51 mg N m $^{-2}$; $n=46$). In the EBS transects, the average C and N deposition was more than 72 times higher ($123 \pm 79\text{ mg C m}^{-2}$ and $29 \pm 18\text{ mg N m}^{-2}$, range: 40–216 mg C m $^{-2}$ and 9.4–51 mg N m $^{-2}$; $n=4$) than in the areas with an undisturbed seafloor ($1.7 \pm 1.8\text{ mg C m}^{-2}$ and $0.40 \pm 0.43\text{ mg N m}^{-2}$, range: 0–6.3 mg C m $^{-2}$ and 0–1.5 mg N m $^{-2}$; $n=26$). The plough-disturbed seafloor had higher levels of pyrosome C ($7.0 \pm 16\text{ mg C m}^{-2}$ and $1.6 \pm 3.8\text{ mg N m}^{-2}$, range: 0–66 mg C m $^{-2}$ and 0–15 mg N m $^{-2}$; $n=16$) than the undisturbed region, but lower than the EBS-disturbed region. This translates to an estimated rate of minimum 2.4 to maximum 20 mg C m $^{-2}$ d $^{-1}$ and of minimum 0.5 to maximum 4.6 mg N m $^{-2}$ d $^{-1}$ during the food fall event (assuming the removal rate of *Phacellaphora camtschatica* at 5,300 m water depth in the western Pacific: minimum 0.6 g wet mass (WM) d $^{-1}$, maximum 5.4 g WM d $^{-1}$; equivalent to 2.6 mg C d $^{-1}$ and 21 mg N d $^{-1}$ or 0.6 mg N d $^{-1}$ and 5.0 mg N d $^{-1}$ or 17% d $^{-1}$ and 144% d $^{-1}$; Sweetman, unpublished data) compared to a total benthic C demand of 6 to 11 mg C m $^{-2}$ d $^{-1}$ in the DEA (Vonnahme et al., 2020).

Faunal associations

Overall, we observed 2,958 individual pyrosome carcasses on the seafloor with OFOS, 184 of which were observed in close proximity to other fauna including taxa of the phyla Echinodermata, Arthropoda and Cnidaria. Close up observations by ROV showed pyrosome colonies to be (putatively) consumed by anemones, brittle stars, seastars and isopods (Figure 5).

Additionally, OFOS and ROV observations showed white organisms inside the pyrosome carcasses, potentially amphipods.

Discussion

Our serendipitous observations of a pyrosome bloom in open ocean waters revealed a pathway for C and N from the photic to the abyssal zone. Our observations, which are among the deepest jelly falls to date, show that pyrosome fluxes may contribute significantly to the local carbon and nitrogen flux of the abyssal plain in the Peru Basin. Additionally, these carcasses provide essential fatty acids to diverse deep-sea scavengers. Temporal analysis showed a peak in seafloor counts approximately 29 days after the first pyrosome observation on the seafloor during the second cruise leg, as well as a gradual decrease in pyrosome carcasses on the seafloor after the peak. The accumulation of carcasses and their subsequent decrease was observed over a relatively broad area of the seafloor. This illustrates the relatively short period of the phenomenon and highlights the challenge associated with capturing biological events relevant to the carbon pump that are stochastic in space and time. Recently disturbed seafloor contained up to twofold higher carbon concentrations than undisturbed surrounding seafloor. While the biological effects of this accumulation require further research, our findings clearly illustrate the urgency for the consideration of pelagic-benthic coupling in the context of deep-sea benthic resource extraction management and impact assessments.

Pyrosomes in the Peru Basin

The pyrosomes collected at the DISCOL site grazed mainly on dinoflagellates as indicated by the specific PLFA ratios 16:1ω7/16:0 and 20:5ω3/22:6ω3 and on Prymnesiophyceae as revealed by the fatty acid markers oleic acid, morotic acid, and 22:5ω6. *Pyrosoma atlanticum* from the Indian Ocean and the Northern California Current have a similar diet and consume predominantly dinoflagellates and Prymnesiophyceae as identified by their fatty acid profiles (Perissinotto et al., 2007; Schram et al., 2020). Additionally, lower concentrations of the calanoid copepod markers gondoic acid and 22:1ω11 in pyrosome tissue from the DISCOL site suggest that these pyrosomes may predate upon zooplankton or ingest their faeces. However, copepod contribution to the overall pyrosome diet is likely very small as implied by the isotope value of $\delta^{13}\text{C} = -22.0\text{‰}$ and $\delta^{15}\text{N} = 2.59$. Though the $\delta^{13}\text{C}$ value of the pyrosomes was in the range of the $\delta^{13}\text{C}$ value of mesozooplankton in the eastern tropical Pacific [range: -19.9 to -22.3‰ (Décima et al., 2019)], where sediment trap samples had a $\delta^{13}\text{C}$ value of -24.1‰ (Décima et al., 2019), the pyrosomes were depleted in ^{15}N compared to *Pyrosoma atlanticum* from the Central Arabian Sea [$\delta^{15}\text{N} = 7.43\text{‰}$ (Gauns et al., 2015)] and from the Northern California Current [$\delta^{15}\text{N} = 7.5\text{‰}$ (Schram et al., 2020)]. This indicates that pyrosomes in the present study had a lower trophic level compared to one in the other studies.

Pyrosome food falls linking epipelagic and abyssal plain food webs

The importance of pyrosomes as vectors of C and N to the deep sea, has been noted in several ocean basins (Kuo et al., 2015; Miller et al., 2019; Schram et al., 2020; Stenvers et al., 2021). Pyrosomes are particularly effective vectors because of efficient feeding and a combination of high fecal pellet production, daily vertical migration (Henschke et al., 2019) and rapidly sinking carcasses with high C content [up to 9% DM in the DISCOL area, but previously reported 35% – 38% DM in the South Atlantic and the Central Arabian Sea (Lebrato and Jones, 2009; Gauns et al., 2015)]. The observed pyrosome flux in the DISCOL area was lower than fluxes of pyrosomes and other gelatinous zooplankton reported in shallower areas in the South Atlantic (5 g C m⁻² at 1,275 m) (Lebrato and Jones, 2009), but comparable to the C flux of pyrosomes off Cabo Verde [North-East Atlantic, 0.6 to 42 mg C m⁻² (Stenvers et al., 2021)] and to the C flux of jelly falls in the North-East Pacific (*Salpa*, >30 mg C m⁻² d⁻¹ at 3,400 m) (Smith et al., 2018) and Norwegian fjords (*Periphylla*, 13 mg C m⁻² d⁻¹) (Sweetman and Chapman, 2015). The pyrosome standing stock on the seafloor in the DISCOL region was likely contributing significantly to the regional carbon pump at the time of this study: The mean regional pyrosome C deposition observed during this study (14 mg C m⁻², ~2 to 20 mg C m⁻² d⁻¹ or 0.7 to 7 g C m⁻² yr⁻¹) would equal 182 to 1543% of the flux of particulate organic C [1.3 mg C m⁻² d⁻¹ or 0.5 g C m⁻² yr⁻¹ calculated based on sediment porosity: 0.93 ± 0.01 , sedimentation velocity: 0.4 cm kyr⁻¹, C content in 0–2 cm sediment layer: $0.67 \pm 0.08\%$ C DM sediment⁻¹ (Haffert et al., 2020)] and 39 to 184% of the total respired benthic C flux [6 – 11 mg C m⁻² d⁻¹ or 2 – 4 g C m⁻² yr⁻¹ (Vonnahme et al., 2020)] in the DISCOL area. Furthermore, we hypothesize that the deposited pyrosomes may increase the so-called ‘priming effect potential’. This potential describes short-term changes in mineralization of sedimentary organic matter after the addition of substances to sediments due to interactions between added substances and the natural element cycles (Kuznyakov et al., 2000; Bianchi, 2011) in the surface sediment. In the Peru Basin, the pyrosomes have a higher lability than the rather refractory sedimentary organic matter. This difference in reactivity is corroborated by the fact that pyrosome total fatty acids and PLFAs were dominated by labile HUFAs with notable contributions of PUFAs compared to more stable MUFAs and saturated fatty acids which comprised the majority of sedimentary fatty acids. This change in relative contribution of HUFAs and PUFAs indicates that more unsaturated fatty acids were preferentially taken up and degraded by organisms scavenging the pyrosome food fall. Hence, these fatty acids are important for organic C cycling in deep-sea sediments and deposition of pyrosomes may also stimulate the overall degradation of organic matter at the seafloor. The pyrosomes additionally provided small amounts of the ‘essential’ fatty acids LA and ALA. However, a short-term (i.e., weeks to months) contribution of 0.001 ± 0.001 mg C m⁻² (range: 0 – 0.005 mg C m⁻²) LA and ALA is equivalent to 0.3% of the LA and ALA stock in the upper 2 cm of surface sediment in the Peru Basin (0.48 ± 0.05 mg C m⁻² LA and ALA; Table 1), so that rapidly deposited phytodetritus might provide a more important

source of these specific fatty acids at undisturbed sites. In comparison, at freshly-trawled and medium-disturbed sites, the stochastically occurring deposition of pyrosomes adds up to $0.10 \pm 0.06 \text{ mg C m}^{-2}$ and $0.006 \pm 0.013 \text{ mg C m}^{-2}$, respectively, of LA and ALA (Table 1). At these disturbed sites, a short-term increase in these ‘essential’ fatty acids might attract mobile deposit feeders, such as holothurians. However, recolonization may be delayed due to reduced food availability. This was observed in the DISCOL area where holothurians recolonized the area, but at a slower pace than expected based on their mobility/moving speed (Stratmann et al., 2018b).

Particulate N flux in the Peru Basin was $0.29 \text{ mg N m}^{-2} \text{ d}^{-1}$ (calculated based on sediment porosity: 0.93 ± 0.01 , sedimentation velocity: 0.4 cm kyr^{-1} , N content in 0–2 cm sediment layer: $0.14 \pm 0.01\% \text{ N DM sediment}^{-1}$). This is in the range of the total PN flux between 3,350 and 3,800 m water depth in the subarctic Pacific (0.4 to $3.2 \text{ mg N m}^{-2} \text{ d}^{-1}$) (Haake et al., 1993; Wu et al., 1999), but two to three orders of magnitude higher than the PN flux at Station M in the North-East Pacific (57 to $165 \text{ mg N m}^{-2} \text{ d}^{-1}$) (Gaye et al., 2009). A pyrosome flux of 0.5 to $4.6 \text{ mg N m}^{-2} \text{ d}^{-1}$ is thus significant (189 to 1601%) to the regional particulate nitrogen flux during the time of the food fall.

Seafloor disturbance and accumulation of detritus

Our study also provides supporting evidence for the effects of physical seafloor disturbances on organic C deposition on the abyssal seafloor. Fine-scale variations in seafloor morphology at the scale of decimeters, physical seafloor disturbance and increased roughness clearly influenced the distribution of deposited pyrosome carcasses and resulted in local accumulations of deposited organic material. This was particularly true for the disturbance by trawling with the EBS, which caused 15 – 20 cm deep marks with steep walls (Vonnahme et al., 2020), resulting in an accumulation of pyrosomes on one side of the track. This accumulation was potentially a result of transport by benthic currents and subsequent hydrodynamic entrapment. Tidal movements dominated current activity in the first half of the expedition (Greinert, 2015). During SO242/2, the bottom current was primarily flowing from the west and with relatively low velocity (3 – 5 cm s^{-1}). The accumulation of pyrosomes along the eastern side of the EBS disturbance tracks corresponded with this hypothesized entrapment, resulting from the prevailing current direction from the west. This may indicate that currents carry the pyrosomes along the seafloor in the bottom boundary waters until they are physically stopped by the track edge. On average, the density of pyrosomes was almost two orders of magnitude greater in the recent EBS deep disturbance marks compared to those observed across the undisturbed surrounding seafloor. Significantly higher pyrosome densities in disturbed areas than those observed elsewhere during the same survey days confirmed that seafloor morphology was the main reason for differences in pyrosome density. Such accumulations of pyrosomes have also been observed along the sides of pipelines and

in depressions off West Africa (Lebrato and Jones, 2009; Lebrato et al., 2022) with salps also having been observed to accumulate in hollows and furrows on the NW Atlantic slope (Cacchione et al., 1978). In order to analyze pyrosome accumulation in disturbed seafloor areas we here pooled undisturbed seafloor areas that included areas with and without nodules. However, we also observed individual pyrosomes trapped against individual manganese nodules. Increased seafloor rugosity as a result of nodule presence may therefore also result in increased organic matter accumulation which may create locally enhanced organic matter availability, potentially attracting fauna and influencing biodiversity in nodule areas. It may also result in flow patterns that prevent the deposition of smaller carcasses. The natural ability of nodule areas to trap pelagic detrital matter needs further investigation.

Although the EBS tracks may be considered a significant disturbance, resulting in reduced biological activities and relatively low organic matter remineralization rates (de Jonge et al., 2020), the greater accumulation of pyrosomes inside the disturbed areas did not appear to be caused by reduced scavenging in these disturbed areas. Previous studies show that the biomass of scavengers and densities of decapods, which can rapidly consume carcasses, were unexpectedly higher inside the plough tracks compared to outside on the undisturbed seafloor (Simon-Lledó et al., 2019a; de Jonge et al., 2020), suggesting higher scavenging rates and shorter residence times would be more likely. Although we do not know the abundance and role of macrofaunal scavenging at DISCOL, the megafaunal scavengers may be responsible for considerable carcass consumption, as has been observed elsewhere (Sweetman et al., 2014). Exact scavenging rates, however, are unknown and directed experiments with known pyrosome carcass numbers deposited on the seafloor would be required to acquire this information. From shallow-water experiments it is known that organic-matter deposition is correlated to sediment surface structure and morphology (Yager et al., 1993) but such biogeochemical knowledge is largely absent for the abyssal plain. While the alteration in organic matter deposition from human-induced physical seafloor disturbances may persist for centuries to millennia (Haackel et al., 2001; Haffert et al., 2020; Volz et al., 2020), it is not well known to what extent such local accumulation of organic matter may impact benthic communities, biodiversity and functioning. Observations of complete shifts from faunal to microbial C cycling processes and spikes in seafloor respiration around gelatinous zooplankton falls suggest functional changes are likely (Sweetman et al., 2016).

Outlook

The significant accumulation of pelagic detritus in high relief features of physically disturbed areas of seafloor represents a rarely considered additional impact of anthropogenic seafloor disturbances on benthic communities, with such physical disturbances likely to result from activities such as benthic trawling or polymetallic nodule mining. Further studies are needed to address how such impacts may alter (e.g. enhance or decrease) biodiversity or biogeochemical

functioning, and whether ecosystem functioning is negatively impacted. Although our observations and calculations were done on a relatively fine scale (1 – 10m), the accumulation of organic matter in anthropogenic tracks is relevant to other types of seafloor disturbance (e.g. dredging, laying of undersea cables and pipelines, shipwrecks, and in particular trawl-fishing) (Martín et al., 2014). Anthropogenic activities taking place on the seafloor are increasing worldwide. Hence, our results are relevant on a global scale. Additionally, the accumulation of pyrosomes in disturbed regions can be illustrative of the deposition of other items from the water column onto the seafloor, including other forms of organic matter, but also plastics and other litter. The accumulation of pelagic detritus and other material in seafloor areas that have lost their natural integrity is likely a general impact (i.e. not pyrosome bloom specific) that requires consideration in future trawling and deep-seabed mining management strategies, i.e. by design of comprehensive (nodule and fish) exploitation technologies. How these locally elevated organic matter accumulations may further impact these already disturbed environments is unknown, but is a relevant aspect of potential mining effects on the ecology of abyssal plains, or benthic trawling on shelf seas, seamounts, and ocean margins.

Data availability statement

Data on pyrosome observations is summarized and provided in [Supplementary Table 1](#). Individual pyrosome annotations in relation to seafloor type will be available in: Purser, Autun; Hoving, Henk-Jan (2022), “Pyrosome corpse observations across the undisturbed and anthropogenically disturbed seafloor of the DISCOL experimental area, Peru Basin”, Mendeley Data, V1, doi: 10.17632/6nyh68vtv9.1. Bathymetric data is available in PANGAEA at <https://doi.org/10.1594/PANGAEA.905616> and <https://doi.org/10.1594/PANGAEA.905579>. Seafloor images are available in PANGAEA at <https://doi.org/10.1594/PANGAEA.890634>. Water column observations and biochemical data are uploaded in PANGAEA and the link will be made available after publication. Data from the biogeochemical analysis are found under the following links: Sediment: <https://doi.org/10.1594/PANGAEA.905377>. Sediment PLFA: <https://doi.pangaea.de/10.1594/PANGAEA.946213>. Pyrosome: <https://doi.pangaea.de/10.1594/PANGAEA.946209>. Pyrosome total fatty acid: <https://doi.pangaea.de/10.1594/PANGAEA.946256>. Pyrosome PLFA: <https://doi.pangaea.de/10.1594/PANGAEA.946259>. Pyrosome NLFA: <https://doi.pangaea.de/10.1594/PANGAEA.946266>.

Author contributions

JG, AB, AP, YM, IS, AS, KD, MH, TS, ES-L performed fieldwork. TS, AP, YM, IS, ES-L, KD, H-JH performed (lab and/or image) analysis. H-JH, AP, DJ and TS drafted the manuscript. All authors contributed to the article and approved the submitted version.

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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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1192242/full#supplementary-material>

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Environmental impacts of shallow water mining in the Baltic Sea

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The discovery of rare metal resources in international waters has raised seabed mining claims for large areas of the bottom. There is abundant scientific evidence of major negative consequences for the maritime environment, such as the destruction of natural landforms and the fauna that depend on them, as well as the production of enormous silt plumes that disrupt aquatic life. This study investigated the environmental risks of seabed mining for metal resources in the Baltic Sea using a combination of hydrodynamic, particle-tracking, and sediment-transport models. The models were applied for ten years i.e., 2000–2009 under prevailing conditions to simulate seabed mining operations. The focus was on sediment concentration near the seabed and its spread. The mean background concentrations were low with small seasonal bed-level variations throughout the Baltic Sea Basin. Late summer and early autumn periods were the most active. Seabed mining significantly alters the dynamics of sediment suspensions and bed level variations. The concentrations increase unsustainably to high levels, posing a serious threat to the ecological health of the Baltic Sea. The Gotland basins in the Baltic Sea are the most susceptible to mining. The bed level variations will be ten-fold, exposing the highly contaminated sediments at the seabed to the flow. In less than a year, 30–60% of the total particles released in each basin reached the thermocline layers. This study suggests that seabed mining in the Baltic Sea is not sustainable.

KEYWORDS

shallow water mining, Baltic Sea, hydrodynamic modelling, sediment transport modelling, sediment suspension

1 Introduction

Seabed mining affects the dynamics of suspended sediment loads near the seabed and in the water column, which is correlated with various environmental problems. It is encouraging to see a growing public awareness of seabed mining as a possible serious environmental threat to the oceans. In this case, the scientific community plays an important role in analyzing and scrutinizing various aspects of seabed mining. To quote Earle and Kammen (2022) on seabed mining “Seldom do we have an opportunity to stop an environmental crisis before it begins. This is one of those opportunities”.

The introduction describes numerous aspects of energy needs, mining technology, and environmental implications of seabed mining, with a focus on sediment plume development, modelling methodologies, and the current state of the Baltic Sea, which is the main area investigated in this study. The goal was to present a brief overview of ongoing efforts on various elements of seabed mining.

There is an exponentially growing worldwide demand for electric transportation to reduce negative trends in global climate change. The details of worldwide energy consumption in different sectors are available from [U.S. Energy Information Administration \(2016\)](#) and [Enerdata \(2022\)](#). Published data indicate 23,845 TWh of energy were used in 2019. Generally, transportation accounts for 25% and industries, including residential housing and commercial businesses, account for 35% of the total energy. Considering the focus of seabed mining on transportation, the present battery demand is 340 GWh, which will grow to 3500 GWh, which is far beyond the total available land resources [IEA \(2022\)](#). The discovery of the majority of rare metal resources in international waters (mostly in the Pacific Ocean) has increased the claims of seabed mining contractors to significant regions of the seafloor ([Sharma, 2015](#)). The International Seabed Authority granted 31 contracts to investigate deep-sea mineral resources as of May 2022. The global seabed designated for resource exploration has a size of 1.45 million km², which is roughly equal to the surface areas of France, Germany, and Spain ([IUCN, 2022](#)).

Mining technology is undergoing further technical developments. Examples of possible technical details and operational modes of deep-sea mining have been discussed by researchers, including [Hengling and Shaojun \(2020\)](#). Mining machinery typically consists of four main units: a water pump, suction pipe, pressure pipe, and collector ([Alhaddad et al., 2022](#)). Mining operations on the seafloor create sediment plumes and turbidity currents. The excavated materials are pumped to a ship and wastewater and debris are dumped back into the ocean to form sediment clouds. The focus of the present study was on sediment plumes.

Most studies have used numerical modelling to investigate the dynamic characteristics of seabed mining (e.g. [Jankowski and Zielke, 2001](#); [Byishimo, 2018](#)). [Jankowski and Zielke \(2001\)](#) developed a complicated mesoscale 3D numerical hydrodynamic and sediment transport model to investigate the impact of deep-sea activity on sediment concentration. The model also accounted for the influence of flocculation on sediment settling velocity. The overall bottom deposition patterns were successfully modelled, in agreement with the measurements. However, no conclusion could be drawn on the prognostic capability of the model because of the lack of complete datasets. [Byishimo \(2018\)](#) presented comprehensive Computational Fluid Dynamics (CFD) and laboratory studies on deep-sea mining, covering different aspects of plumes released at different water depths. It was found that particle settlement in the plume released near the bed was limited by the ambient currents that were carried far from the released currents in the same direction as the local ambient currents. The foregoing and similar studies show the strengths and weaknesses of numerical models for studying the impact of seabed mining on sediment transport. Numerical models can provide detailed results that are difficult to achieve using point

measurements at preselected sites. However, they require field data for validation, which is currently scarce. Based on the success of these and similar models, a numerical approach was used to investigate the impact of SWM in the Baltic Sea.

A key difficulty in studying the impact of seabed mining is a lack of complete and consistent field data. Data are required to validate numerical models and to realistically represent the overall impact of mining machinery. This void can be filled in part by experimental and full-scale machinery tests. There have been several experimental and on-site studies. [Muñoz-Royo et al. \(2022\)](#) conducted an extensive field study on seabed mining in which the dynamics and spreading of sediment plumes created by collector vehicles were investigated in the Clarion Clipperton Zone in the Pacific Ocean at a depth of 4500 m. In a recent study, [Peacock and Ouillon \(2023\)](#) provided a comprehensive summary of the fluid mechanics in deep-sea mining. In situ, deep-sea plume experiments conducted on the Tropic Seamount by [Spearman et al. \(2020\)](#) showed that the suspended sediment concentration increased by 2.5 over the background concentration at 1.5 m above the bed. [Muñoz-Royo et al. \(2022\)](#) reported a value of 200 g/m³ within a 2-meter layer above the seabed in the Pacific Ocean for a deep-sea mining track of 100 m. The extent of the area “affected” was 100 m from the collector vehicle. However, the foregoing findings should not be considered universal as they are subject to many limitations and uncertainties. One is the lack of data on the long-term temporal and spatial variations of sediment plumes that are driven by the complicated hydrodynamic characteristics of the deep sea. These studies were also conducted at a few specific sites that might not be representative of the water body.

There is overwhelming scientific evidence of serious adverse effects on the sea environment. These include the destruction of natural landforms and the wildlife they support and the formation of large sediment plumes that disrupt aquatic life (for example, [Ahnert and Borowski, 2000](#); [DFO, 2000](#); [Halfar and Fujita, 2007](#); [Sharma, 2011](#); [Van Dover et al., 2017](#); [Miller et al., 2018](#); [Orcutt et al., 2020](#); [Scales, 2021](#); [Farran, 2022](#)).

The ocean receives each year extremely large quantities of trash and other pollutants through human activities from both point and nonpoint sources ([NOAA, 2020](#)). In recent decades, anthropogenic inputs of contaminants such as heavy metals into the marine environment have significantly increased ([Ansari et al., 2004](#)). These harmful substances settle and are deposited on the seabed sediments. The release of polluted suspended sediments through mining operations causes further contamination of seawater, posing a serious threat to ecosystems and marine habitats. The related changes in the water quality that support these habitats are mostly irreversible.

The habitats of benthic animals are also disturbed by the removal of portions of the seafloor depending on the type and location of mining and the formation of plumes. Sediment plumes can be detrimental to seabed ecology when mining operation creates clouds of floating particles. There are potentially two types of plumes: the plume created by the collector near the seabed and the plume created by discharge from the mining operation. In the latter case, near-bottom and mid-water plumes are formed, depending on where the discharge water from the mining

platform would be released. Another important aspect is the amount of sediment removed during the mining operations. Sharma (2011) provided a sample calculation of the rate and extent of deep-sea mining. It is stated that nodule mining occurs at a rate of 3 million tons per year and a penetration depth of 10 cm, and the actual area scraped will be 600 km²/year (2 km²/day). However, according to Weaver et al. (2022), a more realistic removal rate is 200 km².

In recent years, the Baltic Sea has received considerable attention owing to the possibility of implementing seabed mining projects for polymetallic nodules in several basins. This presents an alarming prospect regarding the vulnerability of its ecosystem. The ecological and marine health of the Baltic Sea is declining rapidly (Shahabi-Ghahfarokhi, 2022). The major problems are eutrophication, the accumulation of heavy metals, and various toxic chemicals in seabed sediments. One consequence is the hypoxia of the deeper waters in the Baltic Sea, mainly in the southern basins (Dargahi, 2022). It has been reported that the oxygen content in the deeper waters of the Baltic Sea has declined to unseen levels over the last 1500 years.

Annually, large amounts of hazardous substances and heavy metals (for example Cd, Hg, and Pb) enter the Baltic Sea through various rivers from neighboring countries (HELCOM, 2021). For example, the Baltic Sea receives 14.6 GBq 210Po annually from the Vistula River, with a mean discharge of 1080 m³/s (Skwarzec and Jahnz, 2007). More information is available from the Baltic Marine Environmental Protection Commission which maintains large databases on the marine environment, and different theme databases.

The available literature data suggest that the Baltic Sea seabed contains polymetallic nodules of iron and manganese that are found in the southern Baltic, around the Gulf of Bothnia, and the Gulf of Finland (Rolf, 1979; Kaikkonen et al., 2019). Currently, there is no accessible information regarding the type and nature of seabed mining in the Baltic Sea. However, recently, a Swedish company has been granted the exploration of mineral deposits in the Baltic Sea in the Bay of Bothnia. They are expecting 6–9 million polymetallic nodules (for example, Fe, Mg, Si, P, and Co) at the seabed in the Bay of Bothnia. In this study, the focus of SWM is on polymetallic nodules that are mined from relatively soft sediment but need to be pumped to a vessel at the surface.

Given the present vulnerable conditions of the Baltic Sea, the first step should be to improve the environmental status of the Baltic Sea without adding additional constraints. It is likely that with time, economic and political pressures will increase in favor of utilizing the available resources by the nine neighboring Baltic countries. It is also alarming that claims are made by commercial interests that mining will reduce eutrophication and hypoxia of the deeper waters of the Baltic Sea by removing the upper sediment layers (stated by the contracted Swedish Company).

In this study, the impact of mining (the collector machinery) in the Baltic Sea was investigated. The main objective was to provide insight into the impact of seabed mining on sediment transport characteristics in all basins of the Baltic Sea induced by seabed mining. Mining in the Baltic Sea is referred to as Shallow Water Mining (SWM) in contrast to deep-sea mining in oceans up to several 1000 meters of water depth. This study is the first attempt to

model near-bed suspended sediment concentrations and the impact of the SWM in the Baltic Sea.

The paper first presents a summary of the study area, and the formulation of the transport models followed by considering the shallow water case of the Baltic Sea. The result section examines the bed shear stress, bed elevation changes, sediment concentrations, and particle tracking.

2 Study area

The Baltic Sea is a brackish sea with a mean salinity of 7 parts per thousand, found in northern Europe between 53°N and 66°N latitudes and 11°E and 26°E longitudes, and connected to the Atlantic Ocean by the Danish Straits. The longest and widest points are 1,600 km and 193 km, respectively. The surface area is 377,000 km², the average depth is 55 meters, and the water volume is 20,000 cubic kilometers. The maximum depth is 459 m which is located between Stockholm and Gotland Island. The Sea is made up of a collection of interconnecting basins that are connected to the Atlantic Ocean via the Danish Straits. The Baltic Sea is characterized by its shallow depth, narrow connection to the ocean and plenty of freshwater supply by more than 70 small to medium-sized rivers with discharges ranging from 20 to 2310 m³/s. The seabed is composed of bedrock, sand, silty clay, clay, and mud. The upper northern basins are mainly composed of silt, clay, and mud, whereas fine sand to coarse sand dominates the southern basins located south of the Åland Sea (Bobertz et al., 2005; Kyrlyuk and Kratzer, 2016; Kyrlyuk and Kratzer, 2019).

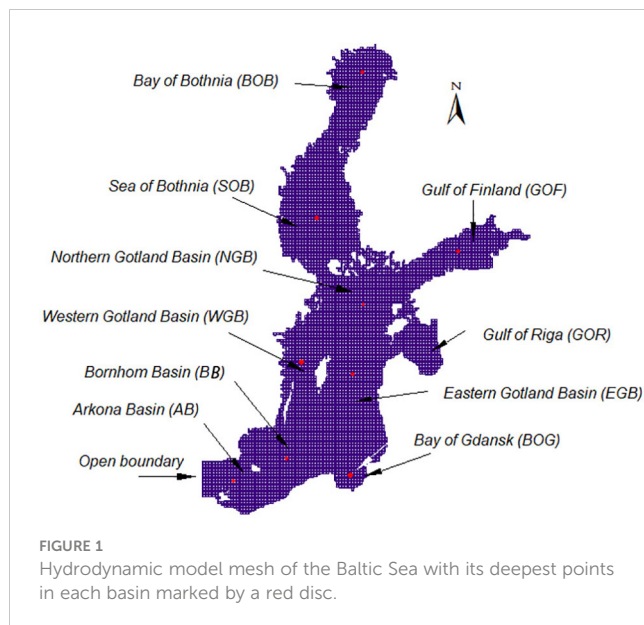
3 Method

To assess the impact of SWM on the Baltic Sea, a combined approach was used that involved 3D hydrodynamic modelling with integrated particle tracking and decoupled computation of the suspended sediment loads near the seabed. The hydrodynamic model provided the velocity vector and shear stress fields required for the sediment computations. A brief description of each method is provided below.

3.1 Hydrodynamic model

The generalized environmental modelling system for surface waters (GEMSS) was used to develop a 3D time-dependent hydrodynamic model for the Baltic Sea. The momentum, continuity, constituent transport equations, and equation of state were used to develop the transport relationships.

A linear ice model was coupled with a non-hydrostatic GEMSS hydrodynamic model. The Mellor–Yamada formulation (Mellor and Yamada, 1982) was used for subgrid parameterization. The various steps in developing the model were reported by Dargahi and Cvetkovic (2014); Dargahi (2022)). Based on this reference, a summary is provided. The grid dimensions in the horizontal plane (x,y) were 240x360, with a cell size of 3.8 km. In the



vertical plane, the domain was divided into 47 non-uniform Z-layers with thicknesses of 9/1.5 8/4 m, 6/5 m, 9/6 m, 9/8 m, and 6/12 m (number of layers per layer thickness).

The boundary conditions of the model include discharges from all main rivers (in total of 70), water heads, precipitation, and meteorological forcing factors. The water level was established at the open boundary with the North Sea, which was approximately 104 km wide and located at the coordinates 54°28'N, 12°50'E and 55°22'N, 13°03'E (Figure 1). The meteorological forcing conditions were defined using gridded data spanning the entire Baltic drainage basin with a resolution of (1×1) ° squares. On January 1, 2000, the model was started using all available profile field data (temperature and salinity). The lowest and maximum simulation time steps chosen were 120 and 240 seconds, respectively. For transportation modelling, the second-order scheme QUICK was used. Hydrodynamic calculations were performed for a period of ten years, from 2000 to 2009. At 1-day intervals, the hydrodynamic outputs were saved.

The temperature and salinity profiles measured at several locations in the Baltic Sea were used to calibrate the model for 2000. The restart files generated by the calibration simulation for 2000 were used to validate the model for ten years. Complete sets of profile field data from all monitoring sites were used. At all monitoring stations, model predictions of water temperature, salinity, and volumetric exchange rates with the North Sea were excellent. The Nash–Sutcliffe coefficients calculated for all monitoring sites ranged from 0.72 to 0.83. Another significant characteristic was the model's capacity to capture 10-year seasonal fluctuations in water temperature and salinity with maximum relative accuracy of ±10%.

3.2 Particle tracking model

The use of particle tracking to investigate the dynamics of sediment transport in various flow regimes is a fairly well-

established method. Krestenitis et al. (2007) and Israel et al. (2017) are examples of this approach. Krestenitis et al. (2007) investigated the sediment transport in the Gulf of Thermaikos, which constitutes the northwestern corner of the Aegean Sea. The model allowed investigation of patterns of sedimentary plume propagation existing in coastal systems. Concentrations of particles were deduced based on the particle mass and location. Israel et al. (2017) recently demonstrated the application of light particle tracking to simulate sediment transport loads in rivers. Their simulations were done using 350,000 particles, allowing for the determination of distinct hydrodynamic situations in which the zones are vulnerable to erosion and siltation at the port entrance in the Magdalena River, Colombia.

The three-dimensional PTM used in this study accounted for both the advection and dispersion processes of massless particles. Appendix A provides the model details. The model was used to simulate spatial and temporal variations in the sediment particle suspension that could not be extracted from the 2D sediment transport model directly. The paths of 1000 particles released from the deepest points in all 12 basins of the Baltic Sea (see Figure 1 for locations), were obtained for a 10-year simulation time (2000–2009).

3.3 Suspended sediment transport model

The formulation of a comprehensive sediment transport model for the Baltic Sea was beyond the scope of this study. The main problems are the inherent empirical nature of the sediment transport equations and unknown boundary conditions. The first is related to the complex nature of interacting sediments with the flow, which does not allow the development of theoretically based equations. Second, such a model would require fully defined river inflow and sediment boundary conditions as well as lateral sediment boundary conditions. The latter consists of fine and often cohesive sediments (i.e., wash load) that enter the sea from the surrounding land by surface land erosion. In rivers, the load can be ignored because it is transported through the river and deposited downstream. The main difficulty is the lack of field data on sediment loads carried by rivers that discharge into the Baltic Sea. There is also no data available on the exchange rate of sediment transport at the model open boundary of the Danish Straits. In this study, a simplified two-dimensional (2D) approach was adopted, which served as the main objective of conducting a comparative study of the impact of SWM on background near-bed sediment concentrations in the Baltic Sea. It was further assumed that the equilibrium state of sediment transport prevailed in the Baltic Sea.

The model was used to simulate the spreading of sediment concentration close to the seabed. Sediment continuity equations for bed-level variations and suspended sediment transport equations were numerically solved. The two-dimensional (2D) form of the Exner equation is given by

$$(1 - \lambda_p) \frac{\partial \eta}{\partial t} + \frac{\partial q_{bx}}{\partial x} + \frac{\partial q_{by}}{\partial y} = w_s(c_a - E) \quad (1)$$

where η is the bed elevation, λ_p is the porosity of the bed material, c_a is the volume concentration of suspended sediment at the reference level, q_{bx} and q_{by} are the bed load transports in the x-y directions, w_s is the particle settling velocity, and E is the volume rate of entrainment of sediment into the suspension, expressed as a fraction of T (dimensionless bed shear parameter, see [Appendix B](#)). In equation 1, $w_s c_a$ denotes the volume flux of suspended sediment settling on the bed and $w_s E$ denotes the volume flux of entrainment of bed sediment into suspension, bed elevation increases due to the net deposition of suspended sediment if $c_a > E$. To account for the influence of flocculation, the particle settling velocity for the cohesive sediment was estimated from the charts provided by [Burt \(1986\)](#) as a function of salinity and sediment concentration. The advection-diffusion equation for suspended sediment transport equation reads (2):

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial x} + v \frac{\partial C}{\partial y} = \frac{\partial}{\partial x} \left(\epsilon_{sx} \frac{\partial C}{\partial x} \right) + \frac{\partial}{\partial y} \left(\epsilon_{sy} \frac{\partial C}{\partial y} \right) \quad (2)$$

where C is the sediment concentration; u and v are the flow velocities in the XY directions, and ϵ_{sx} and ϵ_{sy} are the sediment diffusion coefficients in the XY directions.

The 2D equations were solved in the XY directions using a finite-difference approach and the Crank-Nicolson scheme ([Dargahi, 2004](#); [Shallal and Jumaa, 2016](#)). The sediment diffusion coefficients were obtained from [Van Rijn \(1993\)](#) equations as a function of the shear velocity, flow depth, and settling sediment velocity. Furthermore, the coefficients were assumed to be equal in the x and y directions. These equations were applied to a unit layer above the seabed to obtain the maximum suspended sediment load, which was the focus of the SSTM model. Reference levels (a) were computed from [Van Rijn \(1993\)](#) relationship as a function of sediment size, that is, D_{50} , D_{90} and shear stresses. A mean value of 0.1 m was used in all simulations.

The model required values for near-bed suspended sediment concentration (c_a), bed load, and suspended sediment transport capacity at all mesh points. Modelling the foregoing parameters presents a real challenge owing to the semi-empirical nature of sediment transport equations, which are mainly based on flume tests. For these models to be useful, they must be verified using field measurements. In this study, the Van Rijn equations were used because of their wide range of applicability and good validation results. These equations have been reported to yield results that are closest to the field data (see [Van Rijn, 1993](#)). Sediment transport capacities were computed for non-cohesive and cohesive soils used in different basins of the Baltic Sea, which contain both soil types. For this purpose, [Van Rijn \(1993\)](#) approach and parameterization formulation for erosion flux were applied, as detailed in the previous paragraphs and [Appendix B](#). The main inputs to the model were sediment size, sediment density, particle settling velocity, and flow characteristics (i.e., flow depth, flow velocity, and bed shear stress). The hydrodynamic model provided the flow velocities and bed shear stresses for the 10-year simulation period. The transient sediment model is decoupled from the hydrodynamic model. The shear stresses and velocities of the hydrodynamic model were used to compute suspended sediment transport at each time

step. The SSTM used the non-cohesive and cohesive sediment sizes reported by [Bobertz et al. \(2005\)](#) in the Baltic Sea basins in agreement with their reported bottom sediment map. They reported the mean sediment sizes of silt = 20 μm , fine sand = 130 μm , and medium sand = 250 μm . They also provided data on the critical shear velocities for each sediment type as 4 cm/s, 1.4 cm/s, and 1.6 cm/s, respectively.

In the northern basins with cohesive soil types, the standard range of particle sizes was used, that is, silty clay (0.03–0.02 mm) and clay (0.001–0.0005 mm). The densities of sand, silt, and clay were 2082 kg/m³, 1300 kg/m³, and 1300 kg/m³ for wet-packed sand, silt, and clay, respectively. Seawater density and viscosity were obtained as functions of water temperature, salinity, and pressure. The computation of cohesive sediment transport (i.e. the rate of erosion) at the bed was done using the classical parameterization formulation for the erosion flux ([Partheniades, 1965](#); [Krestenitis et al., 2007](#) and [Partheniades, 2009](#)). [Appendix B](#) provides the details of the sediment transport equations used in this study.

No data were available on the sediment loads supplied by the rivers to the Baltic Sea. Thus, the boundary condition for the sediment transport model was set as a net sediment flux of zero.

3.4 Shallow water mining model

The SWMM model aimed to investigate the suspension and dispersion of the increased suspended sediment load by the operation of the SWM collector (polymetallic nodules) within a one-meter layer of the seabed.

The main difficulty was to replicate the flow characteristics at the seabed realistically induced by the mining operation (i.e. collector). There is limited literature data on seabed mining that is mainly obtained from CFD simulations and small-scale and a few full-scale tests ([Hengling and Shaojun, 2020](#); [Chung 2021](#); [Alhaddad et al., 2022](#); [Muñoz-Royo et al., 2022](#)). However, these data apply only to deep-sea cases and cannot be applied directly to the shallow waters of the Baltic Sea. Presently, no accessible information is available on the type of technology to be utilized in the Baltic Sea.

The Lagrangian spreading formulation for a plume ([Lee and Chu, 2003](#); [Elerian et al., 2021](#)) was used to estimate the mean flow field characteristics induced by a collector. The formulation is based on the momentum and mass conservation equations. The discharge characteristics were taken as a velocity of 3 m/s, diameter of 0.25 m and collector speed of 0.25 m/s. These values were based on information available on various types of nodule mining systems (e.g. [Elerian et al., 2021](#)). According to this plume calculation, the mean entrainment conditions occurred at a velocity of 0.15 m/s and a bed shear stress of 6.35 N/m² using the silt-clay density. The latter sediment type is the bed type found at the selected SWM locations in the Baltic Sea (see [Figure 1](#) for locations). The collector with a speed of 0.25 m/s covers an area of about 4.6 km² in one day. The selected variables were mining area within a mesh unit of 3.8x3.8 km, flow velocity 0.15 m/s and shear stress 6.35 N/m². The SWMM used the cohesive sediment transport model that requires only the

TABLE 1 Validation sediment parameters for 2002.

Parameter	Non-cohesive	Silt-clay	clay
Sediment density (kg/m^3)	1950	1200	1200
Sediment size (mm)	0.02-0.25	0.04	0.005
Sediment size ratio (D_{90}/D_{50})	3.2		
Sediment size ratio (D_{35}/D_{50})	0.8		
Critical shear velocity (m/s)	0.01-0.035		
M_1 northern basins (kg/sm^2)		2×10^{-3}	
M_1 southern basins (kg/sm^2)		5×10^{-3}	

value of applied shear stress. Furthermore, it was assumed that the SWM collector operated at the deepest locations in the Baltic Sea. The model was applied to all 12 basins in the Baltic Sea, as shown in Figure 1, where the SWM locations are marked by small circles. The sediment transport computations were then repeated with the increased shear stress value. The shear stress was applied to the sediment transport model on the first day during the first month of each summer for the years 2000-2009 using dynamically set time steps.

The strength of the proposed model lies in its generality that neither required a presumed penetration depth nor the type of collector or machinery that may be applied to the case of the Baltic Sea.

4 Validation of SSTM

The validation process of the suspended sediment transport model was based on the field data published by Ohde et al. (2007). *In-situ* measured suspended loads were reported for 2002 for the Arkona, Bornholm, and Gotland basins (see Figure 1 for locations). The sediment transport model was run in 2002 to obtain suspended sediment loads close to the seabed. The model was run for different sets of input parameters to obtain the closest agreement with field

data. The main input parameters for the model were sediment size characteristics and density, sediment critical shear stress, and erosion rate parameters for cohesive soil (M_1). The non-cohesive sediment sizes D_{50} (Bobertz et al., 2005) were kept constant, whereas the range of variation for the cohesive material was silt-clay (0.03-0.02 mm) and clay (0.001-0.0005 mm). The sediment densities varied by ± 10 of 2082 kg/m^3 , and 1300 kg/m^3 for the non-cohesive and cohesive soils, respectively. The sediment critical shear values (τ_c) were obtained from the Shield Diagram as functions of the boundary Reynolds number (Re_*). The diagram is valid in the range of $0.1 < Re_* < 1000$. The simulated minimum value of Re_* is 0.2, which is within this range. The values of τ_c changed only slightly, that is, by 5% of the value found in the shield diagram for each D_{50} size. The validation process involved many transient sediment transport simulations to cover the range of input parameters. The input parameters are listed in Table 1.

Table 2 compares the 2002 simulated SSC (suspended sediment concentration) values with the 2002 *in-situ* measurements for the three basins of the Baltic Sea (Kyrlyuk and Kratzer, 2019). It could not be determined with certainty when the measurements were taken, or the details of the actual method. They appear to have been taken from August 16 to September 3, 2002, which is late summer. The range of average values for the autumn period was closest to the measurements. However, the upper value for the Gotland Basin was overestimated by a factor of 1.5.

The SSTM could not be fully validated due to a lack of sufficient field data on sediment concentrations as a time series across the Baltic Sea basins. Because of this constraint, the model was only used for a comparative study of the impact of seabed mining on sediment concentrations near the seafloor. Many inaccuracies exist in the actual measurements and the empirical nature of sediment transport equations, which are addressed in the discussion section.

5 Results

The instantaneous hydrodynamic outputs were obtained daily during the 10-year simulation period. The outputs consisted of bed shear stresses and suspended sediment loads with and without SWM at the seabed. MATLAB was used to process the data and to create various contours and map plots. The daily model outputs were used to create 7,200 plots for the bed shear stresses and suspended loads and 210 plots for the SWMM simulations. The results and discussion are based on these plots and actual numerical values. An attempt was made to illustrate the results through a few typical representative plots.

5.1 Bed shear stresses

The bed shear stresses exhibited significant spatial and seasonal variations in the range of $0.1\text{--}1.8 \text{ N/m}^2$. Shear stresses were higher in the Gotland Basin than in other basins of the Baltic Sea. The maximum values were attained during late summer and autumn. The minimum values were in winter periods in the range of $0.01\text{--}0.5 \text{ N/m}^2$. It should be noted that during the winter to early spring

TABLE 2 Comparison of in-site and simulated SCC (g/m^3).

Study	Arkona basin	Bornholm Sea	Gotland Sea
In-situ data 2002: Ohde et al. (2007)	0.7-9	0.4-5	3-6
Present study 2002 (winter mean)	0.3	0.4	2.5
Present study 2002 (spring mean)	0.25	0.6	3.2
Present study 2002 (summer mean)	4.9	5.8	9.46
Present study 2002 (autumn mean)	10.5	4.2	6.55
The present study (range: mean 2002)	0.3-10.5	0.4-5.8	2.5-9.46

The yellow highlights values compare present study with the in-situ measurement.

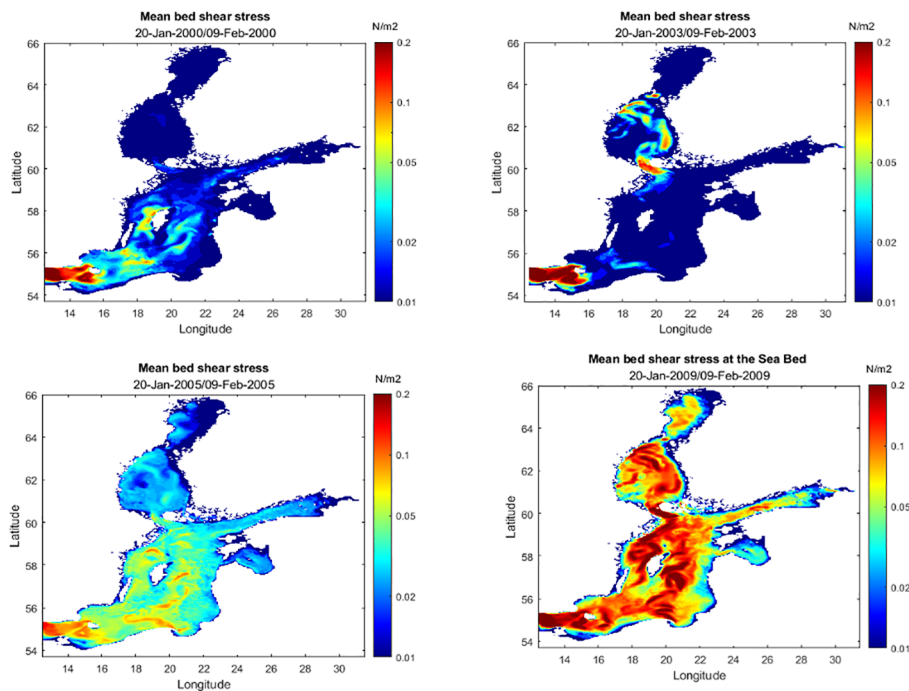


FIGURE 2

Filled contours of bed shear stress showing mean winter values in 2000, 2003, 2005, & 2009 (January 20– February 9).

periods, the northern basins in the Baltic Sea were covered by ice, which explains the lower shear stress values. Figures 2 and 3 show summary plots of the average winter and summer periods for the years 2000, 2003, 2006, and 2009. The average values were for 21

days from January 20 to February 9 and June 29 to July 19. The figure clearly shows two important features: significantly reduced shear stresses in winter, and consistently higher shear stresses in the Gotland Basin throughout the year. Figure 2 depicts a significant

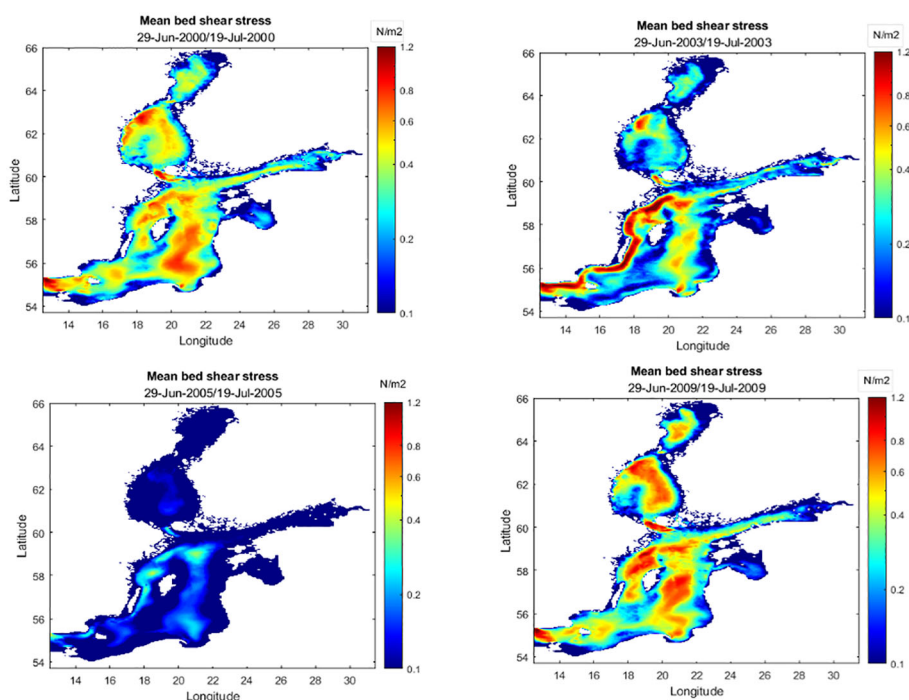


FIGURE 3

Filled contours of bed shear stress showing mean summer values in 2000, 2003, 2005, & 2009 (June 29 – July 19).

TABLE 3 3-years (2007-9) min and max simulated suspended sediment loads (g/m^3) in the basins of the Baltic Sea with reference values of [Kyrlyuk and Kratzer \(2019\)](#) data.

Period														
Period	Winter			Spring			Summer			Fall			Range (summer)	Reference values
Name	1	2	3	4	5	6	7	8	9	10	11	12	(min-max)	(min-max) Summer values
BOB	0.1-3.3	0.1-1.0	0.1-1.37	0.1-1.22	0.1-2.11	0.1-1.51	0.1-7.6	1-12.26	1-24.12	1-64	1-28.12	0.1-14.66	0.1-24.12	(The Quark) 0.15-27.33
SOB	0.1-4.1	0.1-1.5	0.1-1.29	0.1-1.01	0.1-2.7	0.1-3.22	0.1-6.3	1-13.32	1-25.10	0.3-63.21	1.1-28.03	0.1-15.01	0.1-28.03	0.13-36.8
GOF	0.1-7.1	0.1-1.1	0.1-1.2	0.1-0.95	0.2-2.23	0.1-2.22	0.1-6.35	0.1-7.06	1-27.54	0.4-77.57	1.2-30.89	0.1-14.82	0.1-27.54	0.17-35.18
GOR	0.1-4.1	0-0.96	0.1-1.09	0.05-0.86	0.05-1.26	0.1-1.55	0.1-6.43	0.1-5.25	2-26.38	0.5-27.09	21-23.11	0.1-12.54	0.05-26.38	0.21-41.23
NGB	0.1-8.8	0.1-1.88	0.1-1.25	0.1-1.5	0.1-3.7	0.1-3.3	0.1-8.01	1-13.13	1-31.70	1-88.95	1-30.66	0.1-16.52	0.1-31.70	0.1-49.60

Table 3b 3-years (2007-9) min and max simulated suspended sediment loads (g/m^3) in the basins of the Baltic Sea with reference values of [Kyrlyuk and Kratzer \(2019\)](#) data

period	Winter			Spring			Summer			Fall			Range (summer)	Reference values
Name	1	2	3	4	5	6	7	8	9	10	11	12	(min-max)	(min-max) Summer values
WGB	0.1-5.1	0.1-2.22	0.1-1.21	0.1-5.5	0.15-9.3	0.2-8.2	0.5-16.71	0.4-15.92	0.9-23.71	1-88.35	1-31.47	0.1-17.75	0.1-23.71	0.1-32.58
EGB	0.1-6.9	0.1-1.61	0.1-1.31	0.1-1.11	0.1-2.99	0.1-2.23	0.1-8.37	1-13.06	1-33.94	1-86.71	2-41.32	0.1-16.42	1.11-33.94	0.34-48.59
BB	0.1-5.5	0.1-1.5	0.05-0.77	0.05-0.88	0.1-1.9	0.1-3.3	0.3-9.37	0.5-11.11	0.4-31.93	0.5-18.30	1-34.20	0.1-3.73	0.1-31.93	0.11-35.11
AB	0.1-6.1	0.1-4.4	0.1-6.3	0.1-18	0.05-2.66	0.1-2.99	0.1-19.58	0.1-12.19	0.2-35.56	1-75.14	1-39.80	0.1-17.65	0.1-35.56	0.09-50.04
BOG	0.1-4.1	0.05-1.0	0.01-1.2	0.01-0.88	0.1-2.11	0.1-2.22	0.1-15.12	1-14.62	1-24.76	1-72.68	1-26.70	0.1-14.77	0.05-24.76	0.47-27.62

rise in bed shear stress over the winter of 2009. One probable cause is the negative trend in ice cover formation throughout the 2000-decade period. Early in the year 2000, the upper basins of the Baltic Sea were regularly covered by a thick ice sheet (i.e., 30-80 cm). The southern basins were also close to freezing point. Because there is no ice cover, the wind can reach deep into the sea and thereby increase the bed shear stresses. The discussion section contains further information.

5.2 Near bed sediment concentration

According to the model results, suspended sediment transport is the primary transport mode, which is about ten times greater than bed load transport in all Baltic Sea basins. The mean value during summer periods is about $10^{-6} \text{ m}^3/\text{m/s}$ (volumetric rate per meter width) with higher values occurring in autumn and in the southern

basins of the Baltic Sea. Aside from a few studies along the coast, there are no measurements of bed load transport in the open sea of the Baltic Sea. The preceding result can be addressed by reference to the measurements of bed load transportation taken over a 10-kilometer stretch of the southern Baltic Sea coastline ([Krek et al., 2016](#)). The mean recorded bed load was 23.8 kg, which may be converted to volume metric units using a density of 1200 kg/m^3 . It gives a value of $2.85 \times 10^{-6} \text{ m}^3/\text{m}$, which is consistent with the current finding. However, due to the differences between coastal and open sea bed load transport modes, this good agreement is inconclusive. As a result, the comparison should be interpreted only as an analysis of order of magnitude. Variations in the mean bed level elevations were $\pm 1 \text{ mm}$, suggesting a state of dynamic equilibrium for the sediment transport. The model results indicated no significant imbalance in the erosion-deposition patterns for the 10-year simulation period. During winter, the bed-level variations were negligible.

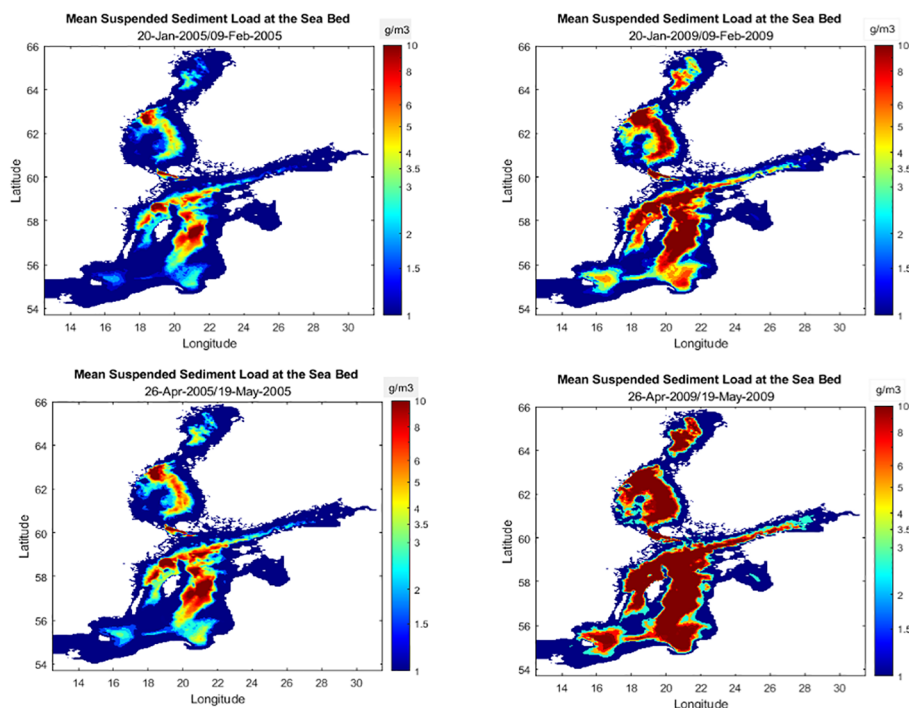


FIGURE 4

Filled contours of mean sediment concentrations in the Baltic Sea, winter (January 21–February 10) and spring (April 26–May 19), 2005 & 2009.

Tables 3A, B list concentration statistics for a 3-year period of 2007–9 where the minimum and maximum seasonal values are given. The maximum suspended sediment concentrations for the simulated period varied in the range of 0.1–90 g/m^3 . The range

corresponds to sediment concentrations during winter and the first month of autumn, respectively. In analogy with the bed shear distributions, the loads showed significant spatial and temporal variations. Higher values were found in the Gotland basins (see

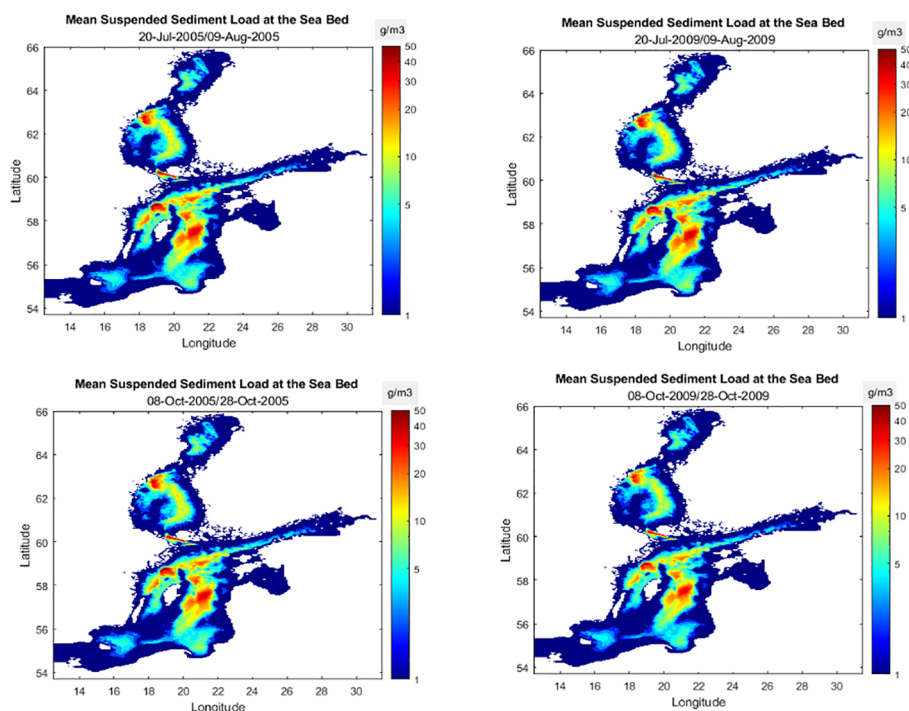


FIGURE 5

Filled contours of mean sediment concentrations in the Baltic Sea, summer (July 20–August 9) and autumn (October 8–28), 2005 & 2009.

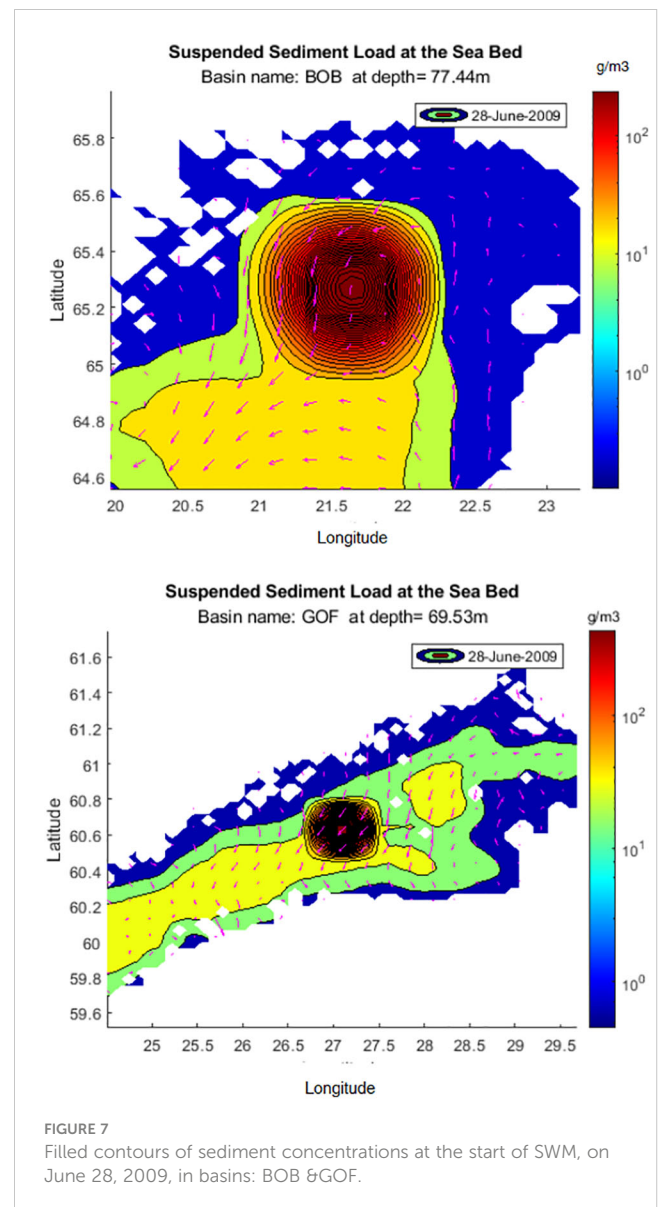
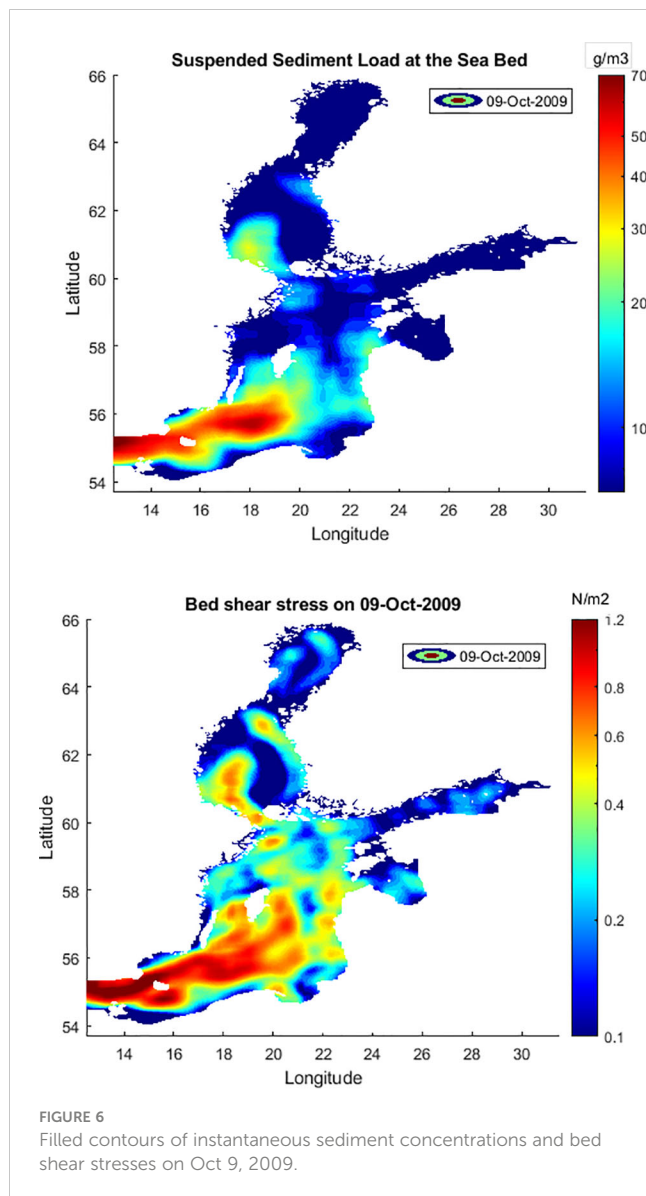
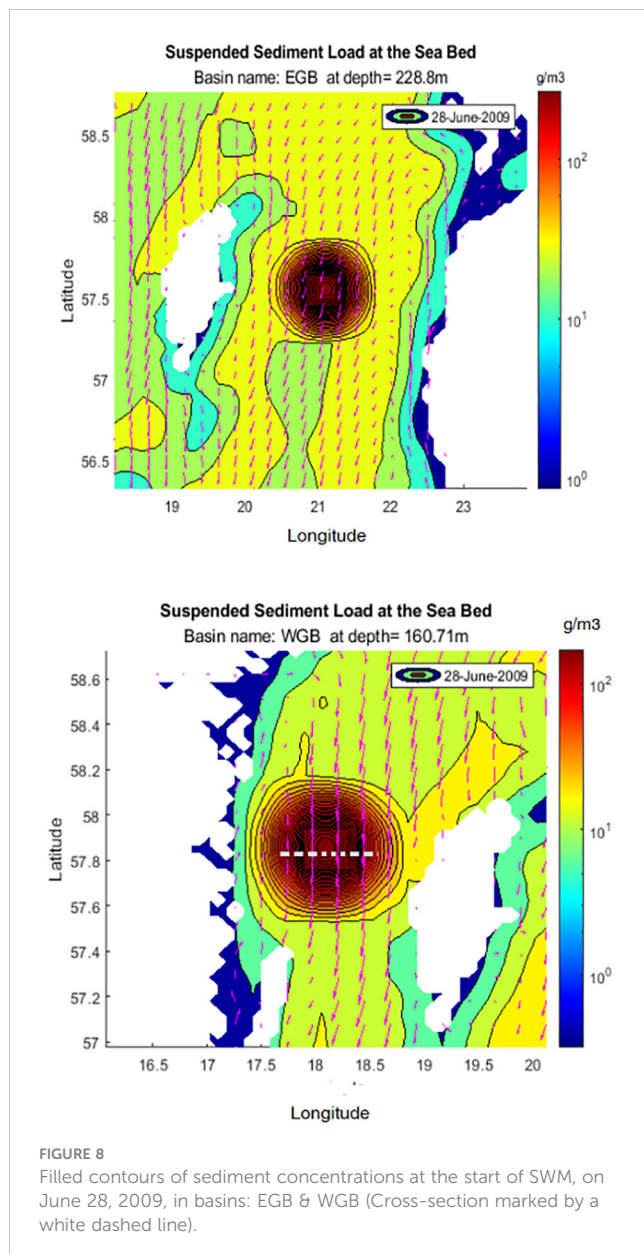


Figure 1 for locations) during warmer periods of the year. The foregoing result agrees with the data reported by Kyryliuk and Kratzer (2019). The winter periods had the lowest values typically $0.1\text{--}1\text{ g/m}^3$. To illustrate some of the main features, Figure 4 shows four plots of the 21-day averaged values of suspended loads for winter and spring for the years 2005 and 2009. The mean concentrations were found to be generally higher in 2009 (see Figure 4). Figure 5 shows four plots of the 21-day averaged values of suspended loads for summer and autumn for the years 2005 and 2009. The mean values during winter and spring periods were $< 0.3\text{ g/m}^3$, and $0.5\text{--}4\text{ g/m}^3$, respectively. Much higher concentrations occurred during the summer and autumn. The corresponding ranges were $1\text{--}8\text{ g/m}^3$ and $2\text{--}12\text{ g/m}^3$. However, maximum values increased by a factor of 10 between winter and summer. Figures 6A, B show a typical plot of the higher sediment concentrations and the corresponding bed shear stresses on October 9, 2009, respectively. Significantly increased concentrations and shear stresses are noticeable in the Arkona Basin which propagates a considerable

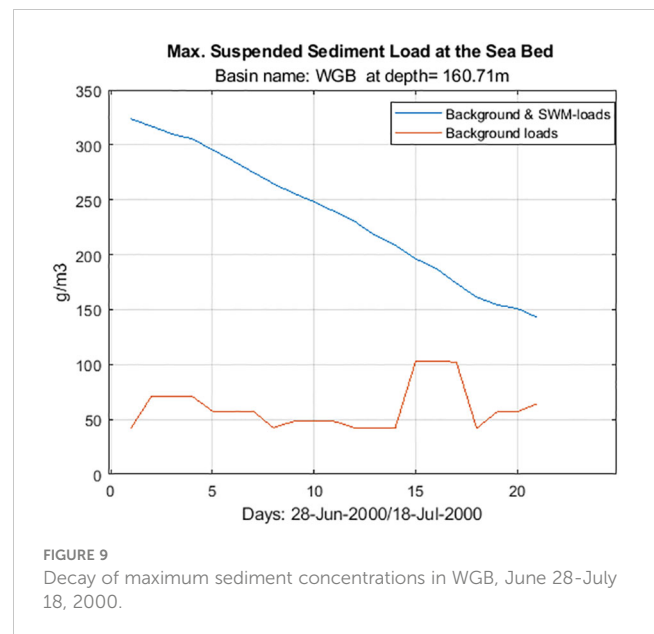
distance into the Bornholm Basin. The increased concentrations could be correlated with high water levels at the open boundary of the model that correspond to inflow events from the North Sea with a typical time scale in the range of 1–7 days that normally occur during late summer and autumn periods (see Mohrholz, 2018; Dargahi, 2022).

The simulated effect of SWM on sediment suspension showed significant temporal and spatial variations from basin to basin. The sediment concentration increased by several factors compared with the background equilibrium conditions. The range of maximum values was $300\text{--}500\text{ g/m}^3$ at the simulated mining locations, which corresponded to one model mesh of $3.8 \times 3.8\text{ km}$. The upper range was detected mainly in the Gotland Basin. The decay rate of sediment concentrations ranged from $10\text{ to }14\text{ g/m}^3$ per day, showing a slower decay in the northern basins of the Baltic Sea. Typically, the concentration of 350 g/m^3 decreased to 100 g/m^3 in the southern basins and 150 g/m^3 in the northern basins. These



values were well above the background sediment concentration. The high-concentration regions were mostly limited to mining areas, although their magnitude decreased over time.

The immediate area affected was approximately 7.6 x 7.6 km, i.e. 2 times the mining area. The main features are illustrated in a set of plots (Figures 7 and 8). They show the filled contours of suspended sediment concentrations at the start of mining on June 28, 2009, in five basins of the Baltic Sea (BOB, GOF, EGB, WGB, see Figure 1). The velocity vectors at the intervals of the four meshes are overlaid. Suspended sediment loads are transported to large areas around the mining locations in the direction of the local velocity field. In the southern basins, the sediment spread to larger areas and exhibited a faster decay in sediment concentration than in the northern basins of the Baltic Sea. Figure 9 shows the decay profile of the maximum concentration in WGB during the same period. The upper and lower curves represent the sum of SWM induced and background



loads, and background loads, respectively. Deducting the two preceding profiles yields the net SWM-induced load. The concentration after 21 days was still about 2 higher than the background maximum concentration. The variation in the local bed level elevations was ± 1 cm, which was ten times greater than the variation in the mean bed level without SWM (i.e., ± 0.1 cm). Figure 10 compares the background and total SWM (SWM plus background) bed level variations in WGB on June 29, 2000. The cross-section was taken at 57°8' N - 17°87' E and 57°8' N - 18°52' E which is drawn with a white dashed line in Figure 8, WGB. The zero position refers to the center point of the mining region. The net SWM-induced profile can be obtained by deducting the two profiles.

5.3 Particle tracking characteristics

The particle tracking results provided insights into the spatial and temporal variations in the suspension of sediment particles. The main aspects to be considered were the Lagrangian spreading of particles, final particle concentrations, and timescales. Generally, particles follow complicated patterns driven by general circulation patterns, secondary flow patterns, and mesoscale vortices (Dargahi, 2022). There are three general circulation patterns in the Baltic Sea: anticlockwise circulation in the upper water layers, clockwise circulation in deeper regions, and cyclonic circulation around the Gotland Island, which is the main circulation pattern in the Gotland Basin. Typical results for the two basins are shown in Figure 11 for BOB and WGB (see Figure 1 for locations). They show plane views of the particle paths colored according to the depth. These figures correspond to the 10-year simulation period. Table 4 summarizes the main results.

Most particles in the three northern basins (BOB, SOB, and GOF, see Figure 1 for locations) remained within the respective basins, with timescales of 700–1289 days. After a simulation period

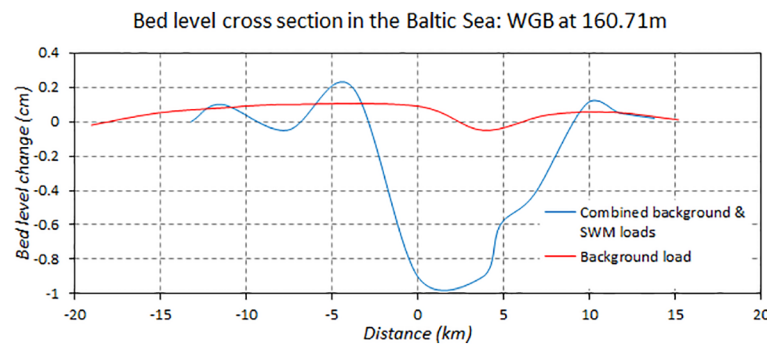


FIGURE 10

Comparison of background and combined SWM-induced and background bed level variations in WGB, June 29, 2000. The cross-section taken at 57°8' N - 17°87' E, 57°8' N - 18°52' E.

of 3650 days, 30% of the particles reached the thermocline layers at approximately half the time scale. The range of the maximum distance travelled was 700-1000 km.

The particles released in the other western basins (BB and BOG) spread to most of the basins in the Baltic Sea, covering the longest distances of up to 1300 km. In these basins, 60% of the particles reached the thermocline layers after 200-350 days. The results indicate a significant difference between the northern and southern basins of the Baltic Sea in terms of both time and length scales. Interpolation of the foregoing results for the dispersion of suspended sediment is presented in the discussion section.

6 Discussion

6.1 Model limitations

There are limitations in both hydrodynamic and sediment transport models. The fully validated 3D hydrodynamic model

showed good performance in comparison with the measured thermodynamic properties of the sea and the recorded water levels, with a maximum error of 10%. However, increasing the vertical resolution of the model produces more detailed and accurate results. The choice of the present model size was limited by the available CPU. The 2D sediment transport model provides the general pattern of sediment transport and bed level variations, but it cannot resolve the 3D characteristics of sediment movements. The latter type of model is more accurate and representative of the prevailing complex flow patterns in the Baltic Sea. However, apart from serious numerical difficulties and a very long computation time, an extensive and detailed set of coherent field data is required to verify and validate the model. Presently, the required data are neither available nor are there any plans to collect them soon. The 2D sediment model is a good starting platform for examining the mean features of sediment transport and addressing the effect of seabed mining. The other aspect is that the shallow water model was based on the estimation of the applied shear stress based on physical principles. As stated in the SWMM section, no information was

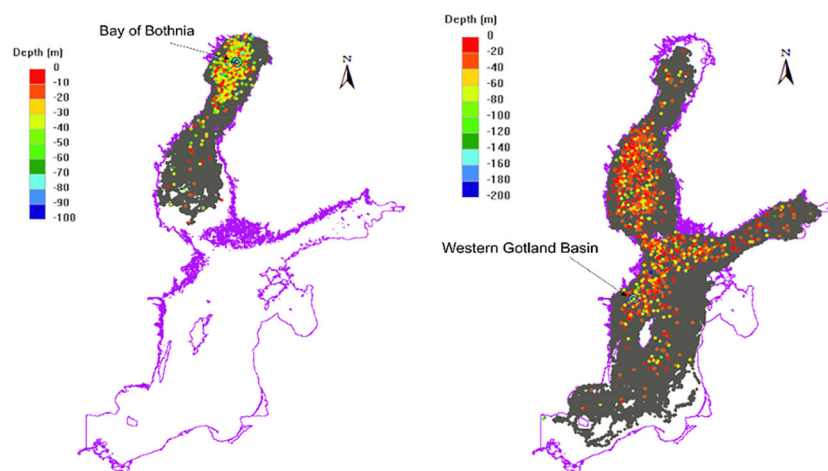


FIGURE 11

Particle paths in different basins of the Baltic Sea colored by depths in two basins: BOB and WGB. Period 2000-2009.

TABLE 4 Particle tracking travel times to shorelines and time to reach the farthest basin.

Basin	Time to reach shore-lines (days)	travelled time (day) to the basins
BOB	50-450	BOB-800
SOB	158-417	SOB-1289
GOF	90-801	GOF-700
WGB	559	GOF- BOB 1799
NGB	50-120	GOF- BOB- 3640
EGB	666	All northern basins-2002
BB	140	All basins-1850
BOG	10	All basins-2445

available to aid in the selection of more realistic values. However, one merit of the SWMM model is the selection of the applied shear stress was estimated independently of the type of mining machinery. The applied value of the shear stress induced by the collector is not unreasonable in comparison with the values reported in the literature. However, the study does not rule out alternative configurations.

There are several uncertainties inherent in the numerical modelling of sediment transport, owing to the semi-empirical nature and uncertainties of sediment data and their characteristics. Models of large water bodies such as the Baltic Sea encounter further difficulties because data on sediment loads are often unavailable at the inlet and outlet boundaries. The current model assumed zero net sediment flux, which did not require actual boundary values. This boundary condition can be understood as a state of dynamic equilibrium that ensures a balance between erosion and deposition. The validity of this assumption is discussed in terms of the study objective. The Baltic Sea is a semi-closed system that exchanges water and masses along its open boundary in the Danish Strait. The main sediment input is through rivers, which are balanced by outgoing sediment transport at the open boundary and the sediment rate of change within the sea. However, the main driving force for sediment transport is bed shear stress, which was obtained from the validated hydrodynamic model. The focus of this study was to compare two cases, with and without SWM, regarding seabed sediment concentrations. In both cases, the acting shear stress field was induced by the hydrodynamics of the sea and not by sediment transport boundary conditions. This implies that the comparison between the two cases was relatively independent of the sediment loads at the boundaries. Furthermore, the seabed elevation changes offshore of a large water body of the order of millimeters, which is several orders of magnitude smaller than the sea depth. This implies a negligible change in the flow field and bed shear stresses.

6.2 SSTM validation

The sediment transport model of the Baltic Sea yielded sediment concentrations that were in reasonable general

agreement with the reported data. However, an accurate comparison requires *in-situ* measurements of sediment concentrations near the seabed, which are currently unavailable. The present data are Kyryliuk and Kratzer (2019) satellite-based data and *in-situ* data obtained from previous studies mainly in the summer of 2002. Tables 3A, B show the 3-year (2007-9) min and maximum simulated suspended sediment loads (g/m^3) in the basins of the Baltic Sea and the reference values of Kyryliuk and Kratzer (2019). There is a general agreement regarding the range of the min-max data in summer periods. However, the maximum values obtained in this study are approximately 20% lower. Kyryliuk and Kratzer stated that, for each basin, their satellite data showed a much higher range than the reported *in situ* range. There are several possible explanations for the deviation between the two datasets. Generally, measuring the maximum values is associated with higher uncertainties and errors depending on the length and duration of the data and the time of measurement. Kyryliuk and Kratzer's data are associated with some additional uncertainties. The data were derived from satellite information obtained using NASA MERIS ocean color sensing and optical transect measurements, which are often dominated by organic matter rather than actual suspended matter. Furthermore, satellite-based data cannot resolve the higher near-seabed sediment concentrations, which is one of the major shortcomings of the method. The details of these satellite-based data regarding the accuracy, exact time, and locations of the measurements are also unclear. Presently, taking extensive sediment concentration measurements in large and deep water bodies is technically and economically not feasible.

Despite these shortcomings, numerical models yield results that cannot be obtained using a limited number of measurements. This is especially true for large open-water bodies such as the Baltic Sea.

6.3 Sediment concentrations

The results are discussed with a focus on two important aspects. One is the state of near-bed sediment concentrations and bed level variations for the decade 2000-2009. The second is the change induced by SWM in near-bed sediment concentrations and the bed level variations (i.e. erosion-deposition).

The instantaneous concentrations in the Baltic Sea showed significant seasonal and spatial variations, analogous to bed shear stress, as indicated in the previous sections. The occurrence of higher concentrations during the late summer-autumn period correlated with increased westerly wind conditions during the same period. During the simulation period, the maximum measured wind speed range was 20-30 m/s. These wind speeds are sufficiently high to reach maximum depths in most basins of the Baltic Sea, inducing higher shear stress and thus increasing sediment transport. Generally, the winds were approximately 1.5-2 higher in the Gotland basins (see Figure 1 for locations), which could explain the higher shear stress and sediment loads in these basins in comparison with the northern basins. In winter, there is a considerable reduction in the shear stress at the seabed and sediment transport activities. This can be attributed to the

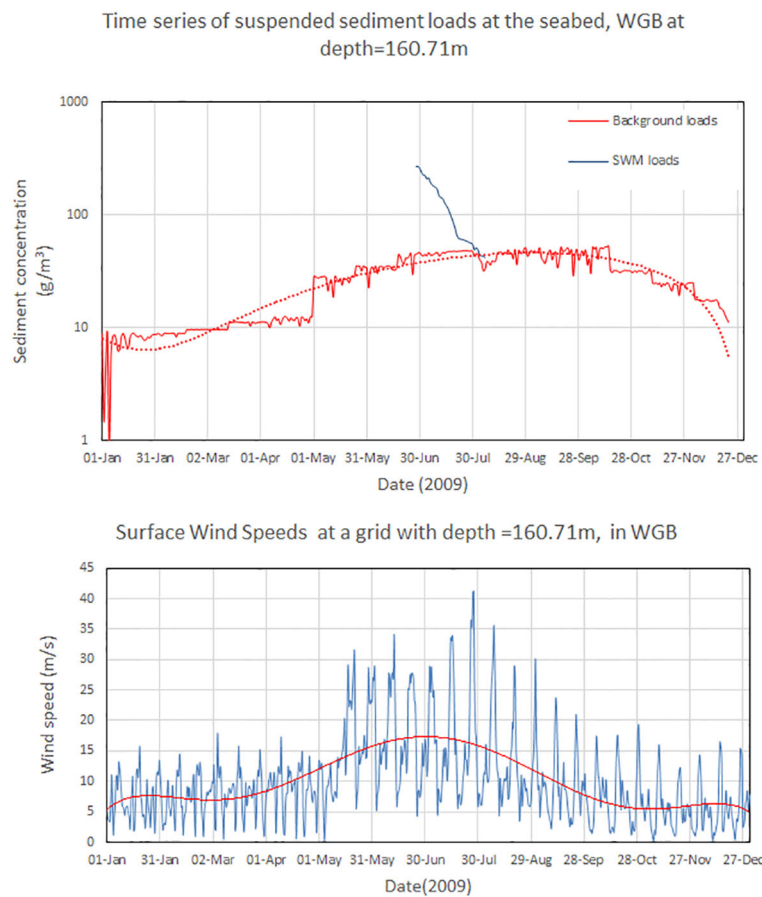


FIGURE 12

Time series of suspended sediment concentration of background and SWM induced loads and wind speeds in 2009, Western Gotland Basin, at the grid with depth 160.71 m.

formation of ice cover in the northern basins and the moderately reduced meteorological loading, specifically wind speed, throughout the basins. Contrary to the significant seasonal variations in sediment concentrations, there was little annual change in sediment concentrations during the period 2000–2008. Sediment concentrations were reduced throughout the winter because the ice cover effectively inhibited wind force from acting on the flow. The formation of ice significantly alters the flow dynamics by converting it into a closed conduit. The vertical shear stress distribution changes to a parabolic profile that divides the total shear stress between the seabed and under the ice cover. The amount of shear stress applied to each surface is determined by the roughness ratio of the ice cover and the seabed. This means that when there is no ice cover, all shear loads are applied to the bottom, increasing sediment concentrations in comparison to ice-cover years. Higher sediment concentrations during the winter of 2009 can be explained by changing ice conditions. The alarming decline in ice conditions has persisted until 2022. Rapid climate change could be the main reason for the altered ice conditions in the Baltic Sea.

Higher values of background sediment concentrations during the summer and early fall seasons may be related to the enhanced forcing meteorological circumstances, particularly wind speed. Figure 12 illustrates the correlation by comparing the evolutions of sediment concentrations with wind speeds in WGB at grid position with depth 160.71 in 2009 (the net SWM-induced loads are also plotted in the same figure). Sediment concentrations increase as wind speed increases, and both variables are characterized by a third-degree polynomial.

Regarding the impact of SWM, there are three important aspects of sediment concentrations, the decay rates and bed-level variations induced by the collector.

High sediment concentrations adversely affect marine habitats. DFO (2000) provides guidelines for protecting fishery resources in terms of sediment concentration risk levels of sediment concentration. The classified risk levels of concentration were low risk 25–100 g/m^3 , moderate risk 100–200, high risk 200–400, and unacceptable ($> 400 \text{ g}/\text{m}^3$). In the case of SWM, the maximum concentrations were 300–500 g/m^3 which are within the high and

unacceptable risk levels. This yielded an increase in concentration by a factor of 3–5 in comparison with the maximum background concentrations.

The decay rates were in the range of 10–14 g/m³ per day in the northern and western basins of the Baltic Sea. The weaker flow field and the difference in the sediment properties in the former basins explain the slower decay (see Figure 9). However, the final decay time to the background levels is approximately 40 days which gives a decay rate of approximately 7.5 g/m³ per day (see Figure 12). The results indicate that the region affected by SWM was limited to ±15 km which is approximately four times the mining area, as shown in Figure 10. However, the impact should be understood in terms of bed-level variations that nearly coincide with the background bed levels within a distance of ±15 km. The net SWM-induced loads are also plotted in the same figure.

Because of the active transport of suspended sediments by currents, the real region affected by SWM may be substantially larger than described by bed level variations. In comparison to silt and sand, cohesive sediments have a very low settling velocity in the absence of flocculation, which keeps the material afloat for extended periods. The creation of coarser aggregates may change their settling velocity and aid in the reduction of mining plume spread, especially in the deep sea, where the surficial sediment is mostly solid clayey sediment (Gillard et al., 2019; Ali et al., 2022). In the former case, the cohesive material could travel further before settling on the seabed. This trend can be confirmed by data reported by Ryabchuk et al. (2017) and the PTM. They reported that during construction work in Neva Bay, fine sediments were transported into the GOF (see Figure 1 for locations) up to tens of kilometers off the coast. The tenfold bed level changes induced by SWM suggest that harmful substances, such as heavy metals have the potential of being transported and spread to all basins.

6.4 Particle tracking

The various limitations of the sediment transport model did not allow investigation of the dynamics of the sediment transport and its 3D nature in the Baltic. However, the particle-tracking model provided useful information on the spatial and temporal characteristics of suspended sediment particles in water.

The applied model accounted for both the advection and dispersion of massless particles. The SSTM accounted for sediment particles larger than 0.005 mm, i.e., very fine silt to coarse clay, with a mean density of 1200 kg/m³. However, the density of the suspended cohesive material can be as low as 1130 kg/m³ which is close to that of seawater 1025 kg/m³. This implies that massless particles provide a reasonable representation of the advection and dispersion of particles with these characteristics. However, compared to heavier particles in the suspension, the dynamic properties of these particles are overestimated in comparison with those of small mass. The limitation on the density implies the spreading of the plume to larger areas.

The particle-tracking model suggested the extensive spreading of particles within all basins of the Baltic Sea. In the southern basins, with strong secondary flows, particles spread into the northern basins over a timescale of 5–7 years. In the southern basins, the particles could reach the shorelines within a relatively short time interval of 10 days to 2 years, and the model-predicted time scale to reach the thermocline layers ranged from 30% to 60% of the total particles released in the northern and southern basins, respectively. The foregoing characteristics, with long spatial-temporal scales, provide some insight into the alarming possible spreading of dislodged particles from the seabed by SWM. The operation will also expose the containment materials which are stored at the top sediment layer of the seabed.

7 Conclusions

The proposed modelling schemes were developed for a comparative study of the impact of shallow water mining in the Baltic Sea.

The application of combined hydrodynamic, particle tracking, and sediment transport models to the Baltic Sea revealed important characteristics of near-bed suspended sediment concentrations.

The main undisturbed transport mode of the Baltic Sea is the suspended transport load, which spreads to all Baltic Sea basins. The advection timescale varied from 10 days to 7 years, depending on the shoreline and basin. The average sediment concentration was approximately 2 g/m³, with peaks up to 90 g/m³ on a single autumn day.

Shallow water mining significantly alters the equilibrium state of sediment transport in the Baltic Sea. The sediment concentrations could reach 300–500 g/m³, which are 3–5 times higher than the background sediment concentrations at the time of mining. These high values are classified as unacceptable risks to fishery resources and sea ecology. The size of the SWM-affected area concerning the bed-level changes was approximately 4 times that of the center point of the mining area. The PTM suggested extensive spreading of lighter particles to all the basins of the Baltic Sea with very large time and length scales. These particles could reach the thermocline layers with a mean concentration of 40%. SWM operation ten folded the bed level variations, posing an additional threat to the ecological health of the Baltic Sea by exposing the contaminated sediment to transport. The Gotland Basin in the Baltic Sea is most vulnerable to SWM.

The environmental and ecological risks of shallow water mining in the Baltic Sea are not sufficiently studied. Consequently, it is prudent to exercise caution and, until then, maintain the Baltic Sea seabed as a region free of mining.

Data availability statement

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

Author contributions

I am the only author.

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Conflict of interest

The author declares 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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Appendix A: Particle tracking

The mobility of particles was determined by using particle equivalence and solving a mass transport equation for a conservative substance (Thompson and Gelhar, 1990). A random-walk particle-tracking scheme was designed after Dimou and Adams (1993) to calculate particle displacement as the sum of an advective deterministic component and an independent random Markovian component that statistically approximated the dispersion characteristics of the environment. By linking the advective and Markovian components to the proper terms in a conservation equation, a strategy has been developed in which a particle distribution turns out to be the same as the concentration resulting from the conservation equation's solution. A conservative substance is moved in a three-dimensional environment by advection.

$$h_1 h_2 \frac{\partial DC}{\partial t} + \frac{\partial(h_2 U_1 DC)}{\partial \xi_1} + \frac{\partial(h_1 U_2 DC)}{\partial \xi_2} + h_1 h_2 \frac{\partial(wC)}{\partial z} = \frac{\partial}{\partial \xi_1} \left(\frac{h_2}{h_1} A_H D \frac{\partial C}{\partial \xi_1} \right) + \frac{\partial}{\partial \xi_2} \left(\frac{h_1}{h_2} A_H D \frac{\partial C}{\partial \xi_2} \right) + \frac{h_1 h_2}{D} \frac{\partial}{\partial z} \left(K_H \frac{\partial C}{\partial z} \right) \quad (\text{A} - 1)$$

where C is the concentration, h_1 and h_2 are the metrics of the unit grid cell in the ξ_1 and ξ_2 directions, respectively, D is the diffusion coefficient, U_1 and U_2 are the velocity components along the ξ_1 and ξ_2 directions, respectively, w is the velocity component in the z -direction, A_H is the momentum dispersion coefficient, and K_H is the diffusion coefficient. The transport problem can also be solved using particle-tracking models by representing the conservative tracer concentration using a collection of particles. The displacement of a particle in a random-walk model is governed by the nonlinear Langevin equation:

$$\frac{d\vec{X}}{dt} = A(\vec{X}, t) + B(\vec{X}, t)Z(t) \quad (\text{A} - 2)$$

where $\vec{X}, t$ is the particle trajectory vector; $A(\vec{X}, t)$ is the deterministic force that advects the particles; $B(\vec{X}, t)$ represents the random force vector that leads to particle diffusion; and $Z(t)$ is a vector of independent random numbers with zero mean and unit variance. A and B are defined by Equations 3 and 4, respectively (Zhang, 1995)

$$A = \begin{bmatrix} \frac{U_1}{h_1} + \frac{1}{h_1 h_2 D} \frac{\partial}{\partial \xi_1} \left(\frac{A_H}{h_1^2} h_1 h_2 D \right) \\ \frac{U_2}{h_2} + \frac{1}{h_1 h_2 D} \frac{\partial}{\partial \xi_2} \left(\frac{A_H}{h_2^2} h_1 h_2 D \right) \\ \frac{w}{D} + \frac{1}{h_1 h_2 D} \frac{\partial}{\partial z} \left(\frac{K_H}{D^2} h_1 h_2 D \right) \end{bmatrix} \quad (\text{A} - 3)$$

$$\frac{1}{2} BB^T = \begin{bmatrix} \frac{A_H}{h_1^2} & 0 & 0 \\ 0 & \frac{A_H}{h_2^2} & 0 \\ 0 & 0 & \frac{K_H}{D^2} \end{bmatrix} \quad (\text{A} - 4)$$

$$\omega = W$$

$$- \frac{1}{h_1 h_2} \left[h_2 U_1 \left(\sigma \frac{\partial D}{\partial \xi_1} + \frac{\partial \eta}{\partial \xi_1} \right) + h_1 U_2 \left(\sigma \frac{\partial D}{\partial \xi_2} + \frac{\partial \eta}{\partial \xi_2} \right) \right] - \left(\sigma \frac{\partial D}{\partial t} + \frac{\partial \eta}{\partial t} \right) \quad (\text{A} - 5)$$

where $D = H\eta$, $\eta(x, y)$ is the water surface elevation defined in Equation 6, and $H(x, y)$ is water depth.

$$\sigma = \frac{z - \eta}{H + \eta} \quad (\text{A} - 6)$$

Appendix B

Computation of suspended load using Van Rijn (1993) equations:

$$C_a = 0.015 \frac{d_{50}^{1.5}}{k_s D_*^{0.3}}$$

$$T = \left(\frac{\tau'_b - \tau_c}{\tau_c} \right)$$

$$\tau'_b = \rho g \left(\frac{u_m}{C'} \right)^2$$

$$C' = 18 \log \left(\frac{12h}{d_{90}} \right)$$

$$D_* = d_{35} \left(\frac{(s-1)g}{\nu^2} \right)^{1/3}$$

where C_a is the reference concentration (kg/m^3) at reference level a , d_{50} , d_{90} , and d_{35} are particle size cumulative percentages of 50, 90, and 35, τ_c is the sediment critical shear stress, k is the roughness height, u_m is the mean flow velocity, h is the mean flow depth, $s = \rho_s / \rho_w$ the sediment and seawater density, g is the acceleration due to gravity, and ν is the kinematic viscosity of seawater.

Particle settling velocities (w_s) were computed using the Rijn (1993) equations valid for different particle sizes.

$$\frac{w_s}{\sqrt{g'd}} = \frac{R_d}{18}$$

$$\frac{w_s}{\sqrt{g'd}} = \frac{10}{R_d} \left(\sqrt{1 + 0.01 R_d^2} - 1 \right)$$

$$\frac{w_s}{\sqrt{g'd}} = 1.1 g' = g \left(\frac{\rho_s}{\rho_w} - 1 \right)$$

$$R_d = \frac{d \sqrt{g'd}}{9}$$

where d is the sediment size (d_{50}). The validity ranges for the three w_s equations were $d < 1000 \mu\text{m}$, $100 \mu\text{m} < d \leq 1000 \mu\text{m}$, and $d > 1000 \mu\text{m}$. The particle settling velocity for the cohesive sediment was obtained from [Burt's \(1986\)](#) charts.

The computation of cohesive sediment transport (i.e., the rate of erosion) at the bed was done using the classical parameterization formulation for erosion flux ([Partheniades, 1965](#); [Krestenitis et al., 2007](#) and [Partheniades, 2009](#)) as follows:

$$Er_0 = M_l \left(\frac{\tau - \tau_c}{\tau_c} \right)$$

where τ is the seabed shear stress (N/m^2), τ_c is the critical sediment particle shear stress, and M_l (kg/sm^2) is given by [Krestenitis et al. \(2007\)](#).

$$M_l = [10^{-5} - 10^{-3}]$$

To convert, Er_0 into (kg/m^3) multiply by velocity^{-1} . The concentration profile was computed using:

$$\frac{C}{C_a} = \left(\frac{a}{h-a} \frac{h-z}{h} \right)^{Z^e}$$

In which the exponent Z^e is defined as:

$$Z^e = \frac{w_s}{bku_*}$$

where C_a is the near-bed concentration at reference level (a), which is computed from [Van Rijn \(1993\)](#) equation, height above the bed, $b=1$, $k=0.4$, and u_* = shear velocity, given by:

$$u_* = \sqrt{\frac{\tau}{\rho_w}}$$



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Developing best environmental practice for polymetallic nodule mining - a review of scientific recommendations

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Best environmental practice (BEP) is a key component of an ecosystem approach to management and is typically a product of practical experience in established industries. For an emerging activity such as deep seabed mining, no such experience will exist at the time of deciding on the permissibility of the first industrial mines. Therefore, experience from deep ocean scientific experiments and research are important to develop a preliminary understanding of BEP for deep seabed mining. This paper offers a detailed review of the scientific literature from which it identifies elements of preliminary BEP for nodule mining. The paper describes the currently envisaged mining process for manganese nodules and its expected effects on the environment and extracts specific recommendations on how to minimise environmental impacts from mining in different layers of the ocean (benthic, benthopelagic, pelagic, and surface waters) as well as from noise and light impacts. In doing so, the paper aims to inform the Mining Code being developed by the International Seabed Authority (ISA). The ISA is the intergovernmental institution mandated to organise and control seabed mining on the international seabed. The ISA is obligated to ensure effective protection of the marine environment from harm likely to arise from mining, with BEP being a core tool to achieve that. This paper provides suggestions for a future ISA Standard on BEP.

KEYWORDS

best environmental practice, deep seabed mining, environment, governance, polymetallic (manganese) nodules

1 Introduction

Deep seabed mining (DSM) is an emerging industry that could carry significant risks for the marine environment. Mining operations will cause biodiversity loss (Van Dover et al., 2017; Niner et al., 2018) and knock-on effects on the food web (Stratmann et al., 2018; Stratmann et al., 2021) due to habitat destruction (Vanreusel et al., 2016; Volz et al., 2020), sediment plumes (Muñoz-Royo et al., 2022) and noise and light pollution (Williams et al., 2022). In aiming to reduce those environmental risks through an ecosystem-based and

precautionary approach (ITLOS, 2011), the International Seabed Authority (ISA), which regulates and manages all DSM-related activities on the international seabed “Area”,¹ requires mining operators to apply Best Environmental Practice (BEP).² In this context, BEP means ‘the most appropriate combination of environmental control measures and strategies’ at any given point in time.³ BEP, by definition, improves over time depending on upcoming environmental challenges as well as advances in knowledge and technology, including Traditional Knowledge by Indigenous Peoples and local communities.⁴

Identifying BEP is never an easy task but may be particularly difficult for an emerging industry that does not yet have a proven track record of safe operations and that uses nascent technologies which are largely untested in the deep ocean environment. Because of these challenges, identifying BEP for DSM will initially have to rely on experience from other offshore industries as well as on long-term experience gained from small-scale scientific disturbance experiments and mining tests. To that effect, this paper seeks to identify elements of preliminary BEP for polymetallic nodule mining, based on a review of scientific recommendations from the literature. This can only be a first approximation, as a multitude of additional, in particular experimental process research (Weaver et al., 2022) and extended test mining with full-size equipment will be necessary to provide knowledge on short- and long-term environmental effects of DSM from *in situ* operational practices (Amon et al., 2022; Singh and Christiansen, 2022; Weaver et al., 2022).

Identifying BEP is important for at least three actors: the ISA as the regulator, the mining companies, States or State enterprises as contractors, and the sponsoring State of the contractor. The ISA is obligated to ensure effective protection of the marine environment from harmful effects of DSM,⁵ with BEP being one important element in achieving this obligation, as specifically recognized in the draft regulations for mineral exploitation which the ISA is currently developing.⁶ Identifying BEP prior to the start of commercial mining is key to enable the ISA to assess whether an applicant has indeed been following BEP. The applicant, in turn, needs to demonstrate that its proposed plan for mineral exploration⁷ or exploitation⁸ applies BEP as

well as best available techniques (BAT).⁹ Lastly, the sponsoring State is required to apply BEP both as a direct obligation¹⁰ and as part of its due diligence obligation to ensure that the contractor complies with its obligations.¹¹ Clarity on what constitutes BEP at any given point in time is thus fundamental for the ISA, its contractors, and sponsoring States to be able to comply with their respective obligations.

Despite the importance of BEP, there is no agreed understanding on what constitutes BEP in the Area. Indeed, in the absence of respective environmental standards, the ISA’s current approach entrusts individual ISA contractors with identifying project-specific and area-specific impact thresholds¹² to determine BEP and other standards based on their baseline data collection. These may be guided by (non-binding) guidelines issued by the ISA (Brown, 2018). However, a common understanding of current BEP, and its links to good industry practice and best available technology,¹³ may be essential to articulate ISA standards, and to help contractors optimize their technology to meet those standards, while reducing financial and legal risks for contractors. Lastly, BEP may help the ISA to assess and compare applications for mineral exploration or exploitation.

Indeed, in 2017, the Netherlands proposed for the ISA Council to develop an assessment and approval methodology to evaluate mining equipment, operational procedures and processes in

1 1982 UN Convention on the Law of the Sea (UNCLOS), Art 1(1)(1).

2 See ITLOS, 2011, paras 122 and 136.

3 ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023, Schedule, page 91.

4 ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023, Schedule, page 91.

5 UNCLOS, Art 145.

6 See e.g. ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023, draft Regulation 44(2 bis)(b), (c).

7 ISA, Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area, ISBA/19/C/17, 22 July 2013, Regulation 31(5).

8 ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023, draft Regulation 44(2 bis)(b), (c).

9 E.g. ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023, draft Regulation 46bis(1), 46ter(1), 48(3)(c). For a discussion paper on BEP and best available technology, see ISA, Key terms: distinguishing between good industry practice and best practices under the draft regulations on exploitation of mineral resources in the Area, ISBA/25/C/11, 15 January 2019.

10 See e.g. ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023, draft Regulation 44(2 bis)(b), (c); ISA, Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area, ISBA/19/C/17, 22 July 2013, Regulation 31(2).

11 Seabed Disputes Chamber, Responsibilities and Obligations of States Sponsoring Persons and Entities with Respect to Activities in the Area (Advisory Opinion, case no 17, 2011) para 136.

12 see ISBA/27/C/4, para 88: ‘Until such time as sufficient data on the Area exists to allow the Authority to establish thresholds for a range of key components that are assessed in the EIA process, an applicant or Contractor should use project-specific and area-specific impact thresholds based on data and analyses commensurate in quality with the importance of the impact.’

13 see e.g. ISBA/25/C/11.

relation to their impact on the marine environment.¹⁴ The proposal was for the ISA to develop technical standards and minimum environmental requirements. Only certified technology would receive approval in prior Environmental Impact Assessments. Certification could be considered early in the technological and operational design process, offering a degree of certainty to mining contractors. In addition, once the criteria exist, the assessment and certification process for equipment could be delegated to external bodies, as is customary for ships and other offshore equipment. Alternatively, an independent advisory body or the future inspection unit of the ISA could fulfil this role.

While no such methodology is being developed yet, the ISA Council has recently decided to take a more active approach and develop environmental thresholds for permissible levels of toxicity, noise, light, and turbidity as well as settling of resuspended sediments from DSM.¹⁵ This will be an important step towards identifying what levels of environmental harm are deemed acceptable, which in turn supports the identification of BEP to meet these thresholds.

This paper starts by outlining the methodology (section 2), followed by a brief overview of potential nodule mining operations (section 3). The paper then addresses the key effects of nodule mining on relevant ocean layers, from the seafloor (section 4), benthic boundary layer (section 5), water column and surface waters (section 6) and noise and light pollution (section 7). Each section discusses the main risks and recommendations for BEP. Section 8 offers overarching recommendations, while section 9 formulates suggestions for a future ISA Standard on BEP, and section 10 offers concluding thoughts.

2 Materials and methods

This paper offers a non-exhaustive list of preliminary recommendations for BEP in nodule mining, expressed as such in relevant scientific publications. The study draws on a broad range of recent studies which provide direct recommendations for managing DSM. Indirect or implied recommendations from the literature were not considered. Overall, the recommendations largely build on each other, although in some cases differences exist, which are reflected in this paper.

We purposefully do not attempt to rank these recommendations in importance, ease of attainability, scales of effects or cost. This will require a broader range of expertise to systematically assess risks and consider all available options for keeping the short- and long-term impacts on of DSM within the limits to be formulated by the ISA in the

future. The present paper merely presents a starting point for developing BEP.

3 Overview of potential polymetallic nodule operations

Of the three minerals, the ISA is concerned with since its creation, polymetallic nodules have been studied most extensively. Polymetallic nodules in the Area are generally between 1 and 12 cm in diameter and lie half-buried in the sediment in abundances amounting up to more than 20 kg/m² in some parts of the Clarion-Clipperton Fracture Zone, CCZ, in the Eastern Tropical Pacific. In certain biogeographic regions of all oceans, the nodules can occur in contiguous fields of similar-sized nodules interspersed with low abundance regions or areas that are almost devoid of nodules (Kuhn et al., 2020). The metal content of the nodules varies with location, size, formation process and weathering state (Kuhn et al., 2020). Nodule abundance and nodule size affect biological communities directly and indirectly (Vanreusel et al., 2016; Smet et al., 2017; Kuhn et al., 2020; Simon-Lledó et al., 2020).

Harvesting nodules means retrieving them from the seafloor and pumping the collected material to a surface vessel through a vertical riser pipe (Jones et al., 2017; Miller et al., 2018). All proposed seabed mineral mining operations are based on a similar concept, which encompasses four major components: (1) extraction tool, (2) riser system, (3) surface platform, and (4) disposal system. Most proposed seabed collection systems envisage the use of remotely operated vehicles, which would extract the nodules from the seabed using mechanical rakes or pressurized water. Although less invasive collection technologies are in the design phase,¹⁶ the nodule collection techniques that are currently being tested all involve self-propelled heavy machinery (with up to 100 t weight under water) operating on the seafloor, and removing up to 10–50 cm of the upper sediment (e.g. reviewed by Paul et al., 2018). So far, the nodule collectors will either consist of one large machine with one or several hydraulic sucking, or raking (e.g. India) intake units, or of several identical modules (e.g. DEMEGSR), mounted together or operating in isolation (Deepak et al., 2001; Handschuh et al., 2001; GSR, 2018; Government of India, 2020). The type of collector will determine the width of the collector path. The machine(s) will strip-mine in a lawnmower pattern, with software ensuring the continuous clearing of nodules (Volkman and Lehnen, 2017). In most cases, the maximum terrain angle that can be cleared of nodules is 3–4% (Kuhn et al., 2011), any steeper or rougher terrain will remain inaccessible. These figures have not changed since the early pilot mining tests, when the National Oceanographic and Atmospheric Administration of the USA (NOAA, 1981) calculated that a nodule collector will traverse 1.9 km² per day, of which 0.8 km² are not mineable.

The future exploitation contracts for minerals in the Area are likely to be valid for a period of three decades.¹⁷ Van Nijen et al.

¹⁴ ISA, Development of environmentally responsible mining technologies: towards an approval process for mining equipment, ISBA/23/C/5, 1 June 2017.

¹⁵ ISA, Decision of the Council of the International Seabed Authority Relating to the Development of Binding Environmental Threshold Values, ISBA/27/C/42, 11 November 2022.

¹⁶ See e.g. <https://impossiblemetals.com/technology/robotic-collection-system/>.

(2018) assume a mine life of 25 years. The “BlueMining” nodule mining concept presented by (Volkman and Lehnen, 2017) assumes an annual production of 1.5–2 Mio t (dry weight, DW) of nodules and expects mining operations to be operational 250 days per year and 20 hours per day. To achieve this production capacity, the nodule collection must reach 400 t DW per hour, and the lifting capacity must achieve 150 kg DW per second. For a mine life of 20 years, the German contractor, BGR, calculates that at a conservative resource abundance of 10 kg of dry material per m² would result in the extraction of nodules from 100 and 200 km² per year or 2300–3600 km² (equivalent to the surface area of Luxembourg) over 20 years (Volkman and Lehnen, 2017).

However, the total seafloor area impacted by individual mining projects will be much larger than suggested by the above figures, particularly as the mine site will rarely be one contiguous area but rather a patchwork of mineable and non-mineable sites (Kuhn et al., 2020). In case of the French contract area in the CCZ, individual mining blocks are expected to be no larger than 100 km² of variable shape (Lenoble, pers. com. in Thiel et al., 2005). Estimates for the total area directly impacted by one 20-year mining operation range from 6500–14,000 km² (Volkman and Lehnen 2017), to 28,000–56,000 km² when considering the sediments plumes generated by the collector vehicle (Sharma et al., 2001).¹⁸ The overall benthic footprint of a single mine over 20 years may be at least 2 to 4 times larger than the mined area itself (Smith et al., 2020). The cumulative impact of several mines in a biogeographic subregion, for example in the eastern CCZ, could degrade substantial portions of the approx. 500,000 km² nodule habitat in the CCZ (Smith et al., 2020).

The nodules collected are separated from the cohesive sediment, both while entering the collector, and inside the collector. Finally, the sediment slurry – possibly ejected by the collector through an exhaust pipe several meters above the seafloor – creates an operational plume of which the larger particle fraction is likely to resettle on the seafloor, while the fine fraction will drift away and remain in the water column for a long time (Baeye et al., 2021; Purkiani et al., 2021; Muñoz-Royo et al., 2022; Weaver et al., 2022).

The nodules may be crushed to be transported as a slurry up to the surface vessel for dewatering, pre-processing and transport to land. Several technologies are under discussion for lifting the ore slurry to the surface ship (Ma et al., 2017), some of which require underwater pumps (Verichev et al., 2012; Ma et al., 2017) likely to cause vibration and noise pollution. The non-mechanical transport systems (pneumatic or hydraulic lifting) require pipes of several kilometers length where the turbulence will cause nodule degradation through particle fragmentation, chipping, attrition, and/or abrasion (Kim et al., 2021). Particles smaller than 8 µm in diameter will be unlikely to be technically recoverable from the slurry and thus likely to be discharged after pre-processing at sea

(Van Wijk and De Hoog, 2020; Kim et al., 2021). As an alternative, Ma et al. (2017) propose a mechanical collection and riser system in open or closed containers, though with a lower production capacity in deep water.

It is yet unclear, whether contractors are aiming at beneficiation of the nodules only, *i.e.*, after dewatering of the nodule slurry for storage, with unrecoverable fractions being discharged at certain depths, or whether a partial treatment of the nodules by chemical and physical means is considered to produce a concentrated intermediate product at sea. A third option would be to carry out the full processing or refining at sea, which seems rather unlikely given the energy requirements.

The ore might have to be watered again for transshipment (inter-ship transfer onto transport barges). This would result in more excess water to be discharged from the transport vessel (likely to surface waters and this time most likely of different physical and chemical composition). A recent publication indicates potential health risks for humans when handling nodule material owing to substantial alpha radiation being emitted from stored and dewatered nodules (Volz et al., 2023).

In addition to vessel-generated noise, the entire mining process from the seafloor to the support vessel will create noise pollution. This includes acoustic telemetry which is employed for positioning, locating, equipment steering and controlling remotely operated vessels (ROV) to support extraction operations. Sound is also used, for example, to communicate with landers, AUVs and other equipment.

An overview of environmental pressures resulting from the excavation process is provided in ISBA/23/C/5, Annex and other reviews (e.g., ICES, 2015; Kaikkonen et al., 2018; Leal Filho et al., 2021, Table 3). In the following, we provide a summary of the currently known likely effects of nodule mining on relevant layers of the ocean, as well as recommendations for BEP from the literature.

4 Seafloor

4.1 Operational effects of collection on the seafloor

Key risks to seafloor integrity result from the removal of the nodules as an essential structural habitat, the destruction of the biogeochemical structure and function of the sediments, and the indirect degradation of adjacent areas through smothering by re-sedimentation, e.g., of operational plumes.

As described above, nodule mining operations target level and soft sediment environments in regions with extremely low sedimentation rates of on average 3.5–5 mm/1000 years in the eastern CCZ (Khripounoff et al., 2006; Mewes et al., 2014; Kuhn et al., 2017). In general, nodule collection will result in the removal of hard substrate, diminished rugosity and qualitative change of large expanses of seafloor for an indefinite time. Effects will be visible from fine-scale to landscape level, e.g. from a vertical removal of the bioactive upper 7 cm of the sediment (Volz et al., 2020), exposing sediments approximately 14,000–20,000 years old, to the mosaic of more rugged or elevated areas related to

17 ISA, Draft Regulations on Exploitation of Mineral Resources in the Area – The President’s Further Revised Draft Text, ISBA/28/C/WOW/CRP.2, 5 October 2023, Draft Regulation 20.

18 These figures are based on old technology. Modern equipment may raise less sediment.

geologically defined horst and graben structures, as well as seamounts remaining after mining (Volkman and Lehnen, 2017; Kuhn et al., 2020).

Nodule mining will cause loss of essential habitats, including the structural habitat required by some faunal groups (Amon et al., 2016; Vanreusel et al., 2016; Leitner et al., 2017; Stratmann et al., 2021). This type of strip-mining will also substantially and persistently impact food web integrity, i.e. through loss of nodules as a structural habitat for ecological keystone taxa, such as stalked sponges (Stratmann et al., 2021).

The clearing of nodules also incurs the loss of a distinct sediment microhabitat (Wu et al., 2013), accompanied by loss of reactive labile total organic carbon (TOC) and bioturbation (Volz et al., 2020), resulting in reduced microbial activity (Vonnahme et al., 2020) and reduced carbon cycling (Sweetman et al., 2018). The most sensitive indicators of sediment disturbance in the deeply oxidised CCZ are (Vonnahme et al., 2020) (i) bioturbation channel connection, (ii) oxygen concentration profiles, (iii) sediment density measurements (via x-ray), (iv) total inorganic carbon, (v) nitrite concentrations, (vi) oxygen utilization/carbon fixation, and (vii) total organic carbon. In the shallow-oxidised Peru Basin, profiles of manganese-oxide concentration in the solid phase are recommended as an indicator of sediment disturbance in addition to the oxygen penetration depth (Paul et al., 2018).

In particular, the following impacts can be expected to affect the sediment and its ecosystems:

First, the nodule collection will disturb the semi-liquid sediment-surface interface and will create a near-bottom plume with substantial amounts of sediment being displaced (Becker et al., 2001). The released sediments will originate from (a) any gear placed on or moved along the seafloor; (b) the separation of nodules from adhering sediments in the collector (Oebius et al., 2001), causing loss of fauna, and (c) nodule crushing either within the collector or in a separate crusher - any leakage will lead to metal slurry polluting the waters (Kim et al., 2021).

Second, equipment that digs into the sediment can have an impact through the direct crushing of buried infauna (animals living in the sediment), by compacting the substrate through increased weight of machinery or equipment (International Seabed Authority, 2010), or conversely, by stirring up the sediment, dislodging animals, and later leaving a suspended sediment cloud that resettles on the seafloor. Effects on the seafloor are likely to be site-specific and depend strongly on the degree of nodule coverage, the type of technology used, the nature of the fauna, the seafloor material (i.e., the sediment), and oceanographic conditions in the area.

Third, intermittent storage dumps of collected nodules or waste material may increase the seafloor area used by the operation [see e.g. Coffey Natural Systems (2008)].

Fourth, the re-sedimentation of the sediment plumes created by operations on the seafloor can cause blanketing and smothering of substantial areas in the vicinity, the size of area depending on discharge and plume characteristics, bottom current strength and seabed morphology (Peukert et al., 2018; Baeye et al., 2021; Purkiani et al., 2021; Haalboom et al., 2022). Aleynik et al. (2017) assume that in the

case of nodule exploitation, such plumes will quickly lead to high levels of suspended sediments and accumulations of > 1 cm thick deposited particulates > 5 km away from the mining activity within 10 days, and 1 mm thick deposits > 50 km away after 1 year, which would equate to >1000 times the background sedimentation rates. However, by taking into consideration gravity currents (Muñoz-Royo et al., 2022) and aggregation (Gillard et al., 2019b), the spatial extent of the blanketing plume may be reduced significantly.

Fifth, if hydraulic pumps are to be used, large volumes of water are required to collect and to pump the extracted nodules to a surface vessel. Most of the animals living near the nodules are not able to avoid the suction flow and will be entrained and die subsequently.

The severity of the ecological effects of mining has to be determined as a function of (estimated) recovery time. As the mined nodules and the associated fauna will only regrow over millions of years, the loss of nodules, but also the loss of biotic structure and organic contents is expected to cause permanent benthic community shifts at mined sites (Haffert et al., 2020; Gollner et al., 2021). The processes will lead to lasting effects on the food web (Orcutt et al., 2018; Stratmann et al., 2021), biogeochemical cycle and carbon-fixation (de Jonge et al., 2020; Hollingsworth et al., 2021). The severity of the effects on ecosystem functioning depends on the amount of labile organic matter removed, i.e. the lower the resulting bacterial activity becomes, the more severe the disturbance will be (Haffert et al., 2020; Volz et al., 2020; Vonnahme et al., 2020). A reduction of organic matter supply to the deep sea due to an expansion of the oxygen minimum zone and related changes in midwater consumer activity may amplify these effects (Wishner et al., 2018).

4.2 Recommendations for best environmental practices on the seafloor

The following recommendations provided in the literature focus on minimising the degradation of the deep seafloor ecosystem integrity and its functioning during the mining operation.

- Safeguard a sufficient amount of representative seafloor habitats in each contract area based on habitat classification (Vanreusel et al., 2016; Hauquier et al., 2019; Uhlenkott et al., 2020; Uhlenkott et al., 2022).
- Minimise the spatial extent of the mined area. Limit the directly mined area within a region to a level that does not threaten ecosystem integrity (MIDAS Consortium, 2016).
- Mine a deposit as completely as possible, including lower-grade areas. This 'whole-of-a-deposit approach' has been recommended to minimise the areal consumption and ecological damage of mining (Volkman, 2014, see also ISA Technical Study No. 11). The assumption is that only mining areas with high-grade deposits may compromise DSM for future generations. As such, the literature recommends lowering the cut-off grade mined to include lower-grade deposits. This approach requires a strong

involvement of the Authority in mine planning and includes a comprehensive resource and reserve assessment of the proposed mining area and the adoption of a sequential mining plan in order to maximize resource utilization (International Seabed Authority, 2013; Volkmann, 2014).

- Arrange the mining tracks so that undisturbed, pristine areas remain to preserve the natural fauna and provide for 'stepping stones' for dispersal (Thiel et al., 2005; Sharma, 2017; Haeckel et al., 2020). Spatial planning should be employed to arrange mined and preserved areas, considering the needs of fauna, the likely dispersal of plumes (Haeckel et al., 2020) and the need not to impact areas outside the contract area. Spatial planning should also avoid conflicts with other human uses of the seafloor, e.g. cable laying (International Seabed Authority, 2015).
- Minimise sediment transport to the surface, i.e. clean the nodules at depth and maximise nodule concentration in the riser pipes (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; Sharma, 2010).
- Minimise the weight of equipment on the seafloor (Cuvelier et al., 2018) and restrict the penetration of the machinery into the sediment to avoid disturbance of consolidated suboxic - or even anoxic - layers (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; Sharma, 2010), i.e. minimise depth of sediment removed/raked to less than 10 cm (Volz et al., 2020; Vonnahme et al., 2020).
- Prevent the mobilisation of heavy metals by not exposing the hypoxic bottom layers, such as in the Peru Basin where the oxygenated zone is limited to a few cm (Koschinsky et al., 2003a; Koschinsky et al., 2003b). Hypoxic situations can be caused also through the discharge of mine tailings or chemical waste (Koschinsky et al., 2003a). The creation of hypoxic situations would increase the concentrations of bio-available metals and might change their speciation, which results in different bioavailabilities and toxicities, eventually leading to levels that would be toxic to animals (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; Koschinsky et al., 2003a).
- Avoid storage piling of nodules which may lead to metal-leaching under reducing conditions (Cuvelier et al., 2018).
- Reduce seawater intake, e.g. by using mechanical collection devices (Weaver and Billett, 2019) instead of hydraulic suction.
- Prevent seafloor blanketing of more than 1 mm thickness (Gillard et al., 2019a) equating to 1000 years of natural sedimentation.
- Plan for recovery. This involves: a) the sequence of mining tracks and succession of mining blocks should consider the predominant current direction to induce re-sedimentation where the seafloor is already disturbed, and thereby avoid disturbance of source populations for recolonisation (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001) and b) the size, spacing, location of mine sites should be known, and subject to spatial and temporal planning by ISA in order to ensure sufficient undisturbed source areas for

recolonisation are available (Thiel et al., 2005; Haeckel et al., 2020).

5 Benthic boundary layer

In addition to the direct effects of nodule removal from the seafloor, the operation of the mining equipment will have 3-dimensional impacts on the seafloor and water column through plumes, vibrations, noise and lighting (Weaver and Billett, 2019). In particular, the effects of increased turbidity from mining plumes may be the most substantial in the benthic boundary layer (BBL), usually the water layer immediately above the seafloor up to a density stratification at approximately 50 m above the seafloor, which is probably the least known deep sea ecosystem (Christiansen et al., 2020). The BBL hosts specialised benthopelagic organisms such as scavenging amphipods, benthopelagic fish and plankton (Christiansen et al., 2010; Vecchione, 2016; Leitner et al., 2017), is an important region for the dispersal of larvae from benthic organisms (Kersten et al., 2017; Kersten et al., 2019; Patel et al., 2020), and is home to a large number of gelatinous organisms (Childress et al., 1989). Benthopelagic coupling is crucial to ecosystem functions from nutrient cycling to energy transfer in food webs and thus particularly sensitive to anthropogenic stressors (Griffiths et al., 2017; Christiansen et al., 2020).

5.1 Operational effects of mining in the benthic boundary layer

Given the near-unknown structure and functioning of the BBL, the multiple stressors from mining will put a particularly high management risk on maintaining ecosystem structure and functioning in the BBL. These risks arise from the following operational effects:

Ambient seawater intake for water jets, suction devices, nodule cleaning and crushing as well as for slurry transport to the surface: Estimates of water removal per single mining operation/collector are > 50,000 m³ per day (Oebius et al., 2001)¹⁹ sourced from the near-bottom water layers and therefore likely to have substantial intake of benthopelagic organisms, including meroplankton larvae and other less mobile species (Christiansen et al., 2020).

Increased turbidity due to unaltered seafloor sediments raised by mining gear: due to the half-fluid nature and extremely small sediment particle sizes, even the slightest touching of the seafloor will raise plumes consisting of the unaltered sediments stirred up from the upper seafloor. In addition, the turbulence behind the vehicle also stirs up sediments. These particles will settle over time

¹⁹ NOAA (1981) calculated 100,000 m³ per day including bottom water and interstitial water for a production of 5500 t DW of nodules, including 783 kg of biota of which 80 % are discharged near the bottom. Weaver et al., 2022. Assessing plume impacts caused by polymetallic nodule mining vehicles. Marine Policy 139, 105011. assumed 30,000 m³ per day.

depending on particle size and degree of aggregation, but over a different area and accumulating differently from the original state. This may overwhelm plankton and seabed organisms in the process, in particular particle filter feeders, and produce chronic impairment (Weaver and Billett, 2019).

Increased turbidity caused by operational exhaust sediment plumes: The extent of plume dispersal depends on the height and momentum of sediment release, seabed morphology, and water column stratification and mixing (Purkiani et al., 2021). Currently no thresholds exist for the environmental significance of turbidity, and impacts on the pelagic fauna are unknown. However, enhanced loads of inorganic and degraded organic particles may impair respiration, decrease food availability, cause higher energy expenditure, and suppress communication and feeding interactions based on bioluminescence and chemical cues. The most prominent effects are the following:

- Clogging of gills and filtration apparatuses of surface-deposit feeding and suspension-feeding organisms, and the raising of energy expenditure and respiratory cost due to unpalatable, mostly inorganic particles of low nutritional value. Organisms with mucus nets (Robison et al., 2005; Christiansen et al., 2020; Katija et al., 2020) are assumed to be highly sensitive to any additional turbidity (Robison, 2009).
- Impairment of chemosensory, olfactory, bioluminescence and other intra- and interspecies light communication is likely to affect populations and propagation.
- Entombment, or burial by sediment plumes.
- Increased oxygen demand in the lower water column because of organic decay may lead locally to reducing conditions which may enhance the bioavailability of heavy metals from sediments (Ozturgut et al., 1981) as well as an increased nutrient flux from the sediments to the water column.

The scale of horizontal dispersal of the operational sediment plume generated by mining along the seafloor depends on several factors: (a) composition and shape of the sediments (e.g. Muñoz-Royo et al., 2022), (b) the degree of flocculation and formation of a turbidity current (Muñoz-Royo et al., 2022), (c) seabed morphology (Peukert et al., 2018; Gausepohl et al., 2020; Purkiani et al., 2021), (d) background currents (Muñoz-Royo et al., 2022), (e) tidal cycles that will keep bottom currents in motion (Baeye et al., 2021); (f) occasional strong events, such as benthic storms or the propagation of surface storms or eddies to the ocean floor resuspend sediments in at least the benthopelagic mixing zone (Aleynik et al., 2017; Purkiani et al., 2020). The latter may be a regular process which will significantly contribute to the spreading of mining-generated plumes both horizontally and vertically. As the density gradient in the water column between the benthic boundary layer and the bathyal and abyssal waters above is small, sediment clouds require little energy to cross and dissipate up in the water column (Purkiani et al., 2021). A significant vertical spreading of operational plumes was observed also by Haalboom et al., (2022); Muñoz-Royo et al., (2022).

Apart from the horizontal and vertical spatial scale of mining, its effects on connectivity patterns of species and the integrity of communities are critical to determine the lasting effects on ecosystem characteristics. Changes will be based on species-specific sensitivity to increased turbidity and blanketing. Jumars (1981) suggests that sensitivity to re-sedimentation depends on the life style or feeding guild of benthic taxa, being highest in sessile suspension feeders tolerating but a thin veneer, medium in suspension and surface deposit feeding species at the sediment-water interface, and lowest in infauna (but see also Mevenkamp et al. (2019) who found short-term responses of meiofauna to an artificial 2 cm thick layer of crushed nodule particles). However, all three guilds will suffer when the sedimented layer grows in thickness due to the reduced organic content and therefore food value (Kim et al., 2014) of the resedimented matter. Differential sensitivities to mining-related and climate change pressures will result in altered or novel species interactions (Levin et al., 2020, and literature quoted therein).

A characteristic of deep-sea sampling is that most of the taxa recovered are either unknown or they occur as singletons in the samples or are otherwise rare (which could also be a sampling artefact) (Amon et al., 2022). Rare species are important contributors to ecosystem functioning (Mouillot et al., 2013). Hauquier et al. (2019) assume that it might be mostly the rare genera and species that will be vulnerable to mining-induced changes in their habitat, based on an investigation of the most abundant meiobenthos component, the nematode communities. Unless the fauna and its life history are better known, mining might thus lead to unforeseen and unnoticed losses of essential functional organisms in the deep sea benthic and pelagic food web.

Even weak chemically reducing conditions in the bottom water will dissolve large amounts of heavy metals from the metal deposit in the upper sediment layers (Koschinsky et al., 2003a).²⁰ These dissolved metals are likely to be bioavailable, depending on the local chemical properties of each metal and of the water, such as pH, alkalinity, amount of organic material and complexing agents, as well as pressure and temperature, and become toxic at a species-specific level (Ramirez-Llodra et al., 2015). The bioavailability of dissolved metals may have lethal or sublethal effects on the fauna manifesting itself in population changes, reduced productivity, respiratory stress, failing reproduction etc. (Hughes et al., 2015; Hauton et al., 2017; Mestre et al., 2017). However, these descriptors are generally unknown, and unlikely to become measurable any time soon.

5.2 Recommendations for best environmental practices in the benthic boundary layer

- The following recommendations provided in the literature focus on minimising the degradation of the benthic boundary layer

²⁰ recent (unpublished) research results indicate that even under fully oxic conditions, the picking up and destruction of the nodules increases the level of bioavailable heavy metals (Haeckel pers. com., JPIO MiningImpact Stakeholder meeting 2023).

ecosystem integrity and its functioning during the mining operation. It is recommended to prevent or minimise operational plume development, its potential for dispersal and redeposition through

- design of shape and arrangement of the mine sites: round or more or less square mining area will have a relatively much smaller plume impact areas compared to elongated and widely spaced mining areas (Volkman, 2014);
 - separation of minerals from sediments (or other debris) as close as possible to the seabed to reduce impacts on the water column (Sharma, 2017);
 - use of buffer containers attached to the collector to catch the mixture of sediment, cut-off nodule fragments and the remains of benthic biota, discharged in the collector path (Abramowski, 2018);
 - horizontal release of sediments close to the bottom (Peukert et al., 2018);
 - inducing a high rate of plume re-sedimentation immediately behind the mining collector (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; Cuvelier et al., 2018; Weaver et al., 2022), e.g. by optimizing particle settling for fast and effective flocculation behind the collector (Peukert et al., 2018). Gillard et al. (2019b) 'suggest that the use of elevated sediment discharge (500 mg L⁻¹) under elevated turbulence results in rapid sediment flocculation' which could be induced by a corresponding mining collector and exhaust pipe design. However, a thorough assessment of operational parameters, expected plume regime, and fraction of discharged sediment is required, as 'the balance of forces in the wake does not vary linearly with operational parameters, and the operational parameters themselves might not vary linearly with scale, which could result in fundamentally different operational regimes' (Muñoz-Royo et al., 2022);
 - choice of optimal location and time windows for sediment releases considering bottom currents and topography and the tidal flow patterns (Baeye et al., 2021).
- Prevent the reduction of oxygen concentration in the near-bottom water.
 - Carefully investigate the potential fate of discharged pre-processing waste material prior to taking decisions about its disposal at any depth. The better option is a zero waste concept (see below).

6 Water column and surface waters

The water column extends from the sunlit upper 200 m (epipelagial) and the mesopelagial (including the faintest light levels down to ca. 1000 m) to the completely dark regions of bathy-, abyss- and benthopelagic waters. The deep pelagic fauna may comprise the largest reservoir of animal diversity on Earth and has crucial roles in ecosystem functioning while providing essential ecosystem services. The deep pelagic habitats have been a

comparatively predictable environment for millions of years, and its physical and biological patterns have led to distinct adaptations of its communities (Robison, 2009, quoting Koslow 2007). Therefore, in bathy-, abyss- and benthopelagic waters, organisms are considered least adapted to environmental changes such as increased turbidity, changes in temperature, food supply as well as light and noise pollution, and small absolute increases may lead to acute effects (van der Grient and Drazen, 2022). Epi- and mesopelagial generate the main organic turn-over and production, feeding the entire foodweb. Surface waters are not only the place where the mining platforms and related vessels operate, but has an ecosystem of its own, the neuston, is the interface to air chemistry and wind forcing, and important for avifauna feeding and all air-breathing animals.

6.1 Operational effects in the water column incl. surface waters

A vessel's presence, its operations and traffic to and from land will not only leave a CO₂ footprint (Heinrich et al., 2019) and likely other effluents, but also cause continuous light and noise disturbances of unknown significance and impact surface and deeper water ecosystems through dumping and discharges.

There are three main hazards to water column organisms from DSM operations: a) The vertical lifting process of the nodule slurry (e.g., 80% water, 20% nodule fragments and likely other sediment) is prone to disaggregate any particle aggregations, abrade nodule fragments through mechanical stress, eventually decrease oxygen saturation through decay and therefore raise the solubility of heavy metals. Breakage of the riser or leakage at pumps and storage facilities may therefore release toxic fluids (Hauton et al., 2017; Kim et al., 2021); b) The discharge of sediment-fluid mixtures from the de-watering and pre-processing onboard the surface platform or vessel will result in a mixture of bottom water, fine sediment < 8µm, organic material, and unrecovered nodule fragments (plus eventual additives) being released back into the ocean (Kim et al., 2021). Whereas larger fragments may quickly sediment to the seafloor, Muñoz-Royo et al. (2021) calculate that the fine sediment fraction of 10 µm diameter and less may remain in the water column for one year, transported in any direction for up to 1000 km. Similar estimates were made for a low concentration deepwater plume of Peru Basin sediment (Rolinski et al., 2001; Baeye et al., 2021), not considering the particle aggregation potential. Depending on the grain size distribution in the discharged waste stream, the material would settle on the bottom within a month (coarse) to 10-15 years (90-95% of the particles, fine particles dominate), and with 2-3% of the sediment mass, corresponding to 25-50% by particle numbers remaining in the water column for 20 years (Baeye et al., 2021); and c) Substantial noise could be generated by the slurry in the vertical transport system, and the necessary pumps. Light may be needed for the remote control of the lifting system.

The discharge plume may intercept with commercially relevant fishes in the epi- and mesopelagic zone, potentially leading to spatial conflict of e.g. tuna fisheries in the CCZ mining in the current

exploration areas (van der Grient and Drazen, 2021). The plume may also result in fertilisation, eutrophication and acidification as well as warming of sea waters, increase of organic input and increased aggregation leading to changes in the water column processes (Cuvelier et al., 2018). In addition, the plume may introduce heavy metals and other potentially toxic substances from the pre-processing which may become bioavailable and cause biological reaction from behavioural change to mortality (Hauton et al., 2017).

It has been discussed that chemicals may be added to the discharges, *i.e.* currently unknown chemical additives to separate the mineral phases from the waste material and water, which ISA requests to be assessed for potential harmful effects (International Seabed Authority, 2020b). Should any chemicals be used, *e.g.* for separating solids from liquids, or to further particle aggregations, utmost attention is needed because of their associated toxicity and bioaccumulative potential (Ramirez-Llodra et al., 2015). Dilution plumes and those formed by the finest particle fraction are persistent, have an unlimited dispersal and are likely to pose the greatest threat to pelagic organisms.

6.2 Recommendations for BEP in the water column incl. surface waters

The water column comprises all waters above the benthic boundary layer, including the surface and epipelagic, mesopelagic, bathypelagic and abyssopelagic waters. Recommended measures and BEP include to

- Prohibit any discharges into epipelagic and mesopelagic waters (Drazen et al., 2020; van der Grient and Drazen, 2021), and if unavoidable, then make permission subject to the Waste Assessment Guidance (WAG) in Annex 2 of the London Protocol (2006);²¹
- Minimise waste (covering all discharges and other waste) towards zero and develop zero-waste concept for the operations of all vessels and platforms in the Area. Schriever and Thiel (2013) review proposals for its secondary use, *i.e.*, waste sediment could be deposited on land. If sediment discharge at sea is necessary, discharged sediments with low toxicity could be compacted into rapidly sinking bricks or lumps, or in biodegradable sediment bags, which will minimise the particle content of the waste water (Cuvelier et al., 2018). The pros and cons of further manipulation techniques need to be discussed.
- Maximise the particle recovery during pre-processing of nodules to decrease particulate volume and metal concentration in effluents (Kim et al., 2021).
- Determine the allowable size range of discharged particles with a focus on reducing the fine fraction (Cuvelier et al., 2018).
- Maximise particulate content of waste water discharged into the water column to promote aggregation and flocculation of particles (without chemical additives), but this increases the risk of leaching heavy metal (Koschinsky et al., 2003a).
- Manage the plume characteristics and spread of fall-out area by optimising the discharge depth and its ambient current velocities, stratification and vertical component (Rzeznik et al., 2019), including also tidal streams (Baeye et al., 2021). Rapid dilution to near-background levels as recommended *e.g.* by van der Grient and Drazen (2022) to reduce acute and chronic effects on the pelagic communities, but limits flocculation of particles and prevents rapid settling of particles (Muñoz-Royo et al., 2021).
- Manage discharge time and frequency as factors which influence environmental effect (van der Grient and Drazen, 2022);
- Use a 'second discharge pipe supported by on-board pumps should be used to return the bottom water and remains of sediment particulates as well as dissolved nutrients and trace metals to the buffer subsystem after on-board separation of nodule fragments' (Abramowski, 2018).
- Instead of a point-release at a certain depth, Cuvelier et al. (2018) propose for the waste water to be discharged along long horizontal or vertical pipes at different points in the water column to increase dilution and decrease overall flow. This would also minimise disturbance of benthic fauna and minimise plume concentrations for pelagic fauna. However, this will result in a low-level contamination of an even larger area with low-value food particles scavenged by *e.g.* flux- and mucous feeders, and as such influence the plankton foodweb (Christiansen et al., 2020).
- Temperature management of the fluids is crucial to minimise turbulence (Rzeznik et al., 2019). The cold bottom water will warm up during transport and processing, and if reintroduced in the deep may cause convection and mixing.
- Prevent transboundary effects by using buffer zones (Billett et al., 2019).
- Prohibit additives from processing in the discharged waste water to prevent chemical contamination of the water column (Hong et al., 2019).

Over the years, scientists have discussed several options for the reintroduction of effluents from ship-board processing into the water column:

- Discharge in surface waters: There is general agreement among the scientific community that epipelagic waters are not suitable for discharge because of possible interaction with phytoplankton, zooplankton, and fish larvae in the epipelagic zone (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; International Seabed Authority, 2010).
- Discharge below the oxygen minimum zone (OMZ): A permanent OMZ is found at depths between 400 and 800 m in all oceans, where low oxygen concentrations

²¹ see <https://www.imo.org/en/OurWork/Environment/Pages/wag-default.aspx>.

and low pH values influence metal binding. The NE Pacific is particularly prone to losing oxygen due to climate change, with the OMZ expanding and deepening (Ross et al., 2020). To avoid dissolution of metals from the particulate phase and their accumulation in the OMZ, discharge is recommended below the thermocline and the OMZ (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; International Seabed Authority, 2010) and only in fully oxygenated waters (Koschinsky et al., 2003a).

- Discharge in midwater (200 to 1000 m) is not recommended (Christiansen et al., 2020; Drazen et al., 2020): The deep mesopelagic and bathypelagic food webs are heavily reliant on low-density, very small organic particles and may be affected through clogging of respiratory and olfactory surfaces, filtering apparatuses and mucous nets (e.g., protists, crustaceans, polychaetes, salps, and appendicularians). Decrease in food value is likely to lead to physiological stress and augmented sinking rates, heavy metal content may be elevated. Light will be absorbed and impact on communication, as will the noise produced by the machinery.
- Discharge close to the seafloor: It has been recommended (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001; Schriever and Thiel, 2013) to discharge the tailings at bathyal or even abyssal depths close to the seafloor to limit the potential ocean-wide dispersion of tailings particles. Cuvelier et al. (2018) and Drazen et al. (2020) propose to (re)deposit the sediments over the mined tracks. Such disposal would need to be temperature-equilibrated to the *in situ* temperature to reduce the spatial footprint.

Any of these options will need comprehensive evaluation of environmental risks. While mining wastes are excluded from the London Convention/Protocol on dumping,²² a mining vessels' operational pollution, anti-fouling systems, ballast waters, and waste disposal is likely subject to established environmental protection rules under the regime of the International Maritime Organisation (International Seabed Authority, 2020a). With respect to dumping, the practices under the London Protocol may be viewed as Best Environmental Practice.

Processing at sea including the dumping of mining waste, incl. sediment, in surface waters was proposed in the 1970s, but is not known to be pursued by any contractor at present. Should this lead to the accumulation of toxic waste on the seafloor from dumping reduced and complexing agents (Koschinsky et al., 2003a), this would require special consideration. Any related assessment of impacts on the water column would require long-term experimental investigations into the composition and individual effects of discharged residues - presumably similar to the assessment

frameworks elaborated for the deep sea disposal of land mine tailings (Ramirez-Llodra et al., 2015; Vare et al., 2018), eventually regulated to meet standards and procedures of the London Protocol, Annex 2 (International Seabed Authority, 2020a).²³ A list of investigations required prior to taking management decisions is provided in Vare et al. (2018).

Transshipment losses of re-watered nodule ore. This will affect surface waters to an unknown degree as the transshipment technology remains unknown. However, re-wetting of the nodule ore on the processing ship and pumping of the slurry over to the transport barge appears very likely. This would result in two separate discharge events, as the transported ore will have to be dewatered again.

Accidents have to be accounted for, such as breakage and leakage of hydraulic lines, riser pipe (slurry dumps), oil pollution from surface vessels, or loss of cargo in heavy seas with subsequent surface plumes.

7 Noise, vibrations and light

The natural environment of the deep oceans may be cold, clear, and dark, but certainly not silent as animals communicate over short and large distances and other geophysical events cause noise (Stocker, 2002; Duarte et al., 2021). Marine animals produce sounds between 10 Hz and 20 kHz for intra- and interspecies purposes, including navigation, foraging, agonistic displays, territorial defense, mate attraction, and reproductive courtship (Duarte et al., 2021). For example, some deep sea fish species use low sound frequencies to communicate (Miller et al., 2018, quoting Rountree et al., 2012), and benthopelagic invertebrates are suspected to use sound to detect food falls up to 100 m away (Stocker, 2002). However, Duarte et al. (2021) demonstrate, that today in many parts of the ocean the sound produced by anthropogenic sources overrules the natural soundscape, affecting marine animals at multiple levels, including their behaviour, physiology, and, in extreme cases, survival.

Natural light only occurs in the upper layers, where it is very dim, monochromatic, and downwelling, and affects vertical migration (Aksnes et al., 2017). Deeper down many taxa produce their own light. Bioluminescence and olfaction are, among other senses, important means of communication in the lightless depths. Bioluminescence has now been detected throughout the oceans (Heger et al., 2008) and is important for mate finding, camouflage,

²³ see ISA Technical Study No. 25, 2022, p. 42: Waste Assessment Guidelines under the London Convention and Protocol (IMO, 2014). The Guidelines provide for eight distinct steps: (1) waste characterization; (2) waste prevention audit and management options; (3) action list; (4) selection of a dump site; (5) impact assessment; (6) permitting system; (7) permit conditions; and (8) monitoring. These include guidelines for dredged materials, which could be relevant depending on technologies used to exploit resources of the Area. See also Revised Specific Guidelines for the Assessment of Vessels, adopted by LC 38/16 (2016).

²² 1996 London Protocol to the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matters, 1972, Article 1(4)(3); 1972 Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matters, Article 3(2)(c).

prey attraction and fending off predators (Robison, 2004). Sensing sound, scent and electromagnetic fields also appears to be common in deep pelagic organisms, but little is known about their functions, and there is urgent need for more studies (Robison, 2004). Most organisms inhabiting this environment possess highly sensitive visual systems, for example, predatory mid-water fish and squid frequently have highly developed eyes, to be functional in even the dimmest illumination (Robison, 2009), which makes them vulnerable to e.g. bright artificial lights of deep sea operations.

7.1 Effects of noise, vibrations and light

Mining operations on the international seabed will require the continuous presence of a fleet of vessels powering the mining operations as well as traffic to and from land. This generates large amounts of carbon dioxide exhaust (Heinrich et al., 2019) and continuous light and noise (McKenna et al., 2012) disturbance of unknown significance in this relatively quiet part of the ocean, which has been described as one of the last wildernesses on Earth (Jones et al., 2018b; Duarte et al., 2021). All industrial activities produce some noise, possibly covering the full frequency bandwidth of animal sounds and traveling long distances under water. Mineral extraction produces some of the loudest noises (Stocker, 2002), and Williams et al. (2022) estimate that a single mine may change the sound environment and elevate noise levels audible up to at least 500 km from the source under favourable weather conditions. Also Duarte et al. (2021) consider the dispersal range of operational noise for subsea mining to reach 100 km. Depending on the frequency and depth, e.g. in the so-called SOFAR channel sound can propagate much longer distances (Duarte et al., 2021), interfering with communication of marine mammals. Furthermore, manmade noise may mask inter- and intraspecies communication (Erbe et al., 2016). The great dispersal range may render a meaningful comparison of Impact Reference Zones, IRZ, with Preservation Reference Zones, PRZ,²⁴ impossible and should thus be a major concern for regulators and mining operators (Williams et al., 2022).

As a first step, Martin et al. (2021) reviewed available knowledge on the likely noise generation of polymetallic nodule mining operations and its documented effects on the marine fauna. They also draw on similar activities in other industries to estimate potential impacts. However, knowledge is scarce, even to predict the actual masking effects of anthropogenic sound on e.g. marine mammals (Erbe et al., 2016). Yet, generally, there is evidence that anthropogenic noise not only impacts marine mammals, but negatively affects both pelagic and benthic fish and invertebrate communities through changes in organisms' behaviour, anatomy, development and physiology (Duarte et al., 2021).

Artificial light in the deep sea may have various effects due to the natural clarity of the water on the one hand, and the high sensitivity of pelagic organisms to light on the other (Douglas et al., 1995; Haddock et al., 2010). Some fishes are known to be attracted to light, whereas others avoid light or do not show any reactions (Widder et al., 2005; Raymond and Widder, 2007; Ryer et al., 2009). Attraction to light may enhance the danger of, for example, entrainment. The ecological function of bioluminescence, e.g. to attract social partners or prey, is likely to be locally masked by bright illumination. A well-lit mine may function as a population sink over a very large area for deep-sea organisms attracted to light. Furthermore, the extremely high intensity of flood lights, as compared to bioluminescence, may irreversibly damage the eyes of organisms in the vicinity, as suggested for vent shrimps by (Herring et al., 1999). A literature review²⁵ confirms that physiological damage to the eyes of a range of taxa may be caused, depending on exposure and light characteristics. However, so far little is known about the quality and quantity of light needed for the mining operations.

Very little is known about the effects of vibrations caused by activities contracting the seabed on marine life, and nothing in relation to deep seabed mining. However, it can be assumed that at least benthic invertebrates can detect and react to vibrations including those from anthropogenic sources (Roberts and Elliott, 2017).

Environmental risk is determined by the severity of the effects and the intensity, duration and probability of noise, light or vibration occurring, however, to date few estimates have been published on emissions of mineral extraction (Kaikkonen et al., 2018; Martin et al., 2021), due also to technological developments being contractor-specific and rapidly emerging. Future mining tests and targeted studies will hopefully reveal some of the characteristics which should be instrumental to developing BEP.

7.2 Recommendations for BEP to minimise light and noise pollution

A BEP Standard for noise, light and vibration emissions during DSM activities needs to be developed. Based on a comprehensive risk assessment, this will require strict mitigation at the source for all elements of the mining chain, including surface operations. A best available technology standard should implement minimum requirements. As a basic requirement, a "rule explicitly stipulating that introduction of energy, including underwater noise, in mined areas and their vicinity should be at levels that do not adversely affect marine life and the marine environment" should be adopted (Martin et al., 2021).

²⁴ PRZ are reference sites which shall inform on the level of disturbance caused by mining in representative mine areas, IRZ. Both zones are to be designated within the contract areas. For details see International Seabed Authority, 2018. Design of IRZs and PRZs in deep-sea mining contract areas. Briefing paper 02/2018. Kingston, Jamaica, pp. 1-8.

²⁵ Kochevar, R.E., 1998. Effects of Artificial Light on Deep Sea Organisms: Recommendations for ongoing use of artificial lights on deep sea submersibles. <https://montereybay.noaa.gov/research/techreports/trkochevar1998.html>.

A BEP noise regime needs to consider all sources of noise inherent with the operations, including during exploration and test mining. The spatial and temporal footprint of the noise planned to be emitted needs to be determined, including its short- to long-term lethal and sublethal effects on the habitat and communities affected (Kaikkonen et al., 2018; Martin et al., 2021; Williams et al., 2022). It is strongly recommended to avoid the introduction of noise at depths of the SOFAR channel (typically at depths of 700 to 1,300 meters) to avoid the spreading of noise over thousands of kilometers (Drazen et al., 2020). This would also have to apply for any riser pipe or pumps at that depth. A temporal scheme of periods of active mining alternating with pauses to facilitate survival, animal condition, animal migration, reproductive success and recruitment of benthic and pelagic communities should be considered (Cuvelier et al., 2018).

Existing technical standards and design requirements may be of some help, as recommended for example by Hitchin et al. (2022) and Martin et al. (2021). However, there may only be limited transferability of knowledge gained from coastal to offshore waters (Kaikkonen et al., 2018). For vessels operating outside areas of national jurisdiction, the IMO voluntary guidelines for reducing underwater noise from commercial ships,²⁶ could be instrumental to develop vessel standards. Best practice may require that ships and underwater operations are to be powered by electric energy which would also minimise noise and vibration emissions (Cuvelier et al., 2018; Duarte et al., 2021).

A BEP light regime should also consider a) the minimum need for light e.g. for the operations, b) its maximum duration and c) maximum intensity as well as a least invasive spectrum (rapidly absorbed light temperature, e.g. around 2000 K), and d) options to minimise light use. Furthermore, the effects of the indispensable light for the operations have to be investigated further to make informed decisions rather than guesses on physiological, behavioural or population changes in affected species or populations. A BEP light regime for surface operations should minimise the disturbance of seabirds and the attraction of other organisms such as cephalopods and marine turtles. This may include the use of red light (Widder et al., 2005).

Ideally, the emission of noise should be restricted to higher frequencies with a fast attenuation, and the use of light should be completely avoided in the deep sea.

8 Overarching recommendations

Overall, a highly precautionary, ecosystem approach to regulating mining operations is essential, focusing on the collection of additional knowledge while avoiding harm as much as possible (Martin et al., 2021; Williams et al., 2022). The authors recommend an iterative approach to noise regulation starting with highly cautious thresholds enabling rapid management responses and full transparency. Acceptable exposure to sediment plumes should also start with concentrations close to background levels

(van der Grient and Drazen, 2022). The thresholds relate to the risks posed to fauna by all mining-related stressors individually and combined (Wedding et al., 2022). This requires strategic environmental goal setting by the ISA, an adaptive governance and a strong scientific programme to reduce scientific and management uncertainties (Jaeckel, 2016; Jaeckel, 2017; Jaeckel, 2020). Existing standards and practices from other offshore industries, e.g. for noise pollution, while indicative, need to be thoroughly tested in the deep sea and offshore context, but may only be applicable to a limited extent (Hitchin et al., 2022).

Before any relevant environmental standards for nodule mining in the Area can be developed, scientifically meaningful and robust environmental baselines have to be established for each contract area and the corresponding region (Billett et al., 2019; Amon et al., 2022; Christiansen et al., 2022a), including the mapping of background noise levels (Martin et al., 2021). Therefore, baseline investigations need to inform the location of the mining area and its corresponding representative reference areas, used to determine mining impacts from the very start of operations in their full ecological context (Jones et al., 2018a; Hao et al., 2020; International Seabed Authority, 2020c). Baselines should also determine habitats, species and ecosystem functions to be preserved. The criteria developed by FAO (2009) and Secretariat of the Convention on Biological Diversity (2009) can guide the positioning of additional no-mining areas of the region(s) and contract areas, in order to preserve essential habitats and communities (Christiansen et al., 2022b). For all BEP standards, it is of utmost importance to observe the pelagic and benthic heterogeneity of the regions in question, e.g. the CCZ, which do not constitute one homogenous region and thus cannot be managed as such (Leitner et al., 2017; Drazen et al., 2021; Leitner et al., 2021; Simon-Lledó et al., 2023).

Temporal or spatial avoidance of certain areas by DSM-related activities as well as the avoidance of especially harmful gear was pointed out as an important precautionary mechanism to deal with uncertainty and as a tool for passive adaptive management (Billett et al., 2019). For example, Cuvelier et al. (2018); Haeckel et al. (2020); Hauquier et al. (2019) and Vanreusel et al. (2016) strongly advocate for the incorporation of additional no-mining sites within each of the contract areas (supplementing the PRZ) for the sole purpose of buffering the inevitable loss of biodiversity. Outside contract areas, the network of 'Areas of Particular Environmental Interest, APEI, in the CCZ has recently been updated to better cover the spectrum of regional and local habitats across the CCZ,²⁷ including the core oxygen minimum zone currently not covered by the APEIs (Perelman et al., 2021).

Wagner et al. (2020) suggest that in line with a precautionary approach to ensuring effective protection, mining activities should not be allowed to proceed as long as it cannot be demonstrated that vulnerable marine ecosystems (VME, FAO, 2009) will not be subjected to lasting harm. For example, VMEs such as corals and sponges occur on hard substrate including nodules, rugged terrain, and seamounts within the contract areas. Such habitats will not be directly mined, however may suffer from activities and sediment blanketing. Therefore, a VME definition adapted to the risks of deep seabed mining is required (Christiansen et al., 2019).

²⁶ www.imo.org/en/MediaCentre/HotTopics/Pages/Nois.aspx.

In addition, Cuvelier et al. (2018) propose to identify seasons or periods during which mining should be restricted or avoided. These “resting periods” (no noise, light and sediment plumes) might help to maintain life functions for the pelagic fauna during critical periods.

Tilot (2019) points to the high importance of preserving the very distinct oldest water masses located in the North-East Pacific between 1800 and 3500 m depth (quoting Talley et al., 2011), which have unique properties due to their perpetual circulation in the deep ocean. The unique biogeochemistry and microbial flora in these waters are presumed to have an impact on the nodule ecosystem (Tilot, 2019). Mining might pollute these waters, for example, through accidents or unregulated leakages from the riser pipes, as well as through wastewater discharge plumes.

Billett et al. (2019) suggest that the full environmental costs should be considered when designing mining equipment, to minimise harm from the start. Additionally, Billett et al. (2019) advocate for the equipment design process to consider mitigation measures at the earliest possible point in time, alongside best available technologies and practices, and cooperation among contractors to advance industry standards and protocols.

Once mining starts, mechanisms should be in place to meticulously observe the intensity and scale of the disturbances, in order to be able to adapt the respective environmental standards and activities to prevent risks and harm to the marine environment (Cormier and Lonsdale, 2020).

9 Towards a standard for BEP

We recommend the ISA to use the scientific recommendations compiled above for starting the development of a first Best Environmental Practice Standard for polymetallic nodule mining operations in the Area. At present, most knowledge and recommendations exist for the Clarion-Clipperton Zone and the Peru Basin. Substantial scientific investigations will be needed for other regions. An ISA Standard for BEP should include operational best practice, maximum permissible emissions, and the environmental framework conditions to be maintained. It would thus build on the environmental goals and objectives to be formulated by the ISA globally and regionally, and the corresponding environmental thresholds, a first set of which are currently being developed.²⁸ A first BEP standard should be available prior to the first applications for exploitation being received, to allow the ISA to assess an applicant's ability to meet BEP. The BEP Standard should be periodically reviewed, based on results from DSM monitoring programmes and scientific progress.

Building on the scientific recommendations on BEP for polymetallic nodule mining cited above, we compile in Box 1 a preliminary list of elements for a management framework that can underlie a future BEP standard (the “what”). This list is probably not exhaustive, but we hope it provides a starting point for developing a detailed and scientifically sound understanding of the BEP for polymetallic nodule mining in the Area.

10 Final considerations

Using best environmental practice for mineral exploration and exploitation in the Area is a legal obligation for the ISA, contractors, and sponsoring states. Whereas environmental standards such as BEP, BAT and GIP,³⁰ are frequently referred to in the ISA draft exploitation regulations (as of 2023)³¹, there is still no agreed understanding of what constitutes current BEP in relation to deep seabed mining, nor are there any agreed processes at ISA to arrive at some (preliminary) conclusions in parallel with the development of the exploitation regulations. This may prevent that the ISA can apply uniform standards to all applications for exploitation contracts. In addition, the development of BEP in the Area and its regions is tightly linked to the as yet undefined global and regional environmental goals and objectives within the ISA to guide the development of a system of precautions in order to avoid unintended, uncontrolled and unnoticed environmental degradation.

Science has a key role to play in informing the development of BEP prior to the commencement of commercial mining. Biological responses to small-scale experimental disturbance events over several decades (Gollner et al., 2017; Jones et al., 2017) provide invaluable indications of what limits need to be set for mining-related interferences in the deep sea. Scientific monitoring and assessment of test mining³² (Boetius and Haeckel, 2018; Haeckel et al., 2020; Amon et al., 2022) brings this knowledge to a new scale with more informed recommendations for a first set of BEPs. To close current gaps in knowledge, Amon et al. (2022) propose a road map for scientific exploration, including better access to contractor environmental data. However, BEP development will also require effective stakeholder participation, e.g. for fisheries (van der Grient and Drazen, 2021) and the cable industry (International Seabed Authority, 2015) as well as harmonization with existing maritime regulation (International Seabed Authority, 2020a).

If mining commences, the monitoring of operations and related environmental effects on various scales are crucial to verify compliance

27 see ISBA/26/C/58. Decision of the Council of the International Seabed Authority relating to the review of the environmental management plan for the Clarion-Clipperton Zone. December 2021. <https://www.isa.org.jm/documents/isba-26-c-58/>.

28 ISA, Decision of the Council of the International Seabed Authority Relating to the Development of Binding Environmental Threshold Values, ISBA/27/C/42, 11 November 2022.

30 Indeed, GIP is referred to most frequently, although such standard may only exist once the industry is mature.

31 ISA, Draft regulations on exploitation of mineral resources in the Area: Parts IV and VI and related Annexes, ISBA/28/C/IWG/ENV/CRP.1, 2 March 2023.

32 A great number of references in the earlier sections derive from the JPIO MiningImpact project (<https://miningimpact.geomar.de/publications;jsessionid=474EFC7BEDC6D8537B80D22A89BE0758>) and some other such scientific studies.

BOX 1 Recommendations on elements for a future BEP Standard for polymetallic nodule mining operations.**Management framework**

1. The overall lack of knowledge on the effects of mining operations on the marine ecosystem requires a highly precautionary, gradual approach, which includes the collection of more knowledge while avoiding harm as much as possible.
2. Comprehensive environmental baseline information of a contract area and its region are essential to developing best operational practice;
3. The spatial and temporal footprint of mining related pressures and needs to be determined, including its short- to long-term lethal and sublethal effects on affected communities.
4. Ecosystem-based, comprehensive risk assessment of the hazards from mining operations is needed for determining BEP to maintain benthic and pelagic processes, taking account of cumulative and synergistic effects, including of climate change.
5. A spatial planning process is recommended to optimise the location and design of the mine sites, the before-after control areas IRZ and PRZ, and other sites in need of protection.
6. Non-mining areas (APEIs, MPAs, VMEs²⁹ and other types) should be established and effectively conserved within and outside the contract areas to safeguard the Area's ecological values in line with the Azores Criteria (Secretariat of the Convention on Biological Diversity, 2009) and others;
7. Seasons or periods during which mining should best be restricted or avoided need to be considered;
8. Consideration of all environmental costs should lead to mitigation measures embedded into equipment design and result in minimising harm; and
9. Adaptive governance is needed to regulate activities in line with environmental goals and thresholds based on high-intensity monitoring of the disturbances and the effects caused.

Operations affecting the seafloor (based on section 4.2)

A best practice standard should determine technical design and operational practice requirements which facilitate to

1. Minimise and control sediment disturbance and removal, including biogeochemical sediment parameters, bioturbation, and oxygen profile of the sediments; and
2. Minimise the extent of the mine site by determining a sustainable cut-off grade for nodule mining, and by implementing a Whole-of-a-deposit policy;
3. Minimise the contact of the equipment with the seafloor;
4. Minimise water intake;
5. Prevent the release or mobilization of heavy metals;
6. Avoid storage dumps.

Operations affecting the Benthic Boundary Layer (based on section 5.2)

A best practice standard should determine technical design and operational practice requirements which

1. Operationalises a zero waste concept including to prevent operational plume discharge and dispersal. Alternatively, minimise operational plume generation;
2. Determines a maximum impact area for sediment plumes, and gives criteria for determining the affected area and the relevant environmental effects
3. Sets operational limits for
 - a) the discharge of sediments at the source (concentration, height above seafloor and volume of release, max. concentrations of metals);
 - b) the maximum blanketing thickness in a mined area;
 - c) allowable re-sedimentation patterns (spatial extent, thickness within and beyond mine site, temporal development, maximum range of particle dispersal, and protection of areas outside contract area);
 - d) the maintenance of oxygen concentration in water and sediment;
 - e) the prevention of heavy metal concentrations in plume and sediment fall-out.

Operations affecting the water column and surface waters (based on section 6.2)

A best practice standard should

1. Provide a BEP framework for ship-based processing/beneficiation waste treatment and transport to shore including requirement to minimize greenhouse gas emissions from the operations;
2. Require fully enclosed riser pipes (with a double hull reducing noise propagation);
3. Develop and operationalise a zero-waste concept, where possible, to prevent/minimise the creation and discharge of waste material into the water column; alternatively,
4. Define minimum discharge water quality standards (e.g., sediment load and composition, time and frequency of discharges, temperature, toxic potential, quantity and quality of additives, if at all permitted) informing the shipboard processing and transshipment of material;
5. Prescribe the documentation needs to prove that no environmental harm will follow from any discharges. Therefore, *a priori* investigations of the quality, quantity and fate of potential waste effluents over appropriate temporal and spatial scales are needed.
6. Recommend technical factors to be considered when discharging waste fluid after shipboard processing (incl. volume, pressure, and maximum dispersal);
7. Require a concept for preventing leakages of hydraulic oil etc. within a contingency plan (as well as all hydraulic oil to be fully biodegradable on all vessels and in all submarine machinery);

Operations as a source of noise, vibrations and light (based on section 7.2)

A best practice standard will have to require strict mitigation at the source for all elements of the mining chain, including surface operations. A Best Available Technology standard should implement minimum equipment standards for minimising noise and light pollution from seafloor to surface.

1. Ships and underwater operations are to be powered by electric energy, to minimise greenhouse gas emissions, noise and vibrations.
2. A light regime should be developed for surface operations to minimise the disturbance of seabirds and the attraction of other organisms such as cephalopods and marine turtles.
3. In addition, it is proposed to establish a temporal scheme of periods of active mining alternating with pauses to facilitate survival, animal condition, reproductive success and recruitment of benthic and pelagic communities. Furthermore,
4. It is strongly recommended to avoid the emission of any noise into the SOFAR channel (typically at depths of 700 to 1,300 meters at mid-latitudes) to avoid the spreading of noise over thousands of kilometers.
5. Maximum allowable light and noise emissions should be quantified and qualified in a stepwise process, for all activities involved in the mining operations, eventually building on existing technical standards and design requirements.

²⁹ "Areas of Particular Environmental Interest", "Marine Protected Areas", "Vulnerable Marine Ecosystems".

but also to check the appropriateness of the *à priori* determined environmental standards to provide for effective environmental protection. Gerber and Grogan (2020) assume it unlikely that the environmental impacts of mining activities, once commenced, can be fully verified by independent monitoring. They propose a staged monitoring approach with a highly intensive, real-time ‘validation monitoring’ in the early phases of the project to ascertain that environmental standards are maintained. In this early phase of mining, uncertainties are highest (Smith et al., 2020), which makes it imperative to validate the *à priori* determined contract conditions (Ginzky et al., 2020), including a review of the appropriateness of the first set of BEP. Subject to the monitoring and assessment results of commercial-scale mining, the standards may need to be adjusted to maintain the desired *in situ* conditions. An alternative approach would be to require an *in situ* test of the mining system of significant scale and duration on the basis of which the standards and BEP can be set. Another alternative would be for contractors to only be allowed to commence with a small-scale project where monitoring should demonstrate the effective protection of the environment.

Unlike in traditional industries, Gerber and Grogan (2020) recommend that required standards for e.g. BEP be limited to those “adopted, approved, or issued by the ISA from time to time.” This allows for some flexibility in adopting new standards. In this way, standards can be applied uniformly to all contractors, while the ISA and sponsoring states can maintain oversight and due diligence. This requires a corresponding highly precautionary and adaptive ISA governance system (Jaekel, 2016; Craik, 2020), which in effect establishes a management cycle that requires contractors to adapt their operations to regularly updated environmental performance standards.

Such an ecosystem-based environmental management framework does not exist yet, and the future standards and thresholds remain vague at present. Yet it is high time to have a discussion about what constitutes BEP and how to get there given that some exploration contractors are considering to venture into commercial-scale mineral exploitation. By offering a review of the recommendations made by scientists on possible BEP in all ocean layers, we hope to set a starting point for a science-based process to developing a BEP Standard for polymetallic nodule mining.

With so much scientific uncertainty remaining about the environmental impacts of seabed mining (Amon et al., 2022), States, NGOs, scientists and others have called for a pause on DSM³³ to give effect to the precautionary principle and improve our understanding of deep ocean ecosystems and their vulnerability to the effects of DSM. One might argue that if and when widespread support for postponing DSM exists, such a delay or pause may itself be regarded as BEP.

Author contributions

SC conceived and created the manuscript and researched the literature. All authors contributed to the article and approved the submitted version.

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Conflict of interest

SB was employed by company BioConsult SH. SB was also employed at the ISA Secretariat as Scientific Affairs Officer from 2013 to 2018.

The remaining author declares 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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³³ For an overview of current actors that support a moratorium or pause on seabed mining, see https://savethehighseas.org/moratorium_2022/.

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The importance of the soluble and colloidal pools for trace metal cycling in deep-sea pore waters

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Physical and chemical trace metal speciation are important for our understanding of metal cycling and potential toxicity to marine life. Trace metals can behave differently in diffusion processes or particle-solution interactions and have different bioavailabilities depending on their physical and chemical forms, which often depend on redox conditions. Here we investigated dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) concentrations of Mn, Co, Ni, Fe, Cu, V, Mo, U, Cd, and As in oxic and suboxic deep-sea sediments of the central equatorial Pacific Ocean. Vanadium, Mo, U, As, and Cd showed no significant concentration differences between their dissolved and soluble forms, suggesting that they are present as inorganic ionic species or organic complexes in the truly dissolved or small colloidal fraction. In contrast, the colloidal fraction ($> 0.02 \mu\text{m} < 0.2 \mu\text{m}$) of Mn, Co, Ni, and Cu increased with depth in oxic pore waters and Fe had the largest but variable colloidal pool. Soluble Mn, Co, and Ni were released in the uppermost 2–4 cm in the sediment because of reductive dissolution. The increasing colloidal fraction with depth suggests a decrease in the concentration of small organic ligands with depth, that are abundant in the surface sediment pore waters, and instead an increasing importance of larger ($> 0.02 \mu\text{m}$) inorganic nanoparticles and colloids such as Mn and Fe (oxyhydr)oxides that control Mn, Fe, and Co cycling at depths > 10 cm. The distribution of Ni and Cu cannot be exclusively explained by inorganic nanoparticles and a shift from low to larger high molecular weight organic ligands might occur. These findings provide new insights into trace metal distributions in the dissolved phase, highlighting the diversity of metal complexes and the need to incorporate these in future calculations of benthic metal fluxes and ecotoxicity assessments, especially in oxic pore waters.

KEYWORDS

colloids, trace metals, deep-sea pore water, biogeochemical cycling, speciation

1 Introduction

The release of potentially toxic metals to the water column during sediment removal and suspension is one of the main environmental concerns related to polymetallic nodule mining (e.g., Amon et al., 2022). To assess environmental impacts, i.e. behavior in the plume and bioavailability, a thorough understanding of natural trace metal cycling and an assessment of baseline conditions is of utmost importance. A detailed chemical and physical characterization of the “dissolved” ($< 0.2 \mu\text{m}$) pool is required to adequately assess impacts: for example, soluble ($< 0.02 \mu\text{m}$) organically complexed metals such as Cu will be less bioavailable compared to free or inorganically complexed Cu (also often $< 0.02 \mu\text{m}$) once released to the bottom seawater (Buck et al., 2007; Paul et al., 2021) and Mn and Fe (oxyhydr)oxide colloids (often $> 0.02 \mu\text{m}$) can scavenge soluble metals in the plume (Koschinsky et al., 2003). The fate and extent of spreading of especially the colloidal metal fraction once released to the seawater remains to be assessed (Hein et al., 2020).

In recent years, the differentiation of physical fractionation classes beyond the simply “dissolved” ($< 0.2 \mu\text{m}$ or $< 0.45 \mu\text{m}$) pool in seawater and pore water has become more widely applied and furthered our understanding of trace metal cycling in the marine environment. Determining the physical fractionation of the dissolved pool (truly dissolved, soluble, colloidal; Figure 1) gives a first indication of metal complexation with e.g., smaller organic ligands or larger inorganic colloids. Roughly three types of colloids or molecules can be distinguished: organic molecules, inorganic colloids, and inorganic colloids associated with organic matter. The definition of dissolved, colloidal, soluble, and truly dissolved is operational and varies between disciplines and no single definition exists so far (Lead and Wilkinson, 2006;

Jensen et al., 2020). For the work presented here, we define particulate as $> 0.2 \mu\text{m}$, large colloidal as between $> 0.02 \mu\text{m}$ and $< 0.2 \mu\text{m}$, and soluble as $< 0.02 \mu\text{m}$ (Figure 1). Our soluble fraction encompasses small colloids and molecules as well as the truly dissolved fraction $< 1 \text{ kDa}$. Colloids are used as a description of a size fraction, as is frequently done in environmental systems (Lead and Wilkinson, 2006). As visualized in Figure 1, many inorganic colloids such as clays and Mn and Fe (oxyhydr)oxides can be largely removed from the soluble phase with a $0.02 \mu\text{m}$ pore size filter. Many organic complexes that interact with metals are, however, smaller than $0.02 \mu\text{m}$ and are found in the soluble pool. Dissolved organic matter (DOM) and consequently metal-DOM complexes can vary considerably in size depending on source material and biogeochemical processes (Aiken et al., 2011). Nanoparticles are particles with a diameter between 5 nm and 100 nm (Raiswell and Canfield, 2012) and therefore fall in the small and large colloidal categories used here, also showing that there is a continuum of colloids and that strict categories are difficult to determine. Nanoparticles are more reactive than larger particles of the same material because of their relatively larger surface area per volume or weight, and the many crystal lattice imperfections (Aiken et al., 2011).

The size fractionation serves as a basis to unravel the complexity of the dissolved pool and the differentiation between the physical species is an important prerequisite for diffusion calculations, flux models, understanding and predicting chemical reactivity, and bioavailability assessments. Depending on the physical and chemical speciation, metals behave differently during biogeochemical cycling and speciation can also influence toxicity. While physical speciation does not directly relate to bioavailability, it can serve as an indication which chemical forms are likely present within the physical size pool (Figure 1). Additionally, exposure

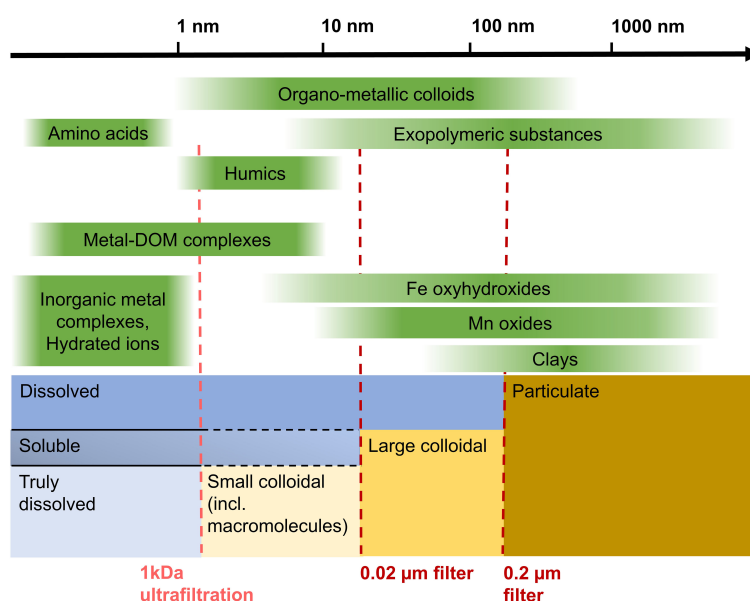


FIGURE 1

Overview of filtration steps, size classes as defined in this study, and examples of organic and inorganic compounds within this size schematic. Size information based on (Lead and Wilkinson, 2006). We use the size class “small colloidal” for all colloids and molecules between 1 kDa and $0.02 \mu\text{m}$.

routes differ for soluble, colloidal, and particulate metals and will affect fauna in different ways (Simpson and Spadaro, 2016; Hauton et al., 2017). It is therefore a useful basis for ecotoxicological studies e.g., in the context of deep-sea mining, to know in which forms the metals are present in the natural system.

A further driver is to understand trace metal diffusion across the sediment-water-interface (SWI) that can impact bottom water trace metal cycling (Huerta-Diaz et al., 2007; Homoky et al., 2011). Depending on their physical and chemical speciation (e.g., free ion, organic complex, colloid), diffusion and reaction properties for metals will differ (Homoky et al., 2016; Baeyens et al., 2018). Diffusion coefficients that have been empirically determined for free ions might not be appropriate for colloiddally bound metals and introduce uncertainty to benthic flux calculations (Homoky et al., 2016; Somes et al., 2021). Scally et al., 2006 discovered that the diffusion coefficients in diffusive gradients in thin film decreased as the ligand size increased and that diffusion coefficients for metal-ligand complexes were 20–30% lower than for simple organic ligands such as diglycolic acid or nitrilotriacetic acid. The much greater retardation of metals complexed by e.g., humic and fulvic substances compared to uncomplexed or inorganically complexed metals has been observed (Zhang and Davison, 1999; Scally et al., 2006; Balch and Guéguen, 2015) and diffusion coefficients are only 10–20% of the values of uncomplexed or inorganically complexed metals. Especially in oxic settings, colloidal species of e.g., Mn and Fe as well as associated metals will play an important role as a source of these elements from the pore water to the water column (Homoky et al., 2021) but are often still neglected in model simulations (Somes et al., 2021). Providing data on the fraction of colloids for a range of metals in abyssal, oxic pore waters will contribute significantly to the improvement of our understanding of benthic fluxes in these areas, especially because the abyssal deep ocean represents the largest area of pore water-seawater exchange (Homoky et al., 2016; Homoky et al., 2021).

Physical speciation of many trace metals is related to the redox conditions in the sediments: Mn and Fe can form (oxyhydr)oxide colloids in oxic pore waters, which are in turn sorbents for many other trace metals. Cationic metals such as Co and Ni are predominantly present as free ions or $\text{CoCl}^+/\text{NiCl}^+$ complexes in seawater when they are not organically complexed (Ćosović et al., 1982; Ellwood and Van den Berg, 2001; Koschinsky and Hein, 2003), and are often associated with Mn oxides (negative surface charge) (Shaw et al., 1990; Koschinsky and Halbach, 1995; Paul et al., 2018). Manganese and Fe colloids are not stable under reducing conditions for these elements (Froelich et al., 1979). The oxyanion-forming metals Mo, V, U and the metalloid As are mostly present as negatively charged complexes and stable in their oxidized, soluble forms: Mo^{6+} (as MoO_4^{2-}), V^{5+} (as H_2VO_4^- or HVO_4^{2-}), U^{6+} (as $\text{UO}_2(\text{CO}_3)_3^{4-}$) and As^{5+} (HAsO_4^{2-}) (Andreae, 1979; Neff, 2002; Beck et al., 2008). Under reducing conditions, As can be desorbed from Mn and Fe oxides or released due to reduction of these host phases (Telfeyan et al., 2017). Arsenic readily adsorbs to positively charged Fe oxyhydroxides (Koschinsky and Halbach, 1995) when present as oxyanions, while adsorption onto negatively charged Mn oxides has mostly

been observed at edge sites of Mn minerals (Schacht and Ginder-Vogel, 2018).

Trace metal size fractionation is increasingly assessed in the oceanic water column (Nishioka et al., 2001; Oldham et al., 2017; Jensen et al., 2020). Less frequently studied are the pore waters, which are traditionally more time-consuming to sample, especially under ultra-clean conditions, and sample volume is limited. The small volumes are a limitation for sequential filtration techniques to assess the different size pools of the dissolved fraction (colloidal, soluble, truly dissolved) because for ultrafiltration (1–10 kDa) usually 100 milliliters to liters are necessary. The typical focus in pore water has been on Fe because there is a large interest in its role as a bio-essential and climate influencing element (Homoky et al., 2011; Homoky et al., 2016; Homoky et al., 2021), but other bio-essential (Mn, Cu, Co, Ni, Cd) or potentially toxic (Cu, Mn, As, Cd) trace metals show a variety of behaviors. In our study, we present a comprehensive overview of the soluble and colloidal distributions of Mn, Co, Ni, Fe, Cu, V, U, Mo, As, and Cd in oxic to suboxic deep-sea pore waters.

2 Methods

2.1 Sampling

Samples were taken during RV SONNE cruise SO268 in the period between February and May 2019 as part of the JPI Oceans MiningImpact project (Haackel and Linke, 2021). The cruise covered the German and Belgian license areas for manganese nodule prospection in the Clarion Clipperton Zone (CCZ), central equatorial Pacific (Figure 2; Table 1).

Surface sediments (ca. 20–30 cm) were collected with a multicorer (MUC) or ROV push core (ROV-PUC) and sediments down to ca. 5 m depth with a gravity corer (GC). Surface sediments up to ca. 75 cm were lost at the top of the GCs. The loss was variable among cores and could not be reconstructed. Therefore, all data shown here for the GCs are sampling depth within the core. Sediment cores were immediately transferred into the cold room of RV SONNE (4–7°C). The MUC bottom waters overlying the collected sediments in the liners were sampled with 0.1 M suprapure HCl and deionized water (DI; 18.2 MΩ•cm) pre-cleaned syringes and directly filtered through 0.2 μm polyethersulfone (PES; Chromafil, Macherey-Nagel) syringe filters that were pre-cleaned with DI during SO268 Leg 1 and with 0.1 M suprapure HCl and DI during SO268 Leg 2 (Table 1). Syringes were rinsed with samples first and the first drops of sample going through the filter were discarded. To sample the pore water, sediment was transferred into acid (technical grade 2% HNO_3 and 0.2% HF) and DI cleaned 50 mL centrifuge tubes with acid (10% HNO_3) and DI cleaned plastic spatulas. MUCs and the ROV PUC were sampled in 2–4 cm layers. For higher resolution dissolved and soluble pool comparisons, such as 136MUC and 171MUC, samples from the same depth intervals of two MUC liners were pooled to acquire sufficient pore fluids. Similarly looking cores were chosen. GCs were sampled upon eye inspection in different layers, approx. 3 samples

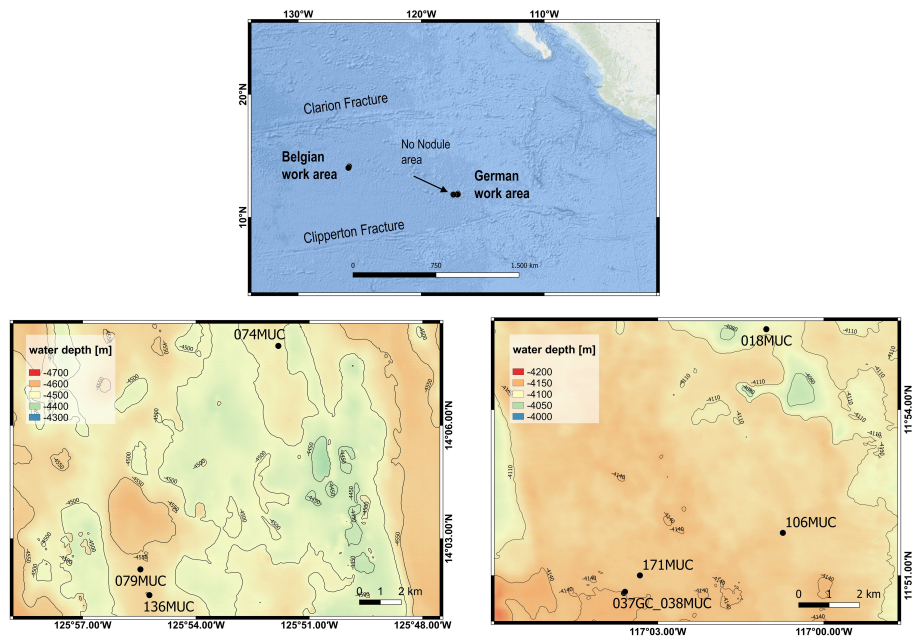


FIGURE 2
Map of sampling locations in the Belgian (left) and German (right) license areas in the CCZ. The ‘No Nodule’ area is not shown in a close-up map. Sources for ESRI Ocean base map: Esri, GEBCO, NOAA, National Geographic, DeLorme, HERE, Geonames.org, and other contributors. Close-up map data is from the multibeam echosounder EM 122 from RV SONNE (Gazis, 2020a; Gazis, 2020b). Maps were created with QGIS.

per 1 m core section. All samples were centrifuged at 4°C, 2465 x g (3500 rpm), for 40 minutes to separate the pore water from the solid phase. The supernatant pore water was subsequently filtered through 0.2 µm PES syringe filters that were pre-cleaned with DI during SO268 Leg 1 and with 0.1 M suprapure HCl and DI during SO268 Leg 2 (Table 1). The syringes were always pre-cleaned with 0.1 M suprapure HCl and DI. Syringes were rinsed with sample first, which was discarded, and the first drops of sample filtered through the syringe filter were also discarded. For 0.02 µm filtration, sample was taken from the 0.2 µm filtrate with a clean syringe after pre-

rinsing with 0.2 µm sample, and filtered through Anotop syringe filters (alumina based Anopore membrane; Whatman) directly after the 0.2 µm filtration was completed. This is well within the 24 h time range recommended by Jensen et al. (2020) to get reliable soluble and dissolved metal concentrations. Anotop filters were cleaned with DI during SO268 Leg 1 and 0.015 M suprapure HCl and DI during SO268 Leg 2. Filtration of oxic samples was performed in a clean bench. Based on the data of onboard ex-situ oxygen measurements (Haeckel and Linke, 2021), samples from suboxic GC sediments were filtered in a glove tent filled with

TABLE 1 Overview of cores sampled for trace metal analyses and MiningImpact project related work areas.

Sample ID	Work area	Latitude [N]	Longitude [W]	Water depth [m]
SO268 Leg 1				
018MUC	German license area – Trial site	11° 55.454'	117° 01.039'	4092
037GC	German license area – Reference site	11° 50.708'	117° 03.593'	4132
038MUC	German license area – Reference site	11° 50.686'	117° 03.612'	4130
074MUC	Belgian license area – Trial site	14° 08.105'	125° 51.819'	4509
079MUC	Belgian license area – Reference site	14° 02.187'	125° 55.471'	4535
SO268 Leg 2				
106MUC	German license area – Dredge site (pre-impact)	11° 51.773'	117° 00.740'	4122
136MUC	Belgian license area – Reference site	14° 01.505'	125° 55.234'	4542
157GC	German license area – No Nodule site	11° 50.976'	117° 22.956'	4275
158ROV-PUC 8	German license area – No Nodule site	11° 50.971'	117° 22.930'	4278
171MUC	German license area – Reference site	11° 51.003'	117° 03.323'	4122

nitrogen. Sediment subsampling of the 1 m GC liner sections was performed in ambient air as is standard procedure for GCs and has been successfully carried out in previous studies (Volz et al., 2018; Paul et al., 2019). Diffusion of oxygen is only a few mm in the 2-3 hours of sampling (Paul et al., 2019) and the few mm of air-exposed surface sediment were removed prior to sampling the sediment into the centrifuge tubes.

Trace metal samples were acidified with 1.5 µL ultrapure HCl per 1 mL sample to ~pH 1.8. Trace metal samples were stored in LDPE bottles or 5 mL PP vials cleaned with detergent, technical grade 2% HNO₃ and 0.2% HF, and 1 M suprapure HCl and DI, at ca. 4-9°C until analysis.

2.2 Analytical

2.2.1 Direct ICP-MS analysis

Cadmium, U, V, Cu, As, and Mo, as well as Mn and Co in pore waters from the GCs, were determined at Jacobs University Bremen (JUB, Germany, now Constructor University) by single quadrupole Inductively-Coupled Plasma - Mass Spectrometry (ICP-MS, Perkin Elmer NexION 350X) coupled to an apex Q desolvating nebulizer (Elemental Scientific, ESI) for increased sensitivity and decreased background. Concentrations of these elements are sufficiently high (in comparison to blanks and the sensitivity of the ICP-MS) to be analyzed directly. Additionally, V, Mn, Co, Cu, As, and Mo were measured in the kinetic energy discrimination (KED) mode using He gas for the minimization of polyatomic interferences. Pore-water and bottom water samples were diluted 80-100 times and Ru was used as internal standard to correct for machine drift. Calibration standards and instrument blanks were matrix matched to the diluted pore-water samples with NaCl (Merck, suprapure). For quality control, certified reference materials (CRMs) for seawater NASS-7 and estuarine water SLEW-3 (both National Research Council of Canada; www.nrc-cnrc.gc.ca) were measured with the samples at the same dilution as the samples. As

no pore-water CRM exists, an in-house pore water mix from the Peru Basin with realistic element concentrations for pore water was measured in every run. Accuracy for NASS-7 and/or SLEW-3 was within 5% for U, V, As, Mo, and Mn, 10% for Cu, 17% for Co and 30% for Cd. Precision was largely within 2-10% for all elements depending slightly on the CRM used, except for Co, Cu and As, which were for some CRMs 12-25%. For details on the detection limit (LOD) and CRM measurements see Table 2. Replicate analyses of 12 samples on different days showed a general agreement within ca. 8%, except for Cu and As (ca. 15%).

2.2.2 SeaFAST offline preconcentration

Selected cores from Leg 2 (Table 1) which were sequentially filtered throughout the core (106MUC, 136MUC, 158ROV PUC8, and 171MUC) were additionally analyzed for Mn, Fe, Co, and Ni using the much more time intensive and costly seaFAST preconcentration. The samples were measured at GEOMAR (Kiel, Germany) with a high-resolution sector field ICP-MS (ElementXR, Thermo Scientific) after offline preconcentration using a SeaFAST (Elemental Scientific, ESI). The method was based on Rapp et al., 2017). In brief, samples were diluted with subboiling HNO₃-DI of pH ~1.6 and loaded onto a chelating resin column (Nobias, ESI CF-N-0200 SeaFAST 200 µL) after buffering to pH 8.5 with acetic acid (HAc, ROMIL UpA) and ammonia (NH₃, ROMIL UpA, 3 M) in the SeaFAST. Samples were subsequently eluted with 1 M HNO₃. Prior to column loading, samples were UV digested for 4 h to destroy organic ligands that may prevent especially Co from quantitative loading onto the chelating resin. All elements were quantified by external calibration. Calibration standards were prepared in seawater from SO268 and preconcentrated with the SeaFAST prior to ICP-MS measurement. For quality control, CRMs for seawater CASS-6 and estuarine water SLEW-3 (both National Research Council of Canada; www.nrc-cnrc.gc.ca) were measured with the samples (Table 3). Accuracy was within 3-6% for Mn, Fe, Co, and Ni for CASS-6 and between 3-13% for SLEW-3. Precision was 3-7% for all elements.

TABLE 2 Overview of LOD and CRM measurements at Jacobs University Bremen.

LOD [µg/kg]	LOD [nmol/kg]		NASS-7 [µg/kg]			SLEW-3 [µg/kg]			In-house [µg/kg]	
			¹ certified ² reference	average measured	n	¹ certified ² information	average measured	n	average measured	n
0.002-0.03	0.018-0.27	Cd	0.0157 ± 0.0016 ¹	0.025 ± 0.007	5	0.047 ± 0.004 ¹	0.061 ± 0.014	9	0.190 ± 0.004	9
0.001-0.04	0.004-0.02	U	2.81 ± 0.16 ¹	2.90 ± 0.22	9	1.8 ²	1.75 ± 0.30	9	2.93 ± 0.08	9
0.007-0.31	0.14-6.09	V	1.27 ± 0.08 ²	1.30 ± 0.09	9	2.54 ± 0.31 ¹	2.63 ± 0.12	9	7.52 ± 0.31	9
0.10-1.14	1.82-20.8	Mn	0.74 ± 0.06 ¹	<LOD		1.59 ± 0.22 ¹	1.58 ± 0.02	2	2.79 ± 0.17	2
0.003-0.017	0.051-0.29	Co	0.0143 ± 0.0014 ¹	<LOD		0.040 ± 0.010 ¹	0.033 ± 0.003	2	0.043 ± 0.011	2
0.03-0.3	0.47-4.72	Cu	0.195 ± 0.014 ¹	<LOD		1.53 ± 0.12 ¹	1.68 ± 0.39	12	4.10 ± 0.35	12
0.01-1.7	0.13-22.7	As	1.23 ± 0.06 ²	1.20 ± 0.16	9	1.34 ± 0.09 ¹	1.38 ± 0.16	8	1.97 ± 0.50	9
0.08-0.32	0.83-3.34	Mo	9.10 ± 0.40 ¹	9.53 ± 0.31	9	5.1 ²	4.90 ± 0.33	9	14.1 ± 0.53	9

Each CRM was measured several times per run and averages from each run were used to calculate the CRM average shown in the table. The in-house standard was measured once per run. n= number of ICP-MS runs.

TABLE 3 Overview of LOD and CRM measurements at GEOMAR.

LOD [nmol/L]	LOD [μ g/L]		CASS-6 [μ g/L]			SLEW-3 [μ g/L]		
			certified	average measured	n	certified	average measured	n
0.013-0.198	0.0007-0.0109	Mn	2.22 $\pm$ 0.12	2.10 $\pm$ 0.47	2	1.61 $\pm$ 0.22	1.66	1
0.082-1.28	0.0046-0.072	Fe	1.56 $\pm$ 0.12	1.48 $\pm$ 0.07	3	0.568 $\pm$ 0.059	0.626	1
0.002-0.009	0.0001-0.0005	Co	0.0672 $\pm$ 0.0052	0.0653 $\pm$ 0.0019	3	0.042 $\pm$ 0.010	0.0474	1
0.081-0.494	0.0048-0.029	Ni	0.418 $\pm$ 0.04	0.394 $\pm$ 0.03	3	1.23 $\pm$ 0.07	1.32	1

Each CRM was measured three times at the beginning of a sequence. The average per sequence was used for the overall calculation shown here. n=number of sequences.

2.2.3 Blanks and reporting

Field blanks (ship DI filtered the same way as the samples) were all considerably below the LOD for all elements except Mn, Fe, and Co and for the Anotop filters for Ni. Blanks were not subtracted from the samples because they were very variable. Anotop 0.02 μ m blanks were generally 10-20 times higher than 0.2 μ m PES blanks. No substantial difference for the filter blanks was noticed for the elements presented here for both cruise legs (acid vs. only DI cleaning), except for Co, for which the blanks of acid cleaned PES syringe filters were about 10 times lower.

The results for 0.02 μ m filtration samples that were considerably higher than for 0.2 μ m filtrations were excluded because the most likely explanation for this was contamination during the sequential filtration. This was mostly the case for Mn and Fe data: Of a total of 23 0.02 μ m filtered samples analyzed for Mn, Fe, Co, and Ni at GEOMAR, 3 samples Mn and 1 sample Fe, Co, and Ni from 106MUC, 5 samples Fe, 4 samples Mn, and 3 samples Co and Ni from 136MUC, and 1 sample Mn and Fe from 171MUC were affected. The remaining 15 Mn, 17 Fe, and 19 Co and Ni samples were determined to be reliable because of the consistency of the data within cores, between cores, and in comparison to the 0.2 μ m filtrates. The 51 0.02 μ m samples analyzed at JUB for Cd, Cu, V, As, U, and Mo in the MUCs and the 8 samples analyzed for Mn, Co, and Cu in the GCs did not show any contamination issues. Solely two of these samples had calibration or interference problems for Mo, Cd, and U and were therefore ignored. Additionally, data for samples that could not be reproduced within the same institute and were extraordinarily high during the second measurement were also discarded due to possible contamination during sample handling (n=2).

Colloidal concentrations ([cMetal]) were calculated from the concentration difference [dMetal]-[sMetal], with [dMetal] being the dissolved metal concentration and [sMetal] being the soluble metal concentration.

3 Results

Dissolved Mn, dCo, dNi, dCu, and dV displayed a peak in the upper 6 cm, with highest concentrations usually at 2 cm depth, just below the SWI (Figures 3–5).

MUC bottom water dMn was 1.7 nmol/kg in the Belgian area and variable within the German area between 2–6 nmol/L and up to

25 nmol/L at the “No Nodule” site (Figure 3). In comparison, pore waters were enriched with 15–21 nmol/L in the Belgian and German areas and up to 177 nmol/L at 2–4 cm depths at the “No Nodule” site. With depth, dMn decreased to ca. 2–3 nmol/L in the Belgian area and to < 1 nmol/L in the German area, but remained highly variable at the “No Nodule” site (Figure 3). Dissolved Co and Ni showed similar trends, with bottom water dCo of 0.11–0.22 nmol/L and dNi of 9–11 nmol/L at the Belgian and German areas, except for higher concentrations at the “No Nodule” site with 0.48 nmol/L dCo and 21 nmol/L dNi. Pore-water concentrations were elevated up to 1.4 nmol/L dCo and 45 nmol/L dNi, with exceptionally high concentrations of 10.5 nmol/L dCo and 726 nmol/L dNi at 2–4 cm depth at the “No Nodule” site. Down to ca. 20 cm depth, concentrations of dCo and dNi remained higher in the Belgian area (~ 0.5 nmol/L dCo, 20–30 nmol/L dNi) compared to the German area (~ 0.1–0.2 nmol/L dCo, 4–10 nmol/L dNi), except for the “No Nodule” site (Figure 3). In the suboxic zone down to 5 m depths, Mn and Co concentrations increased substantially, Mn to the μ mol/kg range and Co to up to 73 nmol/kg (Figure 3). Concentrations in the suboxic zone are considerably higher at the “No Nodule” site (157GC) compared to the other core from the German area (37GC). The “No Nodule” site therefore clearly deviates from the general trends in the German and Belgian work areas.

Manganese, Co, and Ni were predominantly present in the soluble phase (< 0.02 μ m) in the bottom water (sMn 96–108%, sCo 82–87%, sNi 94–101%) and upper 10 cm of the oxic pore waters (sCo 88–105%, sNi 84–107%), with Mn showing the largest variability in the pore waters (sMn 57–97%, 158ROV PUC8 2–4 cm as an outlier with only 4% sMn) (Figure 3). Below 10–15 cm, the concentrations of sMn decreased to 54%, sCo and sNi to ca. 70%. The large peaks below 5 cm were largely colloidal while the soluble profiles were smooth (e.g., 136MUC at 8 cm, Figure 3). In the suboxic zone, however, dissolved and soluble concentrations of Mn and Co were indistinguishable (GCs Figure 3). Nickel was not determined for the GCs.

Bottom water dCu concentrations were on average 12.3 $\pm$ 9.3 nmol/kg and 8.5 $\pm$ 0.3 nmol/kg in the German and Belgian area, respectively (Figure 4). Concentrations for dCu maxima in the subsurface pore water were between 103 and 232 nmol/kg at the German area and slightly lower between 91 and 172 nmol/kg at the Belgian area. Copper concentrations decreased with depth and dCu was generally below the detection limit (ca. 3 nmol/kg) for the GCs

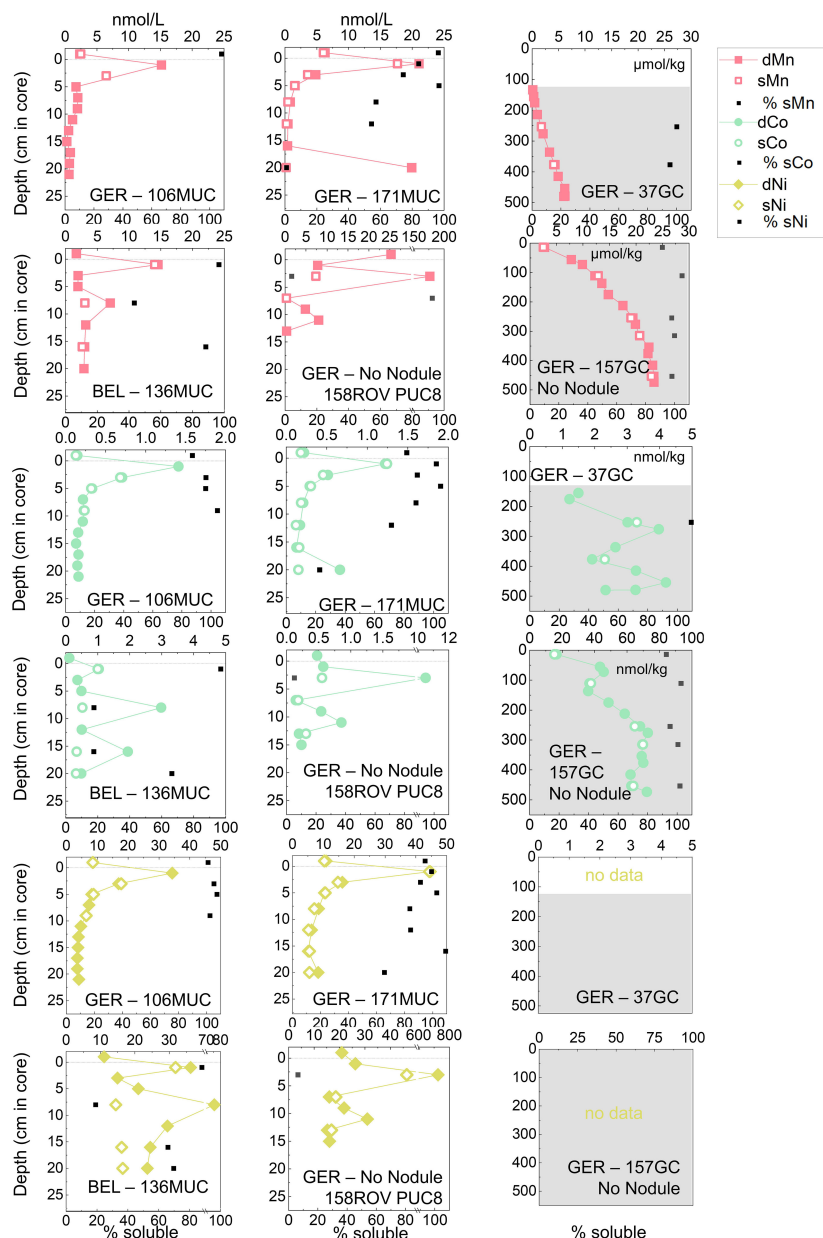


FIGURE 3

Dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) Mn, Co, and Ni concentrations in pore waters of the German (GER) and Belgian (BEL) license areas. The difference between dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) is shown as the percentage of sMetal of dMetal. The uppermost value of the MUC/PUC is the bottom water. The dotted line indicates the SWI. Note different y-axis for GCs and MUCs/PUC. The grey areas in the GC plots indicate suboxic conditions, correlating with the increase of dMn concentrations. For 37GC, Mn and Co in the oxic pore water were below the detection limit 20.8 and 0.29 nmol/kg, respectively.

in the Belgian area and below 20–50 nmol/kg in the German area GCs. While ca. 80–100% of dCu was sCu in the top 5–10 cm at all sites, at around 10 cm depth sCu made up only ca. 60% of the dCu pool. The colloidal pool between 0.02 and $0.2 \mu\text{m}$ gained importance and was the dominant dCu pool with over 50% cCu below ca. 15 cm in the Belgian area and ca. 1 m in the German area (Figure 4). The bottom water cCu pool was in the same range as the cCu pool in the upper ca. 10 cm with sCu 76–104% of dCu except for one outlier of 131% for 79MUC, which needs to be treated with

caution and was therefore also not shown in the % soluble plot in Figure 4.

Vanadium concentrations in the bottom water were 43.1 ± 5.6 nmol/kg in the German area and 38.7 ± 0.7 nmol/kg in the Belgian area. Subsurface maxima of dV in the upper 2 cm were considerably enriched with 185–334 nmol/kg and 195–239 nmol/kg in the German and Belgian areas, respectively. Dissolved V concentrations then decreased to 70–90 nmol/kg with depth in the German area and to 55–75 nmol/kg in the Belgian area (Figure 5).

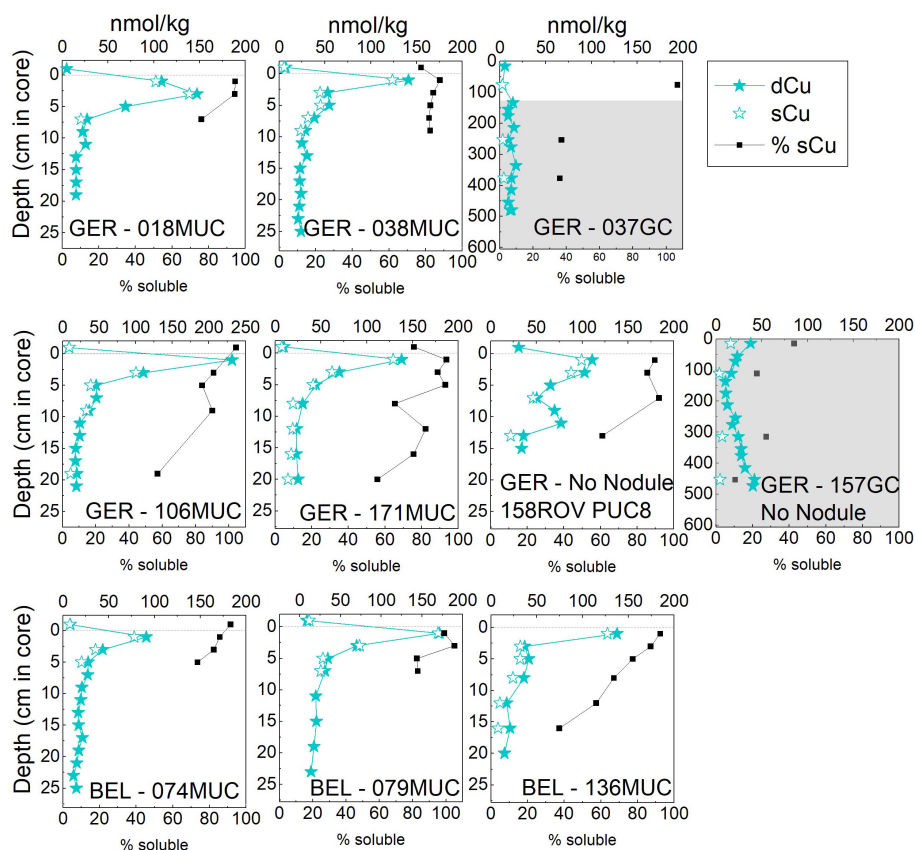


FIGURE 4

Dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) Cu concentrations in pore waters of the German and Belgian license areas. The difference between dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) is shown as the percentage of sCu of dCu. The uppermost value of the MUC/PUC is the bottom water. The dotted line indicates the SWI. Note different y-axis for GCs and MUCs/PUC. The grey areas in the GC plots indicate suboxic conditions. dCu data for 074MUC and 079MUC from (Paul and Koschinsky, 2021a; Paul and Koschinsky, 2021b).

The subsurface peak was less clearly visible for dFe, but all cores showed a peak in the upper 2–4 cm (Figure 6). Dissolved Fe concentrations generally varied between 4–40 nmol/L. The two maxima of ca. 350 nmol/L (171MUC) and 3.5 $\mu\text{mol/L}$ (158ROV PUC8) were only present in the dissolved fraction but not in the soluble fraction, therefore they must be due to Fe colloids present at specific layers (Figure 6).

The subsurface peak was less pronounced for As, but all cores except the one from the ‘No Nodule’ site also show a peak-trend in the upper 2–4 cm (Figure 7). Dissolved and soluble Cd pore-water concentrations were considerably enriched (ca. 1.3–2 nmol/kg) throughout the upper ca. 25 cm compared to bottom seawater [average 1.01 ± 0.04 nmol/kg and 0.88 ± 0.04 nmol/kg in the German and Belgian areas, respectively (Figure 8)]. The pore-water Cd concentrations in the German area were on average higher (1.66 ± 0.17 nmol/kg) compared to the Belgian area (1.33 ± 0.12 nmol/kg).

At all sites, Mo and U displayed straight pore-water profiles in the surface pore waters (upper ca. 25 cm) (Figures 5 and 7); Mo in the same concentration range (ca. 90–120 nmol/kg) as MUC bottom water (ca. 112–114 nmol/kg) in both work areas and U at slightly lower concentrations (ca. 12 nmol/kg) compared to seawater (ca. 13 nmol/kg). However, Mo concentrations decreased steadily with

depth by 13–35%, most strongly at the ‘No Nodule’ site and the Belgian area.

The dissolved and soluble concentrations for V, As, Cd, Mo, and U were the same and within analytical precision and no major differences in concentration and profile pattern could be discerned in the oxic surface pore waters (Figures 5, 7, 8).

Overall, most metals presented here (Mn, Co, Ni, Fe, Cu, V, Cd) were enriched in oxic pore waters compared to bottom seawater. The enrichment was slightly more variable in the German area but the upper 2 cm peak concentrations were also usually higher in the German compared to the Belgian area. The “No Nodule” site in the German area displayed most variable concentrations for Mn, Co, Ni, and Cu. The colloidal pool (0.02–0.2 μm) increased with depth for Mn, Co, Ni, and Cu.

4 Discussion

4.1 Subsurface Mn, Co, and Ni peaks

The pore waters in surface sediments (< 25 cm) were oxic and aerobic respiration largely controlled organic matter degradation in the sediments shallower than 1 m (Volz et al., 2018). Suboxic

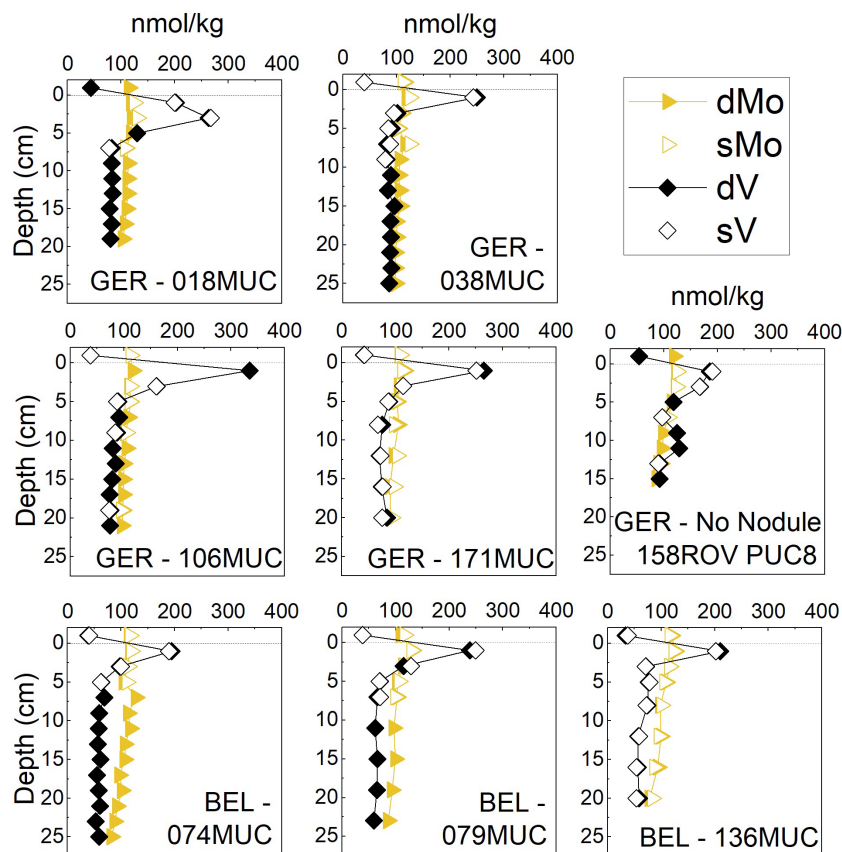


FIGURE 5

Dissolved ($<0.2\mu\text{m}$) and soluble ($<0.02\mu\text{m}$) concentrations of oxyanion forming metals V and Mo in the German (GER) and Belgian (BEL) license areas surface pore waters (ca. 25 cm). The uppermost value of the MUC/PUC is the bottom water. The dotted line indicates the SWI.

conditions (absence of oxygen, co-occurrence of nitrate and elevated (μM range) pore-water Mn, absence of sulfide) occurred at depths > 1 m and were variable between the study sites (Volz et al., 2018). The area was characterized by low but variable inputs of organic matter and the total organic carbon (TOC) content in surface sediments was 0.2–0.6 wt.% (Volz et al., 2018). Reductive dissolution of Mn oxides only occurred at depths of ca. 1 to 10 m (Volz et al., 2018) which is also indicated by increasing Mn concentrations to the $\mu\text{mol/kg}$ range for two examples from the German area (GCs Figure 3). Manganese and Fe (oxyhydr)oxide colloids must therefore be stable in the oxic CCZ surface pore waters.

Manganese, Co, and Ni showed similar depth profiles (Figure 3) because Co and Ni are often adsorbed or incorporated onto or into Mn oxide minerals and then these elements are cycled together between the solid and dissolved as well as between the colloidal and soluble phase (Klinkhammer, 1980; Sawlan and Murray, 1983; Shaw et al., 1990; Koschinsky and Halbach, 1995). All three elements displayed a peak in the upper 6 cm in the dissolved and soluble fractions, with highest concentrations at ca. 2 cm, just below the SWI (Figure 3). This is due to the release of these metals during organic matter degradation, which has been shown for Cu and V in oxic pore waters (Klinkhammer, 1980; Heggie et al., 1986; Shaw et al., 1990; Paul et al., 2018), but rarely for Mn, Co, and Ni, and the

exact mechanisms were not discussed in these previous publications. In the case of Mn, Co, and Ni, this peak was often not discoverable, potentially due to a lack of analytical sensitivity or because studies were mostly conducted in areas with a more condensed redox zonation (e.g., shelves) where the Mn, Co, and Ni peaks at the oxic-suboxic boundary dominated the profiles (Klinkhammer, 1980; Sawlan and Murray, 1983; Shaw et al., 1990). Heggie et al. (1986) observed Mn and Ni peaks in oxic pore waters at the SWI. They attributed the Mn peak to the reduction of solid-phase Mn oxides through dissolved organic matter (Heggie et al., 1986). In the solid phase of the cores presented in this study, Mn was also enriched in the upper ca. 5 cm (Supplementary Figure S1), sometimes considerably in the upper ca. 3 cm, suggesting the presence of Mn micronodules. The solid-phase Mn oxide enrichment can therefore be a source for the reductive Mn (and associated metal) release to the pore waters. Alternatively, in anoxic microenvironments, different forms of Mn oxides that are more easily reduced, or different types of organic matter have been suggested as possible reasons for the metal release at the SWI (Kalhorn and Emerson, 1984; Burdige, 1993). For some organic matter break-down processes, MnO_2 might be the preferred electron acceptor even though O_2 is present (Kalhorn and Emerson, 1984). Additionally, nitrite maxima of up to $0.6\mu\text{M}$ in the uppermost cm were observed at the same sites (Volz et al., in

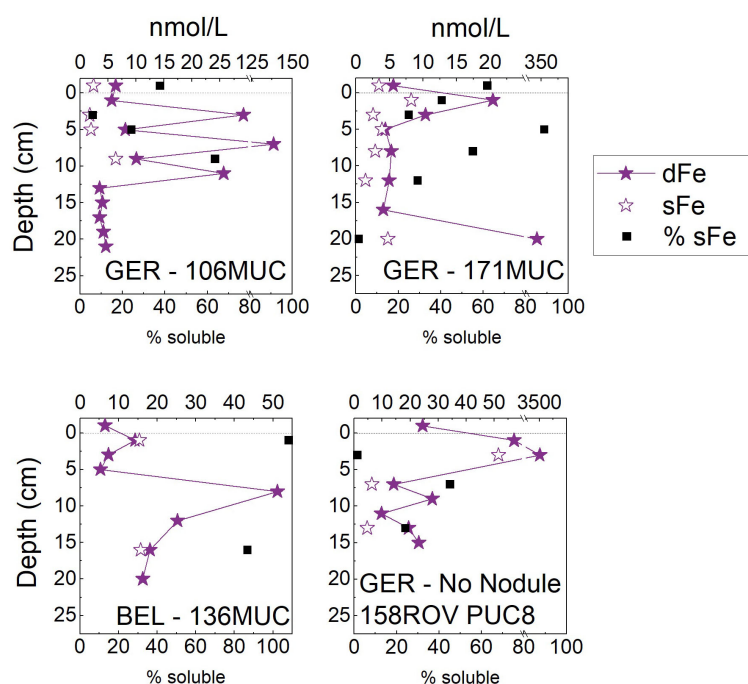


FIGURE 6

Dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) Fe concentrations in pore waters of the German (GER) and Belgian (BEL) license areas. The difference between dissolved ($< 0.2 \mu\text{m}$) and soluble ($< 0.02 \mu\text{m}$) is shown as the percentage of sFe of dFe. The uppermost value of the MUC/PUC is the bottom water. The dotted line indicates the SWI. Iron was not determined for the GCs.

prep; Haeckel and Linke, 2021), suggesting that nitrification or denitrification are occurring, releasing nitrite into the pore water at the same depth as the metals are released. The co-occurrence of nitrite and dMn peaks have previously been reported for the oxygen minimum zones of the Pacific and Arabian Sea, linking denitrification and Mn reduction based on the same noticeable profiles we observed in the CCZ pore waters (Klinkhammer and Bender, 1980; Saager et al., 1989). In the pore waters, nitrite is, however, usually produced by oxidation of ammonia and not by denitrification (Volz et al., 2018). The coinciding nitrite and Mn peaks could therefore also suggest that Mn oxides are utilized for ammonia oxidation (“Mn-anammox”) in these Mn oxide-rich sediments (Mogollón et al., 2016) (Supplementary Figure S1), subsequently releasing nitrite and sMn into the pore waters. The oxidation of ammonia by Mn oxides in the presence of oxygen has been previously described for surface sediments at the Nova Scotia margin (Luther et al., 1997).

Manganese mobilization has also been observed as reprecipitation of Mn oxide on burrow walls from mobilization of oxygen-depleted microenvironments rich in organic matter (Jung and Lee, 1999) and the enrichment of Mn in the shallow subsurface proves this (recent) Mn mobilization, likely in local microenvironments.

In summary, the release of predominantly soluble Mn, Co, and Ni just below the SWI is related to specific conditions and types of Mn oxides or organic matter that allow for reductive dissolution of Mn oxides in this shallow layer of high reactivity.

Once the labile organic matter has been consumed or Mn oxides are not the preferred electron acceptor anymore, the “usual” electron acceptor sequence consisting of O_2 , NO_3^- , and MnO_2

continues downcore (Froelich et al., 1979). The data from Heggie et al. (1986), redrawn in Burdige (1993) at a higher resolution, clearly show the difference between Mn reduction at the SWI in the oxic sediments and in the “main” Mn reduction zone. As the “normal” Mn reduction zone is reached below 30 cm or at several m in the CCZ (Volz et al., 2018), the small Mn reduction zone at the SWI may play an especially important role in trace metal cycling between the pore water and water column, as diffusion from high concentration suboxic pore waters is not occurring – in contrast to sediments underlying more productive surface waters with a narrow redox zonation. However, the peak at 2 cm suggests a benthic flux to the overlying bottom water, likely fueling polymetallic nodule formation as the oxic diagenetic flux. This flux and its contribution to polymetallic nodule formation has been previously suggested for Mn (Kalhorn and Emerson, 1984; Heggie et al., 1986) but we could here also show it for Co and Ni, two of the major metals of interest in polymetallic nodules (Hein et al., 2013) – to our knowledge for the first time.

4.2 Downcore increase of the colloidal fractions of Mn, Co, and Ni in oxic pore waters

Manganese, Co, and Ni were predominantly present in the soluble phase (sMn 57–97%, sCo 88–105%, sNi 84–107%) in the upper 10 cm of the oxic pore waters (Figure 3). Soluble Mn, Co, and Ni can either be present as the free ions or bound in the small colloidal fraction $< 0.02 \mu\text{m}$ e.g., to organic ligands. The formation of e.g., Mn

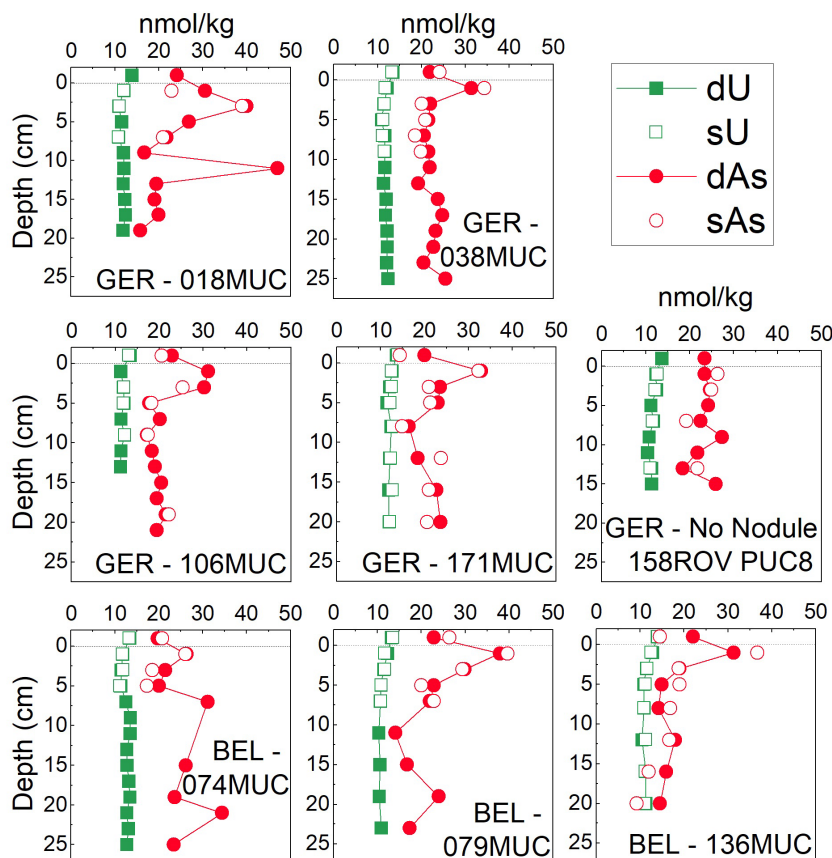


FIGURE 7

Dissolved ($<0.2\mu\text{m}$) and soluble ($<0.02\mu\text{m}$) concentrations of oxyanion forming metal(loid)s U and As in the German (GER) and Belgian (BEL) license areas surface pore waters (ca. 25 cm). The uppermost value of the MUC/PUC is the bottom water. The dotted line indicates the SWI.

(III)-ligands which have been previously found in the $< 0.02\mu\text{m}$ fraction could result from the interaction of Mn with organic matter (Oldham et al., 2017). These Mn(III)-ligands can be produced during ligand-promoted reductive dissolution of Mn oxides (Oldham et al., 2015; Oldham et al., 2020), potentially another pathway for Mn oxide dissolution at the SWI.

Below 10–15 cm, the concentrations of sMn decreased to 54%, sCo and sNi to ca. 70%, indicating that with increasing depth, colloids are becoming more important. Moreover, there were single layers (e.g., 171MUC 20 cm, 136MUC 8 cm) with up to 99% colloidal fraction, which were special microenvironments, potentially in the form of Mn oxides or adsorbed to Fe nanoparticles (also see Figure 6) originating from buried polymetallic nodules in the surrounding sediment. Overall, the dissolved concentrations of all three metals were, however, decreasing with depth. The smallest decrease was visible for sNi and dNi concentrations were higher than dMn and dCo concentrations, indicating that dNi cannot only be controlled by Mn (oxides). A large fraction of dNi is likely controlled by organic ligands, which can be up to 50% in seawater of the tropical South East Pacific Ocean (Boiteau et al., 2016) and might also be important in controlling pore-water Ni. Besides from Mn oxides, Ni is also released during organic matter degradation and silica

dissolution at the sediment water interface (Heggie et al., 1986), which would explain the higher concentrations here.

Our results for the colloidal pool in the top 10 cm corroborate findings from a study of contaminated beach sands in which ca. 80–85% Mn and ca. 70% of Co were soluble (Huerta-Diaz et al., 2007) but it needs to be recognized that the setting was considerably different. The dominance of the soluble pool in the top 10 cm also reflects observations for seawater in which only ca. 4% of Mn is in the colloidal phase (1 kDa– $0.2\mu\text{m}$) (Wells et al., 2000), in line with our bottom water results (sMn 96–108%). Our results for colloidal Mn at depths > 10 cm were in line with results from Homoky et al. (2011) who found on average 61% of Mn in the colloidal phase in oxic deep-water sediments around the Crozet Islands (Indian Ocean sector of the Southern Ocean). The occurrence and extent of colloidal Mn is therefore likely related to the prevalent lithology. The increase of the colloidal fraction with depth might be related to a decrease of organic ligand concentrations that can bind Mn, Co, and Ni and the substitution by larger inorganic colloids or for Ni also a shift from low to high molecular weight (HMW) organic ligands. Additionally, as suggested above, there might be a thin layer of Mn oxide reduction at the SWI, where the Mn oxide colloids are not thermodynamically stable, but below this ca. 2–6 cm layer, Mn oxide colloids are more stable again. In deeper suboxic pore waters,

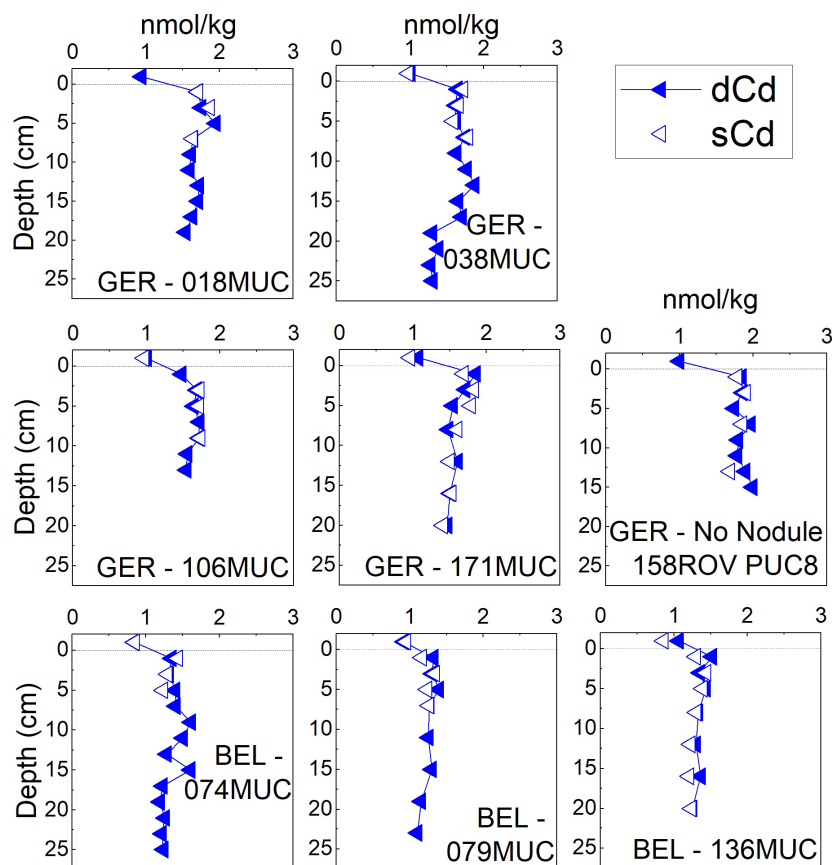


FIGURE 8

Dissolved ($<0.2\mu\text{m}$) and soluble ($<0.02\mu\text{m}$) concentrations of Cd in the German (GER) and Belgian (BEL) license areas surface pore waters (ca. 25 cm). The uppermost value of the MUC/PUC is the bottom water. The dotted line indicates the SWI.

Mn oxide colloids are not stable ($\text{sMn}=\text{dMn}$ and $\text{sCo}=\text{dCo}$; Figure 3 GCs) due to reductive dissolution of MnO_2 , as has also been shown e.g., in Homoky et al. (2011).

The suboxic Mn and Co concentrations are especially high at the “No Nodule” site, up to $23\mu\text{mol/kg}$ and 73 nmol/kg , respectively. Oxygen penetration is shallowest leading to elevated pore-water Mn (and Co) concentrations below 20 cm (Figure 3). Similar trends have been observed at another “No Nodule” site in the German area (Mewes et al., 2014), highlighting that the geochemistry of the “No Nodule” site is different from the nodule covered areas in the Belgian and German areas. The reasons could, however, not be unraveled yet and there are no clear trends from TOC or sedimentation rate data (Mewes et al., 2014) on what the reasons for the different redox zonation could be.

4.3 Abundant Fe colloids

The dFe profiles (Figure 6) showed some exceptionally high peaks for oxic pore waters (up to $3.5\mu\text{mol/L}$). These maxima were only present in the dissolved fraction but not in the soluble fraction, therefore they must be due to Fe colloids present in specific layers (see 158ROV PUC8 and 171MUC, Figure 6). The sFe profiles were smoother (Figure 6), showing the same subsurface peak as Mn, Co,

Ni, Cu, and V. Hence, Fe must also be released during organic matter degradation at the SWI through co-release with other metals or by reductive dissolution of Fe (oxyhydr)oxides in this microenvironment – similar to Mn (section 4.1). Reductive dissolution is likely because sFe was also present but non-reductive dissolution has been suggested to release dFe to oxic pore waters (Homoky et al., 2021). On average, only 40% of dFe was sFe (excluding the peaks where we suspect nanoparticles where the colloidal fraction is even larger), showing that the dominant fraction of dFe in these oxic pore waters was colloidal. This is consistent with findings from Homoky et al. (2021); Homoky et al. (2011), who found a large range of 17–99% of colloidal Fe in oxic pore waters. For the water column, it has been shown that dFe can be mainly colloiddally bound, e.g., up to 50% in the North Pacific predominantly below 200 m depth, but this is seasonally and spatially variable throughout the oceans (Nishioka et al., 2001).

Similar to MnO_2 colloids, Fe (oxyhydr)oxides and nanoparticles can form and persist in oxic pore waters. In the CCZ sediments investigated here, Fe (oxyhydr)oxides are not even reduced at several meters depth, as only Mn reduction is occurring but not Fe reduction (Heller et al., 2018). Hence, Fe (oxyhydr)oxide colloids can persist even in the suboxic pore waters. In oxic sediments with low organic C supply and a gradually declining O_2 gradient [as is the case for the CCZ (Volz et al., 2018)], and therefore slow

diagenetic reactions over years/decades/centuries, goethite is the favored Fe (oxyhydr)oxide (Homoky et al., 2011) with higher stability than the less crystalline Fe (oxyhydr)oxide ferrihydrite, which is often assumed to be the dominant Fe (oxyhydr)oxide in marine sediments (Thamdrup, 2000). As this goethite is nanoparticulate with an average size of 5 nm (van der Zee et al., 2003), it is in the colloidal range and can even occur in the operationally defined soluble phase < 20 nm. As colloids tend to aggregate (Honeyman and Santschi, 1988; Jensen et al., 2020), colloidal aggregates exceeding 20 nm, i.e. the 0.02 μm filter pore size, including colloidal goethite, can be found in our 0.02–0.2 μm fraction. In contrast to redox sensitive Fe (oxyhydr)oxides, Fe-bearing clay colloids can exist in suboxic pore waters irrespective of redox conditions. Fe-bearing smectite is a common clay colloid and especially stable in pore waters with high Si (> 500 μM) (Florindo et al., 2003; Homoky et al., 2011). The CCZ pore waters reach concentrations of 400–550 $\mu\text{mol/kg}$ dSi at depth in the GCs from the German area (37GC and 157GC), and around 300–400 $\mu\text{mol/kg}$ in the Belgian area (84GC) (data not shown). These concentrations are sufficiently high to make smectite colloids likely, especially at several meters depth in the GCs. Fe-bearing clay colloids might therefore be important inorganic sorbents for other trace metals once organic ligands are less abundant and MnO_2 is not stable anymore. Mineralogical data (not shown) for the sediments revealed that smectite was present in the CCZ surface sediments. The persisting Fe colloids can scavenge metals, some of which (e.g., Co) might have been previously adsorbed to MnO_2 colloids. These inorganic Fe colloids are also competing with organic ligands, which become less abundant with depth in the pore water (Paul et al., 2021).

Fe-binding organic ligand analyses in seawater detected 99% Fe (III) bound to organic ligands in the dissolved phase at analytical pH 6.9, which could be slightly less at pH 8 because of different behavior of especially inorganic Fe at the slightly lower analytical pH (Gledhill and van den Berg, 1994). Combined approaches using electrochemical analyses and sequential filtrations (0.4 and 0.02 μm) indicated that the organic ligands are found in the soluble and colloidal pools, but that an additional significant amount of inorganic Fe colloids must be present, which could potentially be Fe (oxyhydr)oxides (Cullen et al., 2006; Fitzsimmons et al., 2015). The differentiation between organic and inorganic ligands/sorbents in seawater has not been straight forward (Boye et al., 2010) and the most prominent and likely explanation is therefore that Fe (oxyhydr)oxides in combination with organic matter are the predominant colloid (Raiswell and Canfield, 2012). There is a continuum of organic-inorganic-nanoparticle-colloid formation and how the metals are bound in each step (Aiken et al., 2011). Processes involving organic and inorganic material include sorption of organic material onto inorganic surfaces and heterogenic precipitation of organic-inorganic clusters/nanoparticles/colloids (Aiken et al., 2011). Adsorption of organic matter onto colloids can change their surface charge and alter their reactivity (Tiller and O'Melia, 1993; Aiken et al., 2011). A complex combination of organic-inorganic ligand complexation of Fe in pore waters is

likely and a lot of questions remain about the nature of Fe colloids. More research is needed to explain the variability of the Fe colloidal pool which was also visible in our sFe and dFe data (Figure 6).

4.4 Copper complexation by organic ligands and inorganic colloids

Copper was predominantly soluble in the upper 10 cm of the pore waters and predominantly colloidal at greater depths (Figure 4). Huerta-Diaz et al. (2007) found that the subsurface peak of Cu was only present in the small colloidal fraction (3 kDa–0.45 μm) but not in the truly dissolved phase (< 3 kDa). Therefore, Cu released during organic matter degradation at the SWI appears to be stabilized in the small colloidal size fraction, but likely smaller than 0.02 μm as our results here suggest.

Dissolved and soluble Cu concentrations generally showed the same trends in the depth profiles (Figure 4), but the percentage of soluble Cu decreased with increasing depth at all sites. While ca. 80–100% of dCu was sCu in the top 5–10 cm at all sites, around 10 cm the sCu made up only ca. 60% of the dCu pool. With increasing depth, the fraction of colloidal Cu became dominant, which was already at ca. 15 cm depth in the Belgian area and below roughly 1 m in the German area. This was similar to the behavior of Mn, Co, and Ni, but the colloidal fraction was larger at depth for Cu.

In the German area the colloidal Cu fraction increased at depth, in the suboxic zone (GCs), to up to 90% (157GC, Figure 4). In the Belgian area (136MUC), sCu was already considerably decreased to 23% within the MUC in the oxic pore water. Therefore, the change in Cu complexation was likely not related to redox conditions but to a decreasing and more refractory organic matter content with depth. The shift from soluble to the colloidal pool might be due to the lack of low molecular weight (LMW) organic ligands that keep Cu in the soluble pool. As shown in Figure 1, organic ligands are predominantly present in the soluble fraction. In surface seawater, Cu is up to 99% complexed by organic ligands (Coale and Bruland, 1988). Recently, it was also shown that deep-sea surface pore water Cu is predominantly complexed by organic ligands (Paul et al., 2021), which had previously been reported for Chesapeake Bay pore waters (Skrabal et al., 1997). The analysis of the upper 20–30 cm pore waters showed, however, that the ligand excess decreases with depth similar to dCu profiles, with highest concentrations in the upper 10 cm (Paul et al., 2021). While still > 99% Cu was complexed by organic ligands, the overall ligand concentration decreased with depth. Our findings here corroborate that Cu complexation changes and that while organic ligands (small colloidal size fraction) might become less available, larger inorganic colloids or HMW organic ligands gain importance as Cu ligands. The shift from predominantly soluble to colloidal Cu does not correspond to the redox zonation, so that the physical speciation of Cu might not be related to differences in redox zonation, as it is partly the case for Mn and Fe, but to the availability of small organic ligands. In the deeper layers, Cu could be partially adsorbed to Fe colloids, which are ubiquitous as shown above

(Figure 6), but dCu concentrations exceed dFe concentrations, requiring another Cu host-phase or HMW ligand.

4.5 Oxyanion forming metal(loid)s (Mo, V, U, As) and Cd

Pore-water concentrations of As and V in surface sediments were elevated by a factor of 1.3 and 6, respectively, in the upper 2 cm compared to bottom seawater. Vanadium is therefore released during organic matter degradation at the SWI, like Mn, Ni, Co, Fe, and Cu. This is in line with results from other studies and which suggested that V is stabilized by organic material in this layer as V^{4+} (Wehrli and Stumm, 1989; Emerson and Huested, 1991). The reduced V species (often present as VO^{2+} or $VO(OH)^+$) is more particle reactive and likely to bind to reactive surfaces (Wehrli and Stumm, 1989), wherefore V could also be released from MnO_2 during reductive dissolution at the SWI as explained in section 4.1. The same is possible for As, which can be cycled with Mn as well (Telfeyan et al., 2017) and usually shows a small peak in the upper 2–4 cm (Figure 7).

The dissolved and soluble concentrations for Mo, V, U, As, and Cd were indistinguishable (Figures 5, 7, 8). They are predominantly present as negatively charged complexes and stable in their oxidized, soluble form: Mo^{6+} (as MoO_4^{2-}), U^{6+} (as $UO_2(CO_3)_3^{4-}$), V^{5+} (as $H_2VO_4^-$ or HVO_4^{2-}), As^{5+} (Andreae, 1979; Beck et al., 2008), therefore not found in the colloidal fraction. Vanadium could also be present in the reduced form stabilized by dissolved organic complexes, as described above (Wehrli and Stumm, 1989; Emerson and Huested, 1991). The consistent concentration of dV and sV with depth below ca. 4 cm – unlike for Cu, for which the sCu fraction decreases with depth – could, however, suggest inorganic speciation as more likely. Vanadium does not seem to be affected by changes in organic ligand availability. Vanadium was also found to be in the soluble phase by Huerta-Diaz et al. (2007), while Mo, U, and As were not studied there. In contrast to Huerta-Diaz et al. (2007), Cd is fully soluble in the pore waters studied here, while Cd was up to 60% colloidal in pore waters in the literature comparison conducted by the authors.

5 Conclusions

The oxic deep-sea pore-water samples from the German and Belgian license areas for polymetallic nodule mining in the CCZ showed variable size fractionation for trace metals. Colloidal Fe was present in the pore waters at variable concentrations, while V, Mo, U, As, and Cd were found predominantly in the soluble ($< 0.02 \mu m$) fraction. Most interestingly, Mn, Co, Ni, and Cu were completely or mainly soluble in ca. the upper 10 cm, while the percentage of the colloidal fraction increased below 10 cm, most strongly for Cu. These metals must therefore experience a change in complexation and we propose that this is due to the decreasing availability of soluble organic ligands with depth (especially for Cu) and an increase of inorganic complexation e.g., with Fe colloids or a shift

to HMW organic ligands. The same might explain the similar distribution of Mn, Co, and Ni. Alternatively, Mn oxides might be less stable in the upper 2–6 cm where a Mn oxide reducing microenvironment might persist. Soluble Mn, Co, and Ni are released to the pore water there and are present at elevated concentrations, fueling the benthic flux of these elements and likely contributing to polymetallic nodule formation. Below this surface layer, Mn oxides incorporating Co and partly Ni form and persist. The distribution of metals between the colloidal and soluble or even truly dissolved phase can have impacts on the diffusion behavior and therefore rates across the SWI (Huerta-Diaz et al., 2007) and needs to be taken into account for flux calculations and further studied. Considering the vastness of the abyssal plains and their large areal contribution to the ocean stresses the importance of these findings to take into account various physical and chemical forms of metals when calculating the global budget of element fluxes across the SWI in the future. To achieve this, diffusion coefficients for various inorganic and organic metal complexes must be experimentally determined first. Furthermore, more research is required in analyzing the organic ligands, nanoparticles, and colloids to fully understand their nature and involvement in particle-solution reactions, which was not possible based on the small sample volumes from this study. The different size fractionations of some metals (e.g., Mn, Cu) in seawater and pore water also showed that the behavior from oxic seawater cannot be easily applied to oxic pore waters and that it is therefore important to thoroughly study pore waters. Understanding the composition of the dissolved pore-water pool is also essential for the assessment of anthropogenic activities – such as deep-sea mining and bottom trawling fisheries – and their impacts on the seafloor environment. Speciation of trace metals will have an influence on the uptake pathways, potential bioavailability, and toxicity of these elements released from the surface pore water by the disturbance. Additionally, the removal of surface sediments during deep-sea mining will impact in which form the metals are released to the bottom seawater: in natural conditions, the here studied metals (except Fe) will be predominantly in the soluble fraction when they diffuse from the pore waters, while post-disturbance an increased colloidal fraction will be part of the benthic flux of Mn, Co, Ni, and Cu until a new equilibrium has been reached because of the increase in the percentage of the colloidal fraction downcore.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Pangaea (Paul et al., 2024).

Author contributions

SP: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. KS: Conceptualization, Investigation, Resources, Writing – review & editing. EA: Writing

– review & editing. AK: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Deep learning–assisted biodiversity assessment in deep-sea benthic megafauna communities: a case study in the context of polymetallic nodule mining

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Introduction: Technological developments have facilitated the collection of large amounts of imagery from isolated deep-sea ecosystems such as abyssal nodule fields. Application of imagery as a monitoring tool in these areas of interest for deep-sea exploitation is extremely valuable. However, in order to collect a comprehensive number of species observations, thousands of images need to be analysed, especially if a high diversity is combined with low abundances such is the case in the abyssal nodule fields. As the visual interpretation of large volumes of imagery and the manual extraction of quantitative information is time-consuming and error-prone, computational detection tools may play a key role to lessen this burden. Yet, there is still no established workflow for efficient marine image analysis using deep learning–based computer vision systems for the task of fauna detection and classification.

Methods: In this case study, a dataset of 2100 images from the deep-sea polymetallic nodule fields of the eastern Clarion-Clipperton Fracture zone from the SO268 expedition (2019) was selected to investigate the potential of machine learning–assisted marine image annotation workflows. The Machine Learning Assisted Image Annotation method (MAIA), provided by the BIIGLE system, was applied to different set-ups trained with manually annotated fauna data. The results computed with the different set-ups were compared to those obtained by trained marine biologists regarding accuracy (i.e. recall and precision) and time.

Results: Our results show that MAIA can be applied for a general object (i.e. species) detection with satisfactory accuracy (90.1% recall and 13.4% precision), when considered as one intermediate step in a comprehensive annotation workflow. We also investigated the performance for different volumes of training data, MAIA performance tuned for individual morphological groups and the impact of sediment coverage in the training data.

Discussion: We conclude that: a) steps must be taken to enable computer vision scientists to access more image data from the CCZ to improve the system's performance and b) computational species detection in combination with a posteriori filtering by marine biologists has a higher efficiency than fully manual analyses.

KEYWORDS

marine imaging, biodiversity, benthic communities, computer vision, deep learning

1 Introduction

Polymetallic nodules occur in all Oceans at abyssal depths (>4000 m), but are most abundant in the Pacific Ocean. Within the north-east Pacific, the Clarion-Clipperton fracture Zone (CCZ) hosts the most extensive nodule fields that cover 6 million km² and stretch from 5° to 20° N and 115° to 160° W, covering over 1% of the world's surface (Jones et al., 2021). Due to their high metal content and the relatively straightforward collection by hydraulic separation from surrounding soft sediment (Oebius et al., 2001), they have spiked an economic interest since the 1960–1970's (Jones et al., 2017). So far, 17 license areas for polymetallic nodule exploration have been approved by the International Seabed Authority (ISA) within the CCZ, each up to 75,000 km² in size (Wedding et al., 2013; Jones et al., 2017) and in 2021, the first polymetallic nodule collector test took place in the BGR and GSR areas (<https://www.demegroup.com/news/metal-rich-nodules-collected-seabed-during-important-technology-trial> published 22 April 2021, accessed 9/02/2022).

Nodules offer hard substrata in an otherwise soft sediment environment and host a specific nodule-associated fauna. More than 60% of megafauna depend on the nodules for attachment and survival (Amon et al., 2016; Vanreusel et al., 2016; Simon-Lledó et al., 2019a). In order to be able to quantify the impact of potential deep-sea mining on nodule associated fauna, there is an urgent need for extensive ecological baselines to understand the natural biodiversity, variation (spatial and temporal) and possible resilience of fauna to mining impacts. With regard to megafauna abundance and diversity assessments, imagery plays an important role to cover large areas. A recently presented CCZ checklist contains 436 named species from a total of 5578 recorded across the CCZ, amounting to an estimated 92% of species new to science (Rabone et al., 2023). The latter study takes into account all size classes including less conspicuous species that are not visible on imagery, but megafauna species make up 28% of the list (Rabone et al., 2023). The larger-sized megafaunal compartment shows quite some variation within license areas (e.g. Simon-Lledó et al., 2019b; Uhlenkott et al., 2023) and across regional scales, depicting a link with depth and its associated variables as well as geomorphology and nodule coverage (Vanreusel et al., 2016; Cuvelier et al., 2020;

Simon-Lledó et al., 2023a). As mining affects extremely large seafloor areas, information on spatial variation in biodiversity is essential to comprehend the ecosystem, its functioning and possibilities to recover. This information is also fundamental to assess the usefulness of refuge areas and the similarity to or representativity of the Areas of Particular Environmental Interest (APEI's), where mining will not be permitted (Lodge et al., 2014). For these areas to be effective, there needs to be an overlap in faunal composition and connectivity, which is likely to be linked to resource availability (i.e. nodule size and density) (Cuvelier et al., 2018).

Imagery is a less biased technique for quantifying biodiversity than sampling since it does not depend on accessibility, is a non-invasive tool and permits covering large surfaces (Cuvelier et al., 2012). In a post-mining scenario, it represents the ultimate monitoring tool in a possible rugged terrain. Moreover, it is cost-effective and easily replicable, on condition that imagery platform deployment techniques and sampling set-up are comparable (Schoening et al., 2020). The counterpart is that a huge amount of imagery is collected, often adding up to tens of thousands of pictures of the seafloor, that need to be analysed to adequately quantify the benthic fauna and its diversity. As illustrated in (Piechaud et al., 2019), a single AUV dive of 22 h can result in 150,000 digital images collected. The main difficulty with images from the abyssal nodule fields is that an image often features multiple morphospecies from different taxonomic groups on an irregular background with alternating hard and soft substrata. Accuracy of biodiversity assessments in these ecosystems tends to increase with sample size, in this case the amount of seafloor visualized; estimates of taxon richness, based on imagery analyses, require larger sample size and were shown to stabilise at 1000–1500 m² of continuous seafloor coverage with >500 individuals (Simon-Lledó et al., 2019b).

To address the issue of having to analyse large image collections for representative biodiversity assessments, be it in marine or terrestrial environments, new computational tools have been developed and proposed over the last 5 to 10 years. The process of detecting objects (such as megafauna and deep-sea benthos) in images and assigning taxonomic labels to them is referred to as annotation. To make this time-consuming and tedious process more efficient, online image and video annotation tools have been

proposed such as SQUIDLE+ (Proctor et al., 2018), VIAME (Dawkins et al., 2017) or BIIGLE (Langenkämper et al., 2017; Zurowietz and Nattkemper, 2021). In the case of benthic biodiversity community assessment, as considered here, the amount of image data collected often greatly exceeds the capacity of visual inspection by domain experts and the potential of machine learning-based annotation has gained interest over the last decade (e.g. Schoening et al., 2012; Möller and Nattkemper, 2021; Mbani et al., 2023; Yamada et al., 2023). To respond to the growing amounts of imagery, the BIIGLE system has been equipped with the MAIA tool (Machine learning Assisted Image Annotation) (Zurowietz et al., 2018; Zurowietz and Nattkemper, 2020) and cloud storage so users can upload their image data into the BIIGLE cloud, and analyse their image data more efficiently, employing machine learning. Automated object detection tools can reduce the time needed to analyse huge volumes of images by an estimated 50% (Zurowietz et al., 2018), allowing to collect biodiversity data more efficiently. This becomes even more important in the context of potential underwater mining activities, where selected areas will be visited repeatedly and more images will be recorded, not only to detect species and describe the

community composition and diversity (baseline) but also to monitor the changes (impact assessment and subsequent surveys). In order to efficiently integrate tools such as MAIA in effective image data analysis workflows it must be investigated how well these machine learning tools are suited to assist the annotation of the accumulating data, how much posterior quality control is required and how performant the species detection is. In contrast to other related works, we mainly focused on the task of detecting fauna rather than the classification of pre-selected image patches. Considering the fact that one single image has an average footprint of 7 m² with more than 24 million pixels, the screening of thousands of images for fauna or objects-of-interest can be considered a crucial part of the bottleneck problem in image annotation.

The performance and effectiveness of the MAIA in BIIGLE system in megafaunal assessments in a deep-sea mining context was tested with data collected at different altitudes from the abyssal nodule fields and with different degrees of disturbance caused by prior dredging (to simulate a mechanical disturbance of a potential mining action). The system was applied in different runs for different tasks, such as general object detection or the detection of one particular taxon and tested for different volumes of training data.

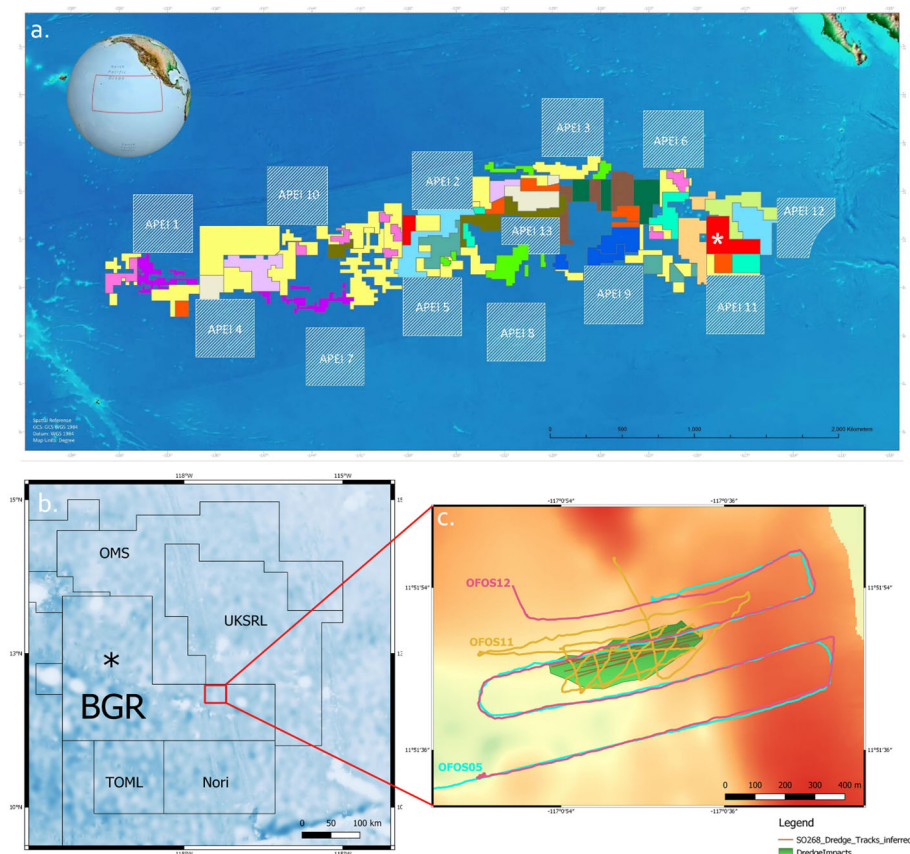


FIGURE 1

(A) Clarion-Clipperton Fracture zone and the exploration and reserved areas from the International Seabed Authority (ISA) (May 2023). BGR area is indicated with *. (B) Localisation of work area (red square) within the BGR license area of the eastern CCZ and (C) Dredge experiment tracks with the shape of the impacted area (green) and the OFOS transects before (SO268/2_100-1 - OFOS05) and after (SO268/2_160-1 - OFOS11) and (SO268/2_164-1 - OFOS12) the dredge disturbance.

2 Materials and methods

2.1 Study site

The Clarion-Clipperton Fracture Zone (CCZ) located in the abyssal equatorial Pacific is the world's largest mineral exploration region featuring polymetallic nodules. Currently, there are 17 contracts for mineral exploration covering 1.2 million km² (Jones et al., 2017) (Figure 1). In April–May 2019, the SO268 expedition (Haeckel and Linke, 2021) to the eastern CCZ carried out a small-scale disturbance experiment with a 1.5 m wide chain dredge in the BGR (Federal Institute for Geosciences and Natural Resources, Germany) license area (Figure 1). This dredge was towed 11 times over an area of ca. 100 x 500 m, creating ~5 cm deep dredge tracks. The area was surveyed with the Ocean Floor Observation System (OFOS) tow-cam before the disturbance (SO268/2_100-1 - OFOS05) (Purser et al., 2021a) and 17–18 days after the impact (SO268/2_160-1 - OFOS11) (Purser et al., 2021b) and 12 (SO268/2_164-1 - OFOS12) (Purser et al., 2021c) (Figures 1C, 2). The OFOS tow-cam was equipped with a Canon EOS 5D Mark IV still camera with 24 mm lens and 3 lasers spaced 40 cm apart. Towing speed was 0.5 knots and one image was taken every 10 seconds at an altitude of ca. 1.5 m above the seafloor with an image resolution of 6720 × 4480 pixels.

2.2 Data collection

In this study, we used the data collected during the three different dives mentioned above (see Table 1 for an overview). One dataset is referred to as image dataset S (source data) (1813

images, from two transects (SO268/2_160-1 - OFOS11) and (SO268/2_164-1 - OFOS12)) and the second dataset is referred to as T (target data) (343 images from dive (SO268/2_100-1 - OFOS05)). The average visual area of the images in the dataset T is 7.1 m². The S dataset was collected in a lower distance to the ground, showing a visual area of average size 6.2 m² and was used in training for automated analysis of dataset T. Image annotation, i.e. location of fauna and taxonomic assignment, was carried out using BIIGLE. Example images from undisturbed and dredged regions are shown in Figure 2 to illustrate the impact of the dredging.

Dataset S was annotated by four different expert users and the smaller dataset T by one expert user. Fauna was annotated at a higher taxonomic level, mostly Classes, e.g. Ophiuroidea, Crinoidea, Holothuroidea, Alcyonacea, some Families (e.g. Deimatidae (Holothuroidea), Isididae (Alcyonacea)) and some with regard to their ecology (e.g. free-living ophiuroids, stalked Porifera) (Figure 3). The same faunal taxon catalogue was used containing *in situ* photographic examples from the CCZ of the taxa to annotate. The full list of semantic labels used for annotating both datasets S and T can be found in the Supplementary Table 1. In dataset S, a number of 67 different labels were attributed and 57 labels in dataset T (see Supplementary Mataterial Table 1). The most abundant higher taxonomic groups/classes (>600 annotations per group) were used to perform follow-up MAIA experiments (Figure 3, Table 2). Holothuroidea with <100 annotations were included for comparison purposes. Selected classes comprised Ophiuroidea, Alcyonacea, Actiniaria/Corallimorpharia, Bryozoa and Holothuroidea. Abundances of both datasets are given in Table 2. Grouped annotations for Porifera were, despite their high abundances, not included here due to high intra-class/intra-phylum variation (Figure 3).

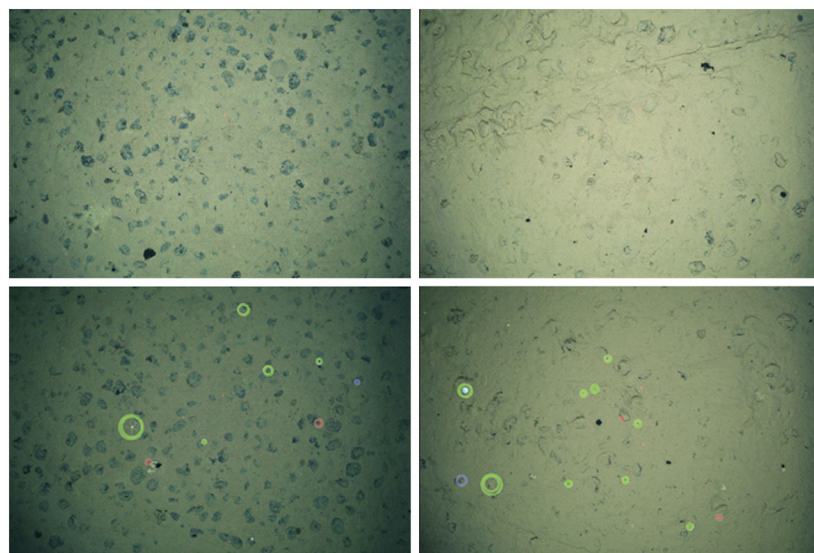


FIGURE 2

The dataset contained images from before and after the dredge experiments; the left panel shows an undisturbed view of a nodule field and its fauna as automatically detected by MAIA (lower panel), while the right panel shows clear disturbance patterns from the dredge experiment (a dredge track) and the sediment (re)deposition. For interpretation of colour codes used, see Figure 4.

TABLE 1 Origin and details about the two image transect datasets T and S used in this study.

Dataset	dive number	# images	# annotations	#taxa	# annotators	average visual area
S	SO268/2_160-1 SO268/2_164-1	1813	12224	67	4	6.2 m ²
T	SO268/2_100-1	343	2879	57	1	7.1 m ²

Dataset S (S = source) is used as the reference data, used for training a machine learning system, in this case MAIA, to detect benthic fauna. The dataset T (T = target) represents a new dataset which is to be processed by the system, without collecting new training data.

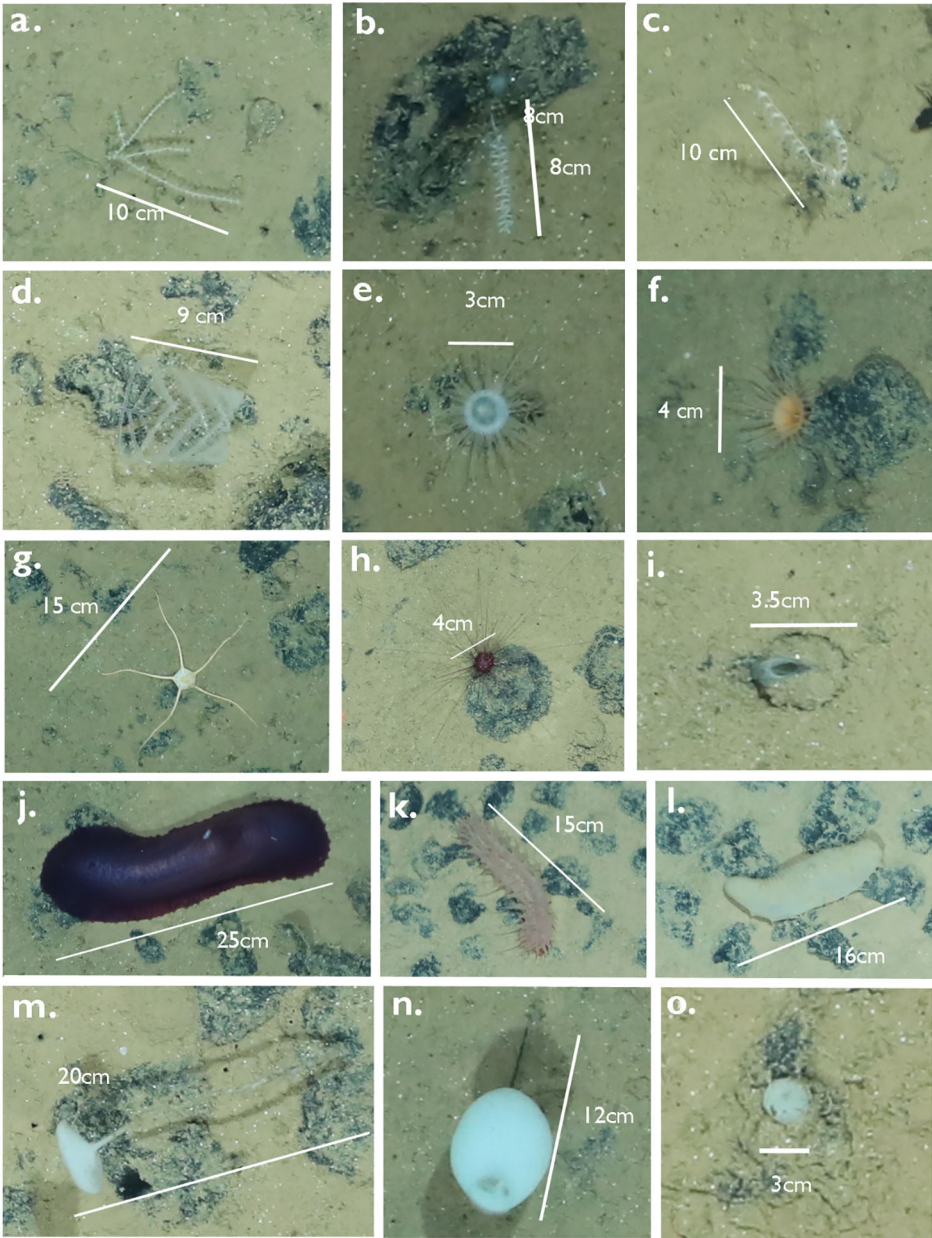


FIGURE 3
Taxa from the BGR license area (images taken during SO268) in the CCZ, demonstrating different taxa and morphological variability. Scales were added for size reference. (A–C): Alcyonacea (Anthozoa, Cnidaria), (D) Antipatharia (Anthozoa, Cnidaria) and (E, F) Actiniaria (Anthozoa, Cnidaria). (G) Ophiuroidea (Echinodermata) and (H, I) Echinoidea (Echinodermata), (J–L) Holothuroidea (Echinodermata), (M, N) stalked Hexactinellida (Porifera) and (O) a non-stalked Porifera.

2.3 MAIA

The MAIA system is designed as a multi-step process that aims to combine the visual experience of domain expert users and machine learning-based computer vision in an efficient way inside the BIIGLE system. One objective in the development was to lower the barrier for machine learning-based marine image analysis for users without a computer science background.

The MAIA system is described in brief here and for a more detailed explanation we refer to the original publications (Zurowietz et al., 2018; Zurowietz and Nattkemper, 2020). MAIA in BIIGLE offers a way to use existing annotations as training data for the machine learning algorithm, skipping some of the steps described in the original publications. The training data is used to train the deep neural network Faster R-CNN (Ren et al., 2015) with pre-trained weights of the COCO dataset, which is then employed for detecting objects of interest in the full image dataset. In the final step of MAIA, the detected objects, referred to as annotation candidates, are presented to the user for rapid manual classification in the LARGO grid view of BIIGLE. Here, the users select and assign labels to image patches showing objects of interest. As the detection task is driven by the selection of the training data, the process is adaptive to different marine image annotation tasks and can reduce the amount of time spent for image object annotation (Zurowietz et al., 2018). Other approaches similar to MAIA have been proposed by other groups such as Yamada et al. (2023) or the CoralNet system (Couch et al., 2019; Chen et al., 2021), however, those were not aiming for detection and classification in deep sea benthic communities for biodiversity assessment. In this study, MAIA was used in two different set-ups by selecting different training data. In the first set-up, MAIA was used to detect any kind of object on the ground, which is referred to as general object detection. This is especially relevant for biodiversity baseline studies, when the researchers must conduct a primary census of the species biodiversity and richness. Here, MAIA was trained with the annotation data from dataset S, referred to as D_{67} (i.e. the dataset including 67 taxa). In the second set-up, MAIA was used to detect five taxonomic groups Ophiuroidea, Alcyonacea, Actinaria/Corallimorpharia, Bryozoa and Holothuroidea individually. To this end, MAIA was trained

with the data from each group in five separate runs. The five individual training datasets were referred to as D_O , D_{Al} , D_{AC} , D_B and D_H (with O = Ophiuroidea, Al = Alcyonacea etc., see Table 2). These experiments addressed the hypothetical case of specific interest in one particular group of organisms with potential indicator value. In addition, MAIA was applied to detecting the union of all five groups, referred to as “5 groups union” or D_{U5} . Subsequently, D_{U5} was filtered for images showing the undisturbed (i.e. non-dredged, see Figure 2) seafloor and this subset, referred to as “5 groups union undisturbed” or D_{U5u} , was used for training to test whether undisturbed images of this subset were better suited for training than the disturbed ones. Based on the results of these two set-ups, we discuss MAIA’s performance in the context of mining-related environmental monitoring.

Since the annotation of training data is an expensive (human resources and time) and tedious procedure, the question of how much MAIA’s performance benefits from increasing amounts of training data was addressed. To study the impact of this factor, we conducted experiments with differently-sized subsets of the training data, i.e. using only 10, 50 100, ... training samples. The effects of these limitations to the training data are shown in the next section. and compared the results.

3 Results

The performance of MAIA in the different set-ups using multiple sets of training data (as described above) was measured counting True Positive detections (TP), False Positives (FP) and False Negatives (FN) using the standard receiver operator characteristics:

$$\text{recall} = \text{TP}/(\text{TP} + \text{FN}) \text{ and } \text{precision} = \text{TP}/(\text{TP} + \text{FP}).$$

True Positives were the detections that matched the annotations (match or found), while False Negatives were the annotations missed by the automated detection tool (miss) (Figure 4). For the general object detection in the first set-up, MAIA was trained with dataset D_{67} from image collection S and applied for general object detection in image collection T. The results from this experiment are shown in the first row of Table 3. Some of the false negative-cases looked rather similar to the true positive-cases, but nonetheless were occasionally overlooked (Figure 4). For the false positive cases, i.e. those that were not manually annotated previously but were detected by the algorithm, often these were either Lebensspuren (past-imprints of organisms’ presence), unidentifiable objects (often unclear if it is an organism or not), organisms which may be dead, or organisms that previously escaped our attention due to any of the above similarities. We report a recall of 90.1% and 13.4% precision for the described experiment, meaning that 90.1% of the manually annotated organisms were also detected by MAIA. The precision shows the percentage of automatically detected objects that were also detected manually. This implies a false positive rate of 86.6% in the general object detection. Indeed, many potentially interesting objects were detected by MAIA, but were considered less relevant by the human annotators since these were mostly non-biological features (e.g. nodules) or non-identifiable objects.

TABLE 2 The five taxonomic groups selected from the list of all taxa and categories (67 in dataset S and 57 in dataset T).

Grouped Taxa	# dataset S	# dataset T
Ophiuroidea (D_O)	1975	369
Alcyonacea (D_{Al})	2433	514
Actinaria/Corallimorpharia (D_{AC})	615	220
Bryozoa (D_B)	705	127
Holothuroidea (D_H)	96	71
5 groups union (D_{U5})	5815	1301
5 groups union undisturbed (D_{U5u})	2949	1301

These five groups comprised subsets of higher resolution identified taxa marked by the annotators. The variable D_O is used to relate to the set of image examples of this group extracted from the data (see below).

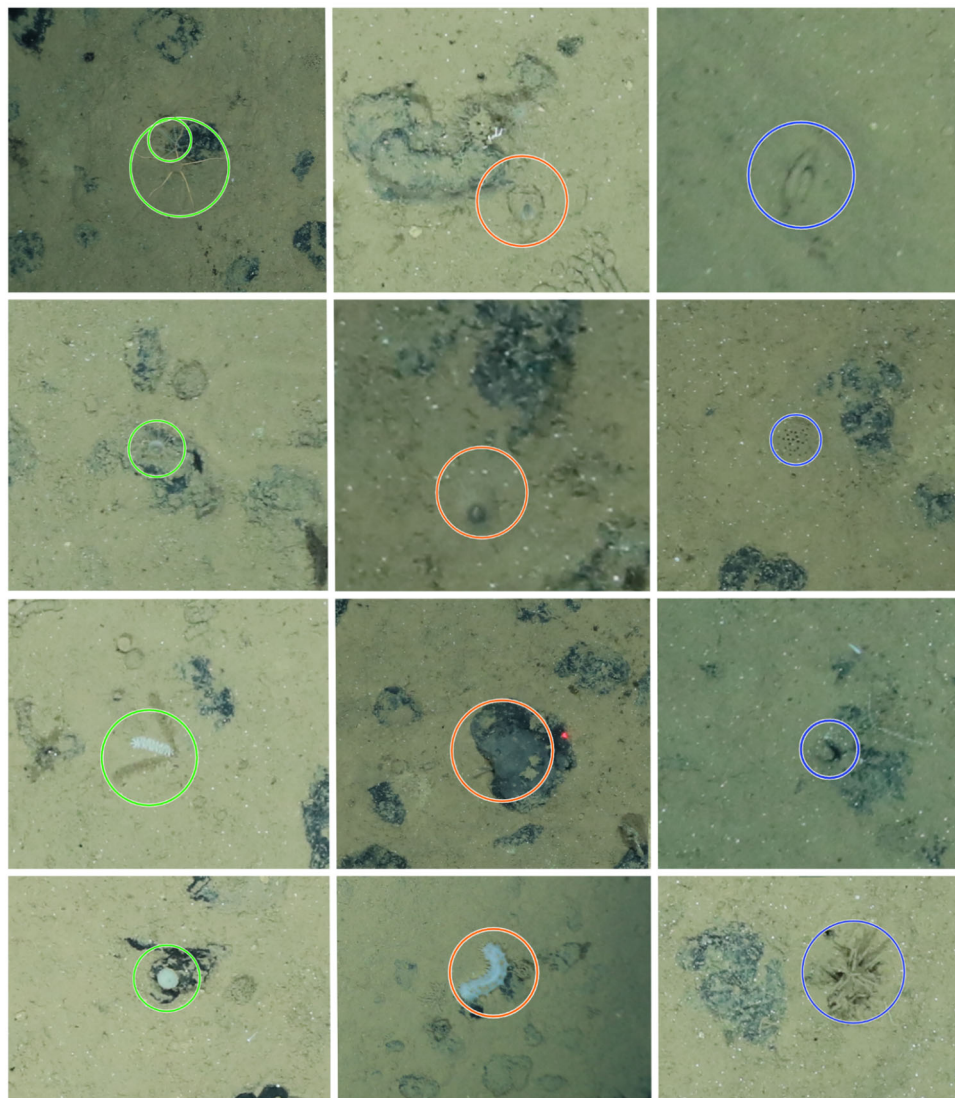


FIGURE 4

Images of correctly found organisms (True Positives, green, left column), missed ones (False Negatives, orange, center column) and newly detected ones (False Positives, blue, right column). Left column from top to bottom feature: Ophiuroidea + Alcyonacea (Primnoidea) (Figures 3A, G), Actiniaria/Corallimorpharia, Alcyonacea (Isidiidae), (possibly stalked) Porifera. Middle column, from top to bottom feature: Echinoidea (Irregularia) (Figure 3I), Actiniaria/Corallimorpharia, encrusting Porifera and Holothuroidea (Synallactidae). Right column from top to bottom feature objects that could be organisms or dead organisms, or Lebensspuren, reason why they were not manually annotated to begin with: Lebensspuren (Holothuroidea fecal casts) or Holothuroidea, *Paleodictyon nodosum* pattern (unknown if it is a Lebensspuren burrow, organism in burrow or a trace fossil (Rona et al., 2009), unknown, carnivorous Porifera (Cladorhizidae) - unclear if the organism is alive or dead due to brown colour.

Next, we analysed the recall of MAIA for each of the 57 label classes in T in this general object detection set-up. For each class, TP and FN were determined by matching the positions of the object detection with their positions as previously annotated by an expert user. In the context of MAIA, all annotations were circles and we defined two annotations to be matching if the Euclidean distance between the center points is less than the greater radius (also taking conflicting matches into account, for details see (Zurowietz, 2022)). As a general object detection was performed without classification, FP cannot be determined for single Classes. Using these counts of TP and FN, the recall for each Class was computed and the results were shown in Figure 5 on the right. 38 out of 57 labels were

detected with a recall > 85% and 16 out of 57 had a perfect recall of 100%. The three most difficult categories with a recall below 40% were “Fossil”, “Jellyfish”, “Pennatulacea” and “Holothuroidea”. These categories were represented with relative low numbers in the training data, i.e. 1 (Fossil), 1 (Jellyfish), 5 (Pennatulacea) or 5 (Holothuroidea) but many other label classes with lower observations in the training set S were observed with much higher recall values in T (see Figure 5), e.g. Actinopterygii (2 training samples/100% recall) or Ophiuroidea (19 training samples/95% recall). In general, we did not observe a clear relationship between the number of training samples of a class and the MAIA performance for the same class in this general object

TABLE 3 The recall and precision values are presented for the general object detection (“All taxa”), the individual MAIA runs for detecting particular groups (second to sixth row), the general object detection trained only the union of the five groups (“All 5 groups”) and the training with the union of the five groups excluding images with disturbed sea floor (“All 5 Groups undisturbed”).

Detection task/training data	counts in S	counts in T	recall (%)	precision (%)
All taxa (D_{67})	12,224	2,879	90.1	13.4
Ophiuroidea/ D_O	1,975	369	96.6	8.8
Alcyonacea/ D_{Al}	2,433	514	94.7	12.4
Actinaria/Corallimorpharia/ D_{AC}	615	220	80.6	3.9
Bryozoa/ D_B	705	127	96.9	2.6
Holothuroidea/ D_H	96	73	71.2	0.9
5 Groups union/ D_{U5}	5,824	1,303	88.1	12.9
5 Groups union undisturbed (D_{U5u})	2,949	1,303	86.6	12.1

detection set-up. Some results were surprising, as some of the top missed label classes consisted of larger organisms that are easily detected with the human eye. For example, Holothuroidea, which are generally recognisable because of their size, shape and colours (Figures 3J–L), had on average a low recall (54%), and were not easily picked up by the algorithm. Other echinoderms performed rather well (Figure 5): Echinoidea at 86% with Infraclass Irregularia (Figure 3I) at 96% recall and, from another Echinoidea Infraclass, the Family Aspidodiadematidae at 100% (Figure 3H); Ophiuroidea attained a 95% recall. Alcyonacea soft coral families were also well detected: Isididae (Figure 3B), Taiaroidea, both at 100% recall, Primnoidea 97% recall (Figures 3A, C) and other Alcyonacea 96% recall. Other Anthozoa classes, such as Antipatharia had a 83% recall (Figure 3D) and Actinaria (e.g. Figures 3E, F), a very abundant and diverse taxon, had a 86% recall. Overall, Porifera were well detected, with the non-stalked (Figure 3O) reaching 88%

and stalked sponges at 96% (Figures 3M, N) and other (not clear if stalked or not) Porifera at 94%.

In the second set-up, we investigated if the performance of MAIA increases when the system is applied to only a single group of taxa. To acquire a more specific model, MAIA was applied to Ophiuroidea, Alcyonacea, Actinaria/Corallimorpharia, Bryozoa and Holothuroidea with the related training datasets D_O , D_{Al} , etc. and the performance was evaluated with recall and precision (see Table 3). The recall for the groups were compared to the results in the general object detection set-up trained with D_{67} . To this end, the macro-average recall for each group was computed (see Supplementary Table 2). The term macro-average recall refers to the average recall which is not computed as the average recall over all individual samples but over the class-specific averages. So label classes with smaller numbers of observations are considered on the same level than classes with larger numbers of observations.



FIGURE 5

On the top left, the abundances for all 57 classes annotated in image dataset T by the experts are shown in decreasing order according to their annotation abundances in the dataset D_{67} from dataset S. On the bottom left, the corresponding recall values computed for results obtained in dataset T are shown for the plot below in the same order. On the top right, the result recall values of the MAIA object detection are shown for all classes in decreasing order. In the bottom right plot the corresponding abundances in the expert annotations in the training dataset D_{67} are shown.

In each of the five runs, no significant increase in performance was observed. Instead, each increase in recall was traded for a significant decrease of precision (see Table 3). When comparing the performances with the first set-up for general object detection (see first row in Table 3 and Supplementary Table 2), we observed that Ophiuroidea, Alcyonacea and Holothuroidea were detected with higher recall but a lower precision (compared to the general object detection D_{67}). Holothurians were detected with a low 54% recall in the general object detection when MAIA was trained with D_{67} and 71.2% (with a very low precision) when MAIA was trained with D_H . Similarly, Bryozoans were detected with 91% recall in the general object detection and 96.9% in the group specific MAIA application. In contrast to that, the recalls for Alcyonacea and Actiniaria/Corallimorpharia decreased from 98% to 94% and 86% to 80%, respectively, compared to the general object detection (see Supplementary Table 2). When MAIA was trained with D_{U5} , for the detection of all five taxonomic groups we observed 88.1% recall and 12.9% precision, so again results similar to the training with D_{67} . No improvement for training with data D_{U5u} which was filtered for undisturbed regions was found, with a 86.6% recall and 12.1% precision for this training data.

Finally, we investigated MAIA's performance (recall and precision) for incrementally growing volumes of training data in all set-ups (Figure 6). In case of learning a general object detection using the complete dataset D_{67} of all taxa, MAIA reaches the best performance with 8,000 or more training samples but does not benefit from further increase in samples (see top left in Figure 6). The precision stays rather stable, slightly above 10% for all training datasets with 500 or more

training samples and does not improve for increasing numbers of samples. Similar results occur when MAIA is again trained for general object detection, but with the data from the union of the five taxonomic groups (D_{U5}) and the subset featuring undisturbed images (D_{U5u}) (result plots are shown in Supplementary Figure 1).

When MAIA is trained for the individual groups, we observe slightly different trends for increasing numbers of training samples in Figure 6. The Ophiuroidea group performance increases until 200–500 annotations, similar to the Bryozoa and the Alcyonacea that show an increase until 500 annotations and then the learning does not improve with more data. The Actiniaria/Corallimorpharia group seem to reach their peak performance quite early, after 200 training samples. Interestingly, we observe a small drop down of MAIA's performance for 50 samples which could be explained by overfitting problems, i.e. the network adapts too much to the low amount of training data and shows low generalization performance.

The trained MAIA system was evaluated to perform with an average annotation time of 111 s/image. For comparison, a traditional purely manual annotation on a subset of 35 images from our data with identification to a comparable taxonomic level as wielded by MAIA, took between 94 and 172 s/image when annotated by a highly trained observer. The discrepancy between minimum and maximum time was related to the changing abundances of faunal organisms per image which is clear by the high standard deviation (an average of 123 ± 30 s/image). There is a significant positive relationship between the time spent annotating an image and the faunal abundance observed ($r=0.91$, $p<0.001$) as well as the richness ($r=0.78$, $p<0.05$).

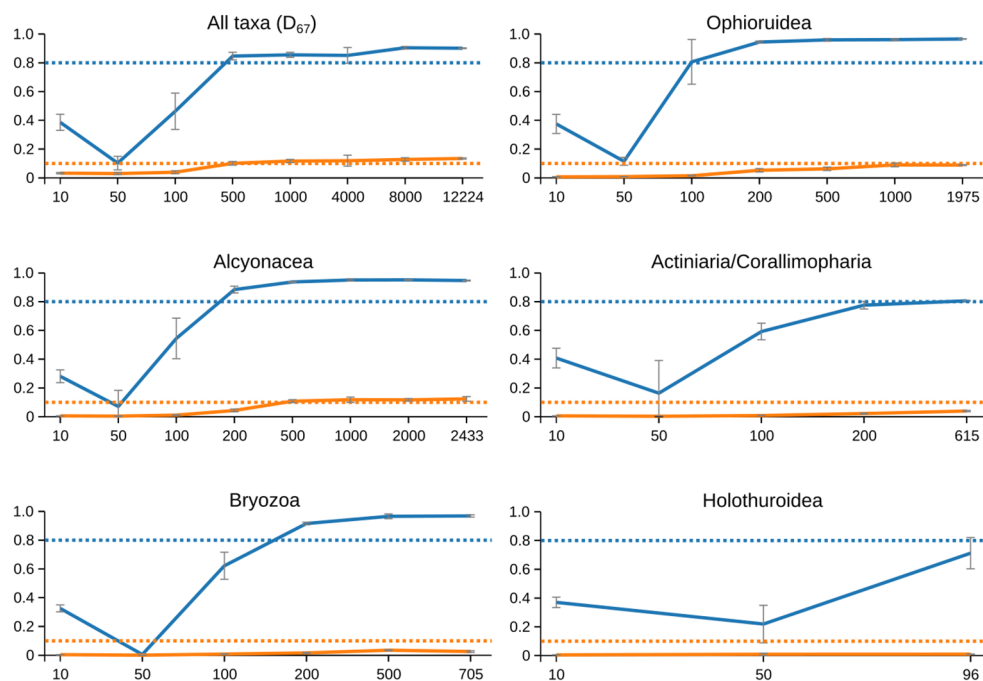


FIGURE 6

MAIA was trained with different data volumes generated as subsets of different sizes from the three datasets D_{67} , D_O , D_{Al} , D_{AC} , D_B and D_H . The orange lines display the precision, the blue lines display the recall. The dotted lines mark the 80% recall and 10% precision as a reference. X-axis=number of annotations, Y-axis=%.

4 Discussion

The vastness of the seafloor areas of interest to deep-sea mining is huge with the CCZ alone spanning 6 million km². A recent checklist for the CCZ region, comprising all size fractions, included 28% megafauna species and showed that over 88% of species sampled in the CCZ region are undescribed (Rabone et al., 2023). While megafauna species are most easily observed based on imagery due to their larger size (>1 cm), they are also the least abundant species and are rarely collected (Rabone et al., 2023). As such, rare taxa (≤3 records) represented one third of the total morphospecies richness in APEI6 (Simon-Lledó et al., 2019b). The recent publication of a morphospecies catalogue for the larger CCZ region shows the difficulties in attributing species names to the megafauna observed (Simon-Lledó et al., 2023b).

In order to allow for quantifiable organism detection and identification, images need to be collected at an optimal altitude ~1.5 m above the seafloor, which limits the area covered by a single image acquisition (Schoening et al., 2020). With 5–10 m² footprints each, tens of millions of images are necessary to cover a potential yearly mining area (200–400 km²/year) (Sharma, 2017; Smith et al., 2020), which is unworkable. Recent studies have shown that estimations of megafauna species richness need at least sample units covering 1000–1500 m² when more than 500 individuals are present to stabilise, i.e. to approach the real species richness, with significantly higher richness detectable in samples >1000 m² (Simon-Lledó et al., 2019b, 2020). Due to high spatial variation observed across the CCZ (Vanreusel et al., 2016; Simon-Lledó et al., 2019b; Cuvelier et al., 2020; Uhlenkott et al., 2023; Simon-Lledó et al., 2023a), replicates remain indispensable, thus adding up to several thousands of images that remain to be analysed to estimate representative biodiversity. Within this context, the usefulness of the MAIA machine learning tool as a detection system can be extremely valuable and is hereby assessed.

Recently, many studies have applied automated workflows and deep-learning techniques to deep sea data and discussed their performances, e.g. (semi-)automated nodule segmentations (Schoening et al., 2016; Peukert et al., 2018; Dumke et al., 2018; Gazis et al., 2018; Gazis and Greinert, 2021) and the classification of nodule associated fauna (Mbani et al., 2023). Our study is the first to address practicality and applicability of computational species detection in assessing biodiversity in mining-related monitoring scenarios. Most related works on machine learning-based underwater biodiversity assessment focus more on the classification of hand selected image patches, which is of course very relevant (Piechaud et al., 2019; Durden et al., 2021). Nevertheless, given the fact that many (rare) species in the images will not be represented in a training data set, the computational general detection of fauna without a detailed taxonomic classification is pertinent and valuable as it would make the time-consuming and error-prone visual screening of the entire images obsolete. This process could be replaced by a much more efficient browsing of image patches (selected by a general object detection) and their manual classification to taxonomic categories. We believe that ongoing contributions aiming at detection or classification of underwater taxa will finally provide the basis for the development of efficient full- or semi-automatic workflows

customised to special scientific contexts. As MAIA is already available to more than 3,000 registered BIIGLE users (on biigle.de), this study gives some insights into the potentials and limitations of machine learning-assisted annotation in deep-sea monitoring.

4.1 Quality assessment

MAIA achieves high recall values when carrying out the general species detection, i.e. detecting any kind of fauna, and as such assessing the overall faunal abundance. While there is still no consensus on what is an acceptable error rate in ecology (Piechaud et al., 2019), we can state that our general species detection happens with satisfactory accuracy (> 90% for all taxa). When increasing the number of training samples, the precision slightly improves (reaching 13.4% with final result obtained using all data). So theoretically, further improvement can be envisioned when adding more image annotation data. Looking at the current trends in machine learning-based computer vision, it seems likely that new strategies such as self-supervised learning methods can make use of huge numbers of non-labelled marine image data for learning purposes in this context. As a consequence, one may conclude that, in our particular ecological context, all underwater and marine image data from the CCZ should be combined in order to provide the best possible training database to develop AI models. A successful combination of data would depend on the application of standards in labeling, metadata and platform (e.g. ROV, AUV) operation protocols.

In general, our levels of recall, sometimes also referred to as accuracy or sensitivity, are comparable to or higher than numbers reported in other studies attempting to automatically detect and classify deep-sea megafauna (Schoening et al., 2012). The precision is a measure for the (mis-)match between automated and manual annotation location and is thus a measure for the False Positives. Our low precision levels can be attributed to the fact that the nodule fields analysed here feature an irregular background pattern due to the differences in soft sediment and hard substrates (nodules), their contrasts, textures and colours. Additionally, the high numbers of rare morphotypes can cause increased False Positives in the more common morphotypes, due to the increased variation, which is thus reflected by a lower precision. However, we observed that within these False Positives, MAIA also finds potentially interesting objects and patterns, including environmental features (e.g. nodules, Lebensspuren) or organisms that were previously left unlabelled because of human doubts or identification issues. In this perspective, MAIA can be used as a re-evaluation tool to narrow down object identification and include additional detections in a morphospecies catalogue.

The comparison of MAIA trained with different training data sets shows that the best recall values are obtained with the largest number of training data. But the performance gain for including more training data diminishes after a certain amount. Interestingly, the MAIA precision for the single taxonomic groups (D_{Al}, D_O, etc.) is lower when trained with this special training data than for the general object detection. So the detection of taxonomic subsets or groups benefits from the additional training data even if these data

points represent other species and morphologies. This observation further supports the necessity to collect or compile as much data as possible, beyond individual license areas and operators (academia and contractors). In contrast to observations reported by [Piechaud et al. \(2019\)](#), we cannot confirm that the performance generally increases for selected smaller subset of classes.

Large differences in morphological shapes of organisms, as observed here for e.g. Echinoidea and Porifera, remain one of the biggest challenges in automated species identification ([Wäldchen et al., 2018](#)). Similarly, and rather surprisingly, because easily detected by the human eye, was the low detection rate for Holothuroidea (<90%). While traditionally hard to identify to species level based on imagery alone, sea cucumbers are generally easily recognisable, occurring in many shapes, sizes and colours and, except for several morphospecies, are rarely inconspicuous. One way to address this in the future is the implementation of feedback loops to prompt users to add additional training samples for particular less represented classes. The challenge for machine learning tools here lies in grouping the considerable differences in morphology, shape, some more sediment-coloured and others brightly coloured. Beside the inter-species morphological differences, intraspecific changes in morphology exist for Porifera and Cnidaria, which show contractile/expansive behaviour when feeding or reacting to stimuli such as tides, pressure and respiration ([Kahn et al., 2020](#)). Individual animal modeled forms with morphometric indices could enhance correct classifications (e.g. [Aguzzi et al., 2011](#)), but represent a disproportionate amount of work in a species rich environment with many rare organisms. Additionally, the performance of these models is still limited by habitat variations and are thus less applicable in a complex and textured, irregular habitat.

The MAIA algorithm appeared to learn quickly for Alcyonacea and Ophiuroidea detections, which might be due to the repetitive patterns of polyp organisation in the former and symmetrical patterns for the latter. Additionally, Alcyonacea are most often whitish in colour which gives an enhanced contrast when compared to the substrate background.

This study also provides a first look into the effect of sediment coverage on the network performance. After removing the sediment-impacted images from the training data, we surprisingly did not see a strong effect on the network performance except a small decrease in classification accuracy which may be explained by a lower number of training data (S).

4.2 Efficiency

The time needed by a highly trained observer to manually annotate a subset of images in BIIGLE was significantly correlated with the abundance of fauna as well as with the richness. While the average manual annotation speed - when restricted to a higher taxonomic level (Class or Family) - was not significantly slower than automated MAIA detections, observer errors and lapses in concentration must be taken into account. Increasing taxonomic resolution, i.e. identification to (morpho-)species level, besides implying a different level of accuracy and difficulty for the annotator, increases taxon richness and thus time necessary for

manual annotation of each image. Similarly, if a lesser trained observer would perform the same task of manual annotation, time spent per image will increase significantly. For comparison, in [Zurowietz and Nattkemper \(2021\)](#) a median gap between two consecutive annotations from one user was reported to be 40.3 seconds according to the records in the entire BIIGLE database. To date, this number is updated to 45.0 seconds, based on the most recent BIIGLE database (11.2 million image annotations as of November 2023). As the average number of annotations for the images in data set T is 8.39, we estimate the predicted annotation time per image to be 378 s/image for an average skilled user. To further decrease the required time, one can a) find ways to further reduce the number of false positives (for instance by improving the image quality) or b) improve the MAIA filtering interface for the users by grouping image patches into clusters of similar morphology, so users can correct or delete image patches in chunks. The annotation time of 123 ± 30 s/image measured in this study falls considerably below the above estimated average annotation time that we have computed from the BIIGLE database. One reason for this considerable difference may be the advanced skill level of the annotators having collaborative experience for working in the area. Another reason could be that the images may show less objects of interest with a higher foreground-background contrast than the average marine image in the BIIGLE database.

4.3 Human factor

Based on the results presented here, we want to highlight the advantages of using an automated detection tool, but with the emphasis that a human factor is indispensable to guide and correct the machine learning tools. Often, computer vision algorithms must be designed specifically to detect and classify particular targets against different background types ([Purser et al., 2009](#); [Aguzzi et al., 2011](#)). Here, we show that for global baseline assessments, MAIA can be used efficiently to quantify overall faunal abundance and assist in assessing the diversity of organisms. In current times, where data is accumulating faster than the processing power or human resources of many research institutions, computational solutions and machine learning tools can prove elemental for effective data exploitation ([Matabos et al., 2017](#)). Accessible automated tools, as those described here, can accelerate time-consuming image analyses and can be used to even the playing field in attributing technical and human capacity to deep-sea research, exploration and monitoring, which is one of the main causes of inequity among institutes and countries ([Bell et al., 2023](#)).

Additional human intervention in the design and subsequent quality control is still required to obtain representative ecological metrics as well as to program or adjust the settings of the imagery collecting platform (e.g. importance of altitude in concept drift: [Langenkämper et al., 2020](#); [Schoening et al., 2020](#)). Regarding imagery annotations, training is crucial both for scientists as well as machine learning tools. Training of deep-sea scientists in recognising and identifying taxa based on imagery and *a posteriori* attribution of morphospecies labels after automated classification in higher taxonomic groups is an important way

forward. Initiatives such as SMarTar-ID (Howell et al., 2020) and open access megafauna atlases (Simon-Lledó et al., 2023b) are therefore indispensable for standardization, uniformisation and learning. A hybrid computer-vision/human approach appears to combine the best of both worlds.

5 Conclusion

In light of the above discussion, we conclude that the annotation of large marine image collections from environmental impact monitoring can be supported by not only deep learning-based taxonomic classification but also by deep learning-based object detection. The free available web tool MAIA in BIIGLE delivers promising results with sufficient recall with a small speed-up compared to a manual annotation by a highly trained expert. For less-experienced users, the speed-up is more significant as well as a gain in usability and consistency. Future research has to address speed up in post processing (i.e. filtering false positives) and increasing precision in object detection in order to further increase the efficiency and effectiveness in this approach.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Annotated imagery data for this study are available in BIIGLE can be accessed upon request, as well as the generated datasets used in Figures 5 and 6. Seafloor images are available in PANGAEA: (Purser et al., 2021a; Purser et al., 2021b; Purser et al., 2021c). Seafloor images of undisturbed and disturbed polymetallic nodule province seafloor collected during RV SONNE expeditions SO268/1 + 2. PANGAEA, <https://doi.org/10.1594/PANGAEA.935856>.

Author contributions

DC: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. MZ: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. TN: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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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.2024.1366078/full#supplementary-material>

SUPPLEMENTARY TABLE 1

label tree for fauna annotations. Labels exclusively occurring in datasets S or T are marked as such.

SUPPLEMENTARY TABLE 2

macro-average recall for taxon groups in for the general object detection.

SUPPLEMENTARY FIGURE 1

MAIA was trained with different data volumes generated as subsets of different sizes from the three data sets D_{67} , D_{U5} and D_{U50} . The red lines display the precision, the blue lines display the recall. The dotted lines mark the 80% recall and 10% precision, which might be considered a minimum performance necessary for an automatic system.

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Industrial mining trial for polymetallic nodules in the Clarion-Clipperton Zone indicates complex and variable disturbances of meiofaunal communities

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Following several small-scale benthic disturbance experiments, an industrial polymetallic nodule collector trial was conducted by the company Global Sea mineral Resources (GSR) in their exploration contract area in the Clarion-Clipperton Zone using the pre-prototype vehicle Patania II (PATII). In this study, meiofaunal (i.e., nematode abundance, ASV diversity and genus composition) and environmental (i.e., grain size, total organic carbon/total nitrogen and pigment) properties are compared between disturbance categories (i.e., Pre-impact, Collector Impact and Plume Impact). One week after the trial, proxies for food availability within the Collector Impact sediments were altered with lower total organic carbon (TOC) and pigment (i.e., CPE: sum of Chlorophyll *a* and phaeopigments) values. Albeit not significant, the observed decrease of nematode abundance and ASV diversity, further indicate the consequences of the removal of the ecologically important surface sediment layer within the PATII tracks. Next to sediment removal, exposed sediments were modified in different ways (e.g., central strips, parallel caterpillar imprints with alternating bands of depressions/ripples and interface patches) and were also subject to heavy collector-induced sediment blanketing. We propose that these cumulative impacts have led to intricate seabed modifications with various levels of disturbance intensity which resulted in the high meiofaunal variability observed. Adjacent nodule-rich areas (i.e., Plume Impact) received considerable levels of sediment deposition (2–3 cm) and were defined by significantly lower food sources (CPE, TOC, carbon to nitrogen ratio) and an observation of meiofaunal enrichment (i.e., higher average nematode abundance and ASV diversity; although statistically non-significant), but mechanisms behind these ecological changes (e.g., suspended material-surface fluxes, passive dispersal

of fauna in the plume vs. active upward migration and “viability” of redeposited fauna) remain unresolved. We conclude that complex benthic pressure-response relationships associated with the PATII trial, combined with the high degree of natural spatial and temporal variability in abyssal meiofaunal communities and sedimentary parameters, complicates the quantitative assessment of deep-sea mining associated disturbances.

KEYWORDS

deep-sea mining, polymetallic nodules, environmental impacts, benthic ecosystem, nematoda, metabarcoding

1 Introduction

Industrial extraction of deep-sea minerals is often put forward as a solution to provide the raw materials needed to fuel the projected future demand for metals needed for the global “green energy” transition, digitalization and future mobility (Hallgren and Hansson, 2021; Miller et al., 2021). Consequently, several countries and entrepreneurs have shown increased interests to explore certain marine areas such as the Clarion-Clipperton Zone (CCZ, eastern equatorial Pacific Ocean), where vast quantities of polymetallic nodules have been found on the abyssal seabed, rich in manganese, iron and economically important metal constituents (e.g., nickel, copper, cobalt and rare earth elements) (Broadus, 1987; Hein et al., 2013; Petersen et al., 2016). So far, a total of 17 contractors have signed exploration contracts for polymetallic nodules with the International Seabed Authority (ISA) in the CCZ (https://www.isa.org/jm/consulted_on_15/09/2023) and great efforts are being made to develop the technology needed to extract these minerals in water depths exceeding 4000 m (Hein et al., 2013; Petersen et al., 2016; Sparenberg, 2019).

Because polymetallic nodules are located at the sediment-water interface, deep-sea mining (DSM) will inevitably affect overall seafloor integrity (i.e., physical, biogeochemical and biological properties) with potential long-term impacts on benthic structure and functioning (Thiel, 2001; Miljutin et al., 2011; Vanreusel et al., 2016; Jones et al., 2017; Mevenkamp et al., 2017, 2019; Volz et al., 2020; Vonnahme et al., 2020). Besides the nodule-associated fauna (e.g., sessile and mobile mega- and macrofauna but also nodule crevice meiofauna), surrounding soft-sediment habitats are also directly impacted as DSM will disturb the ecologically important surficial sediment layers by the removal of nodules and sediment combined with sediment reworking/redistribution (König et al., 2001; Stratmann et al., 2018; Haffert et al., 2020; Volz et al., 2020; Vonnahme et al., 2020). An additional stressor is the deposition of material from the suspended sediment plumes that will be created during nodule mining through the release of removed surface sediments and nodule debris behind the collector vehicle and the chain-drive of the vehicle on the seabed (Oebius et al., 2001; Muñoz-Royo et al., 2022;

Gazis et al., submitted)¹. Considering the low natural background sedimentation levels in the CCZ of less than 0.5 cm/ka (e.g., Volz et al., 2020), substantial negative effects on benthic ecosystems are expected such as altered environmental conditions (Haeckel et al., 2001; König et al., 2001; Volz et al., 2020) and (sub)lethal animal responses (e.g., behavioral changes, impaired respiration/feeding, increased mortality and toxicity) (Wilber and Clarke, 2001; Mevenkamp et al., 2017, 2019).

A full understanding of these effects is hampered by incomplete scientific baseline knowledge (Amon et al., 2022) and the fact that available data mainly come from small-scale and short-term disturbance experiments (Jones et al., 2017; Volz et al., 2018). While significant progress has been made to survey plume dispersal patterns (e.g., Gillard et al., 2019; Purkiani et al., 2021; Muñoz-Royo et al., 2022), investigating faunal responses to enhanced sediment exposure remains challenging within these areas. However, science-industry collaboration enables to investigate the nature, extent and mechanisms of DSM stressors at a representative scale (MI2, 2018; Clark et al., 2020; Amon et al., 2022). During Spring 2021, the pre-prototype nodule collector vehicle, Patania II (PATII), was successfully deployed in the Belgian (GSR) exploration contract area. PATII was able to traverse along the seabed and collect approximately 6.6×10^5 kg of polymetallic nodules (Gazis et al., submitted)¹.

In this study, impacts under an industry-style DSM scenario were investigated within the GSR exploration contract area with a focus on soft-sediment infauna. Sediment samples were collected within non-impacted and impacted (Collector and Plume Impact) sites. The Collector Impact site refers to the area where the PATII nodule collector removed the nodules and surface sediment, whereas the Plume Impact site refers to the area adjacent to the collector test area where the nodule habitat was blanketed by a sediment cover of 2–3 cm (Vink et al., 2022). The main objective was to assess immediate (i.e., one week after the trial) effects of nodule collection on the

¹ Gazis, I. Z., de Stigter, H., Mohrmann, J., Heger, K., Diaz, M., Gillard, B., et al. (2024). Monitoring deep-sea mining benthic plumes, sediment redeposition and nodule collector imprints. [Manuscript submitted for publication].

sediment properties (i.e., grain size, total organic carbon, total nitrogen and pigments) and metazoan meiofaunal communities. Nematoda represent the dominant phylum in terms of abundance within abyssal soft sediments, typically comprising > 90% of all metazoan meiofauna, and have valuable bio-indicator properties (Rex et al., 2006; Pape et al., 2017; Hauquier et al., 2019; Hasemann et al., 2020; Danovaro and Gambi, 2022). As such, nematode community attributes (i.e., abundance, diversity and genus composition) were used as a proxy to evaluate the impact of the nodule collector, using a combination of morphological and metabarcoding methods. Besides the immediate-disturbance study, pre-impact samples obtained within the Trial site (i.e., Collector and Plume Impact sites) during two consecutive sampling campaigns (2019: SO268 and 2021: IP21) are compared to establish natural conditions of the abyssal nodule fields. Finally, recommendations for future DSM impact assessments on soft-sediment habitats are provided, based on our findings.

2 Material and methods

2.1 Study area

The Clarion-Clipperton Zone (CCZ) is an area of approximately $5.1 \times 10^6 \text{ km}^2$ in the eastern equatorial Pacific Ocean, which has been designated by the ISA as a potential region for DSM due to its high abundance of high-grade (i.e., enriched in copper, nickel and cobalt) polymetallic nodules (Hein et al., 2013; Petersen et al., 2016). The exploration contract area of the Belgian company GSR is located in the north-eastern part of the CCZ, and the 72.728-km² large area is subdivided into three zones (Figure 1). The Trial site of the pre-prototype collector vehicle PATII was located in the central zone B4

(Figure 1), at the south-eastern flank of a 140-m high seamount in water depths of 4390–4530 m, exhibiting a nodule abundance of 20–24 kg/m² wet weight with a size of more than 4 cm. During the SO268 sampling campaign in 2019, baseline samples were also obtained within the Trial site (Haeckel and Linke, 2021).

2.2 Sediment plume monitoring

Prior to the PATII test (14–17 April 2021), an extensive sensor array for monitoring the sediment plume was installed on the seabed in the Trial area (Vink et al., 2022). It consisted of 20 sensor platforms with a total of 50 optical and acoustic sensors to monitor and quantify the spatiotemporal dynamics of the sediment plume created by PATII, together with two additional Sedimentation Level Indicator (SLIC) boxes to collect and measure the amount of resettled sediment. These devices were placed on the seabed by means of a Remotely Operated Vehicle (ROV) around the collector impact area (in a circle on a northwest-southeast axis) and at various distances (200 to 1000 m) along the south-southeast axis (Vink et al., 2022). This positioning corresponded with the expected sediment plume dispersal orientation, downslope of the dominant bathymetry gradient and downstream of the prevailing bottom currents, which was based on *a priori* predictive modelling as described by Purkiani et al. (2021).

2.3 Nodule mining trial

The nodule collection trial with PATII (dive number PAT009) in the GSR exploration contract area started on the 18th of April 2021. The vehicle was built at sub-industrial scale (1:4, $25 \times 10^3 \text{ kg}$ in

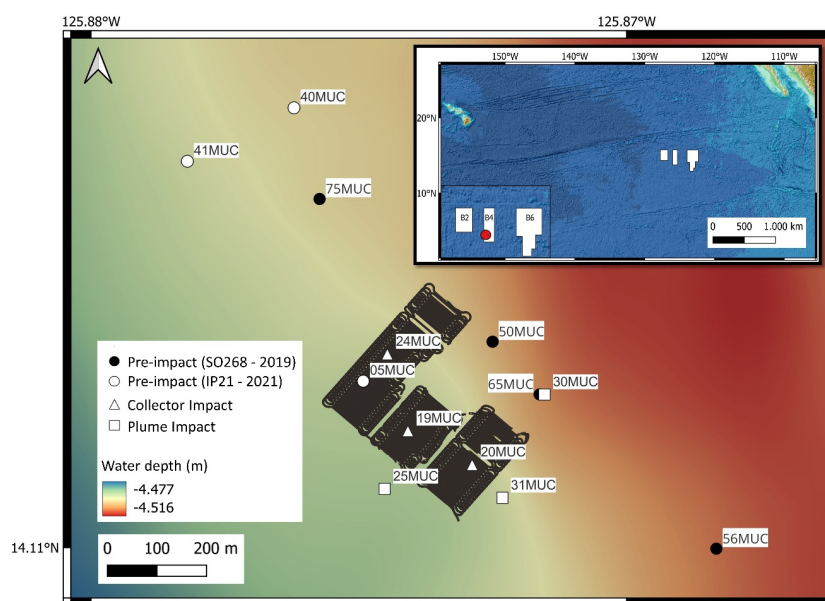


FIGURE 1

Map depicting the PATANIA II (PATII) tracks following a "fishtail" trajectory along three strips within the GSR exploration contract area, covering > 30,000 m². Symbols represent the location of the multicorer (MUC) sampling positions taken in the different categories: Pre-impact (SO268 and IP21), Collector Impact and Plume Impact cover. Upper right corner: location of the three zones (B2, B4 and B6) of the GSR exploration contract area in the northeastern part of the Clarion-Clipperton Zone. The red dot indicates the position of the PATII Trial site within B4.

water, L8 m x W4.8 m x H4.2 m) and is composed of a tracked propulsion (i.e., caterpillar tracks) and a hydraulic nodule collector system (<https://deme-gsr.com/>). The nodule collector vehicle was equipped with several sensors and cameras to monitor environmental conditions such as turbidity, current velocity and sedimentation. Nodules were collected in three strips along parallel 50-m long lanes following a continuous “fishtail” trajectory (Figure 1). Due to local seabed topography, the southernmost strip 1 and middle strip 2 could not be fully completed, resulting in a total of 55 and 31 lanes, respectively. The northernmost strip 3 had a more favorable topography and a total of 85 lanes were completed. Since no riser system was present to bring the nodules to the surface, collected nodules were stored in a container at the back of the PATII vehicle and deposited in the turns of the tracks. Discharge waste, composed of sediments and fragmented nodules, was returned to the seabed by a diffuser at the rear end of the vehicle. The mining trial ended on the 20th of April 2021 after a deployment on the seabed of approximately 40 hours in which approximately 6.6×10^5 kg of nodules were removed, from an area larger than 30.000 m² (Gazis et al., submitted)¹.

2.4 Soft sediment sample collection

A 20-core multicorer (MUC, Oktopus GmbH), equipped with 60 cm-long cores (inner diameter of 96 mm), was used to obtain soft sediment samples. Pre-impact or “no-impact” samples were obtained within the GSR Trial site throughout two consecutive sampling campaigns. During the SO268 campaign in 2019, four MUC deployments were performed along the northwest-southeast axis (Haeckel and Linke, 2021, Figure 1, Table 1). During the IP21 campaign (2021), one MUC deployment was performed prior to the

trial (Figure 1; 05MUC). In addition, two deployments were taken after the trial, located at 500 m northwest of the impact site. This area was also considered as “undisturbed” due to the south-southeast dominated dispersal of the produced sediment plume, based on preliminary data from a turbidity sensor mounted on the AUV and a stationary sensor (NIOZ_PFM_05) placed at 200 m distance in between the MUC locations and the PATII trial site (Gazis et al., submitted)¹. As such, both samples were categorized and further referred to as “Pre-impact” within this paper. This correct categorization could be confirmed after analyzing the AUV-based photo mosaics for plume deposition on the seafloor (Gazis et al., submitted)¹ and numerical modelling of the plume dispersion matching the sensor data (Purkiani and Haeckel, unpublished).

In addition, three MUC deployments were performed after the PATII trial during the IP21 campaign within the tracks of the PATII vehicle (Collector Impact, 1-3 days post-impact, Figure 1, Table 1). Samples were also obtained in the area adjacent to the collector test site (Plume Impact), subject to sedimentation (3-5 days post-impact, Figure 1, Table 1). The post-trial impact categorization (Collector Impact and Plume Impact) and positioning of the MUC deployments were chosen on board based on sensor readings (e.g., AUV and CTD-mounted sensors) and inspection of AUV/ROV derived imagery (Vink et al., 2022). From each deployment, 1-2 cores and 2-3 cores were allocated for meiofaunal morphological and metabarcoding analysis, respectively. Sediment was also collected from 2-3 cores during each deployment for environmental analyses. The exact amount of cores allocated for each type of analysis are provided in Appendix Table 1.

Processing of Pre-impact samples from the SO268 campaign are described in Lefaible et al. (2023) and are comparable to the methods used for the samples collected in IP21. The IP21 cores for meiofaunal analyses were photographed and stored on board at

TABLE 1 Multicorer (MUC) samples collected for environmental and meiofaunal analyses.

Sampling campaign	Sampling category	MUC deployment	Water depth (m)	Latitude (N)	Longitude (W)
SO268 (2019)	Pre-impact	50MUC	4507	14° 6.840′	125° 52.341′
SO268 (2019)	Pre-impact	56MUC	4501	14° 6.601′	125° 52.091′
SO268 (2019)	Pre-impact	65MUC	4495	14° 6.788′	125° 52.295′
SO268 (2019)	Pre-impact	75MUC	4495	14° 6.999′	125° 52.537′
IP21 (2021)	Pre-impact	05MUC	4495	14° 6.787′	125° 52.495′
IP21 (2021)	Pre-impact	40MUC	4496	14° 7.094′	125° 52.573′
IP21 (2021)	Pre-impact	41MUC	4493	14° 7.034′	125° 52.692′
IP21 (2021)	Collector Impact	19MUC	4493	14° 6.731′	125° 52.445′
IP21 (2021)	Collector Impact	20MUC	4498	14° 6.693′	125° 52.373′
IP21 (2021)	Collector Impact	24MUC	4491	14° 6.8173′	125° 52.468′
IP21 (2021)	Plume Impact	25MUC	4489	14° 6.666′	125° 52.471′
IP21 (2021)	Plume Impact	30MUC	4505	14° 6.772′	125° 52.292′
IP21 (2021)	Plume Impact	31MUC	4491	14° 6.656′	125° 52.339′

Samples are reported for each sampling campaign (2019: SO268 and 2021: IP21) per sampling category (Pre-impact, Collector Impact, Plume Impact), MUC deployment, water depth (m), and geographical location (coordinates in degrees, minutes).

4°C. The upper 0–5 cm layer were used for further morphological and metabarcoding analyses. Sediments were collected in plastic containers and fixed with 4% formaldehyde-seawater solution and 98% denatured ethanol for morphological and metabarcoding samples, respectively. Cores obtained for environmental analyses were sliced at different intervals depending on the type of variable under study. For the pigment measurements, cores were sliced at a 1 cm depth resolution (down to 20 cm depth), whereas cores were sliced at 0–1 cm, 1–5 cm and 5–10 cm sediment depth for the grain size, total organic carbon (TOC) and total nitrogen (TN) measurements. Sub-samples of 5 ml were taken for pigment analyses from each depth layer using a cut-off syringe, which was then sealed with a cap and wrapped in aluminum foil. Morphological samples were stored at room temperature, whilst metabarcoding and environmental samples were preserved at -20°C until transported to the Max Planck Institute for Marine Microbiology for pigment analysis (MPI-MM Bremen) and Ghent University for morphological, metabarcoding and environmental (grain size, TOC, TN) analyses.

2.4.1 Environmental samples

Granulometric data were obtained through laser diffraction using a Malvern Mastersizer HYDRO 2000. Sediment fraction classifications were done following Wentworth (1992) and granulometric measurements included the median grain size (MGS, μm), together with the percentages of sand ($> 63 \mu\text{m}$), clay ($< 4 \mu\text{m}$) and silt ($> 4 \mu\text{m} < 63 \mu\text{m}$). Prior to nutrient analysis, sediments were homogenized and pre-treated with 1% HCl solution to remove inorganic carbon compounds. Percentages of total organic carbon (TOC, %) and total nitrogen (TN, %) were measured using an Element Analyzer Flash 2000 and were also used to calculate TOC : TN ratio's (C/N ratio) for each sample.

Pigment analysis for Chlorophyll-*a* (Chl-*a*) and phaeopigment concentrations was done using an acetone extraction method (Boetius and Damm, 1998). The sediment and 8 ml of cold acetone (90%) were transferred into Greiner tubes, containing six glass beads, and shaken in a cell mill for 3 min. The samples were then centrifuged at 1000 rpm for 10 min at 0°C, and the supernatants were transferred into new 15-ml tubes. Acetone was added two more times, and the supernatant was added to the 15-ml tube each time. After a last centrifugation step of the extract for 2 min at 1000 rpm and 0°C, 1 ml of the extract was transferred into a cuvette and measured at a Turner Trilogy fluorometer at an excitation wavelength of 428 nm and an emission wavelength of 671 nm. For the phaeopigment measurement, one drop of HCl (20%) was added to the extracts, which were then remeasured. The Chloroplast Pigment Equivalents (CPE, sum of Chl-*a* and phaeopigment concentration) was calculated for each sample, together with the percentage of Chl-*a* (Chl-*a* %, percentage of Chl-*a*:CPE ratio).

2.4.2 Meiofaunal morphological samples

The 0–5 cm sediment layer was rinsed with filtered tap water over a 32- μm sieve. Next, colloidal silica polymer Ludox HS-40 (density of 1.18 g/cm³) was added to the remaining sediment and meiofaunal organisms were extracted through density-gradient centrifugation (3

x 12 min, at 1905 rcf) using a Hettich ROTANTA 460 R centrifuge. The resulting supernatant with meiofaunal organisms was then collected on a 32- μm sieve, fixed with 4% formaldehyde and stained with Rose Bengal. Further processing included the sorting, counting and higher-taxon identification of the meiofauna by means of a stereomicroscope. Meiofaunal counts were converted to abundance per surface area (ind./10 cm²).

2.4.3 Meiofaunal metabarcoding samples

For a detailed description of the library preparation, we refer to the text box provided in the [Supplementary Material](#).

2.4.3.1 Bioinformatic analyses

Gene-specific adapters were removed with Cutadapt v4.4 (Martin, 2011) as non-internal adapters with the following parameters: minimum length=1, maximum error rate=0.1, minimum overlap=5. Amplicon sequence variants (ASVs) were generated following the DADA2 tutorial (<https://benjjneb.github.io/dada2/tutorial.html> (Callahan et al., 2016)) with the exception that forward and reverse reads were truncated at 250 bp and 200 bp, respectively. Taxonomy was assigned to the ASVs with the ribosomal database project (RDP) classifier (Wang et al., 2007) in two steps: 1st a large reference sequence database was used containing 18991 sequences of eukaryotes and marine nematodes (from Silva release 123 99% Operational Taxonomic Units (OTUs) and UGent nematode Sanger sequences). In the second step, all ASVs receiving a “Nematoda” label where extracted, to these taxonomic assignment was completed using a smaller, marine-nematode-exclusive reference sequence database (971 sequences from Silva release 123 99% OTUs and UGent nematode Sanger sequences). The data were stored in a phyloseq object (McMurdie and Holmes, 2013) and rarefied to the lowest sequence count (n=9244) for further analyses. The R package Ampvis2 (Andersen et al., 2018) was used to visualize taxonomic composition. From the rarefied dataset, two alpha diversity indices were calculated, namely the number of ASVs corresponding to the ASV Richness and the ASV Shannon diversity.

2.5 Statistical analyses

Samples from one deployment (40MUC) were removed from the final biological IP21 dataset because sediment cores appeared highly disturbed after sampling. Final environmental datasets for the all the sampling categories were based on the mean of the pooled 0–5 cm sediment layers. Comparisons between the Pre-impact samples obtained during the two campaigns (SO268 vs. IP21) in terms of univariate environmental and meiofaunal properties were performed by means of independent t-tests. Since pigment measurements were done with different methods during each campaign, these variables were not included for the Pre-impact analysis.

For the immediate-disturbance study, univariate environmental and biological differences between the sampling categories from the IP21 campaign (i.e., Pre-impact, Collector Impact and Plume Impact) were assessed through a one-way analysis of variance

(ANOVA). Data normality and homoscedasticity were assessed by means of a Shapiro and Levene's test, respectively. If the ANOVA analysis was significant, Tukey post-hoc tests were performed to compare all possible pairs of groups/categories. In addition, power analyses were performed for the univariate community descriptors under study for the PATII impact assessment (IP21). Omega squared (ω^2) was chosen as a measure of effect size to quantify the magnitude of an effect, as it is considered to be a reliable estimate when sample sizes are small (Field, 2013). Firstly, ω^2 values were calculated based on the one-way ANOVA models of each variable, through the `omega_squared()` function and were classified as very small ($\omega^2 < 0.01$), small ($0.01 \leq \omega^2 < 0.06$), medium ($0.06 \leq \omega^2 < 0.14$) or large ($\omega^2 \geq 0.14$) according to Field (2013).

A one-way permutational analysis of variance (PERMANOVA, distance metric: unweighted UniFrac (Lozupone and Knight, 2005)) was also applied to test for differences in terms of nematode ASV genus composition between Pre-impact sampling campaigns (SO268 vs. IP21) and impact categories during the IP21 campaign. Permdisp tests were performed to assess the homogeneity of multivariate dispersions. All statistical analyses and graphs were produced with R Statistical Software (v4.1.2; R Core Team 2021) using the following R packages: `car` (Fox and Weisberg, 2019), `tidyverse` (Wickham et al., 2019), `vegan` (Oksanen et al., 2019) and `pwr` (Champely, 2020).

3 Results

3.1 Trial site baseline conditions

3.1.1 Environmental variables

Average MGS was significantly higher (Figure 2, t-test, $p = 0.003$, Appendix Table 2) for the IP21 (2021) Pre-impact samples ($20 \pm 2 \mu\text{m}$) compared to those from SO268 (2019) ($17 \pm 1 \mu\text{m}$) and a similar pattern was found for the average sand fraction (IP21: $14 \pm 3\%$, SO268: $12 \pm 1\%$, Figure 2, t-test, $p = 0.03$). Clay fractions were also significantly lower for the IP21 Pre-impact samples (IP21: $13 \pm 1\%$, SO268: $16 \pm 1\%$, Figure 2, $p < 0.001$), while average silt fractions were comparable between both campaigns (Appendix Table 2, $p > 0.05$). Significant differences were also detected for the average TOC values and C/N ratio's, which were both higher in the IP21 Pre-impact samples (TOC: $0.55 \pm 0.01\%$, C/N: 4.80 ± 0.23) relative to the Pre-impact samples from SO268 (TOC: $0.42 \pm 0.01\%$, C/N: 2.73 ± 0.46) (Figure 2, TOC: $p = 0.002$ and C/N: $p < 0.001$). In contrast, average TN values were significantly higher (Figure 2, $p = 0.001$) for the SO268 sediments ($0.16 \pm 0.03\%$) compared to sediments obtained during IP21 ($0.11 \pm 0.02\%$). However, we would like to highlight that within-group variability was also observed, especially in terms of TOC values for the IP21 sediments and that granulometric differences between the campaigns were mainly

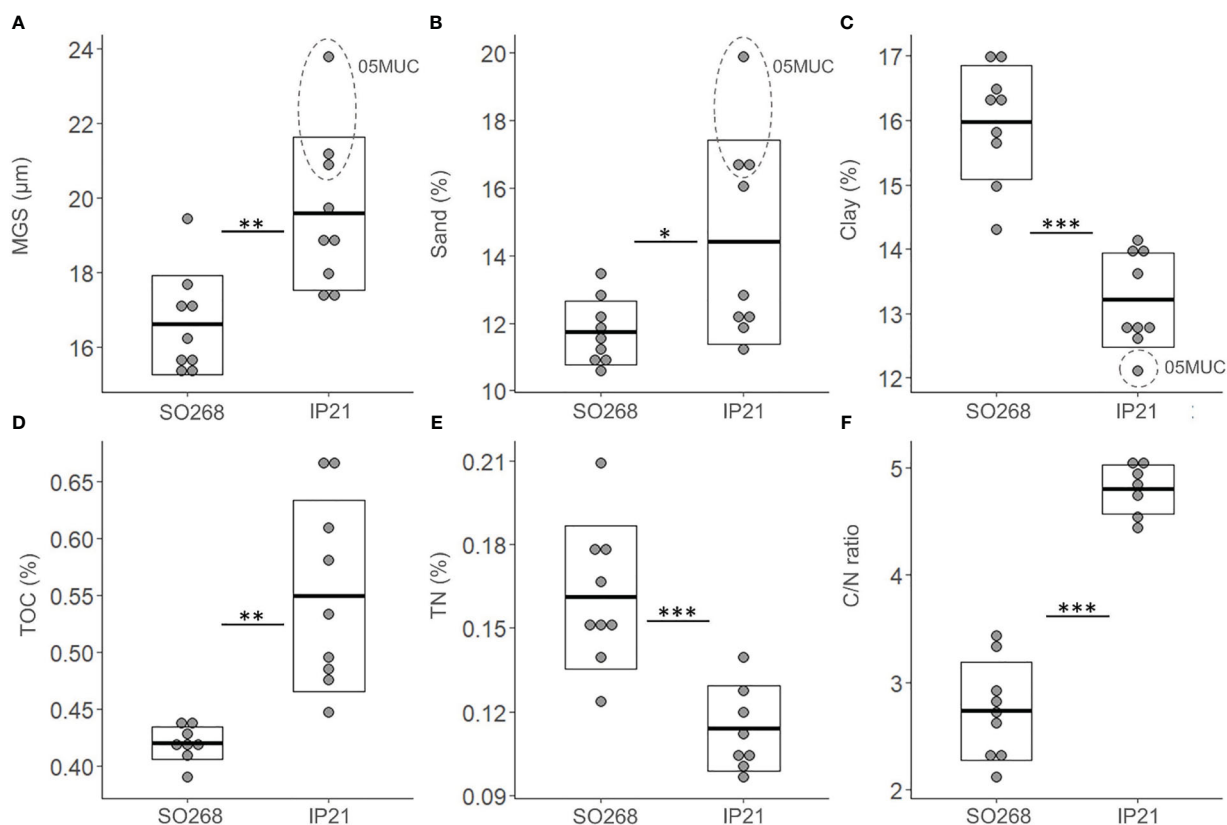


FIGURE 2

Environmental variables; (A) Median grain size (MGS, μm), (B) Sand fraction (Sand, %), (C) Clay fraction (Clay, %), (D) Total organic carbon (TOC, %), (E) Total nitrogen (TN, %) and (F) Total organic carbon to nitrogen ratio (C/N ratio) for the Pre-impact samples collected within the Trial site during the SO268 (2019) and IP21 (2021) campaigns. The crossbar and extent of the box represent the mean and standard deviation, respectively. Asterisks were added to visualize the p-values ($p < 0.05$ '*', $p < 0.01$ '**' and $p < 0.001$ '***') from the t-tests.

driven by measurements from one of the IP21 Pre-impact samples (05MUC, Figure 2).

3.1.2 Nematoda

Results from the t-tests showed that average total nematode abundance, ASV Richness and ASV Shannon diversity were comparable between samples from both campaigns (Appendix Table 2, $p > 0.05$, Appendix Figure 1). The genera *Acantholaimus* ($> 20\%$), *Halalaimus* ($\geq 16\%$), *Desmoscolex* ($> 13\%$) and *Phanodermopsis* ($> 10\%$) were the most abundant in the Pre-impact samples (Appendix Figure 2). However, nematode community composition differed significantly between the SO268 and IP21 campaign (one-way Permanova, $p = 0.002$ and Permdisp test, $p = 0.265$, Appendix Figure 3).

3.2 Impacts of the PATII trial

3.2.1 Environmental variables

All the granulometric variables showed comparable average values between categories (Table 2, one-way ANOVA's, $p > 0.05$). Sediments from the Collector Impact contained lower average TN relative to the Pre-impact and Plume Impact categories (Table 2), but these differences were not statistically significant ($p > 0.05$). In contrast, average TOC values showed statistically significant differences (Table 2, Figure 3, $p = 0.001$) and Tukey HSD post-hoc tests revealed that TOC was significantly lower in the Collector

Impact ($0.43 \pm 0.04\%$) and Plume Impact ($0.47 \pm 0.03\%$) compared to the Pre-impact samples ($0.55 \pm 0.08\%$) (Figure 3, Pre-impact vs. Collector Impact, $p = 0.001$ and Pre-impact vs. Plume Impact, $p = 0.03$). The C/N ratios also differed between impact categories ($p = 0.021$) and were significantly lower for the Plume Impact samples (3.86 ± 0.28) compared to the Pre-impact samples (4.80 ± 0.23) (Figure 3, Tukey HSD test: pre-impact vs. Plume impact, $p = 0.016$).

Average Chl-*a* concentrations were similar for the Pre-impact and Plume Impact samples, while lower average values were found in the PATII tracks (Collector Impact) (Table 2), but no statistically significant difference was detected ($p > 0.05$). In terms of CPE, average values were significantly lower for the Collector Impact ($0.030 \pm 0.011 \mu\text{g/ml}$) and Plume Impact ($0.052 \pm 0.022 \mu\text{g/ml}$) samples compared to the Pre-impact samples ($0.085 \pm 0.029 \mu\text{g/ml}$) (Figure 3) ($p = 0.001$ and Tukey HSD tests: Pre-impact vs. Collector Impact, $p = 0.001$ and Pre-impact vs. Plume Impact, $p = 0.01$). Average Chl-*a* % was highest within the Plume Impact (Table 2), but differences between categories were not statistically significant ($p > 0.05$).

3.2.2 Meiofauna

Considering all impact categories, Nematoda represented the dominant taxon in terms of relative abundance (94%), followed by Copepoda (4%). Polychaeta and nauplii (i.e., Crustacea larvae) both comprised about 1%, while other taxa such as Gastropoda, Isopoda, Tanaidacea, Halacarida and Kinorhyncha contributed to a lesser extent ($< 0.1\%$). Differences between impact categories for

TABLE 2 Minimum (Min), maximum (Max), average and standard deviation ($\text{Av} \pm \text{SD}$) of the abiotic and biotic variables in the 0-5cm sediment depth layer for each category (Pre-impact, Collector Impact and Plume Impact).

Environmental variables	Pre-impact			Collector Impact			Plume Impact		
	Min	Max	$\text{Av} \pm \text{SD}$	Min	Max	$\text{Av} \pm \text{SD}$	Min	Max	$\text{Av} \pm \text{SD}$
MGS (μm)	17	24	20 ± 2	18	21	19 ± 1	17	23	19 ± 2
Sand (%)	11	20	14 ± 3	12	18	15 ± 2	13	24	17 ± 4
Silt (%)	68	75	72 ± 2	68	76	72 ± 3	63	72	69 ± 4
Clay (%)	12	14	13 ± 1	13	16	13 ± 1	12	15	14 ± 1
TN (%)	0.10	0.20	0.12 ± 0.03	0.07	0.13	0.10 ± 0.02	0.11	0.14	0.12 ± 0.01
TOC (%)	0.44	0.67	0.55 ± 0.08	0.38	0.47	0.43 ± 0.04	0.41	0.50	0.47 ± 0.03
C/N ratio	4.48	5.02	4.80 ± 0.23	3.48	5.84	4.37 ± 0.79	3.59	4.35	3.86 ± 0.28
Chl- <i>a</i> ($\mu\text{g/ml}$)	0.009	0.039	0.018 ± 0.010	0.002	0.035	0.011 ± 0.012	0.005	0.049	0.017 ± 0.012
CPE ($\mu\text{g/ml}$)	0.045	0.132	0.085 ± 0.029	0.012	0.045	0.030 ± 0.011	0.024	0.083	0.052 ± 0.022
Chl- <i>a</i> /CPE (%)	13	21	21 ± 7	4	98	39 ± 31	10	92	39 ± 29
Biological variables	Pre-impact			Collector Impact			Plume Impact		
	Min	Max	$\text{Av} \pm \text{SD}$	Min	Max	$\text{Av} \pm \text{SD}$	Min	Max	$\text{Av} \pm \text{SD}$
TNAb (ind./10 cm^2)	7	145	71 ± 57	12	98	35 ± 34	9	168	90 ± 61
ASV Richness	35	297	145 ± 97	27	278	108 ± 73	50	256	143 ± 83
ASV Shannon diversity	3.18	4.05	3.55 ± 0.37	2.08	4.21	3.30 ± 0.69	3.29	4.43	3.85 ± 0.40

Granulometry: Median grain size (MGS, μm) and percent Silt/Clay (%). Nutrients: Total nitrogen (TN, %), Total organic carbon (TOC, %) and Total organic carbon to Nitrogen ratio (C/N ratio). Pigments: Chlorophyll-*a* (Chl-*a*, $\mu\text{g/ml}$), Chloroplastic Pigment Equivalents (CPE, $\mu\text{g/ml}$) and percent of Chl-*a*/CPE ratio (Chl-*a* %, %). Biotic indices: Total Nematoda Abundance (TNAb, ind./10 cm^2), Number of Nematoda Amplicon Sequence Variants (ASVs) corresponding to the ASV Richness and ASV Shannon diversity.

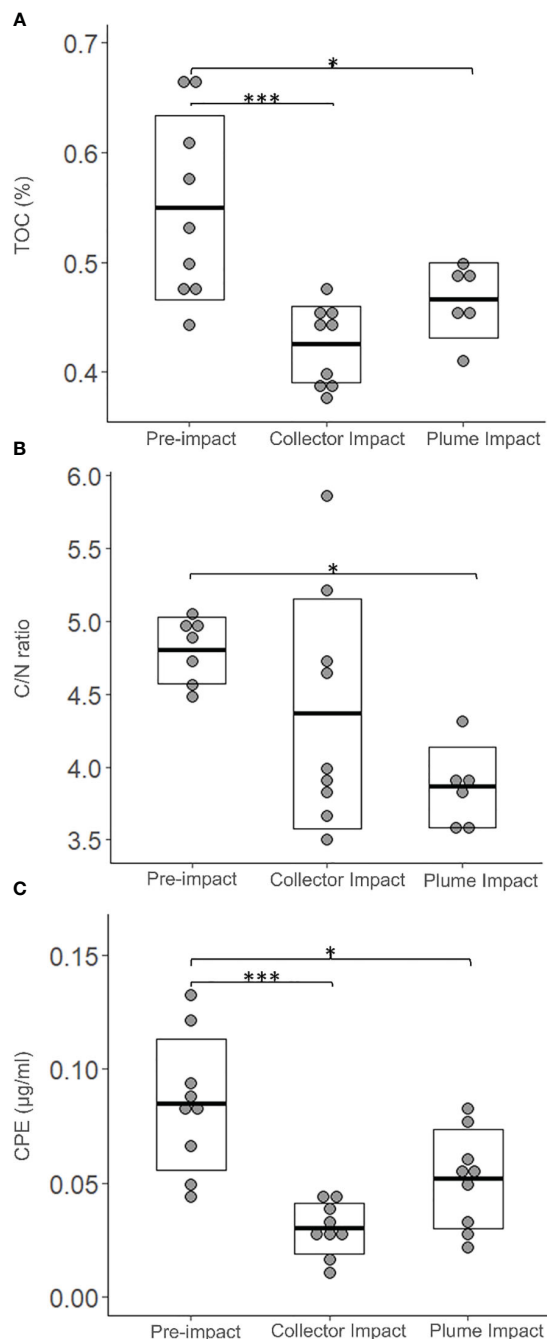


FIGURE 3
Environmental variables; (A) Total organic carbon (TOC, %), (B) Total organic carbon to Nitrogen ratio (C/N ratio) and (C) Chloroplast Pigment Equivalent (CPE, µg/ml) for each category sampled during the IP21 campaign (Pre-impact, Collector Impact, Plume Impact) along the 0–5 cm sediment depth layer. The crossbar and extent of the box represent the mean and standard deviation, respectively. Asterisks were added if Tukey HSD post-hoc tests were significant ($p < 0.05$ * and $p < 0.001$ ***).

Nematode abundance, diversity and genus composition are described in detail within the following section. For the remaining more important taxa, Copepoda and Polychaeta were found to be more abundant in the post-impact categories compared to the Pre-

impact samples, while an opposite patterns was seen for nauplii (Appendix Figure 4). However, no statistically significant differences were detected for these three taxa in terms of average abundances between impact categories ($p > 0.05$).

3.2.2.1 Nematode abundance and ASV diversity

Average total Nematoda abundance was highest for the Plume Impact samples and a comparable result was found for the ASV Shannon diversity (Table 2, Figure 4). In terms of ASV Richness, averages values were quite similar for the Pre-impact and Plume Impact samples, while lowest average ASV Richness was observed within the Collector impact samples. However, all biological indices showed considerable variability within each category (Table 2, Figure 4) and differences between the three categories were not statistically significant ($p > 0.05$). Post-hoc power analyses for the statistical test for the difference in total Nematoda abundance between impact categories (one-way ANOVA with factor “category”) revealed that the test had a medium to large effect size and that a total of 33 samples would be required within each category to achieve a desired statistical power of 0.80 ($\alpha = 0.05$, $\omega^2 = 0.09$, groups = 3). For the Nematoda ASV Shannon diversity, comparable results were found with a medium to large effect size and a required sample size of $n = 30$ for each category to obtain a statistical power of 0.80 ($\alpha = 0.05$, $\omega^2 = 0.10$, groups = 3). In contrast, power analysis was not possible for the Nematoda ASV species richness, as the calculated effect size was equal to zero.

3.2.2.2 Nematoda community composition

The most abundant nematode genus across all three impact categories was *Acantholaimus*, representing $\geq 15\%$ of the assemblage by relative read abundance (Figure 5). *Halalaimus*, *Desmoscolex* and *Chromadorita* also represented abundant genera in all categories, albeit with varying relative read abundance (4–15%). Other important genera included *Phanodermopsis* (4–11%) and *Theristus* (3–4%) in the Pre-impact and Plume Impact samples, while they showed much lower read numbers in the Collector Impact samples ($< 0.5\%$) (Figure 5). It has to be mentioned that both genera were not shared among all the category samples, with higher relative abundance restricted to one or two MUC deployments. The genera *Syringolaimus* and *Tripyloides* also showed considerable relative read abundances (5–6%) within the Collector Impact samples. *Tripyloides* was exclusively found in this impact category, but was only present in 20MUC. Within the Plume Impact samples, the genus *Anticoma*, represented 5% of the assemblage, while much lower read numbers ($< 0.5\%$) of *Anticoma* were found for the other categories (Figure 5). This genus occurred in the majority of the Plume Impact samples, except for 25MUC. Many other genera contributed to a lesser extent ($< 5\%$) to the overall nematode assemblage (Figure 5). However, no statistically significant differences were found in terms of Nematode ASV genus composition between impact categories (one-way Permanova, $p > 0.05$ and Permdisp test: $p = 0.03$).

4 Discussion

4.1 Abyssal nodule-rich sediments

Abyssal sediments in the CCZ area are characterized as fine-grained, mainly consisting of clay and silt, rich in manganese-oxide (MnO_2) (Pape et al., 2017; Hauquier et al., 2019; Volz et al., 2020). Primary productivity in the surface waters of the CCZ is oligotrophic, resulting in low particulate organic carbon (POC) fluxes to the seafloor ($< 2 \text{ mg C}_{\text{org}}\text{m}^{-2}\text{d}^{-1}$) (Lutz et al., 2007; Volz et al., 2018a). Pre-impact upper sediments (0–5 cm) within our study site exhibited environmental properties (e.g., silt $> 70\%$, clay $< 20\%$, TN $< 0.2\%$ and TOC $< 0.6\%$), comparable with previously described baseline conditions in the GSR exploration contract area (De Smet et al., 2017; Pape et al., 2017; Volz et al., 2018; Hauquier et al., 2019). Deep-sea nematode assemblages tend to be characterized by a few prevailing and many rare taxa (Lambshead and Boucher, 2003; Pape et al., 2017; Hauquier et al., 2019; Lefaible et al., 2023). This pattern was also observed in our study, with highest relative read abundance for the genera *Acantholaimus* and *Halalaimus*, which are commonly reported as dominant in nodule-bearing abyssal sediments (Pape et al., 2017; Hauquier et al., 2019; Macheriotou et al., 2020; Lefaible et al., 2023). Previous metabarcoding studies further corroborated that *Desmoscolex*, *Chromadorita* and *Phanodermopsis* are also important genera in terms of relative read abundance ($> 5\%$) in the CCZ (Macheriotou et al., 2020; Lefaible et al., 2023).

Nevertheless, significant geochemical and biological differences were found between baseline sediments taken at the Trial site during two consecutive campaigns (i.e., SO268 and IP21). Stations sampled immediately prior to the PATII trial (Pre-impact IP21) were characterized by significantly higher TOC contents and labile organic matter fractions (C/N ratios) than those sampled during SO268. Differences in food availability may be a consequence of temporal variation in seafloor POC flux but lateral heterogeneity in TOC has also been attributed to interactions between bottom-water currents, topographic relief (e.g., basins, ridges) and the degree of organic matter degradation (Lutz et al., 2007; Volz et al., 2018a; Vonnahme et al., 2020). Since benthic fauna in abyssal sediments is driven by sediment composition and food availability, it can be expected that spatial differences in these variables will also result in spatially patchy meiofaunal communities (Lambshead and Boucher, 2003; Pape et al., 2017; Hauquier et al., 2019; Kuhn et al., 2020; Washburn et al., 2019). Next to this natural heterogeneity on a larger spatial scale (i.e., between sampling stations), abyssal sediments and especially meiofaunal communities are known to exhibit small-scale heterogeneity even between cores of the same deployment (Rosli et al., 2018; Uhlenkott et al., 2021; Lefaible et al., 2023), which was also observed within this study.

For example, average nematode abundances of the Pre-impact IP21 samples varied considerably, ranging from 7 up to 145 ind./10 cm^2 . This high degree of variability regarding nematode abundance is probably also linked to the presence of polymetallic nodules, as they represent the only natural hard substrate in these areas and will

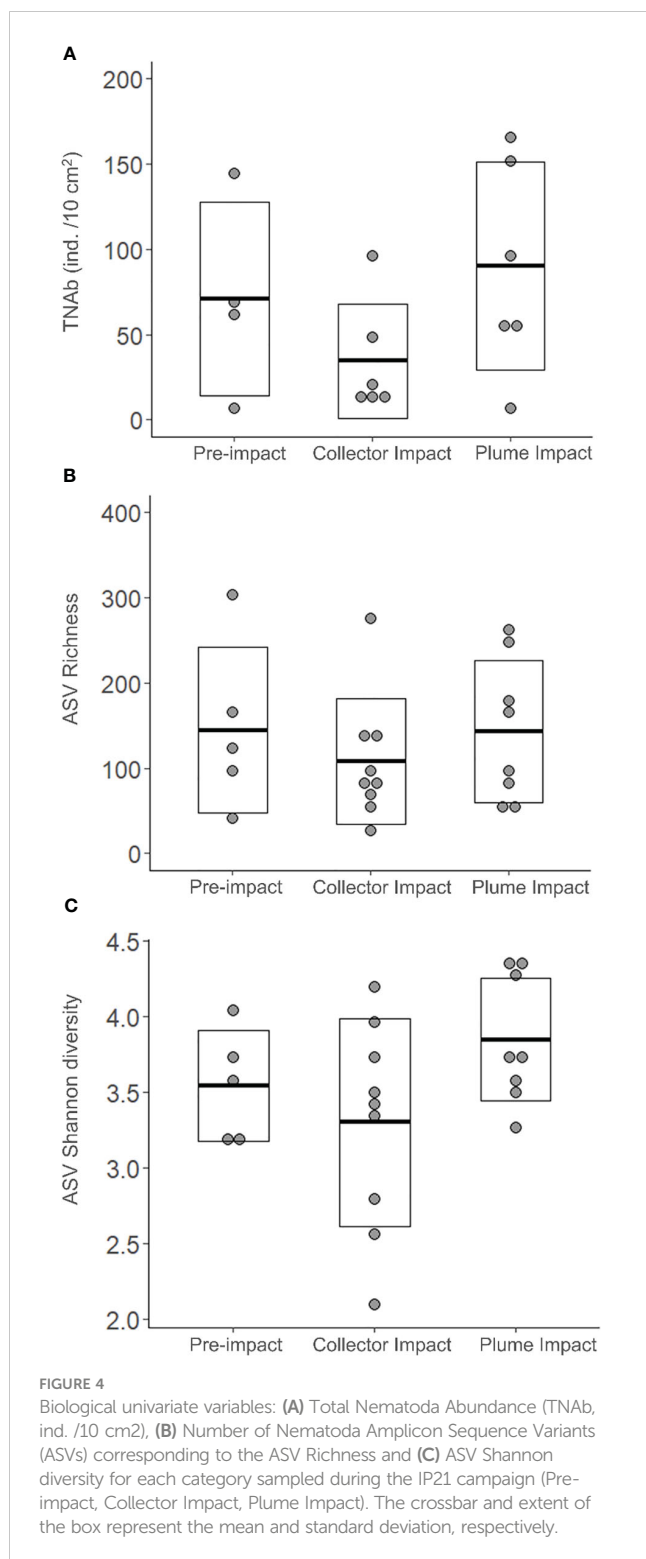
therefore influence local habitat complexity, which is considered to be an additional driver of benthic faunal distribution (Vanreusel et al., 2010; Zeppilli et al., 2016; Uhlenkott et al., 2021). A very low number of nematodes (7 ind./10 cm^2) occurred in one core sample that contained a large, superficial nodule, whereas higher (≥ 140 ind./10 cm^2) to moderate (60–70 ind./10 cm^2) abundances were observed in cores containing smaller nodules, located in deeper sediment layers (Appendix Figure 5). Reduced nematode abundance has consistently been found in nodule-rich areas compared to nodule-free areas (e.g. Miljutina et al., 2010; Singh and Ingole, 2016; Pape et al., 2017; Hauquier et al., 2019; Pape et al., 2021; Uhlenkott et al., 2021) and it has been proposed that a negative relationship between nodule presence and faunal abundance exists due to reduced sediment volume for nematodes (Pape et al., 2017; Hauquier et al., 2019). While the current increase in deep-sea research will provide better understanding of baseline conditions in proposed mining areas, we would like to emphasize that the high level of natural heterogeneity observed in our study has strong implications for effective impact assessments and distinguishing anthropogenic disturbances from natural variability (see section 4.3).

4.2 DSM-induced pressures

Studies such as ours, in which a sub-industrial scale vehicle was used, will be crucial to perform detailed DSM impact assessments and quantify the spatio-temporal extent of the effects that are induced during nodule collection. Therefore, the most pronounced DSM effects in the form of sediment removal, seabed alterations and enhanced sediment deposition are discussed separately throughout the following sections (4.2.1, 4.2.2) in an attempt to identify the mechanisms behind the different stressors and the associated meiofaunal responses. Based on our findings, we also provide methodological recommendations for future DSM research on benthic habitats (4.3).

4.2.1 Sediment removal and seabed alterations

The productive surface sediments in abyssal areas contain the bulk of food-associated compounds such as the labile organic matter pool and biota including meiofaunal organisms (Boetius and Haeckel, 2018; Volz et al., 2020; Vonnahme et al., 2020). Consequently, removal of this ecologically important bioactive layer during nodule mining is considered as one of the most severe DSM pressures, expected to alter meiofaunal distribution, biogeochemical processes and overall ecosystem functioning (Thiel, 2001; Boetius and Haeckel, 2018). Comparable to previous small-scale benthic impact experiments (Volz et al., 2020; Vonnahme et al., 2020; Lefaible et al., 2023), TOC and CPE concentrations in our study were significantly lower in the Collector Impact tracks relative to undisturbed Pre-impact samples, which suggests that sediment removal resulted in the reduction of food sources for benthic organisms in the form of fresh and more refractory organic matter (Volz et al., 2020). Although not significant, average nematode abundance and ASV diversity was also observed to decrease in parallel with the environmental changes in the directly impacted



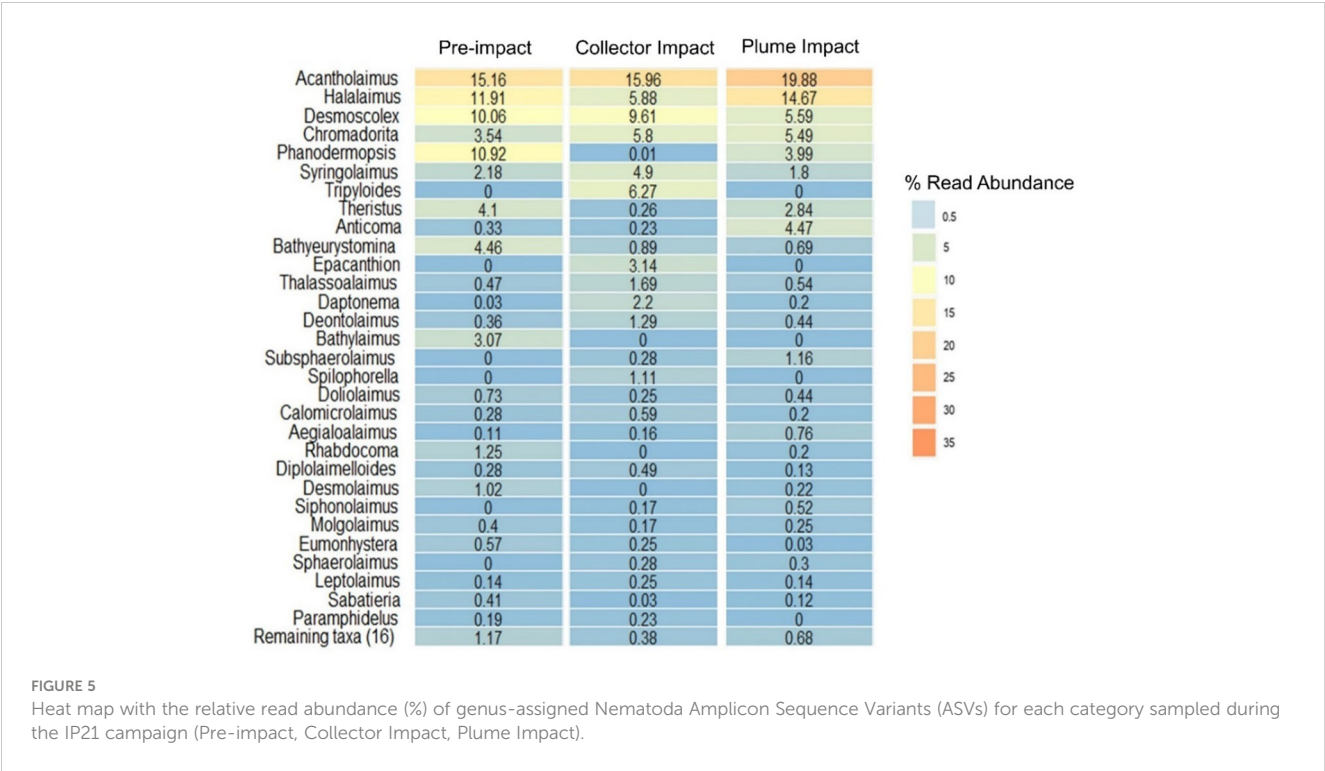
sediments, which is accordance with previously reported meiofaunal responses in most of the available benthic impact studies (Jones et al., 2018).

Despite the fact that there were no significant differences between categories in terms of nematode ASV genus composition, our results suggest that sediments within the PATII tracks contained less *Halalaimus* while *Syringolaimus* was more abundant. Both genera are commonly found in the deep-sea, but

Syringolaimus has been observed more frequently in environmentally extreme environments (e.g., sediments close to hydrothermal vents) and is believed to be more resistant to stress compared to *Halalaimus* (Zeppilli et al., 2015). *Syringolaimus* has also been described as nodule-associated with higher relative abundances inside nodule crevices compared to surrounding soft-sediment (Pape et al., 2021). Consequently, individuals of this genus might also be released into the exposed sediments due to nodule breakdown during the collection process. Moreover, *Triplyloides* was exclusively found in the Collector Impact samples. This observation is difficult to explain as this group is less common within deep-sea abyssal sediments and occurrences were restricted to one sampling station. Shallow-water studies state that this genus is an epistrate feeder, preying on ciliates (Bongers et al., 1991; Moens and Vincx, 1997; Moens et al., 2013) and has also been shown to show a negative correlation with Chlorophyll *a* concentrations (Moens et al., 1999). This finding, in combination with the fact that this genus is virtually absent in Pre-impact sediments, may indicate that it represents a colonizing taxon in the freshly disturbed sediments. However, whether this represents an active response must be confirmed by results of the follow-up study (SO295 campaign, 2022).

Our findings confirm the hypothesis that the hydraulic system used during nodule collection resulted in the removal of considerable fractions of the upper sediments (a minimum of 5 cm, Gazis et al., submitted)¹. However, our study also shows that in addition to sediment removal, complex and variable seabed alterations were created by the propulsion system of the collector vehicle. Seabed imagery obtained after the PATII trial revealed that the sediment patches in the wake of the collector vehicle are characterized by two main topographic features (Figure 6). First, there is a centrally located sediment strip, defined by relatively homogeneous sediment removal and upper layer topography. Secondly, the edges of tracks are manifested as parallel sediment strips with caterpillar imprints composed by alternating bands of depressions and ripples with the presence of unstable sediment clumps on top of the ripples and at the interface between the depressions and ripples (Figure 6). Vonnahme et al. (2020) already differentiated several microhabitats such as outside the track, subsurface/exposed patches, ridges and furrows based on visual observations in a 26-year old plough and a fresh (2.5 weeks) epibenthic sledge (EBS) track in the DISCOL Experimental Area in the Peru Basin. Several classes of disturbances were described, representing a gradient of environmental impact ranging from low (outside the tracks) to severe (removal of active surface layer) with variable microbial responses in each microhabitat (Vonnahme et al., 2020).

This variability in environmental disturbance implies that the degree of impact and associated meiofaunal responses will depend on the exact sampling position within the Collector Impact tracks, which was confirmed by a visual inspection of the high-resolution images of the sediment cores taken for morphological analysis (Appendix Figure 6). Sample 24MUC was TV-guided and one of the cores contained the lowest nematode abundance (12 ind./10 cm²), corresponding to a highly impacted area in the caterpillar depressions, where the dark, MnO₂-rich layer was almost



completely removed (Appendix Figure 6 and Table 3). In contrast, another core from the same MUC deployment had relatively high nematode abundance (98 ind./10 cm²) and showed an irregular surface relief with sediment clumps, indicating that this sample may have been taken within a ripple of the caterpillar profile (Appendix Figure 6 and Table 3). For the remaining deployments, exact sampling positions remain uncertain, but the qualitative description of the high-resolution images and AUV-derived photomosaics suggest that samples from 20MUC might have been taken at an interface patch at the edge between a depression/ripple with low nematode abundances (≤ 20 ind./10 cm²). Moreover, varying nematode abundances (15–50 ind./10 cm²) were observed in samples with relatively homogenous sediment

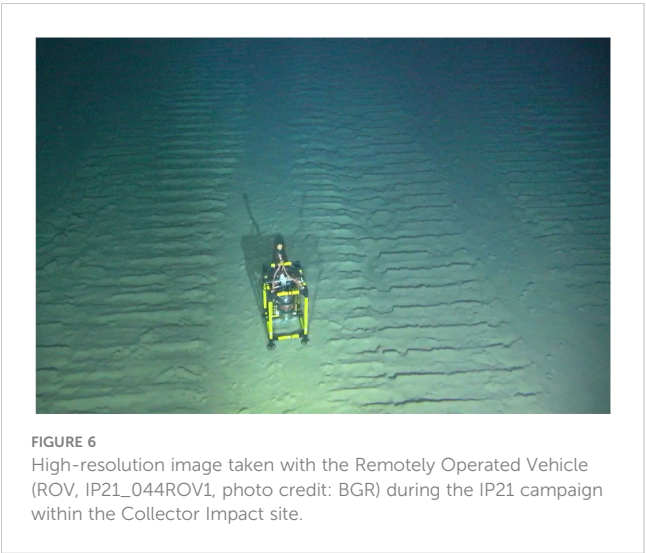
removal corresponding with the sediment strips between caterpillar imprints. These findings demonstrate that nodule collection will induce different sediment modification types on relatively small spatial scales (~meter), with biological responses dependent on the type and intensity of disturbance.

4.2.2 Collector-induced sediment plumes

Investigating the impacts of enhanced sedimentation on benthic habitats represents one of the biggest challenges within DSM research. This is mainly due to the dynamic character of the collector-induced sediment plumes, as they are influenced by many factors such as the nodule collection and separation methods, local seabed topography, sediment properties and hydrodynamic conditions (e.g., background turbidity, currents and near-bottom mixing) (Purkiani et al., 2021; Haalboom et al., 2022; Helmons et al., 2022; Muñoz-Royo et al., 2022; Gazis et al., submitted)¹. Nevertheless, enormous progress has been made in recent years in the techniques needed to characterize plume dispersal patterns (Purkiani et al., 2021; Haalboom et al., 2022; Helmons et al., 2022; Muñoz-Royo et al., 2022; Gazis et al., submitted)¹. Preliminary results from the plume monitoring and geochemistry analyses during the PATII trial showed that sediment dispersal occurred in a relatively narrow band along the predominant current direction. Sediment blanketing (2–3 cm) was observed up to ~100 m from the Collector Impact site, becoming thinner with distance and was not visible on AUV photos anymore beyond 2 km (Vink et al., 2022; Gazis et al., submitted)¹.

4.2.2.1 Blanketing outside collector tracks

Compared to Pre-impact conditions, samples collected within the Plume Impact site showed significantly higher CPE values and



enhanced nematode abundance and ASV diversity, although these increases in community metrics were not significantly different. These patterns are consistent with a small-scale disturbance (i.e., modelled sedimentation < 1 cm) experiment in the CCZ, where most pronounced meiofaunal enrichment was reported for limited modelled blanketing (< 3 mm) (Lefaible et al., 2023). Moreover, visual inspection of the high-resolution images of the sediment cores obtained for morphological analysis allowed us to approximate the level of sediment deposition in the form of changes in grain/flocculation size, or presence of black grained nodule material, and link this with the observed nematode abundances. This further confirmed that thickness of the deposited sediment was around 2–3 cm for the majority of the samples, with nematode abundance ranging from relatively high (≥ 150 ind./10 cm²) to moderate (60–100 ind./10 cm²). While our results verify that surrounding areas were subject to considerable blanketing, we want to emphasize that there are still many uncertainties regarding the geochemical and biological changes associated with anthropogenically-induced sedimentation.

For example, it was expected that the redeposition of the reactive sediment surface layers from the Collector Impact sites in the Plume Impact sites would result in higher or comparable food availability. In contrast, average CPE, TOC values and C/N ratios were significantly lower within the Plume Impact areas compared to Pre-impact sediments. It is known that sediment blanketing occurs in a gradual manner, with fractionation of the resettling particles with rapid deposition of heavier particles (Gillard et al., 2019; Purkiani et al., 2021; Muñoz-Royo et al., 2022). However, it remains unclear for now what influence the aggregate type or flocculation material of the plume (e.g., grain size and organic particle composition) will have on vertical element fluxes such as organic carbon absorption with the surface matrix and concomitant animal-sediment interactions (e.g., microbial-mediated degradation, benthic bioturbation and burrowing) (Haeckel et al., 2001; Arndt et al., 2013; Bianchi et al., 2021).

Mechanisms driving the observed nematode enrichment in the Plume Impact area are also currently unresolved but probable scenarios include either active upward migration of organisms from the original surface sediment now covered by the sediment from the plume, or deposition of suspended surface sediments present in the plume, or a combination of both (Mevenkamp et al., 2017, 2019; Lefaible et al., 2023). In the latter case, it is, however, unknown whether upward migration or deposition is the main contributor to the overall faunal fraction in adjacent sediments and to what extent the resettled fauna within the so-called “blanket” represent damaged/dead or alive organisms (Lefaible et al., 2023). Answering these important questions is further complicated by the sampling interval of 0–5 cm used in our study. Since the collector-induced sediment deposition thickness was approximately 2–3 cm, environmental and biological patterns in this study represent an integrated result from the blanketing layer and exposed, natural sediments.

4.2.2.2 Blanketing inside collector tracks

Rapid redeposition of the suspended particle plume was detected after the PATII trial (Gazis et al., submitted)¹ within the tracks, which corroborates findings from a numerical modelling

analysis after a small-scale disturbance experiment (Gillard et al., 2019; Purkiani et al., 2021). Layers of discharged sediment and ground nodule material were also observed for the majority of our high-resolution images of the Collector Impact samples (Appendix Figure 6). However, the level of blanketing thickness appears to vary depending on the exact position in the collector lanes with stronger sediment accumulation in the caterpillar depressions compared to the ripples (Gazis et al., submitted)¹. Depressions generated during a plough disturbance experiment, showed a low-density layer of deposited sediment and it was suggested that these furrows might act as an accumulation spot for particle resettlement (Vonnahme et al., 2020). The fact that these sediments within the Collector Impact site are subject to a sequence of multiple pressures (sediment removal, intricate seabed modification by collector vehicle and heavy blanketing) enhances the complexity with which mined areas will be affected. However, examining cumulative stressors is very difficult and it remains unclear whether the combination of collector-induced physical seabed disturbances and sediment deposition will result in additive, antagonistic or synergistic impacts on meiofaunal communities (Carrier-Belleau et al., 2021).

4.3 Future challenges: quantifying multiple sources of variability

The comparison of environmental conditions within the Pre-impact and post-impact categories of our study confirm the hypothesis that a considerable fraction of the upper sediment layer within the collector impact site was removed (a minimum of 5 cm, Gazis et al., submitted)¹ during nodule collection and that surrounding areas were subject to sediment blanketing (± 2 –3 cm). Although average values (i.e., reduced levels in Collector Impact sites, enhanced in Plume Impact sites) for the Nematoda community indices support that environmental changes will also have impacts on the associated biota, differences between categories were not statistically significant. Post-hoc power analyses did, however, reveal that nematode abundance and ASV Shannon diversity tests were characterized by moderate to strong effect sizes, indicating the biological relevance of changes in these variables (Field, 2013). Moreover, univariate biological tests had low statistical power and a substantial increase in samples size ($n = 30$ per category) would be required to achieve appropriate statistical sensitivity (power level 80%). As such we propose that the lack of significant biological results might be attributed to the insufficient replication combined with noticeable within-category variability. This conclusion further illustrates what can be considered as the main challenge of DSM research, namely: quantifying multiple sources of variability associated with i) the intricate collector-induced seabed disturbances and ii) the natural heterogeneity of abyssal sediments (Clark et al., 2020).

Nodule collection by the PATII vehicle induced a sequence of pressures (i.e., sediment removal, intricate seabed modification by collector vehicle and sediment blanketing), resulting in complex seabed modifications with different disturbance types such as i) central lanes of sediment removal, ii) alternating bands of

depressions/ripples in caterpillar tracks, ii) microscale variations in topography due to unstable sediment “clumps” and varying levels of sediment deposition in the Collector Impact site. It will therefore be necessary to distinguish and integrate these disturbances into the data collection of future benthic DSM studies. A proposed solution would be to apply a nested sampling design within the “Collector Impact” category, where sufficient replication is performed for the major subgroups (e.g., central lanes, depressions and ripples of caterpillar profile). It could also be opted to combine MUC samples with ROV push cores, which are taken in a more targeted manner owing to the live camera feed. As shown in this study, *a priori* methods like the visual inspection of high-resolution images from the collected samples can help to determine specific seabed alterations (e.g., estimation of position in PATII tracks, exact sedimentation level). Whereas our results indicate that both post-impact categories were affected by enhanced sedimentation, many uncertainties remain regarding the effects of sediment blanketing on overall seabed integrity. Regarding meiofaunal responses, one very important aspect will be to find methods (e.g., staining, DNA/RNA techniques) to determine whether the assemblages were alive or not at time of sampling. Moreover, continued monitoring will be crucial to identify the long term viability of the present populations.

Environmental and biological properties of the Pre-impact samples in our study fell within the range of previously described baseline conditions in the same area. Nevertheless, heterogeneity across small and large spatial scales has been a consistent pattern in the CCZ. As such, an important implication is that environmental variables should preferably be obtained from the same samples used for biological analyses. Furthermore, the sediment depth resolution (i.e., entire 0–5 cm layer) used in this study was not appropriate to distinguish redeposited from natural sediments in adjacent areas (i.e., Plume Impact category) where sediment blanketing was up to 3 cm. Therefore, it should be explored if it is feasible to adjust the slicing depth resolution of the core samples, depending on the expected sediment deposition thickness. Furthermore, we want to emphasize that findings in our study reflect immediate (i.e., one week after trial) benthic responses, and results from the follow-up SO295 campaign will provide additional insights on how the benthic environment has evolved over a period of 1.5 year (MI2, 2018). Lastly, given the observed inter-annual variability in terms of environmental and faunal baseline conditions in our study. Understanding natural temporal variability based on adequate time series and a sufficiently high number of replicates of abyssal benthic communities is therefore crucial for establishing and predicting the impacts of mining activities in deep-sea ecosystems.

5 Conclusions

Significantly lower total organic carbon, CPE (i.e., sum of Chlorophyll *a* and phaeopigments), and patterns of impoverished nematode assemblages within the Collector Impact area were observed in our study, which supports the hypothesis that nodule collection by PATII removed the ecologically important bioactive

surface layer. Besides this seabed alteration, impacted areas also experienced sediment blanketing (2–3 cm), with higher accumulation in caterpillar depressions, indicating different spatial intensities of disturbance. Collector-induced sediment blanketing occurred in adjacent areas outside the Collector Impact area, with sediment deposition up to 3 cm. These Plume Impact sediments exhibited lower food availability, and further research on the plume composition’s role in biogeochemical processes is needed. Mechanisms driving observed meiofaunal enrichment at Plume Impact sites (albeit not statistically significant), and the extent to which deposited fauna represent living individuals remain unclear. We conclude that DSM activities will affect benthic communities in a complex manner and with varying biological responses. These conclusions, together with the spatial and temporal variability in terms of Pre-impact conditions, highlight that future impact studies should incorporate increased replication, larger spatial coverage and long-term monitoring to address uncertainties and distinguish anthropogenic disturbances from natural variability in abyssal polymetallic nodule fields.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.1594/PANGAEA.941222>, <https://doi.org/10.1594/PANGAEA.941216>, <https://doi.org/10.1594/pangaea.de/10.1594/PANGAEA.942156>, <https://doi.org/10.1594/PANGAEA.942177>, <https://doi.org/10.1594/PANGAEA.952416>, <https://doi.org/10.1594/PANGAEA.952333>.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

NL: Methodology, Formal analysis, Writing – original draft. LM: Methodology, Formal analysis, Writing – review & editing. EP: Writing – review & editing. MM: Methodology, Writing – review & editing. MH: Project administration, Funding acquisition, Writing – review & editing. DZ: Writing – review & editing. AV: Project administration, Funding acquisition, Writing – review & editing.

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Macrobenthic communities in the polymetallic nodule field, Indian Ocean, based on multicore and box core analysis

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Managing deep-sea mining is challenging due to limited data on species and habitat distribution, hindering decision-making. In less-explored polymetallic nodule sites in the Indian Ocean, management approaches are deemed unfeasible due to a substantial lack of data. In this study, we aim to bridge the knowledge gap through a detailed analysis of the macrobenthos (fauna > 300 µm) obtained with two sampling gears (multi- and box corer) in potential impact, preservation, and reference zones in the Central Indian Ocean Basin. In total, 337 individuals were categorized into 106 morphospecies and 18 higher taxa. The lowest abundance was reported in the box corer. Nematodes and copepods dominated the macrobenthos community, whereas polychaetes and tanaidaceans were the more prevalent macrofaunal taxa. Carbohydrates and total organic carbon showed a notable positive correlation with polychaetes, isopods, and bivalves, indicating their influence on larger-sized taxa. Various functional traits, i.e., less motile, discretely motile, biodiffusors, and upward/downward conveyers, were dominant in the study area. The comparative analysis of multi- and box corer sampling reveals distinct patterns in macrobenthic diversity, emphasizing the importance of employing both gears for accurate functional analysis. The paper underscores the significance of including large meiofaunal taxa in macrofauna analyses for assessing deep-sea ecosystem functioning and services. Additionally, the findings contribute significantly to environmental baseline and impact assessment, a crucial aspect of sustainable management in a highly promising polymetallic nodule mining area, aligning with the goals of exploring.

KEYWORDS

macrobenthos, functional trait, biodiversity, sampling comparison, deep sea, ecology

1 Introduction

The blue economy is focused on sustainably harnessing the vast natural resources found in the depths of the ocean, including abundant reserves of oil, gas, and minerals in specific deep-sea regions. Of particular interest are its seafloor mineral deposits, which remain untapped (Hein et al., 2020) and encompass three primary types of economically significant deposits: polymetallic nodules (PMN), cobalt-rich ferromanganese crusts, and seafloor massive sulfides (SMS). Such deposits hold the potential to diversify the mineral supply to global industries (Petersen et al., 2016). In an era where the global trajectory is firmly set toward sustainability and technology-driven progress, the availability of critical minerals such as nickel and cobalt contained in PMN assumes greater importance as a lifeline for emerging technologies, including renewable energy systems, electric vehicle batteries, and advanced electronics (Hein et al., 2013). Thus, seafloor mineral deposits can contribute significantly to the sustainable development of the maritime domain and the broader global economy.

Globally, a total of four seafloor areas with abundant polymetallic nodule fields are known, i.e., the Clarion–Clipperton Zone (CCZ), Peru Basin, Penrhyn Basin (including the Cook Islands EEZ), and Central Indian Ocean Basin (CIOB) (Hein et al., 2013). These fields fall under the jurisdiction of the International Seabed Authority (ISA), as they extend beyond the national control of any single country. However, the granting of mining permissions has been stalled due to undefined complexities in environmental, social, and governance aspects related to deep-sea mining (Kung et al., 2021). Among the 31 exploration licenses with 22 nations, India has been allocated a license area for exploring polymetallic nodules and polymetallic sulfide areas in CIOB.

Deep-sea mining has the potential to cause significant environmental impacts, including biodiversity loss, slow recovery, and enormous spatial scales of mines for certain mineral resources (Van Dover et al., 2017). The impacts of deep-sea mining on microbial ecosystem services are also a concern and are rarely considered explicitly (Orcutt et al., 2020). Bottom trawling and mining activities can cause significant physical disturbance to the seafloor, leading to the loss of benthic habitats and the associated ecosystem services (Snelgrove, 1999; McLaverty et al., 2023). These services are critical for the ecological functioning of the deep ocean and provide a range of ecosystem services, including nutrient cycling, carbon sequestration, and habitat provision (Armstrong et al., 2010; Thurber et al., 2014). Therefore, it is crucial to assess the significance of potential large-scale environmental impacts, define “serious harm”, and consider ecosystem service benefits, resilience, postmining restoration costs, and cumulative impacts associated with deep-sea mining activities (Levin et al., 2016; Chen et al., 2022).

Moreover, the nodule fields of the abyssal plain are one of the deep sea’s biologically diverse and unique habitats. This habitat is mainly covered with solid substrate (mostly nodules) and otherwise soft sediment, which supports various benthic life forms (Smith and Demopoulos, 2003). These nodules boost biodiversity at regional scales, underscoring the importance of preserving regions subjected to nodule mining for biodiversity conservation (Smith et al., 2008). Furthermore, some experimental studies have revealed

predominantly negative disturbance effects (Bluhm, 2001; Ingole et al., 2001; Radziejewska, 2002; Miljutin et al., 2011; Jones et al., 2017). Predicting the impact of nodule mining remains challenging due to the limited ecological baseline knowledge of the area (Bridges et al., 2023). Therefore, to assess the potential consequences of future deep-sea mineral resource mining, a comprehensive baseline understanding of the fauna associated with nodule-rich regions is imperative. Earlier studies of the benthic fauna along the nodule areas of CIOB showed a high diversity with the dominance of foraminifera, sponges, anthozoans, mollusks, polychaetes, crustaceans, nematodes, and bryozoans in these habitats (Parulekar et al., 1982; Pavithran et al., 2009b; Simon-Lledó et al., 2019; Singh et al., 2019; Chuar et al., 2020; Washburn et al., 2021), but a relatively lower mean density of macrofauna was recorded compared to other PMN areas in the global ocean (Parulekar et al., 1992; Ingole and Koslow, 2005; Pavithran and Ingole, 2005; Ingole et al., 2005b; Pavithran et al., 2009b). These studies contributed to high species richness and species rarity; however, many deep-sea species are still undescribed by taxonomists (Smith et al., 2006). Recently, a few newly described species and first reports have emerged from CIOB, including species of Copepoda, Bivalvia, Bacteria, and Cirripedia (Chan et al., 2020; Jang et al., 2020; Lee et al., 2020; Lim et al., 2020).

Various sampling techniques and methodologies can be used to sample the benthic fauna, raising questions about the comparability of the data, especially when the objectives involve discerning spatial or temporal differences. In accordance with the recommendations for environmental assessment set forth by the Legal Technical Commissions (LTC) issued by ISA (ISBA/16/LTC/7, 2010; ISBA/25/LTC/6/Rev.1, 2020), most studies on macrofauna have been conducted using box corers (Supplementary Table S1), with the prevalent use of pseudoreplicates, especially in CIOB (Parulekar et al., 1982; Ingole et al., 2005b; Pavithran et al., 2009b). Thus, macrofaunal biodiversity and community structure in the abyssal fields of the CIOB remain poorly characterized, representing just one aspect of a larger picture. Determining the optimal choice for sampling gear or sample processing method is not always straightforward and is often constrained by available equipment and cost considerations. This gives rise to challenges related to efficiency, comparability, and data integrity. Despite recent advancements in sampling equipment and analytical approaches, benthic ecologists frequently adhere to methods employed in earlier studies. However, recent investigations have brought to light that the multicorer offers certain advantages over the box corer, with the latter exhibiting a 2.9-fold underestimation in macrofauna density (Montagna et al., 2017). Additionally, the multicorer proves effective in capturing undisturbed sediment layers, addressing concerns related to sampling accuracy.

In the present study, we undertook an inquiry to address crucial questions regarding the macrobenthic community within the framework of a potential polymetallic nodule mining area: (1) Are there noticeable differences in community structure, density, species/family richness, evenness, and functional composition between potential reference and mining sites? (2) What environmental factors play a role in shaping communities? Additionally, we delve into the intriguing question

of (3) whether the choice of sampling gear can influence the derived univariate indices and ultimately contribute to shaping the observed community pattern and functional structure. For these purposes, we conducted a comparison between the utilization of the box corer and multicorer in proposed mining areas within the CIOB, focusing particularly on the low-productivity zone where each individual holds significant importance. While we aim to answer the questions raised above, the primary aim of this study is to establish baseline information on macrobenthic communities and their interactions with the environment along the PMN region of the CIOB.

2 Materials and methods

2.1 Study area

The CIOB has a northward opening in the Bay of Bengal. The approximate dimensions of CIOB are 6,000 km long and 1,600 km wide, and its depth reaches 6,090 m (MoES, 2020). Indian Ocean Nodule Field (IONF) is situated in the CIOB, extending approximately from 9°S to 16°30'S and 72°E to 80°E, stretched across 739,260 km², and refers to a commercially exploitable portion of the nodule field (Mukhopadhyay et al., 2008).

In the IONF, a number of seamounts of various characteristics and dimensions were identified, along with abyssal hills. Afanasiy Nikitin seamount in the central part of the basin is well known for cobalt crust deposits (Glasby, 2010), which reach a maximum height of 1,549 m (Krishna, 2003). These seamounts and abyssal hills make the habitat more complex for the faunal component and enhance the diversity of the region. The Ganges–Brahmaputra fan appears to be a long distance from the CIOB, but the fan extends up to about 10°S and influences the nutrient chemistry in the IONF (Krishna, 2003; Mukhopadhyay et al., 2008).

The average primary productivity in CIOB is 3.72 mg C m⁻³ day⁻¹ at the surface and a mean of 51.23 mg C m⁻² day⁻¹ in the water column (0–150 m), respectively (Matondkar et al., 2005), which is considerably lower than in other oceans. Subsequently, the low organic content in surface sediments (0.07%–0.41%) is attributed to low sediment accumulation rates in the CIOB (Pattan et al., 2005). CIOB exhibited a high microbial diversity (Shah et al., 2024), and abundance was found to be three times greater than the earlier reported range of 10⁸ to 10⁹ CFU g⁻¹ dry sediment in other oceanic waters around the globe (Raghukumar et al., 2001). Thus, the IONF and adjacent area in CIOB are very complex, and it is important to understand their full biological resources with respect to geomorphology and geophysical parameters.

In the year 2013, a block of ~ 13.875 km × 13.875 km (7.5° × 7.5°) was designated as the Impact Reference Zone (IRZ) and Preservation Reference Zone (PRZ) for conducting the trials of mining in CIOB (MoES, 2020). Within this area, the sediment samples were collected in July 2019 on board R/V Sindhu Sadhana (cruise No. SSD062) at three sites in the licensed area for PMN exploration, approximately 100–120 km apart (Figure 1A; Supplementary Table S2, panel A). The PRZ represents a

reference site and should not be influenced by any mining activities or impacts in the future. The IRZ is designated as an Impact Reference Zone, where the benthic impact experiments and possible mining will take place. Site BC20 will also be used as a reference site located in the north of both sites.

2.2 Sampling strategy

Two sampling gears were used to collect seafloor sediment, viz. multi-corer (Bowers & Connelly Corer, USA; 10 cm inner diameter; Figures 1B, C) and USNEL spade box corer (0.25 m²; Figure 1D). The multicorer with eight core liners and box corer were deployed thrice and twice at each site, respectively. Due to time constraints and logistical limitations, the number of samples collected had to be restricted. The lowering speed of the spade core was about 0.6 m s⁻¹ until the 100-m altimeter reading, then after the spade corer was lowered at 0.1 m s⁻¹ to minimize the impact (i.e., bow wave). Each deployment of gear retrieved relatively undisturbed sediment from the sea floor. The depth of vertical sediment penetration varied between ~ 15 cm and 35 cm, depending on the sediment surface and nodules present. The upper 0–10 cm of sediment layer only was used. The overlaying water in multi- and box corer was also added to the topmost layer of the sediment. Each box corer (0–10 cm) was completely used for macrobenthic faunal extraction according to ISBA guidelines (ISBA/25/LTC/6/Rev.1, 2020). Among the three operations of multicorer deployment, two core liners from the first two operations and a single core liner from the third operation were used for macrobenthic faunal extraction, bringing it all together five core liners from each site (Supplementary Table S2, panel A). Thereafter, two core liners taken from the same operation were analyzed separately, but the data were merged (averaging), making them single core liner data as per the ISBA guidelines (ISBA/25/LTC/6/Rev.1, 2020). Therefore, the used replicates will be three and two per site for multi- (0.039 m² area coverage) and box corers (0.5 m² area coverage), respectively. Another two core liners were taken for estimation of environmental parameters. The remaining core liners were used for separate biological, chemical, and geological studies (not included in this study). Thereafter, the core liner samples for macrobenthic fauna and environmental analysis were vertically sectioned at depths of 0–1 cm, 1–2 cm, 2–3 cm, 3–4 cm, 4–5 cm, 5–6 cm, 6–8 cm, and 8–10 cm below the sediment surface. The polymetallic nodules brought with gears were carefully removed with minimum disturbance and washed to remove any associated fauna. The final abundance data merged these faunal contents with the 0–1-cm layer of multicorer samples. The macrobenthic samples of the single core liner from each operation ($n = 3$) and the first box corer were fixed and stored in 10% borax-buffered formalin and 0.5% Rose Bengal. Other samples of the single-core liner from the first two operations ($n = 2$) and the second box corer were directly preserved in 80% denatured ethanol and stored in a – 80°C deep freezer along with all samples of environmental parameters until further laboratory analysis.

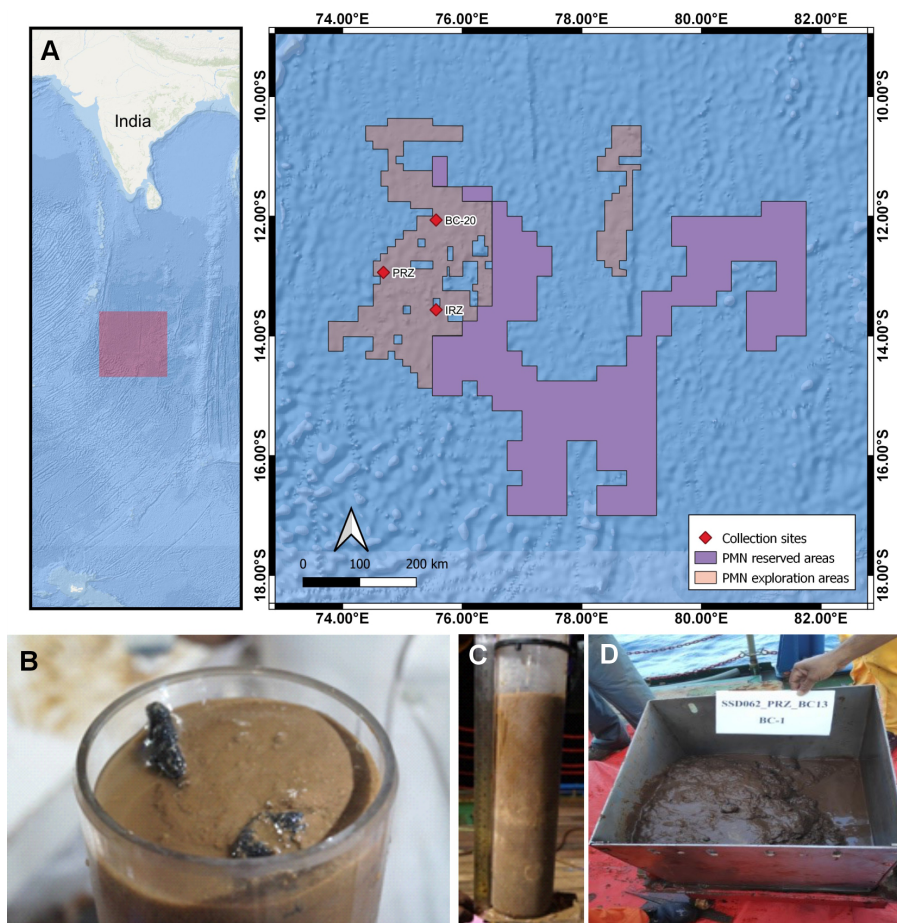


FIGURE 1

Geographic map of sampling sites and sample retrieval in the PMN exploration area, Central Indian Ocean Basin. Multi- and box corer samples were obtained from each site (see [Supplementary Table S2](#)). The map was generated using QGIS open-source software and bathymetric data used from GEBCO 2020. (A) Map showing the location of sampling sites. (B, C) Onboard-taken multicorer retrieved with full undisturbed sediment layers along with polymetallic nodules. (D) Box corer with uneven surfaced sediment sample after siphoning the overlaying water.

2.3 Data analysis and processing

2.3.1 Environmental parameters

The physical properties of the water column (i.e., conductivity-based salinity, temperature, depth) were measured with a CTD profiler (SBE 25, Seabird, USA) equipped with a dissolved oxygen (DO) sensor (RINKO, ALEC, 108 Japan). The seawater was collected for measuring DO and salinity using Niskin bottles connected to a CTD rosette for calibration and standardization of CTD probes.

Two core liners of the multicorer were used for pore water extraction and one liner each for geochemical and sedimentological analysis. All cores were sub-sectioned at 2 cm intervals up to 10 cm and subsequently at a 5-cm interval from 10 cm to 20 cm and stored in a deep freezer at -20°C for further analysis in the laboratory. The porewater for the sediment was extracted onboard using a refrigerated centrifuge at 5,000 rpm under 2°C temperature. The supernatant was filtered through $0.42\ \mu\text{m}$ syringe filters (Merck, Germany) and stored in the refrigerator/deep freezer till further analysis.

The pH, $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_2^{-}\text{-N}$, $\text{NO}_3^{-}\text{-N}$, $\text{SiO}_2^{-}\text{-Si}$, and salinity were estimated for the pore water of each sediment layer, including sediment–water interface (SWI). The pH and salinity were measured using a multiparameter (Hanna, HI98194) calibrated with pH and salinity-known solutions before use. DO in seawater was fixed with the addition of Winkler A and Winkler B reagents, following [Grasshoff et al. \(1999\)](#). Care was taken to ensure that there were no air bubbles while collecting the seawater. The precipitate was dissolved in H_2SO_4 , which was later titrated against sodium thiosulfate using Dosimat (Metrohm, Switzerland). The endpoint was determined by adding starch as an indicator. A precision of $\pm 0.6\%$ was obtained.

The nutrients such as $\text{PO}_4^{3-}\text{-P}$ ($\mu\text{mol L}^{-1}$), $\text{NO}_2^{-}\text{-N}$ ($\mu\text{mol L}^{-1}$), $\text{NO}_3^{-}\text{-N}$ ($\mu\text{mol L}^{-1}$), and $\text{SiO}_2^{-}\text{-Si}$ ($\mu\text{mol L}^{-1}$) were analyzed using 25 mL of seawater and 5 mL of porewater (diluted five times) for each nutrient by following the standard procedure of [Grasshoff et al. \(1999\)](#). The calibration was carried out using several working standards with respect to each nutrient's concentration range. A precision of $\pm 3\%$ was obtained.

The second stored (deep freezer at -20°C) sediment liners retained for geochemical and sedimentological analysis were subjected to estimations of total organic carbon (TOC; %), sand (%), silt (%), clay (%), total carbohydrate (CHO; mg g^{-1}), total protein (PRO; mg g^{-1}), total lipid (LIP; mg g^{-1}), and labile organic matter (LOM; mg g^{-1}). The total organic carbon was analyzed on bulk powdered samples by a flash dynamic combustion method using the Thermo Scientific FLASH 2000 Series Nitrogen and Carbon (CN) analyzer at CSIR-NIO, Goa, India. The total inorganic content was determined using the UIC CM 5014 Coulometer. The CaCO_3 weight percent was calculated by multiplying the values of inorganic carbon with the ratio of molecular weight of CaCO_3 to C (i.e., 8.33). $\text{CaCO}_3\% = \text{total inorganic carbon} \times (\text{molecular weight of } \text{CaCO}_3 / \text{atomic weight of carbon})$ (Johnson et al., 2014). The total organic carbon content was calculated by subtracting the total inorganic carbon from the total carbon. The sand contents were determined after wet sieving through a $62\text{ }\mu\text{m}$ sieve, and silt ($63\text{--}2\text{ }\mu\text{m}$) and clays ($< 2\text{ }\mu\text{m}$) were determined by standard pipette analysis (Buchanan, 1984).

LOM was calculated as a sum of PRO, CHO, and LIP per gram of dried sediment. For this, approximately 5 g of sediment samples from each subsection were air-dried and stored at room temperature. These air-dried samples were then used to estimate the concentration of PRO, CHO, and LIP. PRO was estimated using Folin–Ciocalteu’s phenol reagent. Bovine serum albumin (BSA) was used as a standard. CHO was estimated using the phenol sulfuric acid method. Glucose was used as a standard. LIP was extracted from dried sediment samples using direct elution with chloroform, methanol, and water in 10:5:2 ratios, respectively. The extracts were oxidized with acid dichromate, and spectrometric readings were recorded. Steric acid was used as standard. All three biochemical compounds were performed by the latest modified methods (Danovaro, 2010).

2.3.2 Macrobenthic analysis

In the laboratory, each soft sediment layer was first sieved through a mesh size of $300\text{ }\mu\text{m}$ using filtered seawater (Pavithran et al., 2009a) for meaningful comparison to previous studies (Wilson, 2017; Yu et al., 2018; Bonifácio et al., 2020). The material retained on the sieves was stored in 10% borax-buffered formalin or 80% denatured ethanol and stained with 0.5% Rose Bengal. The stained specimens were entirely picked up for identification purposes. In many deep-sea studies, macrofauna typically refers to organisms retained on a $300\text{ }\mu\text{m}$ sieve and often includes a substantial number of meiofaunal taxa (e.g., Nematoda, Harpacticoida, and Ostracoda) (Hessler and Jumars, 1974). These taxa, including various species and life stages, easily pass through the sieve and are frequently overlooked in macrofauna analyses (Hessler and Jumars, 1974; Wilson, 2017; Washburn et al., 2021). While some studies classify certain meiofaunal taxa as macrofauna, this inconsistency complicates comparative analyses (Ingole et al., 2001; Choi et al., 2004; Yu et al., 2018; Lins and Brandt, 2020; Kaiser et al., 2024). Exclusive meiofaunal taxa, i.e., Nematoda, Copepoda, Ostracoda, Acari, and Kinorhyncha, retained on the $300\text{ }\mu\text{m}$ sieve, are referred to as meiofauna taxa in

macrofauna in the current study. The rest is considered macrofauna “*sensu stricto*”. All fauna retained on a $300\text{ }\mu\text{m}$ sieve are thus called macrobenthos here (Macrobenthos = Macrofauna + Meiofauna; “*sensu lato*”).

An attempt has been made to record and identify all the macrobenthic fauna to the highest possible taxonomic resolution (phylum/class/order). Polychaeta, Copepoda, Isopoda, Tanaidacea, and Nematoda were further identified to family/genus/species level were given morphospecies numeric labels (Supplementary Table S3). Some specimens were damaged or broken and therefore could not be properly identified at the family/genus level. Such specimens were identified to the lowest possible taxonomic level, included in a group of many species (denoted by “.spp”), otherwise included in a single group (denoted by “.sp”), with the same morphological features. The biomass was estimated in wet weight by weighing the taxa in each replicate collectively using the high-precision microweighing balance. The macrobenthic density and biomass were standardized and represented in individuals per square meter (ind. m^{-2} ; $\pm \text{SE}$) and grams per square meter (g m^{-2} ; $\pm \text{SE}$), respectively, after averaging the core liner data to a single core for the same multicorer operation. All the specimens were deposited in the institutional museum at the CSIR-National Institute of Oceanography (CSIR-NIO) under Dr. Sabyasachi Sautya.

2.3.3 Functional trait analysis

The traits of mobility and reworking capability were used to determine the bioturbation potential (BP) (Queirós et al., 2013). Although bioturbation potential is not a direct measure of the bioturbation process, this allows us to understand the capability of the community to perform bioturbation. BP used in time series observations may help in understanding the coupled impact of sediment and organisms and may complement managemental policies (Painting et al., 2013; Van Hoey et al., 2013). Mobility (Mi) and sediment reworking (Ri) scores were calculated after Queirós et al., 2013 (Table 1; Supplementary Table S4), although other studies were also taken into consideration if the information was lacking (Fauchald and Jumars, 1979; Jumars et al., 2015). Where scores were not available for any species, the scores of the closest relative species were used, or the highest possible taxonomic level. Finally, these traits were weighted by species abundance for representation. Furthermore, the community-weighted mean (CWM) matrix of the trait data was computed using site $\times$ species (abundance data) and species $\times$ traits matrices (trait data). The CWM was then employed to assess differences in functional structure between sites and sampling gears.

2.3.4 Statistical analyses

The small sample size just spanning three sites, each over 100 km apart, can restrict the significance of statistical analysis substantially. However, the use of robust statistical methods (e.g., Anderson, 2001; McCune and Grace, 2002) allows for valuable insights even with limited samples. The spatial distribution of sampling sites, although far apart, provides a broad environmental gradient that is essential for understanding regional biodiversity patterns. Therefore, a robust statistical

TABLE 1 Traits, categories, and scores used for functional analysis of macrobenthos in the Central Indian Ocean Basin (CIOB) following Queirós et al. (2013).

Score	Categories	Characteristics
Mobility (Mi)		
1	Sessile/fixed tubes	Living in a fixed tube or sessile likewise
2	Less motile	Limited movement
3	Discretely motile	Slow, free movement through the sediment matrix
4	Motile	Free movement, that is, via burrow system
Sediment reworking (Ri)		
1	Epifauna	Epifauna that bioturbated at the sediment-water interface; living on nodules
2	Surficial modifiers	Diffusive sediment on the surface or just below the surface; moving particles horizontally on the surface
3	UC/DC	Upward and downward conveyors
4	Biodiffusors	Fauna that performs diffusive transport of sediment; move particles in a random manner over short distances
5	Regenerators	Regenerators that excavate holes, transferring sediment at depth to the surface

analysis approach was considered in the present study. The macrobenthic community structure (macrobenthos; “*sensu lato*”) and functional community weighed mean (CWM) were tested for significant differences between and within sites and sampling types using PERMANOVA (Anderson et al., 2008). Prior to this, the Bray–Curtis similarity matrix was calculated on square root-transformed abundance data and the Euclidean distance matrix on abundance-weighted sum of traits. Permutation of residuals under a reduced model method was implemented with Type III (partial). A Monte Carlo test was also performed in parallel to confirm the test results due to the small dataset. The univariate indices were calculated as a measure of diversity, i.e., species richness (S), total abundance (N), Shannon–Wiener index (Shannon and Weaver, 1949) for diversity (H') using $\log_e$, Margalef’s index (Margalef, 1968) for species richness (d), and Pielou’s index (Pielou, 1969) for species evenness (J').

The significant differences in the indices between the sites were analyzed with generalized linear models (*glm* function) using the “stats” package (R Core Team, 2021), negative binomial generalized linear models (*glm.nb* function) using the “MASS” package (Venables and Ripley, 2002), and Generalized least-squares models (*gls* function) using the “nlme” package (Pinheiro et al., 2021) in R. Prior to this, the data were checked for normal distribution using the “ggplot2” package (Wickham et al., 2024), where frequency was plotted against each parameter. Multicollinearity and homogeneity of variance were checked with the *vif* and *levene.test* functions using the “car” (Fox and Weisberg, 2019) and “Lawstat” packages (Hui et al., 2008), respectively. The overdispersion statistic was used for each model using the “deviance

divided by *df.residual*” formula to select the type of link function in the model. The *glm.nb* and *gls* were performed for overdispersion and heterogeneity of variance. For underdispersion, the *log* link function was used in the model. The AIC was also used to select the best-fitted model among others. An ANOVA test (type II sum of squares) was performed to conduct the *F*-test on the *glm* model outputs and the Wald Chi-squared test on the *gls* model outputs (Henseler et al., 2019).

The Spearman’s rank correlation was used to test the relationship between univariate indices and environmental variables. The statistical significance of the correlation coefficient was tested using a significance level of $p < 0.05$. Prior to this, the Shapiro–Wilk test was used for the data normality test, and all variables were found to have a nonnormal distribution. The relationship between the environmental parameters and macrobenthic community structure was ascertained by canonical correspondence analysis (CCA). The data (multi- and box corer) used for CCA were untransformed data for both the macrobenthic community and environmental parameters. Only environmental factors with significance were displayed with a high frequency of morphospecies. The CCA was performed with the *cca* function using the “Vegan” package in R (Oksanen et al., 2020).

The univariate indices and PERMANOVA were performed using PRIMER v6 (Clarke and Gorley, 2006; Anderson et al., 2008). The CWM data were estimated with the *cwm* function using the “BAT” package (Cardoso et al., 2015). The environmental parameters were depicted in a line graph using the “ggplot2”, “dplyr”, and “tidyr” packages (Wickham et al., 2023; Hadley et al., 2024; Wickham et al., 2024). This approach facilitates a clear visualization of sediment depth variations through color gradients. Each color signifies the actual values at specific intercepts (e.g., PRZ, 0–2 cm), while values between these points lack numerical representation on the axis and thus cannot be compared directly. The Venn diagram using the “Venn” package (Dusa, 2021) and the CCA plot were created using R programming software (R Core Team, 2021). The map was accomplished in QGIS 3.10 (QGIS Development Team, 2020) with an open-source bathymetric data gradient (Weatherall et al., 2019).

3 Results

3.1 Environmental settings

The analysis of the sediment–water interface at the three analyzed sites yielded several noteworthy findings. Temperature remained consistent at 1.45°C among all sites with no variability (Supplementary Table S2, panel B), while DO levels were highest at PRZ ($7.50 \pm 0.95 \text{ mL L}^{-1}$), followed by IRZ ($6.04 \pm 0.56 \text{ mL L}^{-1}$) and BC20 ($5.61 \pm 0.40 \text{ mL L}^{-1}$; Supplementary Table S2, panel B). The mean salinity displayed minimal variation, with values ranging from 31.49 ± 0.31 psu (IRZ) to 31.76 ± 0.08 psu (PRZ). The highest pH (8.17 ± 0.04) was recorded at PRZ, while the lowest was observed at BC20 (8.01 ± 0.03), with subsurface pH exhibiting lower values in sediment layers below 6–8 cm vertical depth (Figure 2). Subsurface salinity, measured from pore water analysis, showed relatively low

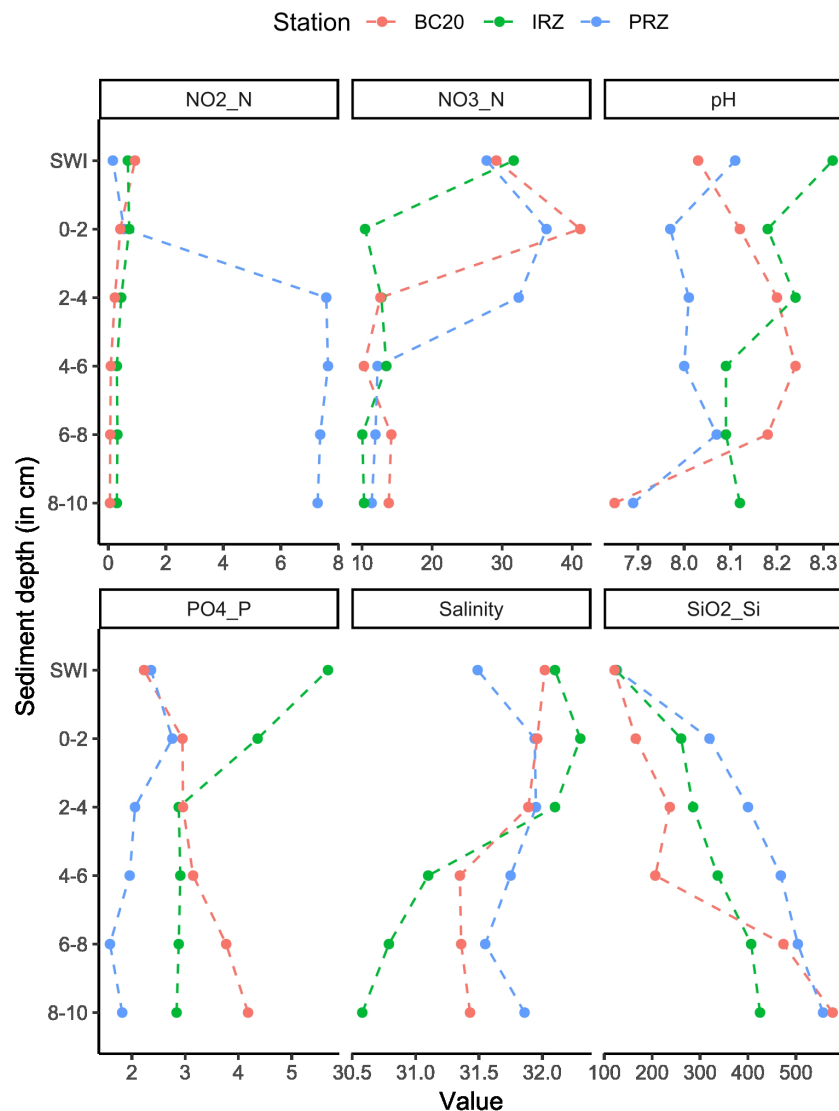


FIGURE 2

Variation in the environment parameters pH, salinity (psu), $\text{PO}_4^{3-}\text{-P}$ ($\mu\text{mol L}^{-1}$), $\text{NO}_2^{-}\text{-N}$ ($\mu\text{mol L}^{-1}$), $\text{NO}_3^{-}\text{-N}$ ($\mu\text{mol L}^{-1}$), and $\text{SiO}_2^{-}\text{-Si}$ ($\mu\text{mol L}^{-1}$) at the sediment–water interface (SWI) and in interstitial waters (pore water) up to 10 cm sediment depth across three sites (BC20, PRZ, and IRZ) in CIOB (see Figure 1 for locations).

variation except for the IRZ's bottom layer, where salinity ranged between 30.5 psu and 31.1 psu. Nutrient profiles varied considerably between sites and vertical depths. Phosphate profiles varied between sites and vertical depths. Phosphate values at the sediment–water interface were similar in PRZ and BC20 ($2.36 \mu\text{mol L}^{-1}$), but much lower than IRZ ($5.68 \mu\text{mol L}^{-1}$). Subsurface pore water phosphate ($\text{PO}_4^{3-}\text{-P}$) profiles indicated the highest values at BC20's 8–10 cm depth ($4.18 \mu\text{mol L}^{-1}$) and less variation ($1.59\text{--}2.36 \mu\text{mol L}^{-1}$) throughout vertical depths in PRZ, whereas IRZ's bottom sediment showed the highest values at surface layers and lower values at 8–10 cm depth ($2.84 \mu\text{mol L}^{-1}$). Nitrite ($\text{NO}_2^{-}\text{-N}$) concentrations were similar throughout the vertical profile of IRZ and BC20; however, higher concentrations were reported in the subsurface layers of PRZ. Nitrate ($\text{NO}_3^{-}\text{-N}$) concentrations were higher at surface layers than subsurface layers. Contrastingly, silicate ($\text{SiO}_2^{-}\text{-Si}$) values gradually increased with the depth sediments at all the sites (Figure 2).

TOC levels in sediments were relatively consistent across sites, with the lowest at PRZ (0.35%) compared to IRZ (0.40%) and BC20 (0.47%). TOC decreased with depth (Figure 3). CHO, protein, and LOM exhibited similar distribution patterns, with higher values in surface sediment layers, while lipid content was higher in subsurface layers around 2–4 cm sediment depth (Figure 3). Surface sediments were primarily composed of clay, ranging from 65.06% to 74.02% in all three sites (Supplementary Table S2, panel B), with silt being the second most dominant grain size (22.96% to 31.7%). Sand particles played a minor role in sediment texture. The sediments were predominantly characterized by pelagic dead radiolarian remains, composed of clay, fine silt, and very coarse silt particles, which exhibited similar properties in BC20 and IRZ compared to PRZ. Subsurface sediments (0–10 cm vertical) displayed greater variability in texture properties, indicating that BC20 and IRZ shared a similar sediment particle origin compared to PRZ. Clay

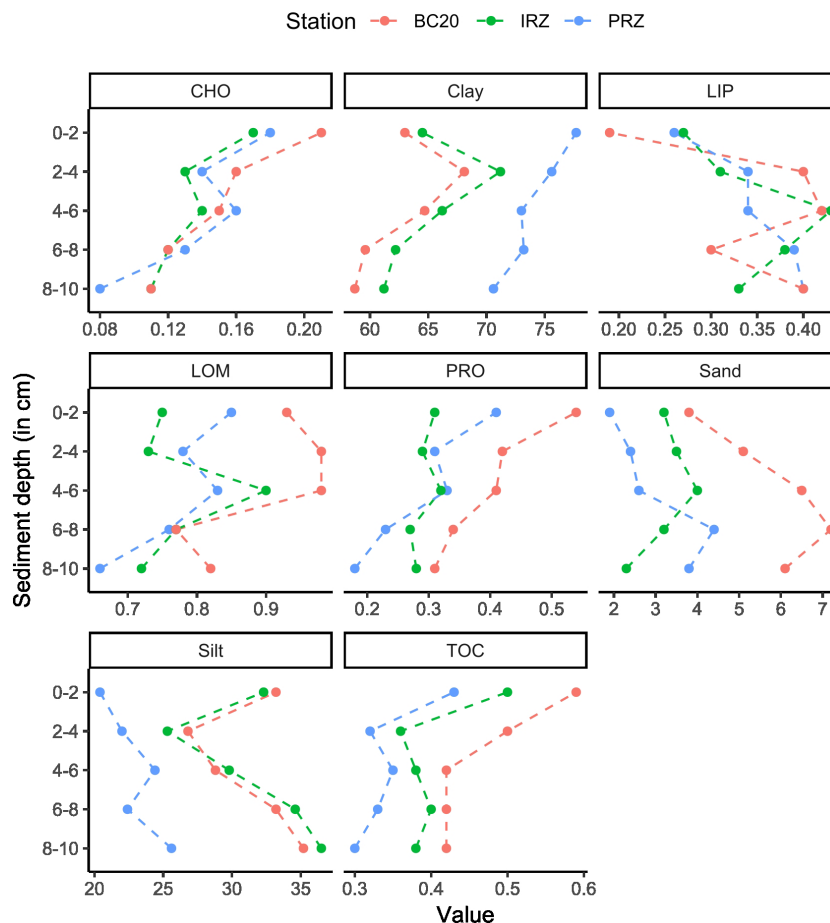


FIGURE 3

Total organic carbon (TOC; %), total carbohydrate (CHO; mg g^{-1}), total protein (PRO; mg g^{-1}), total lipid (LIP; mg g^{-1}), labile organic matter (LOM; mg g^{-1}), sand (%), silt (%), and clay (%) in the top 10 cm sediment layers across three sites (BC20, PRZ, and IRZ) in CIOB.

content was notably higher in the upper fractions (0–2 cm, 2–4 cm, and 4–6 cm), while the bottom fractions (6–8 cm and 8–10 cm) exhibited less clay dominance in all three sites.

3.2 Macrobenthos assessment

3.2.1 Abundance, taxon composition, diversity, and biomass (multi- vs. box corer)

Our findings encompass the macrobenthic community sampled using both the multi- and box corers, presented together for the purpose of comparison. A total of 337 macrobenthic individuals ($> 300 \mu\text{m}$) were observed and analyzed from nine replicates of multicore liners and six box corers in the current study. The fauna was categorized into 106 morphospecies and 18 higher taxa, spanning nine phyla (Supplementary Table S3).

As per the multicorer sampling method, the highest macrobenthic density was recorded from IRZ ($1,953.31 \pm 375.63 \text{ ind. m}^{-2}$) and PRZ ($1,953.31 \pm 588.77 \text{ ind. m}^{-2}$), while the lowest abundance was at BC20 ($1,762.2 \pm 173.79 \text{ ind. m}^{-2}$). Overall, the community structure did not differ much between the sites (Table 2). Across all taxa, Nematoda emerged as the most prevalent group, constituting 52.06% of the total,

followed by Harpacticoida (27.72%), Polychaeta (9.74%), Calanoida (3.37%), Isopoda (1.50%), Ostracoda (1.12%), and Tanaidacea (1.12%). Other taxa contributed 3.31% to the total fauna, and each taxon contributed less than 1% (Supplementary Table S5). In macrofauna (*sensu stricto*), Polychaeta (66.67%) was the most dominant taxon, followed by Isopoda (10.26%) and Tanaidacea (7.69%). The other taxa (Ophiuroidea, Bivalvia, and Porifera) contributed equally, 5.13% each.

The box corer deployment revealed that the highest macrobenthic abundance was recorded from BC20 ($110 \pm 10 \text{ ind. m}^{-2}$), followed by PRZ ($64 \pm 40 \text{ ind. m}^{-2}$), while the lowest abundance was at IRZ ($60 \pm 0 \text{ ind. m}^{-2}$). The Nematoda was the dominant taxon, contributing 23.08% of total abundance in box corers (Supplementary Table S5). The following dominant taxa were Harpacticoida (20.51%), Polychaeta (10.26%), Acari (9.40%), Isopoda (9.40%), Tanaidacea (5.98%), and Ostracoda (5.13%); Bivalvia, Sipuncula, and Clitellata contributed equally 3.42% each and other taxa (i.e., Calanoida, Amphipoda, Decapoda, and Nemertea) contributed collectively 5.98% and less than 2% each (Supplementary Table S5). The highest contribution to the macrofauna (*sensu stricto*) was made by Polychaeta (25.53%), followed by Isopoda (23.39%) and Tanaidacea (14.90%). The Clitellata, Bivalvia, and Sipuncula contributed equally with 8.52%, and other taxa (Amphipoda, Decapoda, and Nemertea)

TABLE 2 The two-way PERMANOVA main test showing difference in abundance- and trait-based macrobenthic community structure (using macrobenthos “*sensu lato*” abundance) between two types of sampling gears and sites in the PMN area, Central Indian Ocean Basin (CIOB).

	Source	df	SS	MS	Pseudo-I	Unique perms	$p^{\text{permonova}}$	$p^{\text{Monte Carlo}}$
Abundance-based	Sites	2	6,289.5	3,144.7	0.95818	997	0.544	0.498
	Gear type	1	11,749	11,749	3.5799	999	0.001*	0.006*
	Sites × gear type	2	6,832.4	3,416.2	1.0409	996	0.416	0.395
	Res	9	29,538	3,282				
	Total	14	54,032					
Trait-based	Sites	2	0.61449	0.30725	3.332	999	0.078	0.070
	Gear type	1	0.15896	0.15896	1.7238	999	0.213	0.192
	Sites × gear type	2	0.21437	0.10719	1.1624	998	0.377	0.350
	Res	9	0.8299	0.092211				
	Total	14	1.7516					

* $p < 0.05$ (significance).

contributed 10.62%. Finally, the macrobenthic community structure did not vary significantly between sites across sampling gears or between sampling gears (Table 3).

In total, 117 individuals were collected in box corer and 220 in multicorer; 39 and 46 morphotypes were unique to multi- and box corer, respectively. However, 21 morphotypes were found commonly in both sampling gears (Figure 4A). Furthermore, BC20 showed the most unique taxa in both gears, followed by IRZ and then PRZ (Figure 4B). The typical meiofaunal taxa were largely captured by the multicorer system, contributing 85.39% of macrobenthos abundance, and box corer captured relatively low (59.82%). Out of 18 higher taxa, 13 and 14 occurred in multi- and box corer sampling types across all the sites, respectively. Only nine taxa (i.e., Polychaeta, Acari, Calanoida, Harpacticoida, Isopoda,

Tanaidacea, Ostracoda, Bivalvia, and Nematoda) occurred in both sampling gears. The mean number of morphospecies (S) was reported 26–39 in multicorer and 22–35 in box corer sampling types. The S was found highest in BC20 (36 and 35) and lowest in PRZ (26 and 22) considering multi- and box corer samples, respectively. The Margalef's richness (d), Pielou's evenness (J'), and Shannon–Wiener diversity (H') showed the lowest values in IRZ and highest in BC20 using multicorer compared to box corer (in BC20). However, the d showed a similar trend of high and low to S in multicorer samples (Supplementary Table S6). The J' and H' are also highest at BC20 but H' was similar in PRZ and IRZ, whereas, J' was found to be lowest in IRZ. Besides these little differences, the sites were not significantly different in any of the indices based on multi- or box corer (Table 4).

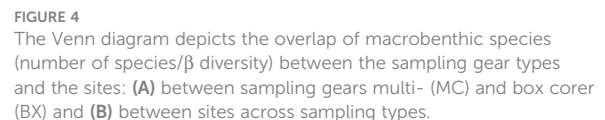
TABLE 3 Two-way PERMANOVA pair-wise test showing difference in density-based macrobenthic community structure (using macrobenthos “*sensu lato*” abundance) between sites across type sampling gears and between type sampling gears across sites in the PMN area, Central Indian Ocean Basin (CIOB).

Sites/gears	Groups	t	$p^{\text{permonova}}$	Unique perms	$p^{\text{Monte Carlo}}$
Sites					
IRZ	MC, BX	1.3565	0.11	10	0.199
BC20	MC, BX	1.3331	0.094	10	0.207
PRZ	MC, BX	1.4362	0.096	10	0.15
Sampling gears					
MC	PRZ, IRZ	0.80923	0.814	10	0.618
	PRZ, BC20	1.1543	0.309	10	0.275
	IRZ, BC20	0.88483	0.607	7	0.509
BX	PRZ, IRZ	0.93121	0.64	3	0.493
	PRZ, BC20	1.0482	0.665	3	0.431
	IRZ, BC20	0.89239	1	3	0.557

MC, multicorer samples; BX, box corer samples.

Indices	Model type	Sum square/ Chi-square	Residuals	F-value	Pr(>F)/ Pr(>Chisq)
Multicorer					
Total morphospecies (S)	glm	2.123	6.045	1.054	0.405
Abundance (N)	glm.nb	0.431	8.172	0.158	0.857
Margalef's richness (d)	glm	0.488	1.225	1.195	0.366
Pielou's evenness (J')	glm	0.048	0.114	1.280	0.345
Shannon-Wiener diversity (H')	glm	0.741	1.168	1.903	0.229
Box corer					
Total morphospecies (S)	gls	2.847	–	–	0.241
Abundance (N)	gls	4.288	–	–	0.117
Margalef's richness (d)	gls	2.499	–	–	0.287
Pielou's evenness (J')	gls	0.466	–	–	0.792
Shannon-Wiener diversity (H')	gls	2.278	–	–	0.320

The present study revealed the presence of 17 morphospecies of polychaetes across 12 families, as detailed in [Supplementary Table S3](#). The collective contribution of polychaetes to macrobenthos was



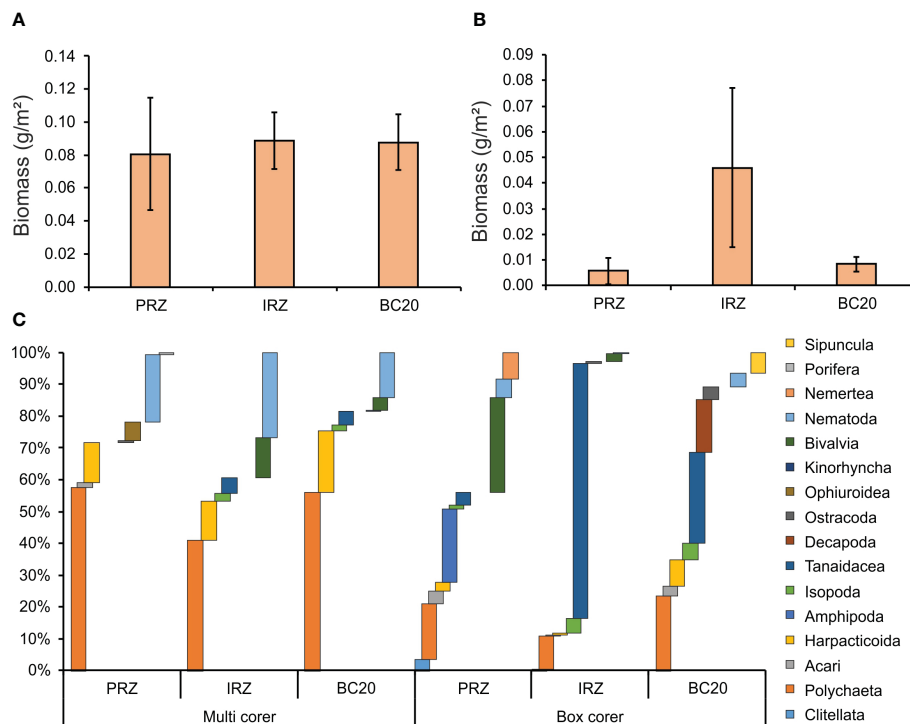


FIGURE 5

The mean estimated biomass multicorer (A); box corer (B) and percentage composition of higher-level taxa (C) across sampling gears and the sites of PMN area.

noteworthy, constituting 9.72% and 10.22% in multi- and box corer samples, respectively. In multicorer samples, Cirratulidae sp., Lacydoniidae sp.1, Opheliidae sp., *Amphicorina bansei*, and *Exogone* sp.1 collectively contributed 6.00% to macrobenthos, which comprised 11 morphospecies and a few unidentified. The analysis of box corer samples revealed the presence of nine morphospecies of polychaetes, with two dominant species, *Aricidea* sp. and Sabellidae sp. of the total polychaete fauna (Supplementary Table S3). In multicorer samples, the morphospecies richness (S) of polychaetes ranged from four to five in multicorer (Supplementary Table S6). Notably, the BC20 site exhibited the highest richness and density, recording values of five and $233.33 \pm 76.59 \text{ ind. m}^{-2}$, respectively, while the lowest values were observed at IRZ for S and PRZ for density. BC20 displayed the highest diversity indices, i.e., d , J' , and H' . In contrast, box corer samples exhibited lower morphospecies richness (2) and density (4 ind. m^{-2}) in PRZ. The highest values of S were reported in IRZ and density in BC20 in box corer samples. Noteworthy variations in diversity indices were observed, with PRZ exhibiting the highest d , PRZ and IRZ showing the highest J' , and IRZ displaying the highest H' .

Within the Malacostraca fauna, the Tanaidacea emerged as the most dominant group in the current study. A total of six morphospecies of Tanaidacea were identified, with two encountered in multicorer samples and five in box corer samples. Collectively, these Tanaidaceans contributed 1.12% (multicorer) and 5.97% (box corer) to macrobenthos (Supplementary Table S5). Analysis of multicorer samples revealed that among Tanaidaceans, Leptognathiidae sp.3 dominated, followed by Leptognathiidae sp.1 (Supplementary Table

S3). In contrast, Tanaidomorpha sp.1 and Leptognathiidae sp.2 were the predominant morphospecies in box corer samples, each contributing 28.48%. In multicorer, tanaids were only reported at the IRZ site (Supplementary Table S6). Contrastingly, IRZ has the highest mean density, S and H' , of tanaids using box corer. It is worth mentioning that diversity indices in multicorer samples were neglected due to the presence of only two morphospecies. Furthermore, Isopoda was also recorded in very low amounts, similar to tanaids, making it difficult to compare between sampling gears.

Copepods were recorded as the second most dominant group among meiofauna ($> 300 \mu\text{m}$), contributing significantly to macrobenthos composition. In the PMN region, a total of 20 distinct morphospecies were identified, with 16 and 12 morphospecies observed in multi- and box corer samples, respectively, collectively constituting 31.44% and 22.19% of macrobenthos (Supplementary Table S5). Harpacticoid copepods within the Copepoda class were particularly diverse and abundant, with 16 morphospecies spanning nine families reported in the study region. Among these, the family Aegisthidae stood out as the most diverse and dominant, followed by Argestidae (Supplementary Table S3), with unidentified harpacticoids exhibiting overall dominance. In multicorer samples, Parameiropsidae sp. made the highest contribution to the harpacticoid fauna, followed by a combination of Aegisthidae sp.3 and Canthocamptidae sp.1, Argestidae sp.2, Argestidae sp.3, and Ectinosomatidae sp.2. In box corer samples, on the other hand, Nannopodidae sp. dominating in copepod community. The diversity indices reflected distinct patterns between the two corer types. Multicorer samples

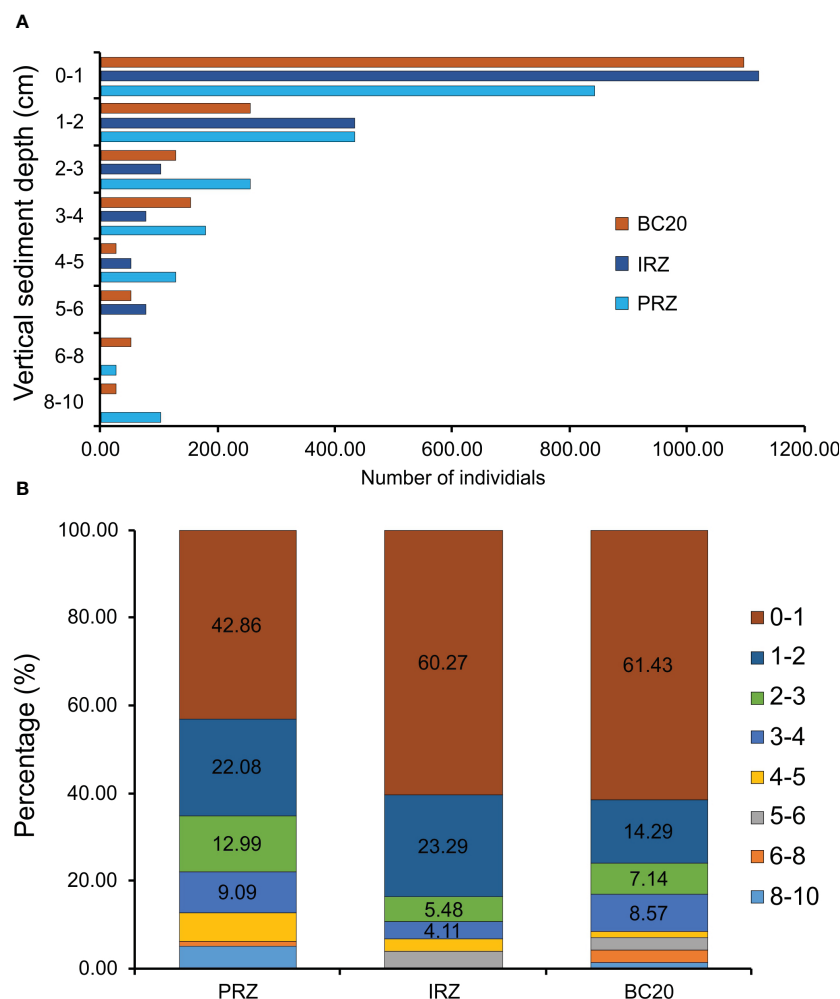


FIGURE 6

Vertical distribution and contribution of each sediment layer to total macrobenthic abundance in multicorer sediment samples across three sites in the PMN area, Central Indian Ocean Basin (CIOB). (A) Vertical distribution; (B) contribution in percentage to each sediment layer.

exhibited the highest species richness (S) ranging from 9 (PRZ) to 11 (IRZ and BC20), whereas box corer samples showed a slightly lower richness ranging from 3 (PRZ and IRZ) to 10 (BC20) (Supplementary Table S6). Density patterns followed a similar trend. Interestingly, the d was lowest in multicorer samples except BC20 using box corer, while H' showed contrasting results. Evenness (J') remained equal in both sampling types. Site BC20 demonstrated the highest copepod density, while IRZ and PRZ exhibited comparable values.

Nematodes were the most dominant and diverse taxa, comprising 31 morphospecies belonging to 14 families, including several unidentified species. Notably, Chromadoridae, Desmodoridae, Ironidae, Oncholaimidae, and Oxystominidae stood out as dominant and diverse families among nematodes in the region. These nematodes contributed 52.02% and 23.03% to macrobenthos in multi- and box corer samples, respectively (Supplementary Table S5). In the multicorer, Desmodoridae sp.2, Oncholaimidae sp.1, and Oxystominidae sp.3 were particularly dominant. While Sphaerolaimidae sp., Dorylaimida sp., Oncholaimidae sp.1, and Oncholaimidae sp.3 held dominance in

box corer samples (Supplementary Table S3), a total of 20 and 15 morphospecies were encountered in multi- and box corer samples, respectively. The morphospecies richness (S) was highest in multicorer samples, ranging from 8 (PRZ) to 17 (BC20), while box corer samples exhibited lower values (5–11). Density peaked in IRZ (multicorer) and BC20 (box corer), while species richness was highest in BC20 across both sampling types. In multicorer samples, H' reached its highest value of 1.1 ± 0.28 , whereas d and J' per site were highest in BC20 and lowest in PRZ. In box corer samples, d was highest in IRZ, while J' was nearly equal in IRZ and BC20 (Supplementary Table S6), and H' was highest at BC20.

3.3 Functional structure (mobility and reworking traits)

Mobility and sediment reworking traits exhibited distinct differences in sampling gear based on the total abundances of morphospecies associated with specific traits. However, the community-weighted mean (CWM) of traits did not significantly

differ in gear types (Table 2). The multicorer method predominantly captured a high abundance of traits, whereas the box corer exhibited considerably less abundance of these traits (Figure 7). This discrepancy could be attributed to the consideration of abundance-weighted traits. However, the variation is much less pronounced or comparable when only the count is considered without weighting. Moreover, relying solely on counts could introduce bias to the results, as abundance serves as a crucial measure of intensity per unit area. Therefore, we considered an abundance-weighted total for each trait. In mobility traits, less motile and discretely motile modalities were dominant among all. Sessile fauna very rarely occurs only in multicorer (PRZ and IRZ) and box corer (BC20). Surficial modifiers emerged as the dominant factor in the PMN region, closely followed by biodiffusors and upward/downward conveyors. Epifauna, on the other hand, was exclusively observed in the PRZ of multicorer samples. In the IRZ multicorer samples, surficial modifiers held sway. These findings illustrate the prevailing presence of fauna with slow and limited mobility, particularly those capable of surface diffusion that moves particles horizontally across the surface in polymetallic nodule fields.

3.3.1 Macrobenthic–univariate indices vs. environment

Results of the CCA (first two axes) show the species–environmental correlations were explained by only two constrained first and second axes (Figure 8). Eigenvalues for axes CCA1 and CCA2 are 0.55 and 0.27, respectively, and the variation explained by these axes are 67.07% and 32.93%, respectively, indicating marginally high relationships with the environmental variables selected. Furthermore, the permutation test for CCA axes under a reduced model (999 permutations) showed that CCA1 was most efficient (near significance) in explaining the variation among parameters ($p = 0.08$). The analysis was performed separately in two groups: firstly, nutrients and pH, and secondly, food-related parameters to decrease the redundancy due to multicollinearity. The variables pH and $\text{PO}_4^{3-}\text{-P}$ were highly correlated with CCA2 (Figure 8A), and CCA1 was correlated with CHO and TOC. CCA showed that the pH and $\text{PO}_4^{3-}\text{-P}$ were high towards the IRZ site as the IRZ and PRZ were aligned with CCA2. However, CHO and TOC were majorly related to the BC20 site.

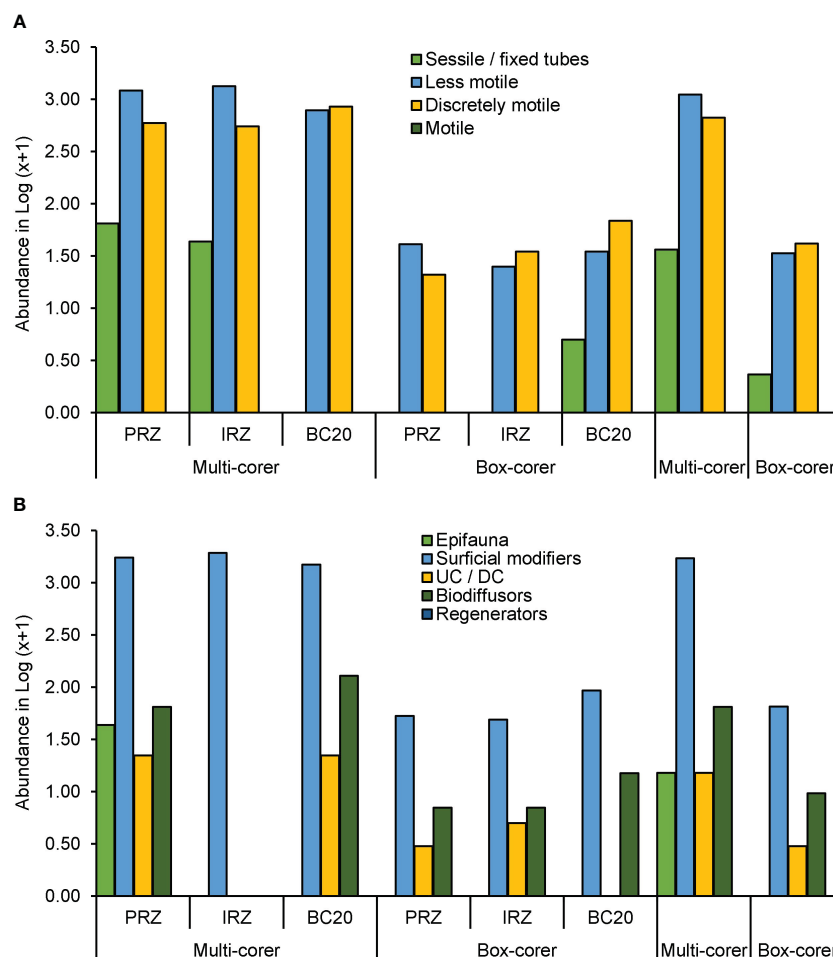


FIGURE 7

Distribution of functional trait associated with bioturbation and abundance weighted by species density represented in Log(X+1): (A) Mobility trait abundance and (B) sediment reworking capability abundance.

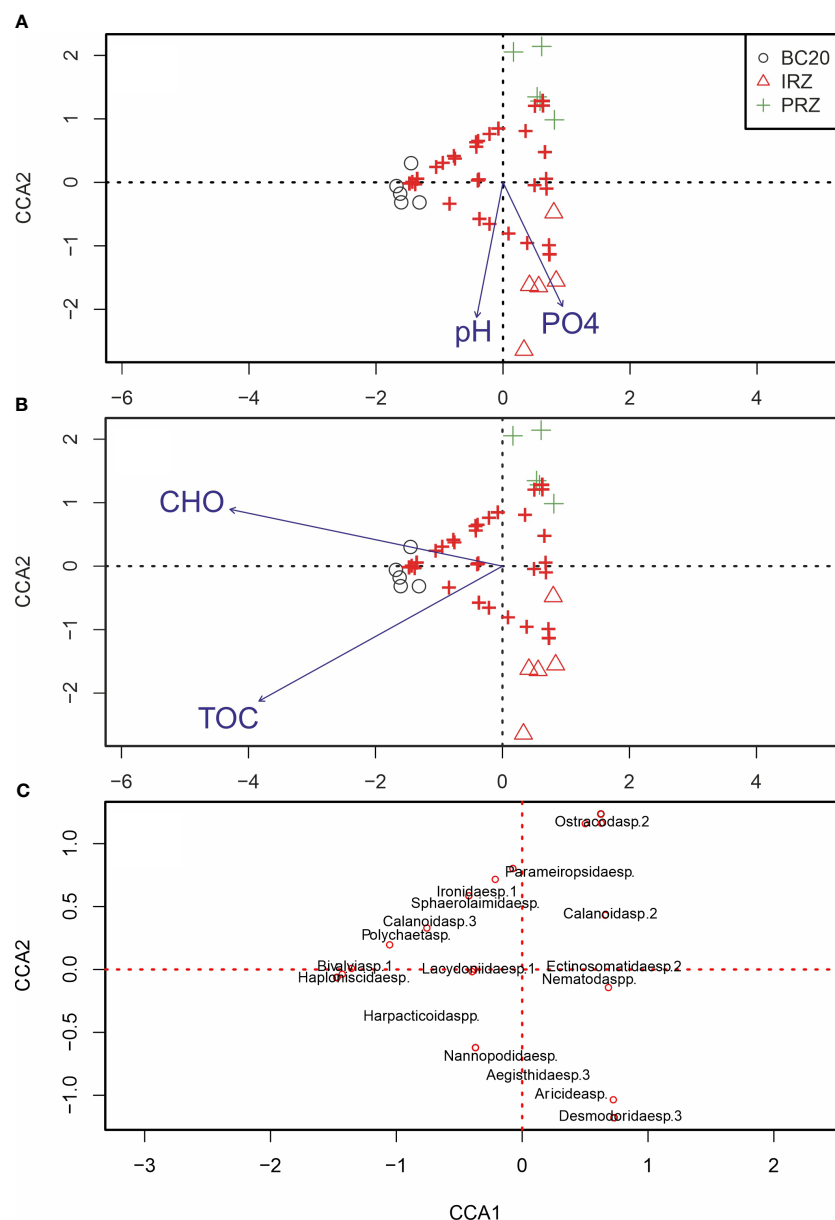


FIGURE 8

Canonical correspondence analysis (CCA) compares the macrobenthic community structure (plus symbols; multi- and box corer data combined) and environmental factors (blue arrows). Environmental factors displayed were selected based on significance calculated from individual CCA results calculated during CCA. Salinity, sand, silt, and clay were removed from the analysis. (A) Pore water chemical parameters (pH, phosphate, nitrate, nitrite, and silicate); (B) sediment characteristics, mainly food-related parameters (TOC, carbohydrates, protein, lipid, and LOM); (C) the majorly influenced species were fitted on the same CCA plot.

The harpacticoid copepods (*Harpacticoida* spp., *Nannopodidae* sp., and *Aegisthidae* sp.3), polychaete *Aricidea* sp. (family *Paraonidae*), and the nematode *Desmodoridae* sp.3 were positively related to pH and $\text{PO}_4^{3-}\text{-P}$. Whereas, ostracods (*Ostracoda* sp.2), harpacticoid copepods (*Parameiropsidae* sp.), and nematodes (*Ironidae* sp.1 and *Sphaerolaimidae* sp.) were negatively related. The copepods (*Harpacticoida* spp. and *Calanoida* sp.3), *Bivalvia* sp.1, isopod (*Haploniscidae* sp.), nematodes (*Ironidae* sp.1 and *Sphaerolaimidae* sp.), and

polychaetes (*Polychaeta* sp. and *Lacydoniidae* sp.1) were significantly positively related to CHO and TOC.

Univariate indices and environment relationship showed *S* was significantly positively related to $\text{NO}_3^- \text{-N}$, CHO, PRO, and LOM (Supplementary Table S7). The negative relations were observed between *S* and $\text{NO}_2^- \text{-N}$ and LIP. The *d* and *H'* were directly proportional to *S*, and *J'* was also proportional to *d* and *H'*. These indirect relations explained that *d*, *J'*, and *H'* were also positively related to $\text{NO}_3^- \text{-N}$, CHO, PRO, and LOM, as well as a similar relation to *S*.

4 Discussion

4.1 Macrobenthic community and diversity pattern

The examination and comparison of available literature data from similar surveys were pivotal in our quest to understand metazoan abundance and distribution within the abyss nodule fields, particularly in different PMN regions. One notable observation was the varying mesh sizes used in macrofaunal studies conducted in the CIOB and the Clarion–Clipperton Fracture Zone (CCFZ) regions. In the CIOB, where studies focused on nodule fields employed large mesh-sized sieves of 500 μm , a unique scenario emerged. This choice of sieve size, while capturing macrobenthic organisms, also contributed to relatively low recorded animal abundance in comparison to studies employing smaller mesh sizes (Ingole et al., 2005b; Pavithran et al., 2007). The utilization of large mesh sizes is not uncommon in the deep-sea research community, as it can aid in the collection of larger and more conspicuous organisms. On the other hand, macrofaunal surveys conducted in the Central North Pacific and Peru Basin within the CCFZ region typically adopted a smaller mesh size sieve of 300 and 250 μm (De Smet et al., 2017; Wilson, 2017; Stratmann et al., 2018; Yu et al., 2018; Sweetman et al., 2019; Chuar et al., 2020; Francesca et al., 2021). This choice was consistent with established deep-sea sampling practices and allowed for the collection of a broader spectrum of metazoan taxa. However, it's important to note that these surveys opted to exclude meiofaunal taxa, i.e., Nematoda, Copepoda, Ostracoda, Acari, and Kinorhyncha, from their analyses to ensure meaningful comparisons with most previous studies in the CCFZ region (De Smet et al., 2017; Wilson, 2017; Francesca et al., 2021; Washburn et al., 2021). In essence, the mesh size utilized in deep-sea sampling can significantly influence the recorded abundance and diversity of metazoan communities. The contrasting mesh sizes used in the CIOB and CCFZ studies reflect the diverse approaches employed by researchers in different PMN regions, each driven by unique research objectives and considerations. Understanding these variations in sampling methodologies is crucial when interpreting and comparing metazoan abundance data from various abyss nodule fields, ultimately advancing our knowledge of these complex deep-sea ecosystems. Few CIOB studies have reported 336 ind. m^{-2} , 376.39 ind. m^{-2} , and 507 ind. m^{-2} , which was the highest faunal abundance among other studies from CIOB, including “meiofaunal” taxa, i.e., Nematoda (Ingole et al., 2001; Ingole and Koslow, 2005; Ingole et al., 2005a). The notion that the inclusion of meiofaunal taxa in the macrofaunal community (macrobenthos) is extremely useful and commendable and is gaining ground recently (Ingels et al., 2021). In the present study, both densities (macrobenthos and macrofauna) were mentioned for a clear understanding of benthic community structure and comparison. It has been recommended that the whole box corer should be used for macrofauna analysis (De Smet et al., 2017). Nevertheless, pseudoreplicates were used in studies from CIOB in the past (Ingole et al., 2005a; Pavithran et al., 2007) and also compared in Supplementary Table S1, thus creating discrepancies

in macrobenthic faunal density. Hence, meaningful comparisons between CIOB and Pacific Ocean studies have been challenging so far. In 2009, a similar study reported a total of 66 species in 120 individuals ($> 500 \mu\text{m}$) from PMN, Indian Ocean (Pavithran et al., 2009a). Contrastingly, 5,199 metazoans ($> 250 \mu\text{m}$) were collected from 12 box corers in CCFZ and encountered 33 higher taxa (Chuar et al., 2020). The present study encountered 117 metazoan ($> 300 \mu\text{m}$) individuals in the box corer. Therefore, the available data strongly suggest that the CIOB nodule field has the relatively lowest macrobenthic faunal abundance and higher taxa diversity.

In the current investigation, our examination of the Shannon–Wiener index (H') revealed a lower diversity compared to other PMN areas within the CCFZ (> 2.0 along the GSR [De Smet et al., 2017]). Polychaetes, widely acknowledged as prominent members of deep-sea macrofaunal communities (Sanders, 1968; Hessler and Jumars, 1974; Wilson, 2017), emerged as the most dominant macrofaunal taxon (macrofauna) in our study, encompassing 17 distinct morphospecies. Surprisingly, tanaidaceans, traditionally recognized as the predominant taxon in the CCFZ located in the northeastern Pacific Ocean (Yu et al., 2018), took a secondary role in our findings. The present study, incorporating nematodes and copepods into the macrobenthos category, witnessed a shift in dominance, with nematodes securing the top position, followed by copepods. This contrasts with the conventional hierarchy, where polychaetes and tanaidaceans typically hold sway. Our observations align with similar dominance patterns reported in the easternmost region of the CCFZ (Chuar et al., 2020; Kaiser et al., 2024).

For vertical distribution, Montagna et al. (2017) reported that more multicorer samples from the top 5 cm sediment depths in various locations of the northern Gulf of Mexico revealed that approximately 90% of faunal communities were concentrated in this uppermost layer. Meiofaunal communities dominated the top 2 cm, while macrofauna extended further down to 10 cm. Similar findings were documented in the Clarion–Clipperton Fracture Zone (CCFZ), with 67% observed in the upper 2 cm, 25% up to 5 cm, and 8% down to 10 cm depths (Chuar et al., 2020). The current study, conducted in the CIOB, aligns with these patterns, highlighting that maximum communities were harbored in the top 5 cm sediment layer across all three sites. Interestingly, previous research in the CIOB contradicted this trend, revealing that benthic fauna was more abundant in the middle (2–5 cm) and lower layers, with traces even in the 20 to 30 cm layer (Pavithran et al., 2009b). This discrepancy may be attributed to the scarce food and surface competition, which may cause the benthic fauna to migrate downward, forming protective tubes that, during sampling, serve as escape routes, leading to inadvertent transport through the sediment layers (Ingole et al., 2005b; Pavithran et al., 2009b).

4.2 Multi- vs. box corer comparison

The methods employed for collection may significantly influence the results of benthic analysis (Jensen, 1981, 1983; Bett, 1994; Hughes and Gage, 2004; Shields and Hughes, 2009). Since 1970, box corers have been used primarily for quantification of deep sediment (Gage and Tyler, 1991); however, few studies have tackled

the issue of inefficiency in box corer sampling for macrofauna (Hughes and Gage, 2004; Shields and Hughes, 2009; Blomqvist et al., 2015; Montagna et al., 2017). In this study, we attempt to investigate the differences in methodologies in the abyssal plain of the world's lowest productive zone (Pavithran et al., 2007). The box corer area coverage at each station was 12.82 times larger than the combined multiple corer sample area. However, a higher mean density of macrofauna (*sensu stricto*) was reported in multicorer (Supplementary Table S6). Polychaetes were dominant in the present study, similarly observed in CCFZ (Bonifácio et al., 2024). Furthermore, total biomass estimates were higher in multicorer samples with less variation between the stations (Figures 5A, B), owing to two possibilities: (1) box corer may have led to a loss of the fauna due to a bow-wave effect before settling on surface sediment and (2) multicorer captured all sized range effectively with much smoother approach near sediment than box corer.

Intriguingly, only multicorer samples were deemed suitable for discerning the densities of small-body-sized fauna (especially Meiofauna). Particularly, nematodes and copepods showed high abundance in the multicorer samples, and many morphospecies captured by the multicorer exclusively did not occur in the box corer samples (Supplementary Table S3; Figure 4). These results indicate the suitability of multicorer usage for macrobenthic fauna collection in the deep sea. Nevertheless, the contribution of smaller organisms retained on a 300 µm mesh to community biomass is relatively minor (Gage et al., 2002). Despite this, there are 11 morphospecies of polychaetes, including motile subsurface deposit feeders, i.e., Cirratulidae sp., Lacydoniidae sp.2, Lumbrineris sp., Opheliidae sp., *Amphicorina bansei*, and *Spiophanes* sp. exclusively occurred in multicorer samples and were associated with increased biomass. In contrast, sessile or less motile and large-sized polychaetes, i.e., *Glyphanostomum* sp., Capitellidae sp.1, *Brada* sp., *Glycera* sp., and Sabellidae sp. were found in the box corer samples. Multicorer showed inefficiency in capturing isopods and tanaids, as represented by the low contribution of these taxa to total abundance. Box corer showed the presence of all isopod and tanaids species listed except Leptognathiidae sp.3.

Morphospecies richness and diversity were higher in the box corer compared to the multicorer samples (Supplementary Table S6). This is not surprising, as diversity tends to increase with the sampling area (Lomolino, 2000), and the area covered by the box corers is larger than that of the multicorer. Margalef richness (d) is three times higher in box corer than in multicorer samples. While Shannon–Wiener diversity (H') was also higher in box corer.

The findings highlighted that smaller fauna, particularly meiofauna, were more likely to be captured by the multicorer, suggesting a potential escape from the box corer. Utilizing box corers for benthic sampling presents a notable advantage by allowing the collection of a more extensive and quantifiable representation of individual organisms over a relatively large area. This comprehensive sampling approach facilitates a nuanced exploration of the diverse benthic community, enhancing the understanding of species diversity within the

study area (Montagna et al., 2017). However, it is crucial to recognize the potential drawbacks associated with the use of box corers. One significant concern stems from the generation of bow waves during the sampling process, which can disturb the seabed, resulting in the resuspension of surface sludge and the potential loss of sediments (Blomqvist et al., 2015). This disturbance may result in the displacement or loss of individual organisms within the sampled area, affecting the accuracy of biodiversity assessments and compromising the integrity of ecological data. Furthermore, the present study underscores the advantages of multicorer sampling in some aspects for investigating the vertical distribution of benthic communities and capturing small-sized faunal fractions (Meiofauna). Particularly, where small-sized fractions could be an important component to understanding the whole ecological perspective in the ecosystems characterized by low food availability, such as open water of deep sea zones and potential mining sites. However, the box corer yielded scant additional information regarding the macrobenthic faunal community compared to the insights gained from the much smaller multiple corer samples. The considerable variation in density and diversity responses to the two sampling methods poses a challenge to confidently recommending one technique over the other (Montagna et al., 2017). Thus, the use of both sampling gears will offer more detailed insights into biodiversity and ecosystem functioning.

4.3 Functional composition

The composition of functional traits did not differ between the sites and gear types, indicating that there was no discernible influence of spatial structure or gear effects. This lack of differentiation could stem from the fact that many species share similar functions across these traits, highlighting redundancy in their functional roles. However, these functions may still impact the local flux of available nutrients in the sediment. Macrobenthos contributes to increased organic matter inputs through biodeposition, a process where organic materials are released into the sediment as metabolic by-products (Graf and Rosenberg, 1997). Simultaneously, these organisms engage in bioturbation, stirring and mixing the sediments, thereby distributing and incorporating organic materials into deeper strata (Kristensen, 2000; Welsh, 2003). These fundamental aspects of the functional traits, including mobility and sediment reworking, of macrobenthic fauna lie in their ability to enhance organic matter (OM) flux within sediments, influencing nutrient cycling, sediment structure, and overall benthic community dynamics. In the present study, the less motile and discretely motile macrobenthic faunal communities were dominant along all the sites, while sessile fauna very rarely occurred in both the sampling gears (multi- and box corer). These findings can be attributed to the burrowing behavior exhibited by a majority of species, namely polychaete families (Ampharetidae, Capitellidae, Cirratulidae, Glyceridae, Lacydoniidae, Lumbrineridae), tanaid families (Leptognathiidae, Neotanaisidae, Tanaellidae), and other taxa. This behavior classifies them as less

motile, with their movements primarily confined to the sediment. Less motile macrobenthic faunal communities in the deep sea typically include organisms with limited mobility, relying on structural adaptations or passive transport for movement (Jones et al., 1998; Billett et al., 2001). Furthermore, less motile organisms may contribute to sediment stability and long-term habitat structure, while discretely motile species actively participate in bioturbation processes, influencing nutrient availability and community composition (Ruhl and Smith, 2004). Discretely motile macrobenthic faunal communities comprise organisms such as mobile predators, scavengers, and deposit-feeders like crustaceans and agile polychaetes, capable of more pronounced and intentional movements within the sediment and across the seafloor to locate food resources and suitable habitats (Thistle et al., 2008). Biodiffusers, organisms that enhance the vertical mixing of sediments through their burrowing and feeding activities, are significant contributors to sediment reworking (Aller, 1982; Aller and Aller, 1992; Aller, 1994), which dominated the macrobenthos population in the present study. Upward and downward conveyors refer to transporting sediment particles vertically within the sediment column. For example, upward conveyors such as certain polychaetes (*Capitellidae* sp.1 and *Capitellidae* sp.2) exhibit upward conveyance by ingesting deeper sediments and excreting processed material at the surface, influencing nutrient distribution (Miri et al., 2023). Furthermore, organisms exhibiting upward and downward conveyance, such as certain burrowing fauna (e.g., *Glyphanostomum* sp., *Brada* sp., and *Spiophanes* sp., see Supplementary Table S3), transport surface sediments into deeper layers and vice versa, affecting sediment structure and biogeochemical cycling (Shull and Yasuda, 2001).

4.4 Environmental relationships

Three key environmental factors, i.e., food availability, sediment texture, and nodule distribution may significantly influence the benthic community structure assemblages in the deep sea. The deep-sea floor is distinguished by exceedingly low organic material inputs, rendering it a food-limited ecosystem (Smith et al., 2008). Similarly, CIOB exhibits low productivity both at the surface and in the water column (Matondkar et al., 2005). Additionally, the region has a diminished organic carbon content attributable to low sediment accretion in the bottom surficial sediments (Pattan et al., 2005), despite the Ganges–Bramhaputra fan has been involved in the changes in nutrient chemistry up to 10°S. The reason attributed to low productivity may be the South Equatorial Counter Current (SECC) and South Equatorial Current (SEC) between 0° and 10°S may have prevented nutrient-rich water of the north Indian Ocean from entering the CIOB (Peng et al., 2015). It progressively diminishes toward the south, leading to a decrease in the flux of particulate organic carbon (POC) from the north to the south. The limited nutrient enrichment potentially holds influential capabilities in shaping benthic communities (Bonifácio et al., 2020, 2024) in the southern region of the CIOB. In the current study, the IRZ and PRZ are situated to the south, while BC20 is positioned to the north within the CIOB. This spatial arrangement may contribute to a slightly higher TOC value in BC20 compared to that in the IRZ and

PRZ. The previous studies from CIOB evidently showed that a relatively higher TOC ranging from 0.52% to 1.31% near 10°S latitude (Nair et al., 2000), whereas the present (12°S–13°S) study reported 0.35%–0.47% of TOC. The presence or absence of TOC exerts a significant (positive/negative depending on feeding behavior) influence on the diversity, population dynamics, and interspecies interactions of macrobenthic fauna. The highest Margalef richness (d) and Shannon–Wiener diversity (H') of macrobenthos at BC20 could have been associated with increased TOC and CHO compared to IRZ and PRZ (Supplementary Table S6). Contrastingly, a negative correlation was observed between food availability and macrofaunal community structure (Kaiser et al., 2024). Polychaete species *Capitellidae* sp.2, *Opheliidae* sp., *Sabellidae* sp., *Lumbrineris* sp., and *Glycera* sp. are exclusively found in BC20, of which *Capitellidae* sp.2, *Opheliidae* sp., and *Sabellidae* sp. were highly dependent on suspended or deposited organic food on surface sediment. Similarly, deposit feeder polychaetes dominated Ifremer and APEI No. 3 sites associated with high POC flux (Bonifácio et al., 2024). However, *Lumbrineris* sp. and *Glycera* sp. were carnivorous in life habits (Jumars et al., 2015), which could potentially be attributed to complex interspecific relationships within the ecosystem. This has been supported by a notable contrast that emerged in the abundance of nematodes at BC20, IRZ, and PRZ sites, with BC20 displaying lower numbers compared to PRZ and IRZ (Supplementary Table S6). This variance in nematode abundance could also support interspecific relationships due to the dependence of macrofauna on meiofaunal components. Interestingly, copepods (including harpacticoid) were more pronounced in BC20, and it is noteworthy that some of the harpacticoid species predominantly follow carnivorous feeding strategies (Bröhlidick, 2005; Shimanaga et al., 2009).

4.5 Conclusion

Despite the limitation of sampling effort (logistical constraints) and a small sample size restricting definitive conclusions, the findings of this study highlight the significant influence of different investigative methodologies on observed macrobenthic faunal assemblages. Future research in the CIOB should indeed aim to increase sample size and use consistent sampling techniques to enhance reliability, but the current approach provides a critical foundation for understanding macrobenthic communities in the polymetallic nodule fields of the CIOB. For the first time, this research on macrobenthos provides quantitative insights into multicorer sampling within the PMN field in the Indian Ocean. The study examined differences in macrobenthic community structure, diversity indices, and functional composition between sites. The results revealed a relatively homogeneous nature at the PMN mining sites, encompassing both PRZ and IRZ areas. Relationships between the macrobenthic faunal community and the environment were analyzed to gain a comprehensive understanding of the ecological drivers. The findings highlight the importance of environmental relationships, particularly the influence of total organic carbon and related components, such as carbohydrates, in extremely low-productive zones. Furthermore, we advocate for the combined use of both multi- and box corers to

achieve more accurate estimates of diversity and abundance, ultimately enhancing our knowledge of species richness and functional understanding. Through this study, we aimed to provide general recommendations for the collection of high-quality data that can be utilized for monitoring purposes. The present research contributes valuable insights into seabed community patterns, encompassing both structural and functional aspects by including large-sized meiofaunal taxa in the macrofauna, which may prove instrumental in managing the mining site in the CIOB. Furthermore, understanding the relationship between benthos and environmental factors is crucial for regulating deep-sea bed mining activities, as they significantly shape community structures. By integrating these insights, the study provides valuable guidance for the sustainable management of deep-sea resources and emphasizes the need for careful consideration of environmental factors in future mining operations. Thus, the scientific endeavor is pivotal for informing and shaping the regulations that will govern future mining exploitations in the deep-sea bed, ensuring a legally sound and ecologically responsible approach to this emerging industry. As the ISA continues its regulatory development, incorporating such scientific findings becomes essential for the sustainable management of deep-sea resources and the preservation of biodiversity in these ecologically sensitive areas.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Ethics statement

The study presents research on animals that do not require ethical approval.

Author contributions

SG: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. SS: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. SD: Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Data curation. MM-P: Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation. VG: Writing – review & editing, Methodology. JP: Writing – review & editing, Methodology, Formal analysis. MN: Writing – review & editing, Validation, Supervision, Resources, Project administration. SB: Writing – review & editing, Validation, Supervision.

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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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1395892/full#supplementary-material>

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Multi-AUV sediment plume estimation using Bayesian optimization

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Sediment plumes created by dredging or mining activities have an impact on the ecosystem in a much larger area than the mining or dredging area itself. It is therefore important and sometimes mandatory to monitor the developing plume to quantify the impact on the ecosystem including its spatial-temporal evolution. To this end, a Bayesian Optimization (BO)-based approach is proposed for plume monitoring using autonomous underwater vehicles (AUVs), which are used as a sensor network. Their paths are updated based on the BO, and additionally, a split-path method and the traveling salesman problem are utilized to account for the distances the AUVs have to travel and to increase the efficiency. To address the time variance of the plume, a sliding-window approach is used in the BO and the dynamics of the plume are modeled by a drift and decay rate of the suspended particulate matter (SPM) concentration measurements. Simulation results with SPM data from a simulation of a dredge experiment in the Pacific Ocean show that the method is able to monitor the plume over space and time with good overall estimation error.

KEYWORDS

plume estimation, plume tracking, AUV, sediment plume, dredge experiment, Bayesian optimization, traveling salesman

1 Introduction

The deep sea contains vast quantities of mineral resources that are currently being explored by mining companies, state entities, and scientists for their economic potential and their value as a habitat to be protected. Three main types of mineral resources exist, namely, polymetallic nodules, cobalt-rich ferromanganese crusts, and seafloor massive sulfides, of which the polymetallic nodules are of specific interest due to the enormous size of the resource and the leap in technology development to mine this resource in recent years. These nodules are small potato-shaped concretions of centimeter to decimeter sizes that lie on the seafloor in 3,000- to 6,000-m water depths and consist mainly of authigenic manganese and iron oxides that during their precipitation incorporated economically

interesting metals such as nickel, copper, cobalt, and REE of a few weight percentages in total (Petersen et al., 2020). Demand for such minerals is growing rapidly as “green industries” producing, e.g., electric car batteries, wind turbines, and solar cells require much larger quantities of such minerals than their fossil counterparts (IEA, 2021). Intensive research has been conducted to study the impact of mining on the environment and ecosystem (Jones et al., 2017; Gillard et al., 2019; Spearman et al., 2020; Baeye et al., 2021; Elerian et al., 2022; Haalboom et al., 2022; Muñoz-Royo et al., 2022; Weaver et al., 2022; Haalboom et al., 2023; Lefaible et al., 2024; Mousadik et al., 2024). Although it is not clear how severe and spatially wide the damage to the ecosystem would be, recent studies suggest that the effects will last for a long period of time, as effects of the 1989 DISCOL disturbance experiment in the Peru Basin are still apparent 26 years later (Drazen et al., 2019; Simon-Lledó et al., 2019; Gausepohl et al., 2020; Vonnahme et al., 2020). One impact on the ecosystem is the sediment plume that is created when nodules are mined with a hydraulic mining vehicle that uses waterjets to lift up the nodules from the seabed, which also sucks up a few centimeters of the sediment (Muñoz-Royo et al., 2022). The generated plume from the exhausted sediment splits into two parts: a gravity current sediment plume and a suspension plume. The gravity current sediment plume occurs in the immediate vicinity of the collector vehicle. This plume contains most of the mobilized sediment but remains very close to the seafloor. The suspension plume forms close to the mining vehicle at the interface between the gravity plume and the undisturbed bottom water. Here, fine particles are detrained from the gravity current and made available for far-field transport by the background water currents. The suspension plume has significantly lower sediment concentrations than the gravity plume but spreads much further and higher (Muñoz-Royo et al., 2022; Mousadik et al., 2024). The suspension plume will affect much larger areas than the mining area itself; therefore, it is important to monitor the spreading of the plume to be able to quantify the impact of the mining on the adjacent environment. Due to the large extent of the plume and changing bottom currents, a stationary sensor network on the seafloor is not able to estimate its full extent, whereas autonomous underwater vehicles (AUVs) are ideal platforms for such tasks as they can operate for long periods of time and do not require constant human input like remotely operated vehicles (ROVs). Some monitoring studies already use AUVs but only in preplanned survey missions that are designed to densely map a specific region. Since this is time consuming and only yields limited information, adaptive mapping approaches should be used (Gazis et al., under revision)¹.

The utilization of adaptive mapping with AUVs to analyze and sample marine plumes is not a novel concept in general. However, the emphasis is frequently not on estimating the concentration or distribution of a measurand over a large, three-dimensional domain. In case of plumes originating from hydrothermal vents,

the task is to detect the plume and track it to its source (see Tian et al., 2014; Hu et al., 2019; von See et al., 2022), whereas for algae blooms, oil spills, or other chemical pollutions, the task is to track and sample its boundary to monitor the extent of the pollution of the environment (see Petillo et al., 2011; Li et al., 2014; Fonseca et al., 2021). While the latter is important in the case of deep sea mining, it is also desirable to obtain an estimate of the suspended particulate matter (SPM) concentration to be able to quantify the amount of sediment. This task can be referred to as field estimation.

A popular approach in this context is to model the field to be estimated as a Gaussian process (GP) and to sample the environment in an intelligent way to estimate the GP hyperparameters. This is always a trade-off between exploring unknown regions and exploiting knowledge in known regions. The authors of Cui et al. (2015) propose a selective basis function Kalman filter to estimate the hyperparameters of the GP, and a mutual information-based multidimensional rapidly exploring random tree (RRT)* algorithm is used to select new sampling locations. Simulation results and an experiment with four robotic fishes in an aquarium for a 2D static field are presented. Zhang et al. (2022) propose a method where some AUVs operate in an exploration mode and others in an exploitation mode. A differential evolution-based path planner is proposed to plan the individual AUV trajectories. Knowledge of the environment is shared via acoustic communication based on a sparse variation GP method. Simulation results for a scalar, static 3D field are presented. A reinforcement learning approach to select new sampling locations is used in Wang et al. (2018). To this end, a long-term reward function, which includes the AUV mobility cost, kinematic, communication, and sensing area constraints, is maximized by the deep deterministic policy gradient algorithm. Simulation results are presented for a static 2D field, and a comparison with a random walk method is performed.

In classical Bayesian optimization, so-called acquisition functions (AFs) are employed to balance between exploration and exploitation. The most common ones are probability of improvement (PI), expected improvement (EI), and upper confidence bound (UCB). In Samaniego et al. (2021), three modifications to these classical AFs are proposed to favor sampling locations close to the agents since the functions do not account for the distance between sample locations. Simulation results for an autonomous surface vessel (ASV) in a lake are presented and compared with classical monitoring approaches as, e.g., lawnmower patterns. Stankiewicz et al. (2021) propose two strategies based on the upper confidence bound (UCB) method to select new sampling locations. The first is based on branch-and-bound techniques, the other on cross-entropy optimization. Simulation results for an underactuated 6-degrees of freedom AUV model in a 3D marine environment as well as results from a field test are presented.

The abovementioned approaches are similar in that they all evaluate their methods on relatively smooth and static data, which is a valid assumption for many natural processes as, e.g., temperature or oxygen distributions in a water body. However, sediment plumes that arise from dredging or mining vehicles yield turbulent, heterogeneous and, due to water currents, also time-variant

¹ Gazis, I., de Stigter, H., Thomsen, L., Mohrmann, J., Heger, K., Diaz, M., et al. Monitoring benthic plumes, sediment redeposition and seafloor imprints caused by deep-sea polymetallic nodule mining. *Nat. Commun.*

concentration distributions (Elerian et al., 2021; Muñoz-Royo et al., 2022; Peacock and Ouillon, 2023). To address these issues, we propose to use a sliding window UCB (SW-UCB) algorithm based on Cheung et al. (2019) for the hyperparameter estimation in combination with a traveling salesman problem (TSP) based approach for the selection of new sample locations. Instead of updating the GP after every sample, we use the $N \in \mathbb{R}$ best sample locations based on the UCB function and calculate the shortest path along these locations by solving the TSP. Additionally, we adopt the split-path method from Samaniego et al. (2021) to also generate samples in specified distances between the sample locations. Furthermore, a simple drift model is proposed to artificially move the measurement samples with the water current to account for the main driver of the time variance of the sediment plume. Finally, a domain reduction scheme is proposed to reduce the search path lengths while making sure that the plume is captured completely.

2 Materials and methods

The objective of the sediment plume monitoring and estimation using AUVs as a sensor network is to sample the plume in an efficient way to estimate the suspended particulate matter concentration and track its change over time. To this end, a combination of Bayesian optimization for time-variant systems and the traveling salesman problem is utilized to achieve a small estimation error.

2.1 Assumptions

The assumptions that have been made for this study are as follows.

AUV dynamics. AUVs that can operate in depth up to 6,000 m are typically torpedo-shaped AUVs that are capable of flying at speeds of up to 5 knots and can reach mission durations of a day and more, making them feasible for long-term monitoring of a plume. They are equipped with one thruster at the back and fins to control the yaw and pitch; thus, they are underactuated. This means that they fly with almost constant velocity during the mission and need to perform turning maneuvers if waypoints lie too close to each other. Due to the large area, we model the AUV dynamics as a point mass. We assume a constant speed of 5 knots between two waypoints and add an extra amount of time T_{turn} for turns that are sharper than 90°.

Acoustic communication. The bandwidth underwater is very limited; thus, a decentralized approach where every AUV receives all information is not feasible. We consider a centralized approach, where all AUVs send their SPM concentration measurements with the corresponding locations to a centralized entity. This could be one of the AUVs or a stationary lander with high computation power and a large battery. The BO and calculation of new waypoints is performed by this entity, and only the new sampling locations are transmitted back to the AUVs. This reduces the communication drastically compared with sharing additionally all hyperparameters among all AUVs.

Current measurements. We assume that current measurements are available for the working area and time of the survey. This can be achieved by a lander (or lander array) equipped with upward-looking profiling acoustic current meters and a small computer and acoustic modem that broadcasts the mean water current values in fixed time intervals. This is an important consideration as the water current values are needed to model the change of the suspension plume over time.

Sensors. We assume that the AUVs are equipped with optical backscatter sensors (OBS), typically turbidity sensors. These have a very limited field of view of a few centimeters and hence provide point measurements. In stationary monitoring scenarios, acoustic backscatter sensors (ABS) are used additionally, typically Acoustic Doppler Current Profilers (ADCPs). Both sensor types do not measure the SPM concentration directly but use pre-calibration data to calculate the concentration from the backscatter signal. In the case of ADCPs, measurements along a line can be obtained which provide a larger coverage than OBS, but at the same time, the analysis of these measurements is more complex (Haalboom et al., 2022), so only OBS are considered here. They are known to work best at low suspended sediment concentrations and small particle sizes. Thus, they are ideally suited for monitoring the suspension plume. Other parameters that affect their accuracy include particle shape, near infrared radiation (NIR) reflectivity, and flocculation/aggregation, but these are less important than suspended sediment concentration and particle size (Downing, 2006). The speed of the AUV and the sampling rate of the sensor can also affect the accuracy of the measurements. In terms of localization, their influence is very limited, since typical sensors, such as those used in Haalboom et al. (2022), have high sampling rates of 8 Hz to 10 Hz, and thus, the measurements can be localized with an accuracy of approximately 30 cm, given the assumed AUV speed of 5 knots. Compared with the large scales of the suspension plume, this error can be neglected. A more detailed discussion of the interactions between the various factors that affect the sensitivity and accuracy of the sensor is beyond the scope of this paper. Here, we assume that the sensor readings are reliable and fast so that the samples can be located with negligible error.

2.2 Bayesian optimization

BO is a global optimization method that dates back to the 1960s and has been used intensively, (see Jones et al., 1998). It is very useful when obtaining new samples from an unknown blackbox function is expensive, e.g., due to complex experiments or computationally intensive simulations. In Figure 1, the general procedure of BO is shown as a flowchart. First, the surrogate model, a GP, is initialized. The AF, which provides a measure of the confidence of the model for all function values $\mathbf{x}$, is evaluated and the next sample location is chosen as the location corresponding to the maximum value of the AF. The model is fitted through a Gaussian process regression (GPR), and the posterior probability distribution of the scalar field can be calculated. Several termination criteria can be defined to evaluate whether the model reached a sufficient level of certainty, e.g., the

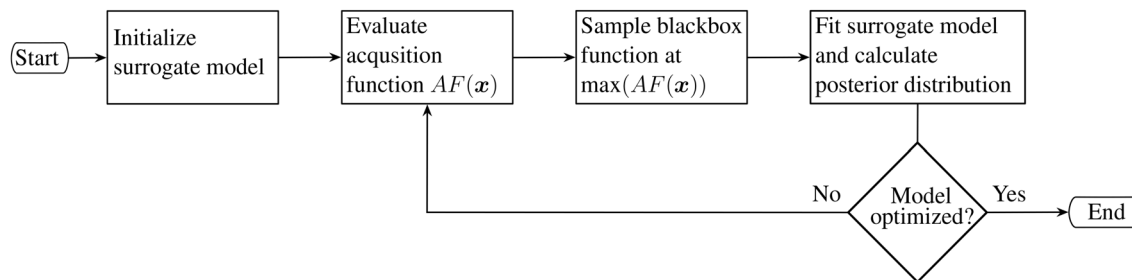


FIGURE 1

Flowchart of the Bayesian optimization without any prior knowledge of the unknown function.

maximum number of iterations or the maximum change in the hyperparameters or in the AF. If such a criterion is met, the procedure ends, and if it is not met, the AF is evaluated again to obtain new samples. In many cases, instead of just initializing the surrogate model, it is advantageous to acquire an initial set of samples and to fit the surrogate model before the optimization loop is started. Especially if taking samples is not only a matter of computational power, but also time or distance traveled between two measurements, it is advisable to acquire initial samples in a coordinated manner. In the context of plume monitoring with AUVs, the function values $\mathbf{x}$ refer to 3D points in the area of the plume. The AF is used to define new sampling locations, and the GPR calculates an estimate of the SPM concentration in the specified region. In the following, the individual parts of BO are explained in further detail.

2.2.1 Gaussian process regression

GPR is a Bayesian statistical technique to calculate an estimate of a function $f(\mathbf{x})$ given some data. In the context of this paper, $f(\mathbf{x})$ refers to the SPM concentration at the location $\mathbf{x}$. The surrogate model used is a GP, which is defined by its mean function $\mu(\mathbf{x})$ and its covariance function, also called the kernel $k(\mathbf{x}, \mathbf{x}')$, given by

$$\mu(\mathbf{x}) = \mathbb{E}[f(\mathbf{x})] \quad (1a)$$

$$k(\mathbf{x}, \mathbf{x}') = \mathbb{E}[(f(\mathbf{x}) - \mu(\mathbf{x}))(f(\mathbf{x}') - \mu(\mathbf{x}'))]. \quad (1b)$$

The blackbox function $f(\mathbf{x})$ can be approximated by the GP as (Williams and Rasmussen, 2006)

$$f(\mathbf{x}) \sim GP(\mu(\mathbf{x}), k(\mathbf{x}, \mathbf{x}')). \quad (2)$$

Kernels can be written as a matrix K where the element $K_{i,j} = k(\mathbf{x}_i, \mathbf{x}_j)$ represents the covariance between the inputs $\mathbf{x}_i$ and $\mathbf{x}_j$. Given a set of training points X and a set of test points X_* , the kernel matrix can be rewritten as

$$K = \begin{bmatrix} K(X, X) + \sigma_n^2 I & K(X, X_*) \\ K(X_*, X) & K(X_*, X_*) \end{bmatrix}. \quad (3)$$

Here, $K(X, X)$ is the covariance of the known data and $\sigma_n^2 I$ is added to account for the noise in the observation data, where σ_n^2 is the variance of the noise and I is the identity matrix. The covariance

between the known and the unknown data is given by $K(X, X_*)$ and $K(X_*, X)$ and the covariance of the unknown data is given by $K(X_*, X_*)$. These submatrices can be used to predict the mean and covariance of unknown data by Williams and Rasmussen (2006)

$$\bar{f}_* = \mathbb{E}[f_* | X, y, X_*] = K(X_*, X)[K(X, X) + \sigma_n^2 I]^{-1} y \quad (4a)$$

$$\text{cov}(f_*) = K(X_*, X_*) - K(X_*, X)[K(X, X) + \sigma_n^2 I]^{-1} K(X, X_*). \quad (4b)$$

Here, y is the vector containing the observation data at the training points X and f_* are the function values at the unknown test data X_* . The quality of this prediction depends on how well the GP parameters match the unknown function. Especially the choice of the kernel is crucial since it defines the shape of the prior and posterior distribution of the GP. Assumptions about the underlying process such as periodicity, smoothness, or (non)linearity are encoded in the kernel. Common choices are the constant kernel, Radial Basis Function kernel, Rational Quadratic kernel, and the Matérn kernel. A detailed discussion of different kernels and their properties is beyond the scope of this paper, and the interested reader is referred to Duvenaud (2014) and Williams and Rasmussen (2006). In this paper, we use the Matérn kernel, which is defined as

$$k_{\text{Matern}}(\mathbf{x}, \mathbf{x}') = \frac{1}{\Gamma(\nu)2^{\nu-1}} \left(\frac{\sqrt{2\nu}}{l} |\mathbf{x} - \mathbf{x}'| \right)^\nu K_\nu \left(\frac{\sqrt{2\nu}}{l} |\mathbf{x} - \mathbf{x}'| \right), \quad (5)$$

where $|\cdot|$ denotes the Euclidean distance, $K_\nu(\cdot)$ is a modified Bessel function, and $\Gamma(\cdot)$ is the gamma function (Williams and Rasmussen, 2006). The positive parameters ν and l are the design parameters of the kernel. The larger ν , the smoother the correlation function will be. Typically, ν is chosen as half-integer $\nu = p + \frac{1}{2}$, $p \in \mathbb{N}$. The length scale l defines how far apart points are still correlated. The regression task is to vary the hyperparameters such that they best fit the data seen so far. This can be done in different ways as pointed out in Williams and Rasmussen (2006). Subsequently, this is done by maximizing the log-marginal-likelihood (LML) via a suitable optimizer, e.g., the L-BFGS-B algorithm (Zhu et al., 1997).

2.2.2 Acquisition functions

The AF provides a measure of how certain the model is about the estimate of the function at each function value $\mathbf{x}$. Several functions have been proposed for this task of which the most

widely used are PI, EI, and UCB (Brochu et al., 2010). They all include the possibility to focus more on exploration or exploitation and are explained below.

PI maximizes the probability of obtaining a function value that is better than the current best. It is computed based on the normalized surrogate model given by

$$Z = \frac{f(\mathbf{x}^*) - \mu(\mathbf{x}) - \xi}{\sigma(\mathbf{x})}, \quad (6)$$

where $\mathbf{x}^*$ is the location of the current best function value, $\sigma(\mathbf{x})$ is the standard deviation of the predicted posterior distribution at $\mathbf{x}$, and ξ is the design parameter that controls the ratio of exploration and exploitation (Snoek et al., 2012). High values for ξ favor exploration and low one's exploitation. The PI is calculated as

$$PI(\mathbf{x}) = \Phi(Z) \quad (7)$$

with $\Phi(\cdot)$ denoting the cumulative distribution function (CDF) Snoek et al. (2012).

EI extends PI in the sense that not only the PI is calculated, but also the magnitude of the potential improvement (Brochu et al., 2010). It reads

$$EI(\mathbf{x}) = (f(\mathbf{x}^*) - \mu(\mathbf{x}) - \xi)\Phi(Z) + \sigma(\mathbf{x})\phi(Z), \quad (8)$$

where $\phi(\cdot)$ is the probability density function (PDF) of the normal distribution.

UCB chooses to sample at the location where the upper bound on the confidence interval of the estimate is the largest. It is calculated by

$$UCB(\mathbf{x}) = \mu(\mathbf{x}) + \kappa\sigma(\mathbf{x}) \quad (9)$$

with $\kappa > 0$ (Brochu et al., 2010). Similar to ξ in PI and EI, high values for κ favor exploration and low one's exploitation.

2.3 Bayesian optimization for time-variant systems

While in many applications the scalar field can be assumed constant or at least very slowly changing, this is not true for sediment plume estimation in the context of dredging or mining activities. Thus, the application of the so far introduced methodology will yield suboptimal results. Some extensions to BO have been proposed to address time-variant systems. These include a regular reset of the learned hyperparameters (Bogunovic et al., 2016), an event-triggered reset of the learned hyperparameters (Brunzema et al., 2022a), separation of the kernel into a stationary and a time-variant covariance function and separate learning of the hyperparameters (Nyikosa et al., 2018), a sliding window for all measurements (Cheung et al., 2019), and weighting of the kernel (Bogunovic et al., 2016; Deng et al., 2022; Brunzema et al., 2022b). For the considered application, a complete reset of the hyperparameters implies gaps in the estimation during the initial sampling phase of the BO due to the large distances the AUVs have to travel. Therefore, these approaches are impractical for the use case. In this paper, we use the sliding-window approach of Cheung

et al. (2019). This means that only the last l_{win} samples are considered in the GPR, where l_{win} is the sliding-window length, which has to be chosen based on the dynamics of the system, here the variability of the bottom currents. This approach has two distinct advantages. First, there is an upper bound for the size of the GPR, which prevents the optimization problem from becoming too large, and second, none of the other steps of the BO have to be adapted; thus, well-established libraries for BO can be used.

2.4 Traveling salesman problem

The traveling salesman problem (TSP) addresses the problem of finding the shortest path along a set of locations, where each location is visited only once. In the proposed approach, we do not only sample at one location per iteration as in classical BO, but at N_{TSP} locations per AUV, which is explained in more detail in the next section. The TSP is used in this context to minimize the AUV path lengths and thus increase the efficiency of the approach. Traditionally, the TSP is formulated as a closed loop so that start and end are the same location, but it can also be formulated as an open loop. The TSP belongs to the class of NP-complete problems (Hoffman et al., 2013). Even though no polynomial-time solution has been found, different algorithms have been proposed that can solve also problems with large numbers of locations in an efficient manner. For this study, the implementation of Mulvad (2022) is used, which implements the Held-Karp algorithm, a dynamic programming algorithm, for computing the exact solution of the TSP.

2.5 Proposed approach

We consider the case that a suspension plume shall be sampled by N_{AUV} AUVs such that a good estimate of the SPM concentration in the working area can be obtained. We extend the classical BO approach as depicted in Figure 1 to address the challenges in this special case. The approach is shown as a flowchart in Figure 2. An initial sampling is performed by flying lawnmower patterns. The patterns are oriented with the long segments parallel to the main water current direction because it can be assumed that this will be the main correlation axis. The GPR, termination criterion and evaluation of the AF are the same as in Section 2.2. In the classical BO, the sampling cost is assumed to be the same for all arguments $\mathbf{x}$ but this is not correct in the considered application because of the distances the AUVs have to travel between measurements. Therefore, instead of sampling the plume only at the location where $\max(\text{AF}(\mathbf{x}))$, we choose the $N_{\text{TSP}} \cdot N_{\text{AUV}}$ locations corresponding to the highest values of the AF. Here, N_{TSP} is the order of the TSP. The sampling locations are clustered into N_{AUV} groups based on their distance to the AUVs. Furthermore, not only the samples at these locations are taken into account but also samples at locations based on the split-path method proposed in Samaniego et al. (2021). In the split-path method samples are taken in equidistant intervals d_{sp} between two waypoints. For the GPR and the successive evaluation of the AF

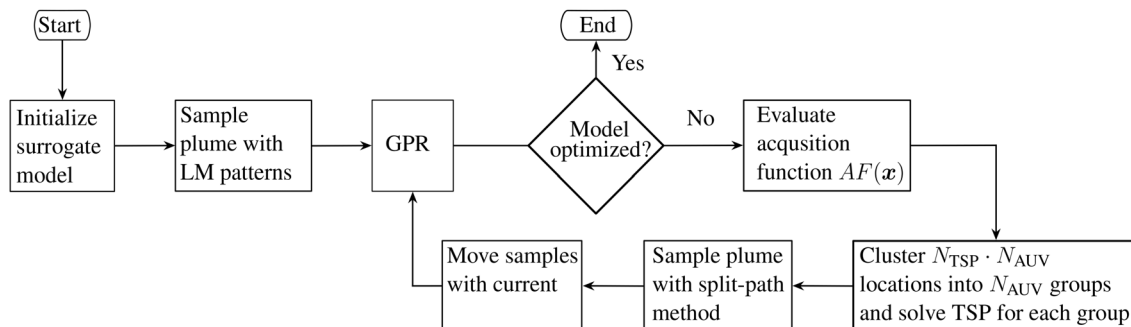


FIGURE 2

Flowchart of the proposed Bayesian optimization scheme for sediment plume estimation with multiple AUVs.

domain bounds have to be specified, which can be hard to estimate *a priori*. It is advisable to overestimate these bound to prevent cutting of the plume, especially in the direction of the bottom near currents. However, this can lead to very large AUV paths whenever the uncertainty of the prediction in the outer parts of the domain is high. Therefore, we adopt and modify the pan and zoom approach presented in [Stander and Craig \(2002\)](#). The aim for this approach is to converge to the optimum of a function by shifting and/or reducing the domain bounds of the optimization problem. However, in the case of plume estimation, the goal is not to converge to a maximum or minimum of the SPM concentration, but to shrink and pan the area so that only parts where no plume is present are discarded. Therefore, we propose a domain reduction by

$$\begin{bmatrix} x_{k,l} \\ x_{k,u} \\ y_{k,l} \\ y_{k,u} \end{bmatrix} = \begin{bmatrix} x_{k-1,min} - d_{tol} \\ x_{k-1,max} + d_{tol} \\ y_{k-1,min} - d_{tol} \\ y_{k-1,min} + d_{tol} \end{bmatrix}, \quad (10)$$

where k refers to the iteration of the optimization and the subscripts l and u refer to the lower and upper bound of the coordinates x and y , respectively. The coordinates $x_{k-1,min}$, $x_{k-1,max}$, $y_{k-1,min}$, and $y_{k-1,max}$ correspond to the smallest and largest coordinates at which the plume was measurable in the last iteration and d_{tol} is a tolerance distance that is added/subtracted to allow not only shrinking of the area but also widening as the plume evolves.

To address the dynamics of the plume, two measures are taken. First, as mentioned above, the sliding window approach proposed in [Cheung et al. \(2019\)](#) is used and second, all measurements are moved with the current to account for the main driver of the plume drift. This is done by the following model

$$\mathbf{p}_i = \mathbf{p}_i + \bar{\mathbf{v}}_{current,i} \cdot C_{trans}(t - t_i) \quad (11a)$$

$$c_i = c_i C_{diff}^{(t-t_i)} \quad (11b)$$

Here, the index i refers to the index in the array, $\mathbf{p}_i$ is a position vector of the SPM measurement, $\bar{\mathbf{v}}_{current,i}$ is the mean current vector in the time interval $[t_i, t]$, where t represents the current time, C_{trans} is a transport coefficient, c_i is the SPM, and C_{diff} is a diffusion

coefficient. Even though this is a simplified model where the diffusion is not modeled by a partial differential equation but only by a decay rate and a decrease of the current velocity, it provides a sufficient approximation on a short time horizon and is much less computationally expensive.

The posterior concentration distribution that can be calculated after the GPR is based on the mean value of the measurement samples μ_{train} and the learned correlation between them. In regions with maximum uncertainty, the predicted concentration will be equal to the mean value of the training samples. This is a valid assumption for many natural processes. However, in the case of sediment plume estimation, it is clear that there are regions without any plume. To account for this, we incorporate the standard deviation $\sigma(\mathbf{x})$, which is calculated during the GPR, and the lower concentration threshold $c_{thr,l}$, which depends on the sensitivity of the considered sensor, to adapt the prediction of the posterior concentration distribution according to

$$\hat{f}(\mathbf{x}) = \begin{cases} 0 & \hat{f}(\mathbf{x}) \leq \mu_{train} \& \sigma(\mathbf{x}) > \sigma_{thr} \\ 0 & \hat{f}(\mathbf{x}) < c_{thr,l} \\ \hat{f}(\mathbf{x}) & else \end{cases} \quad (12)$$

Herein, $\hat{f}(\mathbf{x})$ is the predicted SPM at the grid point $\mathbf{x}$ and σ_{thr} is the upper threshold for the standard deviation. This threshold is a design parameter that should be chosen based on the maximum value of $\sigma(\mathbf{x})$. The first case in (12) describes the situation where the model is uncertain about a relatively small concentration, thus a situation where it is likely that there is no plume present but only background noise.

2.6 Model data

The data used in this study originate from a numerical simulation of sediment plume transport induced by a dredge experiment in the northeastern equatorial Pacific described in [Purkiani et al. \(2021\)](#). The dataset has been published by [Purkiani et al. \(2024\)](#). In the following, the dredge experiment and the data are briefly explained; for further details, the reader is referred to [Purkiani et al. \(2021\)](#) and [Haalboom et al. \(2022\)](#). The dredge experiment was conducted in the northeastern equatorial Pacific,

and the sediment plume was created by towing a 1-m-wide geological chain dredge across the seafloor at velocities between 0.2 ms^{-1} and 0.5 ms^{-1} along 11 tracks between 450 m and 610 m in length. The modeled area for the plume dispersal is approx. $1,175 \times 1,350 \times 114 \text{ m}$ (northing, easting, height/ x, y, z) in steps of $20 \times 23 \times 6 \text{ m}$. The model uses an internal simulation time step length of 5 s and produces data at 300 time steps with intervals of 300 s so that the complete experiment can be covered. Even though the model area in the z -direction is relatively large, the main part of the measurable plume is present in the lowest three grid layers corresponding to a 12-m layer close to the seafloor. In the model

data, the SPM is divided into three sediment classes based on the grain size. Purkiani et al. (2021) call these class 1 (D_{25} : $70 \mu\text{m}$), class 2 (D_{50} : $340 \mu\text{m}$), and class 3 (D_{75} : $590 \mu\text{m}$). In the simulation, we use the sum of all three classes as we assume measurements from an OBS, which cannot differentiate between these classes. To obtain continuous data from the discrete model grid, an interpolation between grid points via the piecewise cubic hermite interpolating polynomial method is used. Similarly, the time resolution is up-sampled to $1/30 \text{ Hz}$ and linearly interpolated. A visualization of the plume and its temporal evolution is shown in Figure 3. Here, a view on the X - Z plane of the plume is provided for the times (A) 0.5 h

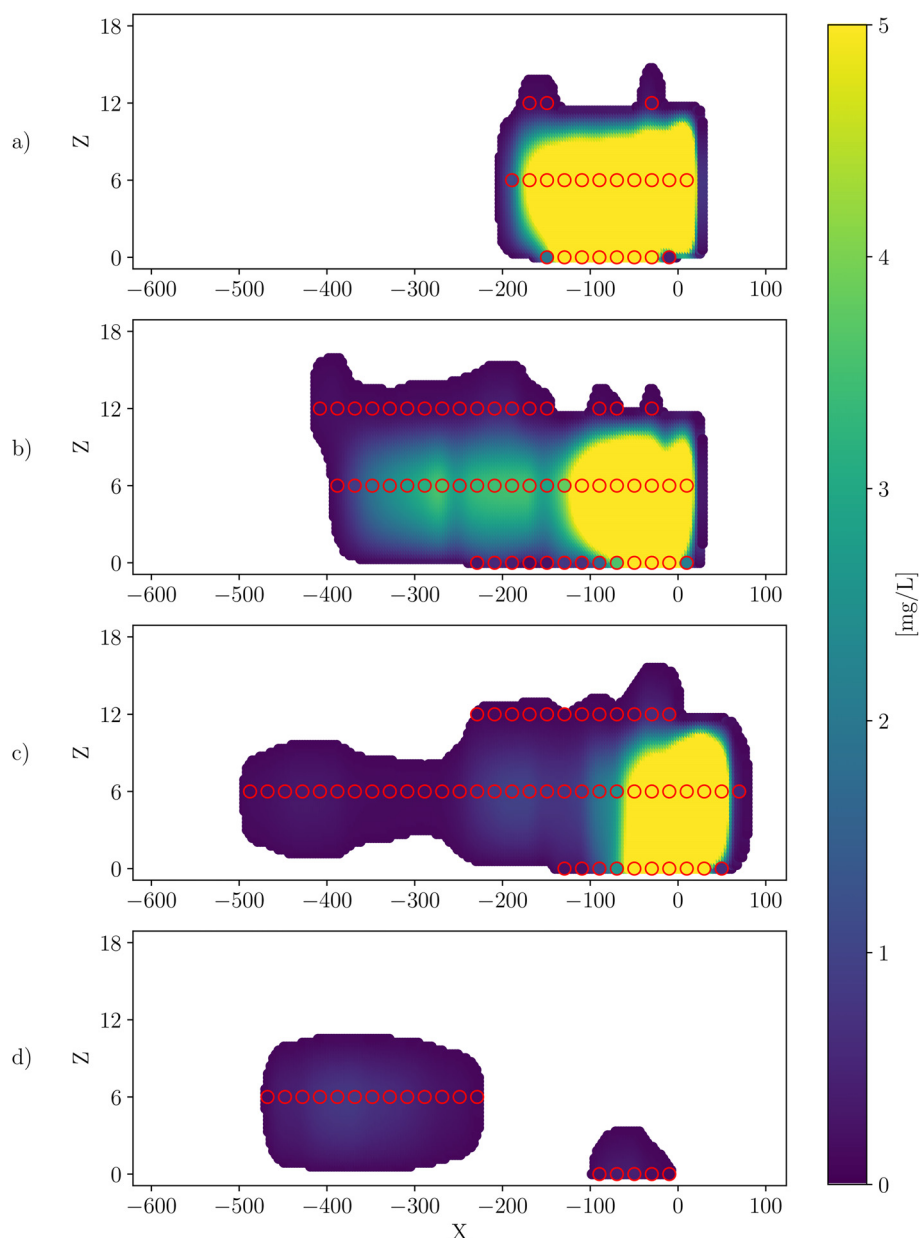


FIGURE 3

View on the X - Z (in meters) plane of the plume for times (A) 0.5 h before and (B) 1 h, (C) 9 h, and (D) 16 h after the AUV mission start. The red circles indicate the original grid points, and the dense points show the pchip-interpolated plume averaged over all y -voxels which contain a measurable suspended particulate matter concentration.

before and (B) 1 h, (C) 9 h, and (D) 16 h after the AUV mission start. The X - Z plane was chosen because the direction of the water current is mainly in the negative X -direction, so the change over time is best seen in this plane. Furthermore, in an interpolated 3D visualization, the high concentration in the inner part of the plume would not be visible because these voxels would be covered by the outer voxels. The SPM concentration is color coded and saturated at $5^{\text{mg}}/\text{L}$. The red circles indicate the original grid points, and the dense, connected points in the background show the interpolated plume. The concentration is averaged over all Y grid points at which the SPM concentration is larger than $c_{\text{thr},l}$. It can be seen that there are large SPM concentrations at $X \in [-150, 50]$, which corresponds to the region where the dredge is towed across the seafloor.

2.7 Quality measures

Finding a good quality measure for the estimation is difficult for this use case. On the one hand, it is important to get as close as possible to the overall SPM concentration, but on the other hand, also the distribution in space is important. A classical measure is the mean squared error (MSE), but this can yield misleading results in case of time-varying systems. Consider, e.g., the (extreme) case where the BO estimates the concentration of all voxels correct but they are shifted by 1 grid index in the opposite direction of the water current. This can be seen as a pure time delay in the estimation, and thus everything would be correct but 20 m off. In terms of the grid-based MSE, this can lead to a large error, depending on the distribution of the plume. Therefore, the MSE alone is not sufficient to measure the quality of the estimation. We propose to use the MSE alongside the ratio of the GP estimated to the ground truth summed SPM concentration, which we in the following call the GP2GT ratio. It is calculated as

$$\text{GP2GT} = \frac{\sum \hat{f}(\mathbf{x})}{\sum f(\mathbf{x})}. \quad (13)$$

A value $\text{GP2GT} = 1$ is the optimal case here. It describes the situation where the model estimates the summed concentration identical to the ground-truth concentration. A value smaller than 1 indicates an underestimation of the summed concentration and a value larger than 1 an overestimation.

3 Results

In Figure 4, the estimated SPM concentrations and those obtained from the model are shown as scatter plots for the time steps 1 h, 9 h, and 16 h after the start. These correspond to the beginning, mid, and end of the dredge experiment. For visualization purpose, the color coding is saturated at $9^{\text{mg}}/\text{L}$ for subplots a) and at $2^{\text{mg}}/\text{L}$ for subplots (b) and (c) in order to also make variations in areas of low concentrations visible. Additionally, the Z -scale is chosen such that there is some space between grid layers so that all three grid layers are fully visible. It can be seen that the rough shape of the plume was correctly estimated at all three time steps

but also that in all three subplots there are some parts of the plume, which are not estimated correctly. The latter occurs mainly in regions with very low concentrations and at the edges of the plume or in the upper and lower depth layer where the plume is only present in small areas. Also, the concentration estimation provides good results in most parts of the plume. The most challenging area for the estimation is close to the upper domain bound of the x coordinate at approx. $x \in [-150, -100]$. The main current direction is in the negative x direction; hence, the plume enters the domain at this bound. Furthermore, due to the irregular swirling of the sediment, the plume entering the domain is strongly time-variable. Therefore, the largest deviations in the concentration estimate are in this region as can be seen in subplots a) and b). In subplots b) at approx. $x \in [-600, -450]$ and in subplots a) at $z = 12$, it can be seen that the BO estimate misses the plume in an area almost completely. This is due to two reasons: first, the concentrations in these regions are smaller than the training mean of the respective BO iterations. Second, less samples in this region are taken because due to the higher uncertainty of the estimate at the upper domain bound of the x coordinate, as mentioned above, this area is sampled more frequently. As this leads to the combination of low expected concentrations and high standard deviation according to Equation 13, these grid cells are discarded.

The quality metrics for the iterations corresponding to each full hour since the start of the estimation as well as the mean values over the complete simulation are listed in Table 1. It can be seen from the GP2GT ratio that there are distinct phases where the BO tends to underestimate or overestimate the concentration. On average, the BO is able to monitor the plume with only a 5% error in terms of the GP2GT ratio. The larger MSE at the beginning of the experiment is mainly related to the higher concentrations in the first hours of the experiment. The average MSE is two orders of magnitude lower than the considered lower concentration threshold $c_{\text{thr},l}$. Both quality measures indicate a very good estimation performance. The slight variation in MSE and GP2GT over the time steps is a consequence of the plume changing and propagating over time due to the irregular sediment release. The parameters used in the simulation are listed in Table 2. A note on the choice of two parameters: First, the sliding window length l_{win} as it might be the least intuitive parameter: It must not be chosen close to or equal to zero as this essentially means that very few or no data are taken into account. On the other hand, it should not be chosen too large because the simple model (11) will not produce accurate results over long time scales. The lower bound of l_{win} should be chosen so that the AUVs are able to cover a significant part of the working area. The upper bound depends on the variability of the water currents. The model (11) works better for laminar flow than for turbulent flow, and thus larger values of l_{win} are feasible in the case of laminar flow. We chose l_{win} such that the measurement data of the last hour are considered but also values between approx. 0.5 h and 1.5 h resulted in reasonable performance metrics. Below and above these values, the performance decreased significantly. Second, the number of AUVs was chosen to be three because we believe that it is possible to operate three AUVs simultaneously and that in large-scale mining scenarios at least three AUVs are needed.

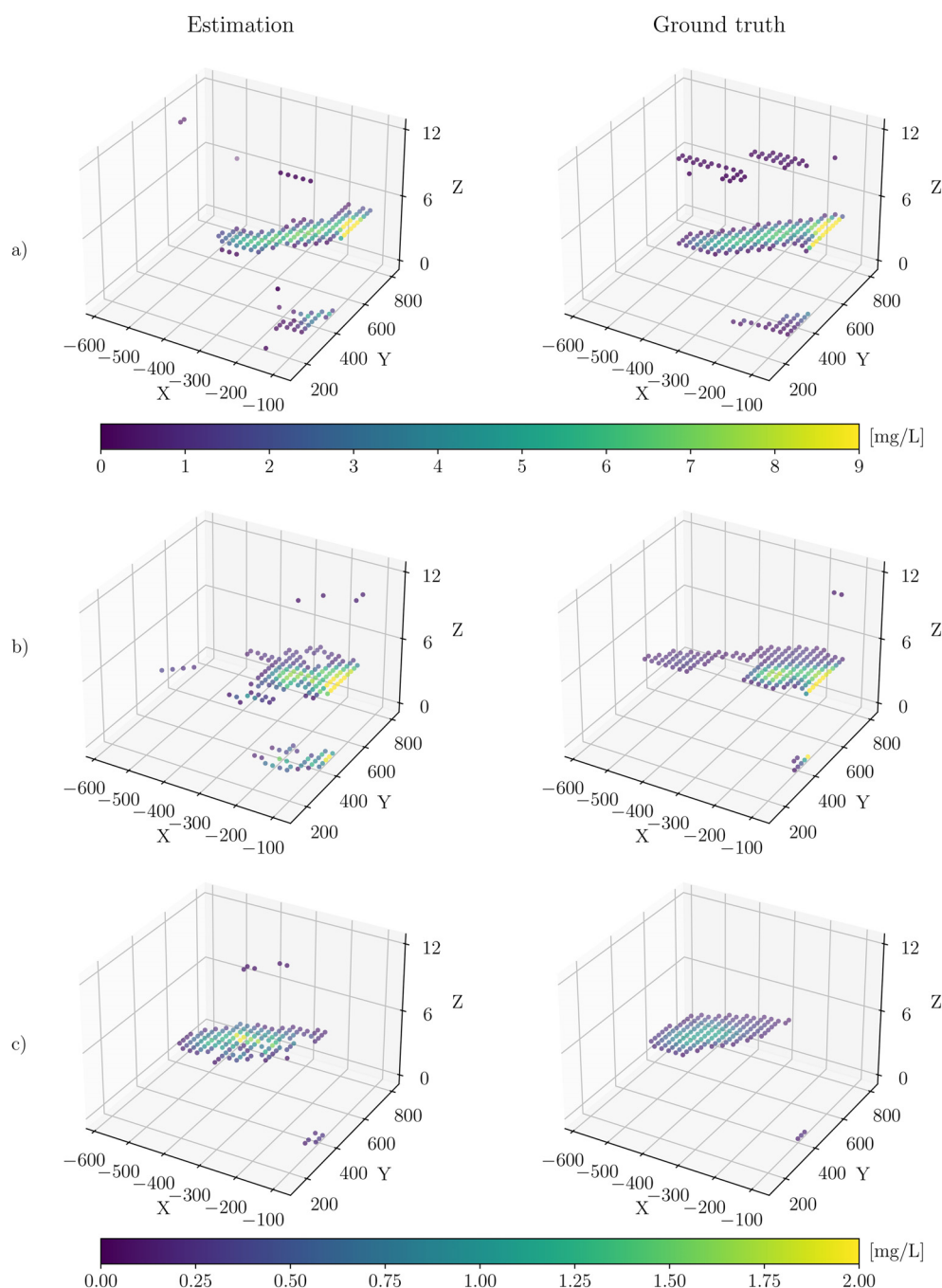


FIGURE 4

Scatter plots of the suspended particulate matter concentration for the time steps (A) 1 h, (B) 9 h, and (C) 16 h after the start. The left column shows the estimated concentration and the right column the ground-truth values. The concentration for the subplots (A) is color coded according to the upper color bar and for subplots (B, C) according to the lower color bar. The concentrations are shown on the original model grid resolution of $20 \times 23 \times 6$ m voxels.

However, the approach also works with one or two AUVs, but with reduced estimation quality. A visualization of the AUV paths is shown in Figure 1 in the Supplementary Files. It can be seen that the region has a higher sampling density at large X-values than at small X-values. At the end of the dredge experiment when the release of sediment stops, the AUV paths move toward smaller X values as is expected.

4 Discussion

The BO domain bounds for the simulation results shown above are chosen as $x \in [-600, -100]$, $y \in [200, 800]$, and $z \in [0, 12]$; thus, they do not include the area of the dredge experiment, which is in the range of $x \in [-100, 50]$. This is due to two reasons. First, in the dredging or mining area, there is at least one instrument or vehicle

TABLE 1 Quality metrics in hourly steps and mean values for the complete simulation.

Time elapsed	MSE	GP2GT
1	$2.1e^{-3}$ mg/L	0.9733
2	$1.0e^{-2}$ mg/L	0.7064
3	$4.5e^{-3}$ mg/L	0.8819
4	$1.2e^{-3}$ mg/L	1.0779
5	$4.9e^{-4}$ mg/L	1.0238
6	$9.1e^{-4}$ mg/L	1.0175
7	$1.1e^{-3}$ mg/L	1.1553
8	$7.6e^{-4}$ mg/L	1.2649
9	$1.7e^{-4}$ mg/L	1.3044
10	$5.3e^{-4}$ mg/L	1.2537
11	$5.1e^{-3}$ mg/L	0.7102
12	$1.0e^{-2}$ mg/L	0.8808
13	$4.7e^{-3}$ mg/L	1.0740
14	$3.6e^{-3}$ mg/L	0.9072
15	$4.1e^{-3}$ mg/L	0.8477
16	$1.7e^{-4}$ mg/L	1.2093
Mean	$3.0e^{-3}$ mg/L	1.0509

operated remotely from the ship via a cable, which can be hard to detect autonomously by an AUV thereby increasing the risk of collision in this area. Second, the SPM concentration in this area will be much higher and much more heterogeneous than further away and will likely include the beginning evolution of near-bottom gravity flows. Since the plume drifting farther away from the dredging or mining is more important for environmental monitoring, the dredge area was discarded in favor of the far-field plume. In addition, the bottom-near gravity plume produced by a mining vehicle is too shallow for monitoring with AUV, and hence also in this scenario, the focus is on the far-field plume.

BO is not designed for discontinuous problems, where the function is equal to zero in large areas, and hence, defining the boundaries of the plume is not straightforward. With Equation 13, we address this issue without losing too much information in areas of low function values. However, as shown in Section 3, a large concentration gradient across the domain may result in the BO not detecting the plume in areas of very low concentrations. In the considered use case, these plume parts can be neglected due to the very low concentrations and the short duration of the dredge experiment. In case of a long-term monitoring, it might be necessary to also monitor these very low concentrations as they add up over time. A possible extension is that an additional BO instance is created and the AUV(s) assigned to this instance operate only in the far field where low concentrations are assumed. The domain bounds of the two BO instances should be chosen such that they do not overlap in order to minimize the risk of AUV collisions.

The plume area considered in this study is relatively small compared with the area of a plume created by a mining vehicle. It is

TABLE 2 Parameters used in the simulation.

AUV parameters	
Number of AUVs N_{AUV}	3
AUV velocity v_{AUV}	$2.5 \frac{\text{m}}{\text{s}}$ (≈ 5 knots)
Time for turning maneuvers T_{turn}	60s
Kernel parameters	
Kernel Type	Matérn
Initial length scale l	[100, 200, 1]
Bounds for l	[(50, 300), (50, 300), (0.2, 3.0)]
Smoothness parameter ν	1.5
Acquisition function parameters	
Function Type	Upper confidence bound
Exploration–exploitation parameter κ	2.8
Bayesian Optimization parameters	
Lower concentration threshold $c_{\text{thr},l}$	$0.1 \frac{\text{mg}}{\text{L}}$
Split path length d_{sp}	30 m
Sliding window length l_{win}	$N_{\text{AUV}} \cdot 3,600 \text{ s} \cdot v_{\text{AUV}}/d_{\text{sp}}$
TSP length N_{TSP}	15
Tolerance distance d_{tol}	50
Transport coefficient C_{trans}	0.2
Diffusion coefficient C_{diff}	0.99985
Standard deviation threshold σ_{thr}	$0.4 \cdot \max(\sigma)$

clear that the number of AUVs cannot be scaled up to yield the same AUV per area ratio as used in this study. However, the plume produced by a mining vehicle will probably be much more homogeneous than the plume produced by the dredge experiment. Therefore, less samples are needed since the correlation of neighboring locations will be much stronger than in the dredge scenario and thus the uncertainty of the prediction will be lower. Furthermore, it can be assumed that the time dependence will decrease because the mining vehicle will constantly bring sediment into suspension. In contrast, the dredge experiment released the sediment very irregularly. Thus, it can be argued that the time dependence of the suspension plume produced by mining will mainly be correlated with the water currents. Therefore, we believe that the method will also be applicable to larger plumes given a limited number of AUVs. Further simulations should be carried out with a dataset of a plume produced by a mining vehicle in order to investigate the influence of these factors in more detail. To the best of the authors' knowledge, such data were not publicly available upon submission of this paper, although Gazis et al. (under revision)¹ describe such a scenario.

The presented study has to be seen as a feasibility study, and before the approach is used on real AUVs, further studies need to be conducted. This would involve implementation of a path planner, proper AUV dynamics, and a collision avoidance system in the

simulation. Even though these aspects are not addressed in this study, we believe that the assumptions made in Section 2.1 are good estimates and the implementation of the mentioned parts will not change the performance of the system significantly. In addition, the clustering of the new sample locations based on the distances to the AUVs does in most cases result in AUV paths that are separated from each other, thus decreasing the probability of collisions.

To further improve the presented approach, not only source measurements from OBS and ABS but also a multibeam echosounder (MBES) could be included. The advantage of MBES systems is that they can sample large areas in a short amount of time, but their use for SPM concentration measurement is not yet as well studied as for OBS and ABS. Fromant et al. (2021) present data, which show a good correlation of MBES data with ABS and OBS for concentrations larger than approx. 40mg/L , which is significantly higher than the concentration considered in this paper. Nevertheless, a combination of both sensor types could be advantageous. An AUV equipped with an MBES system could, e.g., be used to fly above the plume to map its extent making use of the larger coverage. This map could be used to initialize the BO in a more intelligent way, or to update the plume boundaries and to check if new plume patches are covered by the new sample locations suggested by the AF, and if not, manually add waypoints in those regions. In addition, the integration of bathymetry in future simulations would be a valuable extension, as it influences the water current patterns and thus the plume behavior.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

TS: Data curation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing, Conceptualization. JG: Resources, Supervision, Writing – review & editing, Conceptualization, Funding acquisition. TM: Supervision, Writing – review & editing, Conceptualization, Funding acquisition.

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Supplementary material

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Connectivity in the Clarion-Clipperton Zone: a review

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Despite its remoteness, the deep sea is not spared from the impact of human activities. The emergence of industrial-scale deep-sea mining of polymetallic nodules on the abyssal plains (4–5 km depth) of the Clarion-Clipperton fracture zone in the equatorial Pacific is potentially the most threatening undertaking at present time. A primary aspect that must be comprehensively assessed in order to safeguard these abyssal benthic communities through the designation of marine protected areas and the spatial organization of prospective mining fields is connectivity, i.e., the continuity of species distributions in space and time. In this review we provide the current state of knowledge on connectivity through an examination of published literature focused on several animal groups from all benthic faunal size classes (megafauna, macrofauna, meiofauna) as well as Foraminifera and microbes. We highlight the main challenges associated with assessing connectivity in the deep sea and outline the key parameters required to achieve the idealised connectivity study for fauna and microbes.

KEYWORDS

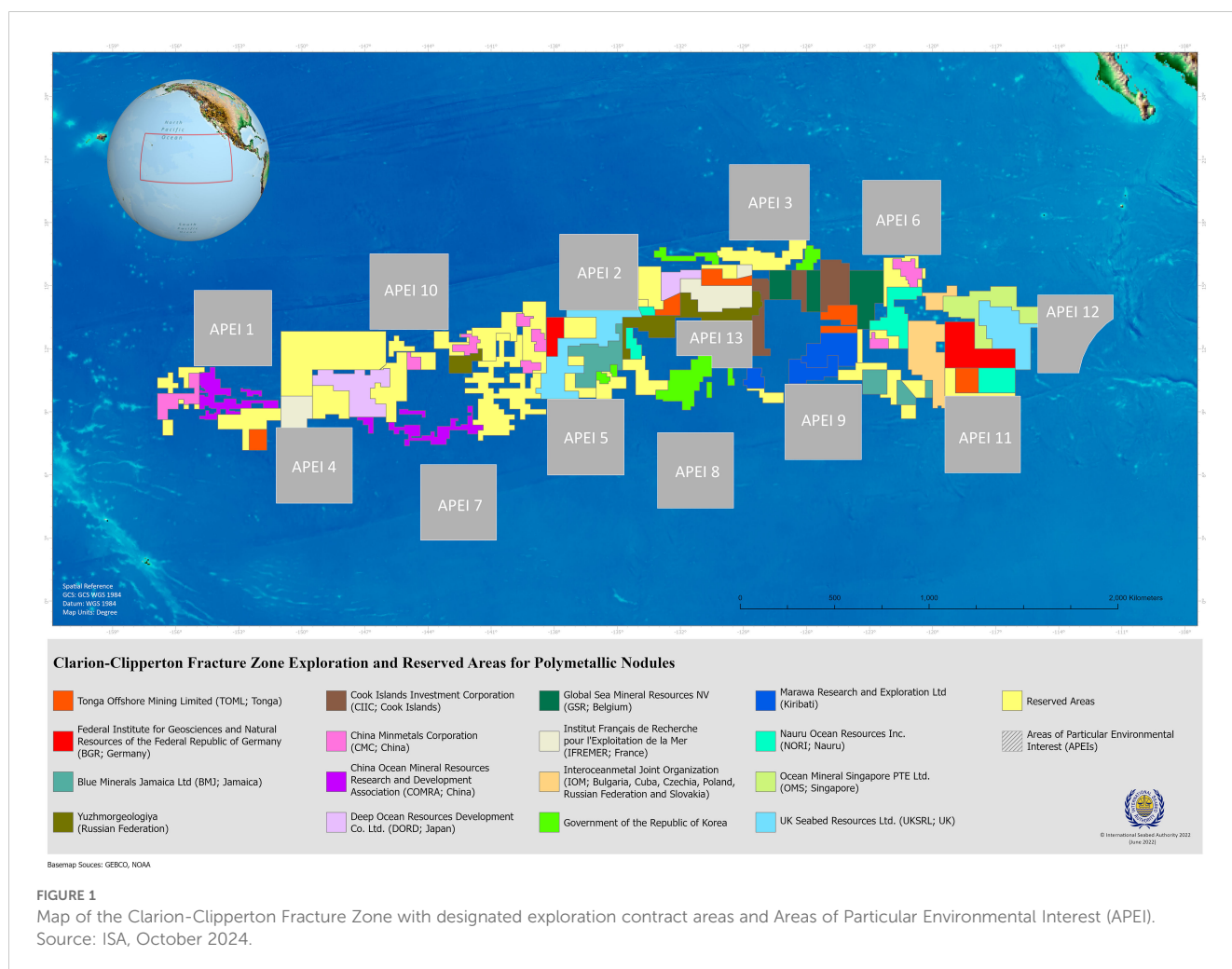
Clarion and Clipperton Fracture Zone, megafauna, macrofauna, meiofauna, microbes, connectivity, review

1 Introduction

The deep sea, defined as marine areas beyond the continental shelf at depths exceeding 200 m, is the largest continuous biome on Earth, amounting to over 95% of the global ocean volume (Danovaro et al., 2020; Webb et al., 2010). While largely inaccessible to most people, all humans benefit from ecosystem services provided by the deep sea, such as climate regulation, carbon sequestration, nutrient cycling and global fish stocks (Thurber et al., 2014; Webb et al., 2010). This largely unexplored environment hosts an astounding amount of biodiversity, potentially unmatched by any other on the planet (Ramirez-Llodra et al., 2010). Abyssal ecosystems are found at depths from 4–6 km, consisting mainly of soft sediments and covering 75% of the global ocean floor, of which less than 1% has been thoroughly investigated (Hughes et al., 2021; Ramirez-Llodra et al., 2010). Despite their remoteness, abyssal plains have not been spared from anthropogenic threats such as climate change, litter and deep seabed mining (Levin and Bris, 2015). Rising ocean temperatures are already altering large-scale deep water mass circulation patterns (e.g., Ditlevsen and Ditlevsen, 2023) while a warmer ocean is expected to cause severe declines in oxygen saturation (Deutsch et al., 2024; Levin, 2017) and nutrient

export from surface waters (Smith et al., 2009; Sweetman et al., 2017), leading to global decreases in abyssal benthic biomass by the end of the century (Jones et al., 2014). Ocean acidification is causing a shallowing of the carbonate compensation depth, an important biogeographic boundary in the abyss (Simon-Lledó et al., 2023) by nearly 100 m since pre-industrial times, and is expected to rise several hundred meters more this century (Sulpis et al., 2018; Harris et al., 2023). These environmental changes have the potential to cause significant shifts in the availability of suitable habitats across large spatial scales, dramatically altering connectivity, i.e., the spatiotemporal continuity of abyssal species distributions.

Halting the current loss of biodiversity is of highest priority in order to ensure the continuation of the provision of the vital ecosystem services and functions that humans depend on. This urgency is reflected in several global initiatives such as the United Nations (UN) 17 Sustainable Development Goals (and notably SDG14, <https://www.un.org/sustainabledevelopment/oceans/>), the Rio Convention of Biological Diversity (<https://www.cbd.int/>) and the UN Agreement on Marine Biological Diversity of Areas beyond National Jurisdiction (BBNJ, <https://www.un.org/bbnjagreement/en>); the latter being especially relevant to deep seabed mining (DSM) since the majority of mineral resources lie beyond national waters. In the case



of the deep-sea realm in particular, which lacks distinctly visible boundaries such as those of terrestrial environments, our ability to conserve biodiversity is inextricably linked not only to the identification of species but also to their distribution and how they are connected between the different locations where they occur. Thus, understanding connectivity is crucial for the assessment of vulnerability and for defining mitigation measures in threatened habitats.

The deep sea is currently being targeted for the potential future extraction of three types of mineral deposits: polymetallic nodules found at abyssal plains (PMN), cobalt-rich ferromanganese crusts on seamounts (CFC), and polymetallic massive sulfides at hydrothermal vents (PMS). In this review we focus on polymetallic nodules, which form via the precipitation of various minerals such as manganese, nickel, copper, and cobalt from the

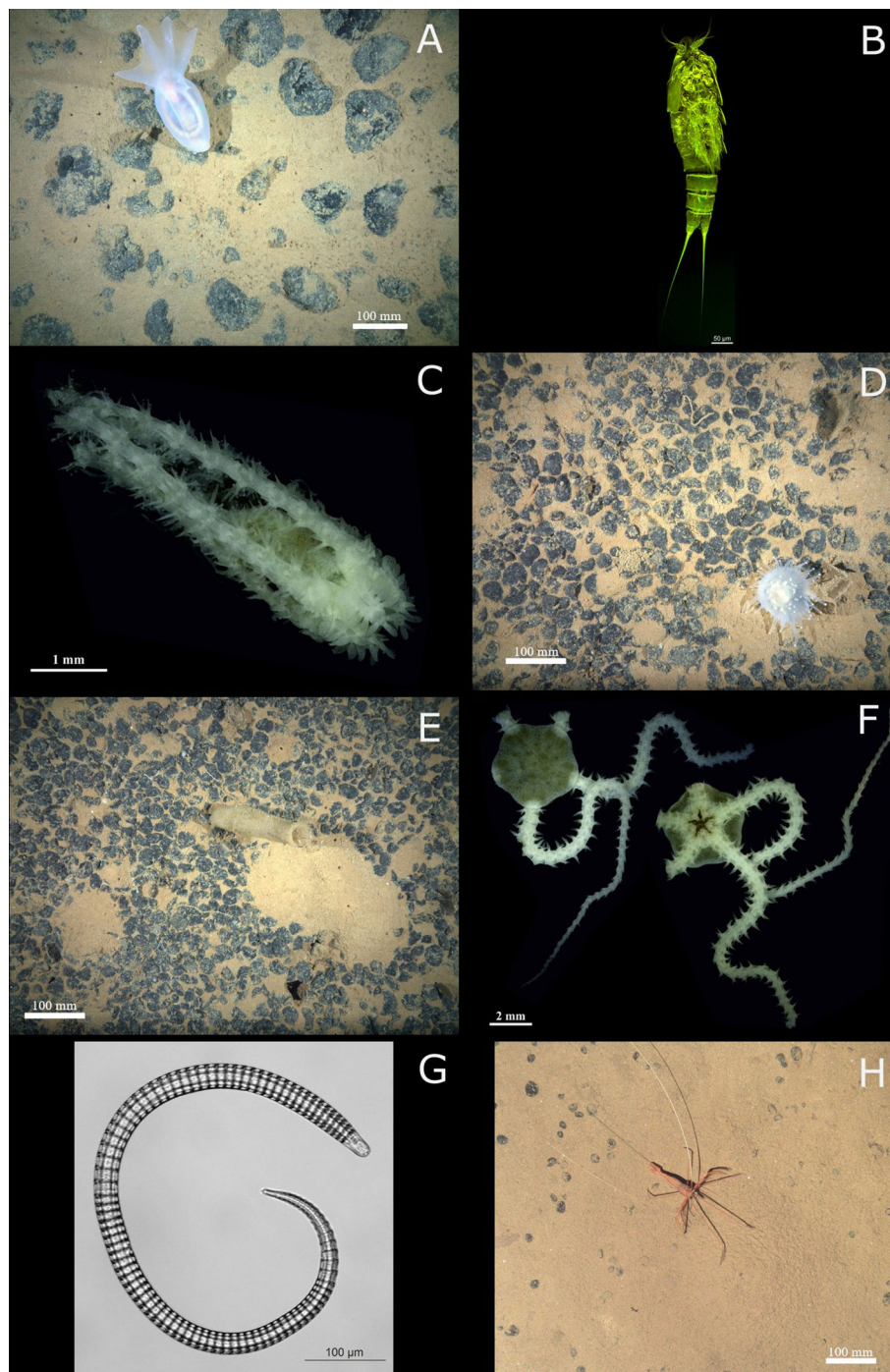


FIGURE 2

Fauna of the Clarion-Clipperton zone. Left to right, top to bottom: (A) *Amperima* sp. indet, (B) *Ectinosomatidae* gen. indet, (C) *Ophiotholia saskia*, (D) *Corallimorphus* sp. indet, (E) *Euplectellidae* gen. indet, (F) *Salix daleus*, (G) *Ceramonea* sp., (H) *Bathystylodactylus echinus*. Image credits: (A, D, E, H) Courtesy of/extracted from Simon-Lledo et al., 2023 (<https://zenodo.org/records/8172728>); (B) Katja Uhlenkott; (C, F): Magdalini Christodoulou; (G): Tania Campinas Bezerra.

seawater (hydrogenetic) and/or the sediment pore water (diagenic) onto an organic nucleus (Hein et al., 2020; Thiel, 2005). Their formation is one of the slowest known geological processes (Dutkiewicz et al., 2020), progressing at a rate of 2–10 mm/million years; effectively, a non-renewable resource by human standards (Boetius and Haeckel, 2018; Verlaan and Cronan, 2022). The nodules are found at the surface of soft sediments on abyssal plains in the Pacific, Atlantic and Indian oceans. The polymetallic nodule fields in the Clarion-Clipperton Fracture Zone (CCZ, Figure 1) in the Pacific cover huge expanses of the seafloor; up to ca. 80% the size of the contiguous United States of America (Wedding et al., 2015). Dispersal barriers such as large and continuous topographical structures (e.g., submarine mountain ridges) and/or steep physical gradients (e.g., temperature, pressure, oxygen) are absent, meaning that the connectivity of benthic communities could operate across thousands of km. Our understanding of fundamental processes, such as dispersal and connectivity, that regulate biodiversity at such large scales, is severely limited; thus, synthesizing our currently available knowledge across all benthic size classes [i.e., microbes (<32 µm), meiofauna (> 32 µm), macrofauna (>300 µm), megafauna (>1 cm), Figure 2], is critical to guide conservation efforts as well as future research.

2 Connectivity in the CCZ

The umbrella-term “connectivity” is used ubiquitously to refer to (i) genetic, (ii) demographic, (iii) biological, (iv) ecological, (v) structural, and/or (vi) functional connectivity (Selkoe et al., 2016). All seek to describe and understand the continuity of species distributions in space and time via different avenues. Beta diversity, which is defined as the shift in species composition among sites (Whittaker, 1960), can be used as an indirect measure of connectivity between communities and more generally to study biogeography (i.e., distribution of taxa across space and time). The biogeography of organisms is controlled by four fundamental processes: dispersal, ecological drift, selection, and mutation (Hanson et al., 2012). It is thought that the scale, direction, frequency and intensity of species dispersal is most influential for deep-sea connectivity (Baco et al., 2016; Hilário et al., 2015). The interaction of these processes manifests in two major biogeographical patterns: the taxa-area relationship, i.e., the positive relationship between the number of taxa in an area and the size of that area (Arrhenius, 1921); and the distance-decay relationship, i.e., the decline in community similarity over increasing geographical distance (Nekola and White, 1999). Several studies have identified taxa-area and distance-decay relationships in marine microbes, with stronger patterns in heterogeneous and island-like habitats than in continuous ones (Zinger et al., 2011, 2014). Microbial dispersal is shaped by intrinsic taxon-specific traits (e.g., spore formation, morphology, habitat specificity) and extrinsic factors such as population size (Anderson et al., 2015), topographic continuity (Schauer et al., 2010; Bienhold et al., 2016), and environmental gradients (Bienhold

et al., 2012; Ruff et al., 2015; Varliero et al., 2019; Ramírez et al., 2019; Orcutt et al., 2021). Although differences in environmental factors (e.g., particulate organic carbon flux to the seafloor, water depth, and sediment composition) that can potentially affect microbial biogeography are known to exist across the CCZ (Washburn et al., 2021a), the study of microbial connectivity is hindered by the paucity of knowledge regarding the diversity of microbes inhabiting the seafloor.

Pelagic larval duration (PLD, the amount of time a larva spends in the water column before settlement), or the more inclusive pelagic propagule duration (PPD, including the embryonic phase) greatly modulates dispersal capacity as currents can transport taxa hundreds of km from their birth place. Although it has been suggested that marine connectivity is typically underestimated (Manel et al., 2019), a significant gap of knowledge with respect to PPD of deep-sea taxa hinders even the most simplistic estimates of dispersal. By knowing the PPD and the speed and direction of currents we can estimate the upper limit of dispersal distance and infer probable trajectories (Hilário et al., 2015). However, due to the scarcity of molecular data, and data on life histories of CCZ fauna, circulation, accurate modelling and precise validation it is currently impossible to accurately predict connectivity in this region. In addition to PPD, knowledge of other biological traits, such as fecundity, spawning periodicity and seasonality are necessary to validate biophysical models. Likewise, we are unable to identify which areas offer high quality habitat for any given taxon and thus able to foster population growth (i.e., sources) and which are low quality habitat, in themselves unable to sustain a population without being linked to a source (i.e., sinks) (Pulliam, 1988).

Inferring potential dispersal trajectories has traditionally relied on Lagrangian particle tracking which models the movement of neutrally buoyant particles in a fluid (Viegas et al., 2024; Young et al., 2012). Generating these models is challenging in deep-sea ecosystems for which water-current and high-resolution bathymetrical mapping is limited (Hilário et al., 2015). In fact, for taxa with long-distance dispersal capacities, connectivity is more important than habitat quality for the successful design of a Marine Protected Area (MPA) network (Berglund et al., 2012). In practice however, connectivity is one of the least frequently applied ecological criteria for the designation of MPAs despite its importance for securing conservation of biodiversity, sustainability, population resilience and persistence (Balbar and Metaxas, 2019). In this context, “ecological connectivity” has now been listed in the BBNJ agreement as an indicative criterium for the establishment of MPAs. Many challenges persist, such as the paucity of genetic data, disagreements on how to apply genetic metrics to inform policy and the aptly-described research-implementation gap between those who generate (geneticists) and analyze data (bioinformaticians) and those who use the data to inform management actions (ecologists) and decision-making (legislators) (Hogg, 2024).

Dispersal and thereby, connectivity, can be inferred through genetic methods. This approach is based on the assumption that genetic differences between specimens from different locations will be small if they are connected by dispersal and vice versa. The various DNA-based techniques (e.g., microsatellites, haplotype

networks, genotypic fingerprinting, Single Nucleotide Polymorphisms [SNPs]) that are used typically require a minimum number of conspecifics from several locations in order to be informative with respect to population structure. Low faunal densities, restricted sampling, limited information on species distributions coupled to habitat heterogeneity, lack of time series covering seasonal and interannual variability, the largely undescribed diversity of the CCZ and cryptic speciation (i.e., distinct species that are morphologically identical) pose significant challenges to achieving population-level resolution. An additional complication arises from the high proportion of taxa found at just a single sampling location (singletons), a consistent pattern in morphological and DNA-based investigations of all size classes in the CCZ (e.g., Christodoulou et al., 2020; Hauquier et al., 2019; Macheriotou et al., 2020; Simon-Lledó et al., 2019; Wilson, 2017). Even though singletons could represent an artefact of under-sampling, by definition, connectivity can only be assessed for taxa that are present in at least two locations, resulting in a substantial gap of knowledge for a large fraction of these assemblages. For meiofaunal taxa, the most abundant metazoans in the CCZ, the inability to achieve species-level resolution significantly hinders investigation into population dynamics. This limitation makes it challenging to determine whether these taxa follow an “island” or “stepping-stone” model of population structure through Isolation-By-Distance (IBD) (Vrijenhoek, 1997), whether alternative modes of differentiation (e.g., “genomic islands” [Malinsky et al., 2015]) predominate, or whether broad dispersal with weak genetic structuring, consistent with the “everything is everywhere” hypothesis (Baas Becking, 1934), is in effect.

The nodule fields of the CCZ (Figure 1) are part of the “Area” (i.e., seabed and ocean floor and subsoil thereof, beyond the limits of national jurisdiction, Article 1, UNCLOS) which along with “its resources are the common heritage of mankind” (Article 136, UNCLOS) and thus “no State shall claim or exercise sovereignty or sovereign rights over any part of the Area or its resources” (Article 137, UNCLOS). The International Seabed Authority (ISA) is “the organization through which States Parties to UNCLOS organize and control all mineral-resources-related activities in the Area for the benefit of humankind as a whole” and “has the mandate to ensure the effective protection of the marine environment from harmful effects that may arise from deep-seabed-related activities.” (<https://www.isa.org.jm/>). To this end, eleven Areas of Particular Environmental Interest (APEIs) measuring 400 x 400 km have been placed along the outer perimeter of the CCZ (Figure 1), and two more in the central region. The latter two were designed taking into consideration boundaries of Exclusive Economic Zones and contract areas and therefore do not meet the size specified in the original criteria of the environmental management plan for the CCZ (ISA, 2021). The APEIs will be excluded from future mining operations (and the impacts thereof) and are meant to be representative of the habitats found in the CCZ in order to safeguard regional biodiversity as well as ecosystem structure and functioning (ISA, 2021; McQuaid et al., 2020). Moreover, the APEIs are intended “to maintain sustainable populations and to capture the full range of habitats and

communities by being large enough to maintain minimum viable population sizes for species potentially restricted to a subregion of the Clarion-Clipperton Fracture Zone, without being affected by mining plumes from any activities immediately adjacent to an area” (ISBA/22/LTC/12, https://isa.org.jm/files/documents/isba-22ltc-12_1.pdf). For this to be possible, it must be proven that the APEIs are large enough to sustain viable populations themselves and/or are linked to another APEI via dispersal. Moreover, we maintain that similar considerations are needed for the areas that will potentially be mined in the future and should thus guide the assignment of the size and spatial layout of these sites. Of equal importance is the concurrent protection of the pelagic realm along with the seabed, as the water column is expected to be highly impacted by sediment plumes generated by the separation of nodules and sediments by the nodule collector (i.e., collector plume) and those derived from nodule washing aboard the surface vessel (i.e., return plume) (Drazen et al., 2020; Wedding et al., 2013). It is well-known that benthic productivity is subsidized by that of overlaying surface waters in the form of detritus, nutrients and prey; thus any effort to protect the former must include the latter in order to be effective (O’Leary and Roberts, 2018).

Investigating any aspect of biodiversity in the CCZ is a challenging endeavor due to the remoteness of the region and the need for large research vessels, sophisticated equipment and trained personnel to obtain samples from such depth. Moreover, connectivity in the marine realm is particularly complicated because dispersal “is arguably the least understood demographic process in the sea” (Sweatner et al., 2019). The transport of an individual from its birth to recruit location, takes place in a three-dimensional space which is influenced by the movement of water masses on a local and global scale as well as behavior, developmental mode, ontogenetic vertical migration, embryo and larval buoyancy, predation, food availability, developmental rate, longevity, and physiological tolerances. These processes have been studied for very few deep-sea animals, and are restricted to species that occur in densities large enough to allow for systematic analyses. Dispersal of less abundant species can only be addressed by assuming similarities to model organisms to which they are related to, or share characteristics with. The vast majority of marine benthic taxa have a free-living dispersive larval stage, while only six out of 40 marine phyla exhibit direct development (i.e., nematodes, chaetognaths, gastrotrichs, kinorhynch, gnathostomulids and tardigrades) (Pechenik, 1999). From the early days of deep-sea exploration, it was assumed that the “extreme” environmental conditions would select for unique larval development and adaptation. However, with few exceptions, the development strategies found in shallow-water systems are also present in the deep sea (Young, 2003). Brooding was once predicted to be the predominant mode of development in the deep sea, particularly in the extensive and nutrient-poor abyssal plains, nevertheless planktonic embryonic/larval development is equally or even more prevalent. Taxa such as peracarid crustaceans, nematodes and many polychaetes, that make up a large proportion of benthic CCZ diversity (Rabone et al., 2023) are phylogenetically constrained to brood embryos and therefore

connectivity depends on adult mobility (Brix et al., 2020). An association between species life-habits and dispersal strategies has been recently hypothesized to explain the more regionally restricted dispersal patterns of nodule-growing species compared to sediment dwelling or swimming fauna (Simon-Lledó et al., 2025). Regardless of potential strategies that may enhance dispersal distance (e.g., increased parental investment, arrested development or long planktonic duration), successful planktonic dispersal and, hence, connectivity, may be reduced in mined areas, as earlier life stages are the most vulnerable to disturbances like the release of contaminants into the water column (Mestre et al., 2019). In addition to active adult migration and embryonic/larval dispersal, episodic disturbances of the seabed or deep-reaching eddies (Aleynik et al., 2017) could resuspend sediment and its infaunal organisms into the water column, and their dispersal from this point would thus be modulated by water currents in the same way as for planktonic life-stages. Other forms of transport include rafting and hitchhiking (Winston, 2012). It is even possible that the nodules themselves serve as stepping stones for dispersal if organisms travel from one nodule to the next. Moreover, it has been shown that population structure patterns can be species-specific, rendering generalizations misleading (Miller and Gunasekera, 2017).

In light of the many anthropogenic impacts that marine ecosystems are being faced with, and the need to preserve threatened biodiversity, connectivity has become a parameter of primary importance in determining resilience, as it helps to define the ability of an ecosystem to recover post-impact (Gollner et al., 2017), and in the design of area-based management tools, including MPAs (CBD, 2009; Lagabrielle et al., 2014). In order to be sustained in the long-term, threatened populations in one locality need to be replenished by a source population to which they are connected to via dispersal or migration. Thus, risk of (local) extinction and the resulting loss of biodiversity can only be realistically assessed when connectivity is understood while each taxon group presents their own challenges when trying to infer connectivity (e.g., difficulties with DNA amplification, complex taxonomy, low abundances, inability to sort bulk sediment samples into individual specimens, etc).

In this review we provide the current state of knowledge of connectivity and address the major impediments to examining it for the major benthic faunal size classes in the CCZ. We examined published literature focused on megafauna, macrofauna, meiofauna, Foraminifera, and microbes with the aim of revealing shared or contrasting beta diversity patterns. Finally, we present the characteristics of an idealized connectivity study to guide future research.

3 Megafauna

The largest animals of the benthos, i.e., megafauna (>1 cm), are also known to be the least abundant, particularly at abyssal depths (Rex et al., 2006). This low abundance, combined with high diversity, has largely constrained the study of connectivity in CCZ megafaunal populations. This is due to the difficulty of sampling

specimens of the same species across locations, as well as the limited baseline knowledge to which newly collected specimens can be compared. Studies based on specimens collected by Remotely Operated Vehicles (ROVs) from multiple phyla in the eastern CCZ (Exploration area UK-1), have shown that typically less than 30% of specimens matched published morphological or genetic descriptions, the remainder being new to science (Dahlgren et al., 2016; Amon et al., 2017a, b; Rabone et al., 2023). Using both morphological and molecular evidence, 55 specimens in the western CCZ (APEI 1, 4, 7) were classified into 48 morphotypes belonging to five phyla, from which only nine were known species, and 39 potentially new to science (Bribiesca-Contreras et al., 2022). With the exception of two ophiuroid taxa (Laming et al., 2021), we still know very little about reproduction strategies or larval typology of CCZ megafauna, as collecting and linking larvae to described adult specimens is extremely challenging at abyssal depths (Kersten et al., 2019). There is a pressing need for detailed taxonomic studies incorporating genetic data (e.g., Bribiesca-Contreras et al., 2022; Christodoulou et al., 2020; Kersken et al., 2019) to expand reference sequence databases and to build robust species inventories to support connectivity research of CCZ megafauna.

To aid molecular connectivity analyses, image-based morphological characterization of megafauna has been applied as a proxy to study community distribution patterns at increasingly larger spatial scales across the CCZ. This approach counters the lower taxonomic resolution of image-based identification, partially addressed using protocols to deal with uncertainty (Horton et al., 2021, 2024), with large numbers of spatially widespread morphospecies occurrences obtained from seabed surveys (Amon et al., 2016; Vanreusel et al., 2016; Simon-Lledó et al., 2019, 2020). From these we know that at short distances (<30 km) communities largely differed between seamounts and adjacent abyssal plains (Cuvelier et al., 2020; Durden et al., 2021); the majority of morphotypes (60-70%) found on CCZ seamounts were typically unique, suggesting limited connectivity between the two. At local scales, distances <10-20 km, community assembly patterns appear to be strongly regulated by niche partitioning mechanisms associated with seabed topography (Simon-Lledó et al., 2019), nodule abundance (Amon et al., 2016; Simon-Lledó et al., 2019) or even the presence of small rock-outcrops (Mejía-Saenz, et al., 2023); features that largely vary within the limits of contract areas and APEIs (see Jones et al., 2021). Even though community structure can differ at distances <300 km (Simon-Lledó et al., 2020; Durden et al., 2021; Jones et al., 2021), ~40% of megafauna morphotypes were typically shared across different abyssal plain locations on a regional scale (<1000 km).

At ocean basin scale (<5000 km), methodological standardization and taxonomical alignment of >50000 megafaunal specimen occurrences from seabed imagery across 14 sites identified a total of 411 distinct megafaunal morphospecies (APSMa catalogue v1, <https://zenodo.org/records/8172728>, <https://zenodo.org/records/8172728>). From these, only five were found across all 14 sites; four sponge and one anemone species, i.e., the carnivorous demosponge *Axoniderma mexicana* sp. inc., the stalked hexactinellid *Hyalonema tylostylum* sp. inc., the discoidal-flat

hexactinellid *Docosaccus maculatus* sp. inc., the nodule encrusting demosponge *Thenea* sp. indet, and the anemone Hormathiidae gen. indet. Eight morphospecies were shared between 13, 12, 11, and 10 locations while 52 were found only in two locations and 124 were singletons. Most remarkably, phylum-level taxonomic replacements revealed the existence of a lower and upper abyssal biogeographic province in the CCZ, likely separated by the large-scale niche-filtering effect of the calcite carbon compensation depth above and below 4500 m, respectively (Simon-Lledó et al., 2023). Additional macroecological analyses of the APSMA dataset suggest an association between species life-habits and regional dispersal patterns in CCZ communities (Simon-Lledó et al., 2025). Sessile taxa that only grow on nodules exhibited significantly higher species turnover than sediment-dwelling or swimming megafauna, suggesting a more restricted dispersal ability in nodule epifauna (Simon-Lledó et al., 2025). However, while these provide an empirical basis to direct future exploration or formulate new hypotheses, outcomes of image-based studies must be coupled with more extensive specimen collection and subsequent molecular analyses (e.g., barcoding) to address the real genetic connectivity among CCZ megafaunal populations, and thereby the congruence between both approaches.

4 Macrofauna

Deep-sea macrofauna is defined as organisms retained on a 300 µm mesh sieve. The macrofauna *sensu stricto* excludes dominant meiofaunal taxa (e.g., nematodes, copepods), while the macrofauna *sensu lato* includes all metazoans (Hessler and Jumars, 1974; Lins et al., 2021). Another distinction is made between the macro-infauna, living in the sediments, and the epibenthic fauna or suprabenthos living within the benthic boundary layer. Macro-infauna and suprabenthos of the CCZ are typically sampled with a box corer and epibenthic sledge, respectively. The macrofauna *sensu stricto* is dominated by polychaetes, tanaids and isopods, although their relative contributions and species composition vary according to sampling gears and habitats (Kaiser et al., 2023).

4.1 Polychaeta

Polychaetes are the most abundant and diverse taxon in macro-infaunal assemblages of the CCZ (e.g., Kaiser et al., 2024; Washburn et al., 2021b). Suprabenthic species are less prominent, and mostly belong to the family Polynoidae (Bonifácio et al., 2021). Polychaete connectivity in CCZ has been addressed at community level through beta diversity patterns, and at species level through spatial genetic structure. In a quantitative study of the macro-infauna including four contract areas in the eastern CCZ (BGR, GSR, IOM, IFREMER) and APEI3 (Figure 1), Bonifácio et al. (2020) found a significant decay of community similarity by distance across contract areas but only when omitting APEI3 from the analysis. As contract areas and the APEI3 are separated by the

Clarion fracture, it may act as a dispersal barrier for polychaetes. Out of 275 macro-infaunal polychaete morphospecies, only one was shared among all five sites, two were shared between APEI3 and at least one contract area, but 104 were shared by at least two contract areas (Bonifácio et al., 2020; Washburn et al., 2021b). A different pattern arose from suprabenthic polychaetes, particularly polynoids (Bonifácio et al., 2021; Bonifácio and Menot, 2018). Contrary to macro-infaunal polychaetes, polynoids were more abundant and diverse in APEI3 than BGR, GSR and IFREMER. From a total of 95 morphospecies, 11 were shared between the APEI and at least one contract area, the same number was shared among contract areas but not with the APEI. Across the contract areas, the rate of morphospecies turnover was estimated between 0.04 to 0.07 morphospecies/km, with corresponding mean geographic range of 14–25 km (Bonifácio et al., 2020), which would fall at the upper limit (5–14 km) of the expected length of potential mining fields (Volkman and Lehn, 2018). Species turnover was largely driven by singletons, which represented about half of the specimens (Bonifácio et al., 2020; Stewart et al., 2023). The study of spatial genetic structure is so far limited to eight widespread and abundant species, including five infaunal species, one nodule-dwelling species and two suprabenthic species (Bonifácio et al., 2021; Stewart et al., 2023). Even for these abundant species, the sample size remained small due to low faunal densities in the CCZ, with number of specimens ranging from 6–146 where the species were most abundant, and 1–3 where the species were least abundant. As species delimitation was the primary purpose of genetic analyses, population connectivity was inferred from haplotype networks of the mitochondrial barcoding genes Cytochrome Oxidase I (COI) and 16S (Bonifácio et al., 2021; Stewart et al., 2023). All eight species showed high haplotype diversity but no phylogeographic structure, suggesting high connectivity across the CCZ and even beyond. The reproductive mode was inferred from egg size for three species, two of which were likely brooders, and one was likely a broadcast spawner (Stewart et al., 2023).

4.2 Isopoda

Few studies have focused on molecular connectivity of isopods in the CCZ. Whereas first attempts provided a general assessment of isopod diversity using the COI fragment (Janssen et al., 2015), a follow-up study investigated select species to specifically address connectivity (Janssen et al., 2019). However, no isopod species were found beyond the margins of a single contract area. Whereas later studies were limited to the BGR, GSR and IFREMER contract areas, a more recent species delimitation approach was carried out including the aforementioned contract areas but also the IOM, the DISCOL experimental area (Peru Basin) and APEI3 using the 16S fragment in addition to COI (Brix et al., 2020). The authors showed that connectivity between areas largely depended life history. Whereas less mobile species tended to show a rather limited distribution range, more mobile species from the families

Munnopsidae and Demosomatidae were found in samples up to 5000 km apart (Brix et al., 2020). They also showed that isopod assemblages in contract areas within the CCZ were more similar to one another than they were to APEI3 or the more distant DISCOL area. Still, the number of shared species between areas was limited; of the 170 Molecular Operational Taxonomic Unit (MOTUs) just three were shared between four areas, seven between three areas and 11 between two areas. The areas with the highest number of shared MOTUs were the BGR and GSR area with 13 and 14 shared MOTUs, respectively (Brix et al., 2020). Only two species were found in all five areas. Notably, one of these, which was morphologically identified as *Macrostylis metallica* (Riehl and Smet, 2020), belonged to the burrowing family Macrostylidae. In general this family showed the narrowest distribution, rarely exceeding the boundaries of a single contractor area. Considering only morphology, *M. metallica* was found in five different areas. However, this species showed high genetic variability resulting in the delimitation into three different MOTUs while exhibiting minimal morphological variation. This suggests *M. metallica* constitutes a cryptic species complex. Another species from the partly-swimming family Nannoniscidae distributed across five contract areas was reported and described as *Nannoniscus hilario* by Kaiser et al. (2021). Bober (2018) showed genetic connectivity of the swimming Munnopsidae between the CCZ and Atlantic waters for *Acanthocope galathea* (Wolff, 1962), which occurs across the Atlantic (Malyutina et al., 2018). Studies on CCZ isopods (reviewed by Kaiser et al., 2023) so far have shown that the majority of species exhibited limited distribution ranges in the CCZ with most being found in a few or a single contract area. Nevertheless, wider distributions of several thousand kilometers have also been recorded, often correlating with isopod lifestyle.

4.3 Ophiuroidea

Ophiuroids are one of the most abundant macro- and megafaunal groups found in the CCZ (Simon-Lledó et al., 2023). The most comprehensive barcoding study using COI to assess ophiuroid diversity was carried out by Christodoulou et al. (2020), analyzing 543 specimens from five contract areas (UKSRL, BGR, IFREMER, GSR, IOM), APEI3 as well as the DISCOL area in the Peru basin. This assessment resulted in 43 species of which 19 were restricted to a single sampling area. Some of these were shown to belong to ancient deep-sea lineages (Christodoulou et al., 2019). The different contract areas shared on average six species ranging from three (IOM and IFREMER) to 14 (BGR and UKSRL) while even the most distant sites (e.g., IFREMER and BGR, IFREMER and UKSRL) shared at least four species. Faunistic assemblages of the contractor areas were surprisingly similar, especially when compared to APEI3 which shared only up to two species with the contractor areas and none with the DISCOL area. The most common species of the study were Ophioleucidae sp., *Silax daleus* (Lyman, 1879) (reported as *Amphioplus daleus* in Christodoulou et al., 2020) and *Ophiosphalma glabrum* (Lütken & Mortensen, 1899), which were

present in all areas except APEI3. *Ophiosphalma glabrum* likely possess lecithotrophic larvae which would allow for long-range larval dispersal independent of resource availability (Laming et al., 2021). One of the species has been described as *Ophiotholia saskia* by Eichsteller et al. (2023) and has since then been collected in the IOM, BGR, UKSRL and NORI contract areas. *Ophiotholia supplicans* (Lyman, 1880) was analyzed and collected from UKSRL, NORI, BGR and OMS contract areas and was also reported from APEI3 and DISCOL. Molecular species delimitation, however, indicated high molecular diversity in *O. supplicans*, resulting in the division of several closely related MOTUs. Thus, it is unclear whether this is one widely distributed species with high genetic variability or several different, locally restricted species.

5 Meiofauna

5.1 Copepoda

Copepods are small crustaceans which make up a significant part of the meiofauna with most of them belonging to the group Harpacticoida. In the CCZ they represent around four to ten percent of the metazoan meiofauna in terms of abundance when including nauplii (Hauquier et al., 2019; Pape et al., 2021; Tong et al., 2022). Despite their high abundance, the diversity and connectivity of benthic copepods in the CCZ is not well understood. Morphological data from Pape et al. (2021) compared copepod species composition and diversity between three stations in the GSR contract area, one nodule-free and two nodule-rich, finding that no copepod species occurred in all three sites, 89% was confined to one site, 63% of all species were singletons, and 12% were doubletons. Very little genetic data were available until a recent study by Rossel et al. (2022) published 1296 COI barcodes. However, this study focused only on the BGR contract area. Even within this dataset, MOTUs were represented by only a few specimens. Irrespective of the applied delimitation method, diversity was high with species numbers ranging from 718 to 794. Notably, over a third of the specimens belonged to singleton MOTUs. Only nine MOTUs were represented by ten or more specimens. Comparing the aforementioned DNA sequences to genetic material from the CCZ that have been published in earlier phylogenetic studies (Khodami et al., 2017, 2019) and species descriptions (Mercado-Salas et al., 2019) revealed only one potential species of hyperbenthic cyclopoids, *Euryte* sp., with a distribution beyond the BGR contract area. It was found in both the BGR and IFREMER contract areas, hundreds of km apart (Khodami et al., 2019). In another study on meroplankton that included genetic analyses from the OMS and UKSRL contract areas using COI, 18S Small Sub-Unit (SSU) V1-V2 and V7-V8 markers, six MOTUs with distributions extending beyond these areas were found (Kersten et al., 2019). Of these, one occurred in the OMS, UKSRL, and BGR areas; another in the OMS and BGR areas; one from the UKSRL area was also found in the BGR area; and another in the IFREMER area. Interestingly, one V1-V2 sequence from the

OMS and UKSRL areas matched a sequence from the Kuril Kamchatka trench with 99.72% identity. A V7-V8 sequence from the UKSRL area had 100% identity with *Cyclopina agilis* from the western Atlantic coast. However, the accuracy of species-level identification using the V7-V8 marker for copepods is questionable (Wu et al., 2015). It may only be reliable for distinguishing genera or families, suggesting the need for further investigation using alternative genetic markers.

The species description of *Siphonis ruehlemani* (Mercado-Salas et al., 2019), included records from the eastern BGR area, and the IFREMER area. The species *Cerviniopsis longicaudata*, originally described from deep waters off Norway, was also found in the CCZ and genetically analyzed by Khodami et al. (2017) from the BGR area. Another specimen with a matching COI barcode was found in the same region years later (Rossel et al., 2022). One new species described by Gheerardyn and George (2019) from the GSR contract area was also in the Porcupine Abyssal Plain in the eastern Atlantic Ocean. Other benthic copepod species described from the CCZ (Republic of Korea area) by Cho et al., 2016 have not been reported elsewhere. Because specimens of new deep-sea species are rare, DNA analyses are sometimes neglected in taxonomic descriptions in order to maintain an intact specimen which would otherwise be consumed during DNA extraction.

Few tentative conclusions can be made regarding the connectivity of benthic copepod species within the CCZ based on DNA barcoding or morphological data. Certain species were shared between distant contract areas, suggesting that the distribution of these microscopic animals across large expanses of abyssal plains is possible, indicative of long range connectivity. Nevertheless, it is currently impossible to conclude on any abiotic factors or life history traits influencing the degree of connectivity. In general, looking at smaller spatial scales, species are rare yet intensified and extended sampling may reveal connectivity to be more extensive.

5.2 Nematoda

Nematodes are the most abundant metazoans in the CCZ, constituting over 80% of the infaunal assemblages (Hauquier et al., 2019; Pape et al., 2021; Pape et al., 2017). While a number of morphological studies of meiofauna have been conducted in the area (Tong et al., 2022; Min et al., 2018; Renaud-Mornant and Gourault, 1990; Uhlenkott et al., 2020) these typically do not provide taxonomic information beyond phylum-level for nematodes. When nematodes are the specific group of study, the resolution is commonly restricted to genus-level (Radziejewska et al., 2001) due to paucity of diagnostic characters and phenotypic plasticity (De Ley et al., 2005; Derycke et al., 2010), with just five studies providing (morpho)species-level identifications (Lamshead and Boucher, 2003; Hauquier et al., 2019; Pape et al., 2017; Pape et al., 2021; Miljutin et al., 2011; Miljutina et al., 2010). Due to the time-consuming process of mounting specimens on microscope slides, typically around 100 nematodes from each sample are chosen at random for genus and species identification. Lamshead and

Boucher, (2003) sorted 3321 specimens into 200 morphospecies. From these, 63% (12/19) of the dominant species (>1% relative abundance) were found across their five sampling stations, spanning a latitudinal gradient from the equator up to 23° North, suggesting that the Clarion and Clipperton fractures do not pose an impediment for connectivity. Miljutina et al. (2010) identified 2114 specimens into 325 morphospecies and reported significant differences between nodule-bearing and nodule-free sites of the IFREMER contract area. Even so, a substantial fraction of morphospecies (44%, 144/325) were shared between the two, meaning that connectivity exists between areas with and without nodules. Similarly, Pape et al. (2021) observed that 6 out of 11 species of the genus *Halalaimus* were shared between nodule-free and nodule-bearing sediments in the GSR contract area. Hauquier et al. (2019) also focused on species of the common deep-sea genus *Halalaimus* which typically represents between 3–11% of the nematode community. Out of 143 specimens, 24 species were identified of which five were found in the APEI3, IFREMER, GSR, IOM, and BGR contract areas. Although this would suggest that connectivity does exist across such large distances, these taxa were represented in low numbers (~ 6 specimens/species) raising the question whether these areas could function as a source of population replenishment following disturbance. Additionally, six of the 14 *Halalaimus* species identified from the GSR contract area had been reported before for the DISCOL area (Pape et al., 2017), indicative of potential connectivity. A consistent finding across all studies was that *Acantholaimus*, *Desmoscolex*, *Halalaimus* and *Thalassomonhystera* were the most widespread and species-rich genera. As in the Peru Basin (Thiel et al., 1993; Bussau et al., 1995), nematodes dominated the metazoan meiofauna inhabiting the crevices of polymetallic nodules of the GSR contract area (Pape et al., 2021). The same dominant taxa were found in both nodule crevices and sediments (i.e., *Monhystrella*/*Thalassomonhystera*), though genus composition differed significantly. About 25% of the genera were unique to either substrate, but none were endemic to nodule crevices. These differences in genus composition corresponded to differential feeding strategies as inferred from buccal morphology, with more tooth-bearing nematodes in the nodule crevices. The divergence between meiofauna communities increased with higher taxonomic resolution (higher taxon → nematode genus), hinting at even greater differences between substrates at the species level, but this remains to be investigated.

The vast majority of deep-sea nematode species are undescribed, leading to significant deficits of taxonomically informed reference sequences in public databases such as GenBank. Resultantly, DNA-based investigations of CCZ nematodes are few and do not resolve beyond phylum- (Laroche et al., 2020; Lejzerowicz et al., 2021) or genus-level (Macheriotou et al., 2020) and tend to have a large proportion of taxonomically unassigned Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs), (Callahan et al., 2017). Macheriotou et al. (2020) targeted the 18S rRNA locus (V1–V2) to generate ASVs; the majority were unique to each area and two (*Acantholaimus_sp* & *Daptonema_sp*) were shared between the BGR, IOM, GSR,

IFREMER and APEI3 while 15% (300/1981) were shared by at least two areas.

Generally, the morphological data point to a degree of connectivity which exceeds that of DNA-based assessments, however one can only speculate whether this would increase or decrease if all specimens in a sample were identified rather than a subset. Moreover, cryptic speciation known for (deep-sea) nematodes (e.g., Smith et al., 2008) may be resulting in an overestimation of shared species between areas while intraspecific variability in morphological characters would result in the reverse (Miljutin and Miljutina, 2016). Cosmopolitanism has been suggested for some nematode species (Bik et al., 2010; Miljutin et al., 2010; Zeppilli et al., 2011); thus high connectivity within the CCZ alone may not be implausible. Similarly, the metabarcoding analyses showed that identical genetic material, most likely belonging to the same species, was found at distances hundreds of km apart, hinting at long range connectivity.

6 Foraminifera

More than 50% of meiofaunal biomass and abundance in abyssal plains is represented by protists (Gooday et al., 2021) with benthic foraminifera being a major contributor. Extensive research over the last decades in the central North Pacific has revealed the striking diversity of this group (Gooday et al., 1992, 2017, 2021). In the different CCZ areas (Figure 1; UK1, OMS, BGR, GSR, IOM, IFREMER, YUZH, APEI1, 3, 4, 6 and 7) on average 75% of foraminiferal species are represented by monothalamiids, characterized by slow growth and metabolic rates. These play an important role in carbon cycling as they feed on refractory organic matter, making it available for higher trophic levels. The ability to feed on sparsely distributed food sources such as bacteria and degraded organic matter is considered to be one of the reasons why these soft-shelled forms are so successful in oligotrophic environments (Gooday et al., 2001). However, rarity, combined with under-sampling, precludes any statements regarding endemism of monothalamiids in the CCZ (Goineau and Gooday, 2019). Xenophyophores are also an abundant component of CCZ megafauna (Gooday et al., 2017; Simon-Lledó et al., 2019) with many species being represented by 1-2 specimens and are known from a single site, rendering any inferences of connectivity impossible (Gooday et al., 2020). Nonetheless, shared occurrences across 3800 km (APEI4 to UK-1/OMS) have been confirmed using barcoding for *Aschemonella monile* and *Moanammia semicircularis* (Gooday et al., 2020). Sessile Foraminifera, especially encrusting forms, are very abundant on polymetallic nodules (Veillette et al., 2007; Gooday et al., 2015) with up to 75 morphospecies reported to occur on just seven nodules (Gooday et al., 2015). Whether these mat-like Foraminifera occur in different CCZ areas with common genetic patterns has not yet been explored, due their complex taxonomy. Most benthic Foraminifera are widely distributed at regional scales (i.e., UK1, OMS, BGR, GSR, IOM, IFREMER, Yuzhmorgeologiya, APEI1, 3, 4, 6 and 7) but not necessarily at a global scale. Both morphological and DNA-based

approaches highlight rarity with singletons often representing up to 30% of the community (Goineau and Gooday, 2017). Their occurrence seems to be positively correlated to nodule abundance and size. Foraminiferal dispersal is driven by propagules which are easily transported by bottom currents after death. Consequently, benthic foraminiferal taxa could hypothetically be transported globally, resulting in cosmopolitan species (Gooday et al., 2021). Distribution similarities at regional scale is often observed despite a large number of singletons. Even though particular taxa have wide ranges, there seems to be a gradual shift in the community composition in relation to environmental gradients, such as productivity and nodule abundance (Gooday et al., 2021). The carbonate compensation depth (CCD) also plays a major role in defining foraminiferal distribution, restricting calcareous species. The CCD is situated at 4500 m in the southern and north Pacific, deepening to ~5000 m near the equator, yet shifting upward in the water column due to global warming (Sulpis et al., 2018), resulting in high dominance of non-calcareous Foraminifera in the CCZ (Gooday et al., 2021). Finally, the ability of Foraminifera to reproduce asexually may allow them to persist at very low abundance without the need for propagules to be introduced from elsewhere. As such, and considering potential patchiness of communities, the observed singletons could reflect pseudo-endemism due to under-sampling (Gooday et al., 2021).

7 Microbiota

Microbial connectivity in the CCZ remains poorly understood, with most studies focusing on microbial communities in seawater, sediments, and polymetallic nodules at local to regional scales (Tully and Heidelberg, 2013; Wu et al., 2013; Shulze et al., 2016; Lindh et al., 2017; Hollingsworth et al., 2021). Larger-scale syntheses have analyzed datasets spanning 10 to 4000 km, revealing substrate-specific microbial compositions (Wear et al., 2021; Orcutt et al., 2024). Different physical properties of nodules and sediments foster distinct habitats; thus, connectivity between these substrates likely results from passive transfer rather than taxon establishment. Given these complexities, the microbial assemblages associated with sediments and nodules are reviewed separately.

7.1 Sediment microbiota

Microbial diversity in CCZ sediments has been studied to establish baselines for mining impact assessments, however, microbial biogeography and connectivity remains poorly understood. Most data come from local to regional scales (10-1500 km) in the eastern CCZ (Shulze et al., 2016; Lindh et al., 2017; Hollingsworth et al., 2021). Their findings suggest high dispersal rates and low compositional turnover, consistent with minimal physical barriers and low sedimentation rates reducing microbial isolation (Washburn et al., 2021a). Community composition showed little variation with distance and was not significantly

influenced by environmental selection, suggesting dispersal mitigates effects of mutation and drift (Hanson et al., 2012). Rare taxa drive community shifts at local scale, indicating spatial and temporal variability in ecological processes (dispersal, drift, selection, mutation) for taxa with different population sizes (Molari, unpublished data). Rare taxa may result from stochastic fluctuations or niche specificity, contributing to microbial adaptiveness (Jousset et al., 2017). Understanding their connectivity and function is key to assessing sediment microbe resilience in polymetallic nodule ecosystems.

Wear et al. (2021) examined benthic samples across 10–4000 km in CCZ, combining new (western CCZ) and existing data. Despite being the most comprehensive study, limitations remain due to differing sampling methods, PCR primers, sequencing techniques, and the absence of central CCZ data. Geographical distance had a weak effect on microbial beta diversity, with shifts mainly in relative taxon abundance rather than composition. The CCZ is characterized by a gradient in primary productivity, organic carbon flux, and sediment organic content decreasing southeastward (Pennington et al., 2006; Lutz et al., 2007; Volz et al., 2018). Microbial abundance shifts may reflect preferential growth under varying trophic regimes. Potential heterotrophs (Woeseiaceae, Dehalococcoidaceae, Acidimicrobiaceae) and nitrifiers (Nitrosomonadaceae, Nitrosopumilaceae, Nitrospiraceae) exhibited habitat preference across the CCZ trophic regimes (Hollingsworth et al., 2021; Wear et al., 2021). Additionally, local microbial community shifts have been observed in presence of different seafloor topographic features (ridges, troughs, deep plains) and nodule coverage (Hollingsworth et al., 2021; Molari, unpublished data), indicating that habitat heterogeneity, alongside trophic regimes at large spatial scale, shapes community structure at local scale.

7.2 Polymetallic nodule microbiota

Polymetallic nodules harbor distinct microbial communities composed of taxa enriched from surrounding seawater and sediments rather than unique species (Wu et al., 2013; Tully and Heidelberg, 2013; Blöthe et al., 2015; Shulze et al., 2017; Lindh et al., 2017; Molari et al., 2020; Wear et al., 2021; Orcutt et al., 2024). Some manganese-oxidizing and -reducing bacteria, potentially involved in nodule formation, have been proposed as core microbiota (Wu et al., 2013; Blöthe et al., 2015), but their presence is inconsistent across studies (Tully and Heidelberg, 2013; Shulze et al., 2017; Lindh et al., 2017; Molari et al., 2020; Wear et al., 2021; Orcutt et al., 2024). It should be noted that this discrepancy might be due to the lack of standards in the study of microbial community associated to the nodules (e.g., part of the nodule investigated, DNA isolation procedure, and sequencing depth). Nodules exhibit higher beta diversity than sediments (e.g., Molari et al., 2020; Wear et al., 2021), but community composition is influenced more by nodule microhabitats than geographic location or time (Tully and Heidelberg, 2013; Blöthe et al., 2015; Wear et al., 2021; Molari, unpublished data). The upper surface, exposed to bottom water and fauna (Vanreusel et al., 2016), differs from the lower side embedded

in the seafloor, where interactions with sediment biogeochemistry and infauna occur. This habitat heterogeneity is likely an important driver for beta diversity. Additionally, the nodules internal structure, mineral composition, and complex pore system (up to 120 m² g⁻¹) provide diverse microhabitats (Blöthe et al., 2015). Microbial cell abundance decreases up to two orders of magnitude from exterior to interior (Shiraishi et al., 2016), further supporting nodule zonation. Thus, it is reasonable to hypothesize that the interplay of ecological processes controlling microbial connectivity may vary across different nodule regions and act at different spatial and temporal scales. For example, microbes on the upper surface of the nodule could follow the biogeographical patterns of sediment communities influenced by trophic regimes, potentially explaining shifts in nitrifying taxa (Nitrosococcaceae, Nitrosomonadaceae, Nitrospiraceae, Nitrosopumilaceae) (Molari et al., 2020; Wear et al., 2021; Molari, unpublished data). In contrast, variations in potential metal-cycling bacteria (Hyphomicrobiaceae, Magnetospiraceae, Methylobacteriaceae, Kiloniellaceae) (Molari et al., 2020; Molari, unpublished data) may be linked to nodule mineralogy, suggesting biogeographical patterns are shaped by nodule formation processes.

8 The ideal connectivity study

As mentioned previously (Section 2), deep-sea research specifically aimed at assessing connectivity is a challenging endeavor requiring a dedicated investment of time and resources. Here we outline the information that would be required to complete the “idealized connectivity study” for fauna and microbes (Table 1).

9 Call to action

Even though the deep sea has not escaped the breadth of human impacts (e.g., oil and gas exploration, litter, radioactive waste disposal, pollution), it remains a largely pristine ecosystem of which only a very small fraction has been thoroughly explored (Mengerink et al., 2014). For it to remain as such and to maintain its ecosystem functions, the deep sea needs to be protected; and in order to be protected, it needs to be understood. This understanding is crucial for marine spatial planning through the designation of areas that will be spared from deep seabed mining and the adoption of a layout of mining operations that reduces harmful effects. Thus, addressing the gaps of knowledge relating to marine connectivity in the CCZ is timely and of paramount importance.

The International Union for the Conservation of Nature (IUCN) has called for a moratorium on deep seabed mining unless and until certain conditions have been met such as conducting rigorous and transparent impact assessments, the projected impacts being understood, livelihoods of interested parties being considered, implementation of the precautionary principle and ensuring the effective protection of the marine environment (IUCN, <https://iucn.org/>, Resolution 122). While a large fraction of the research devoted to MPA design has been

TABLE 1 Information required for a comprehensive connectivity study for fauna and microbes.

FAUNA			
Parameter		Required input	Target
Sampling	Locality	TOC, C to N ratio, phytopigments, porosity, nodule abundance, POC fluxes, DOC, nutrients, depth, seafloor topography, granulometry, current regime	Specimen/eDNA/ Community DNA
	Scale	Local: 0-5 km apart Regional: 100-1000 km apart	
	Frequency	Seasonal and multi-annual	
Diversity	Occurrence	Minimum 2 localities	
	Abundance	Minimum 10 individuals per species per locality	
Ecology	Life history	Propagule pelagic duration	
		Fecundity	
		Spawning periodicity and seasonality	
		Lifespan	
		Age at maturation	
MICROBES			
Parameter		Required input	Target
Sampling	Locality	TOC, C to N ratio, phytopigments, porosity, nodule abundance, POC fluxes, solid and dissolved metals, DOC, nutrients, depth, seafloor topography	eDNA/eRNA
	Scale	Local: 0-5 km apart Regional: 100-1000 km apart	
	Frequency	Seasonal and multi-annual	
	Sediment Polymetallic nodule	Minimum 1 st cm of sediment Upper (exposed to bottom water), inner (core) and lower (in contact with sediment) part of polymetallic nodule	
Diversity	Occurrence	Minimum 5 biological replicates per locality	eDNA/eRNA
	Abundance	Minimum >0.01% of the total reads per lowest taxonomic unit (e.g., ASVs) per polymetallic nodule or sediment slice and locality	eDNA
		Minimum 10 reads per lowest taxonomic unit (e.g. ASVs) per sediment or polymetallic nodule or sediment slice and locality	eRNA
Ecology	Activity	Identification of live taxa (e.g., based on rRNA) or viable cells (e.g., based on membrane integrity)	eDNA
	Habitat	Habitat specificity inferred from relationship with environmental variables	eDNA/eRNA
	Life history	Metabolism and physiology inferred from genomic and transcriptomic investigations	eDNA/eRNA
Sequencing	Amount Quality	>10 ng/μL in > 25 μL after purification	eDNA
		>1 ng/μl in > 50 μL after purification and removal of DNA Integrity value (RIN) > 5	eRNA
	Depth	Minimum 50000 reads per gene target and sample	eDNA
		Minimum 30 million reads per sample for ribosomal RNA (rRNA) based study or for messenger RNA (mRNA) based study	eRNA

eDNA, DNA extracted from a sample without isolating any organisms; eRNA, RNA extracted from a sample without isolating any organisms; Community DNA, DNA extracted from mixtures of organisms isolated from their substrate (e.g.: sediment; water); TOC, Total Organic Carbon; C, Carbon; N, Nitrogen; POC, Particulate Organic Carbon; DOC, Dissolved Organic Carbon.

derived from fisheries sciences and thus focused on fish species or coral reefs (e.g., [Krueck et al., 2017](#); [Gorud-Colvert et al., 2021](#)), the guidelines and recommendations originating from these works can be used to inform spatial management of the CCZ. The APEIs in this context are surrogates to MPAs.

Within this context, we urge scientists and stakeholders to address the aforementioned gaps of knowledge and most importantly, to distribute the insights gained.

- Marine connectivity must be included in spatial management of the CCZ.

Connectivity within and between individual APEIs as well as between APEIs and mined sites should be verified. The size and spatial arrangement of the mined sites should be such that they do not enclose (larval) source areas and do not intercept dispersal routes in order to allow post-mining recolonization of fauna. Likewise, the planning of mining operations should be guided by the timing of spawning or migration events of taxa if known.

- An appropriate sampling design must be applied.

To comprehensively assess connectivity, it is necessary to overcome the limitations that have marred previous studies: (i) restricted geographical range and limited number of samples from each site; (ii) lack of characterization of the relationship between biodiversity and habitat heterogeneity on continuous spatial scales; (iii) absence of time series covering seasonal and interannual variability. The distribution of samples must be widespread enough to incorporate habitat heterogeneity and the number of samples must be informed by statistical analysis to determine the number of replicates required to achieve the desired level of statistical power. Environmental parameters should be derived from the same samples as the biological data. Finally, the same sites should be re-visited across seasons and years to capture temporal variability. These requirements must be included in the ISA mining code to ensure license holders compliance.

- Detailed habitat characterization is needed.

A comprehensive understanding of the CCZ environment in combination with species occurrences is needed to predict species distribution based on habitat suitability models. This requires high-resolution bathymetric maps, including seafloor topography and nodule density to resolve local spatial heterogeneity, as well as particulate organic carbon (POC) flux to characterize the trophic regime. Bottom water-current data need to be mapped to identify where biota and sediment plumes from mining activities will be transported to. Time-series observations of currents and fluxes will be required to capture temporal variability.

- Efficient, state-of-the art sampling methods must be used.

The boxcorer and multiple corer are the most widely used tools to sample macrofauna and meiofauna respectively, and the only ones recommended by the ISA (ISBA/25/LTC/6/Rev.1). ROVs allow for targeted and opportunistic sampling of mainly megafauna, while Epi-Benthic Sleds (EBS) are needed in order to capture macrofauna in large numbers as well as various life-stages, especially those which are under-sampled. Moreover, the EBS can sample benthic boundary layer communities impacted by the sediment plumes, which are not sampled by corers. Where possible, minimally invasive methods shall be employed (e.g., by combining imaging methods with sample-based validation). A disadvantage of imaging methods is that the small faunal elements caught by EBS are not visible in the image data. Thus, even though it leaves a long-lasting impact on the seafloor, the EBS is the only gear that provides a deeper insight into the “sediment secrets”, the macrofauna not visible by naked eye.

- Genetic tools should be comprehensively applied.

Both nuclear (e.g., 18s rRNA) and mitochondrial genes (e.g., COI) should be applied when investigating population structure in order to be most informative. Although the methodologies described herein do

not represent an exhaustive list of what can be used to investigate connectivity, we can provide suggestions as to which strategies would serve this objective, such as barcoding, metabarcoding and Restriction site associated DNA markers (RADseq). In order for sequence data to be interoperable in time and between different institutions requires that the laboratory and bioinformatic workflows are reported in sufficient detail to be reproducible. Given the rapid development of novel methods, archiving following FAIR principles (Wilkinson et al., 2016) is needed for samples to be suited for future re-analysis (Mayer et al., 2021).

- Ensure the accuracy of DNA-based methods.

It is of the utmost importance to validate and standardize (environmental) DNA extraction methods in order to ensure the comparability and reliability of DNA-based diversity data. The presence of dormant or deceased organisms represents a challenge for DNA-based approaches, which are unable to discriminate between the presence of an individual and successful establishment or between one that is living or dead. Consequently, the application of methods (e.g., based on RNA) for determining the active versus dormant fractions is crucial to better resolve the mechanisms controlling connectivity in CCZ, including the ecological role of rare taxa.

- Distribution of knowledge and data sharing.

Data (e.g., species inventories, genetic sequences, image catalogues), metadata, and protocols must be shared amongst researchers, contractors and the public domain following FAIR principles in order to facilitate comprehensive investigations and robust, reproducible inferences (e.g., consistent morphospecies designations). A good example is the CCZ atlas for megafauna identification (Simon-Lledó et al., 2023) and the Clarion-Clipperton Zone Species Checklist embedded in the World Register of Marine Species (<https://www.marinespecies.org/deepsea/CCZ/>).

10 Conclusions

10.1 Megafauna

Despite their large size, megafaunal taxa in the CCZ remain among the most challenging groups to investigate due to low abundances as well as constraints associated with deep water sampling. Unlike smaller fauna, which can be collected in large numbers during a single expedition, ROV or EBS-based surveys typically retrieve fewer than ~50 megafaunal specimens per cruise, making it difficult to conduct robust genetic analyses or build comprehensive reference databases. As a result, our current understanding of connectivity at large spatial scales relies primarily on image-based numerical ecology. Such investigations have revealed the presence of a strong biogeographic boundary across the CCZ likely driven by the carbonate compensation depth and that life history is linked to dispersal potential. To rigorously assess population structure and dispersal dynamics, it is critical to expand molecular analyses on specimens, integrating barcoding and genomic tools with image-based approaches. This will help determine the extent to which observed distributional patterns

correspond to real genetic connectivity and advance our understanding of the evolutionary and ecological processes shaping megafaunal populations across the CCZ.

10.2 Macrofauna

Macrofaunal taxa exhibited a wide range of connectivity patterns, which may be partly driven by dispersal potential as brooding isopods tend to have the narrowest distribution range with 86% of species unique to a contract area, while the most widespread species tend to be those with the highest adult motility. Polychaetes and ophiuroids whose varied reproductive traits and larval forms are generally unknown or at best inferred for CCZ taxa, reported 65% and 56% of species unique to a contract area, respectively. Many of the species that were unique to a contract area occurred as singletons, suggesting either under-sampling of small local populations or the importance of source-sink dynamics in the abyss. Few species were abundant and widespread enough to investigate genetic connectivity; our results nonetheless suggest the absence of genetic structure across the CCZ. However, mitochondrial genes do not have the resolution needed to assess contemporary genetic connectivity, and highly polymorphic markers such as microsatellites, SNPs or RAD sequencing would be better suited.

10.3 Meiofauna

The available data for meiofaunal taxa (primarily Nematoda and Copepoda) in the CCZ thus far suggest that connectivity does exist between geographically distant regions with an apparent lack of “endemic” taxa. Whether this would remain true given exhaustive sampling remains an open question. Concurrently, there is evidence that contract areas in the eastern CCZ have higher connectivity between them than with nearby APEIs which may be harboring distinct assemblages. To comprehensively study connectivity in nematodes will require the time-consuming effort of firstly sorting specimens into species using morphology prior to any further molecular investigation; unfortunately, this action cannot be bypassed. From this point onward, the application of state-of-the-art methods such as SNPs and RAD-sequencing would be possible to obtain detailed population structure. Moreover, such an approach will enable the discovery of species-specific population structures. Comparative studies between metabarcoding and morphological identifications in copepods suggest that the use of COI can match morphological species occurrences; thus, connectivity can in fact be studied using metabarcoding. However, only a short fragment of the usual barcoding region was used, meaning that this would need to be expanded to allow the assessment of ongoing gene flow. The highest resolution would be provided by the aforementioned SNP or RAD-sequencing approaches.

10.4 Microbiota

Studies of microbial beta-diversity in the CCZ suggest high dispersal capacity and low compositional turnover in sediment communities, likely due to minimal physical barriers and low sedimentation rates. Geographical distance exerts a weak effect, with community shifts primarily driven by trophic regimes at large spatial scales (>1000 km) and by seafloor heterogeneity, resource partitioning, and nodule coverage at finer spatial scales (<30 km). Rare taxa contribute to community shifts at local scales, emphasizing the need to understand their role in microbial resilience within polymetallic nodule ecosystems. Nodules host distinct microbial communities enriched from surrounding sediments rather than unique species, with higher beta diversity than sediments due to microhabitat heterogeneity. Ecological processes controlling microbial connectivity likely interact and operate across multiple spatial and temporal scales within different parts of the nodule. Microbial diversity assessments predominantly rely on genetic markers such as the 16S rRNA gene, with taxa defined either as unique sequences or clustered units based on arbitrary similarity thresholds. While higher taxonomic resolution enhances connectivity assessments, lower-resolution clustering facilitates ecological interpretation but risks biasing evolutionary and ecological distances. The harmonization and standardization of sampling and molecular methods are essential to improve cross-study comparisons, mitigate methodological biases, and enhance the robustness of microbial biogeography assessments.

Overall, the benthic communities of the CCZ are characterized by high diversity, low abundance with predominantly undescribed species. By default, connectivity studies focus on abundant and widespread taxa, leaving knowledge gaps regarding the rare and localized fauna. Across the taxa examined (except for Foraminifera), a core set of species exhibits a wide spatial distribution, whereas the majority appear to have restricted geographic ranges, often dominated by singletons and doubletons. This pattern is not unique to the CCZ but is commonly observed in the deep sea, however, the extent to which it is an artefact of under-sampling remains unknown. Spatial variability in community composition across benthic size classes is at least partially driven by environmental gradients, including nodule abundance, seafloor topography, and food availability while the carbonate compensation depth plays a major role for taxa with a calcareous skeleton. Taxa inhabiting both nodules and sediments, such as microbes and nematodes, exhibit clear segregation in community composition between the two substrates, likely driven by the differing physical properties and ecological conditions. Life-history traits, such as mobility and reproductive strategy, which are more readily assessed for mega- and macrobenthic taxa, play a crucial role in determining connectivity. Even though many studies rely on physical samples or seafloor imagery for morphological identifications, genetic data are necessitated by the presence of cryptic speciation in deep-sea taxa.

Concurrently, the main impediment to using metabarcoding in connectivity research is lack of taxonomically informed DNA reference sequences. Finally, this review is of course not exhaustive; firstly it mainly includes data from the eastern CCZ while a comprehensive understanding of connectivity in this extensive region requires data from the central and western areas. Secondly, not all benthic taxa are included herein (e.g., Porifera, Tanaidacea, Anthozoa, Crinoidea, Holothuria and others); nonetheless, we hereby provide the current state-of-the-art for at least a subset of the CCZ benthic fauna.

Author contributions

LMa: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Methodology. SR: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. MM: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. PM: Funding acquisition, Writing – review & editing, Project administration, Resources, Conceptualization. SB: Visualization, Writing – original draft, Writing – review & editing. MC: Visualization, Writing – original draft, Writing – review & editing. PD: Writing – original draft, Writing – review & editing. AH: Conceptualization, Writing – original draft, Writing – review & editing. FJ: Funding acquisition, Project administration, Writing – review & editing. ES: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. LMe: Funding acquisition, Project administration, Writing – review & editing. EP: Writing – original draft, Writing – review & editing. GR: Writing – original draft, Writing – review & editing. AV: Conceptualization, Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing.

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Conflict of interest

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